Gene expression profiling of adult female tissues in feeding *Rhipicephalus microplus* cattle ticks

Christian Stutzer[a], Willem A. van Zyl[a], Nicholas A. Olivier[b], Sabine Richards[c], Christine Maritz-Olivier[c,*]

[a]*Department of Biochemistry, Faculty of Natural and Agricultural Sciences, University of Pretoria, South Africa, 0002.*

[b]*ACGT DNA microarray facility, Department of Plant Sciences, Faculty of Natural and Agricultural Sciences, University of Pretoria, South Africa, 0002.*

[c]*Department of Genetics, Faculty of Natural and Agricultural Sciences, University of Pretoria, South Africa, 0002.*

[*]Corresponding author:.Tel: +27 012 420 3945; fax: +27 012 362 5327

E-mail address: christine.maritz@up.ac.za

Note: Supplementary data associated with this article.

Note: The microarray and sequence data related to this work is available via the NCBI GEO database, GEO accession no. **GSE35867.**

Abstract

The southern cattle tick, *Rhipicephalus microplus*, is an economically important pest, especially for resource-poor countries, both as a highly adaptive invasive species and prominent vector of disease. The increasing prevalence of resistance to chemical acaricides and variable efficacy of current tick vaccine candidates highlight the need for more effective control methods. In the absence of a fully annotated genome, the wealth of available expressed sequence tag (EST) sequence data for this species presents a unique opportunity to study the genes that are expressed in tissues involved in blood meal acquisition, digestion and reproduction during feeding. Utilizing a custom oligonucleotide microarray designed from available singletons (BmiGI Version 2.1) and EST sequences of *R. microplus*, the expression profiles in feeding adult female midgut, salivary glands and ovarian tissues were compared. From 13,456 assembled transcripts, 588 genes expressed in all three tissues were identified from fed adult females 20 days post infestation. The greatest complement of genes relate to translation and protein turnover. Additionally, a number of unique transcripts were identified for each tissue that relate well to their respective physiological/biological function/role(s). These transcripts include secreted anti-hemostatics and defense proteins from the salivary glands for acquisition of a blood meal, proteases as well as enzymes and transporters for digestion and nutrient acquisition from ingested blood in the midgut, and finally proteins and associated factors involved in DNA replication and cell-cycle control for oogenesis in the ovaries. Comparative analyses of adult female tissues during feeding enabled the identification of a catalogue of transcripts that may be essential for successful feeding and reproduction in the cattle tick, *R. microplus*. Future studies will increase our understanding of basic tick biology, allowing the identification of shared proteins/pathways among different tissues that may offer novel targets for the development of new tick control strategies.

Keywords: Rhipicephalus microplus; DNA microarray; Salivary gland; Midgut; Ovary

1. Introduction

The southern cattle tick, *Rhipicephalus microplus*, is a major pest of cattle in the tropical and sub-tropical regions of the world including the South Americas, southern Asia, Madagascar and the southern and eastern coasts of Africa (Lynen et al., 2008). This species is regarded as the most economically devastating tick species worldwide due to three main factors (Guerrero et al., 2006). Firstly, *R. microplus* is a highly adaptable species that is spreading rapidly to occupy previously unaffected areas, such as the Ivory Coast in western Africa and the Limpopo province in South Africa, even displacing endemic tick species (Tønnesen et al., 2004). Secondly, *R. microplus* is a well described vector for the causative agents of Asiatic redwater (*Babesia bovis*) and bovine anaplasmosis (*Anaplasma* spp.) (de la Fuente et al., 2007; Madder et al., 2007; Lynen et al., 2008). Thirdly, an increase in resistance to all major classes of acaricides has been reported for this species, as well as the occurrence of a strain resistant to the Bm86-based tick vaccine (de la Fuente et al., 2000; Rajput et al., 2006; Li et al., 2007).

Due to the tremendous impact that this species has on animal health, rationales were provided for whole genome sequencing of *R. microplus* (Guerrero et al., 2006). The genome size of the cattle tick has been estimated at 7.1 $x10^3$ megabases with approximately 60% constituting repetitive sequences (Ullmann et al., 2005; Pagel van Zee et al., 2007) and assembly of the genome is currently in its primary stages (Moolhuijzen et al., 2011; Bellgard et al., 2012). However, the BmiGI (*Boophilus microplus* Gene Index) database of 42,512 expressed sequence tags (ESTs) that was derived from various tissues, life stages and geographical strains as well as larvae exposed to various temperatures, host odors and acaricides is available for analysis (Wang et al., 2007).

The BmiGI has been used successfully in high-throughput DNA microarray analyses investigating acaricide-induced gene expression in larvae (Saldivar et al., 2008), organ-specific responses to pathogen infection in male adults (Mercado-Curiel et al., 2011), the transcriptional effects of RNA interference (RNAi)-mediated gene silencing in adult females (Lew-Tabor et al., 2011) as well as responses in feeding larvae and adult females on different cattle species (Rodriguez-Valle et al.,

2010). The vast number of genes that are unannotatable, however, limit the full impact of these studies. The latter is largely due to two factors. Firstly, there is considerable evolutionary distance between ticks and other model organisms, limiting homology-based gene predictions, and secondly limited functional protein data of tick protein sequences is available (Hill and Wikel, 2005; Pagel van Zee et al., 2007).

Papers have been published describing the various sialomes of both hard and soft tick species (reviewed by Mans et al., 2008). However, little is known about the gene expression profiles in the various tissues of feeding adult ticks, especially those involved in blood meal acquisition (salivary glands), digestion (midgut) and reproduction (ovaries). A study using EST sequencing showed that a number of genes are uniquely expressed in both salivary glands and ovaries of feeding, female *R. microplus* (de Miranda Santos et al., 2004). More recently, DNA microarray analyses of the transcriptomes of adult male salivary glands and midgut showed a significant response in gene expression during feeding (Mercado-Curiel et al., 2011). This shows that DNA microarrays are a valid high-throughput approach that can be used to elucidate the underlying molecular processes and biochemical pathways that are involved during tick feeding.

To date, transcriptome analysis has been performed using North and South American, as well as Australian, strains of *R. microplus* (Saldivar et al., 2008; Rodriguez-Valle et al., 2010; Mercado-Curiel et al., 2011). However, gene expression profiling of African strains of *R. microplus* on African cattle breeds is lacking. The latter is of vital importance as control of *B, microplus* may vary dramatically in different geographical areas as seen for the Bm86 vaccine (de la Fuente et al., 2000). Therefore, two aims were addressed in this study. Firstly, a custom oligonucleotide microarray platform was designed for the comparison of gene expression in the salivary glands, midgut and ovaries of feeding adult *R. microplus* females ticks from a Mozambique reference strain on a South African *Bos indicus* cattle breed. Global analyses of gene expression in these tissues demonstrated that a total of 588 transcripts were shared between tissues during feeding, while a number of up-regulated transcripts displayed tissue specificity. Secondly, multiple database sequence similarity

searches and extensive manual curation were employed to functionally annotate transcripts. This study provides a combined functional genomics overview of tissues involved in feeding and reproduction, offering new insights into the complex gene expression profiles related to tissue function and basic *R. microplus* tick biology.

2. Materials and methods

2.1. Tick rearing and sample collection

Rhipicephalus microplus larvae (Mozambique strain, provided by ClinVet Pty. Ltd, South Africa) were allowed to feed on Holstein-Friesian cattle under controlled conditions at the University of Pretoria Biomedical Research Centre (UPBRC), South Africa. Ethical and relevant Section 20 clearances were obtained from the South African Department of Agriculture, Forestry and Fisheries (ethical clearance number: EC022-10), as well as the University of Pretoria Animal Use and Care Committee. Larvae were allowed to advance through their life stages until mature females dropped off the host animal. Ticks were sampled on days 4, 5, 7, 13, 15 and 20. Adult tissues were collected and processed according to the method of Nijhof et al. (2010). The various tissues were stored in TRI REAGENT® (Molecular Research Center, Inc., USA) at -70°C.

2.2. Microarray probe design from R. microplus sequences

Using available EST data from GenBank (http://www.ncbi.nlm.nih.gov/nucest/) and the gene index of *R. microplus* (BmiGI release 2.1) from the Harvard gene index project (http://compbio.dfci.harvard.edu/tgi/tgipage.html), a sequence dataset was assembled from some 60,000 ESTs and 13,643 unique sequences to a final sequence database consisting of 13,456 contiguous sequences, using the online bioinformatic tools, cd-hit-est (http://www.bioinformatics.org/cd-hit/) and cap3 (http://pbil.univ-lyon1.fr/cap3.php). Detection and removal of vector sequences from EST data were performed with the VecScreen tool (http://www.ncbi.nlm.nih.gov/VecScreen/VecScreen_docs.html), using the UniVec database

(http://www.ncbi.nlm.nih.gov/VecScreen/UniVec.html). The final sequence dataset was submitted online for array design using the Agilent 8x15k microarray and eArray microarray design platforms (https://earray.chem.agilent.com/earray/). A set of 60 mer probes, incorporating a 3' bias for possible incomplete cDNA synthesis, was designed for complete representation of all assembled transcripts. Probe quality was assessed from base composition scores and the probes were randomly distributed across the array. Additional quality control probes and housekeeping controls were included. These are elongation factor 1 alpha (GenBank accession no. **EW679365**), H3 Histone family 3A (GenBank accession no. **CV442167**), Ribosomal protein L4 (GenBank accession no. **CV447629**), TATA box binding protein (GenBank accession no. **CV453818**) and subolesin (GenBank accession no. **EU301808**). Sequences of assembled transcripts used for custom array design, as well as microarray data related to this work, are available from the NCBI GEO public database (GEO accession no. **GSE35867**). The customized array slide was manufactured by Agilent technologies (USA).

2.3. Isolation of total RNA and microarray analyses

Total RNA was isolated according to the manufacturer's guidelines for TRI REAGENT® and purified with the RNeasy kit (QIAGEN, USA). Final RNA concentrations, purity and integrity were assessed with the Nanodrop-1000 (Thermo Fisher Scientific, USA) and the Bioanalyzer 2100 micro-fluidics systems (Agilent technologies). A reference RNA pool consisting of equivalent amounts of RNA from each life stage and adult tissue was prepared in order to allow the independent analysis of both immature and mature life stages. Test groups for the current study consisted of tissues (salivary gland, midgut and ovary) collected from 15 partially fed females (day 20), from two biological replicates.

High quality DNase I-treated total RNA was used for cDNA synthesis using SuperScript™ III (Invitrogen™ life technologies, USA), an oligo (dT_{25}) primer (5'-$(T)_{25}$VN-3'; N=ATGC; V=AGC), random nanomers and aminoallyl dUTP for Cyanine 3-dCTP/Cyanine 5-dCTP dye coupling. Template was labeled with Cy3 (reference pool) or Cy5 (test sample), purified using the QIAquick PCR purification kit

(QIAGEN, USA) and the concentration and coupling efficiency determined using the Nanodrop-1000 system (Thermo Fisher Scientific Inc., USA). Equivalent picomoles of Cy3-labeled cDNA from the common reference pool were hybridized with Cy5-labeled individual test cDNA. Both biological and technical replication were employed for each test sample. Hybridization was performed at 65^{o}C for 17 h. Prior to scanning, each slide was washed, rinsed and dried in Stabilization and Drying Solution (Agilent Technologies). Following a drying step, slides were scanned with the GenePixTM 4000B microarray laser scanner (Molecular Devices Inc., USA).

2.4. Microarray data analysis and functional annotation

Fluorescence intensities of hybridized Cy3- and Cy5-labeled probes were extracted using default parameters in the GenepixPro microarray feature extraction software (v6.0, Axon Molecular Devices, USA). Following manual inspection to evaluate spot quality, flagged features were given a zero weight and not considered for further analysis. Normalization within slides was performed using the Limma package in R (hhtp://cran.r-project.org/), employing locally weighted scatterplot smoothing (LOWESS), followed by Gquantile normalization between slides. The Limma package was subsequently used to calculate the $\log_2$ fold change between each group pair-wise comparison, using an empirical Bayes ANOVA to identify significantly differentially expressed transcripts with a *P* value adjusted for multiple comparison false discovery rates. Comparisons with *P* values ≥ 0.001 were ignored. In order to identify transcripts that are organ-specific, only genes with M values > 0, a Cy5 intensity >1,000 and a $\log_2$ fold expression of one or greater in a single organ relative to all other tissues examined, were considered. All transcripts with a $\log_2$ fold expression higher than 2 were considered as highly up-regulated under the current chosen conditions. Transcripts with M values > 0 and Cy5 expression intensity > 1,000, in all of the tissues tested, were considered shared (non-differentially expressed) under the current chosen conditions. In this regard, all transcripts with expression intensity higher than 2,000 were considered strongly shared.

In order to functionally annotate transcripts, the desktop cDNA Annotation System (dCAS) (v.1.4.3) was used (Guo et al., 2009). BLAST searches were done for each transcript against the following databases: GO, KOG, Mit-Pla, NR, Pfam, RRNA and SMART (http://exon.niaid.nih.gov). BLAST search results were stored in a tab-delimited file by dCAS; these were manually inspected and final annotations were based on consensus between two or more databases, using an expected value (E value) cutoff of less than $1x10^{-6}$. All transcript descriptions were based on consensus with database entries from Uniprot (http://www.uniprot.org/uniprot/) and BRENDA (http://www.brenda-enzymes.org/) in the case of enzymes. Transcripts were finally classified into functional groups based on the eukaryotic orthologous group terms for gene ontology (Tatusov et al., 2003).

2.5. Validation of microarray results using relative quantitative reverse transcriptase-PCR (qRT-PCR)

For the verification of gene expression results, transcripts encoded by Contigs 1, 5396, 5672 and 8723 were selected and primer sets were designed for use in relative qRT-PCR analysis (Supplementary Table S1). Using the same RNA isolated for microarray hybridizations, cDNA was synthesized using the iScript™ cDNA synthesis kit (BioRad, Hercules, USA). The cDNA was utilized for relative qRT-PCR using the KAPA SYBR® -FAST qPCR kit (KAPA Biosystems, USA) in a 10 µl final reaction volume containing 2.5 pmoles of oligonucleotide primer pairs corresponding to selected sequences that were differentially expressed. For each primer set a no template negative control reaction was performed. The mRNA levels were normalized against Contig 8723, as well as elongation factor 1 alpha that was previously validated as a reference gene (Nijhof et al., 2009). Relative semi-quantitative PCR was performed in triplicate on a LC480® light cycler (Roche Applied Science, USA) using cycling parameters consisting of an activation step at 95°C for 5 min, followed by 45 cycles of 95°C for 3 s, 55°C for 7 s and 72°C for 1 s. Melt curve analysis consisted of a 95°C hold for 30 s, followed by incubation at 55°C for 30 s and a slow increase to 95°C, with continuous signal measurement. The relative transcript levels for selected genes were evaluated using the extracted Cq values and expressed as a fold change (on a $\log_2$ scale) relative to the reference genes using qBase

(Hellemans et al., 2007). Normalized values were used to calculate the fold change for selected transcripts and linear regression analysis was performed to correlate fold changes calculated by qRT-PCR to the fold changes determined from microarray data.

3. Results and Discussion

3.1. Gene expression in feeding R. microplus adult female tissues

Expression analysis identified 5,175 transcripts differentially expressed among all tissues above threshold, representing 38% of the total number of contigs (Table 1). Less than 13% (1,707 transcripts) displayed significant expression in any given tissue or comparison (Table 1, Fig.1A). A set of 588 transcripts was found to be expressed in all three tissues (Supplementary Table S2). Of these, 135 transcripts were identified with more than double the intensity threshold for expression. In addition, several tissue-specific transcripts were identified that were uniquely up-regulated in salivary glands (171 transcripts), midgut (310 transcripts) and ovaries (417 transcripts) (Fig.1A, Table 1, Supplementary Tables S3-S5). Therefore, a total of 1,005 transcripts were expressed in ovaries, followed by 898 transcripts in midgut and 758 transcripts in salivary glands when shared transcripts are included (Tables 1, 2).

3.2. Microarray data validation by qRT-PCR

For verification of microarray results, relative qRT-PCR was performed on randomly selected transcripts from the tissues tested (Supplementary Table S6). These transcripts were: a putative angiotensin-converting enzyme (Contig 1), a putative mucin (Contig 5396) and an unknown secreted protein (Contig 5672). An additional stably expressed 60S ribosomal protein (Contig 8723) was identified (Supplementary Table S6). This transcript was used as an additional reference gene for data normalization (results not shown). When individual fold change values determined from qRT-PCR analysis were expressed relative to each other, a high degree of correlation (0.96) to microarray data was determined (Supplementary Table S6). An overall concordance was observed in the direction and

magnitude of fold change values obtained. However, it has been stated previously that quantitative gene expression analyses require a large set of reference genes to obtain a stable fold change (Rodriguez-Valle et al., 2010). As only a few reference genes are currently known that could correlate with gene expression data (Nijhof et al., 2009), there is a great need for the identification of new reference genes of different abundance classes (low, medium and high) for use in expression analyses.

3.3. Unique transcripts highly regulated in adult female salivary glands

To obtain a blood meal successfully, evasion of host defences against tick attachment and prolonged feeding time is of primary importance. Around 171 transcripts were identified that were salivary gland-specific, of which 70% could not be functionally annotated or classified into a specific ontology (Fig. 1B, Tables 1, 2, and Supplementary Table S3). The major functional classes that were represented related to defence mechanisms (6.4%), protein turnover (4.7%), amino acid transport and metabolism (3.5%), as well as extracellular structures (3.5%).

3.3.1. Defense mechanisms and blood meal acquisition

Defense proteins represent the major functional class identified for salivary glands comprising 6.4% (11 transcripts) of the total complement of tissue-specific transcripts (Fig. 1B, Table 2, Supplementary Table S3). These include putative protease inhibitors such as serine protease inhibitors or serpins (Contig 1520, Contig 6586, CK178656 and Contig 688) that are similar to those described in other tick species (Anderson et al., 2008; Francischetti et al., 2010; Chalaire et al., 2011). Trypsin inhibitor-like cysteine rich domain-containing (TIL) proteins (Contig 8207) with putative anti-elastase, as well as antimicrobial activities, were also up-regulated (Fogaça et al., 2006; Sasaki et al., 2008). Other protease inhibitors related to the thyroglobulin type-1 repeat containing proteins or thyropins (Contig 300) and the alpha-2-macroglobulin family (Contig 2131 and Contig 1745) were

identified. Thyropins have been proposed to act as inhibitors of cysteine proteases, as well as binding partners for heparin (Anatriello et al., 2010; Francischetti et al., 2010). Alpha-2-macroglobulins are universal protease inhibitors involved in clearance of exogenous proteases and innate immunity. These proteins have been identified in both hard and soft tick species (Saravanan et al., 2003; Buresova et al., 2009). Additional well described defense transcripts that were highly up-regulated in salivary gland tissues included putative lipocalins or tick histamine binding proteins (Contig 2493 and Contig 2529) (Mans et al., 2008).

Antimicrobial defense proteins specific to salivary glands were identified that are orthologous to *R. microplus* cysteine-rich microplusin and defensin peptide proteins (Contig 5482 and CK177092). Defensins have been described from the midgut of *Ornithodoros moubata* (Nakajima et al., 2002), as well as various tissues including the salivary glands of *Amblyomma americanum* (Todd et al., 2007). However, microplusin and defensin proteins have only been localized previously to the fat body, ovaries and hemocytes in *R. microplus* female ticks (Fogaça et al., 2004). An additional novel *R. microplus* transcript (Contig 5501), sharing ~30% sequence similarity with invertebrate astakine cytokines, was uniquely up-regulated in salivary glands. A similar protein sharing 38% sequence identity was previously identified from the sialome of *Amblyomma variegatum* (Ribeiro et al., 2011). Astakines are well described effectors in hematopoiesis in crustaceans, whereas related vertebrate prokineticin homologues have also been implicated in a number of biological processes including smooth muscle contraction (Lin et al., 2011). A putative serine proteinase (Contig 486) was identified as a transcript that may be involved in amino acid transport and metabolism, however this transcript showed high sequence identity (~80%) to a factor D-like transcript identified from *Dermacentor variabilis* that has been suggested to have antimicrobial activity (Simser et al., 2004).

3.3.2. Nutrient transport and metabolism

A group consisting of six transcripts with putative functions in amino acid transport and metabolism, representing 3.5% of the salivary gland-specific transcripts, was identified (Fig. 1B, Table

2). Of these, two transcripts encoding putative amino acid and peptide converting enzymes were highly up-regulated (Supplementary Table S3): an aspartate amino transferase (Contig 1049), as well as an angiotensin-converting enzyme (ACE) (Contig 1) identical to the previously characterized Bm91 protein from *R. microplus* (Riding et al., 1994). ACE is an exopeptidase that plays a role in mediation of extracellular volume and vasoconstriction in mammals, as well as in reproduction in insects (Isaac et al., 1999; Macours et al., 2004). The ACE transcript has one of the highest log expression values of the salivary gland-specific transcripts, correlating with previous findings that this enzyme represents a major membrane component of *R. microplus* salivary glands (Jarmey et al., 1996). The Bm91 protein also showed an improved efficacy in subsequent vaccination trails when used in combination with Bm86 (Willadsen et al., 1996).

Proteases have been implicated in acquisition of the blood meal as anti-hemostatics and anti-inflammatories, extracellular matrix degradation molecules for blood pool formation, as well as possible pre-oral digestive enzymes (Ribeiro et al., 2006; Batista et al., 2008). In line with the latter, putative cysteine proteases containing signal sequences for secretion from the C1 peptidase family (Contig 6614 and Contig 1050) were identified (Supplementary Table S3). Similar proteins have only been identified from midgut tissues of *R. microplus* (Kongsuwan et al., 2010).

A transcript with one of the highest log fold expressions of salivary gland transcripts (3.78), encodes a putative aquaporin involved in water and small neutral solute transport (CV443183). Aquaporins have been shown to be closely involved in salivation (Bowman and Sauer, 2004). However, knockdown experiments in *Ixodes ricinus* did not display a significant phenotype, indicating that additional factors are present (Campbell et al., 2010).

3.3.3. Structural proteins, signal transduction and other proteins

Similar to other studies in salivary glands, we identified putative glycine-rich (GGY) cuticle-related (CV437645) and cement (Contig 2328) proteins that may be involved in structural interactions of the peritrophic matrix between the cuticle and salivary gland tissues, in addition to cement cone

formation during attachment (Francischetti et al., 2010; Maruyama et al., 2010), as well as putative mucins (CV452616 and CV444691) (Francischetti et al., 2010). A vaccination trial using a mucin isolated from female *R. microplus* homogenates could not demonstrate greater protection against infestation compared with that of Bm86 (McKenna et al., 1998).

A unique signal transduction transcript was up-regulated in salivary glands, sharing sequence similarity to mitogen-activated protein kinase kinase kinase 1 (MEKK1) (Contig 4877) (Table 2). Similar transcripts have been identified from other tick sialomic data, however not *R. microplus* (Francischetti et al., 2010). MEKK1 is a signal transducer protein of c-Jun N-terminal protein kinase (JNK) and extracellular signal-regulated kinase (ERK) pathways that are involved in cytoskeleton regulation, cell migration and focal adhesion (Uhlik et al., 2004).

A number of putative proteins involved in protein synthesis and trafficking (CK177403, Contig 5601, CV454094 and Contig 470) were also highly up-regulated in salivary glands that have been previously described from sialomic data of ixodid ticks, including *R. microplus* (Supplementary Table S3) (Francischetti et al., 2010).

3.4. Unique transcripts highly regulated in adult female midgut

The midgut represents the first major internal tick tissue barrier encountered by ingested blood and microbes. Unfortunately over 50% of midgut-specific transcripts could not be functionally annotated (Fig. 1C), while 123 annotatable transcripts were highly up-regulated (Tables 1, 2, and Supplementary Table S4). The major functional classes relate to lipid transport and metabolism (7.1%), amino acid transport and metabolism (6.1%), signal transduction (5.8%), protein modification and turnover (5.8%), as well as defense mechanisms (4.8%).

3.4.1. Defense mechanisms and blood meal acquisition

A total of 18 midgut-specific transcripts with putative defense functions were identified, representing the largest subset of defense transcripts for all tissue comparisons (Table 2,

Supplementary Table S4). Of these, 11 transcripts involved in anti-coagulation belonging to four families of protease inhibitors were identified: the reversible papain-like cysteine protease (cystatin) inhibitor family (Contig 5662, CV444905 and Contig 1698), the Kunitz-type serine proteinase inhibitors (TC22004, CK192299 and CK192837), serpins (Contig 1086, CV442792 and Contig 165) and TIL proteins (CV443795 and CK188782) (Supplementary Table S4). These protease inhibitors have been recently identified from the mialome of *D. variabilis* and have been well described from tick siolomic data (Anderson et al., 2008; Francischetti et al., 2010).

Four putative antimicrobial transcripts that share sequence similarity with soft tick antimicrobial proteins were identified in midgut tissues (Contig 4731, Contig 4907, Contig 5243 and Contig 5493). The antimicrobial peptide, microplusin, was previously localized to the fat body, hemocytes and ovaries of engorged females, but not the midgut (Fogaça et al., 2004). Therefore, the transcripts identified in this study represent a unique set of related proteins that are midgut-specific. Interestingly, no defensins were identified in the midgut, which correlates with previous findings by Fogaça et al. (2004).

It has been suggested that digested blood is a source of oxidative stress as heme, a pro-oxidant, is released following hemolysis (Citelli et al., 2007). Glutathione S-transferases or GSTs (Contig 8822, Contig 336 and Contig 706) and cytochrome P450 enzymes (Contig 894, U92732.1 and CV443756) were identified that were up-regulated in gut tissues (Supplementary Table S4). These enzymes are known to be involved in detoxification and management of oxidative stresses and have been found to be highly expressed during feeding in previous comparative studies of *R. microplus* larvae and adults on different cattle breeds (Rodriguez-Valle et al., 2010). Furthermore, GSTs have also been shown to play a vital role in acquired acaricide resistance in *R. microplus* larvae (Saldivar et al., 2008). Numerous proteins involved in the management of oxidative stress, anti-microbial defense, inhibition of host immune responses and maintenance of the blood meal fluidity have been described in the midgut of various tick species, including *R. microplus* (Uhlik et al., 2004; Horn et al., 2009; Kongsuwan et al., 2010; Mercado-Curiel et al., 2011).

3.4.2. Nutrient transport and metabolism

Catabolism and acquisition of nutrients for basic metabolism and oogenesis from the blood meal are essential functions of the digestive tract. It therefore requires expression of a number of genes involved in the processing and transport of proteins, carbohydrates and lipids.

3.4.2.1. Proteins and amino acids

Numerous putative proteases involved in protein and amino acid metabolism were identified including: chymotrypsin-like serine proteases (Contig 598, Contig 3850, Contig 1821), asparaginyl peptidases or legumain-like proteases (Contig 1863), cathepsin peptidases (Contig 6100, Contig 953, Contig 3558, Contig 1506, Contig 3719, Contig 432 Contig 2640 and TC15264), elastase-like peptidases (Contig 6406) aminopeptidases (Contig 5171), serine-type endopeptidases (Contig 5340), carboxypeptidases (Contig 4941 and Contig 5462) and non-specific dipeptidases (Contig 506) (Supplementary Table S4). Considering the most prominent class of up-regulated proteases, the aspartic and cysteine (cathepsin) peptidases, these have been implicated in the hemoglobinolytic pathway in *I. ricinus* and *D. variabilis* midgut (Anderson et al., 2008; Sojka et al., 2008; Horn et al., 2009). Similar enzymes have been implicated in the production of potent antimicrobial hemocidins in *R. microplus* (Cruz et al., 2010). Proteome analysis of the midgut of partially fed female *R. microplus* ticks also identified type L and B-like cathepsins (with ~60% sequence identity to midgut-specific transcripts), as well as other metalloproteases (Kongsuwan et al., 2010). A putative pantetheine hydrolase (CV440582) involved in cellular recycling of pantothenic acid (vitamin B5) and oxidative stress was also highly up-regulated (Supplementary Table S4) (Pitari et al., 2000). This enzyme type has been identified in other ixodid ticks from comparative sialomic data only (Francischetti et al., 2010).

Novel putative amino acid converting enzymes have also been identified as highly up-regulated in midgut tissues that may function in asparagine (Contig 1748), cysteine (Contig 8186),

glycine (Contig 2855), ornithine (Contig 6808), methionine (Contig 3884) and arginine (Contig 8859) metabolism (Supplementary Table S4). Similar enzymes have been identified from sialomes ESTs for *R. microplus* (Francischetti et al., 2010).

3.4.2.2. Carbohydrates

A group of novel midgut up-regulated enzymes involved in carbohydrate metabolism were identified (Supplementary Table S4): glucosylceramidase (Contig 7972) involved in sphingolipid metabolism (Hannun and Obeid, 2008), a hexokinase (Contig 245B) that functions in fructose and mannose metabolism (glycolysis and gluconeogenesis) (Wilson, 2003), putative alpha-L-fucosidases (Contig 8580 and Contig 4273) that hydrolyze the carbohydrate moieties in glycoproteins (Johnson and Alhadeff, 1991), as well as glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase (CV448648) involved in glycoprotein synthesis (Ju et al., 2002). Similar enzyme activities were identified from the midgut homogenates of the blood-feeding phlebotomine sand fly, *Lutzomyia longipalpis*, but not in *R. microplus* (Gontijo et al., 1998).

Additional novel transporters were identified that were higly up-regulated: a putative sialic acid cotransporter, sialin (membrane glycoprotein HP59, Contig 6465), as well as a non-specific monocarboxylate carbohydrate transporter (CV455491). The former is involved in monosaccharide import across the lysosomal membrane and may function in the clearance of free cytosolic oligosaccharides derived from the breakdown of glycoproteins and other catabolic products (Fu et al., 2001; Winchester, 2001).

3.4.2.3. Lipids

Some 22 midgut-specific transcripts were identified that have putative functions in lipid metabolism and represent the largest subset of this class for all comparisons (Table 2). It has been suggested that secreted phospholipases may play a role in hemolysis of the ingested blood cells (Zhu et al., 1997). In this regard, putative lipases (Contig 2297, TC17614 and Contig 1290) were highly up-

regulated in the midgut (Supplementary Table S4). Additional novel lipid carrier proteins were identified, including a carrier protein apolipophorin (Contig 156) with the highest log_2 fold expression of midgut-specific transcripts (5.59) (Supplementary Table S4). This glycoprotein is involved in the transport of various lipids in the hemolymph, as well as heme (Duncan et al., 1999; Weers et al., 2006). A related transcript involved in oogenesis as a yolk protein precursor, vitellogenin (Contig 8127), was also identified (Supplementary Table S4). This protein has been identified in the midgut and fat body of *Haemaphysalis longicornis* and has been shown to play a role in heme sequestration (Thompson et al., 2007; Boldbaatar et al., 2010). Vitellogenin was also found to be regulated in response to ubiquitin-63E knockdown in gene expression microarrays of female *R. microplus* ticks (Lew-Tabor et al., 2011). Several proteins sharing sequence similarities to the Niemann-Pick C family proteins (NPC1 and 2) from hard and soft ticks were highly expressed in midgut (TC17851, Contig 1508, CV443743 and Contig 727). These proteins have been proposed to be involved in intracellular cholesterol cycling and have been identified from the midgut of *D. variabilis* and *I. ricinus* (Vanier et al., 2004; Anderson et al., 2008; Horácková et al., 2010). A unique cytosolic fatty acid-binding protein (Contig 4249), not previously described for *R. microplus*, was also highly up-regulated and may play a role in intracellular transport of long-chain fatty acids,

Other midgut-specific proteins with putative functions in lipid degradation (Contig 7995, TC22152 and Contig 1729), as well as fatty acid biosynthesis (Contig 2638, Contig 6832, Contig 6561, Contig 2086, Contig 2302, Contig 3708 and Contig 2303) were identified (Supplementary Table S4). Of these proteins, prosaposin (Contig 1729) and ATP-dependent CoA ligase (Contig 3708) have been identified previously from the mialome of *D. variabilis* but not from *R. microplus* midgut (Anderson et al., 2008).

3.4.3. Other midgut-specific proteins

During digestion a large amount of heme is produced and as a result high levels of cytotoxic iron are released into the midgut. Furthermore, *R. microplus* lacks the biosynthetic pathway for heme

production and therefore acquires it from the blood meal (Braz et al., 1999). A novel ferritin transcript (Contig 3919), similar to that of *I. ricinus*, was identified among the unique up-regulated midgut transcripts (Supplementary Table S4). Ferritin is a cytosolic storage protein that sequesters ferric iron for heme and iron metabolism. This protein has been cloned from the gut of *O. moubata* and *I. ricinus* (Kopácek et al., 2003). More recently, ferritin has been cloned from *R. microplus* and pilot trials in rabbits using recombinant protein showed promising efficacy as a vaccine target (Hajdusek et al., 2010).

Some 15 putative signal transduction transcripts were highly up-regulated (Supplementary Table S4). These included two membrane associated proteins that are uniquely expressed in midgut tissues, namely ATAQ (Contig 3639) and Bm86 (Contig 8501) (Supplementary Table S4). Bm86 and ATAQ are both glycoproteins of unknown function containing epidermal growth factor domains that have been localized to the midgut tissues of a number of tick species (Nijhof et al., 2010). To date, Bm86 remains the only protein antigen that has been successfully used in the production of a commercial tick vaccine. Additional acetylcholinesterases (Contig 4226 and Contig 8297) were identified that have been characterized for *R. microplus* and implicated in acquiring acaricide resistance (Baxter and Barker, 1998; Guerrero et al., 2012). A calmodulin binding protein (Contig473) and a putative GTP-binding protein (Rab) (Contig 2938), were also highly up-regulated in midgut tissues (Supplementary Table S4). Calmodulin proteins have been identified from midgut tissues of *D. variabilis* and are well known effectors in signal transduction mechanisms, affecting a number of cellular processes including membrane trafficking mediated by Rab GTPases (Stenmark and Olkkonen, 2001; Anderson et al., 2008). These proteins have also been suggested to play a role in smooth muscle contraction of the midgut during feeding.

3.5. Unique transcripts highly regulated in adult female ovaries

Transcriptional analysis of *R. microplus* female ovaries identified 417 unique transcripts (Tables 1, 2, Supplementary Table S5). The major functional classes that could be identified according

to KOG classification related to transcription (7.4%), protein modification and turnover (6.7%), signal transduction (4.6%) and cell cycle control (3.6%), while approximately 52.8% of transcripts could not be functionally assigned (Fig. 1D). A total of 72 annotatable transcripts were highly up-regulated (Tables1, 2, Supplementary Table S5).

3.5.1. Chromatin structure, replication, cell cycle control and signal transduction

Following DNA replication (or S phase), the meiotic cell cycle development occurs in two consecutive cell divisions called M phases (Nebreda and Ferby, 2000). Transcriptional analysis identified some highly up-regulated ovary-specific transcripts that are involved in chromosomal organization. These include several nucleosome core histones of the H2A (TC21623, Contig 4221 and Contig 2123), H3 (Contig 6410, Contig 5900) and H4 (Contig 5362) families, a putative maintenance of chromosomes (SMC) ATPase (CV457670) and microcephalin (Contig 6899) (Supplementary Table S5). These proteins are involved in maintenance of the nuclear architecture, especially during DNA replication (S phase) in other organisms such as *Drosophila* (Kornberg and Lorch, 1999; Hirano, 2005). Two groucho-related transducer proteins (CK189050 and Contig 4005) were identified, presumably involved in transcriptional regulation by interaction with the histone core in response to cyclin-dependent kinases (Turki-Judeh and Courey, 2012). In this regard, a putative cyclin-dependent kinase (CV446249) was also identified (Supplementary Table S5).

Various proteins serve as checkpoints during cell division and tightly control progression through the cell cycle. Ovary-specific transcripts such as a B-type cyclin (CV451547), a serine/threonine protein kinases (Contig 347) and a polo-like protein kinase (Contig 4236), as well as a putative coiled-coil domain-containing protein (Spindly) (Contig 6020) were up-regulated (Supplementary Table S5). Cyclins play key roles in cell cycle development during early mitotic divisions in *Drosophila*, where B-type cyclins are involved in activation of several key proteins involved in spindle formation and progression through metaphase and anaphase (McCleland et al., 2009; Yoshitome et al., 2012). In this regard, the related protein, Spindly, is involved in chromosome

segregation during mitosis/meiosis by mediating the recruitment of dynactin to the kinetochore during spindle formation (Chan et al., 2009). Aurora kinases and polo-like kinases can associate with the centrosome and are involved in regulation of spindle formation via activation of proteins such as Spindly and histone phosphorylation during oogenesis (Ding et al., 2011; Song et al., 2012; Yoshitome et al., 2012). An additional putative repressor of embryonic development via the Wnt/beta-catenin pathway, protein bicaudal C homolog 1 (Contig 383), was also found to be highly up-regulated (Supplementary Table S5).

3.5.2. Transcription and control

Transcriptional initiation and control can be achieved via expression of ovary-specific transcription factors and degradation of the mRNA by the endogenous RNAi machinery, respectively. In this regard, zinc-finger proteins represent the largest family of DNA-binding transcription factors present in a number of bilaterians, including *Homo sapiens, Drosophila melanogaster, Caenorhabitis elegans* and more recently in the arthropod, *Daphnia pulex* (Seetharam et al., 2010). Numerous novel zinc-finger proteins, such as C2H2-type zinc-finger proteins (CV457291, Contig 4738 and CK173279), were identified that were highly up-regulated in ovaries (Supplementary Table S5). Putative RNA processing proteins involved in post-transcriptional gene regulation were also identified as highly up-regulated in ovaries, similar to transcripts previously identified from *R. microplus* ESTs (Kurscheid et al., 2009) (Supplementary Table S5). In addition, a putative proliferating cell nuclear antigen (PCNA)-associated factor (Contig 5833) and a DNA excision repair protein (Contig 7899) were identified (Supplementary Table S5). These proteins are important processing factors involved in post-replication repair (Essers et al., 2005).

3.5.3. Metabolism and other ovary-specific proteins

Only a few highly up-regulated transcripts have been identified that are involved in amino acid, carbohydrate and lipid metabolism (Supplementary Table S5). These include several ovary-specific

proteases with limited description in *R. microplus*. Firstly, is an astacin (Contig 1441) that has been shown to be expressed in *R. microplus* female reproductive tissues (Barnard et al., 2012). Secondly, are novel metalloproteases such as the thrombospondin type-1 domain-containing (ADAMTS)-like protein (Contig 8445) suggested to play a role in modulating microfibril functions, as well as a neprilysin (Contig 6628) similar to *Drosphila* spp. neprilysin 2 that has been suggested to play a role in signaling during embryogenesis (Bland et al., 2007; Bader et al., 2012). Thirdly, a cathepsin L1 cysteine peptidase (Contig 1209) was identified that has only been described in midgut tissues of *R. microplus* (Kongsuwan et al., 2010). Finally, together with other serine proteases, a transcript encoding putative oviductin (Contig 6991) was up-regulated in ovaries (Supplementary Table S5). This secreted protease is involved in egg envelope glycoprotein alteration for fertilization in the oviduct epithelia of amphibians (Hiyoshi et al., 2002). However, its role in tick oogenesis remains to be elucidated.

Two novel up-regulated carbohydrate metabolizing enzymes were identified: an alpha 1,3-fucosyltransferase enzyme (Contig 2393) and an N-acetyllactosaminide beta-1,3-N-acetylglucosaminyltransferase (Contig 46A). These enzymes are involved in N-glycan synthesis of glycoprotein moieties and unsaturated fatty acids biosynthesis, respectively (Fabini et al., 2001; Okajima et al., 2008).

During tick oogenesis and egg maturation, major egg yolk proteins (vitellogenin) are produced by the fat body and the midgut (Tufail and Takeda, 2009; Boldbaatar et al., 2010). These hemoglycolipoproteins are taken up together with lipid-binding lipophorin into the ovaries by low-density lipoprotein receptors via receptor-mediated endocytosis (Tufail and Takeda, 2009). A putative low-density lipoprotein receptor-related protein (Contig 276) was highly up-regulated in ovaries (Supplementary Table S5). These results correlated well with transcripts involved in lipid transport that were up-regulated in midguts.

Several transporter proteins were identified that were highly up-regulated in ovaries and included two novel ABC transporters (Contig 2240 and Contig 1691) (Supplementary Table S5). ABC

transporters are proteins involved in detoxification processes and have been identified from midgut tissues of female *R. microplus*, where transporter activity has been implicated in acaricide resistance (Pohl et al., 2011, 2012).

3.6. Gene expression of transcripts occurring in all adult female tissues during feeding

A key objective of this study was to identify proteins and potential biochemical processes that are shared between the different adult tissues. Following transcriptional analysis, 588 expressed transcripts were identified that were shared among all of the tissues tested (Fig. 1A, Tables 1, 2, Supplementary Table S2). Considering transcripts that have a minimum intensity above the threshold of 1,000 (M value > 0), only 4.4% of the total transcripts used in microarray analysis showed transcriptional regulation (Table 1). Of these transcripts, 38.8% could not be functionally annotated (Fig. 1E). The major functional classes that could be identified involved protein modification and turnover (11.2%), translation, ribosomal structure and biogenesis (10.2%), as well as RNA processing (6.3%) and intracellular trafficking and transport (5.8%) (Fig. 1E). Only 90 annotatable transcripts with a minimum two-fold threshold intensity (Cy5 > 2,000) were identified (Tables 1, 2, Supplementary Table S2).

3.6.1. Metabolism, energy production and transport

Shared transcripts were identified that are related to amino acid, nucleotide, carbohydrate and lipid metabolism (Supplementary Table S2). These transcripts include several putative metabolic enzymes: phosphoglycerate dehydrogenases (Contig 387 and Contig 8440) involved in L-serine biosynthesis, GDP-L-fucose synthase (Contig 4405) and GDP-mannose 4,6 dehydratase (Contig 497) involved GDP-L-fucose biosynthesis and polysaccharide breakdown, a glucan 1,3-alpha-glucosidase (Contig 835) involved in glycan metabolism for glycoprotein synthesis, a tau-protein kinase (Contig 8022) involved in glycogen metabolism, a 3-hydroxyacyl-CoA dehydrogenase (Contig 1246) involved in cholesterol degradation and fatty acid beta-oxidation, acetyl-CoA C-acetyltransferases (Contig 8253

and Contig 2916) involved in ketone body metabolism and finally a dUTP diphosphatase (Contig 8942) involved in the biosynthesis of dUMP. The large number of transcripts identified, unique or shared, across all tissues involved in carbohydrate and lipid metabolism, may imply that metabolic energy is mainly derived from carbohydrate and fatty acid sources.

Transcripts related to mitochondrial transport and energy metabolism were identified and these include components associated with oxidative phosphorylation: a cytochrome c oxidase (Contig 2837), NAD(P)(+) transhydrogenase (Contig 4420) and a ADP/ATP translocase 2 (Contig 4681). A putative isocitrate dehydrogenase (Contig 424), a component of the citric acid cycle, was also identified as a shared transcript (Supplementary Table S2). Cytochrome c oxidases and other associated proteins involved with energy metabolism have been identified from the mialome of *D. variabilis*, as well as sialomes of both hard and soft tick species (Anderson et al., 2008; Francischetti et al., 2010; Kongsuwan et al., 2010).

A number of shared transcripts encoding components of the ubiquitin/proteasome pathway, involved in the degradation of unneeded or damaged proteins, were identified: two proteins involved in ubiquitination of target proteins for degradation, a RING-box protein 1A (Contig 5214) and a ubiquitin-protein ligase (Contig 8175), as well as several subunits of the proteasome endopeptidase complex (Contig 2948, Contig 903 and Contig 887). Subunits of the proton-pumping V-ATPase complex were also identified (Contig 8631, Contig 2275 and Contig 8181), which are involved in acidification of lysosomal lumen necessary for digestion of macromolecules or exocytosis-mediated secretion (Bowman and Sauer, 2004; Mindell, 2012). Similar transcripts have only been identified from comparative sialome data for *R. microplus* (Francischetti et al., 2010).

3.6.2. Transcription, translation and protein synthesis

Following transcriptional analysis, some key shared transcripts were identified that function in gene expression and control (Supplementary Table S2). Of these, some novel transcripts involved in control of transcription activation that were shared between female tissues include: a RuvB-like DNA

helicase (Contig 5083) that is part of the NuA4 histone acetyltransferase complex involved in transcription activation via modification of core histones H4 and H2A (Doyon et al., 2004; Lu et al., 2009), a DPY30 domain-containing protein (Contig 346) that is part of the mixed lineage leukemia protein -1 (MLL1) core complex involved in methylation of histone H3 (Vardanyan et al., 2008; Patel et al., 2011), as well as an arginine N-methyltransferase (Contig 137) that is the main enzyme involved in mono-methylation of histone H4 (Boulanger et al., 2004; Bachand, 2007). Additional transcription factors BTF3 (Contig 1649) and putative X-box-binding protein 1 (Contig 6772) were identified (Liou et al., 1990; Zheng et al., 1990). These proteins have not been characterized for *R. microplus*.

Alternative splicing of mRNA is regarded as one of the main steps in regulation of gene expression and five transcripts encoding splicing factors of the SR family (Contig 825, Contig 8997, Contig 1956, Contig 5832, Contig 8773 and Contig 5369) and a thioredoxin-like protein (Contig 5832) were identified (Supplementary Table S2). These proteins are essential in constitutive pre-mRNA splicing and can also act as regulators in other aspects of mRNA metabolism (Long and Caceres, 2009). A related U2 small nuclear ribonucleoprotein (snRNP) (Contig 1192) which was also identified is a component of the spliceosome complex (Supplementary Table S2). Similar proteins have only been identified from sialome data that include transcripts from *R. microplus* (Francischetti et al., 2010)

Assembly of a functional ribosome is vital for successful protein synthesis. In this regard, a number of ribosome associated proteins were identified that included components of the 40S (Contig 7181 and EW680164.1) and 60S (Contig 1238, Contig 3228, EW680050.1, Contig 6473, Contig 1078, Contig 9000, CK177858 and Contig 7496) ribosomal subunits, as well as the 29S (TC20332) and 39S (Contig 2946) mitochondrial ribosomal subunits. Additional transcripts encoding subunits of the H/ACA small nucleolar ribonucloeprotein complex (Contig 1577) involved in pseudouridylation of rRNA and the eukaryotic translation initiation factor 3 complex (Contig 2574) that associates with the 40S ribosomal subunit for initiation of translation, were also found to be shared (Pestova et al., 2001; Watkins and Bohnsack, 2012). These proteins have been identified from sialome and midgut proteome data of *R. microplus* (Francischetti et al., 2010; Kongsuwan et al., 2010)

Proper folding of translated proteins is predominantly mediated by chaperones of which the alpha subunit of the nascent polypeptide-associated complex (Contig 8810) was identified from shared transcripts (Supplementary Table S2). This complex functions as a molecular chaperone that generally associates with newly synthesized nascent polypeptides from the ribosomes to prevent inappropriate interactions with cytosolic proteins such as signal recognition particles (SRP) (Preissler and Deuerling, 2012). In this regard, a putative SRP14 protein (Contig 4224) was identified from the shared tissue transcripts (Supplementary Table S2). These proteins are involved in polypeptide translocation to the endoplasmic reticulum for intracellular trafficking (Preissler and Deuerling, 2012). Additional shared molecular chaperones (Contig 5499, Contig 210, Contig 2441, Contig 2805, Contig 790, Contig 2759 and Contig 2634) were identified (Supplementary Table S2). Similar chaperones have been identified in other ixodid ticks, as well as sialomes, transcriptome and midgut proteome data for *R. microplus* (Guilfoile et al., 2004; Anderson et al., 2008; Francischetti et al., 2010; Kongsuwan et al., 2010; Rodriguez-Valle et al., 2010). A putative mitochondrial chaperone involved in import and insertion of inner membrane proteins, TIM13 (Contig 6255), was also identified as a shared transcript (Supplementary Table S2).

3.7. Tick biology and future perspectives

Transcriptional profiling of feeding adult *R. microplus* female tissues identified distinct subsets of genes that aid in blood meal acquisition (salivary glands), digestion (midgut) and reproduction (ovaries) (Table 2, Fig. 2). For salivary glands, a large repertoire of anti-hemostatics and immunomodulatory transcripts maintain the fluidity of the blood meal, while additional secreted proteases aid in blood pool formation and pre-digestion of the blood meal prior to ingestion. The midgut in turn is highly specialized to break down the components of the blood meal with wide arrays of proteases involved in digestion, where additional metabolic enzymes and transporters enable the engorging female to acquire the necessary nutrients in preparation for reproduction. Some additional highly up-regulated transcripts are also involved in alleviation of oxidative stress, defense against

microbial invasion and host immune responses, as well as maintenance of blood meal fluidity via anti-coagulants. As the female prepares for egg production, unique genes are highly up-regulated to control cell cycle development, DNA replication, post-transcriptional and post-translational modification. Some transcripts related to metabolism, transport and signaling were also differentially expressed.

Global comparison of transcripts shared between all tissues revealed a broader functional distribution (according to KOG annotation) than any single tissue comparison (Table 2). These include components involved in protein, carbohydrate and lipid metabolism necessary for biosynthetic pathways and energy production that indicate fundamental processes that are ubiquitous in all tissues during feeding. Notable are transcripts involved in signal transduction events that control the cell cycle, transcription and translation, making them an important component of basic tissue biology (Fig. 2).

As is the case with other comparative studies, numerous genes that were expressed in *R. microplus* female tissues during feeding could not be functionally annotated. Further similarity searches against the *Ixodes scapularis* genome database could not confer more informative annotation of unknown genes, highlighting the uniqueness of these transcripts (Hill and Wikel, 2005). Moreover, the vast majority of the predicted genes currently available for *R. microplus* in the BmiGI (v2.1) and the Cattle Tick sequence databases remain un-annotated (Wang et al., 2007; Bellgrad et al., 2012). Therefore, a meta-analysis approach that combines all available sequence databases (nucleotide, protein and structural) and involves extensive manual curation is needed. Such an approach has been successfully employed in the annotation of various tick sialomes (Francischetti et al., 2010). However, a unified nomenclature for sequence annotation is necessary to avoid confusion between similar entries. In this study, final annotation was based on reviewed sequences published in the Uniprot protein and Baunschweig enzyme (BRENDA) databases (Apweiler et al., 2011; Scheer et al., 2011). A central database containing all available tick sequences (Genome, ESTs, transcriptome, sialome and mialome) would also simplify comparative analysis but is still lacking. In this regard, the recently established Cattle Tick Database could become an invaluable resource, as the basis for a

systematic attempt at annotating the full complement of genes and proteins of *R. microplus* (Bellgard et al., 2012).

Application of high-throughput techniques such as deep RNA sequencing (RNA-seq), as well as interactome analysis, will enable verification of open reading frames and aid in functional annotation of transcripts that share little to no sequence identity with other organisms (Brückner et al., 2009; Wang et al., 2009).

In conclusion, a catalogue of tissue-specific and shared genes was identified in major tissues involved in feeding and reproduction of adult *R. microplus* females using available sequence data and transcriptome analysis. This study presented here is, to our knowledge, the first global transcriptomic analysis via DNA microarrays of *R. microplus* female tissues and represents an additional resource that will be further exploited to study proteins and pathways that may be useful for future tick control.

Acknowledgements

This work was funded by the Red Meat Research Development Trust, University of Pretoria Research Development Programme and the Technology and Human Resources for Industry Programme, South Africa. Additional funding and student support was obtained from the National Research Foundation of South Africa. We would also like to thank Mrs. Santa Meyer and Dr. Tamsyn Pulker (University of Pretoria Biomedical Research Center) for cattle management, Prof Fourie Joubert (University of Pretoria, Computational Biology Unit) for bioinformatic support, as well as Prof. Dave Berger (University of Pretoria Department of Plant Science and the ACGT Microarray Facility) for advice during design the of this study.

References

Anatriello, E., Ribeiro, J.M., de Miranda-Santos, I.K., Brandão, L.G., Anderson, J.M., Valenzuela, J.G., Maruyama, S.R., Silva, J.S., Ferreira, B.R., 2010. An insight into the sialotranscriptome of the brown dog tick, *Rhipicephalus sanguineus*. BMC Genomics. 11, 450.

Anderson, J.M., Sonenshine, D.E., Valenzuela, J.G., 2008. Exploring the mialome of ticks: an annotated catalogue of midgut transcripts from the hard tick, *Dermacentor variabilis* (Acari:Ixodidae). BMC Genomics. 9, 552.

Apweiler, R., Jesus Martin, M., O'onovan, C., Magrane, M., Alam-Faruque, Y., Antunes, R., Barrera Casanova, E., Bely, B., Bingley, M., Bower, L., Bursteinas, B., Mun Chan, W., Chavali, G., Da Silva, A., Dimmer, E., Eberhardt, R., Fazzini, F., Fedotov, A., Garavelli, J., Castro, L.G., Gardner, M., Hieta, R., Huntley, R., Jacobsen, J., Legge, D., Liu, W., Luo, J., Orchard, S., Patient, S., Pichler, K., Poggioli, D., Pontikos, N., Pundir, S., Rosanoff, S., Sawford, T., Sehra, H., Turner, E., Wardell, T., Watkins, X., Corbett, M., Donnelly, M., van Rensburg, P., Goujon, M., McWilliam, H., Lopez, R., Xenarios, I., Bougueleret, L., Bridge, A., Poux, S., Redaschi, N., Argoud-Puy, G., Auchincloss, A., Axelsen, K., Baratin, D., Blatter, M.C., Boeckmann, B., Bolleman, J., Bollondi, L., Boutet, E., Braconi Quintaje, S., Breuza, L., deCastro, E., Cerutti, L., Coudert, E., Cuche, B., Cusin, I., Doche, M., Dornevil, D., Duvaud, S., Estreicher, A., Famiglietti, L., Feuermann, M., Gehant, S., Ferro, S., Gasteiger, E., Gerritsen, V., Gos, A., Gruaz-Gumowski, N., Hinz, U., Hulo, C., Hulo, N., James, J., Jimenez, S., Jungo, F., Kappler, T., Keller, G., Lara, V., Lemercier, P., Lieberherr, D., Martin, X., Masson, P., Moinat, M., Morgat, A., Paesano, S., Pedruzzi, I., Pilbout, S., Pozzato, M., Pruess, M., Rivoire, C., Roechert, B., Schneider, M., Sigrist, C., Sonesson, K., Staehli, S., Stanley, E., Stutz, A., Sundaram, S., Tognolli, M., Verbregue, L., Veuthey, A.L., Wu, C.H., Arighi, C.N., Arminski, L., Barker, W.C., Chen, C., Chen, Y., Dubey, P., Huang, H., Kukreja, A., Laiho, K., Mazumder, R., McGarvey, P., Natale, D.A., Natarajan, T.G., Roberts, N.V., Suzek, B.E., Vinayaka, C., Wang,

Q., Wang, Y., Yeh, L.S., Zhang, J., 2011. Reorganizing the protein space at the Universal Protein Resource (UniProt). Nucl. Acids Res., 40(Database issue), D71-75.

Bachand, F., 2007. Protein arginine methyltransferases: from unicellular eukaryotes to humans. Eukaryot. Cell. 6, 889-898.

Bader, H.L., Wang, L.W., Ho, J.C., Tran, T., Holden, P., Fitzgerald, J., Atit, R.P., Reinhardt, D.P., Apte, SS., 2012. A disintegrin-like and metalloprotease domain containing thrombospondin type 1 motif-like 5 (ADAMTSL5) is a novel fibrillin-1-, fibrillin-2-, and heparin-binding member of the ADAMTS superfamily containing a netrin-like module. Matrix Biol. 31, 398–411.

Barnard, A.C., Nijhof, A.M., Gaspar, A.R., Neitz, A.W., Jongejan, F., Maritz-Olivier, C., 2012. Expression profiling, gene silencing and transcriptional networking of metzincin metalloproteases in the cattle tick, *Rhipicephalus (Boophilus) microplus*. Vet. Parasitol., 186, 403-414.

Batista, I.F., Chudzinski-Tavassi, A.M., Faria, F., Simons, S.M., Barros-Batestti, D.M., Labruna, M.B., Leão, L.I., Ho, P.L., Junqueira-de-Azevedo, I.L., 2008. Expressed sequence tags (ESTs) from the salivary glands of the tick *Amblyomma cajennense* (Acari:Ixodidae). Toxicon. 51, 823-834.

Baxter, G.D., Barker, S.C., 1998. Acetylcholinesterase cDNA of the cattle tick, *Boophilus microplus*: characterisation and role in organophosphate resistance. Insect Biochem. Mol. Biol. 28, 581-589.

Bellgard, M.I., Moolhuijzen, P.M., Guerrero, F.D., Schibeci, D., Rodriguez-Valle, M., Peterson, D.G., Dowd, S.E., Barrero, R., Hunter, A., Miller, R.J., Lew-Tabor, A.E., 2012. CattleTickBase: an integrated Internet-based bioinformatics resource for *Rhipicephalus (Boophilus) microplus*. Int J Parasitol. 42, 161-169.

Bland, N.D., Thomas, J.E., Audsley, N., Shirras, A.D., Turner, A.J., Isaac, R.E., 2007. Expression of NEP2, a soluble neprilysin-like endopeptidase, during embryogenesis in *Drosophila melanogaster*. Peptides. 28, 127-135.

Boldbaatar, D., Umemiya-Shirafuji, R., Liao, M., Tanaka, T., Xuan, X., Fujisaki, K., 2010. Multiple vitellogenins from the *Haemaphysalis longicornis* tick are crucial for ovarian development. J. Insect Physiol. 56, 1587-1598.

Boulanger, M.C., Miranda, T.B., Clarke, S., Di Fruscio, M., Suter, B., Lasko, P., Richard, S., 2004. Characterization of the *Drosophila* protein arginine methyltransferases DART1 and DART4. Biochem J. 379(Pt 2), 283-289.

Bowman, A.S., Sauer, J.R., 2004. Tick salivary glands: function, physiology and future. Parasitology 129 (Suppl), S67-S81.

Braz, G.R., Coelho, H.S., Masuda, H., Oliveira, P.L., 1999. A missing metabolic pathway in the cattle tick *Boophilus microplus*. Curr. Biol. 9, 703-706.

Brückner, A., Polge, C., Lentze, N., Auerbach, D., Schlattner, U., 2009. Yeast two-hybrid, a powerful tool for systems biology. Int J Mol Sci. 10, 2763-2788.

Buresova, V., Hajdusek, O., Franta, Z., Sojka, D., Kopacek, P., 2009. IrAM-An alpha2-macroglobulin from the hard tick *Ixodes ricinus*: characterization and function in phagocytosis of a potential pathogen *Chryseobacterium indologenes*. Dev Comp Immunol. 33, 489-498.

Campbell, E.M., Burdin, M., Hoppler, S., Bowman, A.S., 2010. Role of an aquaporin in the sheep tick *Ixodes ricinus*: assessment as a potential control target. Int J Parasitol. 40, 15-23.

Chalaire, K.C., Kim, T.K., Garcia-Rodriguez, H., Mulenga, A., 2011. *Amblyomma americanum* (L.) (Acari: Ixodidae) tick salivary gland serine protease inhibitor (serpin) 6 is secreted into tick saliva during tick feeding. J Exp Biol. 214(Pt 4), 665-673.

Chan, Y.W., Fava, L.L., Uldschmid, A., Schmitz, M.H., Gerlich, D.W., Nigg, E.A., Santamaria, A., 2009. Mitotic control of kinetochore-associated dynein and spindle orientation by human Spindly. J. Cell Biol. 185, 859-874.

Citelli, M., Lara, F.A., da Silva Vaz, I. Jr, Oliveira, P.L., 2007. Oxidative stress impairs heme detoxification in the midgut of the cattle tick, *Rhipicephalus (Boophilus) microplus*. Mol. Biochem. Parasitol. 151, 81-88.

Cruz, C.E., Fogaça, A.C., Nakayasu, E.S., Angeli, C.B., Belmonte, R., Almeida, I.C., Miranda, A., Miranda, M.T., Tanaka, A.S., Braz, G.R., Craik, C.S., Schneider, E., Caffrey, C.R., Daffre, S., 2010. Characterization of proteinases from the midgut of *Rhipicephalus (Boophilus) microplus* involved in the generation of antimicrobial peptides. Parasit. Vectors. 3, 63.

de la Fuente, J., Almazán, C., Canales, M., Pérez de la Lastra, J.M., Kocan, K.M., Willadsen, P., 2007. A ten-year review of commercial vaccine performance for control of tick infestations on cattle. Anim. Health Res. Rev. 8, 23-28.

de La Fuente, J., Rodríguez, M., García-García, J.C., 2000. Immunological control of ticks through vaccination with *Boophilus microplus* gut antigens. Ann N Y Acad Sci. 916, 617-621.

de Miranda Santos, I.K.F., Valenzuela, J.G., Ribeiro, J.M., de Castro, M., Costa, J.N., Costa, A.M., da Silva, E.R., Neto, O.B., Rocha, C., Daffre, S., Ferreira, B.R., da Silva, J.S., Szabó, M.P., Bechara, G., 2004. Gene discovery in *Boophilus microplus*, the cattle tick: the transcriptomes of ovaries, salivary glands, and hemocytes. Ann. N.Y. Acad. Sci. 1026, 242-246.

Ding, J., Swain, J.E., Smith, G.D., 2011. Aurora kinase-A regulates microtubule organizing center (MTOC) localization, chromosome dynamics, and histone-H3 phosphorylation in mouse oocytes. Mol. Reprod. Dev. 78, 80-90.

Doyon, Y., Selleck, W., Lane, W.S., Tan, S., Côté, J., 2004. Structural and functional conservation of the NuA4 histone acetyltransferase complex from yeast to humans. Mol Cell Biol. 24, 1884-1896.

Duncan, T., Osawa, Y., Kutty, R.K., Kutty, G., Wiggert, B., 1999. Heme-binding by *Drosophila* retinoid- and fatty acid-binding glycoprotein (RFABG), a member of the proapolipophorin gene family. J. Lipid Res. 40, 1222-1228.

Essers, J., Theil, A.F., Baldeyron, C., van Cappellen, W.A., Houtsmuller, A.B., Kanaar, R., Vermeulen, W., 2005. Nuclear dynamics of PCNA in DNA replication and repair. Mol. Cell Biol. 25, 9350-9359.

Fabini, G., Freilinger, A., Altmann, F., Wilson, I.B., 2001. Identification of core alpha 1,3-fucosylated glycans and cloning of the requisite fucosyltransferase cDNA from *Drosophila melanogaster*. Potential basis of the neural anti-horseadish peroxidase epitope. J. Biol. Chem. 276, 28058-28067.

Fogaça, A.C., Almeida, I.C., Eberlin, M.N., Tanaka, A.S., Bulet, P., Daffre, S., 2006. Ixodidin, a novel antimicrobial peptide from the hemocytes of the cattle tick *Boophilus microplus* with inhibitory activity against serine proteinases. Peptides. 27, 667-674.

Fogaça, A.C., Lorenzini, D.M., Kaku, L.M., Esteves, E., Bulet, P., Daffre, S., 2004. Cysteine-rich antimicrobial peptides of the cattle tick *Boophilus microplus*: isolation, structural characterization and tissue expression profile. Dev. Comp. Immunol. 28, 191-200.

Francischetti, I.M., Sa-Nunes, A., Mans, B.J., Santos, I.M., Ribeiro, J.M., 2010. The role of saliva in tick feeding. Front Biosci. 14, 2051-2088.

Fu, C., Bardhan, S., Cetateanu, N.D., Wamil, B.D., Wang, Y., Yan, H.P., Shi, E., Carter, C., Venkov, C., Yakes, F.M., Page, D.L., Lloyd, R.S., Mernaugh, R.L., Hellerqvist, C.G., 2001. Identification of a novel membrane protein, HP59, with therapeutic potential as a target of tumor angiogenesis. Clin Cancer Res. 7, 4182-4194.

Gontijo, N.F., Almeida-Silva, S., Costa, F.F., Mares-Guia, M.L., Williams, P., Melo, M.N., 1998. *Lutzomyia longipalpis*: pH in the gut, digestive glycosidases, and some speculations upon *Leishmania* development. Exp. Parasitol. 90, 212-219.

Guerrero, F.D., Lovis, L., Martins, J.R., 2012. Acaricide resistance mechanisms in *Rhipicephalus (Boophilus) microplus*. Rev Bras Parasitol Vet. 21, 1-6.

Guerrero, F.D., Nene, V.M., George, J.E., Barker, S.C., Willadsen, P., 2006. Sequencing a new target genome: the *Boophilus microplus* (Acari:Ixodidae) genome project. J. Med. Entomol. 43, 9-16.

Guilfoile, P.G., Packila, M., 2004. Identification of four genes expressed by feeding female *Ixodes scapularis*, including three with sequence similarity to previously recognized genes. Exp. Appl. Acarol. 32, 103-110.

Guo, Y., Ribeiro, J.M., Anderson, J.M., Bour, S., 2009. dCAS: a desktop application for cDNA sequence annotation. Bioinformatics. 25, 1195-1196.

Hajdusek, O., Almazán, C., Loosova, G., Villar, M., Canales, M., Grubhoffer, L., Kopacek, P., de la Fuente, J., 2010. Characterization of ferritin 2 for the control of tick infestations. Vaccine. 28, 2993-2998.

Hannun, Y.A., Obeid, L.M., 2008. Principles of bioactive lipid signalling: lessons from sphingolipids. Nat Rev Mol Cell Biol. 9, 139-150.

Hellemans, J., Mortier, G., De Paepe, A., Speleman, F., Vandesompele, J., 2007. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol. 8, R19.

Hill, C., Wikel, S.K., 2005. The *Ixodes scapularis* Genome Project:an opportunity for advancing tick research. Trends Parasitol. 21, 151-153.

Hirano, T., 2005. SMC proteins and chromosome mechanics:from bacteria to humans. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 360, 507-514.

Hiyoshi, M., Takamune, K., Mita, K., Kubo, H., Sugimoto, Y., Katagiri, C., 2002. Oviductin, the oviductal protease that mediates gamete interaction by affecting the vitelline coat in *Bufo japonicus*: its molecular cloning and analyses of expression and posttranslational activation. Dev. Biol. 243, 176-184.

Horácková, J., Rudenko, N., Golovchenko, M., Grubhoffer, L., 2010. Der-p2 (*Dermatophagoides pteronyssinus*) allergen-like protein from the hard tick *Ixodes ricinus* - a novel member of ML (MD-2-related lipid-recognition) domain protein family. Parasitology. 137, 1139-1149.

Horn, M., Nussbaumerová, M., Sanda, M., Kovárová, Z., Srba, J., Franta, Z., Sojka, D., Bogyo, M., Caffrey, C.R., Kopácek, P., Mares, M., 2009. Hemoglobin digestion in blood-feeding ticks: mapping a multipeptidase pathway by functional proteomics. Chem. Biol. 16, 1053-1063.

Isaac, R.E., Ekbote, U., Coates, D., Shirras, A.D., 1999. Insect angiotensin-converting enzyme. A processing enzyme with broad substrate specificity and a role in reproduction. Ann. NY Acad. Sci. 897, 342–347.

Jarmey, J.M., Riding, G.A., Pearson, R.D., McKenna, R.V., Willadsen, P., 1995. Carboxydipeptidase from *Boophilus microplus*: a "concealed" antigen with similarity to angiotensin-converting enzyme. Insect Biochem Mol Biol. 25, 969-974.

Johnson, S.W., Alhadeff, J.A., 1991. Mammalian alpha-L-fucosidases. Comp Biochem Physiol B. 99, 479-488.

Ju, T., Brewer, K., D'Souza, A., Cummings, R.D., Canfield, W.M., 2002. Cloning and expression of human core 1 beta1,3-galactosyltransferase. J Biol Chem. 277, 178-186.

Kongsuwan, K., Josh, P., Zhu, Y., Pearson, R., Gough, J., Colgrave, M.L., 2010. Exploring the midgut proteome of partially fed female cattle tick (*Rhipicephalus (Boophilus) microplus*). J. Insect Physiol. 56, 212-226.

Kopácek, P., Zdychová, J., Yoshiga, T., Weise, C., Rudenko, N., Law, J.H., 2003. Molecular cloning, expression and isolation of ferritins from two tick species--*Ornithodoros moubata* and *Ixodes ricinus*. Insect Biochem. Mol. Biol. 33, 103-113.

Kornberg, R.D., Lorch, Y., 1999. Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. Cell. 98, 285-294.

Kurscheid, S., Lew-Tabor, A.E., Rodriguez Valle, M., Bruyeres, A.G., Doogan, V.J., Munderloh, U.G., Guerrero, F.D., Barrero, R.A., Bellgard, M.I., 2009. Evidence of a tick RNAi pathway by comparative genomics and reverse genetics screen of targets with known loss-of-function phenotypes in *Drosophila*. BMC Mol. Biol. 10, 26.

Lew-Tabor, A.E., Kurscheid, S., Barrero, R., Gondro, C., Moolhuijzen, P.M., Rodriguez Valle, M., Morgan, J.A., Covacin, C., Bellgard, M.I., 2011. Gene expression evidence for off-target effects caused by RNA interference-mediated gene silencing of Ubiquitin-63E in the cattle tick *Rhipicephalus microplus*. Int. J. Parasitol. 41, 1001-1014.

Li, A.Y., Chen, A.C., Miller, R.J., Davey, R.B., George, J.E.,2007. Acaricide resistance and synergism between permethrin and amitraz against susceptible and resistant strains of *Boophilus microplus* (Acari:Ixodidae). Pest Manag. Sci. 63, 882-889.

Lin, X., Söderhäll, I., 2011. Crustacean hematopoiesis and the astakine cytokines. Blood. 117, 6417-6424.

Liou, H.C., Boothby, M.R., Finn, P.W., Davidon, R., Nabavi, N., Zeleznik-Le, N.J., Ting, J.P., Glimcher, L.H., 1990. A new member of the leucine zipper class of proteins that binds to the HLA DR alpha promoter. Science. 247, 1581-1584.

Long, J.C., Caceres, J.F., 2009. The SR protein family of splicing factors: master regulators of gene expression. Biochem. J. 417, 15-27.

Lu, P.Y., Lévesque, N., Kobor, M.S., 2009. NuA4 and SWR1-C: two chromatin-modifying complexes with overlapping functions and components. Biochem. Cell Biol. 87, 799-815.

Lynen, G., Zeman, P., Bakuname, C., Di Giulio, G., Mtui, P., Sanka, P., Jongejan, F., 2008. Shifts in the distributional ranges of *Boophilus* ticks in Tanzania:evidence that a parapatric boundary between *Boophilus microplus* and *B. decoloratus* follows climate gradients. Exp. Appl. Acarol. 44, 147–164.

Macours, N., Poels, J., Hens, K., Francis, C., Huybrechts, R., 2004. Structure, evolutionary conservation, and functions of angiotensin- and endothelin-converting enzymes. Int. Rev. Cytol. 239, 47-97.

Madder, M., Thys, E., Geysen, D., Baudoux, C., Horak, I., 2007. *Boophilus microplus* ticks found in West Africa. Exp. Appl. Acarol. 43, 233-234.

Madder, M., Thys, E., Geysen, D., Baudoux, C., Horak, I., 2007. *Boophilus microplus* ticks found in West Africa. Exp Appl Acarol. 43, 233–234.

Mans, B.J., Andersen, J.F., Francischetti, I.M., Valenzuela, J.G., Schwan, T.G., Pham, V.M., Garfield, M.K., Hammer, C.H., Ribeiro, J.M., 2008. Comparative sialomics between hard and soft

ticks:implications for the evolution of blood-feeding behaviour. Insect Biochem. Mol. Biol. 38, 42-58.

Maruyama, S.R., Anatriello, E., Anderson, J.M., Ribeiro, J.M., Brandão, L.G., Valenzuela, J.G., Ferreira, B.R., Garcia, G.R., Szabó, M.P., Patel, S., Bishop, R., de Miranda-Santos, I.K., 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, 363.

McCleland, M.L., Farrell, J.A., O'Farrell, P.H., 2009. Influence of cyclin type and dose on mitotic entry and progression in the early Drosophila embryo. J. Cell Biol. 184, 639-646.

McKenna, R.V., Riding, G.A., Jarmey, J.M., Pearson, R.D., Willadsen, P., 1998. Vaccination of cattle against the *Boophilus microplus* using a mucin-like membrane glycoprotein. Parasite Immunol. 20, 325-336.

Mercado-Curiel, R.F., Palmer, G.H., Guerrero, F.D., Brayton, K.A., 2011. Temporal characterisation of the organ specific *Rhipicephalus microplus* transcriptional response to *Anaplasma marginale* infection. Int. J. Parasitol. 41, 851-860.

Mindell, J.A., 2012. Lysosomal acidification mechanisms. Annu. Rev. Physiol. 74, 69-86.

Moolhuijzen, P.M., Lew-Tabor, A.E., Morgan, J.A., Valle, M.R., Peterson, D.G., Dowd, S.E., Guerrero, F.D., Bellgard, M.I., Appels, R., 2011. The complexity of *Rhipicephalus (Boophilus) microplus* genome characterised through detailed analysis of two BAC clones. BMC Res. Notes. 4, 254.

Nakajima, Y., van der Goes van Naters-Yasui, A., Taylor, D., Yamakawa, M., 2002. Antibacterial peptide defensin is involved in midgut immunity of the soft tick, *Ornithodoros moubata*. Insect. Mol. Biol. 11, 611-618.

Nebreda, A.R., Ferby, I., 2000. Regulation of the meiotic cell cycle in oocytes. Curr. Opin. Cell Biol. 12, 666-675.

Nijhof, A.M., Balk, J.A., Postigo, M., Jongejan, F., 2009. Selection of reference genes for quantitative RT-PCR studies in *Rhipicephalus (Boophilus) microplus* and *Rhipicephalus appendiculatus* ticks and determination of the expression profile of Bm86. BMC Mol. Biol. 10, 112.

Nijhof, A.M., Balk, J.A., Postigo, M., Rhebergen, A.M., Taoufik, A., Jongejan, F., 2010. Bm86 homologues and novel ATAQ proteins with multiple epidermal growth factor (EGF)-like domains from hard and soft ticks. Int. J. Parasitol. 40, 1587-1597.

Okajima, T., Matsuura, A., Matsuda, T., 2008. Biological functions of glycosyltransferase genes involved in O-fucose glycan synthesis. J. Biochem. 144, 1-6.

Pagel Van Zee, J., Geraci, N.S., Guerrero, F.D., Wikel, S.K., Stuart, J.J., Nene, V.M., Hill, C.A., 2007. Tick genomics: the Ixodes genome project and beyond. Int. J. Parasitol. 37, 1297-1305.

Patel, A., Vought, V.E., Dharmarajan, V., Cosgrove, M.S., 2011. A novel non-SET domain multi-subunit methyltransferase required for sequential nucleosomal histone H3 methylation by the mixed lineage leukemia protein-1 (MLL1) core complex. J. Biol. Chem. 286, 3359-3369.

Pestova, T.V., Kolupaeva, V.G., Lomakin, I.B., Pilipenko, E.V., Shatsky, I.N., Agol, V.I., Hellen, C.U., 2001. Molecular mechanisms of translation initiation in eukaryotes. Proc. Natl. Acad. Sci. USA. 98, 7029-7036.

Pitari, G., Malergue, F., Martin, F., Philippe, J.M., Massucci, M.T., Chabret, C., Maras, B., Duprè, S., Naquet, P., Galland, F., 2000. Pantetheinase activity of membrane-bound Vanin-1: lack of free cysteamine in tissues of Vanin-1 deficient mice. FEBS Lett. 483, 149-154.

Pohl, P.C., Klafke, G.M., Carvalho, D.D., Martins, J.R., Daffre, S., da Silva Vaz, I. Jr., Masuda, A., 2011. ABC transporter efflux pumps: a defense mechanism against ivermectin in *Rhipicephalus (Boophilus) microplus.* Int J Parasitol. 41, 1323-1333.

Pohl, P.C., Klafke, G.M., Júnior, J.R., Martins, J.R., da Silva Vaz, I. Jr., Masuda, A., 2012. ABC transporters as a multidrug detoxification mechanism in *Rhipicephalus (Boophilus) microplus*. Parasitol Res. 111, 2345-2351.

Preissler, S., Deuerling, E., 2012. Ribosome-associated chaperones as key players in proteostasis. Trends Biochem. Sci. 37, 274-283.

Rajput, Z.I., Hu, S.H., Chen, W.J., Arijo, A.G., Xiao, C.W.,2006. Importance of ticks and their chemical and immunological control in livestock. J. Zhejiang Univ. Sci. B. 7, 912-921.

Ribeiro, J.M., Alarcon-Chaidez, F., Francischetti, I.M., Mans, B.J., Mather, T.N., Valenzuela, J.G., Wikel, S.K., 2006. An annotated catalog of salivary gland transcripts from *Ixodes scapularis* ticks. Insect Biochem. Mol. Biol. 36, 111-129.

Ribeiro, J.M., Anderson, J.M., Manoukis, N.C., Meng, Z., Francischetti, I.M., 2011. A further insight into the sialome of the tropical bont tick, *Amblyomma variegatum*. BMC Genomics. 12, 136.

Riding, G.A., Jarmey, J., McKenna, R.V., Pearson, R., Cobon, G.S., Willadsen, P., 1994. A protective "concealed" antigen from *Boophilus microplus*. Purification, localization, and possible function. J Immunol. 153, 5158-5166.

Rodriguez-Valle, M., Lew-Tabor, A., Gondro, C., Moolhuijzen, P., Vance, M., Guerrero, F.D., Bellgard, M., Jorgensen, W., 2010. Comparative microarray analysis of *Rhipicephalus (Boophilus) microplus* expression profiles of larvae pre-attachment and feeding adult female stages on *Bos indicus* and *Bos taurus* cattle. BMC Genomics 11, 437.

Saldivar, L., Guerrero, F.D., Miller, R.J., Bendele, K.G., Gondro, C., Brayton, K.A., 2008. Microarray analysis of acaricide-inducible gene expression in the southern cattle tick, *Rhipicephalus (Boophilus) microplus*. Insect Mol. Biol. 17, 597-606.

Saravanan, T., Weise, C., Sojka, D., Kopácek, P., 2003. Molecular cloning, structure and bait region splice variants of alpha2-macroglobulin from the soft tick *Ornithodoros moubata*. Insect Biochem Mol Biol. 33, 841-851.

Sasaki, S.D., de Lima, C.A., Lovato, D.V., Juliano, M.A., Torquato, R.J., Tanaka, A.S., 2008. BmSI-7, a novel subtilisin inhibitor from *Boophilus microplus*, with activity toward Pr1 proteases from the fungus *Metarhizium anisopliae*. Exp Parasitol. 118, 214-220.

Scheer, M., Grote, A., Chang, A., Schomburg, I., Munaretto, C., Rother, M., Söhngen, C., Stelzer, M., Thiele, J., Schomburg, D., 2011. BRENDA, the enzyme information system in 2011. Nucleic Acids Res. 39(Database issue): D670-D676.

Seetharam, A., Bai, Y., Stuart, G.W., 2010. A survey of well conserved families of C2H2 zinc-finger genes in *Daphnia*. BMC Genomics, 11, 276.

Simser, J.A., Mulenga, A., Macaluso, K.R., Azad, A.F., 2004. An immune responsive factor D-like serine proteinase homologue identified from the American dog tick, *Dermacentor variabilis*. Insect Mol Biol. 13, 25-35.

Sojka, D., Franta, Z., Horn, M., Hajdusek, O., Caffrey, C.R., Mares, M., Kopácek, P., 2008. Profiling of proteolytic enzymes in the gut of the tick *Ixodes ricinus* reveals an evolutionarily conserved network of aspartic and cysteine peptidases. Parasit. Vectors. 1, 7.

Song, B., Liu, X.S., Liu, X., 2012. Polo-like kinase 1 (Plk1): an Unexpected Player in DNA Replication. Cell Div. 7, 3.

Stenmark, H., Olkkonen, V.M., 2001. The Rab GTPase family. Genome Biol. 2, reviews 3007.1–3007.7.

Tatusov, R.L., Fedorova, N.D., Jackson, J.D., Jacobs, A.R., Kiryutin, B., Koonin, E.V., Krylov, D.M., Mazumder, R., Mekhedov, S.L., Nikolskaya, A.N., Rao, B.S., Smirnov, S., Sverdlov, A.V., Vasudevan, S., Wolf, Y.I., Yin, J.J., Natale, D.A., 2003. The COG database: an updated version includes eukaryotes. BMC Bioinformatics. 4, 41.

Thompson, D.M., Khalil, S.M., Jeffers, L.A., Sonenshine, D.E., Mitchell, R.D., Osgood, C.J., Michael Roe, R., 2007. Sequence and the developmental and tissue specific regulation of the first complete vitellogenin messenger RNA from ticks responsible for heme sequestration. Insect Biochem. Mol. Biol. 37, 363-374.

Todd, S.M., Sonenshine, D.E., Hynes, W.L., 2007. Tissue and life-stage distribution of a defensin gene in the Lone Star tick, *Amblyomma americanum*. Med. Vet. Entomol. 21, 141-147.

Tønnesen, M.H., Penzhorn, B.L., Bryson, N.R., Stoltsz, W.H., Masibigiri, T., 2004. Displacement of *Boophilus decoloratus* by *Boophilus microplus* in the Soutpansberg region, Limpopo Province, South Africa. Exp. Appl. Acarol. 32, 199–208.

Tufail, M., Takeda, M., 2009. Insect vitellogenin/lipophorin receptors: molecular structures, role in oogenesis, and regulatory mechanisms. J. Insect Physiol. 55, 87-103.

Turki-Judeh, W., Courey, A.J., 2012. Groucho: a corepressor with instructive roles in development. Curr. Top. Dev. Biol. 98, 65-96.

Uhlik, M.T., Abell, A.N., Cuevas, B.D., Nakamura, K., Johnson, G.L., 2004. Wiring diagrams of MAPK regulation by MEKK1, 2, and 3. Biochem. Cell Biol. 82, 658-663.

Ullmann, A.J., Lima, C.M.R., Guerrero, F.D., Piesman, J., Black, W.C.,2005. Genome size and organization in the blacklegged tick, *Ixodes scapularis* and the Southern cattle tick, *Boophilus microplus*. Insect Mol. Biol. 14, 217-222.

Vanier, M.T., Millat, G., 2004. Structure and function of the NPC2 protein. Biochim Biophys Acta. 1685, 14-21.

Vardanyan, A., Atanesyan, L., Egli, D., Raja, S.J., Steinmann-Zwicky, M., Renkawitz-Pohl, R., Georgiev, O., Schaffner, W., 2008. Dumpy-30 family members as determinants of male fertility and interaction partners of metal-responsive transcription factor 1 (MTF-1) in *Drosophila*. BMC Dev Biol. 8, 68.

Wang, M., Guerrero, F.D., Pertea, G., Nene, V.M., 2007. Global comparative analysis of ESTs from the southern cattle tick, *Rhipicephalus (Boophilus) microplus*. BMC Genomics. 8, 368.

Wang, Z., Gerstein, M., Snyder, M., (2009). RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet. 10, 57-63.

Watkins, N.J., Bohnsack, M.T., 2012. The box C/D and H/ACA snoRNPs: key players in the modification, processing and the dynamic folding of ribosomal RNA. Wiley Interdiscip. Rev. RNA. 3, 397-414.

Weers, P.M., Ryan, R.O., 2006. Apolipophorin III: role model apolipoprotein. Insect Biochem. Mol. Biol. 36, 231-240.

Willadsen, P., Smith, D., Cobon, G., McKenna, R.V., 1996. Comparative vaccination of cattle against *Boophilus microplus* with recombinant antigen Bm86 alone or in combination with recombinant Bm91. Parasite Immunol. 18, 241-246.

Wilson, J.E., 2003. Isozymes of mammalian hexokinase: structure, subcellular localization and metabolic function. J Exp Biol. 206(Pt 12), 2049-2057.

Winchester, B.G., 2001. Lysosomal membrane proteins. Eur. J. Paediatr. Neurol. 5 (Suppl A), 11-19.

Yoshitome, S., Furuno, N., Prigent, C., Hashimoto, E., 2012. The subcellular localization of cyclin B2 is required for bipolar spindle formation during *Xenopus* oocyte maturation. Biochem. Biophys. Res. Commun. 422, 770-775.

Zheng, X.M., Black, D., Chambon, P., Egly, J.M., 1990. Sequencing and expression of complementary DNA for the general transcription factor BTF3. Nature. 344, 556-559.

Zhu, K., Dillwith, J.W., Bowman, A.S., Sauer, J.R., 1997. Identification of hemolytic activity in saliva of the lone star tick (Acari:Ixodidae). J. Med. Entomol. 34, 160-166.

Figures

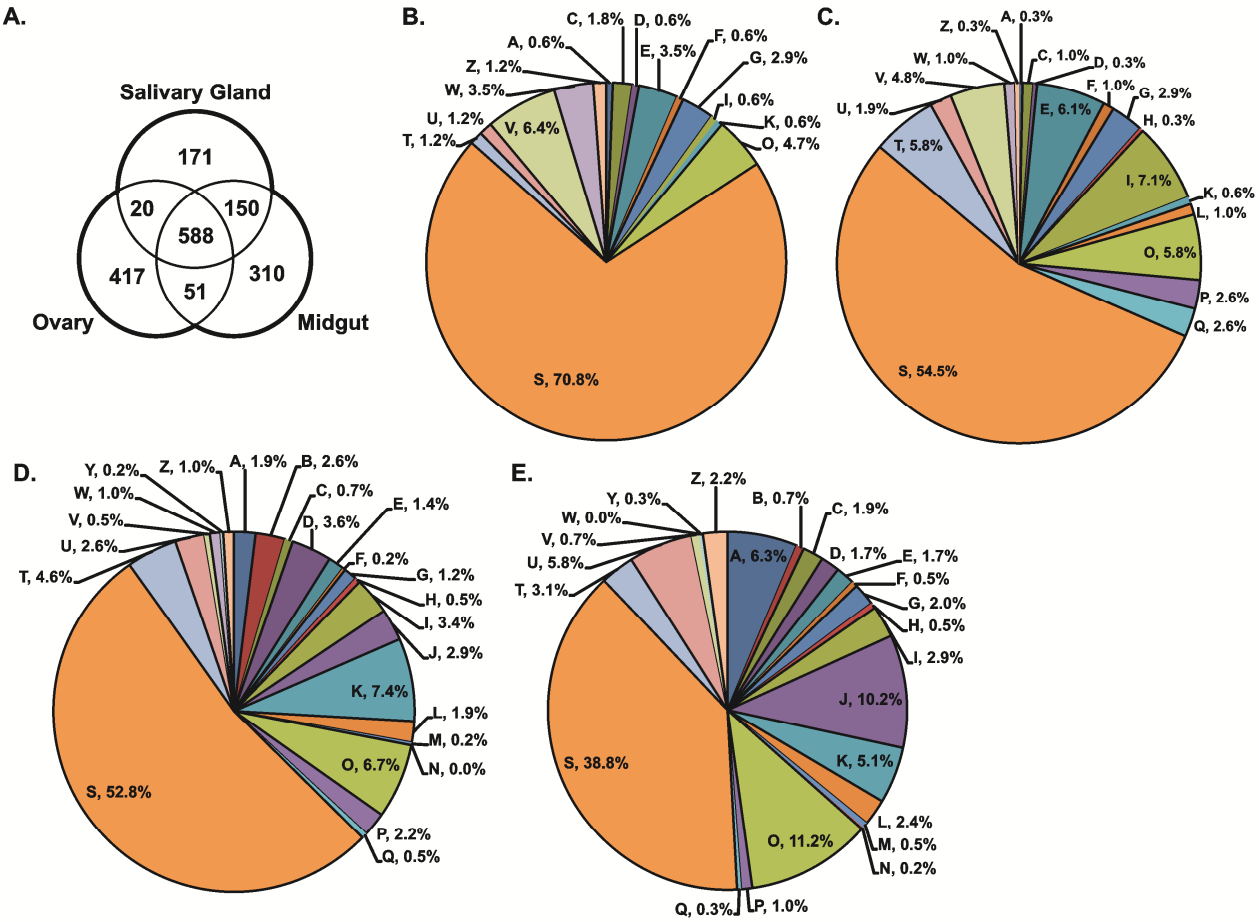

Fig. 1. Distribution and classification of genes regulated between tissues of feeding female *Rhipicephalus microplus* ticks. Indicated are the number of unique genes shared between midgut, salivary glands and ovaries with M values > 0, an intensity threshold above 1,000 and *P* values ≤ 0.001. (A) Venn diagram indicating the number of genes that are unique to or shared between the different tissues. Regulated transcripts in all tissues are classified according to their eukaryotic orthologous functional groups (KOGs). The percentages of unique transcripts regulated in (B) salivary glands, (C) midgut, (D) ovaries and (E) shared between all tissues of female *R. microplus* ticks during feeding are indicated. The functional classifications that are represented include: A, RNA processing and modification; B, chromatin structure and dynamics; C, energy production and conversion; D, cell cycle control, cell division, chromosome partitioning; E, amino acid transport and metabolism; F, nucleotide transport and metabolism; G, carbohydrate transport and metabolism; H, coenzyme transport and metabolism; I, lipid transport and metabolism; J, translation, ribosomal structure and biogenesis; K, transcription; L, replication, recombination and repair; M, cell wall/membrane/envelope biogenesis; N, cell motility; O, posttranslational modification, protein turnover, chaperones; P, inorganic ion transport and metabolism; Q, secondary metabolites biosynthesis, transport and catabolism; S, function unknown (also includes transcripts with only general functional predictions); T, signal transduction mechanisms; U, intracellular trafficking, secretion and vesicular transport; V, defense mechanisms; W, extracellular structures; Y, nuclear structure; Z, cytoskeleton.

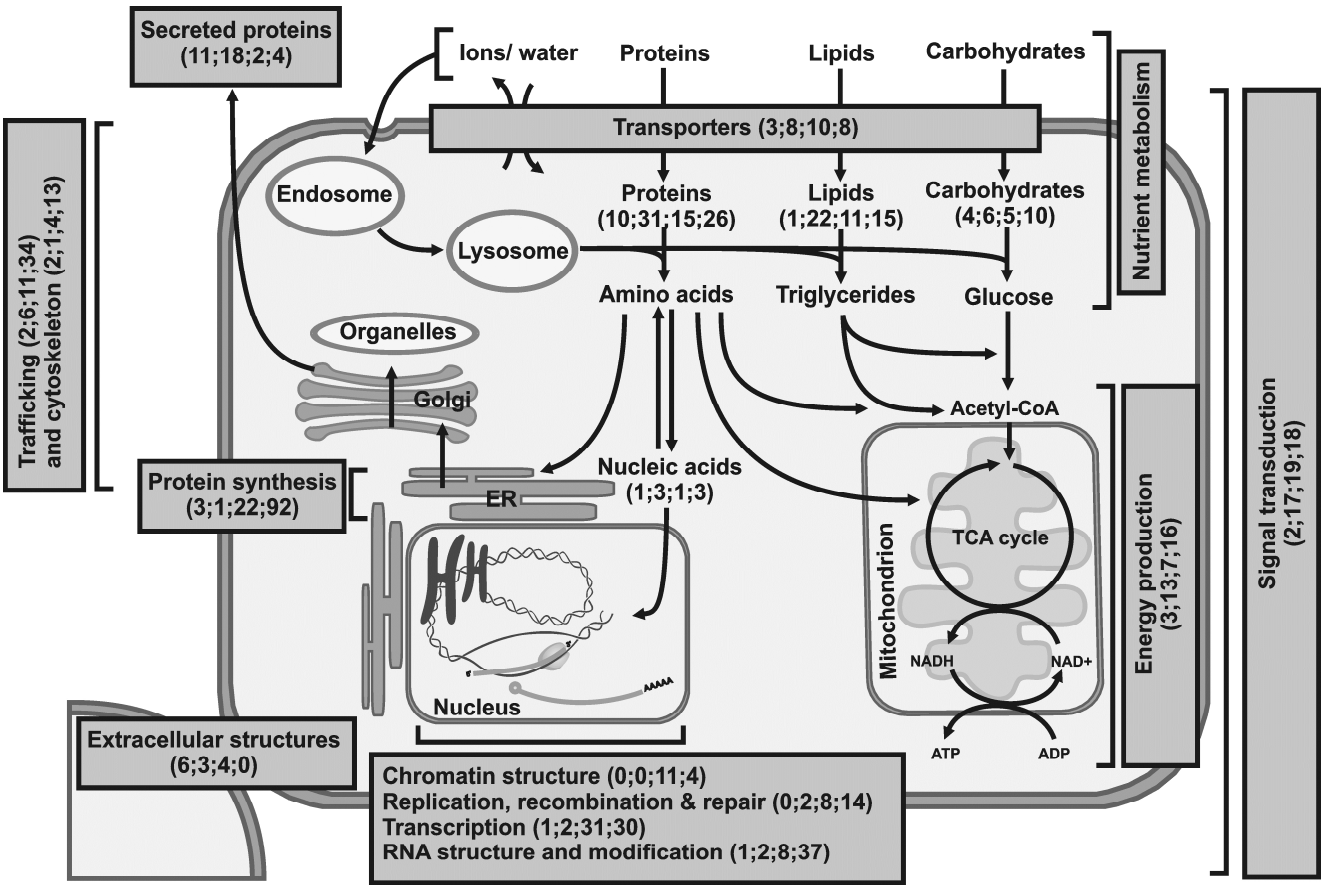

Fig. 2. Overview of regulated transcripts in the tissues of feeding female *Rhipicephalus microplus* ticks. A simplified cell and biological pathways with key processes in blocks are indicated, as well as the relative number of transcripts (in brackets) involved in each process representing salivary glands, midguts, ovaries and shared between all tissues, respectively. The key functional processes represented are: secreted proteins (including anti-coagulants and antimicrobials) that could also include enzymes involved in nutrient acquisition (proteases and lipases); nutrient metabolism that includes both intracellular and extracellular enzymes and transport proteins; enzymes and proteins involved in energy production; enzymes and proteins involved in chromosome structure, replication, transcription, as well as RNA processing and modification; proteins and enzymes involved in protein synthesis, proteins involved in intracellular trafficking and the cytoskeleton, extracellular structures involved in cell-cell contact; receptors, enzymes and accessory proteins involved in signal transduction events throughout the cell. ER, endoplasmic reticulum; TCA, tricarboxylic acid.

Table 1. Summary of unique transcripts expressed in tissues of feeding *Rhipicephalus microplus* female ticks ($P \leq 0.001$). Numbers of transcripts were determined using an intensity threshold of a 1,000 and an average absolute two-fold change relative to other tissues.

	Regulated	Fold change [b]	Salivary gland	Midgut	Ovary	All tissues
Regulation of significant genes [a]	Up	>3	14	130	8	
		>2	62	210	102	
		>1	171	310	417	
	No change	>5,000*	-	-	-	74
		>2,000*				135
		>1,000*				588
Total regulated genes above intensity threshold [c]	5,175		520	552	994	588
Total regulated genes with *P* value ≤ 0.001 [d]	1,707		171	310	417	588[f]
% Regulated genes [e]	12.7		1.3	2.3	3.1	4.4

[a] Data corresponds to fold change values using an empirical Bayes method to identify differentially expressed transcripts with a *P* value ≤ 0.001 adjusted for multiple comparison false discovery rates.

[b] Fold change corresponding to the Log_2 expression ratio of genes with at least a greater than two-fold absolute expression for any given tissue comparison.

[c] Total number of genes regulated above an intensity threshold of 1,000 and an M value (Cy5/Cy3) > 0.

[d] Total number of genes regulated with a *P* value ≤ 0.001 adjusted for multiple comparison false discovery rates.

[e] Percentage of genes regulated from 13,456 total transcripts used on the microarray.

[f] Number of non-differentially expressed genes for all tissue comparisons based on M value (Cy5/Cy3) > 0 and a minimum threshold intensity of 1,000.

Table 2. Summary of unique and shared transcripts expressed in feeding adult *Rhipicephalus microplus* females.

Transcript category	Salivary gland [b]				Midgut [b]				Ovary [b]				Shared transcripts [c]			
(KOG classification) [a]	Quantity [d]	Enzyme [e]	Transport [f]	Average logFC [g]	Quantity [d]	Enzyme [e]	Transport [f]	Average logFC [g]	Quantity [d]	Enzyme [e]	Transport [f]	Average logFC [g]	Quantity [d]	Enzyme [e]	Transport [f]	Intensity threshold >2000 [h]
A. RNA processing and modification	1			2.81	2	1		4.36-3.47	8	6		3.32-1.48	37	13		8
B. Chromatin structure and dynamics									11			4.94-1.55	4			
C. Energy production and conversion	3	3		1.44-1.2	3	3		3.39-2.64	3		3	1.95-1.5	11	4	7	7
D. Cell cycle control, cell division, chromosome partitioning	1			1.89	1	1		4.1	15	7		2.84-1.1	10	3		
E. Amino acid transport and metabolism	6	5	1	3.26-1.27	19	19		5.48-1.28	6	5	1	4.80-2.27	10	9		2
F. Nucleotide transport and metabolism	1		1	1.86	3	3		3.46-1.56	1	1		1.69	3	3		1
G. Carbohydrate transport and metabolism	5	4	1	3.78-1.7	8	6	2	4.72-1.71	5	5		2.62-1.3	12	9	2	4
H. Coenzyme transport and metabolism					2	2		3.35-2.89	2	2		1.30-1.19	3	3		
I. Lipid transport and metabolism	1	1		1.82	22	13		5.59-1.27	14	11	3	4.24-1.57	17	11	2	3
J. Translation, ribosomal structure and biogenesis									12	1		3.42-1.14	60	9		16
K. Transcription	1			1.66	2			4.03-1.27	31	6		3.18-1.07	30	6		6
L. Replication, recombination and repair					2	2		5.18-4.47	8	1		2.94-1.1	14	9		
M. Cell wall/membrane/envelope biogenesis									1			3.11	3			
N. Cell motility													1			
O. Posttranslational modification, protein turnover, chaperones	8	4		2.89-1.55	16	15		5.55-1.44	28	17		4.01-1.04	66	31	1	19
P. Inorganic ion transport and metabolism					8	2	6	5.56-1.9	9		6	3.09-1.37	6		4	1
Q. Secondary metabolites biosynthesis, transport and catabolism					8	6	2	4.01-1.76	2	1	1	1.87-1.56	2	1		
S. Function unknown	121				169				220				228			
T. Signal transduction mechanisms	2	1		2.66-1.72	17	4		5.17-1.38	19	6		3.8-1.1	18	6		3
U. Intracellular trafficking, secretion, and vesicular transport	2			2.54-1.85	6	3	1	3.68-1.31	11	1		3.53-1.17	34	4	6	11
V. Defense mechanisms	11			3.49-1.26	18			5.15-1.55	2	1		1.56-1.18	4	2		1
W. Extracellular structures	6			5.99-1.44	3			4.02-2.51	4			3.67-2.63	0			
Y. Nuclear structure									1		1	1.22	2		2	
Z. Cytoskeleton	2			1.12-1.09	1			3.01	4			2.51-1.01	13			7
Total	171	18	3		310	80	11		417	71	15		588	123	24	90

[a.] Classification of transcripts according to eukaryotic orthologous group terms for gene ontology (Tatusov et al., 2003).

[b.] Unique transcripts expressed in tissues of feeding *R. microplus* female ticks. Transcripts determined using an M value > 0, a minimum threshold of 1,000 and an absolute two-fold change regulation of genes relative to other tissues, *P* value ≤ 0.001.

[c.] Shared transcripts expressed in all tissues of feeding *R. microplus* female ticks. Transcripts determined using an M value > 0 and a minimum intensity threshold of 1,000.

[d.] Total number of transcripts per category.

[e.] Total number of transcripts identified with putative enzymatic function.

[f.] Total number of transcripts identified with putative transporter function.

[g] Highest and lowest average Log_2 expression for transcripts per category. Average Log_2 expression obtained for up-regulated genes across all tissue comparisons, using the arithmetic mean to obtain an overall change in expression for each gene. Log_2 expression ratios (LogFC) calculated for group pair-wise comparisons, to identify significantly differentially expressed transcripts with *P* values adjusted for multiple comparisons false discovery rates.

[h.] Total number of transcripts with M values > 0 and Cy5 intensities > 2,000 in all tissues tested that are considered to be shared.

Supplementary Table S1. Primer sequences (5' to 3') used in quantitative PCR (qPCR) analysis for validation of microarray results including reference gene primer sets used for normalization.

Primer set	Forward	Reverse
Contig1	TGGCGTTCATCCTTCAGTTC	GCGTTCTTCTCTCCGTAAATGTC
Contig5396	AACTTCCCGAAGATTCTGTGTG	CGTATCCTTTCTCCTTGTGTTTG
Contig5672	CTTCCGTGACGATGATACACC	CTTCACATTTATCCCATCCATCC
Contig8723	ATGATCGGCAAGAAGCGTCT	GGAAACCCTTTGTGACACCCTT
Elongation factor 1 alpha (Contig8418)	CGTCTACAAGATTGGTGGCATT	CTCAGTGGTCAGGTTGGCAG

Supplementary Table S3. Unique transcripts expressed in salivary gland tissues of feeding *Rhipicephalus microplus* female ticks ($P \leq 0.001$) identified from microarray analysis. Transcripts were chosen using an intensity threshold of 1,000 and an absolute two-fold change regulation of genes relative to other tissues tested.

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	MG/SG [e] logFC [g]	O/SG [f] logFC [g]	Average logFC [h]
Amino acid transport and metabolism	Contig1	Putative peptidyl-dipeptidase A, angiotensin-converting enzyme (ACE), Peptidase M2 family	*Rhipicephalus microplus*	-3.06	-3.46	3.26
	Contig486	Putative Serine proteinase stubble (Sb), Peptidase S1 family	*Dermacentor variabilis*	-2.91	-1.26	2.09
	Contig1049	Putative aspartate aminotransferase (GOT2), Class-I pyridoxal-phosphate-dependent aminotransferase family	*Ixodes scapularis*	-1.99	-2.16	2.07
	Contig6588	Putative amino acid transporter (AVT1), Amino acid/polyamine transporter 2 family	*Ixodes scapularis*	-1.99	-1.95	1.97
	Contig7971	Putative beta-ureidopropionase (BUP1), CN hydrolase family, BUP subfamily	*Ixodes scapularis*	-1.64	-1.18	1.41
	CV446779	Putative Kynureninase (Kynu), Kynureninase family	*Ixodes scapularis*	-1.54	-1.01	1.27
Carbohydrate transport and metabolism	CV443183	Putative aquaporin, MIP/aquaporin family	*Ixodes scapularis*	-2.32	-5.25	3.78
	Contig3556	Putative chitinase 5 (Cht5), Glycosyl hydrolase 19 family, Chitinase class IV subfamily	*Rhipicephalus sanguineus*	-2.98	-2.93	2.95
	Contig1658	Putative glucose-6-phosphate isomerise (Gpi), GPI family	*Melitaea cinxia*	-2.51	-1.26	1.88
	Contig1253	Putative phosphoglycerate mutase (GPMI), Phosphoglycerate mutase family, BPG-dependent PGAM subfamily	*Ixodes scapularis*	-1.24	-2.25	1.75
	Contig2118	Putative phosphoglycerate kinase (PGK), Phosphoglycerate kinase family	*Ixodes scapularis*	-1.31	-2.09	1.70
Cell cycle control, cell division, chromosome partitioning	Contig2775	Putative BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3), NIP3 family	*Ixodes scapularis*	-1.78	-2.00	1.89
Cytoskeleton	CV441126	Putative microtubule-associated proteins 1A/1B light chain 3A (MAP1LC3A), MAP1 LC3 family	*Monodelphis domestica*	-1.03	-1.20	1.12
	CV436085	Putative thyroid receptor-interacting protein 11-like (TRIP11)	*Strongylocentrotus purpuratus*	-1.04	-1.14	1.09
Defense mechanisms	Contig1520	Putative serpin, Serine protease inhibitor, Serpin family	*Rhipicephalus appendiculatus*	-3.76	-3.23	3.49
	Contig6586	Putative serpin, Serine protease inhibitor, Serpin family	*Ixodes scapularis*	-3.02	-2.77	2.89

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	MG/SG [e]	O/SG [f]	Average logFC [h]
				logFC [g]	logFC [g]	
	Contig5482	Putative antimicrobial peptide, Defensin	*Rhipicephalus microplus*	-2.96	-2.64	2.80
Defense mechanisms	Contig2493	Putative histamine binding protein (HBP), Lipocalin, Calycin superfamily	*Rhipicephalus sanguineus*	-2.85	-2.65	2.75
	CK178656	Putative serpin, Serine protease inhibitor, Serpin family	*Ixodes scapularis*	-2.90	-2.58	2.74
	CK177092	Putative antimicrobial peptide, Microplusin-like protein	*Amblyomma hebraeum*	-2.23	-2.64	2.43
	Contig2529	Putative histamine binding protein (HBP), Lipocalin, Calycin superfamily	*Rhipicephalus sanguineus*	-2.26	-2.09	2.18
	Contig5501	Putative astakine, Prokineticin family, innate immunity	*Acyrthosiphon pisum*	-2.56	-1.66	2.11
	Contig300	Putative thyropin, Thyroglobulin type-1 repeat containing protein	*Rhipicephalus sanguineus*	-2.74	-1.46	2.10
	Contig688	Putative serpin, Serine protease inhibitor, Serpin family	*Ixodes ricinus*	-2.16	-1.20	1.68
	Contig8207	Putative trypsin inhibitor like cysteine rich domain-containing protein (TIL), TIL superfamily	*Ixodes scapularis*	-1.15	-1.38	1.26
Energy production and conversion	Contig240A	Putative NADH dehydrogenase (ubiquinone) 1 alpha subcomplex subunit 10 (ND42)	*Ixodes scapularis*	-1.11	-1.78	1.44
	Contig1269	Putative malate dehydrogenase (MDH2), LDH/MDH superfamily, MDH type 1 family	*Ixodes scapularis*	-1.34	-1.33	1.34
	Contig211	Putative succinate dehydrogenase (ubiquinone) (SDHB), Succinate dehydrogenase/fumarate reductase iron-sulfur protein family	*Ixodes scapularis*	-1.06	-1.34	1.20
Extracellular structures	CV452616	Putative mucin/peritrophin-like protein	*Drosophila melanogaster*	-5.30	-6.67	5.99
	Contig2328	Putative cement protein, Glycine-rich protein	*Ixodes scapularis*	-2.83	-4.04	3.43
	CV437645	Putative cuticle collagen 155 (col-155), Cuticular collagen family, Glycine-rich protein	*Coccidioides posadasii C735 delta SOWgp*	-1.69	-2.79	2.24
	Contig4354	Putative collagen alpha-5(IV) chain (COL4A5), Type IV collagen family, Glycine-rich protein	*Ixodes scapularis*	-1.17	-1.74	1.45
	Contig642	Putative cement protein, Glycine-rich protein	*Ixodes scapularis*	-1.65	-1.24	1.44
Intracellular trafficking, secretion, and vesicular transport	Contig470	Putative Rab5 GDP/GTP exchange factor (RABGEF1)	*Ixodes scapularis*	-3.07	-2.01	2.54
	CK187367	Putative trafficking protein particle complex subunit 9 (TRAPPC9), NIBP family	*Ixodes scapularis*	-1.77	-1.93	1.85
Lipid transport and metabolism	CV454720	Putative phospholipase A2, Phospholipase A2 family	*Ixodes scapularis*	-1.54	-2.10	1.82
Nucleotide transport and	CV438968	Putative solute carrier family 23 member 2 (Slc23a2), Xanthine/uracil permease family, Nucleobase:cation symporter-2 (NCS2)	*Branchiostoma floridae*	-1.38	-2.34	1.86

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	MG/SG [e] logFC [g]	O/SG [f] logFC [g]	Average logFC [h]
metabolism		subfamily				
Posttranslational modification, protein turnover, chaperones	Contig5601	Putative heat shock proteins, Small heat shock protein (HSP20) family	*Ixodes scapularis*	-3.02	-2.77	2.89
	CV454094	Putative heat shock proteins, Small heat shock protein (HSP20) family	*Ixodes scapularis*	-2.39	-3.32	2.86
	Contig6614	Putative cathepsin B, Peptidase C1 family, Longipain-like protein	*Haemaphysalis longicornis*	-2.48	-2.98	2.73
	Contig1050	Putative cathepsin L, Peptidase C1 family	*Rhipicephalus haemaphysaloides haemaphysaloides*	-2.84	-2.18	2.51
	Contig2131	Putative alpha-2-macroglobulin-like protein (A2ML1), Protease inhibitor I39 (alpha-2-macroglobulin) family	*Amblyomma americanum*	-3.22	-1.64	2.43
	Contig1745	Putative alpha-2-macroglobulin-like protein (A2ML1), Protease inhibitor I39 (alpha-2-macroglobulin) family	*Ixodes ricinus*	-2.93	-1.68	2.31
	TC23043	Putative protein-L-isoaspartate(D-aspartate) O-methyltransferase (Pcmt1), Methyltransferase superfamily, L-isoaspartyl/D-aspartyl protein methyltransferase family	*Ixodes scapularis*	-1.64	-2.13	1.89
	Contig6519	Putative peptidylprolyl isomerase (FKBP2), FKBP-type PPIase family, FKBP2 subfamily	*Ixodes scapularis*	-1.34	-1.77	1.55
RNA processing and modification	CK177403	Putative RNA-binding protein Musashi (Rbp6), Musashi family	*Tribolium castaneum*	-3.07	-2.55	2.81
Signal transduction mechanisms	Contig4877	Putative mitogen-activated protein kinase kinase kinase 1 (MAP3K1), Protein kinase superfamily, STE Ser/Thr protein kinase family, MAP kinase kinase kinase subfamily	*Ixodes scapularis*	-2.35	-2.98	2.66
	Contig5314	Putative tetraspanin 5 (TSPAN5), Tetraspanin (TM4SF) family	*Ixodes scapularis*	-1.91	-1.52	1.72
Transcription	Contig663	Putative cyclin L2 (CCNL2), Cyclin family, Cyclin L subfamily	*Ixodes scapularis*	-1.74	-1.59	1.66

[a] Classification of transcripts according to eukaryotic ortologous group terms for gene ontology (Tatusov et al., 2003).

[b] Assigned contiguous sequence identification for transcripts following assembly of all available expressed sequence tags and the *R. microplus* Gene Index version 2.1.

[c] The functional annotation of genes based on comparison among BLAST outputs from seven databases outlined in Section 2.4. All transcript descriptions are based on consensus with database entries from Uniprot (http://www.uniprot.org/uniprot/) and BRENDA (http://www.brenda-enzymes.org/), in the case of enzymes.

[d] Species indicated that showed the highest sequence similarity to *R. microplus* sequences following non-redundant database (NR) BLAST analysis, independent of final assignment.

[e] Log_2 expression ratios (LogFC) calculated for group pair-wise comparison, to identify significant differentially expressed transcripts with *P*-values adjusted for multiple comparisons false discovery rates.

[f] Log_2 expression for group pair-wise comparison between midgut (MG) and salivary glands (SG). Values of MG/SG > 1 correspond to genes that are more expressed in midgut, whereas MG/SG < 1 correspond to genes that are more expressed in salivary glands.

[g] Log_2 expression for group pair-wise comparison between ovaries (O) and salivary glands (SG). Values of O/SG > 1 correspond to genes that are more expressed in midgut, whereas O/SG < 1 correspond to genes that are more expressed in salivary glands.

[h] Average Log_2 expression obtained for up-regulated genes across all tissue comparisons. All negative values have been treated as positive for the calculation of the arithmetic mean, in order to obtain an overall change in expression for each gene.

Supplementary Table S4. Unique transcripts expressed in midgut tissues of feeding *Rhipicephalus microplus* female ticks ($P \leq 0.001$) identified from microarray analysis. Transcripts were chosen using an intensity threshold of 1,000 and an absolute two-fold change regulation of genes relative to other tissues tested.

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	MG/O [f] logFC [e]	MG/SG [g] logFC [e]	Average logFC [h]
Amino acid transport and metabolism	Contig6406	Putative serine protease elastase 2 like (ela2l), Peptidase S1 family	*Rhipicephalus appendiculatus*	6.76	4.21	5.48
	Contig598	Putative serine protease chymotrypsinogen B (CTRB1), Peptidase S1 family, secreted peptidase	*Haemaphysalis longicornis*	6.74	4.19	5.46
	Contig3850	Putative serine protease chymotrypsinogen B (CTRB1), Peptidase S1 family, secreted peptidase	*Ixodes scapularis*	5.84	4.14	4.99
	Contig1748	Putative L-Asparaginase, Asparaginase 1 family	*Drosophila mojavensis*	5.28	4.25	4.76
	Contig8186	Putative betaine-homocysteine S-methyltransferase (BHMT2)	*Branchiostoma floridae*	5.44	3.80	4.62
	Contig5340	Putative serine protease atrial natriuretic peptide-converting enzyme (corin), Peptidase S1 family	*Rhipicephalus appendiculatus*	5.24	3.49	4.37
	Contig1821	Putative serine protease chymotrypsinogen B (CTRB1), Peptidase S1 family, secreted peptidase	*Ornithodoros moubata*	4.28	4.05	4.17
	Contig4941	Putative carboxypeptidase A2 (CPA2), Peptidase M14 family	*Ixodes scapularis*	5.10	3.04	4.07
	Contig5462	Putative serine carboxypeptidase (CPVL), Peptidase S10 family	*Ixodes scapularis*	4.63	3.47	4.05
	Contig2855	Putative glycine N-methyltransferase (GNMT), Class I-like SAM-binding methyltransferase superfamily, Glycine N-methyltransferase family	*Ixodes scapularis*	3.85	3.22	3.53
	Contig5171	Putative aminopeptidase, Peptidase M17 family	*Ixodes scapularis*	4.72	2.12	3.42
	CV440582	Putative pantetheine hydrolase (vanin), CN hydrolase family, BTD/VNN subfamily	*Ixodes scapularis*	3.08	3.16	3.12
	Contig8859	Putative arginase (ARG1), Arginase family	*Ixodes scapularis*	3.72	2.39	3.05
	Contig3884	Putative acireductone dioxygenase (iron(II)-requiring) (ADI1), 1,2-dihydroxy-3-keto-5-methylthiopentene dioxygenase.	*Selaginella moellendorffii*	3.03	2.15	2.59
	Contig506	Putative cytosol non-specific dipeptidase (CNDP), Peptidase M20A family	*Ixodes scapularis*	2.64	2.25	2.45
	Contig6808	Putative ornithine decarboxylase (ODC1)	*Ixodes scapularis*	2.19	1.81	2.00
	CK175263	Putative glycine dehydrogenase (decarboxylating) (GLDC)	*Ixodes scapularis*	1.89	2.05	1.97
	Contig172	Putative aminomethyltransferase (AMT)	*Ixodes scapularis*	1.81	1.59	1.70

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	MG/O [f]	MG/SG [g]	Average
				logFC [e]	logFC [e]	logFC [h]
	CK175193	Putative glycine hydroxymethyltransferase (shmt1)	*Ixodes scapularis*	1.02	1.54	1.28
Carbohydrate transport and metabolism	Contig7972	Putative glucosylceramidase (Gba)	*Ixodes scapularis*	5.16	4.27	4.72
Carbohydrate transport and metabolism	Contig6465	Putative sialin (Sodium/sialic acid cotransporter) (SLC17A5), Solute carrier family 17 member	*Ixodes scapularis*	4.78	3.68	4.23
	Contig8580	Putative Alpha-L-fucosidase (FUCA2)	*Ciona intestinalis*	4.59	3.20	3.90
	Contig4273	Putative Alpha-L-fucosidase (FUCA2)	*Ixodes scapularis*	3.66	2.66	3.16
	CV455491	Putative monocarboxylate transporter 1 (SLC16A1)	*Ixodes scapularis*	2.95	2.87	2.91
	Contig245B	Putative hexokinase	*Ixodes scapularis*	2.80	2.53	2.66
	CV448648	Putative glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase 1 (C1GLT)	*Nasonia vitripennis*	3.17	2.03	2.60
	Contig299B	Putative adenosine kinase (ADK)	*Ixodes scapularis*	1.82	1.59	1.71
Cell cycle control, cell division, chromosome partitioning	Contig4156	Putative caspase-7 (Casp7)	*Haemaphysalis longicornis*	4.54	3.66	4.10
Coenzyme transport and metabolism	Contig392	Putative acid phosphatise (Papl), Metallophosphoesterase superfamily, Purple acid phosphatase family	*Branchiostoma floridae*	4.01	2.70	3.35
	Contig2589	Putative gamma-glutamyl hydrolase (GGH)	*Saccoglossus kowalevskii*	3.05	2.74	2.89
Cytoskeleton	Contig5040	Putative microtubule-associated proteins 1A/1B light chain 3C (MAP1LC3C)	*Hydra magnipapillata*	3.02	3.00	3.01
Defense mechanisms	Contig4907	Putative antimicrobial peptide, Microplusin-like	*Ornithodoros coriaceus*	5.82	4.48	5.15
	Contig4731	Putative antimicrobial peptide, Microplusin-like	*Argas monolakensis*	6.21	4.00	5.11
	TC21607	Putative histamine binding protein (HBP), Lipocalin, Calycin superfamily	*Rhipicephalus sanguineus*	5.92	4.20	5.06
	Contig5662	Putative cystatin, Reversible papain-like cysteine protease inhibitor family	*Haemaphysalis longicornis*	5.93	4.08	5.00
	Contig1086	Putative serpin, Serine protease inhibitor, Serpin family, Ov-serpin subfamily	*Amblyomma americanum*	5.59	4.23	4.91
	CV444905	Putative cystatin, Reversible papain-like cysteine protease inhibitor family	*Haemaphysalis longicornis*	4.97	4.06	4.52
	Contig5243	Putative antimicrobial peptide, Microplusin-like	*Ornithodoros coriaceus*	5.51	3.33	4.42
	TC22004	Putative serine proteinase inhibitor, Boophilin, Kunitz family	*Boophilus microplus*	4.92	3.61	4.26

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	MG/O [f] logFC [e]	MG/SG [g] logFC [e]	Average logFC [h]
	Contig5493	Putative antimicrobial peptide, Microplusin-like	*Ornithodoros coriaceus*	4.80	3.48	4.14
	CV443795	Putative trypsin inhibitor like cysteine rich domain-containing protein (TIL), TIL superfamily	*Rhipicephalus microplus*	3.27	3.42	3.34
Defense mechanisms	CK192299	Putative serine proteinase inhibitor, Kunitz family	*Dermacentor variabilis*	4.18	2.46	3.32
	Contig1698	Putative cystatin, Reversible papain-like cysteine protease inhibitor family	*Rhipicephalus sanguineus*	3.82	2.43	3.13
	CK192837	Putative serine proteinase inhibitor 2 (SPINT2), Kunitz family	*Tribolium castaneum*	3.46	1.52	2.49
	CK188782	Putative trypsin inhibitor like cysteine rich domain-containing protein (TIL), TIL superfamily	*Rhipicephalus microplus*	2.01	2.59	2.30
	CV442792	Putative serpin, Serine protease inhibitor, Serpin family	*Rhipicephalus appendiculatus*	2.44	1.88	2.16
	Contig165	Putative serpin, Serine protease inhibitor, Serpin family	*Ixodes scapularis*	2.70	1.37	2.04
	Contig173B	Putative gallectin-like protein, Galactoside-binding lectin	*Ixodes scapularis*	1.67	1.43	1.55
Energy production and conversion	Contig5385	Putative electron-transferring-flavoprotein dehydrogenase (ETFDH), ETF-QO/fixC family	*Branchiostoma floridae*	2.85	3.92	3.39
	Contig791	Putative retinal dehydrogenase 2 (ALDH1A2)	*Ixodes scapularis*	2.63	3.09	2.86
	CK186476	Putative malate dehydrogenase, Malate/L-lactate dehydrogenase family	*Ixodes scapularis*	3.00	2.27	2.64
Extracellular structures	Contig2856	Putative mucin	*Drosophila melanogaster*	4.19	3.84	4.02
	TC22078	Putative chitin binding peritrophin-A domain protein	*Harpegnathos saltator*	4.38	2.40	3.39
	Contig4613	Putative nidogen (Nid1)	*Pediculus humanus corporis*	3.18	1.83	2.51
inorganic ion transport and metabolism	CV439517	Calcium-activated chloride channel regulator protein (CLCA), CLCR family	*Ixodes scapularis*	6.34	4.78	5.56
	Contig8877	Calcium-activated chloride channel regulator protein (CLCA), CLCR family	*Saccoglossus kowalevskii*	6.13	4.47	5.30
	Contig7035	Putative gluconolactonase (GNL), Regucalcin, SMP-30/CGR1 family	*Ixodes scapularis*	3.93	3.41	3.67
	Contig3919	Putative ferroxidase (Ftn-1), Ferritin	*Ixodes ricinus*	3.37	2.85	3.11
	Contig1796	Putative P protein (OCA2), Citrate (CitM) symporter family	*Ixodes scapularis*	3.18	2.92	3.05
	Contig728	Putative calsequestrin (CASQ2)	*Ixodes scapularis*	2.63	2.94	2.78
	CK174450	Putative potassium channel (KCNK), subfamily K, Two pore domain potassium channel family	*Ixodes scapularis*	2.14	2.14	2.14

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	MG/O [f] logFC [e]	MG/SG [g] logFC [e]	Average logFC [h]
	CV438087	Putative calcium-transporting ATPase, Cation transport ATPase (P-type) family	*Ixodes scapularis*	2.01	1.78	1.90
Intracellular trafficking, secretion, and vesicular transport	CV453190	Putative innexin (inx2), Pannexin family	*Ixodes scapularis*	4.41	2.95	3.68
	Contig1623	Putative atlastin (ATL), GBP family, Atlastin subfamily	*Ixodes scapularis*	3.49	2.08	2.78
	CV456519	Putative vesicular amine transporter 2 (SLC18A2), Major facilitator superfamily (MFS), Vesicular transporter family	*Tribolium castaneum*	2.15	3.38	2.76
	Contig2797	Putative receptor expression-enhancing protein 5-like (REEP), DP1 family	*Ixodes scapularis*	2.22	2.98	2.60
	CK179783	Putative atlastin (ATL), GBP family, Atlastin subfamily	*Ixodes scapularis*	3.06	2.03	2.54
	CV448133	Putative Ras-related protein Rab-30, Small GTPase superfamily, Rab family	*Ixodes scapularis*	1.09	1.53	1.31
Lipid transport and metabolism	Contig156	Putative apolipophorin (Rfabg)	*Ixodes scapularis*	6.74	4.43	5.59
	TC17851	Putative Niemann-Pick type C1 domain-containing protein (NPC1), Patched family	*Ixodes scapularis*	6.69	3.90	5.30
	Contig1508	Putative mite group 2 allergen Tyr p 2-like, Niemann-Pick disease type C2 protein (NPC2) family	*Ixodes ricinus*	6.44	3.94	5.19
	CV443743	Putative mite group 2 allergen Tyr p 2-like, Niemann-Pick disease type C2 protein (NPC2) family	*Ixodes ricinus*	6.29	3.89	5.09
	Contig2297	Putative triacylglycerol lipase, AB hydrolase superfamily, Lipase family	*Ixodes scapularis*	5.35	4.22	4.78
	Contig2302	Putative phosphoethanolamine N-methyltransferase (NMT), Methyltransferase superfamily	*Branchiostoma floridae*	5.98	3.52	4.75
	Contig8127	Putative lipoprotein, Vitellogenin (Yp2), AB hydrolase superfamily. Lipase family	*Haemaphysalis longicornis*	6.27	3.10	4.69
	TC17614	Putative triacylglycerol lipase, AB hydrolase superfamily, Lipase family	*Tribolium castaneum*	5.02	3.71	4.36
	Contig7995	Putative sphingomyelin phosphodiesterase (SMPD1), Acid sphingomyelinase family	*Pediculus humanus corporis*	3.95	3.79	3.87
	Contig2638	Putative estradiol 17-beta-dehydrogenase 8 (HSD17B8), Short-chain dehydrogenases/ reductases (SDR) family	*Ixodes scapularis*	4.16	3.45	3.81
	Contig2086	2-acylglycerol O-acyltransferase (MOGAT), Diacylglycerol acyltransferase family	*Danio rerio*	3.82	3.49	3.65
	Contig3708	Putative long-chain-fatty-acid-CoA ligase, ATP-dependent AMP-binding enzyme family	*Ixodes scapularis*	3.72	3.06	3.39
	Contig1729	Putative proactivator polypeptide (PSAP), Prosaposin	*Ixodes scapularis*	3.32	3.38	3.35
	Contig4249	Putative fatty acid-binding protein (FABP), Lipocalin, Calycin superfamily	*Ixodes scapularis*	3.59	2.43	3.01

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	MG/O [f]	MG/SG [g]	Average
				logFC [e]	logFC [e]	logFC [h]
	Contig6832	Putative 15-hydroxyprostaglandin dehydrogenase (NAD+), Short-chain dehydrogenases/reductases (SDR) family	*Ixodes scapularis*	2.98	2.20	2.59
	Contig6561	Putative estradiol 17-beta-dehydrogenase 8 (HSD17B8), Short-chain dehydrogenases/ reductases (SDR) family	*Ixodes scapularis*	2.49	2.61	2.55
Lipid transport and metabolism	TC22152	Putative phosphatidate phosphatase, PA-phosphatase related phosphoesterase family	*Drosophila virilis*	2.54	2.41	2.47
	Contig2303	Putative fatty acyl-CoA elongase, Long chain fatty acid elongase, ELO family	*Ixodes scapularis*	2.60	2.14	2.37
	Contig727	Putative epididymal secretory protein E1-like, Niemann-Pick disease type C2 protein (NPC2) family	*Dermacentor variabilis*	2.29	2.40	2.34
	Contig2020	Putative ethanolamine kinase 1 (ETNK1), Choline/ethanolamine kinase family	*Ixodes scapularis*	2.04	1.90	1.97
	Contig1102	Putative microsomal triglyceride transfer protein	*Branchiostoma floridae*	1.81	1.04	1.43
	Contig6287	Putative acetate-CoA ligase, ATP-dependent AMP-binding enzyme family	*Nasonia vitripennis*	1.51	1.03	1.27
Nucleotide transport and metabolism	Contig4532	Putative dihydropyrimidine dehydrogenase (NADP+) (DPYD),Dihydropyrimidine dehydrogenase family	*Ixodes scapularis*	3.91	3.01	3.46
	CV455555	Putative GMP synthase (glutamine-hydrolysing) (GMPS)	*Ixodes scapularis*	1.90	1.59	1.75
	Contig7966	Putative IMP dehydrogenase (IMPD), IMPDH/GMPR family	*Camponotus floridanus*	1.84	1.28	1.56
Posttranslational modification, protein turnover, chaperones	Contig8822	Putative glutathione transferase 1 (GST1), GST superfamily, Theta family	*Dermacentor variabilis*	6.84	4.26	5.55
	Contig6100	Putative cathepsin L-like proteinase, Peptidase C1 family	*Rhipicephalus appendiculatus*	6.38	4.61	5.49
	Contig336	Putative glutathione transferase (GST), GST superfamily	*Dermacentor variabilis*	6.75	4.21	5.48
	Contig706	Putative glutathione transferase 1 (GST1), GST superfamily, Theta family	*Dermacentor variabilis*	6.67	4.30	5.48
	Contig953	Putative cathepsin B, Peptidase C1 family	*Ixodes scapularis*	6.40	4.57	5.48
	Contig3558	Putative cathepsin B, Peptidase C1 family	*Ixodes scapularis*	6.01	4.69	5.35
	Contig1506	Putative dipeptidyl-peptidase I (CTSC), Cathepsin C, Peptidase C1 family	*Ixodes ricinus*	6.07	4.55	5.31
	Contig3719	Putative cathepsin B, Peptidase C1 family	*Ixodes scapularis*	6.44	4.16	5.30
	Contig432	Putative cathepsin L, Peptidase C1 family	*Rhipicephalus microplus*	6.08	4.41	5.25
	Contig2640	Putative cathepsin D (CTSD), Peptidase A1 family	*Rhipicephalus microplus*	5.48	3.15	4.31

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	MG/O [f] logFC [e]	MG/SG [g] logFC [e]	Average logFC [h]
	Contig861	Putative gamma-interferon-inducible lysosomal thiol reductase, GILT family	*Amblyomma americanum*	4.81	3.47	4.14
	TC15264	Putative cathepsin D (CTSD), Peptidase A1 family	*Rhipicephalus microplus*	4.33	3.47	3.90
Posttranslational modification, protein turnover, chaperones	Contig2925	Putative gamma-interferon-inducible lysosomal thiol reductase, GILT family	*Amblyomma americanum*	3.30	3.53	3.42
	Contig1863	Putative legumain (LGMN), Peptidase C13 family	*Dermacentor variabilis*	3.93	2.70	3.32
	Contig1303	Putative major heat shock 70 kDa protein (Hsp70), Heat shock protein 70 family	*Ixodes scapularis*	2.98	2.72	2.85
	Contig974	Putative oligopeptidase A, Peptidase M3 family	*Ixodes scapularis*	1.83	1.04	1.44
Replication, recombination and repair	Contig1765	Putative deoxyribonuclease II, DNase II family	*Ixodes scapularis*	5.88	4.49	5.18
	Contig3627	Putative deoxyribonuclease II, DNase II family	*Ixodes scapularis*	5.38	3.56	4.47
RNA processing and modification	Contig3372	Putative ribonuclease T2 family (RNASET2), RNase T2 family	*Ixodes scapularis*	5.35	3.36	4.36
	Contig7833	Putative protein Dom3Z (DOM3Z), Nuclear 5'-3' exoribonuclease-interacting protein, Dom3Z family	*Ixodes scapularis*	3.80	3.14	3.47
Secondary metabolites biosynthesis, transport and catabolism	Contig8732	Putative solute carrier organic anion transporter family member 1C1 (SLCO1C1), Organo anion transporter family	*Ixodes scapularis*	4.01	4.01	4.01
	Contig894	Putative cytochrome P450 (Cyp3a24), Unspecific monooxygenase, Cytochrome P450 family	*Rhipicephalus microplus*	4.18	3.21	3.69
	U92732.1	Putative cytochrome P450, Cytochrome P450 family	*Rhipicephalus microplus*	3.99	3.12	3.55
	CV443756	Putative cytochrome P450 (Cyp3a18), Unspecific monooxygenase, Cytochrome P450 family	*Ixodes scapularis*	2.94	3.12	3.03
	Contig1118	Putative flavin-containing monooxygenase 5 (FMO5), FMO family	*Ixodes scapularis*	4.02	2.03	3.02
	CV451047	Putative ATP-binding cassette sub-family G member 1, ABC transporter superfamily, ABCG family	*Ixodes scapularis*	2.70	3.32	3.01
	Contig3731	Putative oxidoreductase, Short-chain dehydrogenases/reductases (SDR) family	*Ixodes scapularis*	2.95	2.28	2.61
	Contig1505	Putative cytochrome P450 (Cyp3a24), Unspecific monooxygenase, Cytochrome P450 family	*Ixodes scapularis*	1.59	1.93	1.76
Signal transduction mechanisms	Contig6280	Putative leucine-rich repeats and immunoglobulin-like domains-containing protein (LIG)	*Ixodes scapularis*	6.20	4.13	5.17
	Contig4226	Putative cholinesterase (CHE1), Type-B carboxylesterase/lipase family	*Ixodes scapularis*	5.80	4.34	5.07

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	MG/O [f] logFC [e]	MG/SG [g] logFC [e]	Average logFC [h]
	Contig1195	Putative granulin (GRN), Granulin family	*Ixodes scapularis*	5.91	4.21	5.06
	Contig4072	Putative leucine-rich repeats and immunoglobulin-like domains-containing protein (LIG)	*Ixodes scapularis*	5.55	4.16	4.85
Signal transduction mechanisms	Contig4369	Putative leucine-rich repeats and immunoglobulin-like domains-containing protein (LIG)	*Ixodes scapularis*	4.94	3.70	4.32
	Contig376	Putative plexin (semaphoring coreceptor), Plexin family	*Ixodes scapularis*	4.75	3.74	4.25
	Contig8297	Putative acetylcholinesterase, Type-B carboxylesterase/lipase family	*Ixodes scapularis*	4.39	3.79	4.09
	Contig473	Putative calcium-binding protein, Calmodulin (Calm4), Calmodulin family	*Drosophila ananassae*	5.63	2.23	3.93
	Contig1290	Putative phospholipase B-like 2-like, Phospholipase B-like family	*Xenopus (Silurana) tropicalis*	4.31	3.51	3.91
	Contig3639	Putative fibrillin-1, ATAQ protein, Fibrillin family	*Rhipicephalus annulatus*	4.62	3.14	3.88
	Contig3838	Putative Low-density lipoprotein receptor-related protein (LRP), LDLR family	*Ixodes scapularis*	4.10	3.45	3.78
	Contig2938	Ras-related protein Rab-38 (Rab38), GTP-binding protein-like, Small GTPase superfamily, Rab family	*Ixodes scapularis*	2.90	3.50	3.20
	Contig7270	Putative granulin (GRN), Granulin family	*Ixodes scapularis*	3.20	2.52	2.86
	Contig3670	Putative leucine-rich repeats and immunoglobulin-like domains-containing protein (LIG), Putative SLIT and NTRK-like protein, SLITRK family	*Sus scrofa*	3.01	2.09	2.55
	Contig8501	Putative neurogenic locus notch homolog 2 like protein (NOTCH2), Glycoprotein antigen BM86, Fibrillin family	*Rhipicephalus microplus*	3.01	2.01	2.51
	Contig946	Putative calcium and integrin-binding protein 1 (CIB 1), Calmyrin	*Branchiostoma floridae*	1.22	1.62	1.42
	Contig1297	Putative CD63 antigen (CD63), Tetraspanin 30, Tetraspanin (TM4SF) family	*Ixodes scapularis*	1.32	1.44	1.38
Transcription	CV448759	Putative GA-binding protein alpha chain (GABPA), ETS family	*Ixodes scapularis*	4.04	4.03	4.03
	TC20502	Putative nuclear factor related to kappa-B-binding protein (NFRKB), NFRKB family	*Ixodes scapularis*	1.52	1.02	1.27

[a] Classification of transcripts according to eukaryotic ortologous group terms for gene ontology (Tatusov et al., 2003).

[b] Assigned contiguous sequence identification for transcripts following assembly of all available expressed sequence tags and the *R. microplus* Gene Index version 2.1.

[c] The functional annotation of genes based on comparison of BLAST outputs from seven databases outlined in Section 2.4. All transcript descriptions are based on consensus with database entries from Uniprot (http://www.uniprot.org/uniprot/) and BRENDA (http://www.brenda-enzymes.org/), in the case of enzymes.

[d] Species indicated that showed the highest sequence similarity to *R. microplus* sequences following non-redundant database (NR) BLAST analysis, independent of final assignment.

[e] Log_2 expression ratios (LogFC) calculated for group pair-wise comparison, to identify significant differentially expressed transcripts with *P*-values adjusted for multiple comparison false discovery rates.

[f] Log_2 expression for group pair-wise comparison between midgut (MG) and ovaries (O). Values of MG/O > 1 correspond to genes that are more expressed in midgut, whereas MG/O < 1 correspond to genes that are more expressed in ovaries.

[g] Log_2 expression for group pair-wise comparison between midgut (MG) and salivary glands (SG). Values of MG/SG > 1 correspond to genes that are more expressed in midgut, whereas MG/SG < 1 correspond to genes that are more expressed in salivary glands.

[h] Average Log_2 expression obtained for up-regulated genes across all tissue comparisons. All negative values have been treated as positive for the calculation of the arithmetic mean, in order to obtain an overall change in expression for each gene.

Supplementary Table S5. Unique transcripts expressed in ovary tissues of feeding *Rhipicephalus microplus* female ticks ($P \leq 0.001$) identified from microarray analysis. Transcripts were chosen using an intensity threshold of 1,000 and an absolute two-fold change regulation of genes relative to other tissues tested.

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	MG/O [f] logFC [e]	O/SG [g] logFC [e]	Avarege logFC[h]
Amino acid transport and metabolism	Contig6991	Putative Ovochymase-2 (OVCH2), Oviductin, Peptidase S1 family	*Tetraodon nigroviridis*	-6.44	3.16	4.80
	Contig2561	Putative transmembrane protease serine 6 (Tmprss6), Peptidase S1 family	*Tetraodon nigroviridis*	-6.09	2.57	4.33
	Contig8156	Putative serine protease (Stubble), Peptidase S1 family	*Ixodes scapularis*	-5.12	2.51	3.81
	Contig3834	Putative serine carboxypeptidase CPVL (CPVL), Peptidase S10 family	*Ixodes scapularis*	-3.87	1.65	2.76
	Contig6628	Putative neprilysin (NEP2), Peptidase M13 family	*Ixodes scapularis*	-3.28	1.31	2.30
	Contig1302	Putative sodium-coupled neutral amino acid transporter 7 (SLC38A7), Amino acid/polyamine transporter 2 family	*Ixodes scapularis*	-2.83	1.72	2.27
Carbohydrate transport and metabolism	Contig2393	Putative glycoprotein 3-alpha-L-fucosyltransferase, Glycosyltransferase 10 family	*Ixodes scapularis*	-3.29	1.95	2.62
	Contig46A	Putative N-acetyllactosaminide beta-1,3-N-acetylglucosaminyltransferase (B3gnt1), Glycosyltransferase Family 49	*Ixodes scapularis*	-3.23	1.67	2.45
	Contig7812	Putative sulfatase-1 (sulf1), Sulfatase family	*Ixodes scapularis*	-1.96	1.19	1.58
	Contig1898	Putative glycoprotein 3-alpha-L-fucosyltransferase, Glycosyltransferase 10 family	*Ixodes scapularis*	-1.49	1.16	1.32
	Contig7288	Putative anhydro-N-acetylmuramic acid kinase-like protein (anmKl), Anhydro-N-acetylmuramic acid kinase family	*Ixodes scapularis*	-1.39	1.21	1.30
Cell cycle control, cell division, chromosome partitioning	CV451547	Putative G2/mitotic-specific cyclin-B2 (ccnb2), Cyclin family, Cyclin AB subfamily	*Ixodes scapularis*	-3.50	2.17	2.84
	Contig347	Putative non-specific serine/threonine protein kinase (AURKA), Aurora kinase A, Protein kinase superfamily, Ser/Thr protein kinase family, Aurora subfamily	*Ixodes scapularis*	-2.83	1.74	2.29
	Contig6020	Putative coiled-coil domain-containing protein 99 (ccdc99), Protein Spindly, Spindly family	*Equus caballus*	-2.33	1.89	2.11
	Contig3041	Putative actin-binding protein anillin (ANLN)	*Ixodes scapularis*	-2.52	1.31	1.92
	Contig4926	Putative RCC1 and BTB domain-containing protein 1 (Rcbtb1)	*Ixodes scapularis*	-2.05	1.44	1.75
	Contig7399	Putative centriolar coiled-coil protein of 110 kDa (CNTRL), Centriolin	*Branchiostoma floridae*	-1.96	1.52	1.74

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	MG/O [f] logFC [e]	O/SG [g] logFC [e]	Avarege logFC [h]
	Contig7206	Putative microtubule-severing ATPase (SPAST), Spastin, AAA ATPase family, Spastin subfamily	*Ixodes scapularis*	-2.02	1.35	1.69
Cell cycle control, cell division, chromosome partitioning	Contig580	Putative protein-tyrosine-phosphatase (CDC25C), M-phase inducer phosphatase 3, MPI phosphatase family	*Ixodes scapularis*	-2.03	1.32	1.68
	Contig7241	Putative G2/mitotic-specific cyclin-B3 (CycB3), Cyclin family, Cyclin AB subfamily	*Ixodes scapularis*	-2.19	1.14	1.67
	Contig1139	Putative microtubule-severing ATPase (SPAST), Spastin, AAA ATPase family, Spastin subfamily	*Ixodes scapularis*	-1.36	1.70	1.53
	CV446249	Putative cyclin-dependent kinase 14 (CDK14)	*Culex quinquefasciatus*	-1.85	1.21	1.53
	Contig2477	Putative piwi-like protein 1, Argonaute family, Piwi subfamily	*Ixodes scapularis*	-1.51	1.08	1.29
	EW679192.1	Putative mediator of DNA damage checkpoint protein 1 (MDC1)	*Rattus norvegicus*	-1.38	1.10	1.24
	Contig5545	Putative mitotic spindle assembly checkpoint protein MAD2B (MAD2L2)	*Ixodes scapularis*	-1.06	1.15	1.10
	Contig662	Putative piwi-like protein 1, Argonaute family, Piwi subfamily	*Ixodes scapularis*	-1.05	1.15	1.10
Cell wall/ membrane/ envelope biogenesis	Contig2246	Putative transmembrane protein 135-like (Tmem135)	*Ixodes scapularis*	-4.09	2.13	3.11
Chromatin structure and dynamics	Contig6410	Putative histone H3.1 (HIST1H3F), Histone H3 family	*Canis familiaris*	-6.65	3.23	4.94
	Contig5900	Putative histone H3.1 (HIST1H3F), Histone H3 family	*Canis familiaris*	-5.84	2.97	4.41
	TC21623	Putative histone H2A, Histone H2A family	*Caenorhabditis remanei*	-4.68	2.15	3.41
	Contig4221	Putative histone H2A type 1-D, Histone H2A family	*Aplysia californica*	-4.44	2.28	3.36
	CK189050	Putative transducin-like enhancer protein 4 (TLE4), WD repeat Groucho/TLE family	*Ixodes scapularis*	-2.61	1.69	2.15
	Contig6899	Putative microcephalin (Mcph1)	*Ixodes scapularis*	-2.51	1.77	2.14
	Contig5352	Putative histone H4 (HIST1H4B), Histone H4 family	*Canis familiaris*	-2.36	1.45	1.90
	Contig4005	Putative protein groucho 2(GRO2), WD repeat Groucho/TLE family	*Ixodes scapularis*	-2.06	1.33	1.70
	Contig2123	Putative histone H2A, Histone H2A family	*Ixodes scapularis*	-1.97	1.22	1.60
	CV457670	Putative Structural maintenance of chromosomes protein 5 (SMC5), SMC family, SMC5 subfamily	*Ixodes scapularis*	-1.52	1.58	1.55
	Contig4071	Putative Histone H2A.V (H2AFV), Histone H2A family	*Dermacentor variabilis*	-1.75	1.35	1.55
Coenzyme transport and	Contig2901	Putativenicotinate phosphoribosyltransferase (NRPT), NAPRTase family	*Ixodes scapularis*	-1.41	1.19	1.30

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	MG/O [f] logFC [e]	O/SG [g] logFC [e]	Avarege logFC[h]
metabolism						
Coenzyme transport and metabolism	Contig7165	Putative all-trans-decaprenyl-diphosphate synthase subunit 1 (PDSS1), FPP/GGPP synthase family	*Gallus gallus*	-1.36	1.02	1.19
Cytoskeleton	CK177355	Putative Gamma-tubulin complex component 2 (TUBGCP2), GCP family	*Xenopus (Silurana) tropicalis*	-2.93	2.09	2.51
	CV437181	Putative KN motif and ankyrin repeat domain-containing protein (KANK)	*Ixodes scapularis*	-3.13	1.35	2.24
	Contig5218	Putative formin 2 protein (FMN2), Formin homology family	*Ixodes scapularis*	-2.28	1.12	1.70
	Contig8247	Putative thymosin beta-4 (TMSB4X), Thymosin beta family	*Dermacentor variabilis*	-1.01	1.01	1.01
Defense mechanisms	Contig104	Putative peptidase inhibitor 16 (PI16), Cysteine-rich secretory protein family (CRISP)	*Ixodes scapularis*	-1.20	1.93	1.56
	Contig1561	Putative sulfiredoxin (SRXN1), Sulfiredoxin family	*Nasonia vitripennis*	-1.09	1.27	1.18
Energy production and conversion	Contig1954	Putative solute carrier protein family 25 member 16 (SLC25A16), Mitochondrial carrier family	*Ixodes scapularis*	-2.09	1.80	1.95
	CV456316	Putative solute carrier family 25 member 36 (Slc25a36), Mitochondrial carrier family	*Nasonia vitripennis*	-1.85	1.52	1.69
	Contig6442	Putative solute carrier family 25 member 42 (SLC25A42), Mitochondrial carrier family	*Ixodes scapularis*	-1.62	1.38	1.50
Extracellular structures	Contig1170	Putative mucin	*Caenorhabditis remanei*	-4.97	2.38	3.67
	Contig2770	Putative mucin	*Rattus norvegicus*	-4.69	2.56	3.63
	Contig344	Putative mucin	*Saccharomyces cerevisiae*	-4.01	2.37	3.19
	Contig4162	Putative interphotoreceptor matrix proteoglycan 2 (Impg2),	*Strongylocentrotus purpuratus*	-3.29	1.97	2.63
Inorganic ion transport and metabolism	Contig4603	Putative transmembrane protein 163 (TMEM163), TMEM163 family	*Ixodes scapularis*	-4.11	2.08	3.09
	Contig6386	Putative Sodium/potassium/calcium exchanger 6 (SLC24A6), Sodium/potassium/calcium exchanger family, SLC24A subfamily	*Ixodes scapularis*	-3.64	1.94	2.79
	Contig166	Putative superoxide dismutase (SOD1), Cu-Zn superoxide dismutase family	*Cu-Zn*	-3.64	1.82	2.73
	CK173943	Putative calcium-activated chloride channel regulator protein (CLCA), CLCR family	*Saccoglossus kowalevskii*	-3.52	1.60	2.56
	CK188814	Putative prestin (Slc26a5), solute carrier family 26 member 5, SLC26A/SulP transporter family	*Ixodes scapularis*	-2.64	1.89	2.26
	Contig180A	Putative selenium-binding protein 1 (SELENBP1), Selenium-binding protein family	*Xenopus laevis*	-2.42	2.05	2.23

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	MG/O [f] logFC [e]	O/SG [g] logFC [e]	Avarege logFC[h]
	CV448303	Putative sodium bicarbonate transporter-like protein 11 (SLC4A11), Anion exchanger family	*Ixodes scapularis*	-2.79	1.44	2.12
Inorganic ion transport and metabolism	CV453301	Putative zinc transporter SLC39A7	*Ixodes scapularis*	-2.09	1.58	1.83
	Contig70	Putative organic cation transporter-like protein (Orct2), Major facilitator superfamily (MFS), Organic cation transporter family	*Ixodes scapularis*	-1.72	1.01	1.37
Intracellular trafficking, secretion, and vesicular transport	Contig443	Putative clavesin-2 (CLVS2), Retinaldehyde-binding protein 1-like 2	*Ixodes scapularis*	-4.54	2.52	3.53
	Contig1785	Putative tether containing UBX domain for GLUT4 (TUG)	*Pediculus humanus corporis*	-1.45	1.34	1.39
	Contig8621	Putative sorting nexin 21 (SNX21), Sorting nexin family	*Ixodes scapularis*	-1.76	1.00	1.38
	Contig932	Putative conserved oligomeric Golgi complex subunit 4 (COG4), COG4 family	*Ixodes scapularis*	-1.39	1.37	1.38
	Contig3868	Putative maspardin (SPG21), AB hydrolase superfamily	*Branchiostoma floridae*	-1.08	1.64	1.36
	Contig4380	Putative Lateral signalling target protein 2 homolog (ZFYVE28), Zinc finger FYVE domain-containing protein 28	*Ixodes scapularis*	-1.30	1.14	1.22
	Contig5056	Putative Ankyrin repeat and FYVE domain-containing protein 1 (ANKFY1)	*Ixodes scapularis*	-1.09	1.20	1.15
	Contig5948	Putative exocyst complex component 6 (SEC6), SEC6 family	*Branchiostoma floridae*	-1.16	1.11	1.14
	Contig8390	Putative sorting nexin-33 (SNX33), Sorting nexin family	*Pediculus humanus corporis*	-1.03	1.20	1.12
	CK177961	Putative CHMP family member 7 (CHMP7), SNF7 family	*Ixodes scapularis*	-1.18	1.03	1.10
	Contig1237	Putative vesicle transport protein (USE1), USE1 family	*Ixodes scapularis*	-1.30	1.04	1.17
Lipid transport and metabolism	Contig276	Putative Low-density lipoprotein receptor-related protein 4 (LRP4), Putative vitellogenin receptor, LDLR family	*Dermacentor variabilis*	-5.45	3.04	4.24
	CK185778	Putative 3-ketoacyl-CoA synthase (Elovl7), ELO family	*Ixodes scapularis*	-5.80	2.66	4.23
	Contig97	Putative 3-ketoacyl-CoA synthase (Elovl1), ELO family	*Ixodes scapularis*	-5.21	2.37	3.79
	Contig2240	Putative ABC transporter A family member 1 (ABCA1), ABC transporter superfamily, ABCA family	*Ixodes scapularis*	-5.07	2.47	3.77
	Contig190A	Putative 3-ketoacyl-CoA synthase (Elovl4), ELO family	*Ixodes scapularis*	-4.89	2.28	3.58
	Contig6191	Putative ABC transporter G family member 20 (abcG20), ABC transporter superfamily, ABCG family	*Ixodes scapularis*	-4.47	2.23	3.35
	Contig1782	Putative 3-ketoacyl-CoA synthase (Elovl1), ELO family	*Ixodes scapularis*	-3.87	1.95	2.91

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	MG/O [f]	O/SG [g]	Avarege
				logFC [e]	logFC [e]	logFC[h]
	Contig969	Putative long-chain-fatty-acid-CoA ligase	*Ixodes scapularis*	-2.72	2.38	2.55
Lipid transport and metabolism	Contig1663	Putative Fatty acyl-CoA reductase 1 (FAR1), Fatty acyl-CoA reductase family	*Ixodes scapularis*	-2.85	1.49	2.17
	CK177193	Putative triacylglycerol lipase, AB hydrolase superfamily, Lipase family	*Ixodes scapularis*	-2.69	1.54	2.12
	Contig2678	Putative peroxisomal multifunctional enzyme type 2 (HSD17B4), 17-beta-hydroxysteroid dehydrogenase 4, Short-chain dehydrogenases/reductases (SDR) family	*Ixodes scapularis*	-1.95	1.49	1.72
	Contig814	Putative peroxisomal multifunctional enzyme type 2 (HSD17B4), 17-beta-hydroxysteroid dehydrogenase 4, Short-chain dehydrogenases/reductases (SDR) family	*Ixodes scapularis*	-2.24	1.18	1.71
	Contig8307	Putative inositol-polyphosphate 5-phosphatase (INPP5A),Inositol 1,4,5-trisphosphate 5-phosphatase type I family	*Ixodes scapularis*	-1.78	1.43	1.61
	CK183268	Putative protein-tyrosine-phosphatase, Myotubularin-related protein 3 (MTMR3), Protein-tyrosine phosphatase family, Non-receptor class myotubularin subfamily	*Ixodes scapularis*	-1.61	1.53	1.57
Nuclear structure	Contig1959	Putative nucleoporin (NDC1), Transmembrane protein 48 (TMEM48), NDC1 family	*Homo sapiens*	-1.41	1.03	1.22
Nucleotide transport and metabolism	Contig18	Putative hypoxanthine phosphoribosyltransferase (HPRT), Purine/pyrimidine phosphoribosyltransferase family	*Ictalurus punctatus*	-2.34	1.04	1.69
Posttranslational modification, protein turnover, chaperones	CK180207	Putative protein disulfide-isomerase (PDI2), Protein disulfide isomerase family	*Haemaphysalis longicornis*	-5.31	2.71	4.01
	Contig5413	Putative protein disulfide-isomerase (PDI2), Protein disulfide isomerase family	*Ixodes scapularis*	-5.29	2.72	4.00
	Contig1209	Putative cathepsin L (CTSL), Peptidase C1 family	*Rhipicephalus appendiculatus*	-5.00	2.88	3.94
	Contig1826	Putative glutathione transferase (GSTE3), GST superfamily	*Dermacentor variabilis*	-3.95	3.20	3.57
	Contig2266	Putative alpha-2-macroglobulin (CD109), Protease inhibitor I39 (alpha-2-macroglobulin) family	*Ixodes scapularis*	-4.71	1.88	3.30
	Contig8445	Putative disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-like protein 5 (ADAMTSL5)	*Xenopus (Silurana) tropicalis*	-3.48	2.17	2.83
	Contig1441	Putative astacin (nas36), Peptidase M12A family	*Ixodes scapularis*	-3.37	1.33	2.35
	CK187103	Putative alpha-2-macroglobulin receptor-associated protein (Lrpap1), Alpha-2-MRAP family	*Ixodes scapularis*	-2.29	1.94	2.11
	Contig8502	Putative chitobiosyldiphosphodolichol beta-mannosyltransferase (ALG1), Glycosyltransferase group 1 family, Glycosyltransferase	*Trichoplax adhaerens*	-2.02	1.82	1.92

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	MG/O [f] logFC [e]	O/SG [g] logFC [e]	Avarege logFC[h]
		33 subfamily				
Posttranslational modification, protein turnover, chaperones	Contig8117	Putative ubiquitinyl hydrolase 1 (Usp47), Peptidase C19 family, USP47 subfamily	*Mus musculus*	-1.98	1.60	1.79
	Contig3354	Putative DnaJ-like protein subfamily A member 1 (DNAJA1), DnaJ superfamily	*Camponotus floridanus*	-2.15	1.27	1.71
	Contig204	Putative ubiquitinyl hydrolase 1 (USP22), Peptidase C19 family, UBP8 subfamily	*Ixodes scapularis*	-2.05	1.24	1.64
	Contig7869	Putative E3 ubiquitin-protein ligase 3 (MARCH3)	*Ixodes scapularis*	-2.15	1.10	1.62
	Contig3925	Putative glutathione transferase (GSTM), GST superfamily, Mu family	*Rhipicephalus appendiculatus*	-1.72	1.51	1.61
	CV436862	Putative ubiquitinyl hydrolase 1 (USP36), Peptidase C19 family	*Ixodes scapularis*	-1.66	1.47	1.57
	Contig3668	Putative S-phase kinase-associated protein 1 (SKP1), SKP1 family	*Ixodes scapularis*	-1.92	1.16	1.54
	Contig3190	Putative E3 ubiquitin protein ligase TRIP12 (TRIP12), UPL family, K-HECT subfamily	*Ailuropoda melanoleuca*	-1.92	1.16	1.54
	Contig8986	Putative small ubiquitin-related modifier 1 (SUMO1), Ubiquitin family, SUMO subfamily	*Ixodes scapularis*	-1.72	1.25	1.48
	CV441669	Putative endoplasmin (HSP90B1), Heat shock protein 90 family	*Ixodes scapularis*	-1.28	1.37	1.32
	CK184440	Putative ubiquitinyl hydrolase 1 (USP10), Peptidase C19 family, USP10 subfamily	*Strongylocentrotus purpuratus*	-1.03	1.20	1.11
	Contig8510	Putative DnaJ homolog subfamily C member 21 (DNAJC21), DnaJ superfamily	*Xenopus (Silurana) tropicalis*	-1.15	1.07	1.11
	Contig2063	Putative endoplasmin (HSP90B1), Heat shock protein 90 family	*Ixodes scapularis*	-1.09	1.10	1.10
	TC15194	Putative Ulp1 peptidase (SENP1), Sentrin-specific protease 1, Peptidase C48 family	*Ixodes scapularis*	-1.13	1.05	1.09
	Contig2862	Putative heat shock protein HSP 90-alpha (HSP90AA1), Heat shock protein 90 family	*Ixodes scapularis*	-1.08	1.01	1.05
	CK180163	Putative E3 ubiquitin-protein ligase KCMF1, KCMF1 family	*Nasonia vitripennis*	-1.76	1.25	1.51
	Contig3713	Putative G2/M phase-specific E3 ubiquitin-protein ligase (G2E3)	*Branchiostoma floridae*	-1.58	1.00	1.29
	Contig8369	Putative Kelch-like protein 20 (KLHL20), Kelch-like protein diablo	*Ixodes scapularis*	-1.31	1.25	1.28
	CV452499	Putative protein cereblon (CRBN), CRBN family	*Ixodes scapularis*	-1.02	1.05	1.04
Replication, recombination and repair	Contig5833	Putative PCNA-associated factor (PAF)	*Ictalurus furcatus*	-3.62	2.26	2.94

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	MG/O [f] logFC [e]	O/SG [g] logFC [e]	Avarege logFC[h]
	Contig7899	Putative DNA excision repair protein ERCC-6 (ERCC6)	*Ixodes scapularis*	-3.14	2.17	2.66
Replication, recombination and repair	CV454785	Putative protein DBF4 homolog A (DBF4)	*Ixodes scapularis*	-2.01	1.91	1.96
	Contig6830	Putative PAP-associated domain-containing protein 5 (PAPD5), DNA polymerase type-B-like family	*Ixodes scapularis*	-2.22	1.42	1.82
	Contig6802	Putative PCNA-associated factor (PAF)	*Ictalurus furcatus*	-1.97	1.25	1.61
	CK189318	Putative Retrotransposable element Tf2 155 kDa protein type 3 (Tf2-11)	*Ixodes scapularis*	-1.51	1.37	1.44
	Contig7868	Putative Replication protein A 70 kDa DNA-binding subunit (RPA1), Replication factor A protein 1 family	*Callithrix jacchus*	-1.53	1.00	1.27
	CK187178	Putative cell cycle checkpoint protein RAD17 (RAD17), Rad17/RAD24 family	*Ixodes scapularis*	-1.13	1.08	1.10
RNA processing and modification	Contig7796	Putative RNA-directed RNA polymerase (RDR), RdRP family	*Ixodes scapularis*	-4.17	2.46	3.32
	Contig383	Putative protein bicaudal C homolog 1 (BICC1), BicC family	*Ixodes scapularis*	-3.81	2.47	3.14
	Contig621	Putative RNA helicase (MOV10), DNA2/NAM7 helicase family, SDE3 subfamily	*Ixodes scapularis*	-3.01	2.17	2.59
	Contig5328	Putative RNA-binding protein MEX3B (MEX3B)	*Apis mellifera*	-2.57	1.44	2.01
	CK191584	Putative RNA helicase (DDX4), DEAD box helicase family, DDX4/VASA subfamily	*Botryllus primigenus*	-2.07	1.74	1.90
	Contig1284	Putative RNA helicase (DDX4), DEAD box helicase family, DDX4/VASA subfamily	*Ixodes scapularis*	-2.08	1.34	1.71
	CV442379	Putative RNA-directed RNA polymerase (RDR), RdRP family	*Ixodes scapularis*	-1.69	1.52	1.61
	Contig4795	Putative ATP-dependent RNA helicase (DDX31), DEAD box helicase family, DDX31/DBP7 subfamily	*Xenopus (Silurana) tropicalis*	-1.63	1.33	1.48
Secondary metabolites biosynthesis, transport and catabolism	CK173094	Putative 11-cis-retinol dehydrogenase (Rdh1) , Short-chain dehydrogenases/reductases (SDR) family	*Ixodes scapularis*	-2.16	1.58	1.87
	Contig6553	Putative mitochondrial substrate carrier family protein, Solute carrier family 25 member 51 (SLC25A), Mitochondrial carrier family	*Ixodes scapularis*	-1.94	1.19	1.56
Signal transduction mechanisms	Contig4236	Putative polo-like kinase (PLK), Protein kinase superfamily, Ser/Thr protein kinase family, CDC5/Polo subfamily	*Gallus gallus*	-4.79	2.82	3.80
	CK181827	Putative CD82 antigen, Tetraspanin-27, Tetraspanin (TM4SF) family	*Ixodes scapularis*	-2.82	1.43	2.12
	CK179648	Putative PTB domain-containing engulfment adapter protein 1 (CED-6), Ced-6 family	*Ixodes scapularis*	-2.34	1.64	1.99
	CV447216	Putative Rho GTPase-activating protein 20 (ARHGAP20)	*Ixodes scapularis*	-2.28	1.66	1.97

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	MG/O [f] logFC [e]	O/SG [g] logFC [e]	Avarege logFC[h]
Signal transduction mechanisms	CK189238	Putative non-specific serine/threonine protein kinase (CHEK1), Protein kinase superfamily, CAMK Ser/Thr protein kinase family, NIM1 subfamily	*Ornithorhynchus anatinus*	-2.42	1.46	1.94
	Contig6669	Putative GTP-binding protein Rit1 (RIT1), Small GTPase superfamily, Ras family	*Ixodes scapularis*	-2.25	1.53	1.89
	Contig551	Putative discs large-associated protein 5 (DAP-5), SAPAP family	*Ixodes scapularis*	-1.97	1.79	1.88
	CV451440	Putative Rho GTPase-activating protein 20 (ARHGAP20)	*Ixodes scapularis*	-2.27	1.43	1.85
	CV444241	Putative Kelch-like protein 2 (KLHL2), Mayven	*Ixodes scapularis*	-2.24	1.38	1.81
	Contig4788	Putative protein kinase R (PRKR)-interacting protein 1 (PRKRIP1), PRKRIP1 family	*Ixodes scapularis*	-1.98	1.48	1.73
	Contig3355	Putative protein-tyrosine-phosphatase (TPTE)	*Ixodes scapularis*	-1.35	2.07	1.71
	Contig6510	Putative GRB2-associated-binding protein 2 (Gab2), Protein daughter of sevenless (dos)	*Ixodes scapularis*	-2.12	1.14	1.63
	Contig5644	Putative docking protein 5 (DOK5), DOK family	*Ixodes scapularis*	-1.60	1.27	1.44
	CV444871	Putative cation-independent mannose-6-phosphate receptor (IGF2R), MRL1/IGF2R family	*Tetraodon nigroviridis*	-1.19	1.57	1.38
	CV447421	Putative protein phosphatase PP2A 55 kDa regulatory subunit (tws), Phosphatase 2A regulatory subunit B family	*Ixodes scapularis*	-1.32	1.31	1.31
	Contig2486	Putative SH3KBP1-binding protein 1 (SH3KBP1), KCTD3 family	*Ixodes scapularis*	-1.36	1.18	1.27
	Contig2490	Putative TNF receptor-associated factor 6 (TRAF6), TNF receptor-associated factor family, A subfamily	*Branchiostoma floridae*	-1.24	1.15	1.19
	TC15335	Putative Kelch-like protein 2 (KLHL2), Mayven	*Ixodes scapularis*	-1.08	1.24	1.16
	Contig2520	Putative phosphatidylinositol 3-kinase regulatory subunit alpha (Pik3r1), PI3K p85 subunit family	*Ixodes scapularis*	-1.01	1.18	1.10
Transcription	CV457291	Putative zinc finger protein, Krueppel C2H2-type zinc-finger protein family	*Ailuropoda melanoleuca*	-4.10	2.27	3.18
	Contig3306	Putative CCCH-type Zn-finger protein (ZFP36L1)	*Ixodes scapularis*	-3.30	1.87	2.58
	Contig4738	Putative zinc finger protein, Krueppel C2H2-type zinc-finger protein family	*Mus musculus*	-2.90	2.05	2.47
	Contig3878	Putative NFX1-type zinc finger-containing protein 1	*Acyrthosiphon pisum*	-2.37	2.43	2.40
	CK173279	Putative zinc finger protein, Krueppel C2H2-type zinc-finger protein family	*Equus caballus*	-2.45	1.79	2.12
	CV455763	Putative polycomb complex protein BMI-1(BMI1), C3HC4 type (RING finger)	*Xenopus (Silurana) tropicalis*	-2.41	1.78	2.09
	Contig6842	Putative bromo adjacent homology domain-containing 1 protein (BAHD1)	*Ixodes scapularis*	-2.57	1.48	2.03
	Contig6925	Putative RING finger protein 17 (Rnf17)	*Ixodes scapularis*	-2.00	1.78	1.89

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	MG/O [f] logFC [e]	O/SG [g] logFC [e]	Avarege logFC [h]
	TC16744	Putative (histone-H3)-lysine-36 demethylase (KDM2B), JHDM1 histone demethylase family	*Gallus gallus*	-2.40	1.37	1.88
Transcription	CK180492	Putative tudor domain containing 1 protein (TDRD1), TDRD1 family	*Ixodes scapularis*	-2.12	1.58	1.85
	Contig7758	Putative zinc finger protein, Krueppel C2H2-type zinc-finger protein family	*Danio rerio*	-2.22	1.48	1.85
	Contig5974	Putative Tudor domain-containing protein 1 (tdrd1), TDRD1 family	*Ixodes scapularis*	-1.95	1.58	1.77
	Contig4371	Putative zinc finger protein, Krueppel C2H2-type zinc-finger protein family	*Danio rerio*	-1.98	1.38	1.68
	Contig8088	Putative zinc finger protein, Krueppel C2H2-type zinc-finger protein family	*Branchiostoma floridae*	-1.45	1.88	1.67
	CK173872	Putative lethal(3)malignant brain tumor-like protein 2 (L3MBTL2)	*Saccoglossus kowalevskii*	-1.68	1.53	1.60
	CK184156	Putative protein E3 SUMO-protein ligase PIAS (PIAS3), PIAS family	*Ixodes scapularis*	-1.81	1.35	1.58
	CV454199	Putative zinc finger protein, Krueppel C2H2-type zinc-finger protein family	*Ixodes scapularis*	-1.73	1.41	1.57
	CV443642	Putative C2H2-type Zn-finger protein, Krueppel C2H2-type zinc-finger protein family	*Ailuropoda melanoleuca*	-1.91	1.20	1.56
	Contig4264	Putative Tudor domain-containing protein 1 (tdrd1), TDRD1 family	*Ixodes scapularis*	-1.36	1.55	1.45
	Contig7985	Putative zinc finger protein, Krueppel C2H2-type zinc-finger protein family	*Ixodes scapularis*	-1.35	1.51	1.43
	Contig1286	Putative supporter of activation of yellow protein (e(y)3), SAYP family	*Ixodes scapularis*	-1.54	1.32	1.43
	Contig312	Putative zinc finger protein, Krueppel C2H2-type zinc-finger protein family	*Ixodes scapularis*	-1.31	1.41	1.36
	Contig5889	Putative transcription initiation factor TFIID subunit 11 (TAF11), TAF11 family	*Ixodes scapularis*	-1.64	1.04	1.34
	Contig2000	Putative transcriptional repressor p66-beta (GATAD2B)	*Ixodes scapularis*	-1.41	1.19	1.30
	Contig268	Putative CCR4-NOT transcription complex subunit 4 (CNOT4)	*Homo sapiens*	-1.42	1.17	1.29
	Contig5555	Putative RNA uridylyltransferase (ZCCHC11)	*Monodelphis domestica*	-1.45	1.07	1.26
	CV453575	Putative lymphoid-specific helicase (HELLS), SNF2/RAD54 helicase family	*Ixodes scapularis*	-1.43	1.09	1.26
	Contig4658	Putative histone-arginine N-methyltransferase (PRMT6), Protein arginine N-methyltransferase family, PRMT6 subfamily	*Ixodes scapularis*	-1.14	1.27	1.21
	Contig2701	Putative DNA-directed RNA polymerase III subunit RPC1 (POLR3A), RNA polymerase beta' chain family	*Ixodes scapularis*	-1.13	1.20	1.16
	CK175516	Putative GA-binding protein subunit beta-2 (GABPB2)	*Saccoglossus kowalevskii*	-1.09	1.04	1.07
	Contig4680	Putative RE1-silencing transcription factor (REST)	*Ixodes scapularis*	-2.56	2.19	2.38
Translation, ribosomal structure and biogenesis	Contig1465	Putative argonaute (AGO2), Eukaryotic translation initiation factor 2c 2, Argonaute family, Ago subfamily	*Ixodes scapularis*	-4.60	2.23	3.42
	Contig7471	Putative argonaute (AGO4), Eukaryotic translation initiation factor 2c4, Argonaute family, Ago subfamily	*Ixodes scapularis*	-4.25	2.38	3.31

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	MG/O [f] logFC [e]	O/SG [g] logFC [e]	Avarege logFC[h]
	Contig1423	Putative protein LSM14 homolog A (LSM14A), LSM14 family	*Ixodes scapularis*	-3.95	2.67	3.31
Translation, ribosomal structure and biogenesis	CK178055	Putative argonaute (AGO4), Eukaryotic translation initiation factor 2c, Argonaute family, Ago subfamily	*Schistosoma mansoni*	-3.67	2.70	3.18
	Contig2465	Putative argonaute (AGO1), Eukaryotic translation initiation factor 2c 1, Argonaute family, Ago subfamily	*Ixodes scapularis*	-2.27	1.85	2.06
	Contig154	Putative argonaute (AGO2), Eukaryotic translation initiation factor 2c 2, Argonaute family, Ago subfamily	*Ixodes scapularis*	-2.05	1.79	1.92
	CV451557	Putative argonaute (AGO2), Eukaryotic translation initiation factor 2c 2, Argonaute family, Ago subfamily	*Ixodes scapularis*	-1.35	1.38	1.37
	CV439653	Putative polyadenylate-binding protein-interacting protein 1 (PAIP1)	*Ixodes scapularis*	-1.00	1.38	1.19
	Contig8368	Putative B-box type zinc finger protein ncl-1 (ncl-1)	*Ixodes scapularis*	-3.04	1.51	2.28
	Contig1543	Putative DNA-directed RNA polymerase III subunit RPC7 (POLR3G), Eukaryotic RPC7 RNA polymerase subunit family	*Ixodes scapularis*	-1.59	1.23	1.41
	Contig8120	Putative U3 small nucleolar RNA-associated protein 15 homolog (UTP15)	*Ixodes scapularis*	-1.50	1.29	1.39
	CV455361	Putative Integrator complex subunit 3 (INTS3), Integrator subunit 3 family	*Ixodes scapularis*	-1.02	1.27	1.14

[a] Classification of transcripts according to eukaryotic ortologous group terms for gene ontology (Tatusov et al., 2003).

[b] Assigned contiguous sequence identification for transcripts following assembly of all available expressed sequence tags and the *R. microplus* Gene Index version 2.1.

[c] The functional annotation of genes based on comparison of BLAST outputs from seven databases outlined in Section 2.4. All transcript descriptions are based on consensus with database entries from Uniprot (http://www.uniprot.org/uniprot/) and BRENDA (http://www.brenda-enzymes.org/), in the case of enzymes.

[d] Species indicated that showed the highest sequence similarity to *R. microplus* sequences following non-redundant database (NR) BLAST analysis, independent of final assignment.

[e] Log_2 expression ratios (LogFC) calculated for group pair-wise comparison, to identify significant differentially expressed transcripts with p-values adjusted for multiple comparisons false discovery rates.

[f] Log_2 expression for group pair-wise comparison between midgut (MG) and ovaries (O). Values of MG/O > 1 correspond to genes that are more expressed in midgut, whereas MG/O < 1 correspond to genes that are more expressed in ovaries.

[g] Log_2 expression for group pair-wise comparison between ovaries (O) and salivary glands (SG). Values of O/SG > 1 correspond to genes that are more expressed in ovaries, whereas O/SG < 1 correspond to genes that are more expressed in salivary glands.

[h] Average Log_2 expression obtained for up-regulated genes across all tissue comparisons. All negative values have been treated as positive for the calculation of the arithmetic mean, in order to obtain an overall change in expression for each gene.

Supplementary Table S2. Shared transcripts expressed in all tissues of feeding *Rhipicephalus microplus* female ticks identified from microarray analysis. Transcripts were chosen using an M value>0 and a minimum intensity threshold of 1,000.

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	Intensity threshold >1,000 [e]	Intensity threshold >2,000 [f]
Amino acid transport and metabolism	Contig387	Putative phosphoglycerate dehydrogenase (Phgdh), D-isomer specific 2-hydroxyacid dehydrogenase family	*Ixodes scapularis*	Yes	Yes
	Contig8440	Putative phosphoglycerate dehydrogenase (Phgdh), D-isomer specific 2-hydroxyacid dehydrogenase family	*Ixodes scapularis*	Yes	Yes
	Contig2665	Putative nitrilase and fragile histidine triad fusion protein NitFhit (NitFhit), UPF0012 family	*Ixodes scapularis*	Yes	No
	Contig632	Putative acireductone synthase, Enolase-phosphatase E1 (enoph1), HAD-like hydrolase superfamily, MasA/MtnC family	*Xenopus (Silurana) tropicalis*	Yes	No
	Contig4269	Putative fumarylacetoacetase (FAH), FAH family	*Ixodes scapularis*	Yes	No
	CV451089	Putative carboxypeptidase Q (CPQ), Peptidase M28 family	*Ixodes scapularis*	Yes	No
	Contig8749	Putative serine hydrolase-like protein 2 (SERHL2), AB hydrolase superfamily	*Ixodes scapularis*	Yes	No
	Contig6115	Putative cytosol alanyl aminopeptidase, puromycin-sensitive aminopeptidase (NPEPPS), Peptidase M1 family	*Pediculus humanus corporis*	Yes	No
	Contig4651	Putative pyrroline-5-carboxylate reductase, mitochondrial (PYCR1), Pyrroline-5-carboxylate reductase family	*Ixodes scapularis*	Yes	No
	Contig3977	Putative peptidase M20 domain-containing protein (PM20D2), Peptidase M20A family	*Trichoplax adhaerens*	Yes	No
Carbohydrate transport and metabolism	Contig4405	Putative GDP-L-fucose synthase (Tsta3), Fucose synthase family	*Xenopus (Silurana) tropicalis*	Yes	Yes
	Contig497	Putative GDP-mannose 4,6 dehydratase (GMDS), GDP-mannose 4,6-dehydratase family	*Ornithorhynchus anatinus*	Yes	Yes
	Contig835	Putative glucan 1,3-alpha-glucosidase (GANAB), Glycosyl hydrolase 31 family	*Dermacentor variabilis*	Yes	Yes
	Contig8022	Putative tau-protein kinase (GSK3A), Protein kinase superfamily, CMGC Ser/Thr protein kinase family, GSK-3 subfamily	*Ixodes scapularis*	Yes	Yes
	CV435786	Putative GDP-fucose transporter 1 (Slc35c1), TPT transporter family, SLC35C subfamily	*Ixodes scapularis*	Yes	No
	Contig2505	Putative glycolipid transfer protein (GLTP), GLTP family	*Ixodes scapularis*	Yes	No
	Contig1250	Putative lactoylglutathione lyase (GLO1), Glyoxalase I family	*Rattus norvegicus*	Yes	No
	Contig7070	Putative malonyl-CoA decarboxylase (MLYCD)	*Branchiostoma floridae*	Yes	No
	Contig5138	Putative Monocarboxylate transporter 3 (SLC16A8), Major facilitator superfamily, Monocarboxylate porter family	*Ixodes scapularis*	Yes	No

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	Intensity threshold >1,000 [e]	Intensity threshold >2,000 [f]
Carbohydrate transport and metabolism	Contig7956	Putative chitinase domain-containing protein 1 (CHID1), Glycosyl hydrolase 18 family	*Taeniopygia guttata*	Yes	No
	Contig3590	Putative glucose-6-phosphate 1-epimerase, Glucose-6-phosphate 1-epimerase family	*Nasonia vitripennis*	Yes	No
	Contig5221	Putative UDP-sugar diphosphatase (NUDT14), Nudix hydrolase family	*Tribolium castaneum*	Yes	No
Cell cycle control, cell division, chromosome partitioning	Contig4133	Putative baculoviral IAP repeat-containing protein 5 (BIRC5), Survivin, IAP family	*Ixodes scapularis*	Yes	No
	Contig8318	Putative cyclin-dependent kinase 7 (CDK7), Protein kinase superfamily, CMGC Ser/Thr protein kinase family, CDC2/CDKX subfamily	*Ixodes scapularis*	Yes	No
	Contig2869	Putative cell cycle control protein 50A (TMEM30A), CDC50/LEM3 family	*Ixodes scapularis*	Yes	No
	Contig8432	Putative non-specific protein-tyrosine kinase (WEE1), Protein kinase superfamily, Ser/Thr protein kinase family, WEE1 subfamily	*Platynereis dumerilii*	Yes	No
	Contig3399	Putative malignant T-cell-amplified sequence 1 (MCTS1), MCTS1 family	*Pediculus humanus corporis*	Yes	No
	EW679736.1	Putative ubiquitin-like protein Nedd8 (NEDD8), Ubiquitin family	*Amblyomma americanum*	Yes	No
	Contig2832	Putative regulator of chromosome condensation (RCC1)	*Ixodes scapularis*	Yes	No
	Contig6A	Putative cyclin-dependent kinase (CDK10), Protein kinase superfamily, CMGC Ser/Thr protein kinase family, CDC2/CDKX subfamily	*Ixodes scapularis*	Yes	No
	Contig3338	Putative septin-1 (SEP1), Septin family	*Ixodes scapularis*	Yes	No
	Contig1039	Putative protein MIS12 homolog (MIS12), Mis12 family	*Taeniopygia guttata*	Yes	No
Cell motility	Contig2605	Putative dynein light chain Tctex-type 1 (Dynlt1)	*Ixodes scapularis*	Yes	No
Cell wall/ membrane/ envelope biogenesis	CV447425	Putative dynamin-like 120 kDa protein, mitochondrial (OPA1), Dynamin family	*Ixodes scapularis*	Yes	No
	Contig5042	Putative coiled-coil-helix-coiled-coil-helix domain-containing protein 3 (CHCHD3)	*Ixodes scapularis*	Yes	No
	Contig527	Putative NSFL1 cofactor p47 (NSFL1C), NSFL1C family	*Ixodes scapularis*	Yes	No
Chromatin structure and dynamics	Contig1194	Putative nucleosome assembly protein 1-like 1 (nap1l1-b)	*Ixodes scapularis*	Yes	Yes
	Contig1337	Putative Inhibitor of growth protein 2 (ING2), ING family	*Ixodes scapularis*	Yes	No

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	Intensity threshold >1,000 [e]	Intensity threshold >2,000 [f]
Chromatin structure and dynamics	Contig3136	Putative something about silencing protein 10 (UTP3), SAS10 family	*Ixodes scapularis*	Yes	No
	Contig6878	Putative male-specific lethal 3 homolog (MSL3)	*Callithrix jacchus*	Yes	No
Coenzyme transport and metabolism	CV456939	Putative protoheme IX farnesyltransferase, mitochondrial (COX10), UbiA prenyltransferase family	*Ixodes scapularis*	Yes	No
	CK174330	Putative ubiquinone biosynthesis monooxygenase COQ6 (COQ6), UbiH/COQ6 family	*Hydra magnipapillata*	Yes	No
	Contig2881	Putative 4-hydroxybenzoate polyprenyltransferase, mitochondrial (coq2), UbiA prenyltransferase family	*Anopheles gambiae str. PEST*	Yes	No
Cytoskeleton	Contig3106	Putative myosin-2 essential light chain (Mlc-c)	*Ixodes scapularis*	Yes	Yes
	Contig3933	Putative myosin regulatory light chain, Protein spaghetti-squash (sqh)	*Ixodes pacificus*	Yes	Yes
	CK173010	Putative Moesin/ezrin/radixin homolog 1 (MOE)	*Glossina morsitans morsitans*	Yes	Yes
	Contig7803	Putative beta-centractin (ACTR1B), Actin family, ARP1 subfamily	*Ixodes scapularis*	Yes	Yes
	Contig1435	Putative actin-related protein 3B (ACTR3B), Actin family, ARP3 subfamily	*Bos taurus*	Yes	Yes
	Contig3798	Putative actin-related protein Arp2/3 complex (ARPC4), ARPC4 family	*Pediculus humanus corporis*	Yes	Yes
	Contig3260	Putative dynein light chain type 2 (DYNLL2), Dynein light chain family	*Ailuropoda melanoleuca*	Yes	Yes
	CV445272	Putative actin-related protein 10 (ACTR10), Actin family	*Monodelphis domestica*	Yes	No
	CV439239	Putative actin-related protein 5 (ACTR5), Actin family, ARP5 subfamily	*Saccoglossus kowalevskii*	Yes	No
	Contig5192	Putative actin-related protein Arp2/3 complex (ARPC5), ARPC5 family	*Ixodes scapularis*	Yes	No
	Contig613	Putative Bridging integrator 3 (BIN3)	*Ixodes scapularis*	Yes	No
	Contig582	Putative Calponin 3 (CNN3), Calponin family	*Ixodes scapularis*	Yes	No
	Contig1979	Putative nuclear distribution protein nudE-like 1 (NDEL1), NudE family	*Saccoglossus kowalevskii*	Yes	No
Defense mechanisms	Contig3339	Putative Bax inhibitor 1 (TMBIM6), BI1 family	*Ixodes scapularis*	Yes	Yes
	Contig3075	Putative Complement component 1 Q subcomponent-binding protein, mitochondrial (C1QBP), MAM33 family	*Ixodes scapularis*	Yes	No
	Contig113	Putative S-formylglutathione hydrolase (ESD), Putative Esterase D, esterase D family	*Ixodes scapularis*	Yes	No

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	Intensity threshold >1,000 [e]	Intensity threshold >2,000 [f]
Defense mechanisms	Contig2904	Putative S-formylglutathione hydrolase (ESD), Putative Esterase D, esterase D family	*Ixodes scapularis*	Yes	No
Energy production and conversion	Contig2837	Putative cytochrome c oxidase, subunit 1 (MT-CO1), Heme-copper respiratory oxidase family	*Rhipicephalus sanguineus*	Yes	Yes
	Contig4422	Putative NAD(P)(+) transhydrogenase (AB-specific), mitochondrial (NNT), AlaDH/PNT family, PNT beta subunit family	*Ixodes scapularis*	Yes	Yes
	Contig4681	Putative ADP/ATP translocase 2 (SLC25A5), Mitochondrial carrier family	*Ixodes scapularis*	Yes	Yes
	Contig424	Putative isocitrate dehydrogenase (NADP(+)), mitochondrial (IDH2), Isocitrate and isopropylmalate dehydrogenases family	*Ixodes scapularis*	Yes	Yes
	Contig8631	Putative V-type proton ATPase subunit H (VhaSFD), V-ATPase H subunit family	*Nasonia vitripennis*	Yes	Yes
	Contig2278	Putative V-type proton ATPase subunit e 1 (ATP6V0E1), V-ATPase e subunit family	*Ixodes scapularis*	Yes	Yes
	Contig8181	Putative V-type proton ATPase subunit G (Vha13), V-ATPase G subunit family	*Ixodes scapularis*	Yes	Yes
	Contig596	Putative pyruvate dehydrogenase protein X component, mitochondrial (Pdhx), 2-oxoacid dehydrogenase family	*Ixodes scapularis*	Yes	No
	Contig1552	Putative H(+)-transporting two-sector ATPase, mitochondrial (beta subunit) (ATP5B), ATPase alpha/beta chains family	*Ixodes scapularis*	Yes	No
	Contig7051	Putative V-type proton ATPase 116 kDa subunit a isoform 1 (ATP6V0A1), V-ATPase 116 kDa subunit family	*Ixodes scapularis*	Yes	No
	Contig91	Putative V-type proton ATPase subunit C 1 (ATP6V1C1), V-ATPase C subunit family	*Amblyomma americanum*	Yes	No
Inorganic ion transport and metabolism	CV447239	Putative sodium-independent sulfate anion transporter (Slc26a11), SLC26A/SulP transporter family	*Ixodes scapularis*	Yes	Yes
	Contig3592	Putative sodium/potassium-transporting ATPase subunit beta-2 (nrv2), X(+)/potassium ATPases subunit beta family	*Ixodes scapularis*	Yes	No
	Contig2982	Putative cation-transporting ATPase 13A1 (ATP13A1), Cation transport ATPase (P-type) family, Type V subfamily	*Ixodes scapularis*	Yes	No
Inorganic ion transport and metabolism	CV454271	Putative cation-transporting ATPase 13A1 (ATP13A1), Cation transport ATPase (P-type) family, Type V subfamily	*Tribolium castaneum*	Yes	No
	Contig3147	Putative Store-operated calcium entry-associated regulatory factor (tmem66), SARAF family	*Monodelphis domestica*	Yes	No
	Contig17	Putative Store-operated calcium entry-associated regulatory factor (tmem66), SARAF family	*Ixodes scapularis*	Yes	No
Intracellular trafficking, secretion, and vesicular transport	Contig6255	Putative mitochondrial import inner membrane translocase subunit TIM13 (TIM13), Small Tim family	*Pediculus humanus corporis*	Yes	Yes
	Contig3747	Putative nuclear transport factor 2 (NUTF2)	*Aedes aegypti*	Yes	Yes
	Contig4224	Putative signal recognition particle 14 kDa protein (SRP14), SRP14 family	*Rhipicephalus sanguineus*	Yes	Yes

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	Intensity threshold >1,000 [e]	Intensity threshold >2,000 [f]
Intracellular trafficking, secretion, and vesicular transport	TC16109	Putative Charged multivesicular body protein 2a (CHMP2A), SNF7 family	*Ixodes scapularis*	Yes	Yes
	Contig7685	Putative vacuolar protein-sorting-associated protein 36 (VPS36), VPS36 family	*Ixodes scapularis*	Yes	Yes
	CV442636	Putative coatomer subunit alpha (COPA)	*Ixodes scapularis*	Yes	Yes
	TC17848	Putative coatomer subunit gamma-2 (COPG2), COPG family	*Ixodes scapularis*	Yes	Yes
	Contig679	Putative golgi reassembly-stacking protein 2 (GORASP2), GORASP family	*Branchiostoma floridae*	Yes	Yes
	Contig8588	Putative ADP-ribosylation factor 1 (Arf79F), Small GTPase superfamily, Arf family	*Argas monolakensis*	Yes	Yes
	Contig3642	Putative ADP-ribosylation factor 5 (ARF5), Small GTPase superfamily, Arf family	*Marsupenaeus japonicus*	Yes	Yes
	CV443072	Putative mannose lectin ERGIC-53 (LMAN1)	*Ixodes scapularis*	Yes	Yes
	Contig1803	Putative peroxisomal membrane protein 11C (PEX11G), Peroxin-11 family	*Oryctolagus cuniculus*	Yes	No
	CK181968	Putative Mitochondrial Rho GTPase 1 (RHOT1), Mitochondrial Rho GTPase family	*Ixodes scapularis*	Yes	No
	Contig966	Putative charged multivesicular body protein 5 (CHMP5), SNF7 family	*Ixodes scapularis*	Yes	No
	Contig2591	Putative protein YIPF5 (yipf5), YIP1 family	*Ixodes scapularis*	Yes	No
	Contig591	Putative GRIP and coiled-coil domain-containing protein 2 (GCC2)	*Macaca mulatta*	Yes	No
	Contig5586	Putative Syntaxin-5 (STX5), syntaxin family	*Saccoglossus kowalevskii*	Yes	No
	Contig4969	Putative Mitochondrial import receptor subunit TOM22 homolog (TOMM22), Tom22 family	*Tribolium castaneum*	Yes	No
	CK189108	Putative mitochondrial import receptor subunit TOM40 homolog (TOMM40), Tom40 family	*Anopheles gambiae str. PEST*	Yes	No
	Contig1350	Putative trafficking protein particle complex subunit 3 (TRAPPC3), TRAPP small subunits family, BET3 subfamily	*Ixodes scapularis*	Yes	No
	Contig4688	Putative protein kish-A (TMEM167A), KISH family	*Argas monolakensis*	Yes	No
	Contig6912	Putative charged multivesicular body protein 1a (CHMP1A), SNF7 family	*Ixodes scapularis*	Yes	No
	Contig7572	Putative coatomer subunit beta (COPB1)	*Xenopus (Silurana) tropicalis*	Yes	No
	Contig6131	Putative protein transport protein Sec24A (SEC24A), SEC23/SEC24 family, SEC24 subfamily	*Ixodes scapularis*	Yes	No
	Contig6855	Putative AP-1 complex subunit mu-1 (AP1M1), Adaptor complexes medium subunit family	*Tribolium castaneum*	Yes	No

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	Intensity threshold >1,000 [e]	Intensity threshold >2,000 [f]
Intracellular trafficking, secretion, and vesicular transport	Contig3246	Putative protein cornichon homolog (cni), Cornichon family	*Strongylocentrotus purpuratus*	Yes	No
	Contig6268	Putative Ras-related protein Rab-6A (RAB6A), Small GTPase superfamily, Rab family	*Ixodes scapularis*	Yes	No
	Contig6711	Putative Importin subunit alpha-6 (KPNA5), Importin alpha family	*Ixodes scapularis*	Yes	No
	CV439557	Putative lipopolysaccharide-responsive and beige-like anchor protein (LRBA)	*Ixodes scapularis*	Yes	No
	Contig101	Putative protein ERGIC-53 (LMAN1)	*Ixodes scapularis*	Yes	No
	Contig201A	Putative phosphoserine phosphatase, Vacuolar protein sorting-associated protein 29 (VPS29), VPS29 family	*Ixodes scapularis*	Yes	No
	Contig8136	Putative E3 SUMO-protein ligase RanBP2 (RANBP2)	*Ixodes scapularis*	Yes	No
	Contig2281	Putative mitochondrial import receptor subunit TOM7 (TOM7), Tom7 family	*Apis mellifera*	Yes	No
	CK185391	Putative exocyst complex component 7 (Exoc7), EXO70 family	*Ixodes scapularis*	Yes	No
Lipid transport and metabolism	Contig1246	Putative 3-hydroxyacyl-CoA dehydrogenase (HADH), 3-hydroxyacyl-CoA dehydrogenase family	*Ixodes scapularis*	Yes	Yes
	Contig8253	Putative Acetyl-CoA C-acetyltransferase (ACAT1), mitochondrial, Thiolase family	*Ixodes scapularis*	Yes	Yes
	Contig2916	Putative Acetyl-CoA C-acetyltransferase (ACAT1), mitochondrial, Thiolase family	*Ixodes scapularis*	Yes	Yes
	Contig6086	Putative methylmalonyl-CoA epimerase, mitochondrial (MCEE), Glyoxalase I family	*Ixodes scapularis*	Yes	No
	Contig1953	Putative acetyl-coenzyme A transporter 1 (slc33a1), SLC33A transporter family	*Ixodes scapularis*	Yes	No
	Contig3679	Putative long-chain-fatty-acid--CoA ligase (ACSL), ATP-dependent AMP-binding enzyme family	*Ixodes scapularis*	Yes	No
	Contig416	Putative fatty-acid synthase (FASN)	*Ixodes scapularis*	Yes	No
	Contig7867	Putative Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial (Ech1), Enoyl-CoA hydratase/isomerase family	*Ixodes scapularis*	Yes	No
	Contig2062	Putative fatty acid-binding protein (FABP3), Lipocalin, Calycin superfamily, Fatty-acid binding protein (FABP) family	*Ixodes scapularis*	Yes	No
	CV436734	Putative (acyl-carrier-protein) S-malonyltransferase, mitochondrial (MCAT), Type II malonyltransferase family	*Ixodes scapularis*	Yes	No
	Contig4098	Putative butyryl-CoA dehydrogenase (ACADS), Acyl-CoA dehydrogenase family	*Ixodes scapularis*	Yes	No
	Contig1172	Putative mitochondrial carnitine/acylcarnitine carrier protein (SLC25A20), mitochondrial carrier family	*Ixodes scapularis*	Yes	No
	TC22535	Putative phosphatidylinositol N-acetylglucosaminyltransferase (PIGP), PIGP family	*Ixodes scapularis*	Yes	No
	Contig2902	Putative phosphatidylinositide phosphatase SAC1 (SACM1L)	*Ixodes scapularis*	Yes	No

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	Intensity threshold >1,000 [e]	Intensity threshold >2,000 [f]
Lipid transport and metabolism	CV441576	Putative protein spinster homolog 1 (SPNS1), Major facilitator superfamily, Spinster family	*Tribolium castaneum*	Yes	No
	Contig167	Putative Niemann-Pick type C1 domain-containing protein (NPC1), Patched family	*Ixodes scapularis*	Yes	No
	Contig3515	Putative glycerone-phosphate O-acyltransferase (GNPAT), GPAT/DAPAT family	*Strongylocentrotus purpuratus*	Yes	No
Nuclear structure	CV439363	Putative nucleoporin NUP53 (NUP35), Nup53 family	*Ixodes scapularis*	Yes	No
	TC15380	Putative nuclear pore complex protein Nup155 (NUP155), Non-repetitive/WGA-negative nucleoporin family	*Ixodes scapularis*	Yes	No
Nucleotide transport and metabolism	Contig8942	Putative dUTP diphosphatase (DUT), DUTPase family	*Ixodes scapularis*	Yes	Yes
	Contig3765	Putative cytidine deaminase (CDA), Cytidine and deoxycytidylate deaminase family	*Ixodes scapularis*	Yes	No
	Contig6308	Putative Nucleoside-triphosphatase, Cancer-related nucleoside-triphosphatase (NTPCR), THEP1 NTPase family	*Ixodes scapularis*	Yes	No
Posttranslational modification, protein turnover, chaperones	Contig5499	Putative heat shock protein 60, mitochondrial (HSP60/HSPD1), Chaperonin (HSP60) family	*Hyalomma marginatum rufipes*	Yes	Yes
	Contig5025	Putative Stress-induced-phosphoprotein 1 (STIP1)	*Harpegnathos saltator*	Yes	Yes
	Contig2747	Putative endoplasmic reticulum resident protein 29 (ERP29)	*Rhipicephalus sanguineus*	Yes	Yes
	Contig5214	Putative RING-box protein 1A (Roc1a), RING-box family	*Ixodes scapularis*	Yes	Yes
	Contig1836	Putative 15 kDa selenoprotein (SEP15), Selenoprotein M/SEP15 family	*Ixodes scapularis*	Yes	Yes
	Contig8175	Putative ubiquitin-protein ligase (UbcD4), Ubiquitin-conjugating enzyme family	*Ixodes scapularis*	Yes	Yes
	Contig2948	Putative proteasome endopeptidase complex, subunit alpha type-2 (PSMA2), peptidase T1A family	*Aedes aegypti*	Yes	Yes
	Contig903	Putative Proteasome endopeptidase complex, subunit alpha type-7 (PSMA7), Peptidase T1A family	*Ixodes scapularis*	Yes	Yes
	Contig887	Putative proteasome endopeptidase complex, subunit beta type-1 (PROS26), peptidase T1B family	*Tribolium castaneum*	Yes	Yes
	Contig2750	Putative alpha-crystallin B chain (CRYAB), Small heat shock protein (HSP20) family	*Dermacentor variabilis*	Yes	Yes
	Contig2634	Putative heat shock protein beta-2 (HSPB2), Small heat shock protein (HSP20) family	*Dermacentor variabilis*	Yes	Yes
	Contig1372	Putative calnexin (CANX), Calreticulin family	*Ixodes scapularis*	Yes	Yes
	Contig390	Putative calreticulin (CALR), Calreticulin family	*Rhipicephalus microplus*	Yes	Yes

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	Intensity threshold >1,000 [e]	Intensity threshold >2,000 [f]
Posttranslational modification, protein turnover, chaperones	Contig210	Putative T-complex protein 1 subunit alpha (TCP1), TCP-1 chaperonin family	*Apis mellifera*	Yes	Yes
	Contig2441	Putative T-complex protein 1 subunit epsilon (CCT5), TCP-1 chaperonin family	*Ixodes scapularis*	Yes	Yes
	Contig2805	Putative T-complex protein 1 subunit gamma (CCT3), TCP-1 chaperonin family	*Ixodes scapularis*	Yes	Yes
	Contig790	Putative T-complex protein 1 subunit theta (CCT8), TCP-1 chaperonin family	*Ixodes scapularis*	Yes	Yes
	Contig9031	Putative peptidylprolyl isomerase (CYP1), Cyclophilin-type PPIase family, PPIase A subfamily	*Ixodes scapularis*	Yes	Yes
	Contig7969	Putative Glutaredoxin-related protein 5, mitochondrial (Glrx5), Glutaredoxin family, Monothiol subfamily	*Ixodes scapularis*	Yes	Yes
	Contig8311	Putative lipoyltransferase 1, mitochondrial (LIPT1), LplA family	*Ixodes scapularis*	Yes	No
	CV436024	Putative ubiquitinyl hydrolase 1 (ATXN3), Ataxin-3	*Ixodes scapularis*	Yes	No
	Contig222A	Putative translocation protein SEC63 homolog (SEC63)	*Ixodes scapularis*	Yes	No
	Contig5188	Putative mitochondrial import inner membrane translocase subunit TIM14 (DNAJC19)	*Ixodes scapularis*	Yes	No
	Contig155	Putative DnaJ homolog subfamily C member 30 (DNAJC30)	*Ixodes scapularis*	Yes	No
	Contig4436	Putative prefoldin subunit 2 (PFDN2), Prefoldin subunit beta family	*Ixodes scapularis*	Yes	No
	Contig5520	Putative prefoldin subunit 5 (PFDN5), Prefoldin subunit alpha family	*Ixodes scapularis*	Yes	No
	Contig7780	Putative Stress-induced-phosphoprotein 1 (STIP1)	*Ixodes scapularis*	Yes	No
	Contig4501	Putative peptidylprolyl isomerase (PIN1)	*Ixodes scapularis*	Yes	No
	Contig5483	Putative Derlin-2 (DERL2), Derlin family	*Ixodes scapularis*	Yes	No
	Contig1808	Putative DnaJ homolog subfamily C member 8 (Dnajc8)	*Ixodes scapularis*	Yes	No
	Contig2583	Putative UBX domain-containing protein 6 (UBXN6)	*Ixodes scapularis*	Yes	No
	CV446781	Putative E3 ubiquitin-protein ligase UBR5 (UBR5)	*Ixodes scapularis*	Yes	No
	Contig2212	Putative Protein l(2)37Cc (l(2)37Cc), Prohibitin, Prohibitin family	*Ixodes scapularis*	Yes	No
	CV440002	Putative protein geranylgeranyltransferase type I, subunit beta (PGGT1B), Protein prenyltransferase subunit beta family	*Ixodes scapularis*	Yes	No
	Contig441	Putative S-phase kinase-associated protein 1 (skp1), SKP1 family	*Ixodes scapularis*	Yes	No
	Contig4781	Putative peptidylprolyl isomerase (PPIL3), Cyclophilin-type PPIase family. PPIL3 subfamily	*Ovis aries*	Yes	No
	CK177789	Putative thioredoxin-disulfide reductase (TXNRD3), Class-I pyridine nucleotide-disulfide oxidoreductase family	*Branchiostoma floridae*	Yes	No

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	Intensity threshold >1,000 [e]	Intensity threshold >2,000 [f]
Posttranslational modification, protein turnover, chaperones	CV438532	Putative DnaJ homolog subfamily C member 10 (Dnajc10)	*Ixodes scapularis*	Yes	No
	CV446584	Putative BTB/POZ domain-containing protein 6-A (btbd6a)	*Ixodes scapularis*	Yes	No
	Contig567	Putative ubiquitinyl hydrolase 16 (USP16), Peptidase C19 family, USP16 subfamily	*Ixodes scapularis*	Yes	No
	Contig7377	Putative ubiquitinyl hydrolase 8 (USP8), Peptidase C19 family	*Saccoglossus kowalevskii*	Yes	No
	Contig6698	Putative ubiquitinyl hydrolase L3 (UCHL3), Peptidase C12 family	*Trichoplax adhaerens*	Yes	No
	Contig896	Putative ubiquitin fusion degradation protein 1 homolog (UFD1L)	*Ixodes scapularis*	Yes	No
	CK188518	Putative UBX domain-containing protein 4 (UBXN4)	*Danio rerio*	Yes	No
	Contig7693	Putative ubiquitinyl hydrolase 5 (USP5), Peptidase C19 family	*Tribolium castaneum*	Yes	No
	Contig6367	Putative gamma-glutamylcyclotransferase B (GGACT B), Gamma-glutamylcyclotransferase family	*Ixodes scapularis*	Yes	No
	Contig8777	Putative 26S proteasome non-ATPase regulatory subunit 12 (PSMD12), Proteasome subunit p55 family	*Tribolium castaneum*	Yes	No
	Contig6300	Putative arginyltransferase (ATE1), R-transferase family	*Monodelphis domestica*	Yes	No
	Contig8200	Putative (phosphatase 2A protein)-leucine-carboxy methyltransferase (LCMT1), Methyltransferase superfamily, LCMT family	*Ixodes scapularis*	Yes	No
	Contig202	Putative T-complex protein 1 subunit delta (TCP-1-delta), TCP-1 chaperonin family	*Ixodes scapularis*	Yes	No
	Contig940	Putative dehydrodolichyl diphosphate synthase (Dhdds),UPP synthase family	*Strongylocentrotus purpuratus*	Yes	No
	CV453090	Putative E3 ubiquitin-protein ligase (HERC3)	*Branchiostoma floridae*	Yes	No
	CK172681	Putative peptidyl-prolyl cis-trans isomerase FKBP8 (FKBP8)	*Ixodes scapularis*	Yes	No
	Contig7865	Putative glutaredoxin-related protein 5 (Glrx5), mitochondrial, Glutaredoxin family, Monothiol subfamily	*Ixodes scapularis*	Yes	No
	Contig9047	Putative glutaredoxin-related protein 5 (Glrx5), mitochondrial, Glutaredoxin family, Monothiol subfamily	*Ixodes scapularis*	Yes	No
	Contig1074	Putative maleylacetoacetate isomerase, GST superfamily, Zeta family	*Ixodes scapularis*	Yes	No
	Contig1596	Putative glycoprotein 6-alpha-L-fucosyltransferase (alpha1-6FucT), Glycosyltransferase 23 family	*Gallus gallus*	Yes	No
	Contig301	Putative glycosylphosphatidylinositol anchor attachment 1 protein (GPAA1)	*Ixodes scapularis*	Yes	No
	Contig625	Putative dolichyl-phosphate beta-glucosyltransferase (ALG5)	*Drosophila simulans*	Yes	No
	Contig3189	Putative GPI transamidase component PIG-T (PIGT), PIGT family	*Ixodes scapularis*	Yes	No

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	Intensity threshold >1,000 [e]	Intensity threshold >2,000 [f]
Posttranslational modification, protein turnover, chaperones	CV443627	Putative GPI transamidase component PIG-S (PIGS), PIGS family	*Ixodes scapularis*	Yes	No
	Contig7928	Putative mitochondrial intermediate peptidase (MIPEP), Peptidase M3 family	*Aedes aegypti*	Yes	No
	Contig1730	Putative DnaJ homolog subfamily B member 11 (DNAJB11)	*Ixodes scapularis*	Yes	No
	Contig4002	Putative DnaJ homolog subfamily A member 3, mitochondrial (DNAJA3)	*Aedes aegypti*	Yes	No
	Contig83A	Putative complex I intermediate-associated protein 30, mitochondria (NDUFAF1), CIA30 family	*Ixodes scapularis*	Yes	No
	Contig3450	Putative tubulin-specific chaperone A (TBCA), TBCA family	*Pediculus humanus corporis*	Yes	No
Replication, recombination and repair	Contig5258	Putative DNA-(apurinic or apyrimidinic site) lyase (APEX1), DNA repair enzymes AP/ExoA family	*Saccoglossus kowalevskii*	Yes	No
	TC18910	Putative DNA ligase (ATP) (LIG1), ATP-dependent DNA ligase family	*Xenopus laevis*	Yes	No
	CK177580	Putative DNA-directed DNA polymerase, alpha catalytic subunit (POLA1), DNA polymerase type-B family	*Ixodes scapularis*	Yes	No
	Contig115	Putative proliferating cell nuclear antigen (PCNA), PCNA family	*Ixodes scapularis*	Yes	No
	CV457249	Putative DNA helicase, Maternal DNA replication licensing factor (mmcm3), MCM family	*Ixodes scapularis*	Yes	No
	Contig3064	Putative DNA helicase, DNA replication licensing factor mcm5 (mcm5), MCM family	*Strongylocentrotus purpuratus*	Yes	No
	Contig6800	Putative vacuolar protein sorting-associated protein 72 homolog (YL-1), VPS72/YL1 family	*Ixodes scapularis*	Yes	No
	Contig2286	Putative DNA primase large subunit (PRIM2), Eukaryotic-type primase large subunit family	*Ixodes scapularis*	Yes	No
	CK182406	Putative DNA mismatch repair protein Msh6 (MSH6), DNA mismatch repair MutS family	*Monodelphis domestica*	Yes	No
	Contig8820	Putative DNA mismatch repair protein Msh6 (MSH6), DNA mismatch repair MutS family	*Ixodes scapularis*	Yes	No
	Contig5918	Putative 8-oxo-dGTP diphosphatase (NUDT15), Nudix hydrolase family	*Ixodes scapularis*	Yes	No
	Contig2417	Putative E3 SUMO-protein ligase NSE2 (NSE2), NSE2 family	*Rana catesbeiana*	Yes	No
	Contig2350	Putative TIP41-like protein (TIPRL), TIP41 family	*Ixodes scapularis*	Yes	No
	Contig4394	Putative ribonuclease H2 subunit B (RNASEH2B), RNase H2 subunit B family	*Strongylocentrotus purpuratus*	Yes	No

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	Intensity threshold >1,000 [e]	Intensity threshold >2,000 [f]
RNA processing and modification	Contig825	Putative Serine/arginine-rich splicing factor 7 (SRSF7), Splicing factor SR family	*Ixodes scapularis*	Yes	Yes
	Contig8997	Putative Serine/arginine-rich splicing factor 7 (SRSF7), Splicing factor SR family	*Ixodes scapularis*	Yes	Yes
	Contig1956	Putative serine/arginine-rich splicing factor 12 (SRSF12), Splicing factor SR family	*Ixodes scapularis*	Yes	Yes
	Contig4932	Putative serine/arginine-rich splicing factor RS2Z33 (RS2Z33), Splicing factor SR family	*Ixodes scapularis*	Yes	Yes
RNA processing and modification	Contig5832	Putative thioredoxin-like protein 4A (Txnl4a),DIM1 family	*Ixodes scapularis*	Yes	Yes
	Contig8773	Putative serine/arginine-rich splicing factor 12 (SRSF12), Splicing factor SR family	*Ixodes scapularis*	Yes	Yes
	Contig1192	Putative U2 small nuclear ribonucleoprotein B" (SNRPB2), RRM U1 A/B" family	*Nasonia vitripennis*	Yes	Yes
	Contig5369	Putative Transformer-2 protein homolog alpha (TRA2A), Splicing factor SR family	*Ixodes scapularis*	Yes	Yes
	Contig6083	Putative RNA-binding protein with serine-rich domain 1 (RNPS1), Splicing factor SR family	*Ixodes scapularis*	Yes	No
	Contig6666	Putative serine/arginine-rich splicing factor 4 (SRSF4), Splicing factor SR family	*Ixodes scapularis*	Yes	No
	Contig8231	Putative RNA helicase (DDX54), DEAD box helicase family. DDX54/DBP10 subfamily	*Ixodes scapularis*	Yes	No
	Contig5357	Putative RNA helicase (DDX55), DEAD box helicase family, DDX55/SPB4 subfamily	*Ixodes scapularis*	Yes	No
	Contig511	Putative RNA helicase (DDX43), DEAD box helicase family	*Ixodes scapularis*	Yes	No
	Contig8707	Putative RNA helicase (DDX52), DEAD box helicase family, DDX52/ROK1 subfamily	*Ixodes scapularis*	Yes	No
	Contig6359	Putative mRNA (nucleoside-2'-O)-methyltransferase (ftsjd2), Methyltransferase superfamily, RrmJ family	*Saccoglossus kowalevskii*	Yes	No
	Contig3779	Putative mRNA (nucleoside-2'-O)-methyltransferase (ftsjd2), Methyltransferase superfamily, RrmJ family	*Danio rerio*	Yes	No
	Contig1373	Putative tRNA (guanine46-N7)-methyltransferase (METTL1), Methyltransferase superfamily, TrmB family	*Ixodes scapularis*	Yes	No
	Contig2428	Putative mRNA (guanine-N(7)-)-methyltransferase (RNMT), Class I-like SAM-binding methyltransferase superfamily, mRNA cap 0 methyltransferase family	*Ixodes scapularis*	Yes	No
	Contig5896	Putative cleavage stimulation factor subunit 3 (CSTF3)	*Ixodes scapularis*	Yes	No
	Contig4704	Putative RNA helicase (DDX1), DEAD box helicase family, DDX1 subfamily	*Ixodes scapularis*	Yes	No
	Contig8035	Putative pre-mRNA processing factor 40 homolog A (PRPF40A), PRPF40 family	*Ixodes scapularis*	Yes	No
	Contig7643	Putative integrator complex subunit 4 (INTS4), Integrator subunit 4 family	*Ixodes scapularis*	Yes	No
	Contig7662	Putative pre-mRNA-splicing factor CWC22 homolog (CWC22), CWC22 family	*Ixodes scapularis*	Yes	No

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	Intensity threshold >1,000 [e]	Intensity threshold >2,000 [f]
RNA processing and modification	CV443093	Putative cleavage stimulation factor subunit 3 (CSTF3)	*Ixodes scapularis*	Yes	No
	CV438199	Putative U4/U6 small nuclear ribonucleoprotein Prp31 (PRPF31), PRP31 family	*Ixodes scapularis*	Yes	No
	CV449623	Putative nuclear cap-binding protein subunit 1 (ncbp1), NCBP1 family	*Ixodes scapularis*	Yes	No
	CV449947	Putative RNA helicase, superkiller viralicidic activity 2-like 2 (SKIV2L2), Helicase family, SKI2 subfamily	*Saccoglossus kowalevskii*	Yes	No
	Contig981	Putative methylosome protein 50 (WDR77)	*Ixodes scapularis*	Yes	No
	Contig3014	Putative ribosomal RNA-processing protein 8 (Rrp8), Methyltransferase superfamily, RRP8 family	*Tribolium castaneum*	Yes	No
	Contig6223	Putative Pescadillo homolog (PES1), Pescadillo family	*Tetraodon nigroviridis*	Yes	No
	Contig5044	Putative U6 snRNA-associated Sm-like protein LSm4 (LSM4), SnRNP Sm proteins family	*Nematostella vectensis*	Yes	No
	TC19865	Putative small nuclear ribonucleoprotein Sm D2 (SNRPD2), SnRNP core protein family	*Ixodes scapularis*	Yes	No
	Contig3309	Putative Small nuclear ribonucleoprotein Sm D3 (SNRPD3), SnRNP core protein family	*Ixodes scapularis*	Yes	No
	Contig7040	Putative tRNA (guanine(37)-N(1))-methyltransferase (TRM5), TRM5 / TYW2 family	*Tetraodon nigroviridis*	Yes	No
	Contig734	Putative U3 small nucleolar ribonucleoprotein protein IMP3 (IMP3), Ribosomal protein S4P family	*Saccoglossus kowalevskii*	Yes	No
	Contig4769	Putative Protein FAM98B (FAM98B), FAM98 family	*Danio rerio*	Yes	No
	Contig3752	Putative RNA ligase (ATP), tRNA-splicing ligase RtcB homolog (rtcB), RtcB family	*Ixodes scapularis*	Yes	No
Secondary metabolites biosynthesis, transport and catabolism	Contig701	Putative MOSC domain-containing protein 1, mitochondrial (MARC1)	*Ixodes scapularis*	Yes	No
	Contig4351	Putative phosphopantothenoylcysteine decarboxylase (PPCDC), HFCD (homo-oligomeric flavin containing Cys decarboxylase) superfamily	*Ixodes scapularis*	Yes	No
Signal transduction mechanisms	Contig426	Putative stromal membrane-associated protein 1 (SMAP1)	*Ixodes scapularis*	Yes	Yes
	Contig615	Putative myosin-light-chain kinase (MYLK), Protein kinase superfamily, CAMK Ser/Thr protein kinase family	*Apis mellifera*	Yes	Yes
	Contig2242	Putative growth hormone-inducible transmembrane protein (GHITM), BI1 family	*Branchiostoma floridae*	Yes	Yes
	Contig5174	Putative Hsp90 co-chaperone Cdc37 (Cdc37), CDC37 family	*Ixodes scapularis*	Yes	No

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	Intensity threshold >1,000 [e]	Intensity threshold >2,000 [f]
Signal transduction mechanisms	Contig7343	Putative COP9 signalosome complex subunit 5 (COPS5), Peptidase M67A family, CSN5 subfamily	*Xenopus (Silurana) tropicalis*	Yes	No
	Contig6843	Putative phosphatidylinositol 4-kinase alpha (PI4KA), PI3/PI4-kinase family, Type III PI4K subfamily	*Ixodes scapularis*	Yes	No
	Contig7509	Putative peptidylprolyl isomerase activator (PPP2R4), PTPA-type PPIase family	*Harpegnathos saltator*	Yes	No
	Contig3367	Putative glucosidase 2 subunit beta (PRKCSH)	*Pediculus humanus corporis*	Yes	No
	Contig5579	Putative non-specific serine/threonine protein kinase (SRPK2), Protein kinase superfamily, CMGC Ser/Thr protein kinase family	*Nasonia vitripennis*	Yes	No
	Contig6537	Putative hepatitis B virus X-interacting protein (HBXIP), HBXIP family	*Ixodes scapularis*	Yes	No
	Contig3503	Putative mitogen-activated protein kinase-binding protein 1 (MAPKBP1)	*Ixodes scapularis*	Yes	No
	Contig264	Putative mitogen-activated protein kinase-binding protein 1 (MAPKBP1)	*Ixodes scapularis*	Yes	No
	Contig7015	Putative DCC-interacting protein 13-alpha (APPL1)	*Ixodes scapularis*	Yes	No
	Contig5766	Putative 1-alkyl-2-acetylglycerophosphocholine esterase, Platelet-activating factor acetylhydrolase IB subunit gamma (PAFAH1B3), 'GDSL' lipolytic enzyme family, Platelet-activating factor acetylhydrolase IB beta/gamma subunits subfamily	*Ixodes scapularis*	Yes	No
	Contig3634	Putative calcyclin-binding protein (CACYBP)	*Xenopus laevis*	Yes	No
	Contig2429	Putative developmentally-regulated GTP-binding protein 2 (DRG2), GTP1/OBG family	*Ixodes scapularis*	Yes	No
	Contig6702	Putative A-kinase anchor protein 1, mitochondrial (AKAP1)	*Pediculus humanus corporis*	Yes	No
	Contig3619	Putative SLIT-ROBO Rho GTPase-activating protein 1 (SRGAP1)	*Ixodes scapularis*	Yes	No
Transcription	Contig5083	Putative DNA helicase (RUVBL2), RuvB family	*Ixodes scapularis*	Yes	Yes
	Contig346	Putative protein dpy-30 homolog (DPY30), Dpy-30 family	*Ixodes scapularis*	Yes	Yes
	Contig1649	Putative transcription factor BTF3 homolog 4 (BTF3L4), NAC-beta family	*Ixodes scapularis*	Yes	Yes
	Contig8810	Putative nascent polypeptide-associated complex subunit alpha (NACA), NAC-alpha family	*Ixodes scapularis*	Yes	Yes
	Contig6772	Putative X-box-binding protein 1 (XBP1), BZIP family	*Ixodes scapularis*	Yes	Yes
	Contig137	Putative protein arginine N-methyltransferase (PRMT1), Protein arginine N-methyltransferase family	*Ixodes scapularis*	Yes	Yes
	Contig6562	Putative zinc finger protein 132 (ZNF132), Krueppel C2H2-type zinc-finger protein family	*Ixodes scapularis*	Yes	No
	Contig2160	Putative zinc finger protein 84 (ZNF84), Krueppel C2H2-type zinc-finger protein family	*Danio rerio*	Yes	No

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	Intensity threshold >1,000 [e]	Intensity threshold >2,000 [f]
Transcription	Contig3540	Putative transcription factor Dp-1 (TFDP1), E2F/DP family	*Ixodes scapularis*	Yes	No
	CV446980	Putative Protein daughterless (da)	*Apis mellifera*	Yes	No
	Contig7438	Putative DNA-directed RNA polymerase II subunit RPB3 (POLR2C), Archaeal rpoD/eukaryotic RPB3 RNA polymerase subunit family	*Ixodes scapularis*	Yes	No
	Contig3039	Putative transcription elongation factor S-II (TfIIS), TFS-II family	*Ixodes scapularis*	Yes	No
	Contig811	Putative polycomb protein EED (EED), WD repeat ESC family	*Ixodes scapularis*	Yes	No
	Contig1466	Putative rRNA-processing protein FCF2 (FCF2), FCF2 family	*Ixodes scapularis*	Yes	No
	TC15275	Putative Huntington disease protein homolog, May play a role in microtubule-mediated transport or vesicle function	*Ixodes scapularis*	Yes	No
	Contig745	Putative zinc finger HIT domain-containing protein 3 (ZNHIT3), HIT-type zinc finger	*Ixodes scapularis*	Yes	No
	Contig4415	Putative mediator of RNA polymerase II transcription subunit 21 (MED21), Mediator complex subunit 21 family	*Ixodes scapularis*	Yes	No
	Contig380	Putative transcription initiation factor IIA subunit 1 (GTF2A1), TFIIA subunit 1 family	*Ixodes scapularis*	Yes	No
	Contig2530	Putative DNA-directed RNA polymerase III subunit RPC6 (POLR3F), Eukaryotic RPC34/RPC39 RNA polymerase subunit family	*Branchiostoma floridae*	Yes	No
	Contig5236	Putative DNA-directed RNA polymerases I, II, and III subunit RPABC2 (POLR2F), Archaeal rpoK/eukaryotic RPB6 RNA polymerase subunit family	*Ixodes scapularis*	Yes	No
	Contig255A	Putative enhancer of yellow 2 transcription factor homolog (eny2), ENY2 family	*Ixodes scapularis*	Yes	No
	CK183629	Putative mediator of RNA polymerase II transcription subunit 10 (MED10), Mediator complex subunit 10 family	*Ixodes scapularis*	Yes	No
	Contig5558	Putative transcription initiation factor TFIID subunit 10 (TAF10), TAF10 family	*Ixodes scapularis*	Yes	No
	Contig4186	Putative activated RNA polymerase II transcriptional coactivator p15 (SUB1), Transcriptional coactivator PC4 family	*Ixodes scapularis*	Yes	No
	CK190403	Putative zinc finger protein 593 (ZNF593), ZNF593/BUD20 C2H2-type zinc-finger protein family	*Ixodes scapularis*	Yes	No
	TC16293	Putative (Histone H3)-lysine-36 demethylase (NO66), MINA53/NO66 family, NO66 subfamily	*Ixodes scapularis*	Yes	No
	CV435794	Putative mediator of RNA polymerase II transcription subunit 17 (MED17), Mediator complex subunit 17 family	*Apis mellifera*	Yes	No
	TC17107	Putative mediator of RNA polymerase II transcription subunit 8 (Med8), Mediator complex subunit 8 family	*Ixodes scapularis*	Yes	No
	U92783.1	Putative PHD finger protein 12 (PHF12)	*Ixodes scapularis*	Yes	No
	Contig1806	Putative PHD finger protein 23B (phf23b), PHF23 family	*Ixodes scapularis*	Yes	No
Translation, ribosomal structure and biogenesis	Contig1577	Putative H/ACA ribonucleoprotein complex subunit 2-like protein (NHP2), Ribosomal protein L7Ae family	*Ixodes scapularis*	Yes	Yes
	Contig7181	Putative 40S ribosomal protein S25 (RPS25), Ribosomal protein S25e family	*Ixodes scapularis*	Yes	Yes

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	Intensity threshold >1,000 [e]	Intensity threshold >2,000 [f]
Translation, ribosomal structure and biogenesis	EW680164.1	Putative 40S ribosomal protein S29 (RPS29), Ribosomal protein S14P family	*Ixodes scapularis*	Yes	Yes
	Contig1238	Putative 60S Ribosomal protein L13 (RPL13), Ribosomal protein L13e family	*Ornithodoros parkeri*	Yes	Yes
	Contig3228	Putative 60s ribosomal protein L27a (RPL27A), Ribosomal protein L15P family	*Ornithodoros coriaceus*	Yes	Yes
	EW680050.1	Putative 60S ribosomal protein L24 (RPL24), Ribosomal protein L24e family	*Hyalomma anatolicum anatolicum*	Yes	Yes
	Contig6473	Putative 60S ribosomal protein L26 (RPL26), Ribosomal protein L24P family	*Lycosa singoriensis*	Yes	Yes
	Contig107B	Putative 60S ribosomal protein L27 (RPL27), Ribosomal protein L27e family	*Ixodes scapularis*	Yes	Yes
	Contig5017	Putative ribosome biogenesis protein RLP24 (RPL24), Ribosomal protein L24e family	*Strongylocentrotus purpuratus*	Yes	Yes
	Contig9000	Putative 60S ribosomal protein L32 (RPL32), Ribosomal protein L32P family	*Hyalomma marginatum rufipes*	Yes	Yes
	CK177858	Putative 60S ribosomal protein L37a (RPL37A), Ribosomal protein L37Ae family	*Ixodes scapularis*	Yes	Yes
	Contig1990	Putative arginine-tRNA ligase (RARS), Class-I aminoacyl-tRNA synthetase family	*Ixodes scapularis*	Yes	Yes
	Contig7496	Putative 60S ribosomal protein L41 (RPL41), Ribosomal protein L41e family	*Vitis vinifera*	Yes	Yes
	Contig2946	Putative 39S ribosomal protein L32, mitochondrial (MRPL32), Ribosomal protein L32P family	*Drosophila willistoni*	Yes	Yes
	TC20332	Putative 28S ribosomal protein S15, mitochondrial (bonsai), Ribosomal protein S15P family	*Ixodes scapularis*	Yes	Yes
	Contig2574	Putative eukaryotic translation initiation factor 3 subunit G (EIF3G), eIF-3 subunit G family	*Ixodes scapularis*	Yes	Yes
	Contig4252	Putative nucleolin (NUCL)	*Ixodes scapularis*	Yes	No
	Contig8886	Putative nucleolar protein 56 (NOP56), NOP5/NOP56 family	*Ixodes scapularis*	Yes	No
	Contig314B	Putative 40S ribosomal protein S24 (Rps24), ribosomal protein S24e family	*Dermacentor variabilis*	Yes	No
	Contig2998	Putative ribosomal protein L1 (mrpl1), mitochondrial, Ribosomal protein L1P family	*Ixodes scapularis*	Yes	No
	Contig5814	Putative 60S ribosomal protein L19 (RPL19), Ribosomal protein L19e family	*Ornithodoros parkeri*	Yes	No
	Contig7903	Putative D-tyrosyl-tRNA(Tyr) deacylase 1 (DTD1), DTD family	*Ixodes scapularis*	Yes	No
	Contig4298	Putative HBS1-like protein (HBS1L), GTP-binding elongation factor family	*Ixodes scapularis*	Yes	No
	Contig5945	Putative exosome complex exonuclease RRP46 (EXOSC5), RNase PH family	*Ixodes scapularis*	Yes	No

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	Intensity threshold >1,000 [e]	Intensity threshold >2,000 [f]
Translation, ribosomal structure and biogenesis	Contig5596	Putative H/ACA ribonucleoprotein complex subunit 1 (GAR1), GAR1 family	*Ixodes scapularis*	Yes	No
	Contig2724	Putative histidine-tRNA ligase (HARS), Class-II aminoacyl-tRNA synthetase family	*Branchiostoma floridae*	Yes	No
	CV454305	Putative isoleucine-tRNA ligase (IARS), Class-I aminoacyl-tRNA synthetase family	*Ixodes scapularis*	Yes	No
	Contig2011	Putative Protein pelota homolog (PELO), Eukaryotic release factor 1 family, Pelota subfamily, Required for normal chromosome segregation during cell division and genomic stability	*Drosophila ananassae*	Yes	No
	Contig8302	Putative methionyl-tRNA formyltransferase, mitochondrial (MTFMT), Fmt family	*Ixodes scapularis*	Yes	No
	Contig1776	Putative elongation factor Tu, mitochondrial (EfTuM), GTP-binding elongation factor family, EF-Tu/EF-1A subfamily	*Ixodes scapularis*	Yes	No
	Contig8438	Putative 39S ribosomal protein L3, mitochondrial (Mrpl3), Ribosomal protein L3P family	*Ixodes scapularis*	Yes	No
	Contig7444	Putative Pre-rRNA-processing protein TSR1 homolog (TSR1), BMS1/TSR1 family. TSR1 subfamily	*Ixodes scapularis*	Yes	No
	Contig3804	Putative ribosome biogenesis protein BOP1 (BOP1), WD repeat BOP1/ERB1 family	*Branchiostoma floridae*	Yes	No
	Contig714	Putative probable RNA-binding protein EIF1AD, EIF1AD family	*Ixodes scapularis*	Yes	No
	Contig2450	Putative 28S ribosomal protein S28, mitochondrial (MRPS28)	*Anopheles gambiae str. PEST*	Yes	No
	Contig1578	Putative 28S ribosomal protein S5, mitochondrial (MRPS5), Ribosomal protein S5P family	*Ixodes scapularis*	Yes	No
	Contig252	Putative Ribosome biogenesis protein BRX1 homolog (BRIX1), BRX1 family	*Tribolium castaneum*	Yes	No
	CV453853	Putative RNA helicase, eukaryotic initiation factor 4A (EIF4A1), DEAD box helicase family, eIF4A subfamily	*Ixodes scapularis*	Yes	No
	Contig7689	Putative aminoacyl tRNA synthase complex-interacting multifunctional protein 1 (AIMP1)	*Ixodes scapularis*	Yes	No
	Contig4817	Putative mitochondrial ribosomal S23 protein (MRPS23)	*Drosophila willistoni*	Yes	No
	Contig6275	Putative mitochondrial 39S ribosomal protein L38 (MRPL38)	*Ixodes scapularis*	Yes	No
	Contig1485	Putative ribosome biogenesis protein WDR12 (WDR12), WD repeat WDR12/YTM1 family	*Ixodes scapularis*	Yes	No
	Contig1046	Putative Box C/D snoRNA protein 1 (ZNHIT6), BCD1 family	*Ixodes scapularis*	Yes	No
	Contig8406	Putative aminoacyl-tRNA hydrolase (PTRH2), PTH2 family	*Ixodes scapularis*	Yes	No
	Contig5024	Putative nuclear nucleic acid-binding protein C1D (C1D), C1D family	*Ixodes scapularis*	Yes	No
	Contig308	Putative 39S ribosomal protein L37, mitochondrial (MRPL37), Ribosomal protein S30/L37 family	*Ixodes scapularis*	Yes	No
	CK189708	Putative 28S ribosomal protein S6, mitochondrial (MRPS6), Ribosomal protein S6P family	*Ixodes scapularis*	Yes	No

Transcript category (KOG classification) [a]	Contig [b]	Description [c]	Species [d]	Intensity threshold >1,000 [e]	Intensity threshold >2,000 [f]
Translation, ribosomal structure and biogenesis	Contig643	Putative 28S ribosomal protein S10, mitochondrial (MRPS10), Ribosomal protein S10P family	*Ixodes scapularis*	Yes	No
	TC19757	Putative 28S ribosomal protein S18b, mitochondrial (MRPS18B), Ribosomal protein S18P family	*Ixodes scapularis*	Yes	No
	Contig1020	Putative 39S ribosomal protein L19, mitochondrial (MRPL19), Ribosomal protein L19P family	*Ixodes scapularis*	Yes	No
	Contig405	Putative 39S ribosomal protein L24, mitochondrial (MRPL24), Ribosomal protein L24P family	*Ixodes scapularis*	Yes	No
	Contig5697	Putative 28S ribosomal protein S14, mitochondrial, mitochondrial (MRPS14), Ribosomal protein S14P family	*Ixodes scapularis*	Yes	No
	Contig7858	Putative 28S ribosomal protein S16, mitochondrial (MRPS16), Ribosomal protein S16P family	*Ixodes scapularis*	Yes	No
	Contig4891	Putative 28S ribosomal protein S18c, mitochondrial (MRPS18C), Ribosomal protein S18P family	*Ixodes scapularis*	Yes	No
	CV440148	Putative nucleolar protein 6 (NOL6), NRAP family	*Ixodes scapularis*	Yes	No
	Contig3182	Putative rRNA-processing protein FCF1 homolog (FCF1), UTP23/FCF1 family, FCF1 subfamily	*Ixodes scapularis*	Yes	No
	TC20881	Putative nucleolar complex protein 4 homolog (NOC4L), CBF/MAK21 family	*Ixodes scapularis*	Yes	No
	Contig4879	Putative 39S ribosomal protein L48, mitochondrial (MRPL48)	*Ixodes scapularis*	Yes	No
	Contig3662	Putative 39S ribosomal protein L35, mitochondrial (MRPL35), Ribosomal protein L35P family	*Drosophila persimilis*	Yes	No
	Contig4524	Putative 39S ribosomal protein L28, mitochondrial (MRPL28), Ribosomal protein L28P family	*Ixodes scapularis*	Yes	No

[a] Classification of transcripts according to eukaryotic ortologous group terms for gene ontology (Tatusov et al., 2003).

[b] Assigned contiguous sequence identification for transcripts following assembly of all available expressed sequence tags and the *R. microplus* Gene Index version 2.1.

[c] The functional annotation of genes based on comparison among BLAST outputs from seven databases outlined in Section 2.4. All transcript descriptions are based on consensus with database entries from Uniprot (http://www.uniprot.org/uniprot/) and BRENDA (http://www.brenda-enzymes.org/), in the case of enzymes.

[d] Species indicated that showed the highest sequence similarity to *R. microplus* sequences following non-redundant database (NR) BLAST analysis, independent of final assignment.

[e] Transcripts with M values > 0 and Cy5 intensities > 1,000 in all tissues tested that are considered to be shared

[f] Transcripts with M values > 0 and Cy5 intensities > 2,000 in all tissues tested that are considered to be shared

Supplementary Table S6. Verification of differential gene expression of selected transcripts by quantitative reverse transcriptase (qRT-PCR).

Contig [a]	Description [b]	Comparison [c]	Microarray fold change [d]	qRT-PCR fold change [e]	Direction of change
Contig8418	Elongation factor 1 alpha	MG/SG	ND	0.94	Unaffected
Contig8723	Putative 60S ribosomal protein L9, Ribosomal protein L6P family	MG/SG	0.93 [f]	1.06 [f]	Unaffected
Contig1	Putative angiotensin-converting enzyme, Peptidase M2 family	MG/SG	0.12 [f]	6.43E-03 [f]	Down
Contig5396	Putative mucin	MG/SG	19.61 [f]	17.66 [f]	Up
Contig5672	Unknown protein	MG/SG	2.81 [f]	7.05 [f]	Up

[a] Assigned contiguous sequence identification for transcripts following assembly of all available expressed sequence tags and the *Rhipicephalus microplus* Gene Index version 2.1.

[b] The functional annotation of genes based on comparison among BLAST outputs from seven databases outlined in Materials and methods. All transcript descriptions are based on consensus with database entries from Uniprot (http://www.uniprot.org/uniprot/) and BRENDA (http://www.brenda-enzymes.org/), in the case of enzymes.

[c] Pairwise tissue comparison indicated for midgut (MG) and salivary glands (SG) .

[d] Fold change (absolute) for microarray calculated from the $\log_2$ expression level for the gene (*P* value≤0.001).

[e] Fold change data identified from qRT-PCR. The data was normalized against Contig 8418 and Contig 8723 and fold changes are expressed according to the current tissue comparison (i.e. midgut to salivary gland, MG/SG).

[f] These values demonstrated a high correlation at 0.96 with the microarray and qRT-PCR data, calculated by using Pearson linear regression (R squared value of 0.93).

ND, not determined