Spring 2015

Exploring the Neuropeptides, Neuropeptide Receptors and Neurotransmitter Receptors in the Synganglia of Part-Fed Females of *Ornithodoros turicata* (Argasidae) and *Ixodes scapularis* (Ixodidae) with Insights Into Their Roles in Contrasting Biology

Noble I. Egekwu
Old Dominion University

EXPLORING THE NEUROPEPTIDES, NEUROPEPTIDE RECEPTORS AND NEUROTRANSMITTER RECEPTORS IN THE SYNGANGLIA OF PART-FED FEMALES OF *ORNITHODOROS TURICATA* (ARGASIDAE) AND *IXODES SCAPULARIS* (IXODIDAE) WITH INSIGHTS INTO THEIR ROLES IN CONTRASTING BIOLOGY

By

Noble I. Egekwu

B.Sc. May 2002, University of Jos, Nigeria
M.S. May 2009, James Madison University

A Dissertation Submitted to the Faculty of Sciences
Old Dominion University in Partial Fulfillment of the
Requirements for the Degree of

DOCTOR OF PHILOSOPHY

BIOMEDICAL SCIENCE

OLD DOMINION UNIVERSITY
May, 2015

Approved by:

Daniel E. Sonenshine (Director)

David T. Gauthier (Member)

Jing He (Member)

Christopher Osgood (Member)

R. Michael Roe (Member)

ABSTRACT

EXPLORING THE NEUROPEPTIDES, NEUROPEPTIDE RECEPTORS AND NEUROTRANSMITTER RECEPTORS IN THE SYNGANGLIA OF PART-FED FEMALES OF *ORNITHODOROS TURICATA* (ARGASIDAE) AND *IXODES SCAPULARIS* (IXODIDAE) WITH INSIGHTS INTO THEIR ROLES IN CONTRASTING BIOLOGY

Noble I. Egekwu
Old Dominion University, 2015
Director: Dr. Daniel E. Sonenshine (Professor emeritus)

The neurobiology of the synganglion (central nervous system) of the Lyme disease tick, *Ixodes scapularis* and the soft tick *Ornithodoros turicata* was evaluated using Illumina GAII high throughput sequencing which generated high coverage cDNA libraries (transcriptomes). These ticks exhibit different biological patterns of feeding, blood meal water, and salt elimination, cuticle plasticity versus cuticle synthesis, development and reproduction. RNA sequencing of *I. scapularis,* and *Ornithodoros turicata* yielded a total of 117,900,476 raw reads which were assembled to 30,838 contigs and a total of 63,528,102 also assembled to 132,258 contigs, respectively. Comparison of Gene Ontology (GO) mapping success for genes in 32 important GO molecular categories showed little difference between the two species.

Functional assignments of transcripts predicting neuropeptides, neuropeptide receptors and neurotransmitter receptors was done, supported by strong e-values (< -6), and high consensus sequence alignments. For the synganglion of *I. scapularis,* transcripts predicting 23 neuropeptides and/or their receptors were identified. For the synganglion of *O. turicata,* 25 neuropeptides and/or their receptors were identified. Both species had

transcripts predicting all of the same neuropeptides and/or their neuropeptide receptors in common except for allatotropin peptide, found only in *I. scapularis,* and allatostatin C, bursicon β, and glycoprotein B, which were found only in *O. turicata.*

If the repertoire of neuropeptide and neurotransmitter messages expressed in the synganglia of *O. turicata* and *I. scapularis* is so similar, how can we explain the very different physiological processes that occur in these two very different tick species? Real time PCR assays were used to study the expression of candidate genes in response to blood feeding. My study shows a strong similarity in gene identity (annotation/alignments) of both species but marked differences in the gene expression, extent of up-regulation or down-regulation, and the timing of their expression in response to feeding. This may indeed help explain many of the differences in the biology of the two different species. The diversity of messages predicting important genes identified in this study and differences in their expression in response to feeding offers a valuable resource useful for understanding how the tick synganglion regulates important physiological functions in ticks.

This dissertation is dedicated to my Lord and savior Jesus Christ who brought me this far. I am ever grateful to You my Lord.

ACKNOWLEDGMENTS

I want to thank all members of my committee with special thanks going to my mentor and committee chair Dr. Daniel E. Sonenshine. Words will not be enough to express my sincere gratitude for your guidance and support. You never stopped seeing the "Champion" in me even in my most trying moments. Your work ethics, positive attitude (both in thoughts and in speech), your drive, and your understanding are attributes I am taking with me to the next level. To all other committee members, thank you for your commitment towards my success.

Many thanks go to my family. First to my lovely wife Chioma Egekwu and my daughter Amarachukwu Praise Noble-Egekwu for their fervent prayers and support. I owe much of this success to you two. I love you so much. May the Lord continue to bless you two. To my mum Dr. Mrs Uloma Egekwu, and all other family members, thank you for your support and encouraging words.

To George and Judy Coffman, my wonderful family. Your prayers, support, and counsel cannot be forgotten. You have indeed been there for my wife and I. I pray that the Lord will reward you greatly. I cannot wait to see your joy when I receive this degree. I still remember your words when I got admitted into the program at Old Dominion University. Thank you again.

I also want to thank faculty members, Biology administrative staff, Biology department colleagues, friends, brethren, and family from BCF ODU, BCF National, RCCG (HOP) Chesapeake, RCCG (AGS) Stafford and all well-wishers both home and abroad.

Finally I am most thankful to God who has seen me through this journey from the very beginning to the end.

NOMENCLATURE

Ast	Allatostatin
ASTA	Allatostatin-A
ASTC	Allatostatin-C
AT	Allatotropin
preproAST A	Preproallatostatin-A
preproAST C	Preproallatostatin-C
JH:	Juvenile Hormone
CA	Corpora allata
CNS	Central Nervous System
I. scapularis	*Ixodes scapularis*
O. turicata	*Ornithodoros turicata*
CC	Corpora cardiaca
PETH	Pre-ecdysis triggering hormone
ETH	Ecdysis triggering hormone
ETHR	Ecdysis triggering hormone Receptor
Crz	Corazonin
Iscap*CrzRec*	*Ixodes scapularis* corazonin
Otur*CrzRec*	*Ornithodoros turicata* corazonin
qPCR	Quantitative Polymerase chain reaction
PCR	Polymerase chain reaction
CCAP	Crustacean Cardioactive Peptide
CAPA	Cardioacceleratory peptide
RC	Retrocerebral complexes
SG	Salivary gland
SK	Sulfakinin

TABLE OF CONTENTS

Page

LIST OF TABLES xi

LIST OF FIGURES xii

1. INTRODUCTION 1
    1.1 PROBLEM STATEMENT 5
    1.2 DISSERTATION STATEMENT 7
    1.3 SPECIFIC AIMS 7
    1.4 CATEGORIES OF TICKS 10
    1.5 BRIEF OVER VIEW OF TICK BIOLOGY AND SYNGANGLION 12
    1.6 TICK BRAIN GENERAL MORPHOLOGY AND BIOLOGY 16

2. MATERIALS AND METHODS 18
    2.1 TISSUE COLLECTION 18
    2.2 RNA EXTRACTION 20
    2.3 QUALITY ASSURANCE OF TOTAL RNA 21
    2.4 SEQUENCING BY ILLUMINA 22
        2.4.1 LIBRARY PREPARATION 22
        2.4.2 SEQUENCING AND BASE CALLING 22
    2.5 ASSEMBLY 23
    2.6 BIOINFORMATICS 23
    2.7 MAPPING & GENE ONTOLOGY 24
    2.8 GENE EXPRESSION STUDY 25
        2.8.1 REVERSE TRANSCRIPTION (cDNA SYNTHESIS) 25
        2.8.2 GENE EXPRESSION PROFILING 26
        2.8.3 PRIMER DESIGN AND VALIDATION 26
        2.8.4 QUANTITATIVE POLYMERASE CHAIN REACTION 27

3. RESULTS 31
    3.1 RNA EXTRACTION QUANTITATION AND QUALITY 31
    3.2 BIOINFORMATICS 35
    3.3 GENE ONTOLOGY 36
    3.4 BLAST2GO SUMMARY 37
    3.5 COMPARISON OF NEUROPEPTIDES AND RECEPTORS IN *I. SCAPULARIS* AND *O. TURICATA* 44
        3.5.1 *I. SCAPULARIS* TRANSCRIPTOME 44
        3.5.2 *O. TURICATA* TRANSCRIPTOME 48
    3.6 COMPARISON OF NEUROTRANSMITTER RECEPTORS IN *I. SCAPULARIS AND O. TURICATA* 50

Page

3.6.1 *I. SCAPULARIS* TRANSCRIPTOME 50
3.6.2 *O. TURICATA* TRANSCRIPTOME 51
3.7 COMPARATIVE GENE EXPRESSION BETWEEN *I. SCAPULARIS* AND *O. TURICATA* FEMALE SYNGANGLIA FOR SELECTED NEUROPEPTIDES, NEUROPEPTIDE RECEPTORS, AND NEUROTRANSMITTER RECEPTORS 53

4. DISCUSSION 71
4.1 GENERAL COMMENTS AND CONCLUSIONS 71
4.2 GENE CATEGORY: GROWTH, DEVELOPMENT, AND REPRODUCTION 74
4.3 GENE CATEGORY: REPRODUCTION 84
4.4 GENE CATEGORY: WATER BALANCE, AND FEEDING BEHAVIOR 93
4.5 GENE CATEGORY: SATIETY AND FEEDING VOLUME 99
4.6 GENE CATEGORY: NEUROTRANSMITTER RECEPTORS 101

5. SUMMARY 105
5.1 FUTURE DIRECTIONS 108

6. REFERENCES 109

APPENDIX
A. LIST OF PRIMERS 125
B. ALIGNMENTS FOR CANDIDATE NEUROPEPTIDES, NEUROPEPTIDE RECEPTOR GENES, AND NEUROTRANSMITTER RECEPTORS 127

VITA 170

LIST OF TABLES

Table Page

1. Bioanalyzer results of total RNA yield extracted from synganglia of part-fed *Ixodes scapularis* 32

2. Bioanalyzer results of total RNA yield extracted from synganglia of part-fed *Ornithodoros turicata* 33

3. CLC-Bio De Novo Assembly summary data for synganglion transcriptomes of part fed *Ixodes scapularis* and *O. turicata f*emales 35

4. Comparison of Gene Ontology (GO) categories between *Ixodes scapularis* and *Ornithodoros turicata*: Similarities and differences in the two different species 39

5. Comparative data for transcripts predicting neuropeptides, transporters and neuropeptide receptor genes as expressed in the synganglia transcriptomes of part-fed female *Ornithodoros turicata* and *Ixodes scapularis* 40

6. Comparative data of transcripts for candidate neurotransmitter receptors and transporter genes as expressed in the synganglia transcriptomes of part-fed female *Ornithodoros turicata and Ixodes scapularis* 43

7. Comparison of gene expression in *Ixodes scapularis* versus *Ornithodoros turicata* as determined by real time quantitative PCR (qPCR). Organized by functional categories 59

LIST OF FIGURES

Figure Page

1. Images comparing *O. turicata* and *I. scapularis* females from the ventral aspect 14

2. Photograph of dissection of a partially engorged ixodid tick *(Dermacentor variabilis)* showing the location of the synganglion in the anterior portion of the tick body 19

3. Experimental design of gene expression study showing number of biological samples and technical replicates and their arrangement on a 96-well plate 29

4. Bioanalyzer imaging of gel results of *Ixodes scapularis* 32

5. Samples 1-4, Agilent 2100 Bioanalyzer electropherogram profiles of total RNA extracted from 4 samples of synganglia of part-fed *Ixodes scapularis* female ticks using the Qiagen RNeasy kits 33

6. Bioanalyzer imaging of gel results of *Ornithodoros turicata* 34

7. Agilent 2100 Bioanalyzer electropherogram profiles of total RNA extracted from synganglia of part-fed *Ornithodoros turicata* female ticks using the Qiagen RNeasy kit 34

8. Gene Ontology (GO) term assignments at molecular level 3 for *I. scapularis* transcriptomes assembled following sequencing by Illumina 37

9. Gene Ontology (GO) term assignments at molecular level 3 for *O. turicata* transcriptomes assembled following sequencing by Illumina 38

10. Comparison of salivary gene expression in the tick synganglia in response to blood feeding 63

11. Comparison of water balance related gene expression in the tick synganglia in response to blood feeding 64

12. Comparison of feeding volume related gene expression in the tick synganglia in response to blood feeding 65

13. Comparison of ecdysis (molting) related gene expression in the tick synganglia in response to blood feeding 66

Figure Page

14. Comparison of cuticle synthesis related gene expression in the tick synganglia in response to blood feeding.....67

15. Comparison of reproduction and development related gene expression in the tick synganglia in response to blood feeding.....68

16. Comparison of neurotransmitter related gene expression in the tick synganglia in response to blood feeding 69

CHAPTER 1

INTRODUCTION

Ticks, having been recognized as vectors of a wide range of infectious diseases, continue to be the focus of numerous studies in the United States and throughout the world. Ticks are second to mosquitoes as vectors of a wide range of infectious pathogens (protozoa, rickettsiae, spirochetes, and viruses) worldwide (Parola & Raoult, 2001; Jongejan & Uilenberg, 2004). In contrast to many other ectoparasites, ticks are obligatory blood sucking arthropods that parasitize a wide range of vertebrate hosts, including mammals, birds and reptiles (Furmam & Loomis, 1984; Sonenshine, 1991). Ticks have been implicated as vectors of the agents of harmful diseases such as Lyme disease, Rocky Mountain Spotted Fever (RMSF), tick-borne encephalitis, and many other diseases responsible for high mortality and morbidity in humans, livestock, companion animals, and wildlife. Lyme disease is the most commonly reported vector-borne disease in the northern temperate zone regions of the northern hemisphere (Lindgren & Jaenson, 2006; Bacon et al. 2008) with 22,014 confirmed cases in the United States in 2012 (CDC 2013). Due to their veterinary, zoonotic, and public health importance, it is no surprise that ticks are among the most widely studied arthropod disease vectors.

Several studies (e.g., Carroll and Schmidtmann, 1996; Randolph & Storey, 1999; Carroll, 2002; Randolph 2014) carried out on various species of ticks have concentrated on behavioral patterns linked to disease transmission. In order to understand the process of disease transmission, feeding and reproduction patterns (including mating & oviposition) were also studied. Much is known about tick semiochemicals, specifically pheromones, kairmones and allomones (e.g., Kiszewski et al., 2001; Wanzala et al., 2004; Gaillard et

al., 2004; Mulenga, 2014) and how they regulate feeding behavior, mating behavior, responses to environmental cues (odorants, mates, etc) (summarized by Sonenshine, 1991). These studies have provided valuable insights into the triggers (stimuli) and corresponding responses/expected responses affecting the regulation of these processes. For example, a *Dermacentor variabilis* (*D. variabilis*) male is able to detect 2, 6-dichlorophenol (a sex attractant) upon approaching a female but will not react to this odorant and proceed to copulate until it has had a blood meal (Sonenshine, 2006). Coordination of these physiological processes and how they interconnect is maintained and driven by the nervous system. The central nervous system (CNS) employs the use of vehicles such as neurotransmitters, neuropeptides, and receptors/receptor sites etc. to achieve its goal. Neurohormones and neurotransmitters play key roles in tick development and physiology. Although we know a lot about the biology and physiology of tick development, feeding, and reproduction, understanding tick neurobiology has been difficult because of their small size and difficulty in tick rearing. Therefore, precisely how the tick's nervous system regulates these fundamental biological processes is largely unknown. However, advances in molecular biology and high throughput sequencing technology has made it possible to advance our understanding of these processes in ticks perhaps similar to what has been achieved in insects and crustaceans. What is needed is a detailed knowledge of the molecular biology of this important system and the processes that it controls. However, little is known about the transcribed genes in the tick CNS (= synganglion), largely because of the difficulty in extracting sufficient amounts of tissue from these tiny organs. Fortunately, advances in sequencing technology now allow researchers to rapidly obtain large amounts of data from small

amounts of tissue (Mardis, 2011).

In the past decade, advances in high throughput RNA sequencing technology (e.g., Solexa/Illumina (HiSeq), has revolutionized the field of transcriptomics (Wang et al. 2009). One of its most important advantages as compared to earlier methods (e.g., Donohue et al. 2010, Anderson et al. 2008) is the exceptional high coverage (millions of base pairs) of gene transcripts (mRNA). Other advantages include the high reproducibility of results and reduced amount of RNA sample needed because of the absence of a cloning step (Wang et al. 2009). Transcriptomes provide a global expression profile of genes active in response to changes in the animal's physiological states, in a developmental stage, or in response to environmental stimuli. With the possibilities made available by next generation sequencing (NGS) platforms, researchers are presented with the advantage of wholly evaluating global gene expression patterns Hurd et al., 2009). The application of comparative transcriptomics for transcriptional profiling in organisms has been reported in several studies. For instance this concept has been employed in discovering novel stem cell regulators affecting cell-associated functions (e.g. tissue homeostasis, stem cell maintenance, regeneration), from about 123 conserved genes identified when transcriptome data obtained from pluripotent adult stem cells of planarians, human, and mouse (sharing close to 4,432 orthologs) were compared (Labbé et al, 2012). This was the first of this kind of study comparing between invertebrate and vertebrate stem cells. A second example (taking into consideration species from same invertebrate family) investigated the detection of pheromone proteins and reproductive genes implicated in cryptic speciation processes by comparing transcriptomes generated from tissues of two earthworm species *Hormogaster samnitica*

and *H. elisae* (Novo et al., 2013). This study was also first of its kind that provided insight into the molecular machinery that governs reproduction in earthworms. With comparative transcriptomics, it is possible to identify messages predicting genes relevant to cellular responses, environmental cues, morphological change, and growth by analyzing the changes in gene expression between different conditions (Whiston et al, 2012).

RNAseq is the preferred method in my effort to identify neuropeptides, neurotransmitters, and receptors and protein families common to two tick species representative of the two major families of ticks i.e. the Ixodidae (hard ticks) and Argasidae (soft ticks). This study will compare differential expression of putative genes/proteins that are specific to individual species as well as investigate molecular functions of the candidate genes identified. This study represents the first use of comparative transcriptomics to determine and evaluate expression of neuropeptides and neurotransmitters in the synganglion of two different tick species with fundamentally different life histories (e.g., one versus multiple nymphal stages) and how they may regulate their different biological processes.

Neuropeptides, otherwise known as neuronal signaling molecules refer to short chain peptide molecules employed by neurons to communicate with each other. Studying neuropeptides in ticks presents a platform for discovery of their individual target receptors, second messenger systems and consequently physiological functions (Hökfelt et al, 2000). Such information when acquired will reveal putative targets useful for vector control of tick –borne diseases by altering feeding, mating, and mate finding behavior. In contrast to ticks, many more studies of neuropeptides and neurotransmitters have been carried out in insects and crustaceans (Van Wielendaele et al. 2013; Caers et al. 2012; Altstein and

Nässel 2010; Christie et al. 2010). In addition, numerous studies in *Drosophila* for instance, have revealed evidence of close to 40 putative peptide G-protein-coupled receptors (GPCRs) (Nassel, 2002).

1.1. Problem Statement

Many tick-borne diseases exist in the United States and other parts of the world. The pathogens responsible for causing these diseases are transmitted by many different tick species, both hard ticks and soft ticks. The order Ixodida comprises the Ixodidae (hard ticks), including both the Prostriates (e.g., *Ixodes hexagonus, I. scapularis,* etc,) and the Metastriates (e.g., *D. variabilis*, *Amblyomma*, and *Rhipicephalus*); the Argasidae (soft ticks, e.g, *Ornithodoros turicata*); and the Nuttallielidae (with only one species, *Nuttalliela namaqua*). These tick categories differ on the basis of morphology, feeding habits and life cycle details. For instance, hard ticks feed slowly over many days, often increasing up to 100 times their pre-feeding body weight. They synthesize new cuticle to accommodate the enormous blood volumes consumed during feeding. Excess blood meal water is eliminated by secretion from the salivary glands. Although prostriate males are able to undergo spermatogenesis as well as to mate prior to a blood meal, metastriate males need a blood meal to complete maturation of their reproductive organs. Soft ticks take frequent small blood meals from hosts within periods extending over weeks or months irrespective of mating. They do not synthesize new cuticle, but merely expand the soft body folds to accommodate the small blood meal. The coxal glands during or following blood feeding eliminates excess blood meal water. The female soft tick adults are able to mate at any time prior to or after feeding while sheltering in caves, burrows and similar niches (Gray et al., 2014). Prostriate females of the genus *Ixodes* may mate prior to feeding or while feeding on the host but cannot engorge to repletion

until copulation and insemination is complete. Metastriate ticks (e.g., genus *Dermacentor*) need a prior feeding on a vertebrate host before maturation of their sexual organs and will only imbibe a blood meal to full engorgement after copulation and insemination is complete. To understand these physiological processes, we need to know what genes or neuropeptides are active or responsible in real time for triggering the responses or activities leading to rapid blood feeding to full engorgement and, subsequently, oogenesis and oviposition. Following mating and insemination, females need to complete a blood meal to stimulate vitellogenesis, oocyte maturation, and subsequently, oviposition. Feeding/acquiring a blood meal is important and common to all tick species. The reason why this activity is of great medical and economic importance is the simple reason that all major pathogens of the dreaded range of tick-borne diseases (Lyme, Rocky Mountain Spotted Fever (RMSF), STARI, Rickettsia, Tick Borne Relapsing Fever (TBRF), etc) like most vector borne diseases, are transmitted during the course of feeding on the hosts. Understanding the molecular basis of how feeding, mate finding, reproduction, and oviposition are regulated, and how they all connect to enhance/maintain the success of ticks in the ecosystem, will open up a new frontier in vector control. In fact molecular triggers of these mechanisms in ticks are poorly understood. Neuropeptides are actively involved in the regulation of many fundamental physiological processes, e.g., homeostasis, feeding, excess water elimination, developmental processes, modulation of neuronal and muscular activity and many others (Nassel, 2002; Alstein & Nassel, 2011). Studying these neurohormonal molecules may help us understand the regulation of major physiological processes in ticks. Using comparative transcriptomics accompanied by reverse transcriptase (RT) - qPCR for both tick species, *I. scapularis* and *O. turicata*, will give a global view of

candidate neurohormonal genes that are being turned on or off in response to host recognition, attachment, and blood feeding. These findings will be useful in identifying targets for regulation of vital aspects of tick biology for control of disease transmission. The discoveries resulting from this work will be useful for developing new approaches for tick control as well as provide a general model that can be applied across all tick categories. Comparative transcriptomics of various mosquito species has already been done to characterize important differences that may be useful for understanding features of their biology that open new opportunities for disease vector control (see Koutsos et al. 2007; Colpitts et al. 2011 for examples). In this study, I be applying advanced molecular tools to identify neuropeptides/receptors, and receptors for neurotransmitters in the transcriptomes of two tick genera: *Ixodes,* and *Ornithodoros*, and also investigate the differential expression profile of the transcripts identified. To this end, a dissertation statement is offered:

1.2. Dissertation Statement

Exploring the neuropeptides, neuropeptide receptors, and receptors for neurotransmitters in the synganglia of part fed females of *Ornithodoros turicata* (Argasidae) and *Ixodes scapularis (*Ixodidae) with insights into their roles in the contrasting biology of these different species.

1.3. Specific Aims

The specific aims of this project are

1) To generate and analyze the transcriptomes of the synganglia from feeding/fed females using Illumina sequencing technologies for each of the two tick species, *Ixodes*

scapularis and *Ornithodoros turicata.*

2) To carry out a comparative analysis of the transcriptomes for the neuropeptides and neurotransmitter receptors expressed in the synganglia of part-fed females of *I. scapularis* and *O. turicata* by using basic bio-informatics tools to perform assemblies, and then submitting the assembled transcripts to BLAST. The BLAST output will generate a list of annotated transcripts of the neuropeptides, neuropeptide receptors and neurotransmitter receptors present in the transcriptomes based on low e-value matches ($\leq$ e-6) in public databases (e.g., BLASTnr). Genes will be selected based on information from similar studies on other arthropods.

3) To support the presumed functional roles of annotated neuropeptides, neuropeptide receptors, and neurotransmitter receptors as predicted in other arthropods using sequence alignments and reverse transcriptase quantitative polymerase chain reaction (RT-qPCR). Gene expression assays of the candidate genes will be performed to compare differential gene expression in unfed versus part-fed females of *I. scapularis* and *O. turicata* ticks synganglia. The results of the alignments and RT-qPCR assays will support tentative gene identifications (annotations). Examination of the differences in gene expression in the two different species in response to feeding may help explain how these genes regulate the different ways in which the two species suck blood, expand their integuments, eliminate excess blood meal water, initiate reproduction, and other fundamental physiological processes. This is the first study comparing the functional roles of neurohormones between a hard tick (*I. scapularis*) and a soft tick (*O. turicata*) using comparative transcriptomics and gene expression assays.

Rationale

Ticks are obligate blood sucking parasites. Blood is their sole nutrient source. Female ticks imbibe large quantities of blood so as provide sufficient nutrients for oogenesis and

oviposition. Prostriate ticks such as *Ixodes scapularis* are considered to be an ancient group, perhaps close to the ancestral species (Durden and Beati, 2014). They may commence mating prior to or during feeding and integumental growth (i.e., new synthesis) which is essential for the large blood meal that they imbibe. Metastriate ticks such as *D. variabilis* are considered to be a more recently evolved group (Durden and Beati, 2014). In these ticks, mating only occurs on the host. In feeding females, integumental growth proceeds slowly until after mating and copulation, when most of the enormous blood meal is imbibed. In both cases, the fully engorged females drop from their hosts and lay thousands of eggs in a single gonotrophic cycle. Soft ticks such as *O. turicata* feed rapidly, often within as little as 30 – 60 minutes, and imbibe relatively small blood meals. No new integument is created. Instead, unfolding of the existing cuticle occurs so as to accommodate the small blood volume (~ 10 times the original body size) and provide sufficient nutrients for the small egg mass that follows. In this and similar soft tick species, multiple feeding and gonotrophic cycles occur, dispersing the progeny over extended periods of time. Females lay only small egg masses, e.g., up to several eggs, after each feeding, and require additional blood meal to oviposit again. Clearly, these are very different life cycle patterns. Very little is known about the molecular pathways that control these differences. The regulation of the feeding and reproductive processes in the soft ticks described above is poorly known (Oliver et al. 1992). Few molecules were reported to function in these processes (Zhu and Oliver, 1991; Mans et al. 2008 a) identified mostly by comparison with (supposedly) similar molecules in insects. Molecules that regulate the ecdysial process (molting) or wing expansion such as bursicon, corazonin, and eclosion hormone that occur in insects were reported to be expressed in some adult ixodid ticks (Bissinger et al. 2011) This is

surprising since adult ticks do not molt nor have wings. Obviously, the function ascribed to a particular molecule when it was first described may differ in another species e.g., tick vitellogenin, a hemelipoglycoprotein incorporated into oocytes, is very similar to other tick storage proteins but have different functions (Khalil et al. 2011). Why such molecules occur in ticks and how they function remain to be discovered. Other insect molecules that regulate diuresis have similar cognate (homologous) molecules in ticks, even though nitrogenous waste excretion differs radically between the two groups (uric acid versus guanine and other purines). Similar questions relate to signaling molecules, kinins, and many others.

To date, the only in-depth investigation of the neuropeptides, neurotransmitters, and receptors in the synganglion of a tick was that done by Bissinger et al. (2011) with female *Dermacentor variabilis*. In view of the great diversity of tick species, knowledge of these molecules from a single species may not be truly representative of the entire suborder Ixodida. Moreover, ixodid and argasid ticks exhibit major differences in their development, blood feeding, reproduction and other biological processes, as noted in sections 1.5 below. Therefore, this study is proposed to address the comparison of these essential regulatory molecules and their gene expression in different species of ticks and particularly, representatives of the two major families of ticks.

1.4. Categories of Ticks

Ticks, in general taxonomical terms, and on the basis of classification, are grouped as stated below:

Kingdom: Animalia

Phylum: Arthropoda

Class: Arachnida

Subclass: Acari

Superorder: Parasitiformes

Order: Ixodida, Argasidae, Nuttalliedae.

Ticks are grouped into three families, namely, Ixodidae, Argasidae and Nutttallielidae. The latter is represented by a single species, (*Nuttalliella namaqua*) and will not be considered further. Ixodidae, or hard ticks, is by far the largest family, and the one with the most economic significance of the two. It comprises 13 genera and well over 650 species (Sonenshine and Roe, 2014). Genera represented in this group include *Ixodes*, *Haemaphysalis*, *Hyalomma, Rhipicephalus*, *Rhipicentor, Anocentor, Aponoma, Amblyomma*, *Anomalohimalaya*, *Cosmiomma*, *Margaropus*, *Nosomma*, and *Dermacentor* (Hoskins, 1991; Durden and Beatii, 2014). The presence of sclerotized or hardened body parts, e.g., capitulum, scutum, and plates, is a distinctive characteristic of the ixodid ticks (Obenchain & Galun, 1982). These ticks exhibit a remarkable ability to take up and concentrate an enormous volume of vertebrate blood during feeding, in some cases increasing to ~ 100 times their original body weight. To accomplish this, feeding ticks must excrete excess blood meal water and electrolytes since their internal osmotic pressure is approximately 1.5 times greater than vertebrate blood. Feeding ticks continuously excrete water and salts via their saliva into the skin of their hosts (Needham & Teel, 1991).

The Argasidae, or soft ticks comprises186 species with 5 genera, *Argas, Ornithodoros, Otobius, Nothoaspis and Antricola.* These ticks have a flexible, folded leathery cuticle which unfolds during feeding, enabling them to expand their bodies rapidly and feed quickly, often within minutes or hours (Sonenshine and Roe, 2014). With few exceptions, sclerotization is absent in this group (Obenchain & Galun, 1982; Sonenshine,

1991; Sonenshine & Roe, 2014). Except in the larval stage, the mouthparts are located under the anterior end of the body. In the absence of a scutum, distinguishing males from females is done by examining the shape of the genital pore. Nymphs are distinguished from adults by the absence of the genital pore. Aside from these specific features, the anatomy of soft ticks is basically the same as the hard ticks (Needham & Teel, 1991). However, major differences in the biology of these two families of ticks exist, especially pertaining to feeding habitats, number of life stages, reproduction, host finding and host preferences, tick-host behavior, tick relationship to their environment, and disease association among species in this group, (Sonenshine, 2005; Sonenshine and Roe, 2014; Needham & Teel, 1991). These differences in tick biology are discussed below.

1.5. Brief Overview of Tick Biology

Feeding, mating, and osmoregulation in ixodid and argasid ticks.

***Ixodes scapularis* biology:** The hard tick (family Ixodidae), *I. scapularis*, has 3 active life stages (excluding the egg), larva, nymph and adult. An example of an adult female is illustrated in the image below (Fig. 1.1). After contacting a suitable host, females attach by cutting into the host skin with their chelicerae and then embed their mouthparts into the freshly created wound site. The ticks secrete a cement compound in their saliva, creating a firm bond to host skin. Subsequently, anti-coagulants and other anti-hemostatic agents enlarge the wound site, allowing blood and tissue fluids to ooze into the wound (Ribeiro et al. 1985; Francischetti et al. 2009). These actions create a feeding pool from which the ticks feed by sucking blood. Females feed slowly, requiring many days to fully engorge. Feeding females feed slowly for many days until they are mated,

whereupon the females engorge rapidly until replete, usually within 1 or at most 2 days. In order to expand to the huge size reached by the females of this species, cuticle synthesis takes place continuously as increasing volumes of blood are imbibed. Nevertheless, the body cuticle remains very flexible so as to accommodate the expansion of the growing body. In this species, mating may occur in the natural environment prior to feeding or while feeding on the vertebrate host. As noted previously, in contrast to metastriate hard ticks, males of *I. scapularis* do not feed. During female feeding, elimination of excess blood meal water and excess salts is done by secretion of saliva into the wound site, i.e., while feeding on the host. Adults mate only once, after which the female deposits a very large egg mass (many thousands of eggs) and dies.

***Ornithodoros turicata* biology:** The soft tick (family Argasidae), *O. turicata*, also has 3 life stages (excluding the egg), namely, larva, nymph and adult. However, there are multiple nymphal stages, each of which must feed and molt again, thereby extending the duration of the life stages by many months or even years. An example of an adult female is illustrated in the image below (Fig. 1.1). In this species, mating usually occurs in the natural environment, independent of feeding, although it may also occur while feeding on the vertebrate host. After finding a suitable host, these ticks use their chelicerae to cut through their host skin and embed their mouthparts. However, in contrast to *I. scapularis* and other ixodid ticks, soft ticks, including *O. turicata,* do not secrete cement. Instead, they commence sucking blood from the wound immediately, salivating anticoagulants and other anti-hemostatic agents to enhance blood flow (Mans et al. 2008b). Feeding occurs very rapidly, usually within 30 – 60 minutes. During or immediately after feeding, the ticks eliminate large quantities of water (and excess salts) via their coxal

glands, enabling them to concentrate the blood meal. In contrast to *I. scapularis,* both males and females feed. Adults can mate and feed numerous times. After each bloodmeal, the mated female deposits a relatively small egg mass (several hundred eggs).

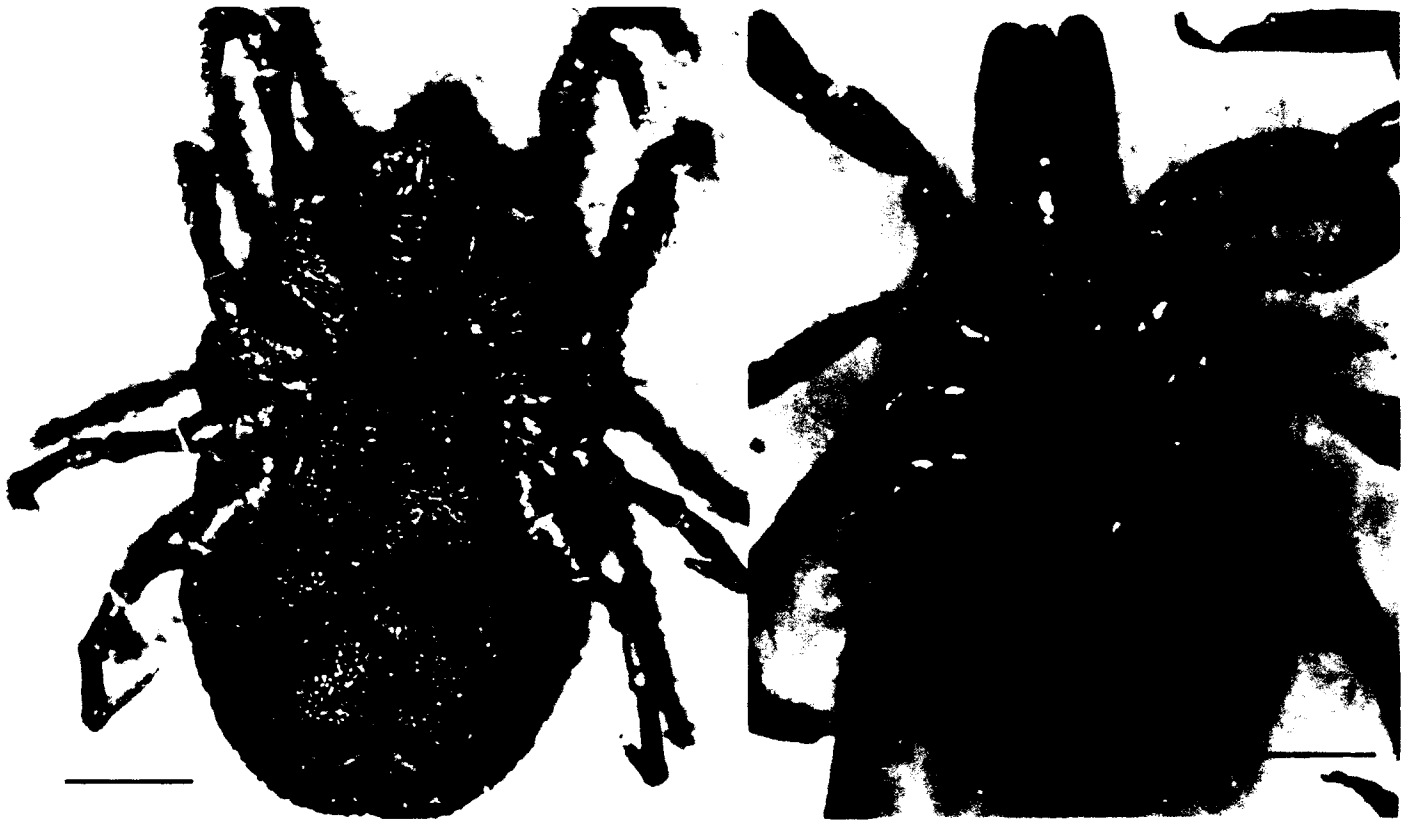

Figure. 1. Images comparing *O. turicata* and *I. scapularis* females from the ventral aspect. Star indicates location of female genital aperture. Photo credit: Dr. David Gauthier, Dept. Biological Sciences, Old Dominion University, Norfolk, Virginia. Bar = 1.0 mm.

1.6. Tick Brain General Morphology & Biology

Tick brain structure: The central nervous system of ticks is consolidated into a single mass, the synganglion, believed to be synonymous with the brain and ventral nerve cord of insects and crustaceans. The synganglion is divided into two major regions, the supra-esophageal region and the sub-esophageal region by the passage of the esophagus between these two sectors. The supraesophageal region contains the protocerebral, cheliceral, and palpal ganglia. Nerves extending from this region innervate the salivary glands, pharynx, esophagus, and optic lobes. Also present on the dorsal side of this region is the retrocerebral organ complex. The subesophageal region contains the 4 pedal ganglia, the opithosomal ganglion and the olfactory lobes. Nerves from this region and and most of the internal organs, innervate the 4 pairs of walking legs, most of the internal organs (reviewed by Simo et al. 2014).

The outer zone of the synganglion comprises the cortex, containing masses of neuron cell bodies clustered into lobes that regulate different functions. Also present are neurosecretory cells grouped into small clusters, known as neurosecretory centers. These centers are the sites where neuropeptides and neurotransmitters are synthesized. Neuropeptides and neurotransmitters pass from these neurosecretory centers via the axons of nerves and neurosecretory tracts to receptors in the target tissues or organs (Simo et al. 2014). A diagram of the synganglion showing the major regions and the neurosecretory centers can be found in Simo et al. (2014). The inner region of the synganglion comprises the neuropile. It consists of a complex system of fibrous tracts of axons and dendrites from the cortical zone neurons, organized into glomeruli, commisures and other connections between the various ganglia (Szlendak and Oliver, 1992). Using immunoreactive staining with antibodies to insect neuropeptides and

neurotransmitters, Simo and colleagues have revealed the existence of a complex peptidergic network in the synganglion of a hard tick, *Rhipicephalus appendiculatus* and identified the neurosecretory centers identified with many of the specific neuropeptides (Simo et al., 2009a). However, immunoreactive staining alone is not conclusive evidence of the same neuropeptides in *I. scapularis* or *O. turicata,* indicating the need for further work to catalogue these molecules and/or their corresponding receptors and to compare their gene expression in the two different species.

In this dissertation, I sought to compare the messages in the transcriptomes of the *I. scapularis* and *O. turicata* synganglion transcriptomes predicting the neuropeptides, neuropeptide receptors and neurotransmitter receptors. I also attempted to compare gene expression assays (RT-qPCR) for these same neuropeptides, neuropeptide receptors, and neurotransmitter receptors. Finally, I reviewed the similarities and differences in expression of these regulatory molecules and suggested possible roles for explaining the biological differences between these two species representative of the two major tick families, Ixodidae and Argasidae.

CHAPTER 2

MATERIALS AND METHODS

2.1. Tissue Collection

The tick strains used were *Ixodes scapularis* (Wikel strain, originally from University of Connecticut, Storrs, CT), and *Ornithodoros turicata* (originally from J.H. Oliver, Georgia Southern University, Statesboro, GA). Adult females, both unfed and fed, were used in the studies. Both tick species were pure line strains maintained in the tick research facility at Old Dominion University Biology building equipped with a functioning walk-in PGC micro pro 2000 Incubator (PGC Inc., Black Mountain, NC) furnished with a Honeywell digital Humidity/Temperature Sensor (Morris Town, NJ) and a reach-in PGC incubator with precise humidity control (PGC Inc., Black Mountain, NC). The parameters for feeding and growing tick colonies as performed in the walk-in incubator were done at a relative humidity of 92 $\pm$ 0.5 % RH and temperature of 18 $\pm$ 1.3 ^{o}F. After feeding, immature ticks were held in the reach-in incubator under 26 $\pm 1^{0}$ C. and 94 $\pm$ 0.5% R.H. for molting. The *I. scapularis* females were partially fed on New Zealand white rabbits, *Oryctolagus cuniculus* (Charles River Labs, MA)) for 5-7 days, then forcibly detached and transferred to the tick lab for dissection. *O. turicata* were fed on albino mice, *Mus musculus* (Charles River Labs, MA) for 1 - 2 hours and then collected immediately from the mice. *O. turicata* females were collected immediately after engorgement because of their rapid feeding behavior. A total of 30-50 part fed females of *I. scapularis* and fully engorged *O. turicata* were used in this study for the RNA sequencing and approximately 500 – 1000 more for gene expression studies achieved by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) experiments.

Ethics Statement: Handling of animals in this project was carried out as specified in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocols were approved by the Old Dominion University Institutional Animal Care and Use Committee (IACUC). The approved protocols (#10-018 and #10-032) are on file at the Office of Research, Old Dominion University, Norfolk, Virginia.

Figure 2. Photograph of dissection of a partially engorged ixodid tick *(Dermacentor variabilis)* showing the location of the synganglion in the anterior portion of the tick body. Similar procedures were used for *Ixodes scapularis* and *Ornithodoros turicata.* Star indicates synganglion (white structure adjacent to asterisk). Bar = 1.0 mm.

Specimen was immobilized on tape and dissected with the aid of a stereoscopic microscope. Tissues of synganglia were collected fresh for RNA extraction. Adult specimens were cleaned with ethanol, followed by RNAase zap (Life Technologies, Carlsbad, CA). Each female tick was attached to carpet tape on glass microscope slides, dorsal side up, and dissected under a stereoscopic microscope (Wild Hebrugg,

Switzerland). Ticks were cut open from their lateral regions with dissecting scissors (Fine Science Tools Inc., Fosterity, CA) and synganglia (see Fig 2) were detached with a pair of fine-pointed Dumont No. 7 forceps [0.1 mm] and Vannas stainless steel irridectomy scissors with 2.5 mm cutting edges (Ted Pella, Inc., Reading, CA). Each synganglion collected was rinsed in a single cavity depression slide containing 200 µl of phosphate buffered saline (PBS buffer), pH 7.4, and extraneous tissues were removed. Next, 10 - 15 tick synganglia were transferred into freshly made 350 µl of RLT Lysis buffer made in accordance with the Qiagen RNeasy Plus Micro Kit instructions (Qiagen, Valencia, CA) in a 1.5 mL Eppendorf microcentrifuge tube. The tube was placed on dry ice, insuring that each synganglion sample remained frozen while the collection accumulated. Next, the samples were thawed and the total RNA extracted immediately in accordance with the kit instructions.

2.2. RNA Extraction

RNA extraction was performed using the Qiagen Rneasy Micro kit and as specified in user manual. Briefly, synganglia collected in RLT buffer as described previously, were disrupted with a Sonic Dismembrator Model 100 (Fisher Scientific, Waltham, MA). Sonic disruption was repeated 3 times for 10 sec and placed for 30 sec on wet ice between each cycle. Next, the disrupted tissues were transferred to a ceramic mortar and hand homogenized with a ceramic pestle (Corstek, Golden, CO). The resulting homogenate was then transferred into the same Eppendorf 1.5 ml microcentrifuge tube and spun in an Eppendorf model 5424 centrifuge (Eppendorf AG, Hauppauge, NY) at $\geq$8,000 × g for 3 minutes. The supernatant was collected and transferred into a gDNA column inserted in a collection tube (provided with the RNeasy kit). RNA extraction was performed as described in the user manual. Purified RNA was collected and quantified

with the Nanodrop 2000 instrument (Thermo Fisher Scientific Co, Waltham, MA). Measurements were documented as concentration and purity (A260/280). RNA samples with A260/280 ratio range of 1.7 to 2.2 were considered to be of acceptable purity (Wilfinger et al., 1997) and any sample with a ratio of <1.7 were discarded. The samples were stored immediately in the -80 ^{o}C freezer.

2.3. Quality Assurance of Total RNA

Quality assurance of total RNA isolated from the synganglia of part fed female *I. scapularis* and *O. turicata* ticks were required prior to Illumina sequencing. Quality assurance assays were accomplished with the Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA), located in the Genome Sciences Laboratory at North Carolina State University, Raleigh, NC. While analysis of total RNA was performed with the RNA 6000 Nano Kit enhanced for use with the Agilent 2100 Bioanalyzer collection of data, visualization, interpretation, and presentation of results were achieved by the 2100 expert software (Agilent Technologies, Inc., Columbia, MD). Total RNA was analyzed as specified in manufacturers manual. Samples were processed on a chip and the chip was then inserted into the Bioanalyzer to initiate the run according to manufacturer's instructions. Results were analyzed accordingly and RNA quality was determined consequently.

RNA extracted from synganglia tissues of part fed females of *I. scapularis* and *O. turicata* ticks yielded > 12 µl for each dissection set. Total RNA yield and purity for each sample was determined with a Nanodrop 2000 spectrophotometer (Thermofisher, Wilmington, DE). Samples with low purity (< 1.7) were discarded. Each sample set was pooled to give a yield of more than > 1 µg of total RNA needed for the Illumina run. The

260/280 ratio was determined to be > 1.8. The samples were submitted to the Genome Sciences Laboratory at the North Carolina State University (Raleigh, NC) and prepared for Illumina sequencing using the Illumina TruSeq RNA Sample Prep Kit v2 (Part No. 15026495, Illumina, Inc. San Diego, CA). The integrity of these RNA samples was evaluated further using a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA); samples that did not meet minimum requirements for Illumina sequencing (RNA integrity ≥ 8) were discarded) (see Fleige and Pfaffl 2006).

2.4. Sequencing by Illumina

2.4.1. Library Preparation

Messenger RNA meeting Bioanalyzer quality assurance standards was prepared for sequencing on the Illumina platform. Library preparation of mRNA was achieved with the TruSeq RNA Sample Prep Kit v2 (Part No.5026495, Illumina Inc., San Diego, CA). Technicians at the Genome Science Facility of North Carolina State University, Raleigh, NC, performed all subsequent procedures, for Illumina sequencing. Paired end libraries were constructed using the procedures highlighted in the manufacturer's manual. To achieve this, messenger RNA were subjected to a series of procedures ranging from mRNA fragmentation, first and second strand cDNA synthesis and clean-up, and ligation of adapter to cDNA strands in accordance with instructions in the sample prep kit noted above. In addition, cluster generation by hybridization and bridge amplification of cDNA libraries to flow cells was performed.

2.4.2. Sequencing and Base calling

Sequencing was performed using the "Sequencing By Synthesis (SBS)" procedure. After the incorporation of FI-NTP (fluorescently labeled nucleotide) to the strands, each dye

was imaged to identify the bases. Base calling is measured by the intensity of the signals emanating from the reaction for each base read out from sequential images. The sequencing reaction cycle was repeated several times. For paired-end sequencing runs, the single read sequenced strands were stripped off and the 3′ ends of the template strands and primers attached which were blocked previously were unblocked to allow template to loop over to form a bridge with primers on the lawn. These primers extend forming a bridge of double stranded DNA molecules. The original template forward strand was cleaved and washed off the flow cell. Next, the 3'ends of the single strands were blocked. Each resulting DNA strand then has a sequencing primer attached to it whereupon sequencing is performed and imaged as previously described.

2.5. Assembly

Adapter and primer sequences, as well as ambiguous bases were first trimmed from the raw reads using default trimming parameters prior to performing assembly to determine coverage and overlapping of reads. The trimmed raw reads were assembled into contigs by using the CLC-BIO Genome *de novo* Assembler software (CLC Bio, Aarhus, Denmark) to perform *de novo* assembly. The program reported average raw read length (trimmed), total raw reads, average contig length and the total number of contigs. The results were documented in FASTA formats (Pearson and Lipman, 1988) as contigs in text files.

2.6 Bioinformatics

Following assembly, the assembled data files (contigs) in fasta format were submitted to non-redundant Basic Local Alignment Search Tool (BLAST) program (Altschul et al, 1990) at the National Institutes of Health, National Library of Medicine, National Center

for Biotechnology Information (NCBI) using the NCBI program Netblast (currently BLAST Plus) in December, 2011 for *I. scapularis* and May, 2012 for *O. turicata.* Parameters selected were e-values set at E-06 and the single best match. Perl script or similar programs were used by the Genome Sciences Laboratory at the North Carolina State University to organize the data into an excel file with columns showing the contig numbers, contig length, matching NCBI sequence numbers, e-values, and annotations. Further analysis to validate these genes was performed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm (Sokal and Michener, 1958) in the Geneious 7.0 program (Drummond et al, 2011) to perform local alignments of the contigs with already annotated conspecific genes from databases. This same program was used to determine relationships and pairwise similarities between the *I. scapularis* and *O. turicata* transcriptomes. Transcripts with pairwise similarities of < 80% with the conspecific *Ixodes* genome were discarded. In cases where strong identities exist with other species for same genes, they were accepted.

2.7. Mapping & Gene Ontology

Mapping, annotation, and gene ontology of *de novo* assembly results for the synganglion transcriptomes of part-fed females of *I. scapularis* and *O.turicata* ticks were performed using Basic Local Alignment Search Tool (BLAST-[blastn]) algorithm (Conesa et al. 2005) in BLAST2GO program (BioBam Bioinformatics S.L., Valencia, Spain). BLAST2GO mapping was done in Spring, 2012 (the program requires several weeks to complete these processes). The program was used to search for similarities by comparing contig sequences against nucleotide sequences deposited in the NCBI Genbank nucleotide collection (nr) and Expression Sequence Tag (EST) databases. The search

was performed at an E-value cutoff of E-06; and blast hit of 3. Gene Ontology (GO) terms related to the hits from the BLAST were retrieved after successful mapping of the transcriptomes to GenBank. The BLAST2GO program assigned the transcripts that were submitted to their different categories for various molecular processes. The GO category assignments at level 3 of molecular processes, was selected for further study. The level 3 GO molecular categories were examined in relation to their importance in understanding their roles in synganglion function, especially those that included neuropeptides, neuropeptide receptors, and neurotransmitter receptors.

2.8. Gene Expression Study

2.8.1. Reverse Transcription (cDNA Synthesis)

RNA isolated as described in 2.1., was reverse transcribed to complementary DNA (cDNA). The kit used to accomplish this was the SuperScript II Reverse Transcriptase kit (Invitrogen Inc, Carlsbad, CA). The cDNA synthesis was performed according to instructions outlined in the manufacturer's manual. An amount of 60-80 ng of total RNA isolated from the synganglia of unfed and part-fed female ticks of *I scapularis* and *O.turicata* were used as starting material for a 20 µl first strand cDNA synthesis reaction. An amount of 60 - 80 ng of RNA was primed with 0.5 µg oligo $(dT)_{12\text{-}18}$, and 1 µl 10nM dNTP brought up to volume with required amount of Nuclease-Free Water (Qiagen) in 0.2 mL (Bio-Rad, Hercules, CA). The tube was transferred into a thermal cycler (TC-312, TECHNE, Duxford, Cambridge) and the mixture heated at 65 ^{O}C for 5 min using a set program to remove secondary structures as described by Bürgmann et al (2003). The mixture was immediately incubated on ice for at least 1 min after which 4 µl 5X first-Strand Buffer, 2 µl 0.1 M DTT, and 40 U RNaseout Recombinant Ribonuclease Inhibitor

was added and incubated in thermal cycler at 42 ^{O}C for 2 min. At this point, 200 U of SuperScript II Reverse transcriptase was added and incubated at 50 ^{O}C for 55 min followed quickly by an inactivation step at 70 ^{O}C for 15 min and held at 4 ^{O}C for ∞.

2.8.2. Gene Expression Profiling

Gene expression was determined by real time quantitative polymerase chain reaction (RT-qPCR) assays using the MyiQ2 Two Color Real-Time PCR Detection System (Bio-Rad, Hercules, CA). The general experimental design approach used for singleplex assay was based on the background of final RT-qPCR data analysis by "Relative Quantification Normalized to a Reference Gene" method described in the Bio-Rad Laboratories Real Time PCR Applications Guide, 2006 (www.bio-rad.com/webroot/Bulletin.5279.pdf; Mathews et al, 1999; Rozen and Skaletsky, 2000). This method requires using a reference gene as a normalizer rather than unit mass. It was also preferred because accurate quantitation and loading of starting material is overlooked in addition to the convenience it presents when working with limited material. The reference genes used after test trials with glyceraldehyde-3- phosphate dehydrogenase (GAPDH), ribosomal protein L8 (RPL8), and Actin, were GAPDH and RPL8 for *I. scapularis* and *O. turicata*, respectively. The procedures for designing, performance, and analysis of RT-qPCR assays generally followed the MIQE (=Minimum Information for qPCR Experiments) guidelines (Bustin et al. 2009) with detailed descriptions presented below.

2.8.3. Primer design and Validation

Forward and reverse primers for singleplex assays were designed for candidate genes coding for neuropeptides, neuropeptide receptors and neurotransmitter receptors of adult females of both *I. scapularis* and *O. turicata*. Primers were designed with Beacon 8.02

software (PREMIER Biosoft, Palo Alto, CA), IDT primer by Integrated DNA Technologies (www.idtdna.com) and supplied by Integrated DNA Technologies (IDT, Coralville, IA). Each primer set was designed to amplify segments of candidate genes to create amplicon lengths of between 80 - 120 base pairs confirmable by gel electrophoresis. Only primers predicting a high probability (>85%) for amplifying the target gene sequence and in which amplicon matched the target gene in Genbank were accepted. A serial dilution of a synganglion cDNA sample (from 10^0 to 10^{-6}) was tested with the GAPDH and/or RPL8 primers and the results evaluated to determine the sensitivity of the qPCR assay (R^2 =0.97 for GAPDH; R^2 =0.98 for RPL8). The sequences of the primer pairs used for the reference genes and for each neuropeptide and/or receptors and each neurotransmitter receptor for *I. scapularis* and *O. turicata* are located in Appendix A.

2.8.4. Quantitative Polymerase Chain Reaction (qPCR)

Real-time quantitative polymerase chain reaction (qPCR) amplification assays were accomplished using the MyiQ2 Two Color Real-Time PCR Detection System built on the quality gradient-enabled icycler thermal cycler (Bio-Rad laboratories Inc., Hercules, CA) and the analysis were performed with the accompanying Bio-Rad IQ5 2.1 Standard edition Optical system software 2.1 (Bio-Rad Laboratories Inc., Hercules, CA). Based on the decision to optimize with reference housekeeping genes, all primer sets designed for the candidate genes and the two reference genes (GAPDH for *I. scapularis* and RPL4/L8 for *O. turicata*) were subjected to gradient real-time qPCR solely for the purpose of determining optimum annealing temperatures for the individual assays. In order to determine expression profiles of each candidate gene in response to feeding, cDNA

samples reversed transcribed from mRNA extracted from unfed and part-fed females of *I. scapularis* and *O. turicata* synganglia were used as templates. The experimental design for the gene expression studies featured three biological samples and 4-6 technical replicates for each candidate gene and reference genes. The design also included no template controls (NTC=all but template) as well as negative controls (water). The SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA) ready-to-use reaction master mix was used to prepare a 10µl PCR reaction assay for each gene expression work. The master mix, which contained antibody-mediated hot-start iTaq DNA polymerase, dNTPs, MgCl2, SYBR Green I dye, enhancers, stabilizers, and fluorescein, was optimized for dye-based quantitative PCR. For a typical 10 µl reaction mixture, 5µl of 2× SYBR Green supermix (final concentration 1×), 1 µl of each forward and reverse primers (final concentration 100 nM), and 1µl template cDNA (final conc. 75 ng/µl) were pipetted into wells of a 96 -well 1Cycler iQ clear PCR plates (BIO-RAD Laboratories), and mixed thoroughly on ice and under dark conditions.

The PCR plate was sealed with PCR Sealers Microseal B'optically transparent film (BIO-RAD Laboratories, Hercules, CA). The plates were centrifuged (1 min) to get rid of bubbles as well as to collect reaction mixture at bottom of well. Once all steps were completed, the plate was placed in the plate compartment of the thermal cycler and qPCR was done using the "2 step Amplification + Melt" default program settings. A representative protocol is as follows:

Cycle 1: Polymerase Activation & Initial denaturation: 95 ^{O}C for 3 min,
Cycle 2: Amplification (×40):
Step 1: Denaturation: 95 ^{O}C for 10 - 15 sec
Step 2: Annealing /Extension: 55 ^{O}C - 60 ^{O}C for 10 sec
Cycle 3: (x81): Melt Curve Analysis: 55 ^{O}C - 95 ^{O}C for 2-5 sec 0.5 sec increment.

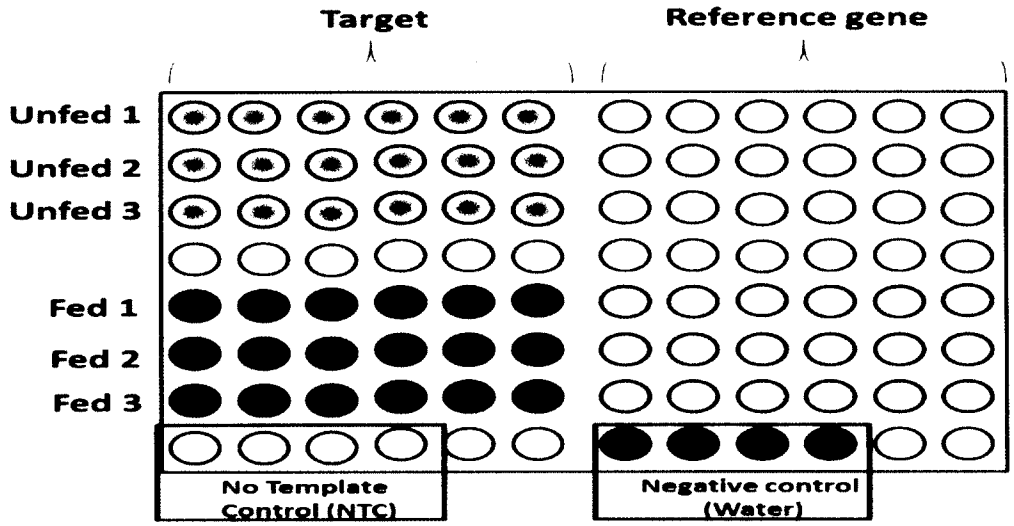

Figure 3. Experimental design of gene expression study showing number of biological samples and technical replicates and their arrangement on a 96-well plate.

Plate reading was taken after the 2nd cycle of annealing/extension step and after the melt curve analysis step. The melt curve analysis was important to confirm that only the specific products were being amplified as well as the purity of template being used; if the melt curve showed abnormalities, e.g., secondary peaks unrelated to the melt temperature for the gene being assayed, the result was discarded. As qPCR progressed, accumulation of products was measured by the amplification curve (determined by relative fluorescence unit [RFU] plotted against the cycle) as the primary output. The threshold cycle commonly referred to as the Ct value was derived from the point at which the amplification curve intersected with the amplification threshold. The software program automatically applies passive reference normalization (Rn) to enforce baseline correction of each curve. Using the delta–delta CT method (Schmittgen and Livak, 2008), the expression levels of each candidate gene were calculated from the threshold cycle by normalizing the amount of target in each sample relative to internal reference genes

which in this case were glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and Ribosomal protein L8 (Rpl8) for *I. scapularis* and *O. turicata* respectively. Each result was reported in fold increase or fold decrease in response to feeding cues. All results were plotted into a bar chart for visualizing the resultsFold differences between the unfed (set to 1.0) versus the fed samples for each gene assayed were reported.

To assure reliability of the qPCR assays, the mean and standard deviations of the Ct values of the 4 – 6 replicates for each biological sample were determined. Replicates deviating by more than 1.0 S.D. were rejected. If insufficient replicates remained for use in the assay, that biological sample was rejected. If not enough biological samples remained that met these standards, then the entire assay was repeated with fresh biological samples. Only those assays were accepted in which the melt curve had a single well-defined peak, free of major secondary peaks (e.g., primer dimers, or non-target amplicons).

CHAPTER 3

RESULTS

3.1. RNA Extraction, Quantitation, And Quality

The tables and figures that follow show the synganglion RNA collections, RNA yield, and bioanalyzer results confirming purity sufficient to proceed to Illumina sequencing. Table 1 shows the contents of four total RNA collections submitted for bioanalyzer assay. The 4 samples were deposited into 4 different wells of the bioanalyzer plate and assayed in the instrument. Figure 4 is an electropherogram of the analysis showing the intense dark bands at (approximately) 43 seconds, which align with the same band in the internal control. Figure 5 provides a graphical display of the electropherogram for each sample and identifies the major peak at 43 seconds as the 28S ribosomal subunit compatible with an RNA integrity number (RIN) satisfactory for Illumina sequencing. The same procedures were used for *O. turicata.* Table 2 shows the contents of four total RNA collections submitted for bioanalyzer assay. Total yields were lower than those obtained from *I. scapularis,* for unknown reasons. Figure 6 shows the same or similar intense bands at 43 seconds as were seen with *I. scapularis* (lanes 4 – 7) of the analysis showing the intense dark bands at (approximately) 41 seconds, which align with the similar bands in other unrelated samples. Figure 7 (graphical display of electropherogram) identifies the peaks (~ 41 seconds) as the 28S ribosomal subunit (RIN > 8) and confirms the suitability of the samples for Illumina sequencing.

Table 1. Bioanalyzer results of total RNA yield extracted from synganglia of part-fed *Ixodes scapularis*

Well	Sample	Concentration (ng/µl)	Total (µg)
4	Is_1_sample 1	27.72	Not recorded
5	Is_2_sample 1	25.50	1.597
6	Is_1_sample 2	30.22	Not recorded
7	Is_2_sample 2	24.86	1.6582

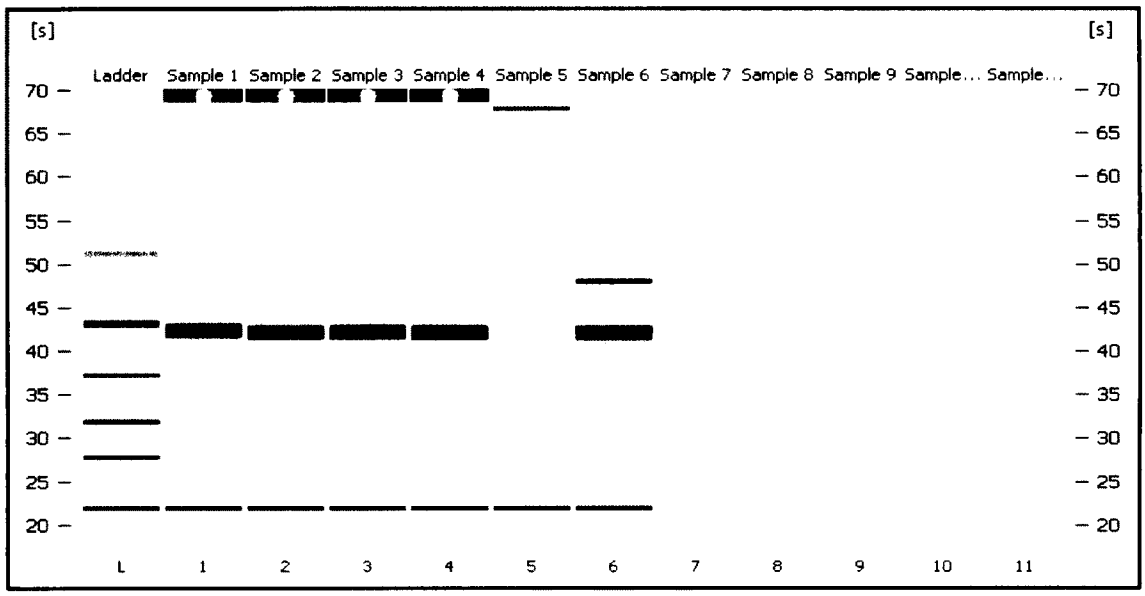

Figure 4. Bioanalyzer imaging of gel results of *Ixodes scapularis*. Samples 1 – 4 represent RNA submitted for bioanalyzer tests. Sample 6 is the internal control.

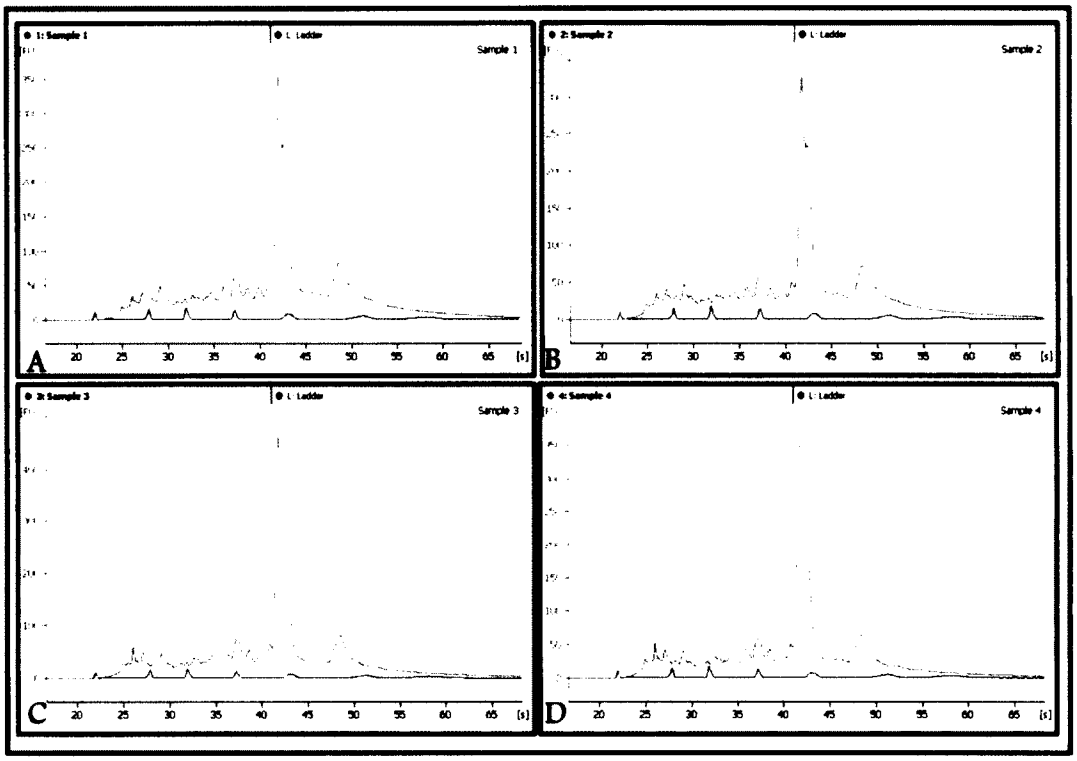

Figure 5. Samples 1 -4. Agilent 2100 Bioanalyzer electropherogram profiles of total RNA extracted from four samples of synganglia of part-fed *Ixodes scapularis* female ticks using the Qiagen Rneasy kit. Tissues were ruptured and sonicated prior to extraction. The first and second dominant peaks at approximately 43 sec and 50 sec are the 18S and 28s ribosomal subunits and is compatible with a satisfactory RIN (RNA integrity number) for use in transcriptomic analysis. See *http://www.genome.duke.edu/cores/microarray/services/rna-qc/documents/Using%20RIN.pdf for details.*

Table 2. *Bioanalyzer results of Total RNA yield extracted from synganglia of part-fed* ***Ornithodoros turicata***

Well	Sample	Concentration (ng/µl)	Estimated (µl)	Total (µg)
4	Ot..5.18.11	24.12	20	0.482
5	Ot..5.24.11	21.40	20	0.428
6	Ot..5.27.11	23.08	20	0.462
7	Ot..6.1.11	28.42	20	0.568

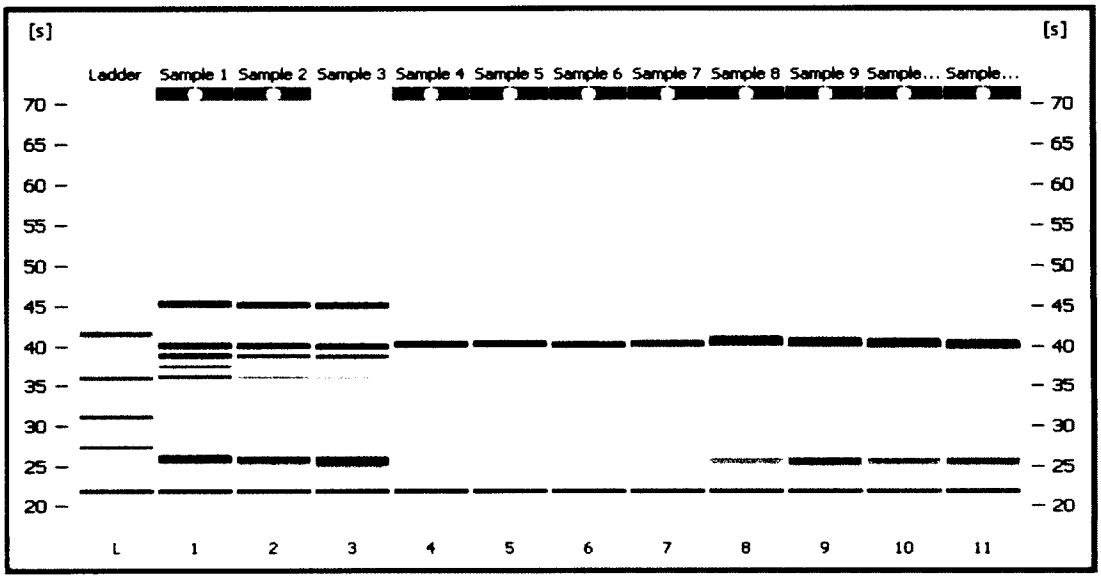

Figure 6. Bioanalyzer imaging of gel results of *Ornithodoros turicata.* Samples 4 – 7 represent RNA submitted for bioanalyzer tests. Other lanes are for unrelated treatments.

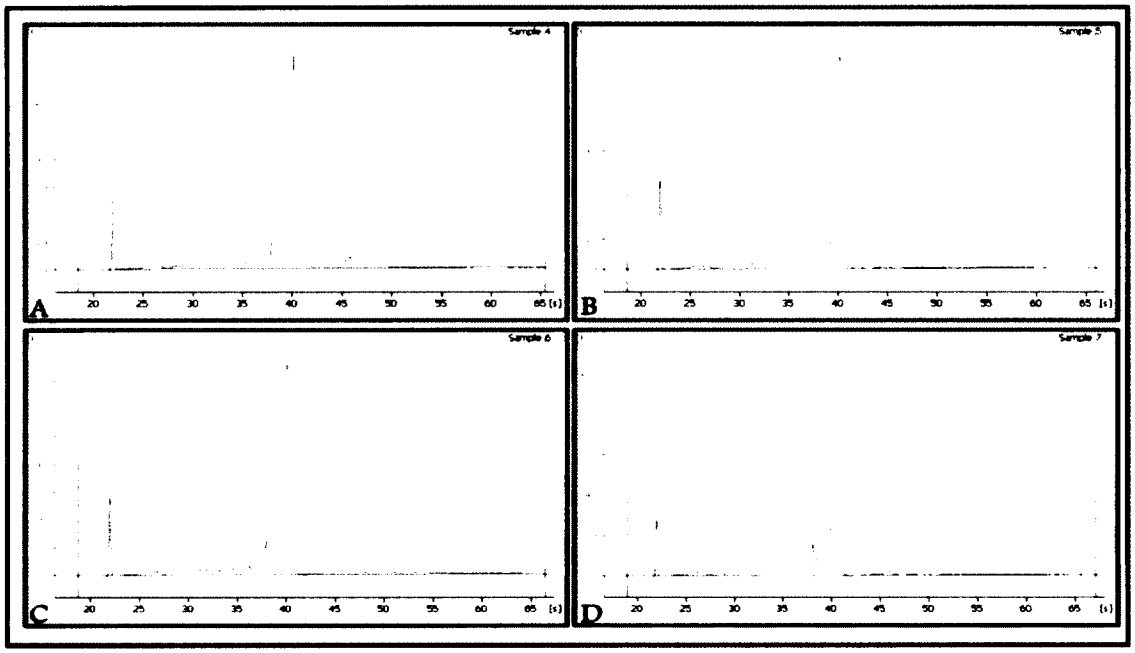

Figure 7. Agilent 2100 Bioanalyzer electropherogram profiles of total RNA extracted from synganglia of part-fed *O. turicata* female ticks using the Qiagen Rneasy kit. Tissues were ruptured and sonicated prior to extraction. The dominant peak at approximately 41 sec is 28S ribosomal subunit and is compatible with a satisfactory RIN (RNA integrity number) for use in transcriptomic analysis. See *http://www.genome.duke.edu/cores/microarray/services/rna-qc/documents/Using%20RIN.pdf for details.*

3.2. Bioinformatics

The results of Illumina *de novo* assembly for *I. scapularis* are shown in Table 3.3. Sequencing (paired end, 2 x a150) yielded a total of 117,900,476 raw reads (not shown in the table). The total mapped read count was 43,351,571, comprising only 3,121,313,112 bp. Following vector trimming, the raw reads were assembled to 30,838 contigs, comprising 20,206,192 bp with an average length of 655 bp. Table 3.3 shows similar data for the Illumina de novo assembly for *O. turicata.* The total mapped read count was 63,528,102 reads with a mean read length of 101 bp. Following vector trimming, the raw reads were assembled to 132,258 contigs, comprising 57,956.605 bp with an average length of 438 bp.

Table 3. CLC-Bio *De novo* Assembly summary data for synganglion transcriptomes of part fed *I. scapularis* and *O. turicata* females

Parameters	***Ixodes scapularis***	***Ornithodoros turicata***
Contig count	30,838	132,258
Type	*De novo* assembly	*De novo* assembly
Total mapped read count	43,351,571	63,528,102
Mean read length (bp)	72	101
Total read length] (bp)	3,121,313,112	6,416,338,302
Mean contig length (bp)	655	438
Total contig length (bp)	20,206,192	57,956,605
GC contents in %	55.72	46.42

3.3. Gene Ontology

The program BLAST2GO was used to map the top BLASTx matches (e-value 1e-06) and to assign gene ontology (GO) term annotations. For biological processes, mapping success showed little difference between the two species (data not shown). However, for molecular processes, GO term mapping success did show some differences. Level 3 mapping was chosen as the level that showed the greatest number of categories, including neurohormones (e.g, neurotransmitter binding) that could be displayed on a single pie chart without extreme crowding. At molecular level 3, a total of 44 mapped gene categories were recognized in *I. scapularis* versus 51 mapped gene categories in *O. turicata.* Table 3.4 presents a tabulation of 32 of the most abundant gene categories as well as those hypothesized to be most relevant to the current study, with comparison of transcripts predicting these genes between the two species. As expected, the most important categories in both tick species comprised transcripts for the common "house keeping" genes, i.e., hydrolase activity, transferase activity, ion binding and nucleotide binding, nucleic acid binding and protein binding. Overall, there was no significant difference between the two different species ($t = 0.277$, $P>0.05$ n.s.). However, several noteworthy differences were observed among three specific categories, namely: 1) nucleoside binding, representing 6.68 % of all mapped sequences in *I. scapularis* versus only 0.08% in *O. turicata,* a 120 fold difference; 2) phosphatase regulator activity, representing 1.00% of all mapped categories in *I. scapularis* versus only 0.08% in *O. turicata,* a 12.5 fold difference; and 3) lyase activity, with 0.68% of all mapped categories in *I. scapularis* versus 1.08% in *O. turicata,* a 1.59 fold difference.

3.4. BLAST2GO Summary

Comparison of GO categories mapped at molecular level 3 for the transcriptomes from *I. scapularis* and *O. turicata*

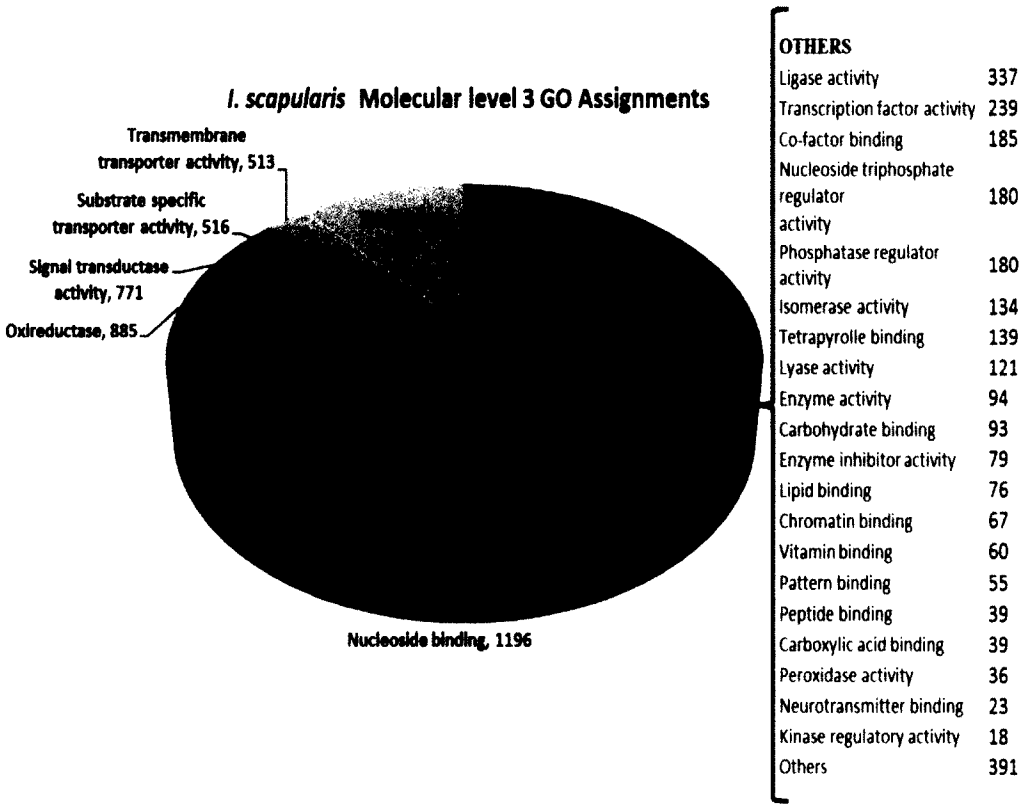

Figure 8. Gene Ontology (GO) term assignments at molecular level 3 for the *I. scapularis* transcriptome assembled following sequencing by Illumina. The figure shows the 34 GO assignments for the part-fed synganglion (17,912 transcripts).

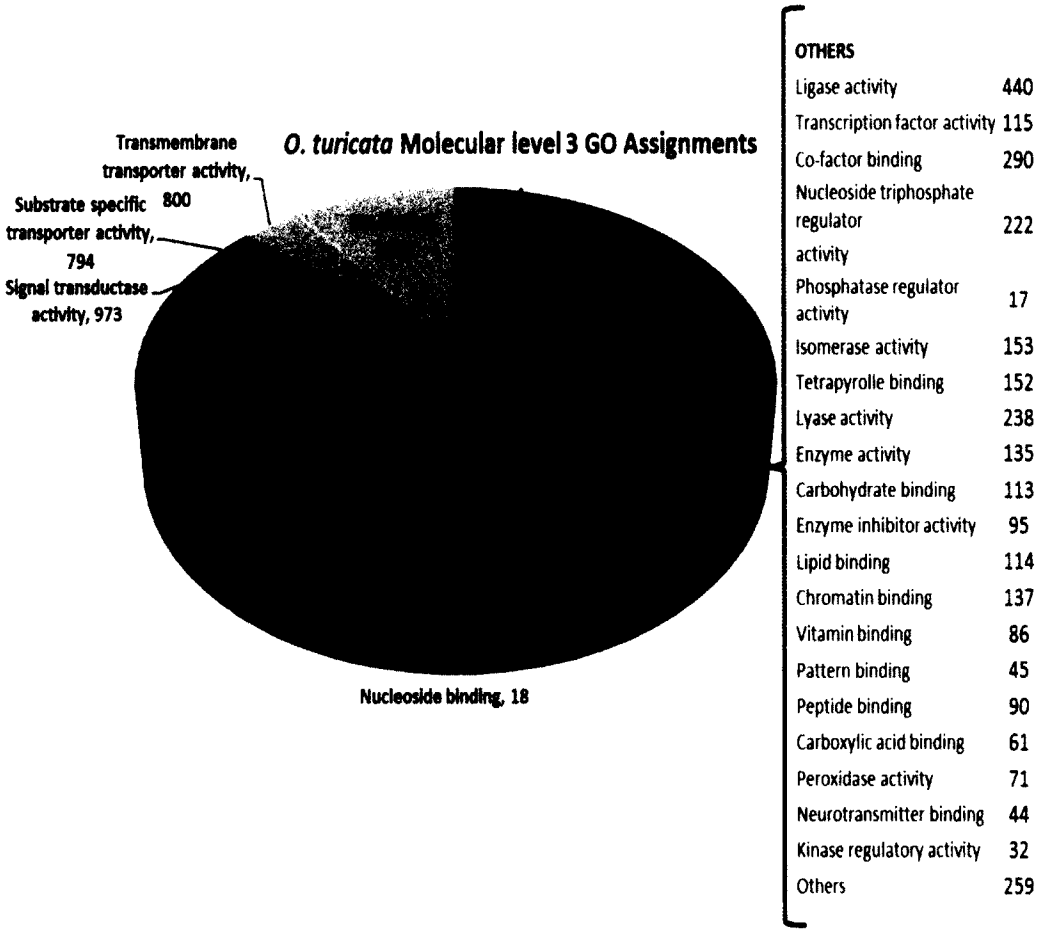

Figure 9. Gene Ontology (GO) term assignments at molecular level 3 for the *O. turicata* transcriptome assembled following sequencing by Illumina. The figure shows the 51 GO assignments for the part-fed synganglion (21,979 transcripts)

Table 4. Comparison of Gene Ontology (GO) categories between *I. scapularis* and *O. turicata*: Similarities and differences in each category in the two different species.

	I. scapularis		*O. turicata*	
GO category	**No. transcripts**	**% total transcripts**	**No. transcripts**	**% total transcripts**
Hydrolase activity	2246	12.54	3293	14.98
Transferase activity	2098	11.71	2778	12.64
Ion binding	1900	10.61	2162	9.84
Nucleotide binding	1625	9.10	2093	9.52
Nucleoside binding	1196	*6.68	18	*0.08
Nucleic acid binding	1815	10.13	2651	12.06
Protein binding	1762	9.84	2335	10.62
Oxireductase	885	4.95	1173	5.34
Signal transductase activity	771	4.30	973	4.42
Substrate specific transporter Activity	516	2.89	794	3.61
Transmembrane transporter activity	513	2.86	800	3.64
Ligase activity	337	1.88	440	2.00
Transcription factor activity	239	1.33	115	0.52
Co-factor binding	185	1.01	290	1.32
Nucleoside triphosphate regulator Activity	180	1.00	222	1.01
Phosphatase regulator activity	180	*1.00	17	*0.08
Isomerase activity	134	0.85	153	0.70
Tetrapyrolle binding	139	0.78	152	0.70
Lyase activity	121	*0.68	238	*1.08
Enzyme activity	94	0.53	135	0.61
Carbohydrate binding	93	0.52	113	0.51
Enzyme inhibitor activity	79	0.44	95	0.43
Lipid binding	76	0.42	114	0.52
Chromatin binding	67	0.37	137	0.62
Vitamin binding	60	0.35	86	0.39
Pattern binding	55	0.31	45	0.21
Peptide binding	39	0.22	90	0.41
Carboxylic acid binding	39	0.22	61	0.28
Peroxidase activity	36	0.20	71	0.32
Neurotransmitter binding	23	0.13	44	0.20
Kinase regulatory activity	18	0.10	32	0.15
Others	391	2.18	259	1.18
Totals (32 selected gene categories)	17,912[1,2]	--------	21,979[1,2]	-------

* Asterisks represent noteworthy differences compared to other gene categories (not tested for statistical significance)

[1]T-test comparing differences in numbers of transcripts in all categories for *I. scapularis* versus the same for *O. turicata;* : $t = 0.277$ (60 df), not significant at 0.05

[2]No. all genes mapped in *I. scapularis* = 17,912 (58.08 %); [2] No. all genes mapped in *O. turicata* = 21,979 (16.6 % of total contigs in the *O. turicata* sample)

Table 5. Comparative data for transcripts predicting neuropeptides, transporters and neuropeptide receptor genes as expressed in the synganglia transcriptomes of part-fed female *O. turicata* and *I.scapularis*[1]

	Ornithodoros turicata				*Ixodes scapularis*			
Genes	**Contig #**	**E-value**	**Accession #**	**Freq**[2]	**Contig #**	**E-value**	**Accession #**	**Freq**[2]
Allatotropin	Absent	----	----	----	8149	4.00E-46	XP_002407036	1
Allatostatin A prepro	Absent	----	----	----	7636	0	XP_002416345	1
Allatostatin B (Bombystatin)	73312	1.60E-07	XP_001850046	1	7636	0	EU620228	1
Allatostatin C	73361	5.80E-10	XP_002433459	1	Absent	----	----	----
Allatostatin receptor	5840	1.00E-55	XP_002433373	3	1184	3.90E-57	XP_002433373	6
Bursicon α	4964	9.70E-74	XP_002407512	1	8916	4.20E-64	XP_002407512	1
Bursicon β	118500	1.90E-08	XP_002407513	1	----	----	----	----
Calcitonin-like receptor	6563	7.00E-51	XP_002436229	12	8281	1.50E-84	XM_00240838	7
Calcitonin-like diuretic hormone	46006	7.20E-04	EU574855	3	9802	5.00E-21	XP_002435658	1
Corazonin prepropeptide	17545	3.10E-18	ACC99609	1	17545	3.12E-18	ACC99609	1
Corazonin receptor	114694	1.80E-42	XP_002402115	2	8130	3.20E-85	XM_002402071	1
Corticotropin-release factor peptide	6630	6.30E-102	XP_00241082 XP_002405845	2	21180	1.50E-109	XP_002410820	3
Corticotropin-releasing factor (CRF) receptor	7646	0	XP_002405845	7	799	2.50E-91	XP_002434070	4
Crustacean cardioacceleratory peptide (CCAP)	112055	2.10E-38	XP_002402276	1	Absent	----	----	----

Table 5 continued.

	Ornithodoros turicata				*Ixodes scapularis*			
Genes	**Contig #**	**E-value**	**Accession #**	**Freq[2]**	**Contig #**	**E-value**	**Accession #**	**Freq[2]**
Crustacean cardio-acceleratory peptide receptor (CCAP)	9516	7.28E-04	XP_002407935	3	19265	6.70E-109	XM_002407891	1
Cardioactive peptide CAPA/pyrokinin receptor	130874	5.80E-35	ACC99623	1	19265	6.70E-109	XM_002407891	1
Diuretic (calcitonin) hormone receptor	76145	9.9E-12	XP_002435658	2	9802	5.00E-21	XP_002435658	1
Eclosion hormone	117001	1.40E-29	XP_002399271	1	16950	1.50E-32	XP_002399271	1
Ecdysis Triggering hormone receptor (ETHR)	121228	4.98E-31	NP_00107692	2	Absent	----	----	----
Glycoprotein A/Tetraspanin	11593	1.00E-94	AE036315	>20	8734	1.40E-102	XP_002401420	>20
Glycoprotein B mannosly transferase[3]	5974	1.50E-145	XP_002411894	7	Absent	----	----	----
Gonadotropin-releasing hormone receptor	112451	6.60E-87	XP_002401870	1	20361	1.81E-51	XP_002435340	2
Insulin-like peptide	5935	4.20E-22	XP_975429	5	29884	5.70E-36	XP_002410559	1
Insulin-like peptide receptor	77139	8.00E-11	XP_002403264	5	7890	0	XP_002416224	2
Ion transport peptide	8959	2.20E-61	XP_002404559	5	7332	1.8 E-151	XM_002399497	7
Neuropeptide F receptor	72103	1.20E-25	XM_002402212	15	3925	3.3 E-56	XP_002402168	1
Myoinhibitory Peptide (MIP)	17453	1.30E-20	XP_002434041	1	38613	2.20E-54	XP_002434041	1
Neurophysin-oxytocin transporter	60859	1.20E-18	XP_003748448	1	3384	8.80E-22	XP_003748448	2
Orcokinin A Precursor	17445	8.70E-19	XP_002401726	2	13501	5.40E-41	XP_002401726	1
Proctolin	Absent	----	----	----	7349	3.30E-35	EFX81804	1

Table 5 continued.

	Ornithodoros turicata			*Ixodes scapularis*				
Genes	**Contig #**	**E-value**	**Accession #**	**Freq[2]**	**Contig #**	**E-value**	**Accession #**	**Freq[2]**
Proctolin	Absent	----	----	----	7349	3.30E-35	EFX81804	1
Pyrokinin receptor	68180	7.90E-93	XP_002401180	2	4791	1.00E-49	XP_002401180	4
FMRFamide precursor	72921	7.50E-06	AEE25641	2	6861	1.80E-105	XM_002413792	1
FMRFamide receptor	9246	2.60E-38	XP_002432135	2	Absent	----	----	----
Thyrotropin receptor	69986	3.50E-97	XP_002435494	1	Absent	----	----	----
SIFamide	17744	2.80E-29	XP_002414623	1	39081	5.70E-30	XP_002414623	1
SIFamide receptor	19513	1.40E-54	XP_970225	1	2800	2.10E-26	XP_001947497	1
Sulfakinin	117562	4.40E-20	ACC99604	1	30943	2.40E-27	ACC99604	1
Sulfakinin receptor	80646	3.20E-21	XP_002413320	2	13955	4.60E-56	XP_002404195	2
Tachykinin receptor	68588	5.30E-100	XP_312088	14	10361	4.60E-109	XM_002411163	2

[1]Also present was proprotein convertase. Proprotein convertase is an enzyme essential for the conversion of neuropeptide hormones to the mature form. This enzyme was found in both *O. turicata* (contig 113357, 3.8E-163, matching Genbank No. XP_002403832) and in *I. scapularis* (contig 23747, 5.7E-104, matching Genbank No. XP_002410536, sequence alignment 100%)

[2] Frequency indicates the number of transcripts recognizing identical annotations in Genbank.

[3] Identity as glycoprotein B in question. Donohue et al. (2010) reported it as a cysteine knot protein with two subunits, alpha and beta. However, when queried in Genbank, it is found not to be a hormone but an enzyme. Full name = alpha-mannosyl glycoprotein beta acetylglucosamine transferase

Table 6. Comparative data of transcripts for candidate neurotransmitter receptors and transporter genes as expressed in the synganglia transcriptomes of part-fed female *Ornithodoros turicata and Ixodes scapularis*.

	Ornithodoros turicata				*Ixodes scapularis*			
Genes (receptors and transporters)	**Contig #**	**E-value**	**Accession #**	**Freq[1]**	**Contig #**	**E-value**	**Accession #**	**Freq[1]**
Acetylcholine (muscarinic) receptor	88863	7.10E-54	XP_002403135	>20	6186	0	XP_0024003135	>30
Acetylcholine (nicotinic) receptor	61851	0	ADG63462	17	21709	0	XP_002409079	>20
Dopamine receptor	48220	7.30E-36	AFC88981	6	6007	3.50E-87	XP_002408422	4
GABA receptor (metabotropic)	116021	0	XP_002406087	7	6166	1.30E-81	XP_002406087	8
Glutamate gated-ion (ionotropic) receptor	61197	0	XP_002415471	10	37672	4.60E-109	XP_002413279	5
Glutamate (Metabotropic) receptor	3710	5.60E-26	XP_002402308	>20	2300	9.80E-69	XP_002407136	>20
Neurotransmitter sodium symporter	113270	1.50E-94	XP_002415798	17	22130	4.10E-14	XP_002411089	21
Norepinephrine/ octopamine transporter	110311	0	XP_002434929	1	12941	0	XP_002434929	2
Octopamine/ Tyramine receptor	41296	1.40E-152	XP_002405020	1	6007	2.00E-169	XP_002408422	11
Serotonin	61315	0	XP_002405023	>20	17069	0	XP_0024050230	5

[1] Frequency indicates the number of transcripts recognizing identical annotations in Genbank.

3.5. Comparison of neuropeptides and receptors in *I. scapularis* and *O. turicata*

The true number neuropeptides in ticks is uncertain. Estimates have varied greatly, from as few as 20, characterized by proteomic methods (Neupert et al. 2008) to as high as 80, characterized by *in silico* searches of publicly accessible EST databases (Christie 2008). Hill et al. (Pers. Commun) identified genes for 56 putative neuropeptides, at least 41 of which are orthologs of genes present in insects and other invertebrates. Similarly, fifty one neuropeptides were reported to occur in another acarine, the spider mite *Tetranynchus urticae* (Veenstra et al. 2012).

Comparisons of the neuropeptides and/or their receptors in the two different tick species are shown in Table 3.5. Evidence supporting these annotations includes the low e-values (in most cases), sequence alignments and RT-qPCR gene expression (for 17 of the candidate neuropeptides or their receptors).

3.5.1. *I. scapularis* Transcriptome

Transcripts encoding for 15 neuropeptides and 15 neuropeptide receptors were recognized in the transcriptomes of the *I. scapularis* synganglion. We found transcripts encoding for allatotropin, allatostatin (A & B), bursicon α, corticotropin-releasing factor (CRF), FMRFamide, glycoprotein A, eclosion hormone, insulin-like peptide, ion transport peptide, myoinhibitory peptide, neuropeptide F, neurophysin/oxytocin, orcokinin A, SIFamide, and sulfakinin. We also found transcripts encoding for 15 different receptors, allatostatin, calcitonin, cardioacceleratory peptide, corazonin, CRF, eclosion hormone, insulin, gonadotropin-releasing hormone receptor, neuropeptide F, perisulfakinin, proctolin, pyrokinin (CAPA), SIFamide, sulfakinin and tachykinin. Although transcripts predicting peptides for eight of these neuropeptides were not

recognized, the finding of transcripts encoding for their receptors suggests that they are also expressed; these include calcitonin, CCAP, corazonin, gonadotropin releasing factor hormone, neuropeptide F, proctolin, pyrokinin (CAPA), and tachykinin. Thus, the total number of neuropeptides in the synganglion transcriptome is predicted to be 23. The actual number of mature peptides is likely even greater since in some cases several neuropeptides can be processed via post-translational modifications from a prepropeptide, e.g., the transcript encoding for allatostatin is processed further into allatostatin A, B and C; version also aligns with *I. scapularis* bombystatin. Seven more transcripts encoding for different neuropeptides were found in the *I. scapularis* synganglion transcriptome than were found in the *D. variabilis* synganglion transcriptome, namely, allatotropin, corticotropin-releasing factor (CRF), FMRFamide, myoinhibitory peptide, neurophysin-isotocin, neuropeptide F, and SIFamide (precursor). Similarly, although transcripts encoding for 4 neuropeptide receptors were found in the synganglion transcriptomes of both species (calcitonin receptor, gonadotropin-releasing hormone receptor, pyrokinin receptor, and sulfakinin receptor), we found transcripts encoding for 9 other neuropeptide receptors, i.e., allatostatin, corazonin, CRF, eclosion, insulin, perisulfakinin, proctolin, SIFamide, and tachykinin. These results greatly extend the number and variety of neuropeptides and/or their receptors in the synganglion of ticks.

Supporting evidence for these gene assignments in the *I. scapularis* synganglion is shown in the sequence alignments Appendix B [Is-1-23]. Comparing the transcripts from the transcriptomes versus the conspecific genes, we found 95.8% pairwise identity for allatostatin (Is-9), 100% pairwise identity for allatotropin (Is-1), 95.7% pairwise identity for glycoprotein A (Is-3), 99.2% pairwise identity for insulin-like peptide (Is-4), 69.7%

pairwise identity for myoinhibitory peptide (Is-15); 98.2% pairwise identity for insulin-like peptide receptor (Is-17); 51.2% pairwise identity for sulfakinin receptor (Is-18); 99.2% pairwise identity for the tachykinin receptor (Is-20); and 89.2% pairwise identity for orcokinin 5 (Is-7). For the transcript encoding for sulfakinin, the closest match, 78.3%, was with a similar neuropeptide in *D. variabilis* (Is-14). Although not a neuropeptide, another noteworthy finding was the occurrence of transcripts encoding for pro-protein convertase in the two Illumina transcriptomes (3 in each, respectively) similar to that found in *D. variabilis*; sequence alignment showed 100% pairwise identity with the conspecific gene (Is-8). Proprotein convertase is essential for the conversion of neuropeptide hormones to the mature form and their subsequent secretions (Wegener et al. 2011) as it performs the function of removing the signal peptide by endoproteolytic cleavage. We did not find a transcript encoding for periviscerokinin, previously identified only by MALDI-TOF mass spectrometry (Neupert et al. 2009). Although we did not find transcripts encoding for the peptides calcitonin, corazonin, gonadotropin-releasing hormone and pyrokinin, neuropeptides reported to occur in the *D. variabilis* synganglion (Donohue et al. 2010), we did find transcripts encoding for their receptors, strongly suggesting that the messages for these peptides are also expressed (Is-13, calcitonin receptor, 95.8% pairwise sequence alignment with conspecific gene; Is-12 corazonin receptor, 100% pairwise identity with the conspecific gene; Is-19, gonadotropin-releasing hormone receptor, 68.1% pairwise sequence alignment with the conspecific gene; and Is-10, pyrokinin receptor, 62.2%, pairwise sequence alignment with the conspecific gene; respectively). In addition, we found transcripts encoding for bursicon α in *I. scapularis* (Is-5, 59.5%) and glycoprotein A, but no evidence of

transcripts encoding for bursicon β or glycoprotein B, in contrast to the *D. variabilis* synganglion where both were present. Transcripts encoding for orcokinin 5, corticotropin-releasing factor (CRF) receptor, CRF-binding protein, eclosion hormone, and FMRFamide were also found in the *I. scapularis* synganglion transcriptome (Is-2, CRF-binding peptide, 100% pairwise sequence alignment; Is-6, eclosion hormone, 97.7% pairwise identity; Is-11, FMRFamide, 62% pairwise identity; Is-16, SIFamide receptor, 100% identity; and neuropeptide F, Is-21, 100% pairwise identity; cardioacceleratory peptide receptor, Is-22, >90% pairwise identity in coding region; and ion transport peptide Is-23, 77.3% pairwise identity). Although no receptor was found for CAPA (it was found in *O. turicata*, see below), it is likely that the pyrokinin receptor is also homologous with the CAPA receptor, since CAPA has been reported to be activated by pyrokinin peptides in anopheline mosquitoes (Olsen et al. 2007). Whether this is true in ticks requires further study.

Analysis of these alignments indicates that 17 out of 23 transcripts had greater than 75% pairwise matching alignments. In addition, 7-transmembrane domains were also identified in many of the receptors, including pyrokinin, sulfakinin, and tachykinin receptors, which had lower % alignments. Finally, the gene expression studies (qPCR results shown in section 3.9) show that the primers recognized these genes in fresh synganglion extracts. In summary, the low e-values, matching sequence alignments and qPCR expression results support the functional assignment (annotations) for these genes presented in this report.

3.5.2. *O. turicata* Transcriptome

Transcripts predicting genes for at least 25 neuropeptides, neuropeptide transporters and/or their receptors were found in the *O. turicata* synganglion transcriptome. These include transcripts encoding for the peptides for allatostatin (including allatostatin B and allatostatin C), bursicon α, bursicon β, diuretic hormone (calcitonin-like peptide), preprocorazonin, corticotropin-releasing factor, crustacean cardioacceleratory peptide, FMRF amide, eclosion hormone, glycoprotein A, glycoprotein B, insulin-like peptide, ion transport peptide, myoinhibitory peptide, neuropeptide F, neurophysin-oxytocin transporter, orcokinin A precursor, proctolin, SIF amide and sulfakinin. In addition, we also found transcripts encoding the receptors for calcitonin/diuretic hormone, CAPA/pyrokinin, thyrotropin and tachykinin, suggesting the occurrence of transcripts predicting the peptides for the same neuropeptides. Together, these findings indicate the expression of 25 different neuropeptides and/or transporters in the synganglion of *O. turicata* during feeding. Supporting evidence for these gene assignments in the *O. turicata* synganglion is shown in the sequence alignments in Appendix B (Ot 1 –22). Comparing the transcripts from the *O. turicata* transcriptome versus the conspecific genes, we found 46% pairwise identity for preproallatostatin C versus *N. vitripennis*, the closest match, when aligning the coding region (Ot-2), 57% pairwise identity for the allatostatin receptor, including 44.2% multiple sequence identity when compared with both *I. scapularis* and the cockroach, *Periplaneta americanum*, as well as recognition of the characteristic transmembrane domain (Ot-2); 25% pairwise identity for cardioacceleratory peptide (CCAP) but with the characteristic peptide domain (Ot-5), 65.7% pairwise identity for the pyrokinin/CAPA receptor when aligned with *D. variabilis*

(CAPA was not present in the *I. scapularis* transcriptome) (Ot-6); 29.2% pairwise identity for the preprocorazonin peptide (Ot-9) but as high as 72% pairwise identity for the corazonin receptor (within open reading frame) (Ot-10; 60% pairwise identity for corticotropin releasing factor (CRF) (Ot-1185.7% pairwise identity for bursicon alpha peptide, which also showed significant alignment with *D, variabilis* and evidence of the characteristic peptide domain (Ot-12); 37% pairwise identity for neuropeptide F (Ot-16); 68% pairwise identity for sulfakinin receptor (Ot-20); 48.4% pairwise identity for bursicon beta, including 35.8% multiple alignment between the *O. turicata* transcript versus *D. variabilis* and *Tribolium castaneum* (Ot-13); 43.3% pairwise identity for the tachykinin receptor (coding region); and 92.2% pairwise identity for the SIFamide receptor (coding region) (Ot-22).

Analysis of these alignments indicates that almost all of these neuropeptide or neuropeptide receptor transcripts analyzed to date had much lower percent identities than what was found for *I. scapularis*; exceptions include alignments for bursicon alpha and SIFamide. This is not surprising since, in most cases, alignments of the *I. scapularis* synganglion transcripts showed high percent identities when aligned with conspecific gene sequences from the *I. scapularis* genome. This was not the case for *O. turicata.* Nevertheless, the presence of the characteristic peptide domain in several of the neuropeptide sequences and the 7-transmembrane domains in the neuropeptide receptors provide additional evidence in support of the gene annotations. Finally, as noted previously for *I. scapularis,* the gene expression studies (RT-qPCR results shown in section 3.10) show that the primers recognized these genes in fresh synganglion extracts.

These findings, along with the low e-values provide support that *O. turicata* transcripts encode for the neuropeptides and neuropeptide receptors described in this report.

3.6. Comparison of neurotransmitter receptors in *I. scapularis* and *O. turicata.*

In ticks as well as other invertebrates, at least 4 different neurotransmitters have been described, namely, acetylcholine (ACh), gamma-aminobutyric acid (GABA), octopamine, and glutamate. In addition, several neuromodulators, i.e., regulate neuronal function, specifically dopamine and serotonin (Osborne, 1996; Egekwu et al 2014).

3.6.1. *I. scapularis* Transcriptome

Transcripts encoding the 4 neurotransmitter receptors and 2 neuromodulators noted above were recognized, namely, ACh, GABA, dopamine, glutamate, octopamine, and serotonin. Transcripts encoding for both muscarinic and nicotinic type acetylcholine receptors were recognized. In addition, transcripts encoding for 2 types of GABA receptors (ion-channel and metabotropic) and 3 different types of glutamate receptors (ionotropic, metabotropic and NMDA) were recognized. Finally, transcripts encoding the enzyme acetylcholinesterase, which degrades acetylcholine, also were recognized. Sequence alignments supporting the functional assignments of these transcripts are shown in Appendix B (Is-24 – 31).

Ach is widely regarded as one of the principal excitatory neurotransmitters (Chapman, 1998) in most eukaryotic animals. Its function is regulated by AChE. ACh and AChE are known to occur in ticks (Lees et al. 2010; Bissinger et al. 2011; Temeyer et al. 2012), including both muscarinic and nicotinic receptors (Is-24). In contrast, GABA, mediates inhibitory synaptic transmission via its ionotropic (GABA-A) and metabotropic receptors (GABA-B) (Is-26) Buckingham et al. 2005).

The transcriptomes of the *I. scapularis* synganglion revealed transcripts encoding for both ionotropic and metabotropic GABA receptors. Transcripts encoding for glutamate receptors were the most abundant (49.4%) of all the neurotransmitter receptors found in the *I. scapularis* synganglion transcriptomes encoding for all three types of glutamate receptors (NDMA, ionotropic and metabotropic) were identified in the transcriptomes of the *I. scapularis* synganglion. In addition, numerous transcripts encoding for glutamate synthase were identified (not shown in Table 6). Transcripts encoding for the neurotransmitter receptors for the monamines dopamine, octopamine and serotonin were also very numerous in the *I. scapularis* synganglion (Table 6). Here we report 11 transcripts encoding for dopamine receptors (Is-25), 10 transcripts encoding for octopamine receptors (Is-30) but only 1 transcript encoding for a serotonin receptor (see Is-31). We also report numerous transcripts predicting receptors for a Na+-transmitter symporter, proteins that transport cations into cells innervated by dopaminergic and/or serotoninergic neurons. Sequence alignments for these *I. scapularis* neurotransmitter receptors ranged from 85% to 99.5% identity (pairwise). Thus, the low e-values, gene expression results (qPCR data shown in the next section) and high percent identities for the sequence alignments support the functional assignment (annotations) for these genes presented in this report.

3.6.2. *O. turicata* Transcriptome

Similar to the findings for *I. scapularis,* transcripts encoding for the same neurotransmitter receptors and associated transporters were found in the transcriptome of the *O. turicata* synganglion (Table 6). Numerous transcripts encoding for both muscarinic and nicotinic acetylcholine receptors were recognized. Similarly, 6

transcripts encoding for a dopamine receptor were recognized. Transcripts encoding for metabotropic GABA receptors (7) 2 types of glutamate receptors (10 ionotropic and>20 for metabotropic). In addition, 10 transcripts were recognized for the NMDA type of glutamate receptor (not shown in the table). Transcripts for octopamine (1) and serotonin (>20) also were identified in the transcriptome. Finally, transcripts encoding for the transporters, neurotransmitter sodium symporter (17) and the norepinephrine/octopamine transporter (1) also were identified. Although the low e-values for these neurotransmitter receptors and transporters support the functional assignments of the transcripts encoding for these genes, the percent identities for the sequence alignments were not as high as they were for *I. scapularis*. It is likely that this reflects the absence of the conspecific genome and relatively few published sequences for other species of soft ticks in Genbank. However, gene expression studies (qPCR results) shown in the next section provide additional support for the expression of these neurotransmitter receptors in the *O. turicata* synganglion.

Sequence alignments supporting the annotations of these neurotransmitter receptors in *O. turicata* are presented in Appendix B.

Alignments: Neuropeptides, Receptors, and Neurotransmitter Receptors.

Alignments are shown comparing (see Appendix B) the putative annotated neuropeptides, neuropeptide receptors and neurotransmitter receptors (contigs) from the transcriptomes versus the corresponding sequence from Genbank. In most cases, alignments are limited to the coding region since most contigs do not represent the full-length message for each of these molecules.

3.7. Comparative gene expression between *I. scapularis* and *O. turicata* female synganglia for selected neuropeptides, neuropeptide receptors and neurotransmitter receptors.

The results of these studies for *I. scapularis* and *O. turicata* are compared in relation to purported functional categories. Data for gene expression are presented as the fold change along with the standard error of the mean (SEM) and probability (P) value based on the student's *t*-test. The fold values were calculated according to method of Livak and Schmittgen (2001); SEM and P values were calculated by student's *t* – test according to method of Yuan et al (2006), the results of these assays are shown in Table 7. Selected examples of RT-qPCR assays are illustrated in section 3.10.

Neuropeptides and neuropeptide receptors: qPCR assays were done for 19 of the 23 neuropeptides and/or neuropeptide receptors recognized in *I. scapularis,* namely, allatostatin A, allatostatin receptor, allatostatin B (myoinhibitory peptide), bursicon α, calcitonin, CAPA/pyrokinin receptor, cardioacceleratory peptide receptor, corazonin receptor, corticotropin releasing hormone receptor, diuretic hormone, eclosion hormone, FMRFamide, neuropeptide F, glycoprotein A, gonadotropin releasing hormone, SIFamide, preprosulfakinin, sulfakinin receptor and tachykinin receptor. Primers designed from contigs in the *O. turicata* transcriptome with the same functional assignments (annotations) amplified transcripts predicting most of the same neuropeptides or neuropeptide receptors in total RNA samples from *O. turicata* female synganglia. This made it possible to carry out qPCR assays for the same neuropeptides and/or neuropeptide receptors in *O. turicata* that were also done in *I. scapularis,* and compare the similarities and differences. Exceptions include allatostatin B (myoinhibitory peptide), cardioacceleratory protein and glycoprotein A. Transcripts

predicting these genes in *O. turicata* showed poor alignments and primers designed from these transcripts failed to amplify the molecules. However, bursicon β, absent in *I. scapularis,* was amplified in samples from *O. turicata.* Thus, I was able to assay reverse transcriptase products from *O. turicata* transcripts for 16 of the same neuropeptides and/or neuropeptide receptors as found in *I. scapularis*, thereby facilitating direct comparisons of the responses of these genes to blood feeding in the two different species.

To facilitate comparison of the responses of the predicted neuropeptides and neuropeptide receptors, the results of these studies are arranged in accordance with their functional roles, as reported in the literature for insects and other arthropods (Simo et al. 2009a; Simo et al. 2011; Simo et al. 2014)

For salivary glands, the neurotransmitter receptor dopamine and two neuropeptides, myoinhibitory peptide (= Allatostatin B) and SIFamide have been reported to regulate gland functions (Simo et al. 2009b). In *I. scapularis,* all three of these genes were upregulated, consistent with their reported roles in regulating salivary secretion in this species. In *O. turicata,* however, although both dopamine receptor and SIFamide peptide were highly significantly upregulated, but myoinhibitory peptide was not amplified. However the difference was not statistically significant, i.e., the difference was essentially unchanged. Possible explanations of these differences between the two species will be considered in the Discussion section.

For diuresis (water balance regulation), 5 predicted neuropeptides and/or neuropeptide receptors identified previously in this report (Table 5) were assayed. In *I. scapularis,* four of these 5 genes were assayed; diuretic hormone was omitted in this species, since excess water and salts are secreted via the salivary glands, believed regulated by

myoinhibitory peptides and SIFamides as noted in the Discussion section. Calcitonin hormone, a more specialized variety of diuretic hormone (Zandawala et al. 2013), corticotropin releasing factor, and CAPA/pyrokinin receptor were upregulated (calcitonin and CAPA/pyrokinin were highly significantly increased), whereas tachykinin was slightly downregulated, essentially unchanged. In *O. turicata,* in which water/salt elimination occurs during or immediately after rapid feeding, calcitonin, diuretic hormone receptor and CAPA/pyrokinin were upregulated. Diuretic hormone is the less specialized neuropeptide, so its strong upregulation is consistent with the rapid elimination of excess water via the coxal glands in this species. Corticotropin releasing factor hormone and tachykinin receptor showed little change in *O. turicata.* The differences in expression of these genes in regulating water, salt, and other wastes in the two different species will be discussed in more detail in the Discussion section.

For regulation of feeding volume (excluding satiety, which is considered separately), 4 neuropeptide and/or neuropeptide receptors were assayed, FMRFamide, neuropeptide F receptor, sulfakinin receptor and tachykinin receptor. In *I. scapularis,* the first 3 genes were upregulated, FMRFamide rather slightly but significantly ($P<0.001$), versus Neuropeptide F receptor and sulfakinin strongly which were strongly (and significantly) upregulated ($P<0.05$ and $P<0.04$, respectively). Tachykinin receptor was downregulated slightly, i.e., essentially unchanged. In *O. turicata,* FMRFamide was strongly upregulated, ($P<0.001$), whereas neuropeptide F and sulfakinin receptor were significantly downregulated ($P<0.03$ and $P<0.02$, respectively). However, tachykinin receptor was upregulated. The significance of these differences in gene expression in

relation to the different blood feeding behaviors in *I. scapularis* and *O. turicata* will be discussed in more detail in the Discussion section.

Satiety function is regulated by sulfakinin. Major differences in sulfakinin peptide expression was observed in the two different tick species, with strong upregulation observed in *I. scapularis* (4.03 fold) consistent with their slow feeding pattern. In *O. turicata,* both sulfakinin peptide and sulfakinin receptor were strongly downregulated (0.47 and 0.66), indicating lack of impediment to rapid blood feeding characteristic of the latter species. The significance of these differences in relation to differences in their blood-feeding habits will be elaborated in the Discussion section.

For regulation of ecdysis, 6 different neuropeptides were assayed; eclosion hormone, preprocorazonin, corazonin receptor, cardioacceleratory peptide (CCAP), ecdysis triggering hormone (ETH), and CAPA/pyrokinin receptor. In *I.scapularis,* eclosion hormone and ecdysis triggering hormone were downregulated significantly, but CAPA/pyrokinin was strongly upregulated (highly significant); corazonin receptor showed weak upregulation, while cardioacceleratory peptide was downregulated, but the fold changes in the expression of these two genes were not significant. In contrast, in *O. turicata*, eclosion hormone, corazonin peptide (=preprocorazonin), ecdysis triggering hormone and CAPA/pyrokinin were all significantly upregulated; corazonin receptor was downregulated. Attempts to assay cardioacceleratory peptide were unsuccessful (primers designed from the annotated contig failed to amplify this gene).

For cuticle synthesis and plasticization, three different neuropeptides were assayed, bursicon α, bursicon β and eclosion hormone. In *I. scapularis,* bursicon α was upregulated significantly, but eclosion hormone was relatively unchanged (although

downregulated, the change was not significant). Bursicon β was not assayed since there was no evidence of this neuropeptide in *I. scapularis* (see Table 3.5). In *O. turicata,* all 3 genes, bursicon α, bursicon β and eclosion hormone were significantly upregulated. The significance of the differences in expression of these genes (as well as their presence or absence) in *I.scapularis* versus *O.turicata* in relation to cuticle expansion during feeding will be discussed later (see Discussion section).

For reproduction, 8 different neuropeptides were assayed, namely, allatostatin A peptide, allatostatin A receptor, myoinhibitory peptide (also known as allatostatin B), gonadotropin releasing hormone, CAPA/pyrokinin (also described as pheromone biosynthesis activating hormone), SIFamide peptide, SIFamide receptor and glycoprotein A. In *I. scapularis,* 4 genes, allatostatin A peptide, allatostatin A receptor, myoinhibitory peptide and CAPA/pyrokinin (= PBAN) were significantly upregulated during the lengthy pre-mating blood feeding period whereas gonadotropin releasing hormone was significantly downregulated; SIFamide peptide, SIFamide receptor and glycoprotein A expression showed little change (not significant). In *O. turicata,* allatostatin A was significantly upregulated but allatostatin A receptor expression was not significantly changed; gonadotropin releasing hormone, SIFamide peptide, SIFamide receptor, and CAPA/pyrokinin were also significantly upregulated. Attempts to amplify myoinhibitory peptide and glycoprotein A were unsuccessful.

Neurotransmitter receptors: qPCR assays were done for 5 of 8 the neurotransmitter receptors recognized in both species, namely, metabotropic and ionotropic glutamate, octopamine, serotonin and dopamine. Primers designed for insulin-like peptide, ion transport peptide and GABA (metabotropic) failed to amplify those

genes in *O. turicata.* In *I. scapularis*, ion transport peptide, GABA, ionotropic glutamate, serotonin, and dopamine were upregulated whereas metabotropic glutamate and octopamine receptors were down regulated. The response for ionototropic glutamate was exceptionally high (fold = 39.95). In *O. turicata,* dopamine receptor was significantly upregulated; ionotropic glutamate and serotonin receptors also were upregulated slightly but these changes were not significant, i.e., they were essentially unchanged. Metabotropic glutamate receptor was downregulated in this species, opposite to what was observed with the same gene in *I. scapularis.* Octopamine receptor was downregulated in *O. turicata,* consistent with what was found in *I. scapularis.* Possible explanations and insights into these differences and in the roles of these neurotransmitter receptors will be considered in the Discussion section.

Table 7. Comparison of gene expression in *Ixodes scapularis* versus *Ornithodoros turicata* as determined by real time quantitative PCR (qPCR). Organized by functional categories.

Functional Category	**Genes expressed**	***I. scapularis*** **(qPCR unfed vs fed) Fold ± SEM & P value[1]**	***O. turicata*** **(qPCR unfed vs fed) Fold ± SEM & P value[1]**
Salivary gland functions	Dopamine	4.82, ± 0.69, T = 0.006, P<0.01	1.87 ±0.24, T=0.0026, P<0.003
	Allatostatin B (= Myoinhibitory peptide)	7.95 ± 0.41, T = 0.019, P<0.02	ND
	SIFamide peptide	1.41 ± 0.45, T = 0.452, p>0.05 NS	3.89, 0.42, T=1.72×10^{-7}, P<0.001
Diuresis	Calcitonin (diuretic hormone) –fluid secretion [collected immediately after blood feeding].	8.77 ± 2.25, T = 0.0004, P<0.001	5.30 ± 0.86, T = 4.65×10^{-5}, P<0.001
Eliminate excess blood meal water	Diuretic hormone (DH) receptor (regulates fluid secretion) [Collected 24 h after feeding].	ND	3.72 ± 0.08, T = 4.72×10^{-7}, P<0.001
	Corticotropin releasing factor (CRF-DH) – eliminates waste from Malpighian tubule (MT).	2.94 ± 0.88, T=0.001, P<0.01	1.44 ± 0.08, T = 0.045. P<0.05
	Tachykinin receptor	0.88 ± 0.25, T = 5.54×10^{-11}, P>0.001	1.44 ± 0.59, T = 0.354, P>0.05 NS
	CAPA/Pyrokinin receptor (periviscerokinin)	2.36 ± 0.65, T = 4.84×10^{-6}, P<0.001	1.59 ± 0.41, T = 1.11×10^{-11}, P<0.001
Feeding volume	FMRFamide-regulates gut muscle contractions.	1.19 ± 0.11, T = 8.33×10^{-9}, P<0.001	3.56 ± 0.56, T = 1.63×10^{-5}, P>0.001
	Neuropeptide F receptor –stimulates feeding.	38.32 ± 0.359, T = 0.014, P<0.02	0.56 ± 0.07, T = 0.03, P<0.05
	Sulfakinin receptor –down-regulation allows >blood volume.	11.96 ± 0.906, T= 0.036, P<0.04	0.66 ± 0.07, T = 5.9×10^{-5}, P<0.001
	Tachykinin receptor ---stimulates gut contractions.	0.88 ± 0.25, T = 0.364, P>0.05 NS	1.44 ± 0.59, T = 0.354, P>0.05 NS

Table 7 continued.

Functional Category	Genes expressed	*I. scapularis* (qPCR unfed vs fed) Fold ± SEM & P value[1]	*O. turicata* (qPCR unfed vs fed) Fold ± SEM & P value[1]
Feeding satiety	Preprosulfakinin –inhibit further feeding (reaches repletion).	4.03 ± 0.25, T = 9.76×10^{-7}, P<0.001	0.47 ± 0.28, T = 0.019, P<0.001
Ecdysis (molting)	Eclosion hormone - presumed regulates ecdysis behavior.	0.32 ± 0.11, T = 0.002, P<0.02	1.96 ± 0.69, T = 0.02, P<0.02
	Prepro-corazonin peptide -regulate release of ecdysis triggering hormone (ETH).	Absent	1.78 ± 1.10, T = 0.01, P<0.01
	Corazonin receptor – receptor for ETH.	1.33 ± 0.61, T = 1.22×10^{-8}, P<0.001	1.01, ±0.77, T=0.246, P>0.05 NS
	Cardioacceleratory peptide (CCAP) –regulates heart rate/ecdysis.	0.97 ± 0.92, T = 0.148, P>0.05 NS	ND
	Ecdysis triggering hormone (ETH) [24 h after feeding].	0.05 ± 0.63, T = 7.5×10^{-7}, P<0.001	6.89 ± 0.33, T = 0.002, P<0.01
	CAPA/Pyrokinin receptor (periviscerokinin)	2.36 ± 0.65, T=4.84×10^{-6}, P<0.001	1.59 ± 0.41, T = 1.11×10^{-11}, P<0.001
Cuticle synthesis/ Plasticization	Bursicon α –insect molting hormone; regulate plasticization.	3.32 ± 1.5, T = 0.001, P<0.002	3.09 ± 0.14, T = 0.02, P<0.05
	Bursicon β – role in plasticization.	Absent	2.89 ± 0.14, T = 0.05m, P<0.05
	Eclosion hormone	0.32 ± 0.11, T = 0.016, P<0.02	1.96 ± 0.69, T = 0.02, P<0.03

Table 7 continued.

Functional Category	Genes expressed	*I. scapularis* (qPCR unfed vs fed) Fold ± SEM & P value[1]	*O. turicata* (qPCR unfed vs fed) Fold ± SEM & P value[1]
Reproduction & Development	Allatostatin A peptide–stimulates JH synthesis (ticks lac±k JH).	6.24 ± 1.55, T = 0.007, P<0.001	6.45 ± 0.33, T = 6.30×10^{-6}, P<0.001
	Allatostatin receptor ----inhibits JH synthesis (ticks lack JH)	21.16 ± 0.31, T = 0.007, P<0.001	0.26 ± 0.70, T = 0.480, P>0.05 NS
	Myoinhibitory peptide (=Allatostatin B) inhibits JH synthesis	7.95 ± 0.71, T=0.035, P<0.02,	ND
	Gonadotropin-releasing hormone receptor (GnRH)	0.39 ± 0.89, T = 7.52×10^{-4}, P<0.001	2.37 1.42, T = 0.028, P<0.05
	CAPA/Pyrokinin (PBAN)—possible pheromone biosynthesis	2.36 ± 0.65, T = 4.84×10^{-6}, P<0.001	1.59 ± 0.41, T = 1.11×10^{-11}, P<0.001
	SIFamide peptide –regulates reproductive behavior	0.18 ± 0.05, T = 0.001, P<0.002	3.89 ± 0.28, T = 1.72×10^{-7}, P<0.001
	SIFamide receptor—regulates reproductive behavior	1.47 ± 0.41, T = 0.452, P>0.05 NS	2.17 ± 0.41, T = 0.008, P<0.01
	Glycoprotein A – presumed regulates reproductive behavior	0.03 ± 2.34, T = 0.05, P≤0.05 NS	Absent

Table 7 continued.

Functional Category	Genes expressed	*I. scapularis* (qPCR unfed vs fed) Fold ± SEM & P value[1]	*O. turicata* (qPCR unfed vs fed) Fold ± SEM & P value[1]
Signaling (Neurotransmitter receptors)	Glutamate (Ionotropic) receptor -major excitatory synaptic transmitter.	0.76 ± 0.188, T = 0.004, P<0.01	0.205 ± 0.186, T= 6.2×10^{-6}, P<0.001
	Glutamate (Metabotropic) receptor -major excitatory synaptic transmitter.	39.95 ± 1.76, T = 3×10^{-5}, P<0.001	4.76±0.25(<1h), T=5.8×10^{-6}, P<0.001 1.76 ± 0.17 (>24h), T=0.349, P>0.05 NS
	Gamma-aminobutyric acid (GABA) receptor—major inhibitor synaptic transmission.	1.93 ± 0.36, T = 0.03, P<0.05	1.20 ± 0.721, T=0.199, P>0.05 NS
	Octopamine/tyramine receptor	0.18 ± 0.05, T = 0.001, P<0.002	0.34 ± 0.07, T = 4.1×10^{-6}, P<0.001
	Serotonin receptor– salivary gland secretion and other functions.	1.43 ±0.53, T= 0.316, P>0.05 NS	1.34 ± 0.21, T = 0.355, P>0.05, NS
	Dopamine receptor	4.83 ± 0.61, T = 0.006, P<0.01	1.59 ± 0.41, T = 1.11×10^{-11}, P<0.001

[1]SEM, T-test and P values were calculated according to the methods from Yuan et al. 2006
ND: Assay not done because primers did not amplify target.
Absent: Transcript was not found in the transcriptomes library generated.

Gene expression

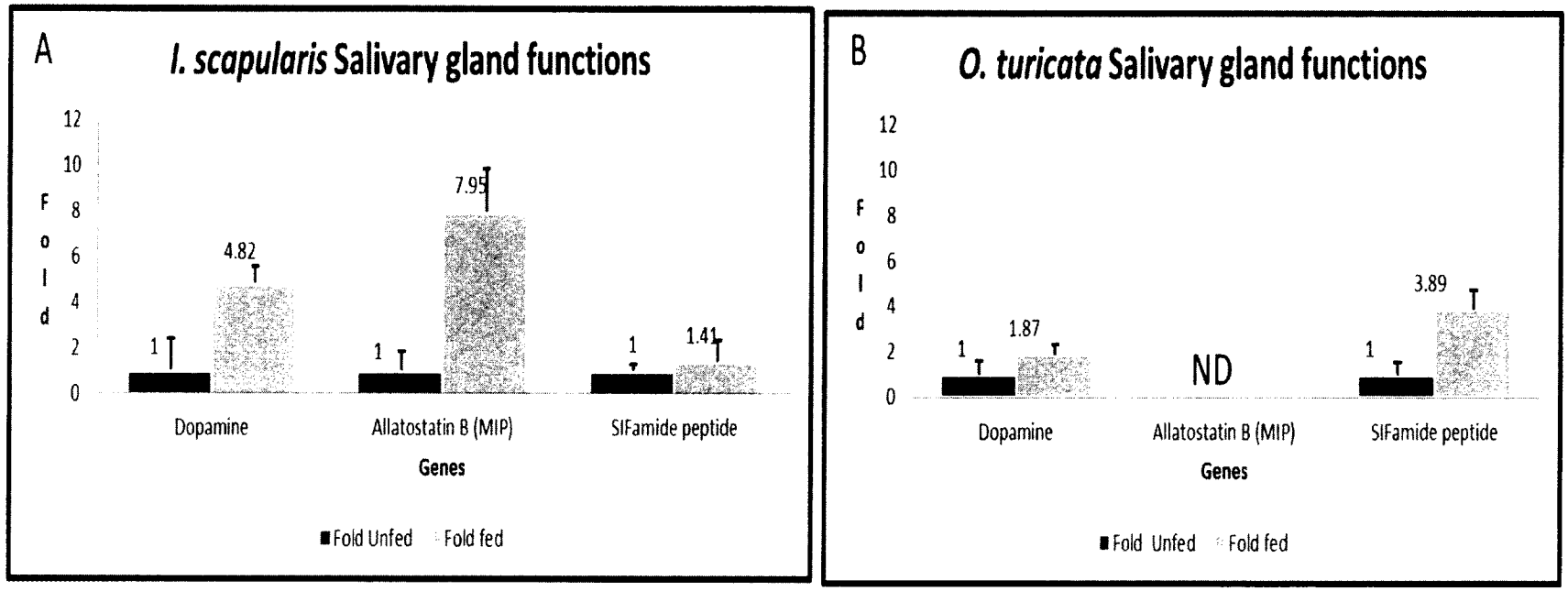

Figure 10. Comparison of salivary gene expression in the tick synganglia in response to blood feeding. **A**) Expression of three salivary gland regulating genes in *Ixodes scapularis* part-fed females. Dopamine and allatostatin B (Myoinhibitory peptide) are strongly (significantly) expressed. Bursicon beta is absent and eclosion hormone is strongly (significantly) down-regulated. **B)** Expression of two salivary gland regulating genes in *Ornithodoros turicata* engorged females. Dopamine and SIFamide peptide were strongly (significantly expressed. Myoinhibitory peptide was not found in the *I. scapularis* transcriptome. ND=Not detected or not assayed as a result no detection by primers.

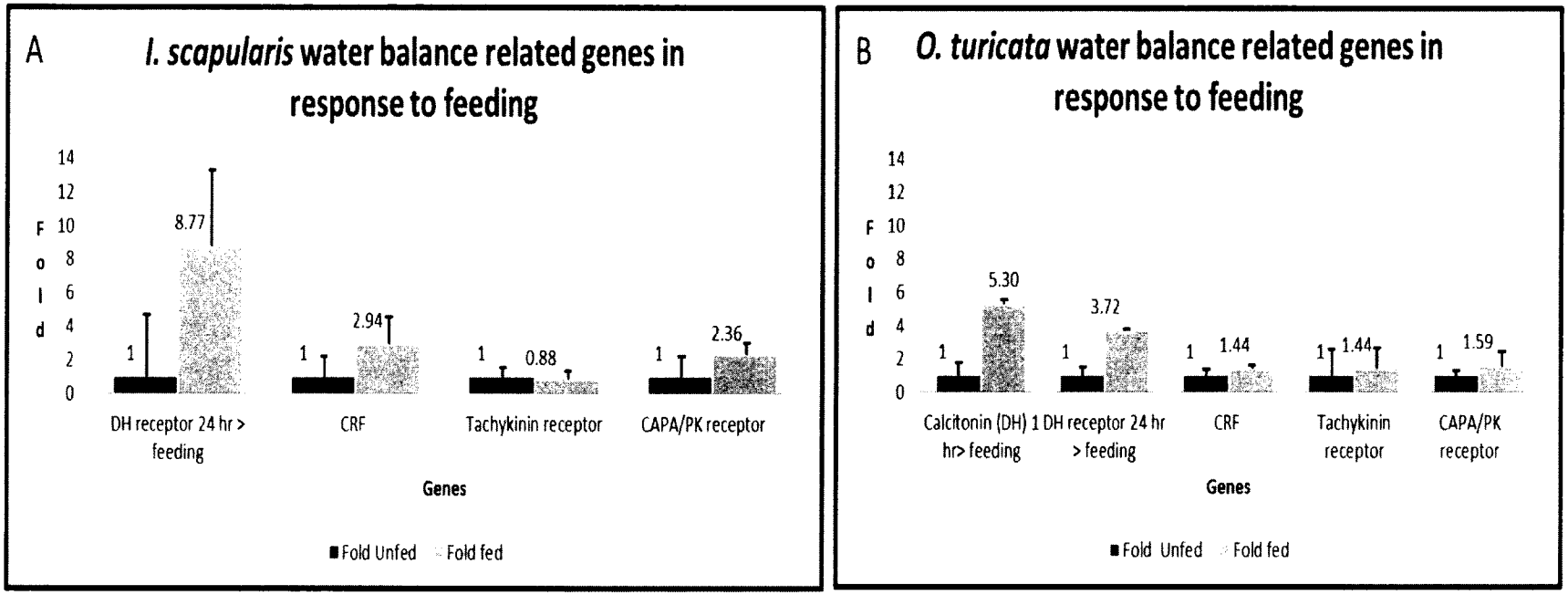

Figure 11. Comparison of water balance-related gene expression in the tick synganglia in response to blood feeding. **A**) Expression of four water balance regulating genes in *Ixodes scapularis* part-fed females. Calrecticulin/diuresis hormone (Cal/DH), CAPA receptor and corticotropin releasing factor (CRF) hormone are strongly (significantly) expressed. However, tachykinin receptor was essentially unchanged. **B)** Expression of four water-balance regulating genes in *Ornithodoros turicata* engorged females. Cal/DH was very strongly (significantly) expressed immediately after feeding, and remained significantly expressed for at least 24 h after feeding. Similarly, both CAPA receptor and CRF hormone genes were significantly expressed immediately after feeding although not as strongly as CAL/DH. Tachykinin receptor was slightly upregulated, but this change was not significant.

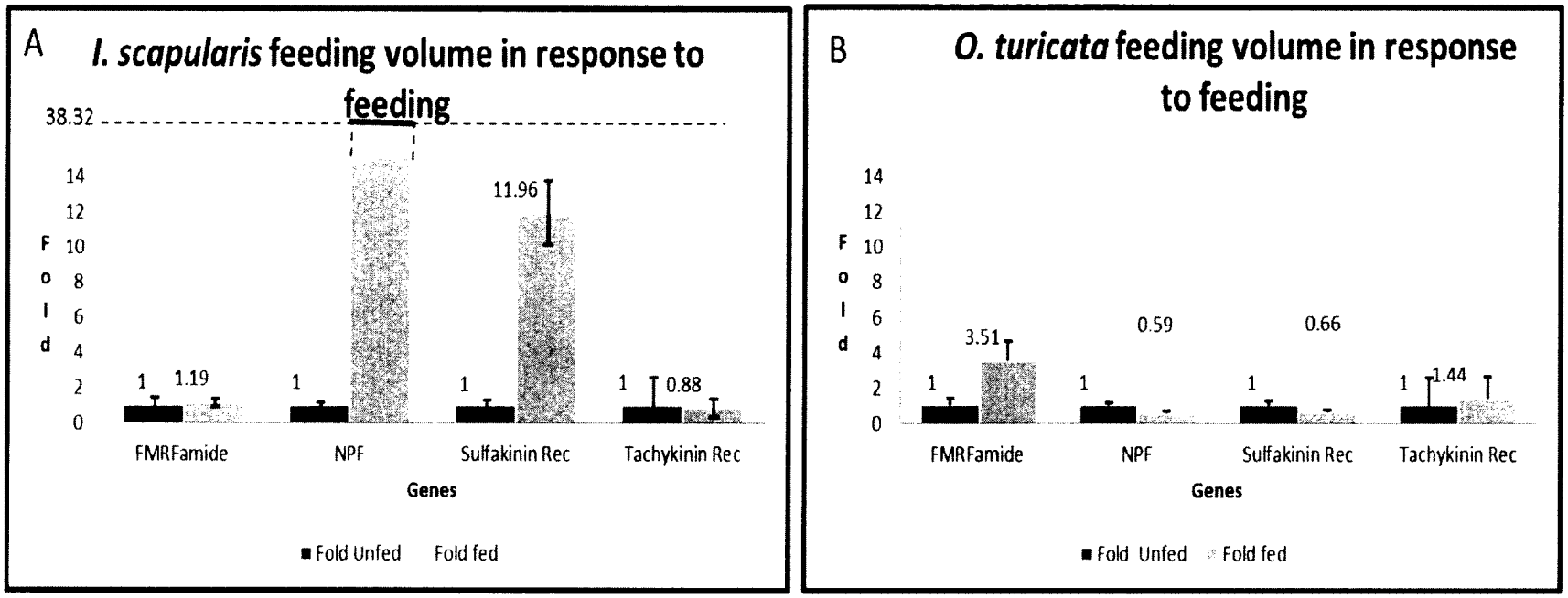

Figure 12. Comparison of feeding volume-related gene expression in the tick synganglia in response to blood feeding. **A**) Expression of blood volume regulating genes in *Ixodes scapularis* part-fed females. FMRFamide receptor is slightly but significantly upregulated while neuropeptide F receptor (NPF rec) and sulfakinin receptor genes are strongly (significantly) upregulated. However, tachykinin receptor was essentially unchanged. Upregulation of satiety factor sulfakinin is believed to prevent rapid increase in blood volume, consistent with slow feeding. **B)** Expression of four blood volume regulating genes in *Ornithodoros turicata* engorged females. FMRFamide was strongly (significantly) upregulated while neuropeptide F(NPF rec) and sulfakinin receptors were downregulated (significantly). In contrast, Tachykinin receptor was slightly upregulated, but this change was not significant. Downregulation of the satiety factor sulfakinin is believed to allow unrestricted blood volume uptake, consistent with rapid feeding in this species.

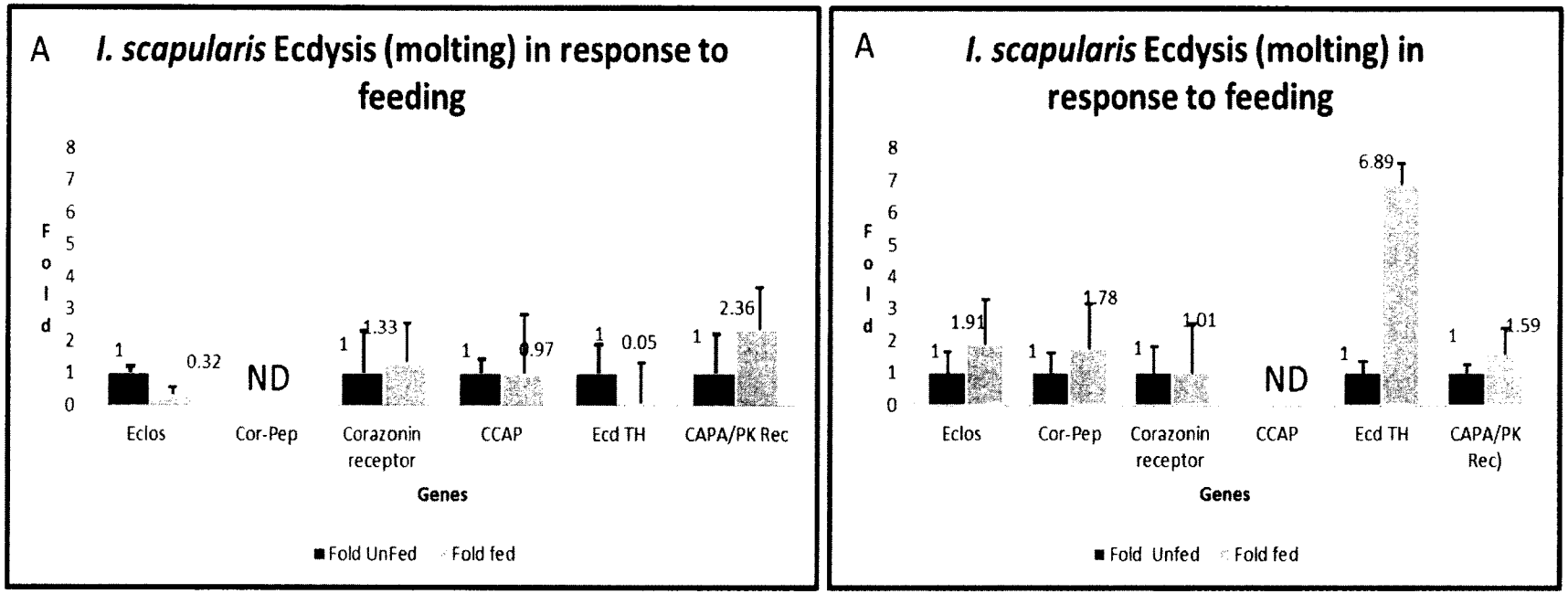

Figure 13. Comparison of ecdysis (molting) gene expression in response to blood feeding. **A**) Expression of five ecdysis regulating genes in *Ixodes scapularis* part-fed females. Eclosion hormone receptor (Eclos) and ecdysis triggering hormone peptides (Ecd TH) are significantly downregulated. Corazonin peptide (Cor-Pep) was not expressed in this species. Corazonin receptor and CCAP receptor (crustacean cardioacceleratory peptide receptor) showed small but ignificant changes. However, CAPA/PK receptor (cardioactive peptide receptor) was strongly upregulated. **B)** Expression of five ecdysis regulating genes in *Ornithodoros turicata* engorged females. Eclosion hormone, pre-corazonin (corazonin peptide), ecdysis triggering hormone and CAPA/PK receptor were strongly (significantly) upregulated. ND=Not detected or not assayed as a result no detection by primers.

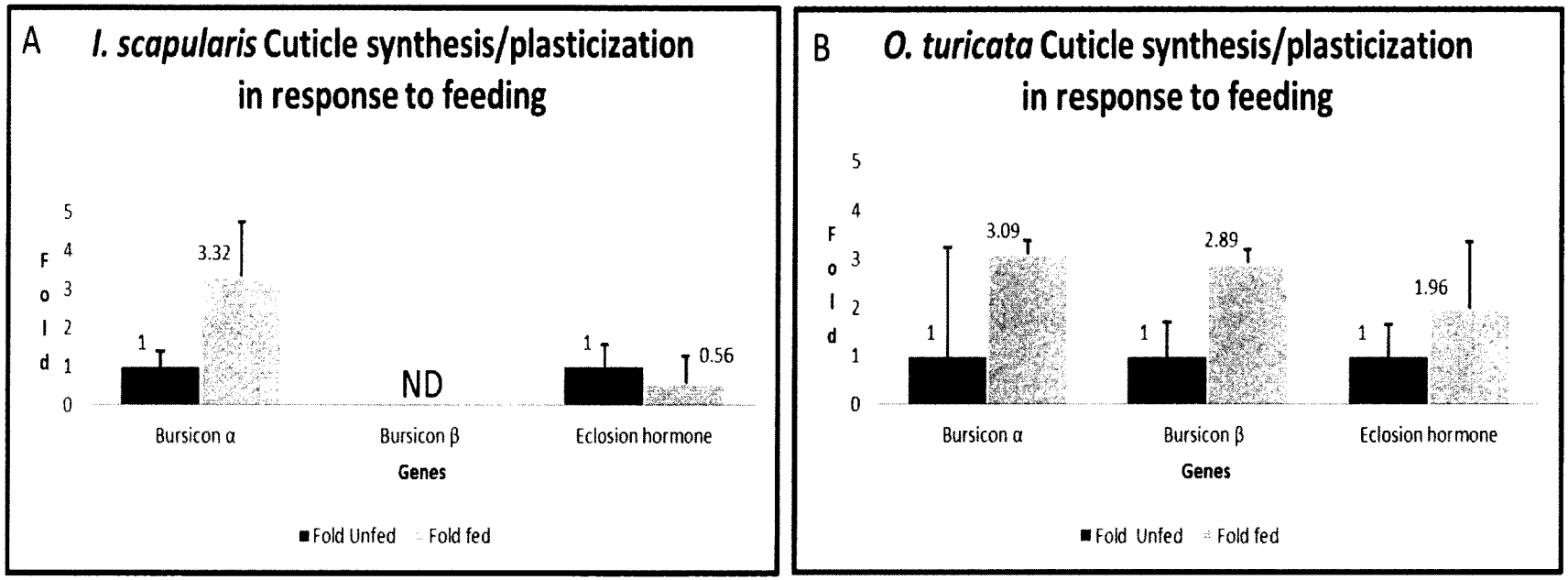

Figure 14. Comparison of cuticle synthesis gene expression in the tick synganglia in response to blood feeding. **A)** Expression of two cuticle synthesis-related genes in *Ixodes scapularis* part-fed females. Bursicon alpha is strongly (significantly) expressed, Bursicon beta is absent and eclosion hormone is strongly (significantly) down-regulated. **B)** Expression of three cuticle synthesis-related genes in *Ornithodoros turicata* engorged females. Both Bursicon α and β are strongly (significantly) expressed, respectively). Eclosion hormone is also strongly (significantly) expressed. The expression of only bursicon α in *I. scapularis,* along with eclosion hormone and possibly also corazonin (not shown in this figure) has been suggested (Bissinger et al. 2011) to be responsible for gradual synthesis of new cuticle needed to accommodate the enormous blood meal while also maintaining its plasticity (i.e., prevent sclerotization). In contrast, the strong upregulation of both bursicon genes along with eclosion hormone in adult *O. turicata* females may be responsible for expression of plasticity of the cuticle needed for rapid engorgement. ND=Not detected or not assayed as a result no detection by primers.

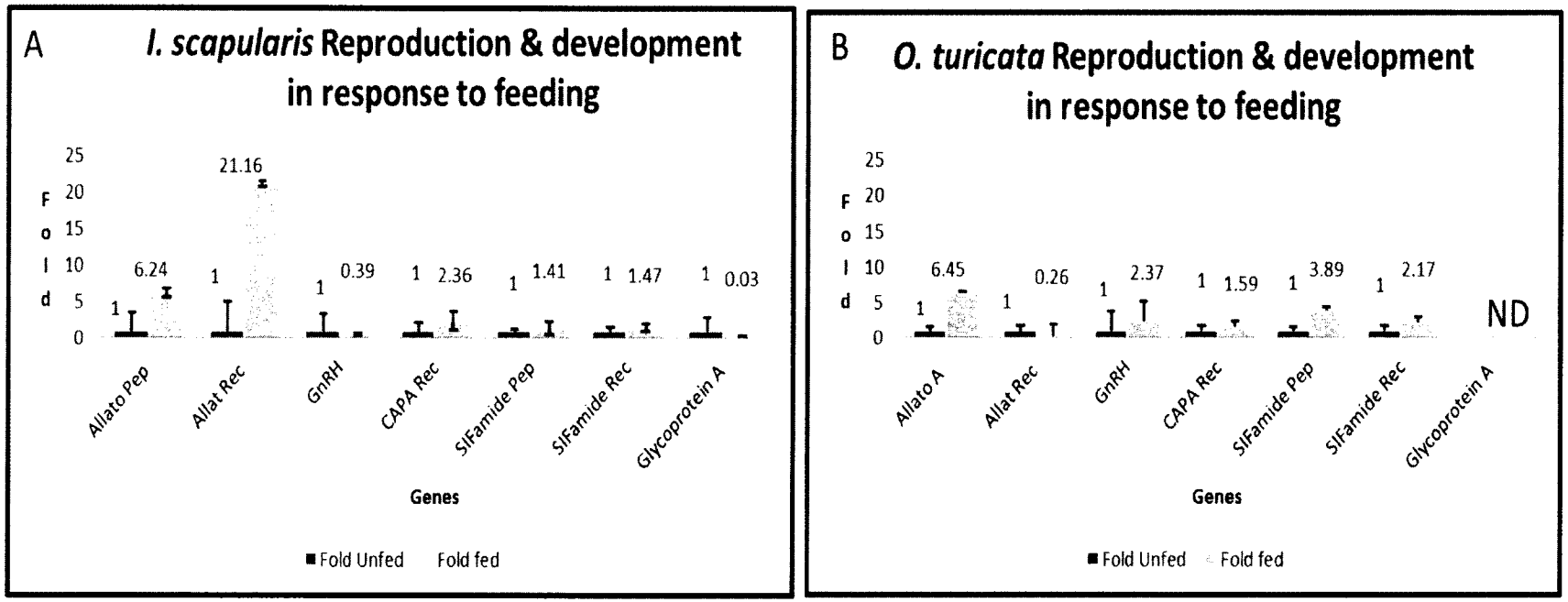

Figure 15. Comparison of reproduction and development-related gene expression in the tick synganglia in response to blood feeding. **A)** Expression of six reproduction/development-related genes in *Ixodes scapularis* part-fed females. Allatostatin peptide (Allato-pep), allatostatin receptor (Allato-rec), and CAPA receptor are strongly (significantly) upregulated, upregulated. However, gonadotropin releasing hormone receptor (GRH rec) was strongly (significantly) downregulated. Expression of the genes SIF peptide and SIF peptide receptor were slightly but not significantly upregulated. **B)** Expression of six reproduction/development-related genes in engorged females of *Ornithodoros turicata* engorged females. Allatostatin peptide, gonadotropin releasing hormone receptor, SIFamide peptide and SIFamide receptor were strongly (significantly) upregulated; CAPA receptor was upregulated slightly (but significantly). Allatostatin receptor was downregulated but this change was not significant.

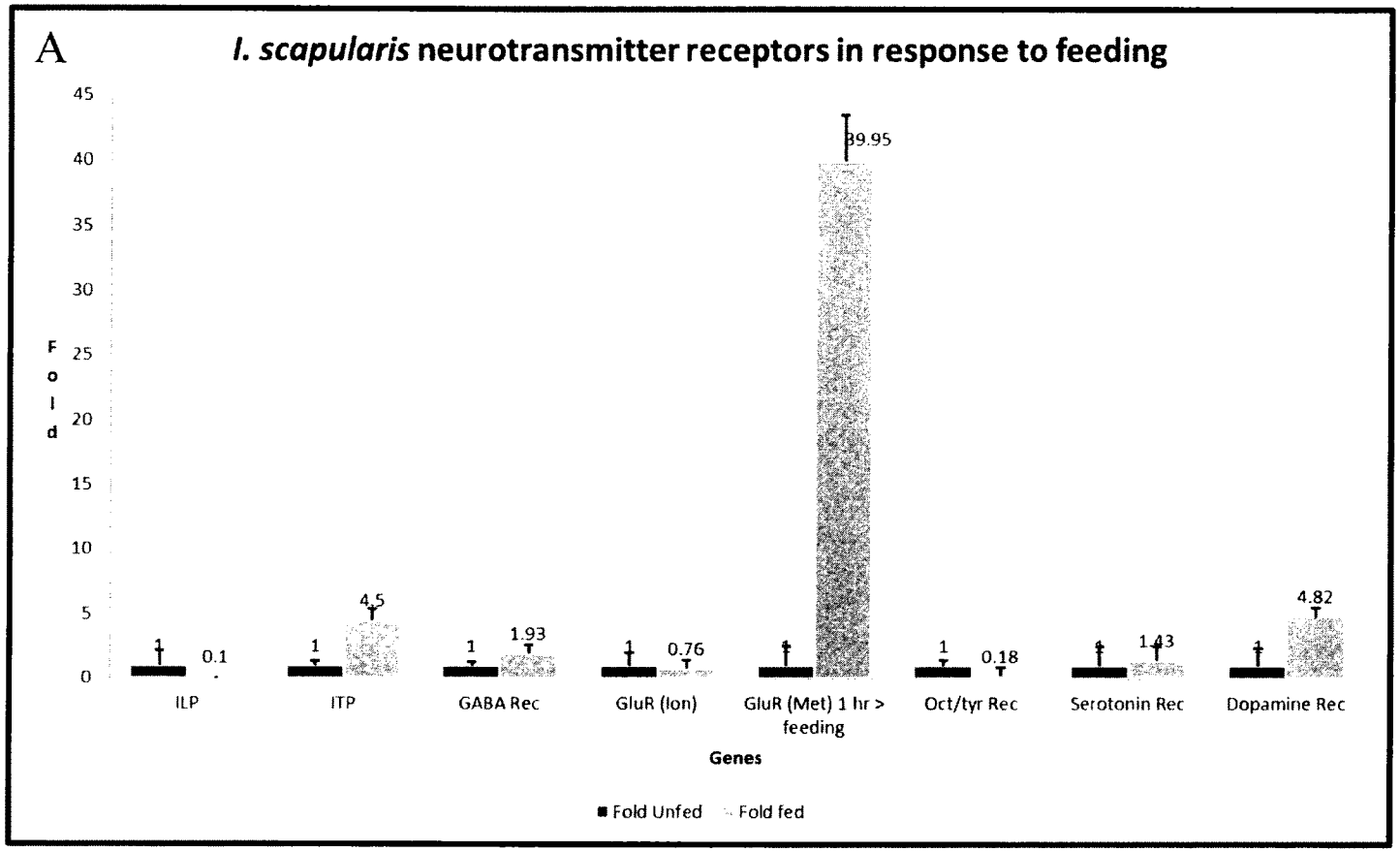

Figure 16. Comparison of neurotransmitter-related gene expression in the tick synganglia in response to blood feeding. Expression of seven neurotransmitter receptor genes in *Ixodes scapularis* part-fed females. Glutamate (metabotropic) was the most strongly expressed of these signaling genes. GABA receptor and dopamine receptor were also upregulated (significantly). Ionotropic glutamate (GluR (Ion)) and octopamine receptor were significantly downregulated, while serotonin was slightly upregulated (not significant). ILP=Insulin-like peptide; ITP=Ion transport peptide; Oct/tyr=Octopamine tyramine receptor.

Figure 16 continued.

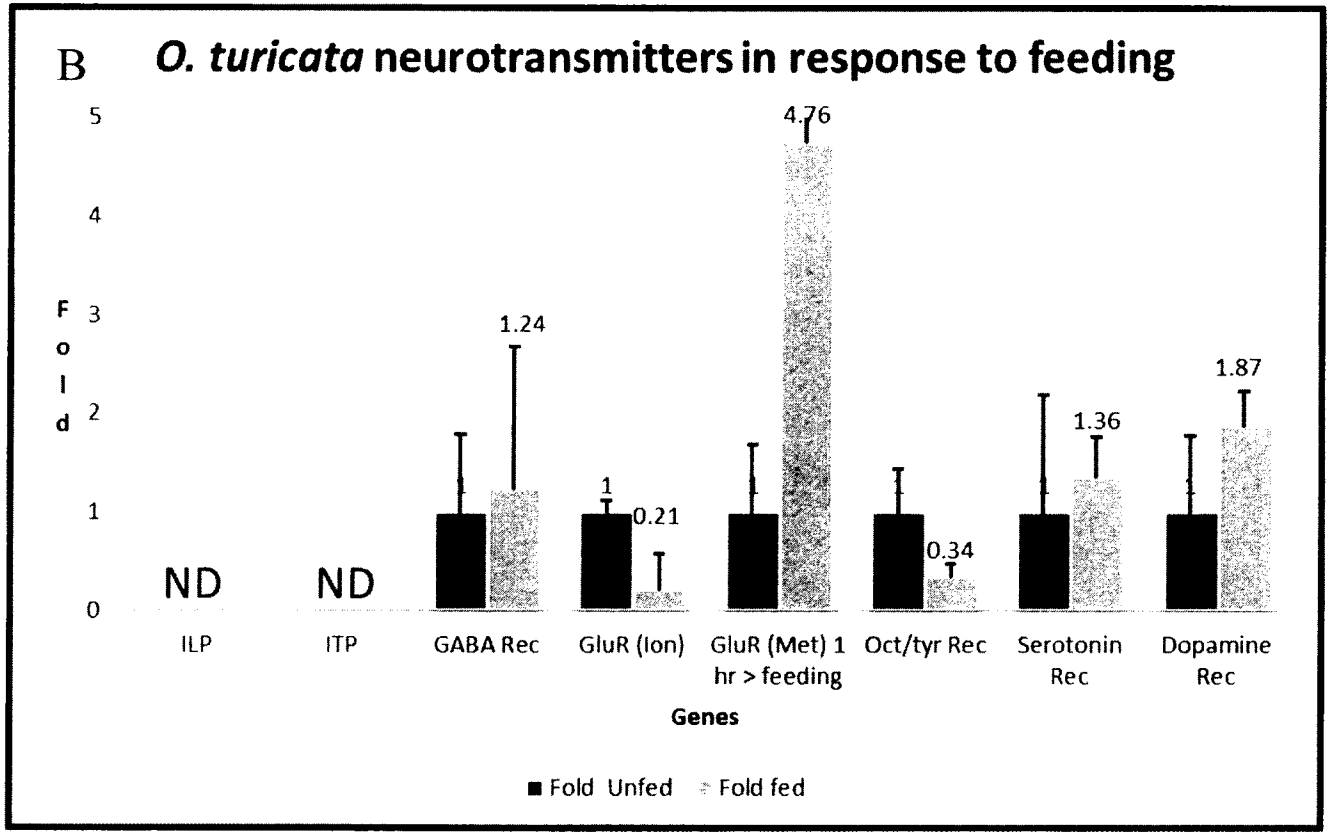

Figure 16. Expression of seven neurotransmitter receptor genes in *O. turicata.* Similar to *I. scapularis,* glutamate (metabotropic) was the most strongly expressed of these signaling genes. Insulin and octopamine receptors were strongly (significantly) downregulated. GABA and serotonin receptors were weakly upregulated but these differences were not significant. ND=Not detected or not assayed as a result no detection by primers.

CHAPTER 4

4.0. Discussion

This study reports the results of comparative transcriptomics of the synganglion of two species of ticks, the hard tick *I. scapularis* and the soft tick, *O. turicata*, with comparisons between the neuropeptides, neuropeptide receptors, and neurotransmitter receptors. Both transcriptomes were created using the same sequencing platforms (Illumina GAII). Quality control (Bioanalyzer) reports, assembly of the raw reads into transcripts (CLC-BIO) and transcriptome analysis suggest that the two transcriptomes generated using these methods present two cDNA libraries with high coverage of the synganglion genes expressed during blood feeding. Annotations for the transcripts predicting the specific genes of interest, namely, the neuropeptide, neuropeptide receptors and neurotransmitter receptors, initially supported by the low Genbank e-values, are further supported by sequence alignments with other species and the results of qPCR assays. Consequently, I believe that meaningful comparisons can be made about the gene categories and specific genes in the two species as discussed below.

4.1. General Comments and Conclusions

Despite the many biological differences in their life cycles, blood feeding, elimination of blood meal water, and cycles of reproduction described in the introduction of this dissertation, the transcriptomes of the two representative species, *I. scapularis* and *O. turicata* are remarkably similar with respect to the gene categories and relative abundance. Comparison of the BLAST2GO charts at molecular level 3 shows these similarities. Although mapping was much more successful for *I. scapularis* than *O. turicata*, examination of 32 gene categories (at molecular level 3) shows little difference

in the number of transcripts expressed for each category, with 3 exceptions, nucleoside binding, phosphatase regulator activity (both much more abundant in *I. scapularis*) and lyase activity (much more abundant in *O. turicata*). Comparison of the transcripts encoding for neuropeptides, neuropeptide transporters and/or their receptors shows virtually the same genes in the transcriptomes of the two different tick species (Table 3.5). Hill et al. (pers. commun.), in their manuscript describing the genome of *I. scapularis*, reported 56 neuropeptides, 41 of which have orthologs in insects and other invertebrates. Of these 41 neuropeptides, transcripts predicting 25 of these genes were recognized in *O. turicata* versus 23 in *I. scapularis*. Included are allatotropin, allatostatin, bursicon α, bursicon β, calcitonin, corazonin, CRF, CCAP, CPA/pyrokinin receptor, diuretic hormone, eclosion hormone, FMRFamide, glycoprotein A, GRF, insulin-like peptide, ion transport peptide, neuropeptide F, myoinhibitory peptide, neurophysin-oxytocin transporter, orcokinin (A version only), proctolin, SIFamide, sulfakinin, thyrokinin and tachykinin.

Few differences were noted between the two species. The *O. turicata* transcriptome showed transcripts for 2 of the 3 major allatostatin genes, allatostatin B (Bombystatin) and allatostatin C, as well as both bursicon α and bursicon β, glycoprotein B, and thyrotropin. In contrast, the *I. scapularis* transcriptome showed transcripts for allatotropin, but only allatostatin B, allatostatin C, only one bursicon, bursicon α, only glycoprotein A, and no transcript for thyrotropin. In several cases, no transcripts predicting the neuropeptide were identified but transcripts for the corresponding receptor were found, indicating that the transcript for the neuropeptide may have been missed during sequencing or assembly, or that these peptides were not expressed under the

sampling conditions used in the study. Similar relationships were noted for the neurotransmitter receptors and transporters. Transcripts predicting 8 neurotransmitter receptors, acetylcholine (muscarinic and nicotinic), dopamine, GABA, glutamate (metabotropic and ionotropic), octopamine, and serotonin were annotated in the transcriptomes of both species, as were transporters neurotransmitter sodium symporter and norepinephrine/octopamine transporter.

The hard tick, *D. variabilis*, is the only other species of Ixodid ticks for which comparable studies on the transcriptomics of the neuropeptides and neurotransmitters were done (Bissinger et al. 2011; Donohue et al. 2010). Comparing the findings with the transcriptome of *D. variabilis*, transcripts encoding for the peptides and/or receptors for 13 of the same genes as were found in *D. variabilis*, namely, allatostatin, bombsystatin (= allatostatin β), bursicon α and β (both in *O. turicata* but only bursicon α in *I. scapularis*), calcitonin, corazonin, eclosion hormone, glycoprotein A, gonadotropin-releasing hormone, insulin-like peptide, ion transport peptide, orcokinin, pyrokinin/CAPA, and sulfakinin. However, I also found transcripts encoding for an additional 9 other neuropeptides and/or their receptors that were not found in the *D. variabilis* transcriptome, namely, allatotropin, corticotropin-releasing factor (CRF), FMRFamide, myoinhibitory peptide, neurophysin-isotocin, neuropeptide F, proctolin, SIFamide and tachykinin.

The reason why so many additional neuropeptides/neuropeptide receptors were found in *I. scapularis* and *O. turicata* as compared to *D. variabilis* is unknown. However, it is possible that the much greater coverage provided by Illumina sequencing as compared to 454 pyrosequencing is the major factor explaining this difference.

If the Gene Ontology categories (molecular level 3) and the neuropeptide and neurotransmitter profiles of the synganglia in these two different tick species are so similar, how can we explain the major physiological differences observed in their biology? Do these genes function in similar roles, or are they expressed at different times and integrated differently in different patterns of neurohormonal regulation. To address this question, the results of quantitative PCR, together with evidence from the literature will be examined as discussed in the following section.

Note: The identities mentioned in this section were arrived at by a separate BLAST performed specifically to validate gene similarities and may not be similar to the information presented in table 3.5-3.6.

4.2. Gene Category: Growth and Development
Bursicon

Bursicon is described as a peptide neurohormone present in arthropods (Honneger et al 2002; Mendive et al 2005). Discovered in 1965 in the blowfly *Calliphora erythocephala* by the famous "neck-ligated blow-fly assay" (Fraenkel and Hsiao, 1965), it is said to be the cuticle tanning and scelerotization factor of insects important in the accumulation of additional cuticular layers after molting has occurred in newly emerged dipteran adults (Honegger et al 2008; Wang et al, 2008; Dircksen et al, 2013). Bursicon has also been implicated in wing expansion activity in insects. These functions are achieved by means of a G-protein coupled receptor (GPCR). Bursicon is found in the central nervous system (CNS) and the hemolymph of newly emerged adult arthropods and chordates (Loy et al, 2007) but not in nematodes (Sharp et al, 2010). Also, cross-species activity of bursicon has been demonstrated among arthropods (An et al, 2008; Song & An, 2011) where

bursicon isolated from a species has produced cuticle sclerotization effect when injected into another species.

Molecular characterization of the protein in several insect species revealed a 30-kDa bioactive heterodimer made up of two bursicon subunits conserved among the different arthropods (Dewey et al, 2004; Honneger et al, 2008; Song & An, 2011). The two subunits bursicon alpha and bursicon beta identified in *Drosophila melanogaster* have molecular weights of 16 kDa and 14 kDa respectively. I identified homologs of bursicon alpha and bursicon beta messages in the *O. turicata* transcriptome sharing 81% and 79% sequence identity with bursicon alpha (XM_002407468.1, e-value 6.00E-83) of the deer tick *I. scapularis* and bursicon beta (XM_002407469.1, e-value 4.00E-67) of *I. scapularis* respectively. Searches from the conserved domain database returned matches to the DAN superfamily cl17734] at e-values of 2.44e-04 and 1.43e-07 respectively, showing the 9 conserved cysteines characteristic of the cysteine knot family (Dewey et al, 2004). Interestingly, we found a transcript for bursicon alpha in the transcriptome of the *I. scapularis* synganglion, but no evidence of bursicon beta.

Biological function of bursicon in ticks

While bursicon has been described previously as a scelerotization and tanning factor as well as wing expansion gene in various insects, very little is known about its function and role in the biology or survival of ticks. This is so because there is no evidence of molting or sclerotization in fully mature adult ticks. However, cuticular plasticization has been described in the cattle tick *Haemaphysalis longicornis* during blood feeding, a phenomenon synonymous to cuticle expansion in Argasids (Okura et al, 1996). We suggest here that an extension of the cuticle occurs during feeding. In insects, bursicon is

the hormone that triggers the process of sclerotization shortly after molting of each instar (Reynolds, 1983). Primers designed to amplify regions of the bursicon alpha/beta in *O. turicata* and *I scapularis* showed interesting results. While bursicon alpha and bursicon beta showed a 3.09 and 2.89 fold increase respectively in *O. turicata* in response to feeding, bursicon alpha showed a 3.32 fold increase in *I. scapularis*. Judging from the feeding behavior of both tick species, the increase in body size in *O. turicata* may be accounted for by their characteristic fast feeding and engorgement that occurs within an hour in argasids. The increase in *I scapularis* may also be attributed to the same plasticization process, which may occur at a very slow rate as a result of slow feeding behavior typical of ixodids usually lasting for days, and not feeding to engorgement until mating has taken place. According to Kaufman et al. (2010), bursicon regulates the increased plasticization that occurs gradually as the *I. scapularis* female feeds, allowing it to increase to up 50 – 75 times its original body size. Dopamine was also implicated in this process (Kaufman, 2014). The implication of this may be that the rate at which bursicon is expressed is relatively lower in ixodids, especially during the initial feeding period prior to copulation. I hypothesize that plasticization is the means by which the *O. turicata* females are able to expand their cuticles for rapid blood feeding, regulated by expression of both bursicon α and β as shown in the high fold values recorded immediately after feeding. In contrast, gradual synthesis of new cuticle, along with plasticization as the cuticle grows, is the mechanism by which *I. scapularis* females expands its body to accommodate the much larger blood meals consumed in this species, regulated by the ecdysial hormones (eclosion, ETH, corazonin) and bursicon functions in a secondary role solely to foster the plasticity of the slowly growing cuticle.

Corazonin

Corazonin (CRZ) a peptidergic neurohormone of insects, initially discovered in extracts of the corpora cardiaca (CC) from the American cockroach *Periplaneta americana*, has been described as multi-functional, accounting for several phenotypes or behavior in diverse insect species (Predel et al, 2007; Choi, 2009). This neuropeptide has been observed to "be a potent cardioactive substance" in the American cockroach controlling heart beat as well as triggering muscle contractions (Veenstra, 1989). It is also known to induce dark coloration in the migratory locust *Locusta migratoria* (Tanaka, 2003), reduce rate of silk spinning, and cocoon formed in the silk worm *Bombyx mori* during transition from larvae to pupae (Tanaka, et al, 2002), to stimulate the initiation of pre-ecdysis triggering hormone ([PETH] (a function that is yet unclear) in the tobacco hornworm/sphinx moth *Manduca sexta* (Kim, 2004; Veenstra, 2009), and has been suggested to be active under nutritional stress in most insects (Veenstra, 2009). Overall, there is not a common function that has been solely attributed to the CRZ neurohormone not even in *Drosophila* (Cazzamali, et al, 2002; Settembrini BP et al, 2011).

I identified messages encoding the corazonin receptor in both transcriptomes of *O. turicata* and *I. scapularis*. I identified contigs in the *O. turicata* and the *I. scapularis* transcriptome both sharing 86% and 100% pairwise sequence identity with corazonin receptor (XM_002435295.1) of the *I. scapularis* tick at e-values of 6e-82 and 0.0 respectively.

Searches from the conserved domain database returned matches to the serpentine type 7TM GPCR Srx superfamily [cl18571] and [cl18179], at e-values of 6.30e-03 and 1.68e-04 respectively. Searches for cleavage sites using the translated nucleotides as query

(using the bioinformatics tool "neuropred"), predicted signal sequences for the immature preprohormone as well as consensus cleavage sites on the receptor, predicting possibly, corazonin activity. In addition, the message for corazonin receptor was identified in the *O. turicata* transcriptome sharing 86% and 77% identity to the corazonin receptor gene (XM_002435295.1; e-value: 9E-82) with *I. scapularis* and (XM_001972489.1; e-value: 1E-35) with *Drosophila erecta* respectively.

Biological function of corazonin in ticks

Like most other neuropeptides, there is little known about the function of corazonin in ticks. Corazonin has been studied to an extent in the Crustacea and the Insecta classes of arthropods and has been observed to be pleiotropic in their functions. This indeed is interesting as the function of this neurohormone in ticks still eludes acarologists. We however, suggest that contrary to phenotypes suggested in other studies, corazonin may not be responsible. One that is worth considering is its action as a neurohormone that responds to nutritional stress (Veenstra, 2009). In our study, nutritional stress was less likely at the time tissue collection was performed. Quantitative PCR data for corazonin receptor supporting this hypothesis showed a little or no upregulation of *O. turicata* corazonin receptor (1.01 fold increase) and slight up-regulation of *I. scapularis* corazonin receptor (1.33 fold increase) in response to feeding in *O. turicata* and *I. scapularis* respectively. If this hypothesis of corazonin's role is correct, we would expect to see down-regulation of this gene in the replete females. In light of this, more study of this neuropeptide in ticks is recommended.

Crustacean Cardio-acceleratory Peptide (CCAP)

The crustacean cardio-acceleratory peptide (CCAP) is one of the numerous peptide-signaling factors present in the nervous system and its surrounding tissues (Loi et al, 2001). A cyclic nonapeptide with a characteristic intramolecular disulfide bridge, CCAP is strongly conserved across several arthropods in which studies have been carried out (Dircksen et al, 2013). Information about the CCAP synonymous to CAP2a gene was first isolated from the central nervous system (CNS) of the tobacco hawkmoth *Manduca sexta* (Tublitz et al, 1991). The CCAP gene has also been described as multi-functional, playing a defined role in the following: ecdysis regulation (ecdysis refers to the shedding of long-standing exoskeleton at the end of the molt); hormone release; muscle contractions in insects and crustacean; gut tissue regulation (as a gut factor); gut contraction (Sakai et al, 2004); and control activity of the anterograde cardiac pacemaker in *Drosophila melanogaster* (Dulcis et al, 2005, Lahr et al, 2012). Expression of CCAP may also play a role in the circulatory physiology of *Anopheles gambiae* (Estévez-Lao et al, 2013).

The CCAP gene has been characterized in several arthropods. The peptide contains 125 amino acid residues as described in *M. sexta* (Loi et al, 2001). We identified messages encoding the crustacean parts of cardio-acceleratory peptide in the transcriptomes of *O. turicata* sharing a 88% and 71% identity with the American cockroach *Periplaneta americana* (AB126034.1; E-value: 2e-05) and a hypothetical protein (XM_002402232.1; e-value: 2e-18). We did not find any matches in the *I. scapularis* transcriptome (except for strong matches to hypothetical proteins), but designed primers for CCAP with sequence information available from the *I. scapularis* genome deposited in the genbank

database. Searches to the conserved domain database revealed motifs matching with the arthropod cardioacceleratory peptide 2a (CCAP) superfamily [cl12661] at an e-value of 9.18e-08.

Biological functions of CCAP

In spite of the importance of this peptide in other arthropods, little is known about the function of CCAP in ticks. Other studies have however, given accounts of the presence of this gene in the synganglia transcriptomes of *Dermacentor variabilis* and mites (Bissinger et al, 2011). Gene expression studies with qPCR assay revealed a 0.97 fold (down-regulation) decrease in response to feeding in *I scapularis* demonstrating little or no activity, further leaving us with questions of the functionality of CCAP in ticks.

Pre-Ecdysis, Ecdysis Triggering Hormone (PETH, ETH) and ETH Receptor (ETHR)

The ecdysis-triggering hormone (ETH) as its name implies is an endocrine factor that triggers or induces the onset and suppression of pre-ecdysis and ecdysis behavior in insects. Ecdysis is a complex periodic process involving the shedding of old exoskeleton, respiratory system, and cuticular lining of the fore gut & hind gut during metamorphosis (Park et al, 2003). Pre-ecdysis and ecdysis triggering hormones (PETH & ETH) are secreted in endocrine Inka cells in most arthropods (Žitňan et al, 2003), and some studies suggest far reaching "lethal and behavioral deficits (non-initiation of ecdysis sequence, inability to inflate new respiratory system, incomplete ecdysis, and mortality)" upon silencing of the eth gene in *Drosophila* (Park et al, 2002). Molecular characterization of the ETHR revealed two splicing variants of the receptor ETHR-A and ETHR-B inferring conserved structures and organization in representative sequences of ETH and ETHR

from *Rhodnius*, *Acyrthosiphon*, *Pediculus*, *Apis*, *Anopheles*, and *Culex* while orthologs of the eth and ethr genes were identified in *Ixodes* and *Daphnia* (Roller et al, 2010). Even though transcripts encoding the ETH-like peptide were not identified in both transcriptomes of *O. turicata* and *I. scapularis*, matches were found sharing 82% identity with variants Ethr-A and Ethr-B of the red floor beetle *Tribolium castaneum* (NM_001083323.1 NM_001083324.1; e-values: 1e-17) in the *O. turicata* transcriptome predicting a likely ETH-ETHR signaling in ticks. The transcriptome generated from the pooled sample of unfed, part-fed and replete *I. scapularis* females revealed the presence of a transcript for ETHR, but no transcript for ETHR was found in the transcriptome generated from the part-fed females (Egekwu et al, 2014). This suggests that ETHR is expressed in the unfed or replete females, but not in the part-fed (virgin) females.

Biological function of ETH, PETH, and ETHR in Ticks

Adult ticks as mentioned in previous sections do not undergo molting or ecdysis. This is known to occur in larvae and nymphal stages prior to molting. However, ticks are unusual in that cuticular expansion occurs during feeding so as to accommodate the huge blood meal. While there was little expression of 0.05 fold of ETHR in the *I. scapularis* part-fed female, we report here qPCR data revealing up-regulation of 6.89 fold for *O. turicata* Ethr in response to feeding. I speculate here further, a strong connection to the biology of both ticks species. If cuticular expansion is the case here, then the data supports the hypothesis that the ETH-ETHR signaling is active in *O. turicata* as a result of its ability to feed to engorgement within 1 to 2 hours and then likely suppressed soon afterwards. This however, is not the case with *I. scapularis* that has a partial feeding phase initially necessary before the onset of copulation and then a second slow phase (5-7

days) of feeding to engorgement after copulation is achieved with male. It is very likely that cuticle expansion is more sustained over a longer period in *I. scapularis* hence the need for ETH activity. We hypothesize that ETH activity will be high in replete females as evidence in previous studies showed highly significant downregulation of ETH in part fed female transcriptome and presence of ETH in transcriptome of mixed synganglia samples of unfed, part-fed, and replete *Ixodes* females (Egekwu et al, 2014). These findings suggest that ETH may be needed only during the post-mating cuticle expansion that occurs when the female feeds to repletion. This is however, subject to further investigation.

Eclosion Hormone (EH)

Eclosion hormone on the other hand has been implicated as a regulator of ETH secretion prior to ecdysis (Zitnan et al, 2007). Eclosion hormone being peptidergic, is one of four peptides (others being (CCAP, PETH, ETH) involved in cell-cell communication leading to activation of pre-ecdysis and ecdysis processes in insects (Park et al, 1999). The interaction of these peptides as studied in *Manduca sexta* describes ETH as an activator of EH and EH as a trigger for CCAP secretion and onset of the ecdysis motor program (Gammie & Truman, 1999).

We identified messages encoding the eclosion hormone in both transcriptomes of *O. turicata* and *I. scapularis* sharing 83%, 99% pairwise sequence identity with eclosion hormone (XM_002399230.1) of the *I. scapularis* tick at e-values of 3.00e-35 and 2.00e-124 respectively. Sequence identity of 78%, 80 % (EU567122.1; e-values: 1e-26, 3e-27) with eclosion of *D. variabilis* identity was also observed in both transcriptomes of *O. turicata* and *I. scapularis* respectively. Searches from the conserved domain database

returned matches to the eclosion superfamily [cl04714] at e-values of 1.20e-13 and 1.20e-16 respectively.

Biological function of Eclosion in Ticks

The role of eclosion hormone has been well documented in insects. The role of this hormone in ticks however, has appeared a bit controversial because of what is known about how eclosion hormone works in tandem with the ecdysis, pre-ecdysis hormone, and CCAP to regulate growth and development behavior through ecdysis and molting in insects (Roe et al, 2013). Adult ticks however, with a different biology from insects, do not undergo molting. But the presence of this neuropeptide in several transcriptomes of adult fed ticks e.g., *D. variabilis* (Donohue et al, 2010; Bissinger et al, 2011) and *I. scapularis* (Egekwu et al, 2014) enables me to suggest some other "feeding activated" roles regulating adult tick behavior, cuticle expansion, and female reproduction as speculated by Roe et al. (2013). However, gene expression from my qPCR data, showed up-regulation of eclosion hormone by 1.96 fold and 0.32 down-regulation in *O. turicata* and *I. scapularis* respectively in response to feeding. Based on these findings, I suggest that eclosion hormone may facilitate the rapid cuticle plasticization needed by *O. turicata* that enables them to blood feed within minutes. In contrast, the fact that it is downregulated in part-fed *I. scapularis* is consistent with the slow feeding process that occurs prior to mating and subsequent rapid engorgement. There is high similarity between the *O. turicata* and *I. scapularis* eclosion sequences (suggesting similarity in functions) but there appears to be important differences in their expression patterns and time of expression. Further studies are needed to test this hypothesis.

4.3. Gene Category: Reproduction

Allatostatins (Ast) and Preproallatostatin (preproAST)

Allatostatins (Ast) have been described as pleiotropic neuropeptides particularly due to multiple phenotypic functions that are affected by the expression of this gene (Stay & Tobe, 2007). Allatostatins have also been implicated in several functions in diverse organisms (Veenstral et al 1997). In insects and crustaceans, they are generally known to obstruct the biosynthesis of a growth and reproduction regulator, the juvenile hormone (JH), by the corpora allata (CA) of the synganglion and mandibular glands in insects and crustaceans respectively. JH is widely responsible for maturation of larvae and nymphal development as well as vitellogenesis in adult virgin females during reproduction (Woodhead et al, 1989; Stay & Tobe, 2007). In some other cases, it has been known to regulate certain phenotypes in insects such as color morphs, caste differentiation, wing length, etc, which are typical examples of polyphenism (Hartfelder & Emlen, 2012; Jindra et al, 2013). And in mammalian cells, allatostatin binds allatostatin receptors to reduce membrane potential and input resistance by opening G-protein coupled inward-rectifying potassium channels (Tan et al, 2007). Allatostatin has also been implicated in mid-gut movement and recently, it has been shown to affect satiety in *Drosophila*. An inhibitory effect on feeding and an enhancement of food aversion was observed in *Drosophila* upon activation of AstA-expressing neuron (Hergarden et al 2012). However, the function of this gene in ticks has not received extensive studies. We identified two allatostatin preprohormones (preproAST), preproallatostatin A & preproallatostatin C, from the *O. turicata* transcriptome that shared 84% sequence identity in each case with preproAST A (AJ302036.1, e-value 0.091) of the cricket

Gryllus bimaculatus and preproAST C (XM_003426036.1, e-value 6e-06) of the jewel wasps *Nasonia vetripennis* respectively. Receptor genes of Ast were also identified in both *O. turicata* and *I. scapularis* sharing 78% and 99% identity with *I. scapularis* allatostatin receptor (XM_002403808.1, e-value 6e-96) and (XM_002433328.1, e-value 0) respectively. Both contigs contained domains belonging to 7TM_GPCR and 7TM

Biological Function of Allatostatin in Ticks

The function of allatostatin in ticks is yet unknown. In fact, several questions still remain as to why it is present in adult ticks. Several studies on insects and crustacea, has revealed that these genes inhibit the biosynthesis of JH by the corpora allata and mandibular glands respectively. Adult ticks do not molt nor undergo vitellogenesis until they mate, leaving us with the sole question of the function of Ast in these two tick species. Is it also possible that the function of these genes may have evolved across the different classes of arthropods? For instance it has been suggested that these neuropeptides may be playing an important role in cuticle expansion (Kaufman, 2014). This question is worthy of investigation. Although Ast A, B or C were not identified in both transcriptomes, the presence of PreproAST A, preproAST C messages, and Ast receptors in the *O. turicata* transcriptome lends credence to the possible synthesis of the mature Ast peptide. Quantitative PCR (see fig 3.12) data showed a 6.45-fold up regulation and 0.26 of PreproAst A in *O. turicata* and the receptors respectively. We can attribute this trend to the feeding behavior of *O. turicata* females that feed within an hour or two and quickly drop off. It is quite possible that the Ast is turned on to inhibit the need for more blood meal. In *I. scapularis*, we went ahead to design primers for preproAst A & B (based on information from Genbank) and gene expression studies

revealed a 6.24 fold increase 7.95 fold increase, and 21.16 upregulation upon feeding for preproAst A, Ast B, and Allastostatin receptor respectively.

Allatotropin (AT)

Allatotropin (AT) is a multi-functional amidated neuropeptide that is critical in Juvenile Hormone (JH) synthesis in insects. The role of JH in insects is mentioned in the previous section (see 4.3.1.). Allatotropin has been described as a regulator of the JH synthesis by the corpora allata (CA) stimulating an increase in the synthesis of JH in retrocerebral complexes (RC) of adult female moths (Teal, 2002; Alzugaray et al, 2013). The peptide in several arthropods is pleiotropic in action performing neural, endocrine and myoactive roles amongst which include mid-gut ion transport inhibition, circadian rhythms control, and cardioacceleratory functions (Alzugaray et al, 2013). The action of Allatotropin and Allatostatin are antagonistic as the secretion of Ast has been demonstrated to inhibit the production of Allatotropin (Teal, 2002).

Contigs sharing sequence identity of 100% with the *I. scapularis* Allatotropin XM_002406992.1; e-value: 1e-166). None was identified in the *O. turicata* transcriptome.

Biological function of Allatotropin

The function of Allatotropin has been related to stimulation of JH biosynthesis in insects. The function in ticks has not received a lot of attention and so remains unclear. And because adult ticks do not undergo molting, we speculate that this peptide may be performing a different function in ticks. Even though we found a good alignment of this gene in *I. scapularis*, qPCR data is not yet available at this point. We are also unable to comment on the similarities between the sequences in *I. scapularis* and *O. turicata*

because of the absence of an allatotropin-like peptide in the *O. turicata* transcriptome. We could however, adduce the differences in expression patterns and possibly the absence of the peptide in *O. turicata* transcriptome to the antagonistic action of the Allatostatin peptide to Allatotropin (Teal, 2002). Quantitative qPCR data for Allastostatin showed up-regulation of the preprohormone and receptors in both *O. turicata* and *I. scapularis* to support our speculations. At this stage, I cannot speculate as to the roles of allatotropin and allatostatin in tick reproduction.

SIFamide

Originally isolated from the flesh fly *Neobelliera bullata* as a myotropic neuropeptide NeobuLFamides, SIFamide is extremely conserved and abundant in arthropods that have been studied (Verleyen et al, 2004; Vázquez-Acevedo et al, 2009; Dircksen et al, 2013). Studies have shown conservation of six isoforms in numerous crtustaceans (gut and brain), several insects (4 neurons in pars intercerebralis), and a tick, implicating the peptide in neuromodulatory functions such as vision, tactile input and olfaction (Verleyen et al, 2008). Other studies have also demonstrated the role of SIFamide in modulation of sexual behavior in fruit flies and control of male agresssive behavior in the lobster *Macrobranchium rosenbergii* (Tehazz et al, 2007; Dircksen, 2013, Kastin, 2013).
We identified sequences sharing 74%, 80% identity with the *I. scapularis* SIfamide precursor (GQ214556.1; e-value: 1e-25) and SIFamide receptor (KC422392.1; e-value: 3e-89) in the *O. turicata* transcriptome. Sequences sharing 100%, 68% identity with *I. scapularis* hypothetical protein (XM_002404070.1; e-value: 1e-80) and *Nasonia vetripennis* SIFamide like receptor (XM_001600048.2; 1e-09) were also identified in the

I. scapularis transcriptome. All sequences revealed typical motifs of 7TM_GPCR_Srx, and 7 transmembrane receptor (rhodopsin) families from the conserved domain database.

Biological Function of SIFamide in Ticks

SIfamide has been implicated in several functions in arthropods. In ticks however, immunohistochemistry patterns have demonstrated activity of the SIFamide receptors in salivary glands (SG) of *I. scapularis* suggesting a pre-existence of SIFamide signaling system in unfed salivary glands (Simo et al, 2013). It was inferred (based on the location of the SIFamide receptor in the SG), that the likely targets and functions of this peptide were the myoepithelial cells (contraction of the acini and/or the control of the valve), basally located dopaminergic granular cells (paracrine and dopamine regulation); and neck cells (control of the acinar duct and its valve). RNAi however, has not verified this finding due to the difficulty in delivering the dsRNA in ticks (Simo et al, 2013; Simo et al, 2014).

Quantitative PCR data showed a 3.89 fold increase of the SIFamide receptor in *O turicata* and 1. 41-fold increase in *I. scapularis* in response to feeding. While the trend in *I. scapularis*, may suggest the role of SIFamide in controlling the SG as proposed in Simo et al, (2013), the trend in *O. turicata* points us in a different direction. We show sequence similarities in both tick species but still see differences in their expression patterns and time of expression of SIFamide peptide. We therefore speculate that SIFamide may be performing other functions in ticks and these functions may be behavior-dependent (biology of both ticks) in *O. turicata* and *I. scapularis*.

Pyrokinin/Pheromone Biosynthesis Activating Neuropeptide (PBAN).

First discovered in the cockroach, *Leucophaea madarae* the pyrokinin/pheromone synthesis activating neuropeptide (PK/PBAN) family is distributed in a wide range of insects and is recognized by a C-terminal pentapeptide, FXPRLamide, which may also be utilized in vast amount of physiological functions in different insect species (Fan et al, 2012; Hellmich et al, 2014). In addition to playing myotropic roles, this peptide neurohormone family, may be involved in melanization, puparial contraction, diapause trigger/inhibition, increased production of sex pheromones, and stimulation of pheromone biosynthesis in different insect species (Paluzzi and O'Donell, 2012; Hellmich et al, 2014). We identified partial sequences from the *O. turicata* transcriptomes sharing 100%, 84%, and 72% identity with an I.scapularis G-protein receptor (XM_002401136.1; e-value: 0.0); a *D. variabilis* pyrokinin-like receptor (EU659109.1; e-value: 2e-124); and a *Plutella xylostella* biosynthesis activating neuropeptide receptor (AY974334.1; e-value: 1e-39). We also identified transcripts in the I. scapularis transcriptomes sharing 76% and 70% identity with a *D. variabilis* pyrokinin-like receptor (EU659109.1; e-value: 1e-109), and a *P. xylostella* biosynthesis activating neuropeptide receptor (AY974334.1; e-value: 8e-30).

Biological Functions of PK/PBAN in Ticks

Like most other neurohormones, the function of pyrokinin/PBAN in ticks is not known. We observed a 0.63 fold decrease in the expression of the peptide in the synganglion of part fed *O turicata* (not shown in the figure) versus a 2.36 fold increase in *I. scapularis* females. The many roles attributed to this peptide in insects and arachnids demands extensive work in researching the functions they play in ticks. We speculate here that

this PK/PBAN might indeed be playing a role in the activation of pheromone biosynthesis neuropeptide. We support this finding for PBAN with qPCR data from the same biological samples of *O. turicata* females used for pyrokinin. We do not have qPCR data for *I. scapularis* PBAN and cannot comment further at this time. We also speculate here, the possibility of this neurohormone playing a vital role in the biosynthesis of sex pheromones in female ticks as it affects the PBAN peptide. While argasids (e.g., *O. turicata*) release this pheromone post-feeding period to attract males, ixodid prostriates (*Ixodes sp*) sex pheromones are released by unfed females as well as feeding females. This may account for the trend in the qPCR results showing 0.63 in PK/PBAN expression in *O. turicata* (collected after drop off) and 2.36 in PK/PBAN expression in *I. scapularis* (which should increase progressively during the course of feeding). The role of sex pheromones in tick reproductive behavior is well documented in Sonenshine (1999, 2006) but little is known about the genes that regulate this process. Again we document here possible differences in the expression pattern and time of expression that may be related to the difference in sexual behavior and feeding biology of both ticks in spite of the substantial similarities in their gene sequences.

Other related peptides in this category

The pheromone biosynthesis activating neuropeptide (PBAN) hormone has been identified in moths as a neuropeptide that regulates biosynthesis of pheromones (Raina et al, 1989; Tillman et al, 1999) and in some cases responsible for induction and termination of diapause (DH-PBAN) as observed in *Bombyx mori* (Zhang et al, 2004). While no transcripts corresponding to these insect neuropeptides were identified in the *I. scapularis* transcriptome, contigs sharing 60% sequence similarity with *I. scapularis* hypothetical

protein (XP_002407526.1; e-value: 4.58E-36) was identified in the *O. turicata* transcriptome. Furthermore, searches from the conserved domain database revealed the PBAN super family ([cl05446]; e-value: 1.77E-06) confirming the presence of this neurohormone in the query sequence. Very little or nothing is known about the biosynthesis of the various pheromones in ticks (such as 2, 6, dichlorophenol [2, 6-DCP] or the Attraction-Aggregation-Attachment Pheromone [AAA]) and there is little known about the PBAN that may be activating biosynthesis of the sex pheromones. We suggest that this peptide, which may be species specific, is involved in the production of sex pheromones in individual tick species to attract males for mating (Sonenshine et al 1984; Sonenshine, 1985; Sonenshine, 2006). Quantitative PCR data also showed a 0.63 down-regulation of the gene in the synganglion of the female *O. turicata*. The reasons for these speculations are explained above (in the PK/PBAN section).

The Gonadotropin Releasing Hormone (GnRH) has been implicated in release of gonadotropins, which in turn initiates the development of the gonads in insects. This hormone has been implicated in ecdysteroid synthesis in invertebrates (Loof et al, 2012). Even though the function of the GnRH is not known in ticks, we identified sequences sharing 77% and 97% identity with *I. scapularis* GnRH-receptors (XM_002401826.1; e-value: 2e-89) and (XM_002407293.1; e-value: 5e-30) in the transcriptomes of *O. turicata* and *I. scapularis* respectively. While the partial sequence identified in *I. scapularis* transcriptome revealed no results upon searches in the conserved domain database (CDD), the sequence from the *O. turicata* transcriptome revealed the 7tm_1 [pfam00001], 7 transmembrane receptor (rhodopsin family). Furthermore, qPCR data revealed a 2.37 fold increase and 0.39 fold decrease in GnRH receptor expression in the

synganglia of female *O. turicata* and *I. scapularis* ticks respectively. These findings are surprising, since I expected that GnRH activity would have been increased since feeding activates spermatogenesis and oogenesis in ticks. The function of this hormone therefore, should be investigated further.

The Pheromone and Odorant Receptor was identified in both the *O. turicata* and *I. scapularis* transcriptomes. Whilst we identified partial sequences sharing 75% with *I. scapularis* pheromone and odorant receptor (XM_002415597.1; e-value: 0.0), partial sequences sharing 99% identity with the same accession number were identified for *O. turica*ta and *I scapularis* respectively. Quantitative PCR data showed down-regulation by 0.39 fold of the receptors as expressed in the synganglion of *O. turicata* females in response to feeding. In spite of insufficient information of the function of these receptors in ticks, they are important and may be species specific in various organisms (ticks inclusive) in functioning as modulators of sexual behavior (Sakai et al, 2011) and food perception (Touhara et al, 2009) depending on ligands that elicits their activation. The sequences identified in *O. turicata* previously in this section revealed alignments to the Major Facilitator (MFS [cd06174]) super family while the sequences identified in I. scapularis revealed alignment to Ligand binding domain of the metabotropic glutamate receptors (PBP1_mGluR [cd06362]). It is possible that this receptor is part of the interpretative repertoire of association neurons in the synganglion. Further research is needed to investigate this hypothesis.

4.4. Gene Category: Water Balance and Feeding Behaviour

Diuretic Hormones

Diuretic hormones (DH) are hormones that play roles in water regulation, fluid secretion and ion balance in Malpighian tubules in arthropods (Li et al, 1997; Nassel, 2002). In insects and crustaceans, two or more genes may encode precursors of putative diuretic hormones (Nassel, 2002; Dircksen et al, 2013). We describe herewith in the transcriptomes of *O. turicata* and *I. scapularis*, the presence of transcripts encoding a corticotropin releasing factor-(CRF/DH) like diuretic hormone receptor and a calcitonin-like diuretic hormone receptor (CT/DH). These diuretic hormones will be discussed individually and as expressed in the ticks. We identified contigs sharing 83% and 78% partial sequence identity to *I. scapularis* DH receptor (XM_002435613.1, e-value: 6e-81) and *I. scapularis* CRF/DH receptor (XM_002403924.1, e value: 6e-30) in the *O. turicata* transcriptome while contigs sharing 77% and 74% with *I. scapularis* DH receptor (XM_002435613.1, e-value: 1e-47) and *I. scapularis* CRF/DH-receptor (XM_002403924.1; e value: 1e-41) were identified in the *I. scapularis* transcriptome. Conserved domain searches with these contigs as queries revealed motifs belonging to the 7 transmembrane receptor (Secretin family) [cl19289] with e values of 2.71e-06 and 1.32e-30 for *O. turicata* and *I. scapularis* respectively.

We also identified sequences sharing 75% identity with *I. scapularis* CT/DH-receptor (XM_002413994.1; e-value: 3e-95) in the *O. turicata* transcriptome and partial sequences sharing 99% identity with *I. scapularis* CT/DH-receptor (XM_002408382.1; e-value: 8e-102) in the *I. scapularis* transcriptome.

Results for *O. turicata* (table 7) show that expression of diuretic hormone is strongly upregulated during the brief feeding period (< 1 hour), but then declines when assayed again 1 – 2 days post-feeding. Results for *I. scapularis* show that diuretic hormone receptor is strongly upregulated during the lengthy tick-feeding period (5 – 6 days post-attachment). These findings help explain one of the major differences in the osmoregulatory functions of these two species.

Corticotropin Releasing Factor-Diuretic Hormone (CRF/DH)

The CRF peptide, also a homolog of the Diuretic hormone (DH) has been studied in several arthropods and suggested to act as neuromodulators in the CNS (Nassel et al, 2002). The CRF/DH regulates fluid secretion rate by stimulating cyclic AMP (cAMP) production in Malpighian tubules (Clottens, et al, 1994). Many studies have also implicated CRF as a "clearance peptide" in blood feeding insects, excreting very high amount of metabolic waste from the haemolymph (Kay et al, 1993) and may also have a hormonal role in the regulation of post feeding diuresis (Coast, 1996).

Biological Function of CRF/DH in Ticks

Although little is known about the function of this peptide in ticks, we can draw speculation from the gene's role in other arthropods. Gene function studies by RNAi may be needed to investigate the function of this peptide in ticks. Quantitative qPCR data of CRF/DH-R revealed weak up-regulation (1.44 fold) in *O. turicata* and up-regulation of 2.94 fold in *I. scapularis* respectively. We propose here that CRF may be playing a role in waste removal from the haemolymph in ticks. The qPCR data supports this hypothesis for *I. scapularis* but not *O. turicata*. However, the timing of the synganglion extractions in the latter may be a factor in explaining this difference

(synganglia were extracted ~ 1-2 days after feeding). The sequence similarities may be an indication of the same function but the time of expression might be different as a result of their respective feeding biology.

Calcitonin-like Diuretic Hormone (CT/DH)

The calcitonin–like diuretic hormone (CT/DH) family was so called because of its role in stimulating salt and water transport by Malpighian tubules (MT) secretion as well as ion homeostasis in some insects (Furuja, et al, 2000; Zandawala et al, 2013). The prepropeptides are quite conserved in many insects where they are found in the cells present in the central nervous system (CNS) and CT/DH receptors are reported in insects and other arthropods (Zandawala, 2012). The CT/DH has been implicated as a powerful heart modulator in the American lobster *Homarus americanus* (Christie et al, 2009) and a stimulator of V-ATPase activity in the fruit fly (Coast et al, 2001) suggesting other possible functions in arthropods.

The role of CT/DH in ticks has not received much investigation. Ticks need to retain or expel water or ions to balance their osmotic pressures after every blood meal. We suggest that CT/DH just like other diuretic hormones is vital in this homeostatic process. The qPCR data for part fed *I scapularis* CT/DH-R female revealed an 8.77 fold up-regulation in response to feeding. In contrast, we observed a strong 5.30 fold up-regulation of CT/DH-R in *O. turicata*, declining to 3.72 folds 2 days post engorgement. We suggest that there are great similarities in the sequence of the CT/DH in both ticks but differences in the duration of their expression appear to be related to the time of feeding, very brief in *O. turicata* versus long periods in *I. scapularis.* These findings are consistent with the very different modes of feeding in the two species, rapid feeding and

rapid water/salt elimination in *O. turicata* versus more gradual water elimination during slow feeding and salivary gland transformation in *I. scapularis*.

Cardioacceleratory Peptide CAPA peptide Pyrokinin/-Periviscerokinin) Receptors

The CAPA peptide family, singled out as one of the known nitridergic peptides in insects has received a lot of attention in the past decade in insects and arachnids. They are presumed "the most abundant neuropeptides in the abdominal neurohemal system" in insects (Predel and Wegener, 2006). The function of this peptide is myomodulatory and osmoregulatory especially important in the induction of calcium signaling via CAPA receptors (CAPA-R), and performing diuretic roles by increasing levels of nitric oxide and cGMP in Malpighian tubules (MT) of insects (Terhza et al, 2012; Davies et al, 2013). The peptide and its receptor (capaR) have also been implicated in the regulation of anti-diuresis in *Manduca sexta* and *Rhodnius prolixus* respectively by inhibiting fluid secretion in the MTs (Quilan et al, 1997; Paluzzi et al, 2010). The CAPA peptides have been grouped into periviscerokinin (PVK/CAP_{2b}) and pyrokinin (PK/CAP_{2b}) peptide families and this nomenclature may be based on the isolation history and the position on the prepropeptide (Predel and Wegener, 2006). The peptide PVK-CAPA has also been isolated from the Gulf Coast tick *Amblyomma maculatum* (Neupert et al, 2008) and *Rhipicephalus (Boophilus) microplus* (Yang et al, 2013).

We identified transcripts encoding the CAPA receptor-like peptide in the O. turicata transcriptome sharing 76% identity with the predatory mite *Metaseiulus occidentalis* capa receptor (XM_003739621.1; e-value: 1e-32) and 70% identity with the *I. scapularis* periviscerokinin/Cap2b receptor (JQ771528.1; e-value: 4e-07). Partial sequences of the same transcripts revealed 89% identity with the red imported fire ant *Solenopsis invicta*

pyrokinin-2 receptor (JX657040.1; e-value: 0.008). We also identified contigs sharing 98% identity with *I. scapularis* conserved hypothetical protein (XM_002435093.1; e-value: 6e-48) in the *I. scapularis* transcriptome. All contigs mentioned revealed the presence of motifs of the 7 transmembrane receptor (7tm_1 [pfam00001]; e-value: 2.04e-08) common to this group.

Biological Function of CAPA peptide in Ticks

Even though the role of PVK/CAP_{2b} and PK/CAP_{2b} peptide family in water retention/diuresis is well documented in insects, its function in ticks is not known. We speculate that these peptides may be performing the same diuretic function in ticks as suggested in Neupert et al (2008). Ticks being blood feeders need to regulate water and ion balance. Quantitative qPCR data showed a 1.59 and 2.36 fold increase of the CAPA-R in synganglia of *O. turicata* and *I. scapularis* respectively in response to feeding. If indeed CAPA has diuretic capabilities, then we would expect such an increase in its expression. We speculate that the upregulation of CAPA found in *O. turicata* is expected to be highest during feeding or soon after feeding, similar to what was reported previously for other diuretic hormones (calcitonin and DH). CAPA expression found in *I. scapularis* however showed a greater increase, perhaps related to the sustained feeding process characteristic of this species. We therefore describe strong similarities in peptide sequences but differences in the time of gene expression patterns of CAPA-R in *O. turicata* and *I. scapularis*. Further work is recommended to determine the function of this peptide family in ticks.

Tachykinin-Related Peptides (TKRP)

This family of peptides present in the brain or guts of mammals and invertebrates represents by far, one of the largest peptide families with about 40 TKRPs isolated from insects, worms, molluscs, and several vertebrate tissues (Severinin et al, 2002). With several isoforms identified in arthropods, TKRPs are said to be multi-functional and are implicated in neuromodulatory roles in the CNS, control of blood flow in mammals, as well as stimulating a large number of visceral muscles. In addition, a recent study has described TKRPs as an ancestral relative of the natalisin gene implicated in the regulation of sexual/mating behavior and fecundity in insects and arthropods (Jiang, et al, 2013). We identified transcripts sharing 77% and 71% identity with the Western predatory mite *Metaseiulus occidentalis* tachykinin-like peptides receptor 99D-like (XM_003744167.1) at e-values of 1e-34 and 3e-109 in the *O. turicata* and *I. scapularis* transcriptomes respectively. Searches in the conserved domain data base revealed matches to the 7 transmembrane receptor GPCRs of the rhodopsin family (7tm_1 [pfam00001]) with e-values of 6.0e-14 and 1.55e-57 for *O. turicata* and *I. scapularis* respectively.

Biological functions of Tachykinin in Ticks

The function of this peptide has not been investigated in ticks although receptors of TKRP have been mentioned in the salivary ducts (Šimo et al, 2011). Quantitative PCR data revealed a 1.44 fold increase in expression of TKRP receptors in the synganglion of *O. turicata* females and a 0.88 fold decrease in expression of TKRP receptors in *I. scapularis* female synganglion in response to feeding. We are unable to offer concrete explanations for these observations. We can however, speculate that this peptide family may be involved in tick feeding behavior especially in gut contraction, an activity which

occurs with much higher frequency in the rapidly feeding argasids as compared to the slow blood sucking activity in ixodids. We report here high sequence similarities and yet differences in patterns of expression of TKRP and its receptors in *O. turicata* and *I. scapularis* ticks

4.5. Gene Category: Feeding Volume; Satiety Neuropeptide F (NPF).

A homologue of its equivalent neuropeptide Y in vertebrates, Neuropeptide F (NPF)-related peptides are abundant in the nervous system of most invertebrates studied (Maule et al 1995; Gonzalez and Ochard, 2009). These peptides have been implicated in several physiological roles. While some studies have revealed the role of NPF-related peptides in feeding, metabolism, reproduction and stress responses (Nässel and Wegener, 2011), other studies have suggested myoinhibitory roles, ability to decrease epithelial membrane potential (Gonzalez & Orchard, 2009); inhibiting activity of the abdominal ganglia (Maule et al, 1995); and a possible role for signaling associated with nutritional status (Garczynski, et al, 2005) in insects and other invertebrates studied.

We identified messages for the NPF receptors sharing partial sequence identity of 84% with the *I. scapularis* neuropeptide F receptor (XM_002402121.1; e-value: 4e-22) in the *O. turicata* transcriptome and messages sharing 79% identity with *I. scapularis* neuropeptide F receptor (XM_002413274.1 ; e-value: 2e-104) in the *I. scapularis* transcriptome. These contigs showed a strong alignment with the 7 transmembrane receptor (rhodopsin family) conserved domain (7tm_1 [pfam00001], at e-values of 1.04e-06 and 9.85e-38 for *O. turicata* and *I. scapularis* respectively.

Biological Function of NPF in Ticks

The biological function of NPF-related peptides has not been thoroughly investigated in ticks. Based on information available from studies in insects, we suggest possible roles for NPF in gut contraction and feeding behavior. Reverse transcriptase-PCR revealed a 0.56 fold decrease and a very high 38.32 fold increase in the expression of the transcripts of NPF-receptors in the *O. turicata* and *I. scapularis* synganglia, respectively. We see here again similarities in the gene sequences and a huge disparity in the level, pattern, and timing of gene expression in both ticks. However, the exact function regulated by this gene in the two different species is unknown.

Sulfakinin (SK)-SK-Receptors

The sulfakinin (SK) family of neuropeptides peptides have been isolated in several insects and are considered as factors that are involved in regulating the feeding and the process of digestion in insects (Liesch et al, 2013). Previously, it was implicated in neurotransmission or neuromodulation in insects and crustaceans (Johnsen et al, 2001); a satiety factor demonstrated to have inhibited feeding in the German cockroach and desert locust (Downer et al, 2006); increase heart beat frequency in cockroaches; and amplitude of hindgut contraction (Kastin, 2006).

Messages encoding SK-receptors (SK-R) were identified in both transcriptomes of the *O. turicata* and *I. scapularis* transcriptomes sharing 78% & 99% sequence identity to *I. scapularis* sulfakinin receptor (XM_002434986.1; e-value: 6e-24) and (XM_002404151.1; e-value: 2e-170) respectively.

Biological Function of SK in Ticks

Like most of the neuropeptides already mentioned, SK has not been investigated for its function in ticks. We therefore infer its function from the information presented in studies performed in insects, also supported by qPCR data from our laboratory. We speculate here that SK in ticks may be involved in feeding regulation as well as may be a satiety factor. The qPCR data revealed a 0.66 down-regulation in SK-R expression in synganglia of female fed *O. turicata* and an 11.96 fold increase of SK-R expression in part fed *I. scapularis* female synganglia. Again we see here a difference in the expression pattern in these two ticks. It is presumed that a decrease in SK-R expression would avoid early termination of blood volume intake, thereby allowing an increase blood volume/intake. This we clearly see in the *O. turicata* females as they feed and engorge within hours. In contrast, *I. scapularis* feeds slowly for 5-6 days, so that the upregulation of SK-R expression observed with the qPCR results is consistent with the need to keep feeding relatively slowly until mating. These ticks do not engorge fully until after mating, hence a reverse in the expression pattern is expected during the slow feeding period. Here again, there are very substantial similarities in the SK/SK-R sequences in both *O. turicata* and *I. scapularis*, but very great differences in gene expression consistent with their different patterns of blood feeding. Further studies are needed to fully explain how SK and SK-R regulates blood feeding in ticks.

4.6. Categories: Neurotransmitter Receptors

Here we describe receptors of 3 biogenic amine neurotransmitters (dopamine, octopamine, serotonin [5-hydroxytryptamine]), 1 acetylcholine, and 2 amino acid neurotransmitters (glutamate and GABA) in both transcriptomes of *O. turicata* and *I.*

scapularis trancriptomes (Osborne, 1996). We report in the *O. turicata* transcriptome, transcripts predicting dopamine activity with 78% identity to *I. scapularis* dopamine D1/beta receptor (XM_002435536.1; e-value: 9e-13; 7tm_1 GPCR), Octopamine with 83% identity to *I. scapularis* octopamine receptor (XM_002408768.1; e-value: 1e-42; 7tm_1 GPCR); Serotonin with 79% identity with *I. scapularis* serotonin receptor (XM_002404954.1; e-value: 3e-54; 7tm_1 GPCR). Similarly, receptors for acetylcholine neurotransmitters were identified in the transcriptomes with the presence of transcripts sharing 75% identity with *I. scapularis* acetylcholinesterase (XM_002402693.1; e-value: 3e-16; Esterase_lipase super family[cl19094]). Finally we report here messages for the metabotropic glutamate receptors sharing 71% identity with the *I. scapularis* metabotropic glutamate receptor 4, 6, 7 (XM_002413234.1 ; e-value: 1e-160; PBP1_mGluR[cd06362]), 83% identity with *I. scapularis* ionotropic glutamate receptor (XM_002409119.1; e-value: 1e-63; Periplasmic_Binding_Protein_Type_1 super family[cl10011), and 87% identity with *I. scapularis* GABA receptor (XM_002411520.1; e-value: 5e-97; Neur_chan_memb[pfam02932]).

We report similarly in the *I.scapularis* transcriptome, transcripts identified predicting dopamine activity with 78% identity to *I.scapularis* dopamine D1/beta receptor (XM_002435536.1; e-value: 2e-137; Rick_17kDa_Anti[pfam05433]), Octopamine with 100% partial sequence identity to *I. scapularis* octopamine receptor (XM_002408768.1; e-value: 0.0; 7tm_1 GPCR); Serotonin with 99% identity with *I. scapularis* serotonin 4 receptor (XM_002406426.1; e-value: 0.0; 7tm_1 GPCR). We document here, the presence of transcripts sharing 99% and 99% identity with *I. scapularis* acetylcholine receptors (XM_002407257.1; e-value: 0.0; Neur_chan_memb[pfam02932]) and

acetylcholinesterase (XM_002413971.1; e-value: 2e-133; Esterase_lipase super family[cl19094]). Finally we also report here, messages for the metabotropic glutamate receptors sharing 96% identity with the *I. scapularis* glutamate receptor (XM_002409118.1 ; e-value: 1e-162; PBP1_mGluR[cd06362]) 100% identity with *I. scapularis* ionotropic glutamate receptor (XM_002401727.1; e-value: 2e-162; PBPb[cd00134]), and 100% identity with *I. scapularis* GABA-B receptor (XM_002433605.1; e-value: 0.0; PBP1_GABAb_receptor[cd06366]).

Biological functions of neurotransmitters in Ticks

For all neurotransmitters mentioned in this work, there exists no clear documentation of their functions in argasid and ixodid ticks. However, working off of concept-building experimentation (Dircksen et al, 2013) based on primary information of structure and function of these molecules extensively studied in most insects, as well as from the high similarity of these molecules with those isolated from insects; I am able to speculate the physiological functions of these neurotransmitters in ticks. These inferences are of course, subject to further investigation by performing functional studies. I hereby suggest putative roles of candidate neurotransmitters and interpretation of gene expression data (in response to feeding) of the respective neurotransmitter receptors in both *O. turicata* and *I. scapularis* ticks.

Gene expression analysis of dopamine receptors implicated by immunochemistry as inducers of salivary secretion of ticks (Simo et al, 2011) revealed an up-regulation by 1.87 and 4.82 fold in engorged *O. turicata* and part-fed *I. scapularis* respectively. Similarly, expression of octopamine receptors of which the neurotransmitter activity is known to be multi-functional in insects (Farooqui, 2012) and to inhibit oviposition in

ticks (Cossío-Bayúgar et al, 2012) revealed a 0.34 and 0.18 fold down-regulation in *O. turicata* and *I. scapularis* respectively. Serotonin neurotransmitters (5-hydroxytrptamine) which bind and activate membrane receptors to influence aggression, sleep, circadian rhythms, visual stimuli response, and associative learning in insects (Blenau and Thamm, 2011) revealed a slight up-regulation of its receptors by 1.34 folds in synganglia of *O. turicata* and slight 1.43 fold up-regulation in *I. scapularis*. metabotropic Glutamate (i-glutamic acid) involved in fast excitatory synaptic transmission (O'Connor, 1999; Olsen, 2002)) revealed expressions of 4.76 fold in *O. turicata* and significant 39.95 fold in *I. scapularis* for the glutamate receptors (mGluR). Finally we show a 1.20 and 1.93 fold expression of γ-aminobutyric acid (GABA) receptors in the synganglia of *O. turicata* and *I. scapularis* respectively. GABA neurotransmitters are the major fast synaptic transmission inhibitors (Olsen, 2002). In spite of the similarities observed in the receptor sequences of the various neurotransmitters, I report some significant differences in their pattern and level of expression in both tick species studied. I recommend further studies on the general characteristics, structure, and function of neurotransmitters in ticks.

CHAPTER 5

SUMMARY

The neurobiology of the synganglion (central nervous system) of the Lyme disease tick, *Ixodes scapularis* and the soft tick *Ornithodoros turicata* was evaluated using Illumina GAII high throughput sequencing which generated high coverage cDNA libraries (transcriptomes). These ticks exhibit very different biological patterns of feeding, blood meal water and salt elimination, cuticle plasticity versus cuticle synthesis, development and reproduction. Two important conclusions have emerged from the comparison of the neuropeptides, neuropeptide receptors and neurotransmitter receptors in two different tick species as found in the synganglion transcriptomes. Briefly, 1) there is relatively little difference in the occurrence of transcripts encoding these peptides/proteins in the synganglia of the different ticks; 2) differences in gene expression, extent of their upregulation or downregulation, and the timing of their expression contribute to explaining many of the differences in the biology of the two different species.

For *I. scapularis, s*equencing yielded a total of 117,900,476 raw reads, which were assembled to 30,838 contigs. For *O. turicata,* sequencing yielded a total of 63,528,102, which were assembled to 132,258 contigs. Comparison of Gene Ontology (GO) mapping success for genes in 32 of the most important GO molecular categories showed relatively little difference between the two species ($t = 0.277$, $P > 0.05$, not significant). Functional assignments of transcripts predicting neuropeptides, neurotransmitter receptors and other genes of interest were done, supported by strong e-values (< -6), and high consensus sequence alignments. For the synganglion of *I. scapularis,* transcripts predicting 23 neuropeptides and/or their receptors were identified. Included were

transcripts encoding for allatotropin, allatostatin (A & B), bursicon α, calcitonin, CAPA/pyrokinin receptor, cardioacceleratory peptide, corazonin, corticotropin-releasing factor (CRF), FMRF amide, glycoprotein A, eclosion hormone, insulin-like peptide, ion transport peptide, gonadotropin-releasing hormone receptor, myoinhibitory peptide, neuropeptide F, neurophysin/oxytocin, orcokinin A, perisulfakinin, proctolin, SIFamide, sulfakinin and tachykinin. For the synganglion of *O. turicata,* transcripts predicting 25 neuropeptides and/or their receptors were identified. Both species had transcripts predicting all of the same neuropeptides and/or their neuropeptide receptors in common except for allatotropin peptide, which was found only in *I. scapularis,* and allatostatin C, bursicon β, and glycoprotein B, which were found only in *O. turicata.* Also present in both species was the enzyme pro-protein convertase, essential for converting neuropeptide preprohormones to their mature form. These results include at least 8 more transcripts encoding for different neuropeptides than were found in the *D. variabilis* synganglion transcriptomes. Transcripts predicting the same 6 neurotransmitter/neuromodulator receptors and the same 3-neurotransmitter transporters were found in the transcriptomes of both *I. scapularis* and *O. turicata.*

If the repertoire of neuropeptide and neurotransmitter messages expressed in the synganglia of *O. turicata* and *I. scapularis* is so similar, how can we explain the very different physiological processes that occur in these two very different tick species? Reverse transcriptase qPCR was used to address this question. Primers designed from the transcript and/or published gene sequences were used to assay the expression of the most of the neuropeptides and/or neurotransmitter receptors in response to blood feeding. Examples include calcitonin-diuretic hormone, bursicon and eclosion hormone. Results

for *O. turicata* show that expression of calcitonin and diuretic hormone receptors were strongly upregulated in females during (pre-drop off) and immediately after the brief feeding period (< 1 hour), when the excess blood meal water and salts are excreted, but then declined when assayed again 2 days post-drp off. In contrast, results for *I. scapularis* show that the calcitonin-like receptor was strongly upregulated during the lengthy tick-feeding period (5 – 6 days post-attachment) when excess blood meal water is secreted frequently in the saliva as the ticks feed. Results for bursicon showing strong upregulation of both bursicon α and β in adult *O. turicata* females suggest that these neuropeptides may be responsible for enhancing cuticle plasticity, i. e., the need for stretching the existing integument during the brief period of rapid blood feeding. Similarly, eclosion hormone, part of the gene pathway that regulates ecdysis, also was strongly upregulated in *O. turicata,* consistent with a role in cuticle plasticization needed by these ticks that enables them to blood feed within minutes. In contrast, the expression of only bursicon α in *I. scapularis,* along with eclosion hormone, ecdysis triggering hormone and corazonin, has been suggested (Bissinger et al. 2011) to be responsible for new cuticle synthesis during feeding. Growth of new cuticle is needed to accommodate the enormous blood meal consumed during the lengthy feeding period in this species. Contrasts in gene expression assays for several other neuropeptide regulatory genes that were done for *O. turicata* and *I. scapularis* are reviewed and their possible roles for explaining the differences the biological differences between the two species are discussed. Similarly, downregulation of sulfakinin, important in regulating satiety during blood feeding, allows the soft ticks to consume large volumes of fluid very rapidly; in contrast, its upregulation in *I. scapularis* which feeds slowly and expands gradually,

insures the opposite, so that they do not consume more volume than the slowly growing cuticle can accommodate.

The diversity of messages predicting important genes identified in this study and differences in their expression in response to feeding offers a valuable resource useful for understanding how the tick synganglion regulates important physiological functions in ticks.

5.1. Future Directions

For future studies on how neuropeptides and neurotransmitters regulate blood feeding and reproduction in the two different tick species, I will collect specimens at different intervals during and after feeding. For the soft ticks, I will collect specimens every 5 – 10 minutes during the brief (approximately 1 hour) feeding period, followed by collections at 24 and 48 hours after feeding. For the hard ticks, I will collect specimens 2, 4 and 6 days after attachment, 24 hours after mating, and 2 and 4 days after drop off (repletion). This will enable me to monitor the time course in changes in gene expression and compare the findings with the different biological patterns for these two species. Gene analysis will be done for the several different gene categories reported in the dissertation. In addition, RNAi of selected genes will be done to assess gene function. In addition single gene knockdown studies, RNAi with pooled gene constructs will be done since it is possible that knockdown of only one gene in a complex regulatory pathway (e.g. calcitonin/DH) may not be sufficient to fully disrupt the process.

REFERENCES

Alstein, M. and Dick, R.N., (2010). Neuropeptide signaling in insects. In: *Neuropeptide systems as targets for parasite and pest contr*ol (Geary GT, Maule AG, eds) Springer Landes Bioscience and Springer Science+Business Media. New York, NY.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) Basic local alignment search tool. *J Mol Biol* **215**: 403-410

Altstein, M. and Nässel, D.R. (2010) Neuropeptide signaling in insects. *Adv Exp Med Biol* **692**:155-165.

An, S., Songjie, W., David, S. and Qisheng, S. (2009) Identification of a novel bursicon-regulated transcriptional regulator, Md13379, in the house fly *Musca domestica*. *Arch. of Insect Biochem. & Physiol* **70**: 1-16. doi:10.1002/arch.20283.

Anderson, J., Sonenshine, D.E. and 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.

Alzugaray, M.E., Mariana. L., Diambra, A.L.A., Hernandez-Martinez, S., Damborenea, C., Noriega, F.G., et al. (2013) Allatotropin: an ancestral myotropic neuropeptide involved in feeding. *PLoS One* **8**: 10 E77520. doi:10.1371/journal.pone.0077520.

Bacon, R.M., Kuegler, K.J. and Mead, P.S. (2008) Surveillance for Lyme disease-United States, 1992-2006. *MMWR Sureill Summ* **57**: 1-9.

Blenau, W. and, Thamm, M. (2011) Distribution of serotonin (5-HT) and its receptors in the insect brain with focus on the mushroom bodies. Lessons from *Drosophila melanogaster* and *Apis mellifera*. *Arthropod Struct & Dev* **40**: 381-94. doi:10.1016/j.asd.2011.01.004.

Buckingham, S., Biggin, P.C., Satelle, B.M., Brown, L.A. and Satelle, D.B. (2005) Insect GABA receptors: splicing, editing and targeting by antiparasities and insecticides. *Mol Pharmacol* **68**: 942-951.

Bürgmann, H., Widmer, Sigler, W.V. and Zeyer, J. (2003) mRNA Extraction and reverse transcription-PCR protocol for detection of NifH gene expression by *Azotobacter vinelandii* in Soil. *App Environ Microbiol* **69**: 1928-935.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M. et al. (2009). The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem* **55**: 611-22.

Bissinger, B., Donohue, K.V., Khalil, S.M., Grozinger, C.M., Sonenshine, D.E., Zhu, R. and Roe, M. (2011) Synganglion transcriptome and developmental global gene expression in 153 adult females of the American dog tick, *Dermacentor variabilis*. *Insect Mol Biol* **20**: 465-91.

Buckingham, S., Biggin, P.C., Satelle, B.M., Brown, L.A. and Satelle, D.B. (2005) Insect GABA receptors: splicing, editing, and targeting by antiparasitics and insecticides. *Mol Pharmacol* **68**: 942-951.

Caers, J., Verlinden, H., Zels, S., Vandersmissen, H.P., Vuerinckx, K. and Schoofs, L. (2012) More than two decades of research on insect neuropeptide GPCRs: an overview. *Front Endocrinol* **3**:151.

Chapman, R. (1998) *The insects*. Cambridge University Press, Cambridge UK.

Carroll, J.F. (2002) How specific are host-produced kairomones to host-seeking ixodid ticks? *Exp Appl Acarol*. **28**: 155–161.

Carroll, J.F. and Schmidtmann, E.T. (1996) Dispersal of blacklegged tick (Acari: Ixodidae) nymphs and adults at the woods-pasture interface. *J Med Entomol* **33**: 554–558.

Cazzamali, G., Saxild, N. and Grimmelikhuijzen, C. (2002) Molecular cloning and functional expression of a *Drosophila* corazonin receptor. *Biochem Biophys Res Commun* **298**:31-6

Centers for Disease Control and Prevention (2013) Lyme disease data. Available: www.cdc.gov/osels/ph/_surveillance/nndss/casedef/lyme_disease_2008.htm.

Choi, M. and Vander Meer, R.K. (2012) Molecular structure and diversity of PBAN/pyrokinin family peptides in ants. *Front Endocrinol* **20**: 323-343.

Christie, A. (2008) Neuropeptide discovery in ixodoidea: an in silico investigation using publicly accessible expressed sequence tags. *Gen Comp Endocrinol*. **157**: 174-185.

Christie, A.E., Stemmler, E.A. and Dickinson, P.S. (2010) Crustacean neuropeptides. *Cell Mol Life Sci* **67**: 4135-4169.

Christie, A.E., Stevens, J.S., Bowe, M.R., Chapline, M.C., Jensen, D.A., Schegg, K.M. et al (2009) Identification of a calcitonin-like diuretic hormone that functions as an intrinsic modulator of the American lobster, *Homarus americanus*, cardiac neuromuscular system. *J Exp Biol* **213**: 118-27. doi:10.1242/jeb.037077.

Clottens, F.L., Holman, G.M., Coast, G.M., Totty, N.F., Hayes T.K., Kay, I. et al. (1994) Isolation and characterization of a diuretic peptide common to the house fly and stable fly. *Peptides* **15**: 971-79. doi:10.1016/0196-9781(94)90059-0. 154

Coast, G.M. (1996) Neuropeptides implicated in the control of diuresis in insects. *Peptides* **17**: 327-36. doi:10.1016/0196-9781(95)02096-9.

Coast, G.M., Webster, S.G., Schegg, K.M., Tobe, S.S., Schooley, D.A. (2001) The *Drosophila melanogaster* homologue of an insect calcitonin-like diuretic peptide stimulates V-ATPase activity in fruit fly Malpighian tubules. *J Expt Biol* **204**: 1795-804.

Colpitts, T.M., Cox, J., Vanlandingham, D.L., Feitosa, F.M., Cheng, G., Kurscheid, S. et al. (2011) Alterations in the *Aedes aegypti* transcriptome during infection with West Nile, dengue and yellow fever viruses. *PLoS Pathog* **7**: e1002189.

Conesa, A., Gotz, S., Garcia-Gomez, J.M., Tero, l.J., Talon, M. and Robles M. (2005) BLAST2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* **21**: 3674-3676.

Cossío-Bayúgar, R., Miranda-Miranda, E., Narváez, P.V., Olvera-Valencia, F. and Reynaud, E. (2012). Perturbation of tyraminergic/octopaminergic function inhibits oviposition in the cattle tick *Rhipicephalus (Boophilus) microplus*. *J Insect Physiol* **58**: 628-33

Davies, S.A., Cabrero, P., Povsic, M., Johnston, N.R., Terhzaz, S. and Dow, J.A. (2013) Signaling by *Drosophila* capa neuropeptides. *Gen Comp Endocrinol* **188**: 60-66

Dewey, E.M., Mcnabb, S.L., Ewer, J., Kuo, G.R., Takanishi, L.C., Truman, J.W., Hans-Willi, D. and Honegger, H. (2004) Identification of the gene encoding bursicon, an insect neuropeptide responsible for cuticle sclerotization and wing spreading. *Current Biol* **14**: 1208-213. doi:10.1016/j.cub.2004.06.051.

Dircksen H, Neupert S, Predel R, Verleyen P, Huybrechts J, Strauss J, Hauser F, Stafflinger E, Schneider M, Pauwels K. et al. (2011). Genomics, transcriptomics, and peptidomics of *Daphnia pulex* neuropeptides and protein hormones. J. Proteome Res. **10**: 4478-4504.

Dircksen, H. (2013) Crustacean bioactive peptides. In: *Handbook of biologically active peptides* (Abba Kastin eds). pp 209-228 Academic Press, Waltham, MA.

Donohue, K.V., Khalil, S.M.S., Ross, E., Grozinger, C.M., Sonenshine, D.E. and Roe R.M. (2010) Neuropeptide signaling sequences identified by pyrosequencing of the American dog tick synganglion transcriptome during blood feeding and reproduction. *Insect Biochem. Mol Biol* **40**: 79-90.

Downer, K.E., Haselton, A.T., Nachman, R.J. and Stoffolano, J.G. (2007). Insect satiety: sulfakinin localization and the effect of drosulfakinin on protein and carbohydrate ingestion in the blow fly, *Phormia regina* (Diptera: Calliphoridae). *J Insect Physiol* **53**: 106-12. doi:10.1016/j.jinsphys.2006.10.013.

Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A. et al. (2011) Geneious V5.5. "http://www.geneious.com.

Dulcis, D., Levine, R.B. and Ewe, J. (2005) Role of the neuropeptide CCAP in *Drosophila* cardiac function. *Dev Neurobiol* **64**: 259-74. doi:10.1002/neu.20136.

Durden, L.A. and Beatii, L. (2014) Modern Tick Systematics. In *Biology of ticks 2nd edition* (Sonenshine, D.E. and Roe, R.M. eds) Vol 1 pp. 17-58. Oxford University Press, New York, NY.

Egekwu, N., Sonenshine, D.E., Bissinger, B.W. and Roe, R.M. (2014). Transcriptome of the female synganglion of the black-legged tick *Ixodes scapularis* (Acari: Ixodidae) with comparison between Illumina and 454 systems. *PLoS One* **9**: e102667.

Estevez-Lao, T.Y., Boyce, D.S., Honegger, H.W. and Hillyer, J.F. (2013). Cardioacceleratory function of the neurohormone CCAP in the mosquito *Anopheles gambiae*. *J Expt Biol* **216**: 601-13. doi:10.1242/jeb.077164.

Fan, Y., Pereira, R.M., Kilic, E., Casella, G. and Keyhani, N.O. (2012). Pyrokinin β-neuropeptide affects necrophoretic behavior in fire ants (*S. Invicta*), and expression of β-NP in a mycoinsecticide increases its virulence. *PLoS One* **7**: E26924. doi:10.1371/journal.pone.0026924.

Farooqui, T. (2012). Review of octopamine in insect nervous systems. Open Access *Insect physiol* **4**: 1-17.

Fleige, S. and Pfaffl, M.W. (2006) RNA Integrity and the effect on the real-time qRT-PCR performance. *Mol Aspects Med* **27**: 126-39.

Francischetti, I.M., Sa-Nunes, A., Mans, B.J., Santos, I.M. and Ribeiro, J.M. (2009). The role of saliva in tick feeding. *Front Biosci* **14**: 2510-2088.

Fraenkel, G. and Hsiao, C. (1965), Bursicon, a hormone which mediates tanning of the cuticle in the adult fly and other insects. *J Insect Physiol* **11**: 513-556.

Furman, D.P. and Loomis, E.C. (1984) *The ticks of California (Acari: Ixodida)* University of California Press, Berkeley, CA.

Furuya, K.R., Milchak, R.J., Schegg, K.M., Zhang, J., Tobe, S.S., Coast, G.M. and Schooley, D.A. (2000) Cockroach diuretic hormones: Characterization of a

calcitonin-like peptide in insects. *PNAS* **9**: 6469-474. doi:10.1073/-pnas.97.12.6469.

Gaillard, I., Rouquier, S. and Giorgi, D. (2004) Olfactory receptors. *Cell Mol Life Sci* **61**:456-469.

Gammie, S.C. and Truman, J. (1999) Eclosion hormone provides a link between ecdysis-triggering hormone and crustacean cardioactive peptide in the neuroendocrine cascade that controls ecdysis behavior. *J Exp Biol* **202**: 343-52.

Garczynski, S.F., Crim, J.W., Brown, M.R. (2005) Characterization of neuropeptide F and its receptor from the African malaria mosquito, *Anopheles gambiae. Peptides* **26**: 99-107. doi:10.1016/j.peptides.2004.07.014. 156

Geary, T.G. and Maule, A.G. (2010) *Neuropeptide Systems as Targets for Parasite and Pest Control.* Springer Science+Business Media, New York, NY.

Gonzalez, R. and Orchard, I. (2009). Physiological activity of Neuropeptide F on the hindgut of the blood-feeding hemipteran, *Rhodnius prolixus. J Insect Sci* **57**, 9

Gray, J.S., Estrada-Peña, A. and Vial, L. (2014). Ecology of nidicolous ticks. P. 39-60. In: *Biology of Ticks 2nd edition* (Sonenshine, D.E. and Roe, R.M. eds.) Vol. 2, pp. 39-60, Oxford University Press, New York, NY.

Harmer, D. (2013). Illumina Genome Analyzer II; The New Gold-standard for next now generation sequencing! Lecture. Accessed 2013. http://ncifrederick.cancer.gov/atp/cms/wp-content/uploads/2010/08/CustomerIlluminaTechExplanation.pdf.

Hartfelder, K. and Emlen, D.J. (2011). Endocrine control of insect polyphenism. In: *Insect Endocrinology* (Gilbert, L.I. eds.) pp. 464-522, Elsevier, Amsterdam.

Hellmich, E., Nusawardani, T., Bartholomay, L. and Russell, J. (2014) Pyrokinin/PBAN-like peptides in the central nervous system of mosquitoes. *Cell Tissue Res* **356**: 39-47. doi:10.1007/s00441-013-1782-8.

Hergarden, A.C., Tayler, T.D. and Anderson, D.J. (2012) Allatostatin-A neurons inhibit feeding behavior in adult *Drosophila. PNAS* **109**: 3967-972.

Hill, C.A., Meyer, J.A., Waterhouse, R.M., Miller, J.R., Walenz, B.P. et al. The genome sequence of the Lyme disease tick, *Ixodes scapularis*. Manuscript for submission to *BMC Genomics*.

Honegger, H., Dewey, E.M. and Ewer, J. (2008) Bursicon, the tanning hormone of insects: Recent advances following the discovery of its molecular identity. *Comp Physiol* [A] **194**: 989-1005. doi:10.1007/s00359-008-0386-3.

Hökfelt, T., Broberger, C., Xu, C.Z.D., Sergeyev, V., Ubink, R. and Diez, M. (2000) Neuropeptides—An Overview. *Neuropharmacology*: **39**: 1337-356.

Hoskins, J.D. (1991) Ixodid and argasid ticks. Keys to their identification. *Vet Clin North Am Small Anim Pract* **21**: 185-87.

Hurd, P. and Nelson, C.J. (2009) Advantages of next-generation sequencing versus the microarray in epigenetic research. *Briefings Functional Genom Proteom* **8**: 174-183. 157

Jiang, H., Lkhagva, A., Daubnerová, I., Chae, H.S., Šimo, L., Jung, S.H. et al. (2013) Natalisin, a tachykinin-like signaling system, regulates sexual activity and fecundity in insects. *PNAS* **110**: E3526-3534.

Jindra, M., Palli, S.R. and Riddiford, L.M. (2013) The juvenile hormone signaling pathway in insect development. *Annu Rev Entomol* **58**: 181-204.

Johnsen, A.H., Hanne, D., Marlene, D., Mike, H. and Thorpe, A. (2001) Sulfakinin neuropeptides in a crustacean isolation, identification and tissue localization in the tiger prawn *Penaeus monodon*. *Eur J Biochem* **267**: 1153-160.

Jongejan, F. and Uilenberg, G. (2004). The global importance of ticks. *Parasitology*. **129** Suppl: S3-14. Review.

Kastin, A.J. (2006) *Handbook of Biologically Active Peptides*. Academic Press, Amsterdam.

Kaufman, R.W. (2014). Integument and Ecdysis.. In: *Biology of Ticks.2nd edition* (Sonenshine, D.E. and Roe, R.M.), vol 2, pp. 99-121Oxford University Press, NY, NY.

Kaufman, W.R., Flynn, P.C. and Reynolds, S.E. (2010). Cuticular plasticization in the tick, *Amblyomma hebraeum* (Acari: Ixodidae): Possible roles of monoamines and cuticular pH. *J Exp Biol* **213**: 2820-831. doi:10.1242/jeb.044412.

Kay, I. and Wheeler, C.H. (1993) Diuretic peptides in the house cricket, *Acheta domesticus* (L.): *A possible dual control of Malpighian tubules. In: Structure and function of primary messengers in invertebrates: Insect diuretic and antidiuretic peptides* (Klaus, W.B. and Coast, G.M. eds.). pp. 38-66, Basel. NewYork, NY.

Kingan, T.G., Gray, W., Žitňan, D. and Adams, M.E. (1997). Regulation of ecdysis-triggering hormone release by eclosion hormone. *J Exp Biol* 200: 3245-256.

Kim, Y.J., Spalovská-Valachová I, Cho, K.H., Zitnanova, I., Park, Y., Adams, M.E. and Zitnan, D. (2004) Corazonin receptor signaling in ecdysis initiation. *PNAS* **101**: 6704-6709. Epub 2004 Apr 19.

Kiszewski, A.E., Matuschka, F.R. and Spielman, A. (2001) Mating strategies and spermiogenesis in ixodid ticks. *Annu Rev Entomol* **46**: 167-82.

Khalil, S.M., Donohue, K.V., Thompson, D.M., Jeffers, L.A., Ananthapadmanaban, U. Sonenshine, D.E. et al. (2011). Full-length sequence, regulation and developmental studies of a second vitellogenin gene from the American dog tick, *Dermacentor variabilis*. *J Insect Physiol* **57**: 400-408.

Koutsos, A.C., Blass, C., Meister, S., Schmidt, S., MacCallum, R.M., Soares, M.B., Collins, F.H. et al. (2007) Life cycle transcriptome of the malaria mosquito Anopheles gambiae and comparison with the fruit fly *Drosophila melanogaster*. *PNAS* **104**: 11304-11309. Epub 2007

Kuschel, M. (2000) Analysis of Messenger RNA using the Agilent 2100 Bioanalyzer and the RNA 6000 LabChip® Kit Application Note. Agilent Technologies. **158**

Labbé, R.M., Irimia, M., Currie, K.W., Lin, A., Zhu, S.J. et al. (2012). A comparative transcriptomic analysis reveals conserved features of stem cell pluripotency in planarians and mammals. *Stem Cells* **8**: 1734-45. doi: 10.1002/stem.1144

Lahr, E.C., Dean, D. and Ewer, J. (2012) Genetic analysis of ecdysis behavior in *Drosophila* reveals partially overlapping functions of two unrelated neuropeptides. *J Neurosci* **32**: 6819-829. doi:10.1523/JNEUROSCI.5301-11.2012.

Lee, C., Kim, J., Shin, S.G. and Hwang, S. (2006) Absolute and relative QPCR quantification of plasmid copy number in *Escherichia Coli*. *J Biotech* **123**: 273-80. doi:10.1016/j.jbiotec.2005.11.014.

Lees, K., Woods, D.J. and Bowman, A.S. (2010) Transcriptome analysis of the synganglion from the brown dog tick, *Rhipicephalus sanguineus*. *Insect Mol Biol* **19**: 273-282.

Li, H., Wang, H., Schegg, K.M. and Schooley, D.A. (1997) Metabolism of an insect diuretic hormone by Malpighian Tubules studied by liquid chromatography coupled with electrospray ionization Mass spectrometry. *PNAS* **94**: 13463-3468. doi:10.1073/pnas.94.25.13463.

Liesch, J., Bellani, L.L. and Vosshall, L.B. (2013) Functional and genetic characterization of neuropeptide Y-like receptors in *Aedes aegypti*. *PLoS Negl Trop Dis* **7**: E2486. doi:10.1371/journal.pntd.0002486.

Lindgren, E. and Jaenson, T.G.T. (2006) Lyme borreliosis in Europe: influences of climate and climate change, epidemiology, ecology and adaptation measures. Copenhagan, Denmark: *World Health Organization* pp. 1-34.

Livak, K.J. and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* **25**: 402-408.

Loof, A.D., Lindemans, M., Liu, F., Groef, B.D. and Schoofs, L. (2012) Endocrine Archeology: Do insects retain ancestrally inherited counterparts of the vertebrate releasing hormones GnRH, GHRH, TRH, and CRF? *Gen Comp Endocrinol* **177**: 18-27. doi:10.1016/j.ygcen.2012.02.002.

Loi, P.K., Emmal, S.A., Park, Y. and Tublitz, N.J. (2001) Identification, sequence and expression of a crustacean cardioactive peptide (CCAP) gene in the moth *Manduca sexta. J Exp Biol* **204**: 2803-2816.

Tublitz, N.J. and Loi, P.K. (2004) Sequence and expression of the CAPA/CAP2b gene in the tobacco hawkmoth, *Manduca sexta. J Exp Biol* **207**: 3681-3691.

Loy, T.V., Matthias, B., Van, H., Peter, H., Poels, V.J., Mendive, F., Vassart, M.F. and Broeck (2007) Evolutionary conservation of bursicon in the animal kingdom. *Gen Comp Endocrinol* **15**: 59-63. doi:10.1016/j.ygcen.2006.12.004.

Mans, B.J., Ribeiro, J.M. and Andersen, J.F. (2008 a) Structure, function and evolution of biogenic amine-binding proteins in soft ticks. *J Biol Chem* **283**: 18721-18733.

Mans, B.J., Andersen, J.F., Francischetti, I.M., Valenzuela, J.G. and Schwan, T.G. (2008 b) Comparative sialomics between hard and soft ticks: implications for the evolution of blood-feeding behavior. *Insect Biochem Mol Biol* **38**: 42-58

Mardis, E.R. (2011) A decade's perspective on DNA sequencing technology. *Nature* **470**: 198–203.

Maule, A.G., Halton, D.W. and Shaw, C. (1995) Neuropeptide F: A ubiquitous invertebrate neuromediator? *Hydrobiologia* **305**: 297-303. doi:10.1007/BF00036410.

Mulenga, A. (2014) Molecular biology and physiology of chemical communication. In: *Biology of Ticks 2nd edition* (Sonenshine, D.E. and Roe, R.M. eds). Vol 2 pp. 368-397, Oxford University Press, NewYork, NY.

Neupert, S., Russell, W.K., David, H., Russell, D.H., Strey, O.F., Teel, P.D., Strey, A. and Nachman, R.J. (2008) Identification of a CAPA-PVK (IXORI-PVK) from single cells of the Gulf Coast tick, *Amblyomma maculatum. Pestycydy* **1-2**: 67-73.

Nassel, D. (2002) Neuropeptides in the nervous system of *Drosophila* and other insects: Multiple roles as neuromodulators and neurohormones. *Progress in Neurobiology* **68**: 1-84.

Nässel, D.R. and Wegener, C. (2011) A comparative review of short and long neuropeptide F signaling in invertebrates: Any similarities to vertebrate neuropeptide Y signaling? *Peptides* **32**: 1335-355. doi:10.1016/j.peptides.2011.03.013.

Needham, G.R. and Teel, P.D. (1991) Host physiological ecology of ixodid ticks. *Annu Rev Entomol* **36**: 659-81. doi:10.1146/annurev.en.36.010191.003303.

Neupert, S., Russell, W.K, Predel, R., Russell, D.H, Strey, O.F. et al. (2009) The neuropeptidomics of Ixodes scapularis synganglion. *J Proteomics* **72**: 1040-1045.

Novo, M., Riesgo, A., Fernández-Guerra, A. and Giribet, G. (2013): Pheromone evolution, reproductive genes, and comparative transcriptomics in mediterranean earthworms (annelida, oligochaeta, hormogastridae). *Mol Biol Evol* **7**: 1614-1629. doi: 10.1093/molbev/mst074

Obenchain, F.D., Galun, R. (1982) *Physiology of Ticks*. Pergamon Press, Oxford (Oxfordshire), UK.

O'connor, V. (1999). Calmodulin dependence of presynaptic metabotropic glutamate receptor signaling. *Science* **286**: 1180-184. doi:10.1126/ science. 286. 5442.1180.

Okura, N., Hiroyasu, K., Takayuk, M. and Satoshi, S. (1996). Cuticular plasticization induced by copulatory stimuli in female *Haemaphysalis longicornis*. *J Med Entomol* **33**: 702-05.

Olsen, S.S., Cazzamali, G., Williamson, M.G. and Hauser, F. (2007) Identification of one capa and two pyrokinin receptors from the malaria mosquito *Anopheles gambiae*. *Biochem Biophys Res Commun* **362**: 245-251.

Osborne, R.H.(1996) Insect Neurotransmission: Neurotransmitters and their receptors. *Pharmacol Ther* **69**: 117-42. doi:10.1016/0163-7258(95)02054-3.

Oliver, J.H. Jr, Zhu, X.X., Vogel, G.N., Dotson, E.M. (1992). Role of synganglion in oogenesis of the tick *Ornithodoros parkeri*. *J Parasitol* **78**: 93-98.

Paluzzi, J. and O'Donell, M. J. (2012). Identification, spatial expression analysis and functional characterization of a pyrokinin-1 receptor in the Chagas' disease vector, *Rhodnius prolixus*. *Mol Cell Endocrinol* **363**: 36-45.

Paluzzi, J.P., Park, Y., Nachman, R.J. and Orchard, R. (2010) Isolation, expression analysis, and functional characteristics of the first antidiuretic hormone receptor in insects. *PNAS* **107**:10290-10295

Park, Y., Zitnan, D., Gill, S.S. and Adams, M.E. (1999) Molecular cloning and biological activity of ecdysis-triggering hormones in *Drosophila melanogaster*. *FEBS Letters* **463**: 133-38. doi:10.1016/S0014-5793 (99)01622-1.

Park, Y., Filippov, V., Gill, S.S. and Adams, M.E. (2002) Deletion of the ecdysis-triggering hormone gene leads to lethal ecdysis deficiency. *Dev* **129**: 493-503. doi:10.1242/jeb.00261.

Park, Y., Kim, Y., Dupriez, V. and Adams, M.E. (2003) Two subtypes of ecdysis-triggering hormone receptor in *Drosophila melanogaster*. *J Biol* Chem **278**: 17710-7715.

Parola, P. and Raoult, D. (2001) Tick-borne bacterial diseases emerging in Europe. *Clin Microbiol Infect* **7**: 80-83.

Pearson, W.R. and Lipman, D.J. (1988) Improved tools for biological sequence comparison. *PNAS* **85**: 2444–8. doi:10.1073/pnas.85.8.2444. PMC 280013. PMID 3162770

Predel, R. and Wegener, C. (2006) Biology of the CAPA peptides in insects. *Cell Mol Life Sci* **63**: 2477-490. doi:10.1007/s00018-006-6187-3.

Predel, R, Neupert, S., Russell, W.K., Scheibner, O. and Nachman, R.J. (2007) Corazonin in insects. *Peptides* **28**: 3-10. doi:10.1016/j.peptides.2006.10.011.

Preparing DNA libraries for sequencing on the MiSeq®. Accessed 2013. www.illumina.com/support/kits. 161

Quinlan, M.C., Tublitz, N.J., O'Donnell, M.J. (1997) Anti-diuresis in the blood-feeding insect Rhodnius prolixus Stal: The peptide CAP2b and cyclic GMP inhibit Malpighian tubule fluid secretion. *J Exp Biol* **200**: 2363-2367.

Raina, A.K., Jaffe, H., Kempe, T.J., Keim, P, Blache, R.W. et al. (1989) Identification of a neuropeptide hormone that regulates sex pheromone production in female moths. *Science* **244**: 796-98. doi:10.1126/science.244.4906.796.

Randolph, S.E. (2014). Ecology of non-nidicolous ticks. In *Biology of Ticks, 2nd edition* (Sonenshine, D.E., and Roe, R.M. eds). pp. 1 -38. Oxford University Press, New York, NY.

Randolph, S.E. and Storey, K. (1999) Impact of microclimate on immature tick-rodent host interactions (Acari: Ixodidae): implications for parasite transmission. *J Med Entomol* **36**: 741-748.

Reynolds, S. E. (1983) Bursicon. In *Endocrinology of insects* (Downer R.G.H., Raufer H.L.eds). pp. 235-248, Alan R. Liss Inc., New York, NY.

Roe, M.R., Donohue, K.D., Khalil, S.M.L., Bissinger, B.W., Zhu, J. and Sonenshine. D.E. (2014) Hormonal Regulation of Metamorphosis and Reproduction in Ticks. In *Biology of Ticks 2nd edition* (Sonenshine, D.E. and Roe, R.M. eds). Vol. 1, pp. 437-439. Oxford University Press, New York, NY.

Roller, L., Žitňanová, I., Dai, L., Šimo, L., Park, Y. et al. (2010). Ecdysis triggering hormone signaling in arthropods. *Peptides* **31**: 429-41. doi:10.1016/j. peptides.2009.11.022.

Ribeiro, J.M.C., Makoul, G., Levine, J., Robinson, D. and Spielman, A. (1985) Antihemostatic, anti-inflammatory and immunosuppressive properties of the saliva of a tick, *Ixodes dammini*. *J Exp Med* **161**:332–344.

Sakai, T., Satake, H., Minakata, H. and Takeda.M. (2004) Characterization of crustacean cardioactive peptide as a novel insect midgut factor: Isolation, localization, and stimulation of α-Amylase activity and gut contraction. *Endocrinol* **145**: 5671-5678 doi:http://dx.doi.org/10.1210/en.2004-0722.

Settembrini, B.P., de Pasquale, D. Postal, M., Pinto, P.M., Carlini, C.R. and Villar. M.J. (2011) Distribution and characterization of corazonin in the central nervous system of *Triatoma infestans* (Insecta: Heteroptera). *Peptides* **32**: 461-468.

Severini, C. (2002) The Tachykinin peptide family. *Pharmacol Rev* **54**: 285-322. doi:10.1124/pr.54.2.285.

Schmittgen, T.D., Livak, K.J. (2008) Analyzing real-time PCR data by the comparative CT method. *Nat Protoc* **3**: 1101-1108.

Sharp, J.H., Wilcockson, D.C. and Webster, S.G. (2010) Identification and expression of mRNAs encoding bursicon in the plesiomorphic central nervous system of *Homarus gammarus*. *Gen Comp Endocrinol* **169**: 65-74. doi:10.1016/j.ygcen.2010.07.006.

Šimo, L., Slovák, M., Park, Y. and Žitňan, D. (2009a) Identification of a complex peptidergic neuroendocrine network in the hard tick, *Rhipicephalus appendiculatus*. *Cell Tissue Res* **335**: 639–655.

Šimo, L. Žitňan, D. and Park, Y. (2009b) Two novel neuropeptides in innervation of the salivary glands of the black-legged tick, *Ixodes scapularis*: myoinhibitory peptide and SIFamide. *J Comp Neurol* **517**:551-563.

Šimo, L, Žitňan, D., and Park, Y. (2011) Neural Control of salivary glands in ixodid ticks *J Insect Physiol* **58**: 459-466. doi:10.1016/j.jinsphys.2011.11.006.

Šimo, L, Koči, J., Žitňan, D. and Park, Y. (2011) Evidence for D1 dopamine receptor activation by a paracrine signal of dopamine in tick salivary glands. *PLoS One* 6: e16158

Šimo, L., Koči, J. and Park, Y. (2013) Receptors for the neuropeptides, myoinhibitory peptide and SIFamide, in control of the salivary glands of the blacklegged tick *Ixodes scapularis*. *Insect Biochem Mol Biol* **43**: 376-87. doi:10.1016/j.ibmb.2013.01.002.

Simo, L., Sonenshine, D.E., Park, Y. and Zitnan, D. (2014) *In Biology of Ticks 2nd Edition*. (Sonenshine, D.E. and Roe, R.M. eds). Vol. 1., pp. 309-367. Oxford University Press, New York, NY.

Sokal, R, Michener, C. (1958). A statistical method for evaluating systematic relationships. *University of Kansas Science Bulletin* **38**: 1409–1438.

Sonenshine, D.E. (1985). Pheromones and other semiochemicals of the Acari. *Annu Rev Entomol* **30**: 1-28. doi:10.1146/annurev.ento.30.1.1.

Sonenshine, D.E. (1991) *Biology of Ticks*. Vol. 1. pp. 1 -447. Oxford University Press, New York, NY.

Sonenshine, D.E. (1999) Pheromones and other semiochemicals of ticks and their use in tick Control. *Parasitology* **129**: S405-425. doi:10.1017/S003118200400486X.

Sonenshine, D.E. (2005). The biology of tick vectors of human disease. In *Tick-borne diseases of humans* (Goodman, J.L., Dennis, D.T. and Sonenshine, D.E. eds). pp 12 – 36. ASM Press, Washington, D.C.

Sonenshine, D.E. (2006) Tick pheromones and their use in tick control. *Annu Rev Entomol* **51**: 557-80.

Sonenshine, D.E. and Roe, R.M. (2014). Overview. Ticks, people and animals. In *Biology of ticks 2nd edition* (Sonenshine, D.E. and Roe, R.M. eds), Vol. 1. pp 3 – 16. Oxford University Press, New York, NY.

Sonenshine, D.E., Silverstein, R.M. and West, J.R. (1984). Occurrence of sex attractant pheromone, 2,6-dichlorophenol, in relation to age and feeding in American dog tick, Dermacentor variabilis (say) (Acari: Ixodidae). *J Chem Ecol* **10**: 95-100. doi:10.1007/BF00987646.

Song, Q. and An, S. (2011). Bursicon, a neuropeptide hormone that controls cuticle tanning and beyond. Recent Advances in Entomological Research: From Molecular Biology to Pest Management, In (Tongxian, L, Kang, L. eds). pp. 132-147. Higher Education Press, Beijing, China.

Stay, B. and Tobe, S.S. (2007) The role of allatostatins in juvenile hormone synthesis in insects and crustaceans *Annu Rev Entomol* **52**: 277-99.

Szlendak, E. and Oliver, J.H. Jr. (1992) Anatomy of synganglia, including their neurosecretory regions, in unfed, virgin female *Ixodes scapularis* (Acari: Ixodidae). *J Morphol* **213**: 349–364.

Tan, E.M., Yamaguchi, Y., Horwitz, G.D., Gosgnach, S., Lein, E.E. et al. (2007) Selective and quickly reversible inactivation of mammalian neurons in vivo using the *Drosophila* allatostatin receptor. *Neuron* **51**: 157-70.

Tanaka, Y., Ishibashi, .J. and Tanaka, S.(2003) Comparison of structure-activity relations of corazonin using two different bioassay systems. *Peptides* **24**: 837-844.

Tanaka, S., Zhu, D.H., Hoste, B. and Breuer, M. (2002) The dark-color inducing neuropeptide, [His (7)]-corazonin, causes a shift in morphometic characteristics towards the gregarious phase in isolated-reared (solitarious) *Locusta migratoria*. *J Insect Physiol* **48**: 1065-1074.

Teal, P.E.A. (2002) Effects of allatotropin and allatostatin on in vitro production of juvenile hormones by the corpora allata of virgin females of the moths of *Heliothis virescens* and *Manduca sexta*. *Peptides* 23: 663-69. doi:10.1016/S0196-9781(01)00660-X.

Technical Workflow. CBot Support: http://support.illumina.com/sequencing/sequencing_instruments/cbot/workflow.ilmn. Accessed 2013.

Terhzaz, S., Cabrero, P., Robben, J.H., Radford, J.C., Hudson, B.D. et al. (2012) Mechanism and Function of *Drosophila* CAPA GPCR: A desiccation stress-responsive receptor with functional homology to human neuromedin receptor. *PLoS One* **7**: E29897. doi:10.1371/journal.pone.0029897.

Terhzaz, S., Philippe, R., Goodwin, S.F. and Veenstra, J,A, (2007). The neuropeptide SIFamide modulates sexual behavior in *Drosophila*. *Biochem Biophys Res Commun* **352**: 305-310. doi:10.1016/j.bbrc.2006.11.030.

Temeyer, K.B., Tuckow, A.P., Brake, D.K., Li, A.Y. and Pérez de León, A.A. (2012) Acetylcholinesterases of blood-feeding flies and ticks. *Chem Biol Interact* **203**: 319-322.

Tillman, J.A., Seybold, S.J., Jurenka, R.A. and Blomquist, G.J. (1999) Insect Pheromones—an Overview of biosynthesis and endocrine regulation. *Insect Biochem Mol Biol* **29**: 481-514. doi:10.1016/S0965-1748(99)00016-8.

Touhara, K. and Vosshall, L.B. (2009) Sensing odorants and pheromones with chemosensory receptors. *Annu Rev Physiol* **71**: 307-32. doi:10.1146/annurev.physiol. 010908. 163209.

Tublitz, N.J., Allen, A.T., Cheungm, C.C., Edwards, K.K., Kimble, D.P., Loi P.K. and Sylwester, A.W. (1992) Insect cardioactive peptides: regulation of hindgut activity by cardioacceleratory peptide 2 (CAP2) during wandering behaviour in *Manduca sexta* larvae. *J Exp Biol* **165**: 241-64.

Van Wielendaele, P., Badisco, L. and Vanden Broeck, J. (2013) Neuropeptidergic regulation of reproduction in insects. *Gen Comp Endocrinol* **188**: 23-34.

Vázquez-Acevedo, N., Rivera, N.M., Torres-González. A.M., Rullan-Matheu, Y., Ruíz-Rodrígue, E.A. and Sosa, M.A. (2009). GYRKPPFNGSIFamide (Gly-SIFamide) 165 modulates aggression in the freshwater prawn *Macrobrachium rosenbergii. Biological Bulletin* **217**: 313-26.

Veenstra, J,A. (1989) Isolation and structure of corazonin, a cardioactive peptide from the American cockroach. *FEBS Lett.* **250**: 231-234

Veenstra, J,A (2009) Does corazonin signal nutritional stress in insects? *Insect Biochem Mol Biol* **39**: 755-62.

Veenstra, J,A, Noriega, F.G., Graf, R. and Feyereisen, R. (1997) Identification of three allatostatins and their cDNA from the mosquito *Aedes aegypti. Peptides* **18**: 937-42.

Veenstra, J.A., Rombauts, S. and Grbić, M. (2012) In silico cloning of genes encoding neuropeptides, neurohormones and their putative G-protein coupled receptors in a spider mite. *Insect Biochem Mol Biol* **42**: 277-295.

Verleyen, P., Huybrechts, J. and Schoofs, L. (2008) SIFamide illustrates the rapid evolution in arthropod neuropeptide research. *Gen Comp Endocrinol* **162**: 27-35. doi:10.1016/j.ygcen.2008.10.020.

Verleyen, P., Huybrechts, J., Baggerman, G., Van Lommel, A., De Loof, A. and Schoofs, L. (2004). SIFamide is a highly conserved neuropeptide: A comparative study in different insect species. *Biochem Biophys Res Commun* **320**: 334-41. doi:10.1016/j.bbrc.2004.05.173.

Wang, Z., Gerstein, M. and Snyde, M.. (2009) RNA-Seq: A Revolutionary Tool for transcriptomics. *Nature Rev Gen* **10**: 57-63.

Wang, S., An, S.and Song, Q. (2008) Transcriptional expression of bursicon and novel bursicon-regulated genes in the house fly *Musca domestica. Arch Insect*

Biochem Physiol **68**: 1-13.
http://onlinelibrary.wiley.com/doi/10.1002/arch.20239/pdf.

Wanzala, W., Sika, N.F.K., Gule, S.and Hasanali, A. (2004) Attractive and repellent host odours guide ticks to their respective feeding sites. *Chemoecol* **14**: 229-232.

Wegener, C., Herbert, H., Kahnt, J., Bender, M., Rhea and J.M. (2011) Deficiency of prohormone convertase dPC2 (AMONTILLADO) results in impaired production of bioactive neuropeptide hormones in Drosophila. *J Neurochem* **118**: 581-595.

White Paper on De Novo Assembly in CLC Assembly Cell 4.0. 2012.
http://www.clcbio.com/files/whitepapers/whitepaper-denovo-assembly-4.pdf.

Whiston, E., Zhang Wise., H., Sharpton, T.J., Jui, G., Cole, G.T.and Taylor, T.W. (2012) Comparative transcriptomics of the saprobic and parasitic growth phases in *Coccidioides Spp. PloS One* **7**: e41034. doi: 10.1371/journal.pone.0041034

Wilfinger, W.W., Mackey, K. and Chomczynski, P. (1997) Effect of pH and Ionic strength on the spectrophotometric assessment of nucleic acid purity. *Biotechniques* **22**: 474-6-78-81.

Woodhead, A.P., Stay, B., Seidel, S.L., Khan, M.A. and Tobe, S.S. (1989) Primary structure of four allatostatins: Neuropeptide inhibitors of juvenile hormone synthesis. *PNAS* **86**: 5997-6001.

Yang, Y., Bajracharya, P., Castillo, P, Nachman, R.J. and Pietrantonio, P.V. (2013) Molecular and functional characterization of the first tick CAP2b (periviscerokinin) receptor from *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae). *Gen Comp Endocrinol* **194**: 142-51.

Yuan, S.J., Reed, A., Chen, F. and Stewart, C.N. Jr. (2006) Statistical analysis of real-time PCR data. *BMC Bioinformatics* **7**: 85 doi:10.1016/j.ygcen.2013.09.001. 166.

Zandawala, M. (2012) Calcitonin-like diuretic hormones in insects. *Insect Biochem Mol Biol* **42**: 816-25. doi:10.1016/j.ibmb.2012.06.006.

Zandawala, M., Li, S., Hauser, F., Grimmelikhuijzen, C.J.P. and Orchard, I. (2013) Isolation and functional characterization of calcitonin-like diuretic hormone receptors in *Rhodnius prolixus*. *PLoS One* **8**: E82466. doi:10.1371/ journal.pone. 0082466.

Zhang, T., Sun, J., Zhang, Q., Xu, J., Jiang R. and Xu,W. (2004) The diapause hormone-pheromone biosynthesis activating neuropeptide gene of *Helicoverpa*

armigera encodes multiple peptides that break, rather than induce, diapause. *J Insect Physiol* **50**: 547-54. doi:10.1016/j.jinsphys.2004.03.011.

Zhu, X.X., Oliver J.H. Jr. (1991) Immunocytochemical localization of an insulin-like substance in the synganglion of the tick *Ornithodoros parkeri* (Acari: Argasidae). *Exp appl Acarol* **13**: 153-159.

Zitnan, D, Žitňanová I, Spalovská, I., Takáč P, Park, Y. and Adams M.E. (2003). Conservation of ecdysis-triggering hormone signalling in insects. *J Expl Biol* **206**: 1275-289. doi:10.1242/jeb.00261.

Zitnan, D., Kim, Y.J., Zitnanová, I., Roller, L. and Adamsm, M.E. (2007) Complex steroid-peptide-receptor cascade controls insect ecdysis. *Gen Comp Endocrinol* **153** 88-96. Epub 2007

Zandawala, M. (2012) Calcitonin-like diuretic hormones in insects. *Insect Biochem Mol Biol* **42:** 816-25. doi:10.1016/j.ibmb.2012.06.006.

Zandawala, M., Li, S., Hauser, F., Grimmelikhuijzen, C.J.P. and Orchard, I. (2013) Isolation and functional characterization of calcitonin-like diuretic hormone receptors in *Rhodnius prolixus. PLoS One* **8**: E82466. doi:10.1371/journal.pone.0082466.

Zhang, T., Sun, J., Zhang, Q, Xu, J., Jiang R, Xu W. (2004) The diapause hormone-pheromone biosynthesis activating neuropeptide gene of *Helicoverpa armigera* encodes multiple peptides that break, rather than induce, diapause. *J Insect Physiol* **50:** 547-54. doi:10.1016/j.jinsphys.2004.03.011.

Zhu, X.X., and Oliver, J.H. Jr. (1991) Immunocytochemical localization of an insulin-like substance in the synganglion of the tick *Ornithodoros parkeri* (Acari: Argasidae). *Exp appl Acarol* **13**: 153-159.

APPENDIX A

LIST OF PRIMERS USED IN GENE EXPRESSION STUDIES

Species	Target	Forward	Reverse
I. scapularis	GAPDH	5'-TGGCATTCGGTTGTTCTTTG-3'	5'-GCCAACCTTTGCCATGCT-3'
I. scapularis	Allatotropin	5'-gTTCCgCAAgATgAAgAT-3'	5'-CCAGCAAGAAAGAGAGA-3'
" "	Allatostatin prepro	5'-CACgAAgTACgATgAgAT-3'	5'-CggCTAgACAACATAgAT-3'
" "	Allatostatin receptor	5'-ACAACCAACATCCTGATA-3'	5'-GCCAGTAGTTAAGAGTGTA-3'
"	Bursicon α	5'-ggATCATgCTCTTCCTACg-3	5' CATCTCCTggCAACACAT-3'
"	Calcitonin receptor	5'-AATCATCTTCTgTTACCA-3'	5'-CCTTCTTCTCCTAATCTT -3'
" "	Cardioacceleratory peptide (CCAP)	5'-CACCAAgAggACCAgTCC-3'	5'-AACACgAAgACgATgATgA-3'
"	Corazonin receptor	5'-CAAGGCCAAGATGAAGTC-3'	5'-CCAGGAAGATGAAGATGAT-3'
"	Corticotropin RF receptor	5'-ACCACCATCTACTACATA-3'	5'- gTAATATAggAATATCCATAgg–3'
"	Eclosion hormone receptor	5'-AAgTAACggATggTTgTC-3'	5'-TAATAggAgCgAAgTTgAC-3'
" "	FMRFamide peptide	5'-ATCACCAgACgCAgACTT-3'	5'-CTCgCTgTATTCgTTgTCAA-3'
" "	Glycoprotein hormone α	5'-TACAcgCTTCgCATgAAC-3' 5'-CTgAATgTgACCTgTgAA-3'	5'-gTCCTCAgTATCCATgATgTT-3' 5'- AggTgAggTAgATgTgAA-3'
" "	Gonadotropin releasing hormone receptor	5'-ATgCTTAggAgACTTgTAA-3'	5'-CAACACTTgAAggTTCTT-3'

Species	Target	Forward	Reverse
" "	Myoinhibitory peptide (=Bombystatin)	5'- ggTTCggCTTCggTCTCg-3'	5'-CAAAgTTgTACCTCCgCTTCAT-3'
" "	Neuropeptide F receptor	5'-AgTACATCCgCTACATCT-3'	5'-gTTCATCCAgCAgTAgAT-3'
" "	Orcokinin peptide	5'-ggAgAACATgAACACCAg -3'	5'-CCgCTCAATTTgTCCAAg-3'
" "	protein convertase type 2	5'-CTCCACgTAgTTCACTTC-3'	5'-AACCAATAACagCCTgAA-3'
" "	Pyrokinin receptor primer set 1	5-gACCTgTCTgTTCTTTgCCTTTC-3'	5'-CCCATgCgCCCgTACA-3'
" "	Pyrokinin receptor Primer set 2	5'-ggCTggCTgTATTACgTTTCCT-3'	5'-CggTACCggTgTgACATCAC-3'
" "	SIFamide receptor (GenBank)	5'-TCCAACggCAgCATATTC-3;	5'-TTCACACATTgCGtATTTCA-3'
" "	Sulfakinin receptor	5'- gACggCTCTAAATTACAT-3'	5'-CACTTg ATT CTT CAC Cag-3'
	Tachykinin receptor	5'-gCACTgTCACCAATTACT-3'	5'–ATgTagATgAAgTTgAAgATgA-3'
O. turicata	RPL8	5'-AAGGTCGGTCTGATTGCT-3'	5'-TCAGTCGTCCTTGTTCTTG-3'
	Dopamine (β)	5'-CgAAgATTgCTTCAAgTT-3'	5'-AAgATgCTgTagATgATTg-3'
"	RPL8	5'-AAGGTCGGTCTGATTGCT-3'	5'-TCAgTCgTCCTTgTTCTTg-3'
"	Histamine receptor	5'-GAATGCATCAGCGCGAAAG-3'	5'-GCGTACAGAAAAGGATTCATCG-3'
"	Pheromone odorant receptor	5'-AACATACTGTTCTCTGTGCTG-3'	5'-AACATGCTAAGATCCACCTCG-3'

APPENDIX B

ALIGNMENTS FOR CANDIDATE NEUROPEPTIDES, NEUROPEPTIDE RECEPTOR GENES, AND NEUROTRANSMITTER RECEPTORS.

1s-1. Allatotropin.

Multiple sequence alignment (ClustalW) of the deduced amino acid sequence of a putative *I. scapularis* allatotropin (contig 8149) from the *I. scapularis* synganglion transcriptome compared with the conspecific *I. scapularis* (Iscap: XP_002406992) and the tobacco horn worm *Manduca sexta* (Msexta: AAB08759). Pairwise identity for contig versus *I. scapularis* sequence from Genbank 100%; multiple identity 43%. Asterisks denote identical residues, dots indicate conservative substitutions.

```
Msexta          ----------------------------MNLTMQLAVIVAVCLCLAEGAPDVRLTRTKQ
Iscap           --------------------------MAALGRTSAL-VAAALFLCLAAAGSETPEASDRQ
Contig8149      RSSDLGGTLGSRRGSPSRARHENPSDMAALGRTSAL-VAAALFLCLAAAGSETPEASDRQ
                                        :::::::*::* *::*::****:::::::::::*
Msexta          QRPTRGFKNVEMMTARGFGKRDRPHPRAERDVDHQAPSARPNRGTPTFKSPTVGIARDFG
Iscap           HR--GGFQKLRLSTARGFGKRIPPGLAFLRQRNQEPADPIIKKG---FRKMKISTARGFG
Contig8149      HR--GGFQKLRLSTARGFGKRIPPGLAFLRQRNQEPADPIIKKG---FRKMKISTARGFG
                :*  :**::::::********::*:::::::::::::::::::    *:::::::**:**
Msexta          KRASQ-----YGNEE--EIRVTRGTFKPNSNILIARGYGKRTQLPQIDGVYGLDNFWEML
Iscap           KREDPDLSFLLENEDIDPVDLK-----------------------------------
Contig8149      KREDPDLSFLLENEDIDPVDLKEKRGIRRLSLSTARGFGKRMSPGFSDDQGPSDAGQSGS
                **::::::::::**::::::::             ::: : :      :     :
Msexta          ETSPEREVQEVDEKTLESIPLDWFVNEMLNNPDFARSVVRKFIDLNQDGMLSSEELLRNF
Iscap           ------------------------------------------------------------
Contig8149      GWLAEEIAKVADISDDGLAYQDSF*QAQ*HTM*S*TSSRLLFSTIVS*PQGQSLYIKSST
                    :       :           :            :    :          :
```

Is-2. Corticotropin Releasing Factor.

Multiple sequence alignment (ClustalW) of the deduced amino acid sequence of a putative *I. scapularis* CRF-binding peptide (contig 21180) from the *I. scapularis* synganglion transcriptome compared with the conspecific *I. scapularis* (Iscap: XP_002410820) and the aphid *Acyrthosiphon pisum* (Acyr: XP_003240961). Pairwise identity for contig versus *I. scapularis* sequence from Genbank 100%. Asterisks denote identical residues, dots indicate conservative substitutions.

```
Contig21180 QEEHPKPMDQRFAEFCGPIKPWKPFTTSQNVGVVMFRVPTRGNYFSVLVTFRRNPKPCNVLLQP
Iscap       -------MDQRFAEFCGPIKPWKPFTTSQNVGVVMFRVPTRGNYFSVLVTFRRNPKPCNVLLQP
                   ************************************************************
```

Is-2 continued.

```
Contig21180 LSESPVYTLRNYGRRVNCSAAFIFPMNFRILATSIGSQGFSGSTNSELETGLITKCKKRGMQDY
Iscap       LSESPVYTLRNYGRRVNCSAAFIFPMNFRILATSIGSQGFSGSTNSELETGLITKCKKRGMQDY
            ************************************************************

Contig21180 AQFLGGNGISTEGMLIVEDICGLDSTASKKAIQIPCGSTAVRLVSSGRYEDSLTFGYEPIIEIE
Iscap       AQFLGGNGISTEGMLIVEDICGLDSTASKKAIQIPCGSTAVRLVSSGRYEDSLTFGYEPIIEIE
            ************************************************************
```

Is-3. Glycoprotein A.

Multiple sequence alignment (ClustalW) of the deduced amino acid sequence of *I. scapularis* Glycoprotein alpha (contig 26726)from from the *I. scapularis* synganglion transcriptome compared with the conspecific *I. scapularis* (Iscap: CAR94694) and *D. variabilis* (Dvar: ACC96601). Pairwise identity contig versus *I. scapularis* sequence from Genbank 95.7%; multiple identity 83.2%. Asterisks denote identical residues, dots indicate conservative substitutions.

```
Cont26726 ----------------------------GCHKVGHTRRVSIPDCVEFDMTTNACRGFCTS
Iscap     CRTSTNSWRSLLLFWSLVKPERIFGSVRAATKSGHTRRVSIPDCVEFDMTTNACRGFCTS
Dvar      MARKQQRVAIVLAMLALAGAEANFWERPGCHKVGHTRRVSIPECVEFDITTNACRGFCTS
                     :    :   :  :        *********:*****:***********
Cont26726 YSIPSPEYTLRMNPNQGVTSFGQCCNIMDTEDVKVQVRCLDGHKDLTFKSAKSCSCFHCK
Iscap     YSIPSPEYTLRMNPNQGVTSFGQCCNIMDTEDVKVQVRCLDGHKDLTFKSAKSCSCFHCK
Dvar      YSIPSPEFTLRMNRNQRVTSFGQCCNIIDTEDVKVQVRCLDGHRDLVFKSAKSCAC----
          *******:*****:**:**********:***************:**:*********::::
```

Is-4. Insulin-like Peptide.

Multiple sequence alignment (ClustalW) of the deduced amino acid sequence of a putative *I. scapularis* insulin-like peptide (contig 28977) from the *I. scapularis* synganglion transcriptome compared with the conspecific *I. scapularis* (Iscap: XM_002402930) and the American dog tick (Dvar: EU616823). Pairwise identity contig versus *I. scapularis* sequence from Genbank Alignment contig28977 versus *I. scapularis* 99.2%; multiple alignment *I. scapularis* and *D. variabilis* 93.9%. Asterisks denote identical residues, dots indicate conservative substitutions. Contains the IIGF-insulin-bombyxin-like superfamily conserved domain.

```
Contig28977 PMVSWALNTVLVALVAASALVAPAAAGSGRRCGKILLEFMEFVCEGEFYDPYENTGPKRS
Iscap       -MVSWALNTVVVALVAASALVAPAAAGSGRRCGKILLEFMEFVCEGEFYDPYENTGPKRS
Dvar        ------------------------------------------------------------
```

Figure 3.7a Is-4 continued.

```
                 :::::::::::::::::::::::::::::::::::::::::::::::::::::::::: :::
Contig28977 LIGQRLFPLVSPGIENTDKAPASGFLRAESASQLLRKRNFQGGIVFECCYKACSIMEAQS
Iscap       LIGQRLFPLVSPGIENTDKAPASGFLRAESASQLLRKRNFQGGIVFECCYKACSIMEAQS
Dvar        ----------------------------------------GIVFECCYKACSIAEAQS
            :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Contig28977 YCPS*RPTIERLTTDTT*IDDKGERPRVSNVTFGTINF*HLYR
Iscap       YCPS-----------------------------------
Dvar        YCLS-
            **:*
```

Is-5. Bursicon alpha.

Multiple sequence alignment (ClustalW) of the deduced amino acid sequence of a putative *I. scapularis* bursicon alpha (contig 8916) from the *I. scapularis* synganglion transcriptome compared with the conspecific *I. scapularis* (Iscap: M_002407468), the American dog tick (Dvar: ACC99596) and the mosquito *Culex quingquefasciatus* (Culex: XM_001851995). Pairwise identity contig 8916 versus *I. scapularis* sequence from Genbank versus 59.5%. Asterisks denote identical residues, dots indicate conservative substitutions.

```
Contig8916- LPAAARHPRAQAAWLPAQAYTVLCLPGIMLFLRPGSGSRYWQVERSCMCCQEMGEREATK
Iscap       CQLRPVIHVLKQPGCQPKPIPSFACQGSCSSYVQVSGSRYWQVERSCMCCQEMGEREATK
Dvar        ---------------------------------SGSRYWQVERSCMCCQEMGEREATK
Culex-      -QVTPVIHVLQYPGCVPKPIPSFACVGRCASYIQVSGSKIWQMERSCMCCQESGEREASV
             :  ::::::   :: :::::::::   :    :::::::**:*********:*******
Contig8916  AVFCPKGP-GPKFRKLITRAPVECMCRPCTAPDEASILPQEFVGL*APAPH---------
Icap        AVFCPKGP-GPKFRKLITRAPVECMCRPCTAPDEASILPQEFVGL---------------
Dvar        AVFCPKGP-GPKFRKLVTRAPVECMCRPCTAPDEASVLPQEFVGL---------------
Culex-      SLFCPKAKNGEKKFKKFGGISMRGHRRECHTRVPQS-----------------------
            :::::::: ::*::*:::::::::::::*:::::::::::::::
```

Is-6. Eclosion Hormone.

Multiple sequence alignment (ClustalW) of the deduced amino acid sequence of a putative *I. scapularis* eclosion hormone (contig16950) from the *I. scapularis* synganglion transcriptome compared with the conspecific *I. scapularis* (Iscap: XP_002399271), the American dog tick (Dvar: ACC99595) and the mosquito *Aedes aegypti* (Aaegypt: XP_001661508). Pairwise identity contig 16950 versus *I. scapularis* sequence from Genbank versus 97.7%; multiple alignment identity 48.6%. Bold indicates conserved domain.

```
Contig16950     YKVIQTIYLVSISRQGQLSKTTMARIIDFPIFLVVSAAAFAVLLSLSSATHTYPS--DPV
Iscap           ----------------------MARILDFPIFLVVSAVAFAVLLSLSSATHTYPS--DPV
Dvar            ------------------------------------------LVAARISTRLP--EAT
Aaegypt         ----------------------MASVRIVALFIAIALVVVLVSEASANPQIDILGGYDMI
                                       **:::::::*:::::::::*:::::::::::::::::

Contig16950     LVCINNCGQCKMIYGEYFNGRQCAEECLSTAGFIQPDCDEADSIVKYLRR----------
Iscap           LVCINNCGQCKMIYGEYFNGRQCAEECLSTAGFIQPDCDEADSIVKYLRRKP--------
Dvar            MICIENCGQCKIMYGDYFDGRKCAEECVSTVGYIQPDCDIADTIIKYLRRKP--------
Aaegypt         GVCINNCAQCKKMFGEFFEGHLCAEACIQFKGKMVPDCEDINSIAPFLNKLN--------
                ::*::**:***:::*::*:*::***:*::::*:::***:::::*:::*::::
```

Is-7. Orcokinin 5.

Multiple sequence alignment (ClustalW) of the deduced amino acid sequence of a putative *I. scapularis* Orcokinin 5 peptide (contig 13501) from the *I. scapularis* synganglion transcriptome compared with the conspecific *I. scapularis* (Iscap: XP_2401726) and the American dog tick (Dvar: ACC99606). Pairwise identity contig 13501 versus *I. scapularis* sequence from Genbank 89.2%; versus *D. variabilis* sequence from Genbank 52.6%; multiple alignment identity 40.0%. Asterisks denote identical residues, dots indicate conservative substitutions.

```
Dvar            ------MTSLFGVLLLVTASLCSALIEVRGEEPGGVAAPASPSSKGARTLDKLSGGEYIR
Contig13501     SIYAYRMTSLLFVFLLAGVSLCSALIDGHEAEPG----------KGVRTLDKLSGGEYIR
Iscap           ---------------MGGENMNTR--DGHEAETG----------KGVRTLDKLSGGEYIR
                      ::::  : :: : ::::::::::::*:*          **:**************
Dvar            GLG--GRRRLDKISGGELLRSADDA------ELLRELVLPYALRGVPASSQGSGSRRGLD
Contig13501     ALHRLGGRRLDKISGGELLRAMPESQDRSSGEVLRSLGGPYALRRLTVP-------RGLD
Iscap           ALHRLGGRRLDKISGGELLRAMPESQDRSSGEVLRSLGGPYALRRLTVP-------RGLD
                :*:::***************:::::::::*:**:*::*****:::::       ****
```

Is-7 continued.

```
Dvar          KIGGGEYIRMAGAFPPGASPAKRQAAFDSLSGLTFGGDQGGLHKRGYGHGEFDEIDHA--
Contig13501   RISGGEYIRAMGPSGFPAGPAAA-----------------------------------
Iscap         RISGGEYIRAMGPSGFSAGPAAAKSLCHTDATYRAPLEDF-------------------
              :*:******::*:::*::*:::*
```

Is-8. Proprotein Convertase.

Multiple sequence alignment (ClustalW) of the deduced amino acid sequence of a putative *I. scapularis* Proprotein convertase (contig 23747) from the *I. scapularis* synganglion transcriptome compared with the conspecific *I. scapularis* (Iscap: XP_002410536), the American dog tick (Dvar: ACD63025) and the marine worm, *Platynereis dumerilii* (Pdumer: E54439). Pairwise identity contig 23747 versus *I. scapularis* sequence from Genbank 100%; pairwise identity contig 23747 versus *D. variabilis* sequence from Genbank 80.6%; multiple alignment identity (all 4 sequences) 51.1%. Conserved peptidase domain present. The S8 family has an Asp/His/Ser catalytic triad similar to but not identical to that found in trypsin-like proteases; Specific hit] pfam01483, Proprotein convertase P-domain ; A unique feature of the eukaryotic subtilisin-like proprotein convertases is the presence of an additional highly conserved sequence of approximately 150 residues (P domain) located immediately downstream of the catalytic domain. Asterisks denote identical residues, dots indicate conservative substitutions.

```
Dvar          TSKRNSLYDSKNRFHWKMNGVGLEFNHLFGFGVLDAGAMVALAKIWKTVPARFHCEAGSY
Iscap         TSKRNSLYDSKNRFHWKMNGVGLEFNHLFGYGVLDAGAMVALAKIWKTVPPRYHCEAGSY
Pdumer        -----HASDPNGEHNWTINGAGLEFNHLFGYGVLDAGDMVDMAREWKNVPDRFHCTAGTV
Contig23747   ------------------------------LDAGAMVALAKIWKTVPPRYHCEAGSY
              :::::::::::::::::::::::::::::::*******::*::** **:*:**:**::

Dvar          VKTSEFKANESLKIYLDTDSCAGTDTEVNYVEHVQAVITLNATRRGDVKLFMVSPSGTRS
Iscap         LKTSEFRTNNSLKIFIDTDSCAGTVTEVNYVEHVQAVITLNATRRGDVKLFMVSPSGTRS
Pdumer        TGDYAYTTKQSLILSIDTDACKGLENQVNYLEHVQSFITLKASRRGDITLYLLSPMNTTS
Contig23747   LKTSEFRTNNSLKIFIDTDSCAGTVTEVNYVEHVQAVITLNATRRGDVKLFMVSPSGTRS
              ::::::::::****::***:*:*::::***:***:::***:::****::*:::**::*:*

Dvar          MILSRRPNDDDSHDGFTKWPFMTTHTWGENPRGRWTLEAHIDRGTGGAKDSGSDDAGGEA
Iscap         MILSRRPNDDDRHDGFTKWPFMTTHTWGENPRGRWSLEARIE----GADPSKSDPK---A
Pdumer        MILSKRPKDDDSTDGFTKWPFMTTHTWAENPRGTWKLFVIFDS---------EEPQ----
Contig23747   MILSRRPNDDDRHDGFTKWPFMTTHTWGENPRGRWSLEARIE----GADPSKSDPK---A
              ****:**:***::**************:*****:*:*:::::     ::::::::::    :
```

Is-8 continued.

```
Dvar         RGFLKEWTLMIHGTRDPPYVDLP--AHDHNSKLAIVKKAHESTRRGAAALKARSRP----
Iscap        RGFLREWTLMVHGTRDPPYVDLP--AHDSNSKLAIVKKAHETTQRGGKAPRP--------
Pdumer       DGVLFEWTLMLHGTQVSPYVHQKNVNLDKHSKLAVVKREHESGANFQF------------
Contig23747  RGFLREWTLMVHGTRDPPYVDLP--AHDSNSKLAIVKKAHETTQRGGKAPRP*RVGGPRG
             :*:*:*********:::***::: ::**::****:***:**:::::::**:::
```

Is- 9. Allatostatin Prepropeptide.

Multiple sequence alignment for preproallatostatin from the fed female synganglion of the *Ixodes scapularis* transcriptome versus the same and other species. Multiple sequence alignment (ClustalW) of the deduced amino acid sequence of a putative *I. scapularis* allatostatin (contig 7636) compared with the conspecific *I. scapularis* (Iscap: EEC 20057), the American dog tick (Dvar: ACC99603). Pairwise identity contig 7636 versus *I. scapularis* sequence from Genbank versus 95.8%; multiple alignment identity 55.5%. Asterisks denote identical residues, dots indicate conservative substitutions.

```
ACC99603     ------------------------------------------------------------
Contig07636  PADKRSFSGQFDGSRYWLRPTTSLFGMDMRRSPCTVSRFMRPCPVTCLLLLFMLAAQYCR
EEC20057_    ---------------------------------------MRPCPVTCLLLLFMLAAQYCR
                                                     :::::::::::::::::::::
ACC99603_    ----------------------------------ADIDEDEDDDAMAEAAAASRTGGYL
Contig07636_AEDASPAQLQENDKRRPPAAMYGFGLGKRAPFLFLAD-DAAEQAAERAEAEDEDPDLNYL
EEC20057_    AEDASPAQLQENDKRRPPAAMYGFGLGKRAPFLFLAD-DAAEQAAERAEAEDEDPDLNYL
             :::::::::::::::::::::::::::::::** *::*::::::***:::::::::**
ACC99603_    EKRG--PREPLRYGFGLGKRRSGQEREYVPFDQEKRERHRFSFGLGKRDKKSKLEDFMKR
Contig07636_DKRGERPQHPLRYGFGLGKRLDREGSYPGSIDHNRRERHRFGFGLGKRGKKSEIEDFMKR
EEC20057_    DKRGERPQHPLRYGFGLGKRLDRDGNYPGSIDHNRRERHRFGFGLGKRGKKSEIEDFMKR
             :***::*::**********:::::::::*:::******:******:***::******
ACC99603_    RYNFGLGKRGIYGDADAGERWKRSF---------------------------------
Contig07636_RYSFGLGRRSAYG-GDDGERWKRSLASDHN*NRNRRSRVGPRWAYLGLIGSDRC*A*RAL
EEC20057_    RYNFGLGKRSAYG-GDDGERWKRSLASDHN----------------------------
             **:****:*::** :*::******::::::
```

Is-10. CAPA/Pyrokinin/PBAN.

Multiple sequence alignment for putative pyrokinin/PBAN receptor from the fed female *I. scapularis* synganglion transcriptome versus the same and other species. Multiple sequence alignment (ClustalW) of the deduced amino acid sequence of a putative *I. scapularis* pyrokinin receptor (contig 570) compared with the conspecific *I. scapularis* (Iscap: XM_002401136), the American dog tick (Dvar: ACC99623). Pairwise identity contig 570 versus *I. scapularis* sequence from Genbank versus 62.2%; multiple alignment identity 55.0%. Box shows the GPCR F1_1 domain (PS00237). The disulfide domain (from position 121 – 206), GPCR F1_2 was also found in all three sequences.

```
Contig570     -MWRKYPYVFGEVFCILRGLTSEMSTNASILTITAFTVERYVAICHPLRAHTMSQLPRAI
Iscap         QLWERHPYVFGEAFCILRGLTSETSTNASILTITAFTIERYVAICHPLRAHTMSKLSRAV
Dvar          QLWQRHPYVFGEAFCVLRGLTSETSTNASILTITAFTIERYVAICHPLRAHTMSKLSRAV
              ::* ::******:**:**********:**********:******:*********:*:**:
Contig571     RSIVGIWIVAGAFAVPLALQFGIVYEKTRDGGALILESAACMLKRPVEHAFTVSTVLFFV
Iscap         KFVVVIWVLSAVCAIPLAVQFGIVHQ-TLDGTTVLPETAACTVKDPLEHAFELSTFVFFL
Dvar          KFVVAIWVLSAVCAIPLAVQFGIVHQ-TLDGTTVLPESAACTVKDPLEHAFELSTFXFFL
              :::* **:::::::*:***:*****:: *:**:::::: ***::*:*:****::**::**:
Contig570     LPISVISVLYVLIGIQLRRSSAATRRD----CSPDMN----------GKS----HRSNTS
Iscap         LPMSVILVLYVCIALQLKRSNALSRQDVDHRCPVVHN----------GKSATAAEKE---
Dvar          LPMSVILVLYVCIALQLKRSDALSRQDVHHKCPSSNNTSSSVVNGKGGADSSSGHKQGSA
              **:***:****:*::**:** *::*:*::::*::: *          *::
Contig570     MRTP---RHTGG--------------GSKRAVVKMLVAVVVSFIICWTPFHAQRLIATYA
Iscap         MVQPV-HKFQRGCQL-----------------------------------------------
Dvar          AVQPLPSKLQRGCQLRKSVRGGAAASSSRKAVINMLIAVVVAFFICWAPFHAQRLMAVYA
              :::*   : ::*:::            :  ::  :: :::: : ::: :::::::: : ::
```

Is-11. FMRFamide.

Pairwise sequence alignment of FMRFamide from the fed *I. scapularis* synganglion transcriptome versus the conspecific sequence from Genbank. Pairwise sequence alignment with the translation of the nucleic acid sequence (contig 6861) versus an *I. scapularis* sequence from Genbank (Iscap: XM_002413792) Pairwise identity between contig 6861 and *I. scapularis* Genbank sequence XM_002413792 = 62%.

```
Contig6861    ---------------------------------QSHTASKRVTNRIMHFGKRESAFSIPL
Iscap:        ----------------MGTDVQQGEPESVNEPKRSAQPLDRVARTADPDNTKDASGRDPY
                                                **
Contig6861    EDQLKRDFLEWKKRYTDRMLHFGKKRPQDRYTDKRITNRIMHFGKRGVIFPLSDETDDSS
Iscap:        SDY--RTLMALMGPRQHRYLHFGRKKALPLYSDVPIDQ------VEGSDDYIGDDYDASS
                   *           * ******     * *  *          *         * **
```

Is-11 continued.

```
Contig6861      GKQKRQLKNSILHFGKRDDELSIEKRTRNRIMHFGKREEGYPHENTLTSDKHLGDRILHF
Iscap:          AESLEDMRWAKPYLGDGLHEDVVLSGAPEDGEVI--REEGYPYENTLTSDMHLGDRILHF
                          *    *                  ****** ******* *********
Contig6861      GKQEPHHQDADSLNKRSTANADLQFDNEDNESPYLVDKKITNRILHFGKRLDGSAEDPGK
Iscap:          GKQEPHHQDADFLNKRSTANADLQFDNEYSESPYLVDKKITNRILHFGKRLDGSAEDPGK
                ************************************************************
Contig6861      ASGKSKQHVPFVNSDIKFEDSFLIEEHKPHNRKKRSLGFDQYDLDETLERVVHQLMDAGY
Iscap:          VSGKSKQDVPSVNSDIKFEDSFLVEEHKPHNRRKRSLGFDQYDLDETLERVVHQLMDAGY
                 ****** *************** ************************************
Contig6861      PKRIALGHPGIPGHLHLPHAFVAAHVYGSELPRMLSRPSRSDRFFPDPYSGEHREAPKGP
Iscap:          PKRVALAHPGIPGHLHLPHAFVAAHVYGSELPRMLSRPSRSDRFFPVPYSGEHREAPKGP
                ****** ***************
Contig6861_     SRNVFLRFG*SLSKTLSRGMKILTPSNTPLSMPASHPLHHDIGRNTYQHLPWTKRWRFSP
XM_002413792    SRNVFLHFG-----------------------------------------------------
                      *********
```

Is-12. Corazonin Receptor.

Multiple sequence alignment of the **corazonin receptor** from the fed female *Ixodes scapularis* synganglion transcriptome versus the conspecific and other species. Multiple sequence alignment with the translation of the nucleic acid sequence (contig 8130) versus an *I. scapularis* sequence from Genbank (Iscap: XM_002402071) and *Anopheles gambiae* sequence (Agam: XM_321555). Pairwise identity for contig 8130 versus conspecific Genbank acquired sequence is 100%; pairwise identity between contig 8130, XM_002402071 and XM_321555 in three-way alignment is 42.1%. Highlighted sequences (gray) represent the GPCR family signature features (F1_2 from 131 – 206 domain) and F1-1 from 146 – 162 domain, bold italics, respectively).

```
contig08130     SCRLGCVVLLAATVLSSVAIALDSGGNSTLPKDECGPDNATCGTEPL-HAPVFQPSSLIR
Iscap:          ------------------------------------------------------------IR
Agam:           SGLVTLVGDIMAQSGATILPREECDRLNISYAFENGTALEIPGLSCYEHAPTLSKSGVIR
                                                                          **
contig08130     VVILVLIGVLSLVGNCATLVSIWKTRLRAR------STVYLLLAHLSVADLLVTFFCVLA
Iscap:          VVILVLIGVLSLVGNCATLVSIWKTRLRAR------STVYLLLAHLSVADLLVTFFCVLA
Agam:           VIVLSAMAIVSLLGNVATMWNIQKNRKSRRVTRHNWSAIYSLIFHLSIADVLVTGFCLIG
                ****      ** ** **   * * *   *      *  & *  *** ** **  **
```

Is-12 continued.

```
contig08130     EAAWTWTVQWTAGDGACKAVKFLQMFSLYLSTFILVVIAFDRFAAIRFPMRRASARRTVV
XM_002402071    EAAWTWTVQWTAGDGACKAVKFLQMFSLYLSTFILVVIAFDRFAAIRFPMRRASARRTVV
XM_321555       EAAWYYTVDWVAGNLFCKLFKLCQMFSLYLSTYVLVLVGVDRWVAVKYPMKSLNTARRCH
                ****::**:*:*** *:::::::::********:***::**::*::::*:::::::::::
contig08130     RMVFGVWALSAMLSLPQVFIFRVQRGPFEEEFYQCVTYGFYSAQWQEQLYTTVSLVLMFL
Iscap:          RMVFGVWALSAMLSLPQVCAHFSRISLFAH------------------------------
Agam:           RFLFVAYLLSFLLSTPQWMIFRVAKGPFVEDFYQCVTHGFYTDRWQEQLYTTFTLVFMFI
                *::*::::**::**:** :::::
```

Is-13. Calcitonin Receptor.

Multiple sequence alignment for calcitonin receptor from the fed female *Ixodes scapularis* synganglion transcriptome versus the conspecific and other species. Multiple sequence alignment (ClustalW) of the deduced amino acid sequence of a putative *I. scapularis* calcitonin receptor (contig 08281) from the *I. scapularis* synganglion transcriptome compared with the conspecific *I. scapularis* (Iscap: XM_002408382) and *Rhipicephalus pulchellus* (Rpul: JAA63077). Pairwise identity contig 08281 versus *I. scapularis* sequence from Genbank 95.8%; multiple alignment identity 79.5%. Asterisks denote identical residues.

```
Contig8281      -----------------LRHPFCYLIFNALLAGGALGQRPSPKKFPGSCRMENQLYVSQA
Iscap:               ----------------------------------------------MENQLYVSQA
Rpul:           PILIASPSLEDSMLLGRLRIHFVVSLLAAAAVCSVSARRNLYEKTADTCRMETQHYLEPH
                              ::  :      :          :    :    ::**:*:*::::
Contig8281      TFNQISCARCYKYMANLSFKNGSRLTYCRSGKLCR-DCETPTECYVPYGNQSDVEVYDTF
Iscap:          TFNQISCARCYKYMANLSFKNGSRLTYCRSGKLCR-DCETPTECYVPYGNQSDVEVYDTF
Rpul:           VFSRLTSARCYKYMPSLAFKNASRLTYCGKGSLCRGDCE--GECFQPYGNDSDVQVLETF
                :::::::*******::*:***:******::*:*** ***:::**::****:***:*::**
Contig8281      KKKLFAERWESCCRAARECCDEMLSEDADATSSPKEGLYCPATWDGWTCYKDTPAGMTVQ
Iscap:          KKKLFAERWESCCRAARECCDEMLSEDADATSSPK------------------------
Rpul:           KKGLYADRWKSCCSAARECCQEMLADNHAGVSAEKDGLHCPATWDGWTCYKDTPAGTTVQ
                **:*:*:**:***:******:***:::::::*::* :: ::::::::::::::::: :::
Contig8281      KPCPFHAYYITEKPQCIKMSKKICWENGTWYYNQEFGKEFTFYDCGSLEYHRSMTVFSIV
Iscap:          ------------------------------------------------------------
Rpul:           KPCPAHAYFITETPQCIKMSKKTCWENGTWFYSQEYGKEYTFYDCGSLEYHRSMTIFSIV
                :::  ::: ::: ::::::::: ::::::: : :: ::: ::::::::::::: :: ::::
```

Is-13 continued.

```
Contig8281      LHSLSVIVLVPAIVIFSVYKQLQVHRISLHKNFCVAMVLYDISVILVDSLFILDHVNEEK
Iscap:          -------VLVPAIVIFSVYKQLQVHRISLHKNFCVAMVLYDISVILVDSLFILDHVNEEK
Rpul:           LHSLSVIVLVPAIAIFSIYKQLQVHRISLHKNFCVAMVLYDISVILVDAVFILDHVSEEK
                :::::::**********:******************************::**********
Contig8281      NIRVHQSPDLCKVLYTLSRYFRLCQYAWMFCEGFYLHKLIASAFAEQKSLLIFYVVGWGC
Iscap:          NIR-----DLCKVLYTLSRYFRLCQYAWMFCEGFYLHKLIASAFAEQKSLLIFYVVGWGC
Rpul:           NIRVNENPNLCKVLYTLSRYFRLCQYAWMFCEGFYLHKLIASAFAEQKSLLVFYVIGWGC
                ****   ::*****************************************:********
Contig8281      PAIFVTISAVLRAMRLGHPCWMDNVEGYNWITLAPGLFCLFANFLFLCNIIRVLVTKLRS
Iscap:          PAIFVTISAVLRAMRLGHP----------------------------------------
Rpul:           PAIFVTISAVLRAVRIGHPCWMDNVEGYNWITLAPGLFCLFANFVFLCNIIRVLVTKLRS
                *************::*********************************************
Contig8281      THANEPSQFRKAVRAVLVLFPLFGMHFLMTVYRSPANCGAWEVYQYISKASDGLQGFFVA
Iscap:          ----------KAVRAVLVLFPLFGMHFLMTVYRSPANCGAWEVYQYISKASDGLQ-----
Rpul:           THANEPSQFRKAVRAVLVLFPLFGMHFLMTVYRSPANCGAWEIYQYISKASDGLQGFFVA
                ::::::::: *********************************************
Contig8281      VIFCYL----------------------------------------------------
Iscap:          ---------VLYLLKRSYGRYRLQRGFSSRRGQSMAMTRMSVSTHVSSVGDNSNPNNGSI
Rpul:           VIFCYLNGEVLYLLRRSYGRYRLQRGFSSRRGNSLAMTRMSVSTHVTSVGDSLNP-NGSI
                ::::::   ************************************:****::** ****
```

Is-14. Preprosulfakinin.

Sequence alignment for preprosulfakinin from the fed female *Ixodes scapularis* synganglioin transcriptome versus the same and other species. Pairwise sequence alignment for preprosulfakinin from the fed female synganglion of the *Ixodes scapularis* transcriptome versus the same from *Dermacentor variabilis*. Pairwise sequence alignment (ClustalW) of the deduced amino acid sequence of the putative *I. scapularis* preprosulfakinin (contig 30943) compared with *D. variabilis* (Dvar:ACC99604). Pairwise identity = 78.3%. Asterisks denote identical residues.

```
Contig30943 RPSASNMRASSWFLLCLLAALVYGSWSSPASMQQRHRMAMGKWLKSVLPGAPSGGDAGSR
Dvar        ----------------------------------------------------SAGDSDSR
                                                                 * **  **

Contig30943 NSGDID-TDMIDPVILANGFAKRRDDDYGHMRFGRSDDYGHMRFGRK-TDGR
Dvar        NTADLDAADMIDPVLLASGFAKRQEDDYGHMRFGRSDDYGHMRFGRK-----
            *  **   ********* *****  **********************
```

Is-15. Myoinhibitory Peptide.

Multiple sequence alignment for myoinhibitory peptide (=Allatostatin B) from the fed female *Ixodes scapularis* synganglion transcriptome versus the conspecific and other species. Multiple sequence alignment (ClustalW) of the deduced amino acid sequence of a putative *I. scapularis* myoinhibitory peptide (contig 38613) compared with the conspecific *I. scapularis* (Iscap: XP_002434041) and *Drosophila melanogaster* (Dmel: NP648971). Pairwise identity contig 38613 versus *I. scapularis* sequence from Genbank 69.7%; multiple alignment identity 17.5%. Asterisks denote identical residues, dots indicate conservative substitutions.

```
Contig38613     GTRELQDGIEPCLAAWDDAR*TRHCSGSTVTTEARGKRPVLRQGA-LLRTPSAPCLPSNR
Iscap:          --------------------------------------------------MSPVESSR
Dmel:           GNN--KRAWQSLQSSWGKRSSSGDVSDPDIYMTGHFVPLVITDGTNTIDWDTFERLASGQ
                :                        :               :                : *::
Contig38613     LGTLEGGPSWRRTASLAAPRPARWS*AGAC*CCSAALLCCGAAEPQPQGGDWNALSGMWG
Iscap:          HAGRRPVVATYGESGRTATSAVVLSRSLLVLLVLAALLCCSAAEPQPQGGDWNALSGMWG
Dmel:           SAQQQQQQPLQQQSQSGEDFDDLAGEPDVEKRAWKSMNVAWGKRRQAQG--WNKFRGAWG
                 :                                ::::::  ::::::::::**:::*:**
Contig38613     KRASDWNRLSGMWGKRAGAYGPYQALLLRAGESNDGAGHGISARAAPPGSPRENHWNDLS
Iscap:          KRASDWNRLSGMWGKRAGAYGPYQALLLRAEESNDGAGHGISARAAPPGSPRENHWNDLS
Dmel:           KREPTWNNLKGMWGKRDQWQKLHGGWGKRSQLPSN------------------------
                **:::**:::******:::::::::::*:::::::::::::::::::::::::::::::
Contig38613     GYWG*RKRAASTHTDARLTEFPSSSSSP
Iscap:          GYWG------------------------
Dmel:           ----------------------------
                ****
```

Is-16. SIFamide Peptide.

Sequence alignment (pairwise) for SIFamide peptide from the fed female synganglion of the *Ixodes scapularis* transcriptome versus the same for this species. Pairwise sequence alignment (ClustalW) of the deduced amino acid sequence of the putative *I. scapularis* SIFamide (contig39081) compared with the conspecific *I. scapularis* in Genbank (Iscap: XP_002414623). Pairwise identity = 100% (excluding non-coding regions). Asterisks denote identical residues.

```
Contig39081 LTEVLRLSTRTEFHRAT*QFQEIRKMNSWKAFFMFGTLLVMAVMMNMACAAYRKPPFNGS
Iscap:      -------------------------MNSWKAFFMFGTLLVMAVMMNMACAAYRKPPFNGS
                                     **********************************
```

Is-16 continued.

```
Contig39081 IFGKRSRADLNNADVKYAMCEAVWDTCTQWFPITQDGAQ*RQIKTK*GHIFDVEPSPLFP
Iscap:      IFGKRSRADLNNADVKYAMCEAVWDTCTQWFPITQDGAQ--------------------
            ************************************
```

Is-17 Insulin-like Receptor.

Multiple sequence alignment Insulin-like receptor from the fed female *Ixodes scapularis* synganglion *transcriptome* versus the conspecific and other species. Multiple sequence alignment (ClustalW) of the deduced amino acid sequence of a putative *I. scapularis* insulin receptor (contig13135) compared with the conspecific *I. scapularis* (Iscap: XP_002416224) and *Apis florea* (Apis:XP_003690408). Pairwise identity contig 13135 versus *I. scapularis* sequence from Genbank 98.2%; versus *Apis florea* sequence from Genbank, identity 51.9%. Asterisks denote identical residues, dots indicate conservative substitutions

```
Contig13135:   ---------------------------------------------DPGVNMQIIAHLKPF
Iscap:         RSLLGYVVYYREAPFQNVTLFDGRDACQGDVYVGLWICLWKTADADPGVNMQIIAHLKPF
Apisflorea:    QQLLSYLLNYIETENENITYE--MNACGGN---NTWQILDVDIPEWTSPVSKHIANLKPY
                 ::       :       :      :: :      :  :       ::::::::::*:***:

Contig13135:   TQYAVYVKAYTLPTAE------QGAQSDITYFKTLPAAPSQPQNLKVTPSKDSKLMISWV
Iscap:         TQYAVYVKAYTLPTAE------QGAQSDITYFKTLPAAPSQPQNLKVTPSKDSKLMISWV
Apisflorea:    TMYAVYVKTFNSRTTNFFVNPNEVGQSRIIFFRTKSTIPSPPMNVISTPLSDTEILVKWE
               *:*****:::::::::       :::**:*::*:*::::**:*:*:::**::*::::::*:
Contig13135:   PPKYPNGDVRFYRVVGIAQPSAPLHHYLGESRDYCVD----------------------
Iscap:         PPKYPNGDVRFYRVVGIAQPSAPLHHYLGESRDYCSEGREGSSPTPEVPDRPVPKAPATP
Apisflorea:    SPLFPNGPIGYYM---LSATMIREDESLISSRDYCNDTLENDLDSEEAAPVTVPEV-TV-
               ::::**:*:::*::::*::::::::::*::***** :  :       :      ::
```

Is-18 Sulfakinin Receptor.

Multiple sequence alignment for sulfakinin receptor from the fed female *Ixodes scapularis* synganglion transcriptome versus the conspecific and other species. Multiple sequence alignment (ClustalW) of the deduced amino acid sequence of a putative *I. scapularis* sulfakinin receptor (Is contig17295) compared with the conspecific *I. scapularis* (Iscap: XM_002413275) and *Culex quinquefasciatus* from Genbank (Culex: XM_001866703). Pairwise identity contig 17295 versus *I. scapularis* sequence from Genbank 51.2%; versus *C. quinquefasciatus* 44.2%. Asterisks denote identical residues, dots indicate conservative substitutions. The transmembrane domain (Pfam00001) is present.

```
Cont17295   GTPG?QRILK-LRGYYQAVRPEARKWARRCSGSAWTRAAEQPSPQGASSWMETCNLSTEG
Iscap:      ------MIRKVISNIPEHTAPSPPSTTSWVFDGVNLLPNVTPTPD-IVEVLTVSNITLED
                  : :          :                  : :           :    :
Cont17295   NGSDARPAYSWWRSDQAVLVAPYTVILLLAVLGNGLVIVTLAVNKRMRTVTNLFLLNLAV
Iscap:      SGGQDIPPEP---EDVILRITLYSIIFVFAVVGNVLVLVTLVQNKRMRTVTNVFLVNLAV
            :    :        :        :   :   :: :: :: :::  ::::::::: :: ::::
Cont17295   SDLLLGVFCMPFTLAGVLLREFVFGELMCRLIPYLQAVSVCVSAWTLMAMSVERYFAICY
Iscap:      SDLLLGVLCMPFTLVGSLLRNFVFGEIMCRLIPYLQE-GYGRQCTRTTGVNDEAY-----
            ::::::: :::::: : ::: ::::: :::::::::                  :
```

Is-19. Gonadotropin releasing hormone (GnRH).

Pairwise sequence alignment for Gonadotropin releasing hormone (GnRH) receptor from the fed female *Ixodes scapularis* synganglion transcriptome versus the published sequence (GenBank) for this hormone of the same species. Pairwise sequence alignment (ClustalW) of the deduced amino acid sequence of a putative *I. scapularis* GRH receptor (contig 12746) compared with the conspecific *I. scapularis* (Iscap: XM_002407293). Pairwise identity = 68.1%. Asterisks denote identical residues.

```
Contig12746   ---------------------------------FSSDSSVYPYELTIRFKGQLFSKCSK
Iscap         FFGARDAKTAPMIVPEVKPVVLPKDKTTVGDNSLFSSD-SVYPYELTIRFKGQLFSKCSK
                                               **** **********************
Contig12746   ARHLPDCSCESCALSGRYGKGNKFGSSSLHDPKQTSSPTNHSM*CPSRTLMERRTSLIET
Iscap         ----------------RYGKGSKFGSSSLHDPKQTSSPTNHSM-----------------
                             *****  ********************
```

Is-20. Tachykinin Receptor

Multiple sequence alignment for the tachykinin receptor from the fed female *Ixodes scapularis* synganglion transcriptome versus the published sequences (GenBank) for the same and other species. Multiple sequence alignment (ClustalW) of the deduced amino acid sequence of a putative *I. scapularis* tachykinin receptor contig 10361 compared with the conspecific *I. scapularis* GPCR receptor (XP_002411208) and *Metaseiulus occidentalis* from Genbank (Moccid: XP_003744215). Pairwise sequence identity (coding region) = 46.2%; multiple sequence identity = 38.3%. Asterisks denote identical residues, dots indicate conservative substitutions. This GPCR receptor includes the Serpentine type 7 TM GPCR chemoreceptor Srsx domain (Superfamily cl18179) characteristic of chemosensory functions.

```
Contig10361:    -*VDMELNESDLGNASG-DAVSLVNLSSEDIMTENVYIMPWWGQAAWTLLFGCMVMVATG
Iscap:          ----MELNESDLGNASG-DAVSLVNLSSEDIMTENVYIMPWWGQAAWTLLFGCMVMVATG
Moccid:         MDALEEMNLSSTFNISEVFRIYGANLTFDD—GDSAFYMPLYIEVLWCVLFSTMIVVAAC-
                    :*:*:*:::*:*: :::::::**:::*:::::::***:::::**:**::::*::*::
Contig10361:    GNLIVIWIVLAHKRMRTVTNYFIVNLSLADTMVSTLNVIFNFIYMLNGNWPFGTAFCKVS
Iscap:          GNLIVIWIVLAHKRMRTVTNYFIVNLSLADTMVSTLNVIFNFIYMLNGNWPFGTAFCKVS
Moccid:         GNLIVIWIVLAHKRMRTVTNYFIVNLSIADTMVSTLNVIFNFTYMLRREWWFGEWYCKFS
                ***************************:**************:***:::*:**:::**:*
Contig10361:    NFIAIVSVAASVFTLMAICIDRYMAII*SPRLSPEPSGQSTQLRVRTHQTPKQVPAIHLC
Iscap:          NFIAIVSVAASVFTLMAICIDR-----------------------------------
Moccid          NFIAVVSVSASVFTLMAISIDRYMAI-----MHPLHPRMSRTMTLNIAVCIWILAGILSC
                ****:***:*********:***::::       :     :                :  :
```

Is-21. Neuropeptide F Receptor.

Sequence alignment (pairwise) for the neuropeptide F receptor from the fed female *Ixodes scapularis* synganglion transcriptome versus the conspecific species. Pairwise sequence alignment (ClustalW) of the deduced amino acid sequence of the putative *I. scapularis* neuroepeptide Y receptor (contig3925) compared with Iscap Genbank XP_002402168) Pairwise identity = 100%. Asterisks denote identical residues. This receptor includes Serpentine type 7 TM GPCR chemoreceptor Srsx domain the (Super family cl18571) characteristic of chemosensory functions.

```
Contig3925  --------------------------------LGYWPFGGVMCVVVTYAQCVTVFISAY
Iscap:      MRTVTNMFIMNLAIGDILMASLCIPFTFVSNLLLGYWPFGGVMCVVVTYAQCVTVFISAY
                                            ****************************
Contig3925  TLIAISVDRYTAIVYPLRPRMTKLRSKIIIGVVWLVALVTPLPTALVTQLVPHPCANQ--
Iscap:      TLIAISVDRYTAIVYPLRPRMTKLRSKIIIGVVWLVALVTPLPTALVTQLVPHPCANQTY
          ************************************************************
```

Is-22. Crustacean Cardioacceleratory Peptide (CCAP).

Sequence alignment (pairwise) for the crustacean cardioacceleratory peptide (CCAP) receptor from the fed female synganglion of the *Ixodes scapularis* transcriptome sample Il-2 versus the conspecific species. Pairwise sequence alignment (ClustalW) of the deduced amino acid sequence of the putative *I. scapularis* cardioacceleratory peptide receptor (contig 19265) compared with Iscap Genbank XM_002407891). Pairwise identity = 52.4%. Pairwise identity for the coding region > 90%. Asterisks denote identical residues. Both the GPCR domain F1.1 and F1.2 characteristic of these transmembrane receptors were identified.

```
Contig19265 MSVPAVFLSREALVRGRLQCWIELELWQWQLYMTLVACSLFFVPALVITACYSVIVYTIW
Iscap:      MSVPAVFLSREALVRGRLQCWIELELWQWQLYMTLVACSLFFVPALVITACYSVIVYTIW
            ********************************************************* ***
Contig19265 TKSKILSYPKLPSAKTGS--NNHKTGEPDSDTKRTSSRGVIPKAKIKTIKMTLIIVFVFIL
Iscap:      TKSKILSYPKLPSVGLCNCLAERLCGWWPSTPRRSWREQTVSRPSECSTMTESLQQQQRSS
            *************            * **      *   *       *    *
Contig19265 CWSPYFVYDLLQVYGYTSQTQTAIAVSTF
Iscap:      TRLQLRQHRRGTLLRSTDSTDAHVRNSHV
                       *  *      *
```

Is-23. Ion transport Peptide.

Sequence alignment (pairwise) for the ion transport peptide from the fed female synganglion of the *Ixodes scapularis* transcriptome versus the conspecific species. Pairwise sequence alignment (ClustalW) of the deduced amino acid sequence of the putative *I. scapularis* ion transport peptide (contig contig7332) compared with Iscap Genbank XM_002399497). Pairwise identity = 77.3%. Asterisks denote identical residues.

```
Contig7332  ----------LLGPVLGFLTAGICLRFYEDPFHDPGITPRDPRWVGAWWMGYILFAMGLA
Iscap       LTLGMGFAFRLLGPVLGFLTAGICLRFYEDPFHDPGITPRDPRWVGAWWMGYILFAMGLA
                     **************************************************
Contig7332  LVALPMMLFPRILPSGKHYKVNNLQKLSKSKSKDGLKTENGPEKHPFINFAICKDIV---
Iscap       LVALPMMLFPRILPSGKHYKVNNLQKLSKSKSKDGLKTENGPEKHPFINFAICKGMKSPS
            ****************************************************:::
Contig7332  -------VIVK--RLGRNPIFMLKTIGAVFVLHATAGYGTAFTKYVE-------FQFGQS
Iscap       MFIPRHGVLPRPLRLGRNPIFMLKTIGAVFVLHATAGYGTAFTKYVDVCVVVRTVHKHCR
                   **::  **********************************:       ::::::
Contig7332  ASKASYYTGAAKVVTTMVGIMVGAVAVHRLRPRPTILAGYSALVEIALTAGFIAMMFIGC
Iscap       SNTVLVPTGAAKVVTTMVGIMVGAVAVHRLRPRPTILAGYSALVEIALTAGFIAMMFIGC
                   **************************************************
```

3.7a-Is-23 continued.

```
Contig7332  DNPIVAGVSPGTNTTTSLVDACNVNCDCTTQIYEPVCSSDKLTSFFSPCHAGCKDVSLSP
Iscap       DNPIVAGVSPGTNTTTSLVDACNVNCDCTTQIYEPVCSSDKLTSFFSPCHAGCKDVSLSP
            ************************************************************
Contig7332  SNKT-----------------------------------------------VYSNCSC
Iscap       SNKTVWHLVCLLSISKSRLNYEDDIEHARLTLFFLHCQRMCKSLTRCGHANLQVYSNCSC
            ****                                                *******
Contig7332  IATAVAEASSYVTPGLCGSTCNKLSLFLMIVIAGQLLG----------------------
Iscap       IATAVAEASSYVTPGLCGSTCNKLSLFLMIVIAGQLLGSTGRIGSMLIYLRLVPSSPGYS
            ************************************
```

(Ot-1 to Ot-22): Alignments for *O. turicata* candidate neuropeptides and neuropeptide receptor genes

Ot-1. RPL-8

Alignment for control genes: Alignment (Pairwise) *O. turicata* Ribosomal Protein L8 (RPL-8) contig 116968 from the fed female synganglion of the *O. turicata* transcriptome versus *Ornithodoros coriaceus* from Genbank (ACB70396. Pairwise alignment 63.5%. Due to its lower e-value and higher alignment success, this gene was used as the control gene for qPCR assays.

```
Contig116968 WLLPF*VHFQTRKVLLEMGRVIRAQRKGAGSVFRAHTKHRKGAPKLRSIDFAERHGYMKG
Ocoriaceus:  ---------------------------------------EGAPKLRAIDFAERHGYMKG
                                                    ***** ************
Contig116968 IIKEIIHDPGRGAPLARVVFRDPYRYKLRRHVHWPVCLLWQEGAAPGWQRVALEC++++L
coriaceus:   IIKEIIHDPGRGAPLARVVFRDPYRYKLRKELFLAAEGMYTGQFVYCGKKAQLQVGNVLP
             ******************************                  *
Contig116968 -NA*RYGHL---------QR*REARRQGQHATVIAHNPDSRKTRVKLPSGAKKVLSSANR
Ocoriaceus:  LSAMPEGTVICNVEEKPGDRGSLARTSGNYATVIAHNPDPRKTRVKLPSGAKKVLSSANR
                   *                ** * *********  ********************
Contig116968 AMVGIVAGGGRVEKPI-------------------------------------------
Ocoriaceus:  AMVGIVAGGGRVEKPILKAGRAYHKYKAKRNCWPKVRGVAMNPVEHPHGGGNHQHIGKAS
             ****************
```

Ot-2. PreproAllatostatin C.

Sequence alignment (pairwise) of the putative preproallatostatin C (Contig 73361) from the fed female *Ornithodoros turicata* synganglion transcriptome versus *Nasonia vitripennis* XP_ XP_003426084 allatostatin prohormone. Pairwise identity= 46% (coding region).

```
Nason:       MMSLRIAVCTAVALVVLVDWSTALPAGDKEALMNGLNMMGDEEGAAVERD--LLNYLVGR
Contig73361 ----------------------------------LVEESGNPGSSGNLDNLLLNYLFAK
                                                          *  *****
Nason:       RFVKRLRNQADVD---DLQRKRNYWRQCAFNAVSCFGK----------------------
Contig73361 QMARRVQ-QRDLEQSMDFQRKRSGWRQCAFNAVSCFGRRK*TPSVRRCCSPEVLHRESHN
                  * *      * ** *  **************
```

Ot-3. Octopamine/tyramine Receptor.

Sequence alignment (pairwise) octopamine/tyramine receptor contig8628 from the fed female *Ornithodoros turicata* synganglion transcriptome versus the *I. scapularis* sequence from Genbank (XM_002408768). Percent identity *O. turicata* versus *I. scapularis* = 58%.

```
Iscap:       VWLCTSSILNLCAISVDRYLAITRPVRYRSLMSSKRAKLLIV--AVWVIAFVICFPPLVG
Contig8628:  ----------------------PPLVGWNDGSEVTVPYAATNGTLHLESRVVTKPSTAN
                                    *       *                 *   *
Iscap:       WNDGTENLSAQCVLINNKGYVIYSALGSFYIPMLFMLFFNYRIYRAAIQTGRALERGFIT
Contig8628:  DTDLPVCESAQCVLINNKGYVIYSALGSFYIPMLFMLFFNYRIYRAAIQTSRALERGFIS
               *     ****************************************** ********
Iscap:       TKSGKIKGRTQEQRLTLRVHRGNDSGLNVKRGSEHVGAETCIDGIVTGRRRPGLKKSRDE
Contig8628:  TKSGKIKGKAEEP-----------------------------------------------
             ********   *
```

Ot-4. Dopamine D1 Receptor.

Sequence alignment (multiple) of the Dopamine D1 receptor contig 42220 from the fed female *Ornithodoros turicata* synganglion transcriptome versus the *Rhipicephalus microplus* and the *Ixodes scapularis* sequences from Genbank. Alignment *O. turicata* dopamine d1 receptor contig 41220 versus *R. microplus* (Rmicro=AFC88981) and *I. scapularis* (Iscap:= XM_002409287). Pairwise % against *R. microplus*: 68.0%; versus *I. scapularis* 44%

```
Contig41220 -----------------TRVVGASQTRTSTMICLSKRLGISLQIAMTTPPSS--------
Rmicro:      NKSLQVSSMVPEADPLTANTGSGSQPDEDEFDDLPTEAGGDKWAECDEEEASFLA--AQS
Iscap:       ----CDIWIAFDVMCSTASILNLCAISMDRFLHIKDPLGYGRWMTKRAVLGTICAIWMLS
                           ::      :     : :  :    :*    :         :
```

Ot-4 continued.

```
Contig41220 APSPKPANIIVVHNRNHDSGYAASHLEETQFLSNVQAPRLPDTKRNGTAVPLLTVSVARD
Rmicro:     ATSGSDPPSHATHNRNHDSGYVDSNVDEVHF-SRQERPN-KEFKKNGTVMPLLTVSVVGG
Iscap:      ALMSFLPISLGWHRPYPDSLLLVNGLTMCAL---DLTPEYAVTSSLISFYMPCVVMVALY
            •     : :   **:::**::  : : :  : :    *     : :::  ::::*:*
Contig41220 APSSTERDAESQTTPVKKKSRFNLGRKHKSSRRKREKASAKRERKATKTLAIVLGVFLIC
Rmicro:     PTL----GASIEDGPARKKSRFNLGRKHKSSRKKREKASAKRERKATKTLAIVLGVFLIC
Iscap:      TRLYLYARKHVQNIRAVTKPMNHKDMSPTKFRSMGQSSLHVMDHKAAITLGIIVGVFLCC
              :     :  :  :: :*:::::::::::::*:::::::::::::**::**:*::****:*
Contig41220 WVPFFTCNVIEAVCMKLQRNDCHLGVTVFLLTTWLGYMNSCVNPVIYTIFNPEFRKAFKK
Rmicro:     WVPFFTCNVVDAVCMKLQSQDCHLGVTVFLLTTWLGYVNSCVNPVIYTIFNPEFRKAFKK
Iscap:      WVPFFCANIVAAFCKTCISEDC------FKFLTWLGYLNSALNPIIYSIFNTEFRDAFRR
            *****::*:: *:*::::: **:::::::*:::***** **::**:**:***:***:**::
```

Ot-5. Cardioacceleratory Peptide (CCAP).

Sequence alignment (pairwise) for cardioacceleratory peptide (CCAP) contig 112055 from the fed female *Ornithodoros turicata* synganglion transcriptome versus same for the *Ixodes scapularis* hypothetical protein (Iscap: XP_002402276,) and *Bombyx mori* (NM001130897) sequences from Genbank. The Cardioacceleratory peptide domain (pssmid 256294) characteristic of this neuropeptide is present. Percent identity 25%.

```
Contig112055   PV*GVSTFRNAPLPSNCTGILKYTRFVIEDSLSFLYGVGLPTREVTMKTELVTLVASIVY
Iscap:         ----------------------------------------------MKPDLSTVISSSLY
Bmori:         ----------------------------------------------------MTSRVLLVLV
                                                                ::  : : : : ::
Contig112055   VLVLSA--CIVRALEKQDD--TSDDSFLTEQKRPFCNAFTGCGGKRS--GPGRRDLLAQI
Iscap:         ILLLSA--CLSAALEKQDD--TADDSYLVEQKRPFCNAFTGCGGKRS--SPNRIDLLARL
Bmori:         VALLCAECCVTATIPRNFDPRSNEEMVTMPKKRPFCNAFTGCGRKRSQTAPGMPNQDLMR
               :::*:*  *  :::::::*  : ::: : ::************:***   *:: ::::
Contig112055   QNRLLNEARVLEFQRRLSENPSQDREAITDEL-RSNRLLMDLLAPSSLRKRIPSTIDAE*
Iscap:         QNRLLSEIRNLELRTRLEEGPSRRHDEYTDNVSRLNKREESISDASSTRDRCP-------
Bmori:         QRQYVDEDT---LGTMLDSESAI--DELSRQILSEAKLWEAIQEASAEIARRKQKEFYNS
               **::::* : ::: ::* : ::   :::::   : ::: : :  :*::: * :
```

Ot-6. CAPA Receptor.

Sequence alignment (pairwise) for the putative CAPA receptor contig 130874 from the fed female *Ornithodoros turicata* synganglion transcriptome versus the *D.variabilis* ACC99623 Pyrokinin/CAPA receptor. Pairwise identity 65.7%.

```
Dvar:       VQPLPSKLQRGCQLRKSVRGGAAASSSRKAVINMLIAVVVAFFICWAPFHAQRLMAVYAK
Iscap:      ----------------------------------------FLCWAPFHAQRLMAVYVT
                                                    ***************
Dvar:       VPTPALEIAFNLLTYVSGVTYYVSATINPILYSIMSLKFRQAFRDTLMRCCGRHRATRHG
Iscap:      STTPALETAFHALTHISGVTYYVSATINPILYSIMSLKFRQAFRDTLMRCCGR-RVPRHE
               **** **  **  ************************************ *  **
Dvar:       KSVVSVTFPSGFRSTFRSSLAFETSDFTLLTDGGPPPPYTVEALLAQRARNVIVSIDECS
Iscap:      WTSADGYVSNHHPHTTPSSV*HAV-----------------------------------
                         *  **
```

Ot-7. Pheromone Biosynthesis-Activating Neuropeptide (PBAN).

Sequence alignment (multiple) for the Pheromone biosynthesis-activating neuropeptide receptor contig 44381 from the fed female *Ornithodoros turicata* synganglion transcriptome versus the *I.scapularis* sequence from Genbank (XM002407482) and the *Plutella xylostella* (AAX99220) from Genbank. Pairwise identity contig 44381 versus *I. scapularis* 34%; contig 44381 versus both Genbank sequences 38%.

```
Contig44381     SVSESSDAYWGDTGATADNDITESPYTDWGILVMPYKRRSSTFTPRIGRKRRSINQGDIN
Iscap:          SAGRDL-----GRGPEELLTLDTELGSDWAFLLLPYKRRSNNFTPRIGAKRRSVSE-DGG
Px:             ------------------------------------------------------------
                :             :              ::  :  ::::::  :::::: ::::    :
Contig44381:    VAK-------------------------------------------------------
Iscap:          HGD-------------------------------------------------------
Px:             ---MLSLFFGKAVCLMTIFNVCSMTSADDLKDEDIQRDARDRASMWFGPRLGKRAMHLAP

Contig44381:    --------------------------------DQDDREMSKP-FWADTGYSYQRDSRQII
Iscap:          --------------------------------SSDMRALSRH-SWPALDWSYPRMSQQMI
Px:             DGDGQAVYRMLEAADALKYYYDQLQYYGAQADDPETKVTKKVIFTPKLGRNADEDQQQSV
                                                : :: : ::  ::: :: :: ::::* :
Contig44381:    PLPRIGR-AFVPRLGKRQDDDLDDMGTDEFDGQRFYTPRIGRAFTPRIGR-A---FTPRI
Iscap:          PVPRNGRGSFVPRLGKRRMGYDDPESWDSREFSASGDPKRG-SFTPRIGRAA---FTPRI
Px:             --------DFTPRLGRRRLKDSGLAPPDEYRTPELLDAR-AQYFSPRLGRGGSMTFSPRL
                : :: ::  *:******:  : :    *:       ::: :  *:**:** :    :**:
```

Ot-7 continued.

```
Contig44381:    GRA-------FTPRIGRTPFTPRIR----------------------
Iscap:          GRT------PFTPRIGRSGDSNKDTMSNDDKAQSASGSDSNSRSSV
Px:             GRNLVYDLYPASIRVARSANKTKST----------------------
                **          :::*:::::    :
```

Ot-8. Calcitonin-related Peptide.

Sequnece alignment (Pairwise) for the calcitonin-related peptide contig 46006 from the fed female *Ornithodoros turicata* synganglion transcriptome versus the *O. coriaceus* EU574856 from Genbank. Pairwise identity 31.8% (coding region).

```
Ocoriaceus:       MKYGMRLICAILLLLVFLAGRAELAVLSPSTGDAEVAPKEDNGGTRVKRTVCRFSTC
Contig46006       ------------------------------------QEEEGTPKPSTSRCSLPTC
                                                      *  *            **
Ocoriaceus:       AGQNLADRLSGGGRGTSPPGSTGSGGYGRRK--------------------------
Contig46006       ---NLASQLA---KPTSPPGSTGSHGFGRRRRRSVPDKKYLLSLVAG----------
                     ***  *      ********* * ***
```

Ot-9. Corazonin prepropeptide

Sequence alignment (pairwise) for the corazonin prepropeptide contig 17454 from the fed female *Ornithodoros turicata* synganglion transcriptome versus the *D. variabilis* sequence (Dvar: ACC99609) from Genbank. Pairwise identity 29.2% (coding region).

```
Contig17545 EAVASGNRAAGIAVVLGQESFTRTTPKYRHESTNGSVLFRVDILPYSQTFQYSRGWTNGK
Dvar:       -------------------------MSRTVATCGVLLACLVMIASCQTFQYSRGWTNGK
                                                 ************
contig17545 RRAEAVASGPSQAAEERSLVEDALAKASLREHLLLEKLGRAFRTLDRVDEEQQEYYGH*Q
Dvar:       RRDGAIVAGPSRVTVEHRLLEEFLSKFAPKDRVVLERLGHLFRTLDR-SEDDQEY--
            **  *   ***    *  * * *          ** **  ******  *  ***
```

Ot-10. Corazonin Receptor.

Sequnece alignment (pairwise) for the corazonin receptor contig 1917 from the fed female *Ornithodoros turicata* synganglion transcriptome versus *I.scapularis* XP_002435340X full sequence from Genbank. Pairwise identity 40%.; pairwise identity within open reading frame 72.2%.

```
Contig1917  YTTISLVLMFLLPLATLVTTYLCTFYTISVQRSFFDPAKSGGSSSGRSAMEDARRKLLHK
Iscap:      ---------------------------------------------MDDARRKLLHK
                                                         * *********
Contig1917  AKMKSLMITVVIVLAFIICWTPYYCMMIIFIFLDPDDQLTEELQAGIFFFGSSTAMINPI
Iscap:      AKMKSLMITVVIVLAFIVCWTPYYCMMIIFIFLDPDDQLTEELQAGIFFFGSSTAMINPL
            ***************** ****************************************
Contig1917  IYGVFHLRRRRRGSSKQYNSSMTSRGVADYPSAVHNKRTLRARRGSSDGVSSSSCVNGGR
Iscap:      IYGVFHLRRRPSRGSKQFNSSVASRGAEN--SVLLTNCPRRTRSGHQPPQQLRMVQQTSH
            **********    *** ***  ***     *        * * *
Contig1917  PLIRLKYSVVSNGCLRQKETALVAVQP-ER*HCNC*PHGALRTFVSLTAALCNNFW*N*I
Iscap:      PVVRLKYSVVSNGCLKQKQATVVDVEDIDR-----------------------------
            *  ************ **    * *    *
```

Ot-11. Corticotropin releasing Factor Receptor.

Sequence alignment (pairwise) of the corticotropin releasing factor receptor contig7646 from the fed female *Ornithodoros turicata* synganglion transcriptome versus the *Ixodes scapularis* (XP _002405845) sequence from Genbank. Pairwise identity 60% (76.5% coding region).

```
Contig7646  FDAGLTEDECIRLRDEQLQVRGSSKILQCEIVWDSFTCWPATELGKVVRKPCSDILATLN
Iscap:      ----------------------MHERLYCRAVWDSFTCWPPAPAGKVLRKPCADIIASLD
                                   * *  *********    ***  *** ** *
Contig7646  VTLDLKRAAQSEGMYAYRACGTNGDWLWGNWTNYTDCVGLIKHQTSGMSH-VSLAVTYIF
Iscap:      ITLDMKQDSLSSGLYAYRVCGPEGNWLWGNWTNYTECLGLINNQPQEMSSVVSLAVSYIL
             *** *    * * **** **  * ********** * ***  *   **  ***** **
Contig7646  IIFSFVSLVFLSASVFIFCYFRSLQCSRTKVHQNLVLALMIHSVMVVVLYLPIVLHSDEP
Iscap:      LLFSLLSLIFLFATMFIFCYFRSLQCSRTRVHQNLVLALMVHAVMLVVLSLPVVLHSDAP
              **  ** ** *  ************** ********** * ****** ** ***** *
Contig7646  TPFAPIRWLCKSILSLKMYAEMASINWMFVEGLLLHSRITICIFRQDAPFKLYYAIGWGL
Iscap:      SPFVQIPPLCKSILSLKMYAAMASINWMFVEGLLLHSRITICIFRQDAPFKLYHAIGWGL
             **  *  ************ ****************************************
```

Ot-11 continued.

```
Contig7646  PMVFVIAWAYMMQQQLGTACWEGYGANQYVWLLIGPRLVALLVNFVFLVNIIRILVTRVR
Iscap:      PLTFILSWAYLMEQTLRTPCWEGYGSNSYVWLLIGPRLVALLVNFVFLVNIIRILVTRVK
            *  *   *** * * * * ****** * ******************************
Contig7646  SAVSVETTQFQKAIKATVLLFPLLGITHLLFCINPKDEDLGLQEAYMIINAILQSSQGIF
Iscap:      SAVSLETTQFRKAIKATVLLFPLLGITHLLFCINPQDEDMGLKEAYMIINAILQSSQGIF
            ********** ************************ *** ** *****************
Contig7646  VSVLYCFMNSEVQTAVRNAYLRAAIRRNPN-DRSFVRGGCSQTSAVFMSHFNGSVTDNG-
Iscap:      VSVLYCFMNSEVQTALRNAYLRAAIRRNPNKERPFLRGNFSQTSTVFLSPFNGSVTEQSP
            *************** **************  * * **  **** ** *  *****
Contig7646  ATRTANKIIRKYPVRTTTITT--------------
Iscap:      SPRASSKVIRKYPLRATAGPQRIPRNNNGAAVAAV
              *   * ***** * *
```

Ot-12. Bursicon alpha.

Sequence alignment (multiple) of the Bursicon alpha peptide contig 4964 from the fed female *Ornithodoros turicata* synganglion transcriptome versus the *I. scapularis* (: XP_002407512) and *D. variabilis* bursicon alpha (EU574002) sequences from Genbank. Pairwise percent identity for the coding region = 85.7%.

```
Contig4964:     STLLWQQKSTMEQTSGMSVLCVLFFLARLVQSTSIGPEESCQLRPVIHVLKQPGCQPKPI
Iscap:          ----------MLICVRSPCSLLASWLILAVLAASMGPEESCQLRPVIHVLKQPGCQPKPI
Dvar:           ------------------------------------------------------------
                           :              :   :   :  ::::::::::::::::::::::::
Contig4964:     PSFACQGSCSSYVQVSGSRYWQVERSCMCCQEMGEREATKAVFCPNSPGPKFRKVVTRAP
Iscap:          PSFACQGSCSSYVQVSGSRYWQVERSCMCCQEMGEREATKAVFCPKGPGPKFRKLITRAP
Dvar:           ---------------SGSRYWQVERSCMCCQEMGEREATKAVFCPKGPGPKFRKLVTRAP
                ::::::::::::::**********************************::*******::****
Contig4964:     VECMCRPCTSPDENSIIPQEFVTL*TSLNAFLTMFISSFLFMYVQCLERSDRNGLVKSDK
Iscap:          VECMCRPCTAPDEASILPQEFVGL-----------------------------------
Dvar:           VECMCRPCTAPDEASVLPQEFVGL-----------------------------------
                *********:***:*::*****:*
```

Ot-13. Bursicon beta.

Sequence alignment (Multiple) of the bursicon beta contig 118500 from the fed female *Ornithodoros turicata* synganglion transcriptome versus the *Dermacentor variabilis* (Dvar: EU616824) and *Tribolium castaneum* (Tcast: Q156997) sequences from Genbank. Pairwise percent identity = 48.4% (coding region); multiple percent identity 35.8%.

```
Contig118500    ----------------------------------EAATCNLQSTSIRITRDQTDEQGAL
Dvar:           MTWRALTVAAAISALWLTTAAVSASLASALEGPGGGVASCRLQETSIRITRDHSDDQGSP
Tcast:          --------------MFDKIILCLVYATCVYSVSEISEETCETLMSDINLIKEEFDELGRL
                                                  ::* :: :::::::: *::* :
Contig118500    QRTCEGTVFVARCEGTCVSQVQPSITLPHGFLKECNCCRETYMNRREIQLQDCFDPNGQK
Dvar:           VRTCEGTVLVSRCEGTCVSQVQPSITLPHGFLK--------------------------
Tcast:          QRICNGEVAVNKCEGSCKSQVQPSVITPTGFLKECYCCRESFLRERTITLTHCYDPDGVR
                :*:*:*:* * :***:*:******:*:*:****:: :::: :  :  : :: :
Contig118500:   IYG-AEGSMTIFLEEPQECACHKCGL*N-
Dvar:           ----------------------------
Tcast:          LTAETVNSMDVKLREPAECKCYKCGDFSR
                        ::   : :: :: : :::
```

Ot-14. Glycogen Synthase Kinase.

Sequence alignment (pairwise) of the contig 35555, glycogen synthase kinase from the fed female *Ornithodoros turicata* synganglion transcriptome versus the *I. scapularis* (*I. scapularis* XM_002401461, glycogen synthase kinase sequence from Genbank. Pairwise % identity 15%. Pairwise identity for open reading frame 53.7%.

```
Contig35555 -------------------------------------QAPRPVFCKDACRSPSQLRVHP
Iscap:      MCSARDGQAQRCHIRPASDPKSATARRRNRTTPGRGVPCLPRPRWYAAAARDEVG---GP
                                                  ***     * *         *
Contig35555 VLLQAFKTETFAGMARKLTAFSTAGSTAMGSGYYHLPISDIVITKRDGQKVKTVQVVKSE
Iscap:      AAGTVPSPQSHKGMAKKLTAFSTAGSNVAGGGYYHLPITDIVITKRDGQKVKTVQVVKAD
                     **************   * ******* ********************
Contig35555 GPDDELQEMSYVSTKVIGQRNFRSRA---------------------------------
Iscap:      GSDDDLIELSYITTKVIGKGTFGVVQQIKLVDTGEVYAVKWVRQDRKFKNRELQIMRHLS
            * ** * * **  *****   *
```

Ot-15. Allatostatin Receptor.

Sequence alignment (multiple) sequence for the allatostatin receptor contig 61571 from the fed female *Ornithodoros turicata* synganglion transcriptome versus the same for *I. scapularis* (XM_002403808) and *Periplaneta americana* (AF336364) sequences from Genbank. Pairwise identity 57.0% for the coding region; multiple sequence identity 44.2%.

```
Pamer:          -------------------------MDVSGTVTAPPPLGVGIGGLRYHACVNISVNTS
Cont61574       MDDYMNDD-DIPFDVRNGVDYPLIDHPDKGQVLTTALAAVLHTAAAQLCNVTRCGNVT
Iscap:          MAYYLRDVMRPPTDGASFEMTTSLPNSDKLEMLSTAMAMLSQTALPESIATSASRLCK
                :  :  :    : :               ::: :: :: : :  :::         : :
Pamer:          ELSAFC--SNSSEQLNGYGLDPPPEPQSLQLIQKIVSIVVPLLFGLIVLVGLFGNALVVL
Cont61574       DLSSL------DPDFSGDD-PDLDPYSGNRTVEEVLAVVVPILFGTIAIVGFFGNALVVM
Iscap:          ENSSLCRANVSGPSYPDSEYEDMYDYPGNRAVEEVLAIVVPILFGAIAVVGFFGNALVVL
                ::*:::    : :   :    :   : ::: :::::::***:*** *: **:*******:
Pamer:          VVAANQQMRSTTNLLIINLAVADLLFIVFCVPFTATDYVLPFWPFGDIWCKIVQYLIVVT
Cont61574       VVLCNPQMRSTTNLLIINLAMADLLFIVFCVPFTGWDYTLSYWPFGDVWCRIVQYLVIVC
Iscap:          VVLCNPQMRSTTNILIINLAMADLLFIVFCVPFTGWDYTLNYWPFGDVWCRIVQYLVIVC
                **:::*:*******:******:*************::**:* :*****:**:*****::*:
Pamer:          AYASVYTLVLMSLDRFLAVVHPITSMSIRTERNAIAAIAVTWVVILLASVPVYLSHGEVT
Cont61574       AYASIYTLVLMSLDRFLAVVHPITSMSIRTERNAYIAICLTWIVILLACVPALFAHGMM-
Iscap:          AYASIYTLVLMSFDRFLAVVHPITSMSIRTERNAYIAIFLTWVVILLACVPALFSHGMVF
                ****:*******:**********************::** :**:*****:**::::**::
Pamer:          YTYSSAEHTACVFLEADPINRPDGYNKPVFQIIFFATSYVTPLALICGLYLWLLVRLWRG
Cont61574       ---THGRDSGCTFRS------DLGYNWAAFQICFFLSSYVVPLSLTFVLYVLMLKRLWFG
Iscap:          ---LDDNYSSCTFLA------EMGYSLAAFQICFFMFSFVVPLALIFILYVLMLKRLWFG
                : *:*:          **: ::***:**  *:*:**:*:: **:::*:***:*
Pamer:          AAPGGHVSAESRRGKKRVTRMVVVVVAIFAVCWFPIQLILVLKSVDKY--EITNTSVMIQ
Cont61574       VAPGGRVSADSVRSKKRVTRLVVVVVVVFAVCWCPVQVVLVLKSVNAYG-KMNPPRIVIQ
Iscap:          VTPGGRVSAESVRSKKRVTRLVVVVVVVFAVCWCPVQIVLVLKSVELYGLPMNPPRIVIQ
                ::***:***:*:*:******:*****::*****:*:* :******  *:  :::::::**
Pamer:          IVSHVLAYMNSCVNPILYAFLSDHFRKAFRKVINC--GSAQRAQPGPRYHR-----ASTI
Cont61574       IASQILAYTNSCVNPFLYAFLSENFRKSFRKIILC---NSRVLGSGPARTR--DDFERTE
Iscap:          IASQILAYTNSCVNPFLYAFLSENFRKSFRKIIFCYQRNASSSSSGPSRTRIGEEGEKTE
                *:*::***:******:******::***:***:* *   :     :** ::*     : *:
Pamer:          QQQPQANGRALNNECVENDNKSGLLNVTKATRANGSSNDIL
Cont615874      RETMAGNCSAGKSTKISNDIL*QPSGA*YTT*PDGPSVPWL
Iscap           RETM-GNCTT-KTSKISNDIL--------------------
                :::: :*: : :  :::**::         :   : :   :
```

Ot-16. Neuropeptide F.

Sequence alignment (multiple) of neuropeptide F Contig72103 from the fed female *Ornithodoros turicata* synganglion transcriptome versus same for *I. scapularis* (XM002402121) and *M.* occidentalis (XM003738374) sequences from Genbank. Pairwise sequence identity contig 72103 versus *I. scapularis* 37%.

```
Contig72103:    -------PEIYELAAIGYIWFICHWLAMSHACYNPIIYFWMNAKFRAGLQSTFR-CLPFV
Iscap:          ILLSDLNPDINSYEYIRYIYFVIHWLAMSHASYNPFIYCWMNAKFREGFGNLTRRCWPPV
Moccid:         NLLS-AYSKLNESHYAKYIYFGSHWIAMSHTCYNPIIYCWLNAKFRQGFYRLFCSRKSRS
                : : :   : ** *  *******::*** ** * ***** *    :  : : :

Contig72103     KPSVQTTTSYVSARK-LTSQRSPI*SSLPCTSYGPSVSVVADDKDDDHRGDRVHAELVP-
Iscap:          CWPGRLRRQTLRKESNEGAALRRVNTYTTYVSVRAAGGGSSLKFNGNRGLKEVNGKIGDY
Moccid:         SQRRNTYTSYVSCNNHHLNTNHVARHQKQPVVHEPLMDNTEF-----------------
                : :::::                    :  :
```

Ot-17. Nitric Oxide Synthase.

Sequence alignemt (multiple) of the nitric oxide synthase brain-like receptor Contig117001 from the fed female *Ornithodoros turicata* synganglion transcriptome versus the same for *Ixodes scapularis* nitric oxide synthase (Iscap: XM002401328 and *B. floridae* (Bf lor: XM002605780) sequences from Genbank.

```
Contig117001    ----------------------------------------LPCSNTICQGSLM--G-GP
Iscap:          ----------------------------------------LPCSNTICQGSLM---CGP
Bflor:          PSSRRNSTTLSPSAKPRYARMKNWLNDKQMTDTLHNKGTPVNPCSGTKCLGSLMRPNAAA
                                                       :***:*:*:****    ::
Contig117001    ---EPRPKEEVLQHAKEFLDQYYTSIKRFHSKVHE------------------
Iscap:          VRRNGK---EPRPTDEVLQHAKEFLDQFYTSIKRFHSKAHEKRWSEVERQVQQRGTYDLT
Bflor:          LAKSGRPAGEVRPKEEVLEHAKEFLDEFFASIKRANTQAHKQRWAEAKAQIEEKGWYELT
                    :    * ::  ::: ::::::: :  *:::    ::  :: :   :    : : ::
```

Ot-18. Diuretic Hormone Receptor.

Sequence alignment (pairwise) of the Diuretic hormone receptor contig 103210 from the fed female *Ornithodoros turicata* synganglion transcriptome versus the same for *I. scapularis (*Iscap: XM002435613) sequence from Genbank. Percent identity for the coding region =88.3%.

```
Cont103210: ------------------------------------IYDCIFICPVVIVLLVNMFFMG
Iscap:      GCRRWWSPCGPPPKPSSVPTTTIRFSRTECECVWQLKDIYDCIFICPVVIVLLVNIFFMG
                                                ***************** ****
Cont103210: EIMWVLITKLRAATTLETQQYRKAAKALLVLIPLLGVTYILVIWTPSHRTAKVIFTYLQV
Iscap:      EIMWVLITKLRAATTLETQQYRKAAKALLVLIPLLGVTYILVIWTPSHKTARIIFTYLQI
            ********************************************** **  *******
Cont103210: TLLSTQGFTVAV-----------------------------------------------
Is Iscap:   TLLSTQVRSTIRHHLERWRASRALRAERLRTGLSYRVGCRQASQERVRSTIRHHLERWRA
            ******
```

Ot-19. Histamine Release Factor.

Sequence alignment (multiple) of the Histamine release factor *O. turicata* contig 127579 from the fed female *Ornithodoros turicata* synganglion transcriptome versus the same for *D. andersoni* (Dand: DQ009480) and *Amblyomma americanum* (Aamer: DQ009481) sequences from Genbank. Percent identity for the coding region 86.7%.

```
Contig127579    -KVLPKLDDYQFFIGESCNAEGIVGLLEYREQDGGGEKAVMMFFKHGLDEEKM*MYQEL*
Dand:           KKVLPKLDDYQFFIGESCNAEGIVGLLEYREQDGGGEKAVMMFFKHGLDEEKM-------
Aamer:          KKVLPKLDDYQFFIGESCNAEGIVGLLEYREQDGGGEKAVMMFFKHGLDEEKM-------
                **************************************************
```

Ot-20. Sulfakinin Receptor.

Sequence alignment (pairwise) for the Sulfakinin receptor contig 17295 from the fed female *Ornithodoros turicata* synganglion transcriptome versus the same for *I. scapularis* (XP_002435031) sequence from Genbank. Percent identity 68.0% (coding region).

```
Cont17295   GTPG?QRILK-LRGYYQAVRPEARKWARRCSGSAWTRAAEQPSPQGASSWMETCNLSTEG
Iscap:      ------MIRKVISNIPEHTAPSPPSTTSWVFDGVNLLPNVTPTPD-IVEVLTVSNITLED
                  * *          *                    * *           *   *
Cont17295   NGSDARPAYSWWRSDQAVLVAPYTVILLLAVLGNGLVIVTLAVNKRMRTVTNLFLLNLAV
Iscap:      SGGQDIPPEP---EDVILRITLYSIIFVFAVVGNVLVLVTLVQNKRMRTVTNVFLVNLAV
             *    *       *       *  *   ** ** ** ***  ********* ** ****
```

Ot-20 continued.

```
Cont17295    SDLLLGVFCMPFTLAGVLLREFVFGELMCRLIPYLQAVSVCVSAWTLMAMSVERYFAICY
Iscap:       SDLLLGVLCMPFTLVGSLLRNFVFGEIMCRLIPYLQE-GYGRQCTRTTGVNDEAY-----
             ******* ****** * *** ***** *********
```

Ot-21. Tachykinin Receptor.

Sequence alignment (multiple) of the tachykinin receptor Contig.110646 from the fed female *Ornithodoros turicata* synganglion transcriptome versus the same for *I.scapularis* GPCR receptor (Iscap: XM002411163) and *Metaseiulus occidentalis* (Moccid: XM003744167) sequences from Genbank. Percent identity (pairwise) 53.3% (coding region).

```
Contig110646   CIST*QELDAAVPPYNGTERNFLRD*ERHSHTGEPHLRRHHGRECVHLAPAAWTLLFGCM
Iscap:         -----------MELNESDLGNASGDAVSLVNLSSEDIMTENVYIMPWWGQAAWTLLFGCM
Moccid:        -------MDALEEMNLSSTFNIS-EVFRIYGANLTFDDGDSAFYMPLYIEVLWCVLFSTM
                           : : :   * : :  :                  ::    :::::**:::

Contig110646   VLVATGGNLIVIWIVLAHKRMRTVTNYFIVNLSLADTMVSTLNVVFNFIFMLNEHWPFGR
Iscap:         VMVATGGNLIVIWIVLAHKRMRTVTNYFIVNLSLADTMVSTLNVIFNFIYMLNGNWPFGT
Moccid:        IVVAACGNLIVIWIVLAHKRMRTVTNYFIVNLSIADTMVSTLNVIFNFTYMLRREWWFGE
               : **::**********************************************::**:  *::*
Contig110646   AYCKVSNFVSIVSVAASVFTLMAICIDRYMAIMRPLRPRMSRAMTLNIAVCIWIASSLIS
Iscap:         AFCKVSNFIAIVSVAASVFTLMAICIDR--------------------------------
Moccid:        WYCKFSNFIAVVSVSASVFTLMAISIDRYMAIMHPLHPRMSRTMTLNIAVCIWILAGILS
               : **:***:::***::********:******** ** ***** **********      *
Contig110646   LPNIIYSTTTQEMFTNGDSRTICLLLWP-DGDASKSPTDYVYNVIIVVVAYLIPLTAMAF
Iscap:         ------------------------------------------------------------
Moccid:        CPQYVYSRTREQ-----DNHTVCYMFFNEDGEITESEEDYIYNVLILIVTYVIPMQAMAF
                    ** *         *  * *      **    *  ** *** *  * * **  ****
Contig110646   TYFRVGRELWGSQSIGECTATQLESINS--------------------------------
Iscap:         ------------------------------------------------------------
Moccid:        TYFRVGRELWGSQSIGEVTRKQTETINSKRKIVKMMIVVVAIFGVCWLPYHLYFLIVHHF
               ****************  *  * * ***
```

Ot-22. SIFamide Receptor.
Sequence alignment (Pairwise) of the SIFamide receptor contig 19513 from the fed female *Ornithodoros turicata* synganglion transcriptome versus the same versus same for *I. scapularis* SIFamide receptor (Iscap: KC422392). Percent identity 92.2% (coding region).

```
Contig19513 ------------------------------------NATDLYSIPSDLWMRYSPG
Iscap       MLGSSLLNADNVTLRWGDSQTDDTSQSETANAVQDNGSWNASDYDLYSIPSDLWMRYSPG
                                                ****************
Contig19513 MVAVFCLAYSLVFMVGLLGNSFVVAVVIRSPRMRTVTNYFIVNLAMADILVCVFCIPATL
Iscap:      IVAVFCLAYSVVFVMGLLGNSFVVAVVARSPRMRTVTNYFIVNLAMADILVVVFCIPATL
             ********* **  *********** ********************** ********
Contig19513 VSNIFVPWVLGWFMCKTMSYLQGVAVSASINTLVAISVDRCLAICYPLKC----------
Iscap:      VSNIFVPWVLGWFMCKTMSYLQGVAVSASINTLVAISMDRCLAICYPLKCQLSTRSVRKI
            ********************************** ************
```

Neurotransmitter Receptors

Is-24. Muscarinic Acetylcholine Receptor.
Multiple sequence alignment for muscarinic acetylcholine receptor from the fed female synganglion of the *Ixodes scapularis* transcriptome Il-2 versus the same and other species. Pairwise sequence alignment (ClustalW) of the deduced amino acid sequence of a putative *I. scapularis* muscarinic acetylcholine receptor (contig6686, Il-2) compared with the conspecific *I. scapularis* (Iscap: XP_0024003135) and the *Rhipicephalus microplus* (Rmicro: AFC88982). Pairwise identity = 99.5%; multiple sequence identity 86.2%. Asterisks denote identical residues; dots indicate conserved residues.

```
Contig6686      RMMTSLWWPCREL*PRGTQTAHACKGRRRNMGAFENKGPTVMR*PEAQVMALLGAAFNAS
Iscap:          -------------------------------------------------MALLGAAFNAS
Rmicro:         ----------------------------------------MGLLETALNASVLLAATEAG
                                                         ::*::*::*:
Contig6686      AASTDAGPLDDV---------TINITNTSLTE-SGGTSSPYSLPEVILIAILAALLSTLT
Iscap:          AASTDAGPLDDV---------TINITNTSLTE-SGGTSSPYSLPEVILIAILAALLSTLT
Rmicro:         GSGGWNGVLDDGSGGASTTSGTGNATNDSVASGSSGHSAPYSLPEVILIAFLAALLSAVT
                ::::::*:***:          *:::**:::::: ***:*:***********:*******:*
```

Is-24 continued.

```
Contig6686      IIGNLMVMISFKLDKQLQTISNYFLLSLAIADFSIGVISMPLFTMYTLYDHWPLGTFICD
Iscap:          IGNLMVMISFKLDKQLQTISNYFLLSLAIADFSIGVISMPLFTMYTLYDHWPLGTFICDD
Rmicro:         IIGNLMVMISFKLDKQLQTISNYFLLSLAIADFSIGVISMPLFTMYTLYDHWPLGPFICD
                *********:**************************************************
Contig6686      TWLAFDYLTSNASVLNLLIISFDRYFSVTRPLTYRARRTTKRAAIMIASAWVISLVLWPP
Iscap:          TWLAFDYLTSNASVLNLLIISFDRYFSVTRPLTYRARRTTKRAAIMIASAWVISLVLWPP
Rmicro:         TWLAFDYLTSNASVLNLLIISFDRYFSVTRPLTYRARRTTKRAAIMIASAWVISLVLWPP
                ************************************************************
Contig6686      WIYSWPYIEGRRSVPVDRCYIQFLETNIYVTFGTALAAFYVPVTVMCILYWRIWRETEKR
Iscap:          WIYSWPYIEGRRSVPVDRCYIQFLETNIYVTFGTALAAFYVPVTVMCILYWRIWRETEKR
Rmicro:         WIYSWPYIEGQRSVPLDRCYIQFLETNIYVTFGTALAAFYVPVTVMCILYWRIWRETEKR
                ************************************************************
Contig6686      QKDLTQLQAGRKD--GSRRSTSSDDPAESEDFRRGRSDSCAPDVETTYVPTSLCVETSKY
Iscap:          QKDLTQLQAGKKD--GSRKSTSSDDPAESEDFRRGRSDSCAPDVETTYVPTSLCVETSKY
Rmicro:         QKDLTQLQAGRKETGGSRKSTSSDDPAESEDFRRGRSDSCPPDVETTYVPTSLCVETSKY
                **********:*:  *************************:**********************
Contig6686      LPPAVPKRRRLKDVLLSWCRIDNDKEDDDSTSHGGSPGTQTPASVETPVQSASMTFRADQ
Iscap:          LPPAVPKRRRLKDVLLSWCRIDNDKEDDDSTSHGGSPGTQTPASVETPVQSASMTFRADQ
RMICRO:         LPPAAPKRRRLRDVLLSWCRIDNDKEDDDSTSHGGSPGTQTPASIETPVQSASMTFRADQ
                ********************************************:***************
Contig6686      LVQLNPAGRQ----------V-----------SIPMTDRNGLRRSDRP-------STSRS
Iscap:          LVQLNPAGRQ--------------------VSIPMTDRNGLRRSDRP-------STSRS
Rmicro:         LVQLNPAGRTGTGTSSAIASIGGGGGGGTGGVSIPMTDRNGLRRNEKTGATAAATSASRS
                *********:                    :************::::       *:***
Contig6686      YSSDSVYTILIRLPTQPSLEGGASQA---------------------------------
Iscap:          YSSDSVYTILIRLPTQPSLEGGASQASIKMILEEDAEKNET---------TTTFARTSSE
Rmicro:         YSSDSVYTILIRLPTQPSLEGETSQASIKMILEEDAEKHEAAGGAASSGAAATFARTSSE
                **********************::***:::::::::::::          :::::::::
```

Is-25. Dopamine Receptor.

Multiple sequence alignment for a putative dopamine receptor from the fed female synganglion of *Ixodes scapularis* transcriptome Il-2 versus the published sequences (GenBank) for this neurotransmitter for the same and other species. Pairwise sequence alignment (ClustalW) of the deduced amino acid sequence of a putative dopamine receptor (contig 6007) compared with the conspecific *I. scapularis* dopamine receptor (XP_002408422). Pairwise sequence identity = 93.4%. Asterisks denote identical residues. Dots indicate conserved residues.

```
Rmicro:          IAFDVMCSTASILNLCAISLDRFLHIKDPLNYGRWMTKRAVLGTICGIWMLSALLSFLPI
Contig3334:      ---------------------------------------------WMLSALMSFLPI
Iscap:           IAFDVMCSTASILNLCAISMDRFLHIKDPLGYGRWMTKRAVLGTICAIWMLSALMSFLPI
                 :::::::::::::::::::::::::::::::::::::::::::::::******:****
Rmicro:          SLGWHRPYPDSLLVVNGLTMCALDLTPEYAVTSSLISFYMPCVVMVALYARLYLYARRHV
Contig:3334      SLGWHRPYPDSLLLVNGLTMCALDLTPEYAVTSSLISFYMPCVVMVALYTRLYLYARKHV
Iscap:           SLGWHRPYPDSLLLVNGLTMCALDLTPEYAVTSSLISFYMPCVVMVALYTRLYLYARKHV
                 ************:*************************************::******:**
Rmicro:          QNIRAVTKPCVVNNKDSGSPTKFRAIGGQSSLHVMDHKAAITLGIIVGVFLCCWVPFFCA
Contig3334       QNIRAVTKP--MNHKDM-SPTKFRSM-GQ-------------------------------
Iscap:           QNIRAVTKP--MNHKDM-SPTKFRSM-GQSSLHVMDHKAAITLGIIVGVFLCCWVPFFCA
                 *********  :*:**  ******::**::::::::::::::::::::::::::::::
```

Is-26. Metabotropic gamma aminobutyric acid (GABA) Receptor.

Multiple sequence alignment for metabotropic gamma aminobutyric acid (GABA) receptor from the fed female synganglion of the *Ixodes scapularis* transcriptome Il-2 versus the same and other species. Pairwise sequence alignment (ClustalW) of the deduced amino acid sequence of a putative *I. scapularis* metabotropic GABA receptor (contig6166, Il-2) compared with the conspecific *I. scapularis* (Iscap: XP_002406087) and the *Rhipicephalus microplus* strain NRFS metabotropic GABA receptor, (Rmicro: JN974907). Pairwise identity = 93.1%; multiple sequence identity 85.8%. Asterisks denote identical residues; dots indicate conserved residues.

```
Rmicro:          VTRSPLANPNEKSRDKEYKTSKSLYIAAVFPMKGHGGWLGGQGCFPAALMALEDVNKRSD
Iscap:           ----------------------SLYIAAVFPMKGHGGWLGGQGCLPAALMALEDVNKRSD
Contig6166       GLIPLQPSSVDKVETVEASTLKSLYIAAVFPMKGHGGWLGGQGCLPAALMALEDVNKRSD
                           :    :  : :**********************:***************
Rmicro:          LLIGYKLEIDWRDSQ-CN-----PGLAATVMYDLLYNEP--QKLMLLGGCSIVCSTVAEA
Iscap:           LLIGYKLEIDWRDSQVCALPSDFPGFRDHARAPNSYGFPYFSSLCMAHSPTRLGRMFSRR
Contig6166       LLIGYKLEIDWRDSQ-CN-----PGLAATVMYDLLYNDP--QKLMLLGGCSIVCSTIAEA
```

Is-26 continued.

```
                ********************* *:     **:::::::::::*: *  ::*:::::::::::: :::
Rmicro:         A-KMWNLVVISYGSSSPALSNRKRFPTFFRTHPSATIHNPTRIKLFQKFSWSRIAIIQEA
Iscap:          PHRRLSFFQISYGSSSPALSNRKRFPTFFRTHPSATIHNPTRIKLFQKFEWSRIAIIQEA
Contig6166      A-KMWNLVVISYGSSSPALSNRKRFPTFFRTHPSATIHNPTRIKLFQKFEWSRIAIIQEA
                : :::::::**********************************************************
Rmicro:         EEVFISTGEDLEARCKEAHIEIVTRQSFLTDPTDAVKNLVRQDARIIVGMFYVAAARRVF
Iscap:          EEVFTSTGEDLEIKCKEAHIEIVTRQSFLTDPTDAVKNLVRQDARIIVGMFYVAAARRVF
Contig6166      EEVFTSTGEDLEIKCKEAHIEIVTRQSFLTDPTDAVKNLVRQDARIIVGMFYVAAARRVF
                ************:::**************************************************
Rmicro:         CEAYKQNVFGKQYVWLLIGWYEDGWYTVQDKGHNCTTEQMKEALEGHFTTEALMLNQGSQ
Iscap:          CEAYKQNVFGKQYVWLLIGWYEDGWYTVQDKGHNCTTEQMKEALEGHFTTEALMLNQGNQ
Contig6166      CEAYKQNVFGKQYVWLLIGWYEDGWYTVQDKGHNCTTEQMKEALEGHFTTEALMLNQGNQ
                ************************************************************
Rmicro:         ETISGMSSQQFLERYERALAEQNGGLQGYKPEGHQEAPLAYDAIWAIALALNKTINTLRE
Iscap:          ETISGMSSQQFLERYELALAEQN-GMNGYRPEGHQEAPLAYDAIWAIALALNKTINTLKE
Contig6166      ETISGMSSQQFLERYELALAEQN-GMNGYRPEGHQEAPLAYDAIWAIALALNKTINTLKE
                 *************************:::**:*****************************:*
Rmicro:         YSMSIEDFTYTNHKIADEIWSAMNATQFLGVSGFVAFSAKGDRMAWTLIEQMIDGNYVKI
Iscap:          YSMSIEDFTYTNKKIADEIWSAMDATQFLGVSGFVAFSALGDRMSWTLIEQMIEGSYVKI
Contig6166      YSMSIEDFTYTNKKIADEIWSAMDATQFLGVSGFVAFSALGDRMSWTLIEQMIEGSYVKI
                ********************************************************:*:****
Rmicro:         GYFDTQTDNLTILNQEKWTDGKPPQDRTIIVRVHRKVSLSLFAGMCAVAFIGVVWAVGLL
Iscap:          GYYDIETDNLTIYTQAKWTDGKPPQDRTIIVRVHRKVSLSLFAGMCAVAFIGVVWAVGLL
Contig6166      GYYDIETDNLTIYTQAKWTDGKPPQDRTIIVRVHRKVSLSLFAGMCAVAFIGVVWAVGLL
                **:*::******::*:*************************************************
Rmicro:         IFNWIFRHSRYIQLSHPMCNNIMLIGIILCLVCVCLLGLDGQFVSEFRYAHICQARSWFL
Iscap:          VFNWIFRHSRYIQLSHPMCNNIMLIGIILCLVCVCLLGLDGQFVSEFRYAHICQARSWFL
Contig6166      VFNWIFRHSRYIQLSHPMCNNIMLIGIILCLVCVCLLGLDGQFVSEFRYAHICQARSWFL
                :************************************************************
Rmicro:         AIGFTLSFGAMFSKIWRVHRLTTKSKSESKGLSFQRVESWRLYGMVGGLVLVDAVILSAW
Iscap:          TIGFTLSFGAMFSKIWRVHRITTMSKSESK-----KVESWKLYGMVGAMVLIDAVILTAW
Contig6166      TIGFTLSFGAMFSKIWRVHRITTMSKSESKGLSFQKVESWKLYGMVGAMVLIDAVILTAW
                :*******************:**:******:::::::***********::**:*****:**
Rmicro:         QLVDPMQRHLEVFPLEPPALSDEDVRIEPALEHCESRNHAIWLGVMYSYKGLLLIFGIFL
Iscap:          QLVDPMKRELEIFPLEDPEMSDEDVKIEPALEHCESKNHAIWLGVMYSYKGLLLIFGIFL
```

Is-26 continued

```
Contig6166      QLVDPMKRELEIFPLEDPEMSDEDVKIEPALEHCESKNHAIWLGVMYSYKGLLLIFGIFL
                ******:*:**:****:*::*****:**********:**********************
R.microplus     AYETRSVKIKQLNDSRLVGMSIYNVVVLCLITAPVTLVIGSQQDATFAFVALAIIFCSFL
Iscap:          AYETRSVKIKQLNDSRLVGMSIYNVVVLCLITAPVTLVIGSQQDATFAFVALAIIFCSFL
Contig6166      AYETRSVKIKQLNDSRLVGMSIYNVVVLCLITAPVTLVIGSQQDATFAFVALAIIFCSFL
                ************************************************************
R.microplus     SMALIFVPKIIELVRRPRERADVRSLMDTITSKEEEERHQRLLAENEDLKKQIAEKEEQI
Iscap:          SMALIFVPKIIELVRRPRERADVRSLMDTITSKEEEERHQRLLAENEDLKKQIAEKEEQI
Contig6166      SMALIFVPKIIELVRRPRERADVRSLMDTITSKEEEERHQRLLAENEDLKKQIAEKEEQI
                ************************************************************
Rmicro:         QVLNQKLQERQRLAHQTALPASGERVFLASVSPWSVLAPDTLVGHSCCAAAPEEYTKTDS
Iscap:          QLLNQKLQER----------------------------------------------------
Contig6166      QLLNQKLQERQRMA--TALPSSGERVRIA-------LPPTYADGMLAPREQIAIDPVSDS
                *:********::    :::: :::::  :       : :    :               ::
```

Is-27. Glutamate (metabotropic) Receptor.

Sequence alignment (pairwise) for the glutamate (metabotropic) receptor from the fed female synganglion of the *Ixodes scapularis* transcriptome Il-1 versus the same and other species. Sequence alignment (ClustalW) of the deduced amino acid sequence of a putative *I. scapularis* glutamate (NMDA) receptor (contig 37672) from the Illumina sample Il- 1 compared with the conspecific *I. scapularis* (Iscap: XP_002413279). Pairwise identity = 94.2%. Asterisks denote identical residues.

```
Contig37672     ----------------------HMSPGSPWAAAAALALASLGALAALAVLAVFLAYRDTPV
XP_002413279    AECAPGTLPDPFHERCLPVPESHMSPGSPWAAAAALALASLGALAALAVLAVFLAYRDTPV
                                       ************************************
Contig37672     VRASGRELSCVLLAGILLCHGTALLLVQRPSAAVCGAQRAALGLCFAVVYSAILAKTDRI
XP_002413279    VRASGRELSCVLLAGILLCHGTALLLVQRPSAAVCGAQRAALGLCFAVVYSAILAKTDRI
                ************************************************************
Contig37672     ARIFRAGRRSAQRPGCISPRSQLALCGALVSVQGLVAALWLALRPPRAVHHHPTREDNQL
XP_002413279    ARIFRAGRRSAQRPGCISPRSQLALCGALVSVQGLVAALWLALRPPRAVHHHPTREDNQL
                ************************************************************
Contig37672     VCLASVQRAGYALALAYPLCLVAVCTVYAVLTRKIPEAFNESKYIGFAMYTTCVIWLAFL
XP_002413279    VCLASVQRAGYALALAYPLCLVAVCTVYAVLTRKIPEAFNESKYIGFAMYTTCVIWLAFL
                ************************************************************
```

Is-27 contnued.

```
Contig37672     PIYLTTWRHVNLNLTSMAVAVSLSASVTLACLFVPKLYIILLHPEKNVRQSIMAKYGTLK
XP_002413279    PIYLTTWRHVNLNLTSMAVAVSLSASVTLACLFVPKLYIILLHPEKNVRQSIMAKYGTLK
                ************************************************************
Contig37672     NQQQHRVESATQSDVDYEMSDKQRSACSLVSTNSRASCATQTDDQDSALWDKDVQL*PGG
XP_002413279    NQQQHRVESATQSDGACFLLPYWRASRRKFTPA--------------------------
                **************
```

Is-28. Glutamate (ionotropic) Receptor.

Sequence alignment (pairwise) for the glutamate (ionotropic) receptor from the fed female synganglion of the *Ixodes scapularis* transcriptome sample Il-2versus the published sequence (GenBank) for this neurotransmitter of the conspecific species. Pairwise sequence alignment (ClustalW) of the deduced amino acid sequence of the putative glutamate (ionotropic) receptor (contig8356) compared with the *Ixodes scapularis* sequence (Iscap: XP_002407641). Pairwise identity = 86.5%. Asterisks denote identical residues.

```
Iscap:          GNVTTVVLSSTLHSSLALQSLFKNTNVPYVATSYQEHCSVNVGGNLNLPSADSLGVSLLP
Contig8356      ---TTVVLSSTLHSSLALQSLFKNTNVPYVATSYQEHCSVNVGGNLNLPSADSLGVSLLP
                   ************************************************************
Iscap:          DYLPAVAEVVDHLAWDTFVYVYDSDNGPSKLQRLLSHQFKNSVSMRYAKRISNSSDANDF
Contig8356      DYLPAVAEVVDHLAWDTFVYVYDSDNGPSKLQRLLSHQFKNSVSMRYAKRISNSSDANDF
                ************************************************************
Iscap:          LRLLETTDRESRKYVLLDCRFETAKRIIIDHVRDIYMGRRNYHFLLVNPVVNELTYEKVP
Contig8356      LRLLETTDRESRKYVLLDCRFETAKRIIIDHVRDIYMGRRNYHFLLVNPVVNELTYEKVP
                ************************************************************
Iscap:          EFVAVNITGLRLVGEDLSQQSSLDPSQEPREKKIT-----------------------
Contig8356      EFVAVNITGLRLVGEDLSQQSSLDPSQEPREKKITVEEALIHDAATLIVNTYKELKLRSL
                **********************************
Iscap:          ------------EEDERRAGPQPRPRLEAEAELLEEGDQLLQADSPSTTTCGDRPSVPQE
Contig8356      VPHRYSYIFEDQEEDERRAGPQPRPRLEAEAELLEEGDQLLQA-----------------
                ****************************************
Iscap:          LGEIITRNLREVRPKDTEVDVCSCNFVLLVPSIVLQTLLCFYVLLQRSFQGLTGSIRFTS
Contig8356      LGEIITRNLRE--------------------------------------------------
                ***********
```

Is-28 continued.

```
Iscap:      DGCRIDYNVHVVQLNVNNEAIKIAEWSDTKGFEPVIKPARVEVNDTGVLDKDKTYVIQSV
Contig8356  DGCRIDYNVHVVQLNVNNEAIKIAEWSDTKGFEPVIKPARVEVNDTGVLDKDKTYVIQSV
            ************************************************************
Iscap:      L-KPSSW-QRVPDAQDRMGNDRYDGYCKDLIDALARELDIKYELRAAEETVYGRRDHKVR
Contig8356  LEKPYLMVKESPDAQDRTGNDRYDGYCKDLIDALARELGIKYELRAAEETVYGRRDHKVR
            ****       ************************************************
Iscap:      GGWTGLIGEVLRKASQNAQSPSTATVINAERKEAVDFSQPFMTTGIAALMLKPSDLPGRG
Contig8356  GGWTGLIGEVLRK---EVDMGVAATVINAERKEAVDFSQPFMTTGIAALMLKPSDLPGRG
            *************          ************************************
Iscap:      MFTFLAPFSLELWIFFVSSFGLVFVIMFFVSFFTTRVSSTKPGMEHSACGTIYKSLCYSL
Contig8356  MFTFLAPFSLELWIFFVSSFGLVFVIMFFVSFFTTRVSSTKPGMEHSACGTIYKSLCYSL
            ************************************************************
Iscap:      EAFTPHYIDSYYARPYEVLRSTGSYRRRYPIFPTLSRSISGRVIGNIWWLFIVFVFSAYT
Contig8356  EAFTPHYIDSYYA-------------------------------------------------
            *************
Iscap:      ASMVPFLSKESRIRPIRSVEDLPLQSQVDYGFSRQSMAKKYFENPNLNSTAHRRMWEVMN
Contig8356  ASMVPFLSKESRIRPIRSVEDLPLQSQVDYGFSRQSMAKKYFENPNLNSTAHRRMWEVMN
            ************************************************************
Iscap:      SKPDVFKNSNAEGVDAVRSSKGNYVFFMEANAVAFVNTQRPCDTMQLGGTFGVRSFAVAV
Contig8356  SKPDVFKNSNAEGVDAVRSSKGNYVFFMEANAVAFVNTQRPCDTMQLGDTFGVRSFAVAV
            ************************************************************
Iscap:      PKGSSLRKHLDEAIAHLSETGELDKLKKKWWTQKSYCQYPERKKDETVMPLDNFIGVFFI
Contig8356  PKGSSLRKHLDEAIAHLSETGELDKLKKKWWTQKSYCQYPERKKDETVMPLDNFIGVFFI
            ************************************************************
Iscap:      LGGGVALGILVGLIEFI-----------------------------------------
Contig8356  LGGGVALGILVGLIEFIYKCCVRSSAAKGTVPETTSTGEATITEKEFAEQALASA**RKR
            *****************
```

Is-29. Glutamate (NMDA) Receptor.

Sequence alignment (pairwise) for the glutamate (NMDA) receptor from the fed female synganglion of the *Ixodes scapularis* transcriptome sample Il-1versus the same for this species. Pairwise sequence alignment (ClustalW) of the deduced amino acid sequence of the putative glutamate NDMA receptor (contig36704) compared with the *Ixodes scapularis* sequence (Iscap: XP_002408667). Pairwise identity = 96.7%. Asterisks denote identical residues. The four transmembrane regions characteristic of the ionotropic and NMDA domains (pfam00060) as well as the N-terminal leucine/isoleucine/valine-binding protein (LIVBP)-like domain of the NMDA (cd06351) are present in this receptor (as well as other domains).

```
Iscap:      GNVTTVVLSSTLHSSLALQSLFKNTNVPYVATSYQEHCSVNVGGNLNLPSADSLGVSLLP
Contig8356  ---TTVVLSSTLHSSLALQSLFKNTNVPYVATSYQEHCSVNVGGNLNLPSADSLGVSLLP
               ************************************************************
Iscap:      DYLPAVAEVVDHLAWDTFVYVYDSDNGPSKLQRLLSHQFKNSVSMRYAKRISNSSDANDF
Contig8356  DYLPAVAEVVDHLAWDTFVYVYDSDNGPSKLQRLLSHQFKNSVSMRYAKRISNSSDANDF
            ************************************************************
Iscap:      LRLLETTDRESRKYVLLDCRFETAKRIIIDHVRDIYMGRRNYHFLLVNPVVNELTYEKVP
Contig8356  LRLLETTDRESRKYVLLDCRFETAKRIIIDHVRDIYMGRRNYHFLLVNPVVNELTYEKVP
            ************************************************************
Iscap:      EFVAVNITGLRLVGEDLSQQSSLDPSQEPREKKIT-----------------------
Contig8356  EFVAVNITGLRLVGEDLSQQSSLDPSQEPREKKITVEEALIHDAATLIVNTYKELKLRSL
            **********************************
Iscap:      ------------EEDERRAGPQPRPRLEAEAELLEEGDQLLQADSPSTTTCGDRPSVPQE
Contig8356  VPHRYSYIFEDQEEDERRAGPQPRPRLEAEAELLEEGDQLLQA-----------------
            ****************************************
Iscap:      LGEIITRNLREVRPKDTEVDVCSCNFVLLVPSIVLQTLLCFYVLLQRSFQGLTGSIRFTS
Contig8356  LGEIITRNLRE-----------------------------------------------------
            ***********
Iscap:      DGCRIDYNVHVVQLNVNNEAIKIAEWSDTKGFEPVIKPARVEVNDTGVLDKDKTYVIQSV
Contig8356  DGCRIDYNVHVVQLNVNNEAIKIAEWSDTKGFEPVIKPARVEVNDTGVLDKDKTYVIQSV
            ************************************************************
Iscap:      L-KPSSW-QRVPDAQDRMGNDRYDGYCKDLIDALARELDIKYELRAAEETVYGRRDHKVR
Contig8356  LEKPYLMVKESPDAQDRTGNDRYDGYCKDLIDALARELGIKYELRAAEETVYGRRDHKVR
            ****       ************************************************
Iscap:      GGWTGLIGEVLRKASQNAQSPSTATVINAERKEAVDFSQPFMTTGIAALMLKPSDLPGRG
Contig8356  GGWTGLIGEVLRK---EVDMGVAATVINAERKEAVDFSQPFMTTGIAALMLKPSDLPGRG
            *************          ************************************
Iscap:      MFTFLAPFSLELWIFFVSSFGLVFVIMFFVSFFTTRVSSTKPGMEHSACGTIYKSLCYSL
Contig8356  MFTFLAPFSLELWIFFVSSFGLVFVIMFFVSFFTTRVSSTKPGMEHSACGTIYKSLCYSL
            ************************************************************
```

Is-29 continued.

```
Iscap:      EAFTPHYIDSYYARPYEVLRSTGSYRRRYPIFPTLSRSISGRVIGNIWWLFIVFVFSAYT
Contig8356  EAFTPHYIDSYYA----------------------------------------------
            *************
Iscap:      ASMVPFLSKESRIRPIRSVEDLPLQSQVDYGFSRQSMAKKYFENPNLNSTAHRRMWEVMN
Contig8356  ASMVPFLSKESRIRPIRSVEDLPLQSQVDYGFSRQSMAKKYFENPNLNSTAHRRMWEVMN
************************************************************
Iscap:      SKPDVFKNSNAEGVDAVRSSKGNYVFFMEANAVAFVNTQRPCDTMQLGGTFGVRSFAVAV
Contig8356  SKPDVFKNSNAEGVDAVRSSKGNYVFFMEANAVAFVNTQRPCDTMQLGDTFGVRSFAVAV
            ************************************************************
Iscap:      PKGSSLRKHLDEAIAHLSETGELDKLKKKWWTQKSYCQYPERKKDETVMPLDNFIGVFFI
Contig8356  PKGSSLRKHLDEAIAHLSETGELDKLKKKWWTQKSYCQYPERKKDETVMPLDNFIGVFFI
            ************************************************************
Iscap:      LGGGVALGILVGLIEFI-------------------------------------------
Contig8356  LGGGVALGILVGLIEFIYKCCVRSSAAKGTVPETTSTGEATITEKEFAEQALASA**RKR
            *****************
```

Is-30. Octopamine Neurotransmitter Receptor.

Pairwise sequence alignment for the octopamine neurotransmitter receptor from the fed female synganglion of the *Ixodes scapularis* transcriptome (Il-2) versus published sequences (GenBank) from the conspecific species. Pairwise sequence alignment (ClustalW) of the deduced amino acid sequence of a putative *I. scapularis* octopamine (contig 6007)from the Illumina sample Il-2 compared with the conspecific *I. scapularis* (Iscap: XP_002408422). Pairwise identity for contig 6007 versus the *I. scapularis* sequence from Genbank 93.4%. Asterisks denote identical residues. The 7-transmembrane receptor (rhodopsin family) is present in this receptor.

```
Contig6007  LLAEASRVSPSTYYYYGDAAVSVMSVLILVFMVLSVLGNAMVVLTVVRHRGMRTRTNMFI
Iscap:      -----------TYYYYGDAAVSVMSVLILVFMVLSVLGNAMVVLTVVRHRGMRTRTNMFI
                       ************************************************
Contig6007  VNLAVADILVAVLDMPVSLATLLRGDWTLGYGFCQFNGFTMALLLMCSIHTLMYMSVHKY
Iscap:      VNLAVADILVAVLDMPVSLATLLRGDWTLGYGFCQFNGFTMALLLMCSIHTLMYMSVHKY
            ************************************************************
Contig6007  VSITRPFSRAMTKRRVGFLIAAAWLWPFFCALTPFLGLTKIVYKIGASQCGPAYPHSMKM
Iscap:      VSITRPFSRAMTKRRVGFLIAAAWLWPFFCAVTPFLGLTKIVYKIGASQCGPAYPHSMKM
            ************************************************************
Contig6007  YAHSALITVTNYFVPLIVMGFCYFNIFRAIGEHMTRVKATSNISLHNSVTQQKRINVTLV
Iscap:      YAHSALITVTNYFVPLIVMGFCYFNIFRAIGEHMTRVKATSNISLHNSVTQQKRINVTLV
            ************************************************************
```

Is-30 continued.

```
Contig6007   LVLLCFLFCWTPYMIYTFVVNSRTSKTKVPYILNPVAYWFGYLNSACNPIIYAFRSPSFR
Iscap:       LVLLCFLFCWTPYMIYTFVVNSRTSKTKVPYILNPVAYWFGYLNSACNPIIYAFRSPSFR
             ************************************************************
Contig6007   HGYKELLFGSGVTVVSCEGSTGQRSSSFQQKVAADAAAVPGSPPAAPTDPKSCRRPKRFL
Iscap:       HGYKELLFGSGVTVVSCEG-----EAPFLW-LPSDAALWVNEAPAV--------------
             ******************             ***      **
```

Is-31. Serotonin Receptor.

Multiple sequence alignment for a putative serotonin receptor from the fed female synganglion of the *Ixodes scapularis* transcriptome (Il-2) versus published Genbank sequences from the conspecific and other species. Multiple sequence alignment (ClustalW) of the deduced amino acid sequence of a putative *I. scapularis* serotonin receptor (contig 17069) from the Illumina sample Il-2 compared with the conspecific *I. scapularis* (Iscap: XP_0024050230) and the cattle tick *R. microplus* (Rmicro:AAQ89933) sequences. Pairwise identity contig 17069 versus the *I. scapularis* sequence from Genbank = 99.5%; versus *R. microplus* sequence from Genbank 91.4%. Asterisks denote identical residues, dots indicate conservative substitutions. The 7-transmembrane receptor (rhodopsin family) is present in this receptor sequence.

```
AAQ89933         ------------------------------------------------------------
Contig17069      KLRSDRCERTECRKLEYD*RWQPDHNDAC*RGMNGDSRLTTLWPDMAGANPHYASVINEA
XP_002405023     ---------------------------------------------MAGANPHYASVINEA
                                                               ***************
AAQ89933         ------------------MQANLSQSWHENLPVVITVSLLLCLVIVATVIGNIFVIAAII
Contig17069      DASRQDNWSSAQESCTNSSIGNSSLSWHENLVVVVTVSILLSLVIVATVIGNIFVIAAIL
XP_002405023     DASRQGNWSSAQESCTNSSIGNSSLSWHENLVVVVTVSILLSLVIVATVIGNIFVIAAIL
                 *****:************:::*:*:******:**:******:******************
AAQ89933         WERNLRTVSNYLVLSLAVADLMVACLVMPLGAVYEVTREWRMPPELCDVWTCCDVLCCTA
Contig17069      MERNLRTVSNYLVLSLAVADLMVACLVMPLGAVYEVTQEWVLAPELCDVWTCCDVLCCTA
XP_002405023     MERNLRTVSNYLVLSLAVADLMVACLVMPLGAVYEVTQEWVLAPELCDVWTCCDVLCCTA
                 :************************************************************
AAQ89933         SILHLLAIAVDRYWAVTIVDYMRQRDVRKVGIMIFLVWSVAFVVSIAPIFGWKDKDSRSR
Contig17069      SILHLLAIAVDRYWAVTCMDYMRQRDVRKVGSMIFLVWSVSFVVSIAPIFGWKDKDSHSR
XP_002405023     SILHLLAIAVDRYWAVTCMDYMRQRDVRKVGSMIFLVWSVSFVVSIAPIFGWKDKDSHSR
                 *****************::************:********:******************
AAQ89933         VLHEKKCLVSQDAAYQVFATCSSFYVPLIMILLLYWRIFKVARQRIRHKPGAKAVLIVHK
Contig17069      VLNEKKCLVSQDAAYQVFATCSSFYVPLIMILLLYWRIFKVARQRIRHKPGAKAVLIVHK
XP_002405023     VLNEKKCLVSQDAAYQVFATCSSFYVPLIMILLLYWRIFKVARQRIRHKPGAKAVLIVHK
                 **:************************************************************
```

Is-31 continued.

```
AAQ89933        EPSTSSAVASNENTPQHNATVASSPVRNSSNQSSPSNGMNKAMHGGIGRLLVLTKREKKH
Contig17069     EPSTSSAPPSNESTPHHGTL-------NSGNQP-PSNGM-KAVHGGIGRLLVLTKREKKH
XP_002405023    EPSTSSAPPSNESTPHHGTL-------NSGNQP-PSNGM-KAVHGGIGRLLVLTKREKKH
                *******:::***:*:*:*:        **:**  ***** **:*****************
AAQ89933        VEETIESRRERKAAKTVAIITGVFVMCWLPFFVMALVMPLCETCDPGKLVFSFFLWLGYA
Contig17069     VEESIESRRERKAAKTVAIITGVFVMCWLPFFMMALVMALCDACDPGKLLFSFFLWLGYA
XP_002405023    VEESIESRRERKAAKTVAIITGVFVMCWLPFFMMALVMALCEACDPGKLLFSFFLWLGYA
                ***:*****************************:*****:**::******:**********
AAQ89933        NSMINPIIYTIFSPDFRNAFNRILCGKKPPMR--------------------------
Contig17069     NSMINPIIYTIFSPDFRNAFSRILCGKKPTFR*QQGASASATVRRTSVNPELEMV*SVTE
XP_002405023    NSMINPIIYTIFSPDFRNAFSRILCGKKPTFR--------------------------
                ********************:********::*
```

Ot-23. Muscarinic Acetylcholine Receptor.

Sequence alignment (multiple) of the muscarinic acetylcholine receptor (contig88863) from the fed female *Ornithodoros turicata* synganglion transcriptome versus the same from *Ixodes scapularis* (Iscap: XM_002403091) and *Rhipicephalus microplus* (Rmicro: JN97419) from Genbank. Pairwise identity versus *I.scapularis* = 82.3%; multiple identity all three sequences = 84.2%.

```
Contig88863:    ------------KTLSAILLAFIVTWTPYNVLVLIKTLSPCEDCIPMGLWNFAYYLCYI
Iscap:          RKQQERKQEKKAAKTLSAILLAFIVTWTPYNVLVLIKTVSSCDDCIPTGLWNFVYYLCYI
Rmicro:         RKQQERKQEKKAAKTLSAILLAFIVTWTPYNVLVLIKTVSSCDDCIPTGLWNFVYYLCYI
                ************************************************:******

Contig88863:    NSTVNPLCYALCNANFRRTYMRILSCKWHNKQRSMNRGYFS*TYAIVRQRKQRSCKCSST
Iscap:          NSTVNPLCYALCNANFRRTYMRILSCKWHNKQRSMNRGYFS-------------------
Rmicro:         NSTVNPLCYALCNANFRRTYMRILSCKWHNKQRSMNRGYFT-------------------
                ****************************************
```

Ot-24. Metabotropic Glutamate.

Sequence alignment (pairwise) for the metabotropic glutamate receptor contig11722 from the fed female *Ornithodoros turicata* synganglion transcriptome versus the same for the *Ixodes scapularis* sequence (Iscap: XP_002400087) from Genbank. Percent identity (coding region) = 64.6%.

```
Contig11722:  --YFGSKHKVITMCLCISFSAMVALVLLFFPKVYIIMFRPEKNNRSAFTTSKDVRCHIGY
Iscap:        PIYFGSKHKVITLCLCISFSAEVALVLLFLPRVYIIIFRPDKNNRSAFTTSKDVRCHIGY
              ********** ******** ******* ****** *** ******************
Contig11722:  VNS----------------AAAAVSRNSSHSASEFSMESPRNHSGE-VLPKSASKSKSLN
Iscap:        MNSAVTGPGGSSAAAAAAAATSAVSRNSSHSTSEFSLESPRNHSGDTVLKPPPTRSKSLN
              **                ** ********* **** ********  **      *****
Contig11722:  LVARFRVSKQDRIAASVAQHIRAVRAAEDLDRRT-------------------------
Iscap:        LLERFRASKQDRIAASVAQHIRAVRAAEALDRRTRLRHSAEPLFGPNKIPATRTSSDSPP
              *  *** ********************** *****
```

Ot-25. Ionotropic Glutamate Receptor.

Sequence alignment (pairwise) for the putative ionotropic glutamate receptor contig 9148 from the fed female *Ornithodoros turicata* synganglion transcriptome versus the same from the *I. scapularis* sequence Glutamate Receptor from Genbank (XP_002404506). Percent identity (coding region) = 58.3%.

```
Iscap:       RFEGFCVDLVRELSLLLGFRYQLRLVRDGAYGTKDSTGRWNGMVRELVDREADLALGDLT
Contig9148:  ----------------------------------PKGAWNGMIRELIDREADLAIADLT
                                               * **** *** *******  ***
Iscap:       ITYVREEAVDFTMPFMTLGIGILFRKPQGDRTLFFFLSPLSSDVWLCVAVSYLGVSFLLC
Contig9148:  ISYIREEAVDFTMPFMTLGISILFRKPELEQTLLFFLTPLSVDVWVSMAMAYVGISVLLF
             * * ************************    ** *** *** ***   *  * * * **
Iscap:       LLARFSPAESGLKRRSCCCEGTLSPCGHSKESELKNQFTLLNSLWFTISAIMQQGCDASP
Contig9148:  LVARFSPYE---WTAAPPCE-LIESSAHG--PILRNHFTLLNSLWFTISAIMRQGCDAS-
             * ***** *          **       *     * * *************** ******
Iscap:       RSASGRLLAASWWFFSFVAISTYTANLASFLTRERLRSPIQSAEDLVKQSDVRYGCVRSG
Contig9148:  -----RIIAAVWWFFSFVIISSYTANLASFLTRERMRSPIENAEDLAKQSEILYGCVKSG
                  *  ** ******* ** ************* ****  **** ***   **** **
Iscap:       STEAFFKAINYTTYERMWQAMKH----SMVESNSEGVSRVLSEAYAFLMESTSIEYVAQR
Contig9148:  STEAFFKESKFETYEKMWQTMASAQPPTLTESNAEGASRVRAGKYAFLMESTSIEYIVER
             *******     *** *** *          *** ** ***    ************   *
```

Ot 25 continued.

```
Iscap:      HCQLNQVGGLLDSKGYGIATPTGSPYRNLLSSAILRLQESGTLQLLKERWWNVDTRGRCP
Contig9148: DCELQQIGGLLDSKGYGIATPSGSPYRTHLSSAILQLQEKGTLQELKDRWWKVDAHHRCA
             * * * ************** *****  ****** *** **** ** *** **   **
Iscap:      EDAGSGVSSLSAASELGLSKVGGVFVVLLAGLGFACIIAVVEFL----------------
Contig9148: DD-GSQVKP-GSASELGLSKVGGVFVVLLAGLGLACVIAFAEFICKTRSMRRPIKVEEET
             * ** *     ********************** ** **  **
```

Ot-26. GABA-A Receptor.

Sequence alignment (pairwise) for the GABA-A receptor (contig 75714) from the fed female *Ornithodoros turicata* synganglion transcriptome versus the same for *Ixodes scapularis* (Iscap: XP_002411565) sequence from Genbank. Pairwise percent identity = 75.8%.

```
Contig75714: ---NYSRLVCEIRFVRSMGYYLIQIYIPASLIVVISWVSFWLHRNATPARVALGVTTVLT
Iscap:       RPRNYSRLVCEIRFARSMGYYLIQIYIPAGLIVVISWVSFWLHRNASPARVALGVTTVLT
             *********** ************** ***************** *************
Contig75714: MTTLMSSTNAALPKISYVKSIDIYLGTCFLMVFASLLEYAAV------------------
Iscap:       MTTLMSSTNAALPKISYVKSIDVYLGTCFVMVFTALLEYATVGYLGKRITMRKTRCQQLA
             ********************** ****** ***  ***** *
```

Ot-27. Serotonin Receptor.

Alignment (pairwise) serotonin receptor *O. turicata* contig34862 from the fed female *Ornithodoros turicata* synganglion transcriptome versus the same for the *Ixodes scapularis* (XP_002404998) sequence from Genbank. Pairwise percent Identity (coding region): 61.7%.

```
Contig34862:-----------------RSVSPTSWGACLVMPLAAVAEVSQEWVLGPALCDVWTCCDVLC
Iscap:      IFMERNLRSVGNYLVLSLGVADLMV-ACLVMPLAAVAEVSQEWALGPALCDVWTCCDVLC
                              *      ***************** ****************
Contig34862 CTASILHLLAIAMDRYRTVSHVDYVRQRNARQVGTMILLVWGVAVV--------------
Iscap:      CTASILHLLAIAMDRYRTVAQVDYVRQRNARQVGFMILLVWAVALAVSVAPVFGWKDPDF
            ******************  ************* ****** **
```

Ot-28. Octopamine/norepinephrine Transporter.

Sequence alignment (multiple) of 3 sequences: Norepinephrine transporter Contig110311 from the fed female *Ornithodoros turicata* synganglion transcriptome versus sequences from for *I. scapularis* (XP_002434929) and *Limulus polyphemus* (OAT1)_KF321729) from Genbank. Pairwise % Identity (coding region): 67.8%.

```
Contig110311: FEYLFEVYQ*DLHHSTMPSNNTVINTTATTQLTPATDAQQQQKNEQHSVADSDAEEGSGD
Iscap:        -------------------------MPTNNSSLVPKSEQLQQQQEQRNSAAGQPLIPAAGS
Lpoly:        ----------------------------------------------MSEHFSSNSKTHDPESPD
                                        *    * *     ***  :* * :  *  * :::
Contig110311: VKAEV------NAKTDTDKPRS----PVSRFFSHHHHSKESPSG-SEKGKWKKLRHAAPR
Iscap:        VTEEYQESLEGGSKTSSPLPDSDKRGPAFRPLVHQPQSKESPL-IFEKSKLKRLRHSAIR
Lpoly:        QETDKSENPRDSQNTTTPSPDSSK-------SIPTANGKGGPNDLRNKNKKRRLRKYLTK
              :  :  :       :* :: *:* :  *  :    :  :*::*    :* * ::*** : :
Contig110311: DDGYCSSTSTPLSSEYVEQLNANDCQSEVVLVDGLATTVPVHKGAHPDFESPKVTTDGTT
Iscap:        DDGYCSSSSTPLSSEYVDHLGSTEGQTEVILADGVASTVPI-------VVDAKAADSKGG
Lpoly:        DEGYYSTSSTPKSLAHKVVLLSG---TSVSTLPGQVSAT----------DSRKLPDLADG
              :*::*:**:::*:****  : *   ***: * *: *****          * :  *   *
Contig110311: GVTSGSQGDSRGGDRPTWKNKADFLLSIIGFAVDLANVWRFPYLCYRNGGGVFLIPYMLM
Iscap:        SVVPSGPHDS--GDRPTWKNKADFLLSIIGFAVDLANVWRFPYLCYRNGGGVFLIPYLLM
Lpoly:        TLTENASEDGEGDDRPTWGKKADFLLSIIGFAVDLANVWRFPYLCYKNGGGVFLIPYLLM
               **     :* **:::::**:::::::::::::::::::::::::::*:::::::::::::
Contig110311: LIFGALPLFYMELVLGQYNRQGPISVWKICPLFKGVGYCAVLVSWYVSFYYNVIIGWALY
Iscap:        LVFGALPLFYMELVLGQYNRQGPISVWKLCPLFKGVGYCSVLVSWYVSFYYNVIIGWALY
Lpoly:        LVFGAMPLFYMELALGQYNRLGPISVWKICPLFKGVGYCAVLISWYVSFYYNVIIGWTVY
              :*:::*::::::::*::::::*:::::::::::::::::::*::*:::::::::::::**:
Contig110311: FMFSSFRSELPWSHCGNPWNTENCYTGSFLDISRSNNTTQISPTNRNSPALEFFNRAVLE
Iscap:        FMFSSFRAELPWARCGNPWNTPSCYSGT-LDDNGTVQDDSMPIENRTSPALEFFNRAVLE
Lpoly:        FIYKSFSSELPWMKCGNEWNTNLCSTGG-LPNSSDVNSTDLNVLNKTSPALEFFDREVLE
              :***::**::::  :::*:::  :**:  :* *   * *     :**:::::::*:*:::
Contig110311: LHSSSGMHDLGVPKWQLVLCVFLVFVILYLALFKGVSSSGKVVWVTATAPYIILTLLLLR
Iscap:        LHTSPGMHDLGVPKWQLLLCVMLVFVILYLALFKGVSSSGKVVWVTATAPYVILTLLLLR
Lpoly:        VHLSTGFHDLGAPRWQLVICVFIVFLILYLSLFKGVKSSGKVVWVTATAPYIILTILLLR
              *: : :*::::*:*:::**::**::*::::*::::*:::::::::::::::*:::*::::
Contig110311: GVLLPGAGQGVMYYLQPNVGKLLETQVWVDAACQVFYSVGVGFGVHLTYASYNPFHNNCY
Iscap:        GVLLPGAGTGVKYYLQPNVDKLLETQVWVDAAVQVFYSVGVGFGVHLTYASYNPFHNNCY
Lpoly:        GVLLPGAINGMNYYLRPDVHKLLDSQVWIDAAVQVFYSVGVGFGVHLTYASYNKFHNNCY
              :::::::* :* :::*:*: :::**:::*:::*:::::::::::::::::::*::::::
```

Ot-28 continued.

```
Contig110311: RDCLMTTIVNSFTSFYSGFVIFVYLGYMAAKQGVPIDRVATEGHGLVFQVYPEAIATLPG
Iscap:        RDCLMTTAVNSFTSFYSGFVIFVYLGYMAAKQGVPIQTVATEGHGLVFQVYPEAIATLPG
Lpoly:        RDCLLTTAINSSTSFFSGFVIFVYLGYMAERQGVPINKVATEGHGLVFQVYPEAIATLPG
              ::::*::**::*:::*:::::::::::::**:::::  ::::::::::::::::::::::
Contig110311: GPIWAVLFFVMLLTLGLDSAMGGLESVITGLMDEFRIYFSRWRFRREIFTAVVLCASFCV
Iscap:        APIWAVLFFIMLLTLGLDSAMGGLESVITGLMDEFKPFFSRWRCRREVFTAVVICASFCV
Lpoly:        APFWAVLFFFMLLTLGLDSAMGGLESVITGLLDEFHTYFTRWRFRREIFTAVIISVSFII
              *:*:::::: :::::::::::::::::::::*:::   :*:::*:::*::::****::**
Contig110311: SLVNVTRGGGFMIHWFDTYSAGISLLCSALFESIGVAWIYGLDRFCGNIHEMLGFRPGIF
Iscap:        SIVNVTRGGGYMITWFDTYSAGISLLCSALFESIGIAWFYGLDRFCGNINEMLGFRPGIF
Lpoly:        GMVNVTRGGGYTMYWFDTYSAGISLLCSALLEAIGVAWFYGLANFCEDIHEMLGFSPGLF
              * :::::::::*** ::::::::::::::::*:*::*::*:::**::**:*:::::*::::
Contig110311: WKLCWKFITPTFLVAVIISAVVNEPRLEYHDYWYPPWAVNMGWALALSSVAMVPIVAVIK
Iscap:        WRLCWKFITPTFLVAVILSGIVNQAPVEYHNYRYPTWAINMGWGLALSSVAMVPIVAAFK
Lpoly:        WRLCWKFISPFFLVAVIASAVATNAPLVYHNYRFPGWAVALGWSFALSSVTMVPVVACYK
              ::::::::*:*:::::: :**** ****::*:**: ::***:: *:::::*:::*:::
Contig110311: LCRAKGTCAERVAYTITPVKEWEESKEKGVIERFKMSHWTYV*GVCTSAKQRERVLHWML
Iscap:        LCRAKGTCAELSHWTYV-----------------------------------------
Lpoly:        LFKARGTCTERLAKVITPEQELEQQTDKVVVNQFHLSHWVYV------------------
              :**:*:::*:* * ****  * *    * *   *  *** **
```

VITA

Noble I. Egekwu

110 MGB, Elkhorn & 43rd
Norfolk, VA 23539

PhD, Biomedical Sciences
Old Dominion University, Norfolk VA May 2015
Dissertation Title: "Comparative Transcriptomics: Identification and Putative Roles of Neuropeptides, Neurotransmitters, and their Receptors as Expressed in the Ttranscriptomes of the Synganglia of a Soft tick and Hard tick species (*Onithodorus turicata* and *Ixodes scapularis*)."

M.S., Integrated Sciences and Technology (Biotech)
James Madison University May 2009
Thesis Title: "Cloning of *bclA*, Expression of the BclA Protein in Both Prokaryotic and Eukaryotic cell systems, And its Use in the Development of a Thermopile-based Biosensor".

B.S. with Honors, Zoology
University of Jos, Nigeria December 2001
Acute Toxicity of Tannery Effluents in two Cichlids: *Oreochromis niloticus* and *Oreochromis aureus*

PUBLICATIONS

Egekwu N, Sonenshine DE, Bissinger BW, Roe MR (2014) Transcriptome of the female synganglion of the black-legged tick *Ixodes scapularis* (Acari: Ixodidae) with comparison between Illumina and 454 systems. PLoS One. 9: e102667

Sonenshine DE, Bissinger BW, **Egekwu N**, Donohue KV, Khalil SM, Roe RM (2011) First transcriptome of the testis-vas deferens-male accessory gland and proteome of the spermatophore from *Dermacentor variabilis* (Acari: Ixodidae). PLoS One.6::e24711.

Egekwu, N, Sonenshine, D.E. and Roe, R.M. "Identification and putative roles of neuropeptides, neurotransmitters and their receptors expressed in the transcriptomes of the synganglia of a soft tick and hard tick species *Ornithodoros turicata* & *Ixodes scapularis*". *In preparation (for submission to the journal* PlosOne)