The Simuliid Bulletin

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Number 54 July 2020

THE SIMULIID BULLETIN

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Cover Image: The larva of *Simulium tribulatum* caught while spinning silk. Photo: Charles L. Brockhouse.

From the Editor

The first half of the year 2020 was for most of us different than we have planned. The COVID-19 pandemics made us change our plans and working schedules, and the Simulid Bulletin is no exception. The current issue should have been dedicated to the International Simuliidae Symposium. Unfortunately, this time the successful tradition of the simuliid meetings was interrupted. Let us hope the situation will improve next year and we could meet in Morocco to share our news.

The current situation inspired us to add a new section of Bulletin "COVID-19 pandemics notes" where we can share our stories related to how the COVID-19 pandemic influenced our work. If you would like to share your experiences with colleagues, we want to invite you to publish your stories also in the next issue of Bulletin.

I wish you to stay safe and healthy during these difficult times.

Tatiana Kúdelová, Editor

FORTHCOMING MEETINGS

19th Annual North American Black Fly Association (NABFA) Meeting

February 4-5, 2021

Greetings NABFA Members, Friends and Colleagues,

The 19th Annual North American Black Fly Association (NABFA) Meeting will be held February 4-5, 2021 at the University of Georgia in Athens, GA.

We invite black fly enthusiasts, researchers, control specialists and students to join us to exchange ideas, information and camaraderie. Students are especially encouraged to participate in the *Mike Spironello Award* competition presented annually to the student giving the best talk. The award was established to honor the memory of former NABFA Secretary and black fly researcher, Mike Spironello, who passed away unexpectedly in 2006.

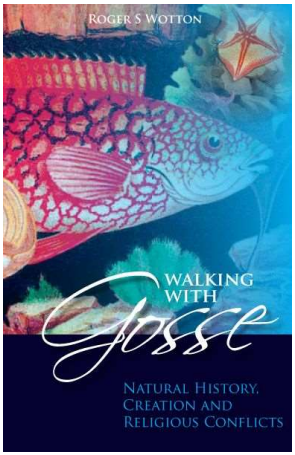
John Walz – NABFA President
Metropolitan Mosquito Control District
St. Paul, Minnesota US 55104
Office: 651-643-8388
Email: johnwalz@mmcd.org
Elmer W. Gray – NABFA Vice-President
Department of Entomology, University of Georgia
Athens, GA 30602
Laboratory (706) 542-1184
Email: ewgray@uga.edu
Carey LaMere – NABFA Webmaster
Metropolitan Mosquito Control District
St. Paul, Minnesota US 55104
Office: 651-643-8389
Email: clamere@mmcd.org

COVID-19 PANDEMICS NOTES

Walking with Gosse

Roger S. Wotton

In her recent e-mail, Tatiana asked us to share our stories of how the COVID-19 pandemic is affecting our work.



Knowing that lockdown was going to last several weeks in the UK, I decided that I needed a desk project to keep me occupied, so began a revision of my book "Walking with Gosse", that first appeared in 2012. Once the revision is completed, I'll publish the second edition as an e-book. So, what is "Walking with Gosse" about? It centres on Philip Henry Gosse, the 19th Century natural historian, best known for his work in popularising aquaria, the use of microscopes, and collecting animals on the sea shore. He was also a creationist and a believer in the literal truth of the Bible.

And what has this to do with blackflies? Henry Gosse always saw the extraordinary adaptations of the creatures he studied to be evidence of the infinite power of God, but, as an evolutionist, I have a similar sense of wonder at so many things in the natural world. In a section of the book headed "When believing in creation seems like an easy option", I give examples of adaptations that it is difficult to believe occurred through evolution. One of them is the mechanism of larval blackfly feeding.

The cephalic fans have rays that extend by hydrostatic pressure and can be folded by the use of a muscle. Each primary ray has microtrichia and there are also secondary rays and a number of other characteristic features (although not all larvae have fans of this kind). The late Doug Craig was able to follow the path of individual particles within the fans and it is complex – the fans act, in part, as sieves, but their shape means that many rays are "side-on" to the prevailing current of water and there is much turbulence

within the "concavity" of the fan as a result. We know that blackfly larvae can capture particles of colloidal size and they also collect flocs, exopolymers, coarse and fine detritus, algal cells, and a whole lot more. It is likely that the current of water passing through, and within, the fans also causes rays to vibrate, and they are thus probably acting as turbulent flocculators. It's not simple then.

When the fans are folded, everything that has been captured is scraped into the gut, without milling, and its passage usually lasts 30 – 45 minutes, with little mixing. Enzymes begin to work on the material around the periphery of the contents and the high pH of the midgut serves to split off adsorbed compounds by alkaline hydrolysis, these passing across the peritrophic membrane for absorption. The high gut pH also creates hostile conditions that cause some bacteria and algal cells to lyse. Those that survive, and there are many, release exudates as a defence and some of these are labile and may also be absorbed. The gut contents now pass through to the hind gut where they are given a squeeze, releasing more compounds in aqueous extract, before faecal pellets are formed and egested. Only a very small fraction of the food taken in is absorbed, but the constant throughput ensures sufficient nutrition for larvae to grow rapidly in most regions.

Going back to my original point – how did all that evolve? Then, after attempting to answer that question, add in the evolution of the secondary aquatic habit, the "miracle" of complete metamorphosis, the genetic control of larval, pupa and adult development, the methods that allow blood meals to be collected by many female adults, and all the other adaptations shown by these fascinating insects. One is left with an extraordinary sense of wonder.

Of course, we cannot comprehend geological time scales, and we don't know just how many acts of reproduction have occurred to allow for the selection of mutations that led to the current forms that are so familiar to us. That makes one's amazement even more profound. I have a different view of Nature to Gosse, but our reactions to what we see, and discover, seem remarkable similar. It's only the ultimate explanation that is different.

Now, back to work on the revision.

SCIENTIFIC PAPERS

Intriguing Genes: Expressed Sequences from the *Simulium vittatum-tribulatum* complex. I. Negative Control of Blood Coagulation.

Julia A. Brockhouse[1,3], Alexie Papanicolaou[2], and Charles L. Brockhouse*[3]

1: Parks College of Engineering, Aviation and Technology, St. Louis University, St. Louis, MO, USA
2: Hawkesbury Institute for the Environment, Western Sydney University, Sydney, Australia
3: Marine and Aquatic Genetics Laboratory, Biology Department, Creighton University, Omaha, NE, 68178, USA

*corresponding author

Introduction:

Simulium vittatum and *S. tribulatum* are a sibling species pair that together range through all North America, and across the north Atlantic Islands as far as the Faroes (Adler, Currie and Wood, 2004). The complex is of special importance in blackfly physiology and molecular biology because *S. vittatum* is the only species currently maintained as a long-term culture. As such, it is a vital source of stage-specific experimental material; the Simulium Genomics Project initially focused on *S. vittatum* for that very reason. Unfortunately, the inherent difficulties of all simuliid genomes (high AT, high repetitive DNA) are magnified by the record-holding number of paracentric inversion polymorphisms shared by both species (Pasternak, 1961; Rothfels and Featherston, 1981) including the colony population (Brockhouse and Adler, 2002). The inversions have frustrated attempts to assemble a reasonable genome by alleles mimicking chromosome block duplications.

While the genome itself is proving highly challenging, we have constructed deep transcriptomes (sequence collections of expressed genes) for several developmental stages of adult colony

S. vittatum and larvae *of S. tribulatum* (collected near Omaha, NE). The transcriptome was assembled and annotated (by AP; in preparation), from cDNA generated by the Brockhouse lab, and sequenced at BGI. The transcriptomes are searchable using Gene Ontology (GO) terms; standardized terms associated with molecular function, cell compartment, enzyme activity or biological role (http://geneontology.org). Each sequence in the *vittatum-tribulatum* transcriptome collection is annotated with GO terms derived from searching standard databanks in which sequences with experimentally verified functions are available (e.g., UniProt). For the next several issues, in advance of full publication of the database, we will be presenting *S. vittatum-tribulatum* cDNA sequences annotated with a variety of GO terms, together with measures of relative transcription levels in several developmental stages.

In this Volume, we present a subset of 13 of the 61 cDNA contigs annotated with the GO term "negative regulation of blood coagulation" (GO 50819). Note that most of these sequences are also annotated with other GO terms, and any one sequence might not serve that specific function in the stage expressed. The sequences presented are representative of some general groups sharing the 50819 GO term (there are many more than space allows).

We intend these sequences as a starting point for further biological investigations. We are happy to consider any specific GO term requests for upcoming issues of the Bulletin. Please contact the corresponding author (CLB) if your work would be helped by *S. vittatum-tribulatum* sequences.

Results:
Of 61 sequences examined, a majority fell into one of four protein categories based on their uniprot matches: trypsin, kallikrein, cytochrome, and snake venom. Two sequences that did not match one of these categories are listed in an "Outliers" category after the other four groups. Representative sequences from each group are listed with its mRNA sequence, protein sequence, and effective FPKM values (a standard measure of expression level: fragments per thousand bases of sequence per million mapped reads) for the various species and stages of development.

Each sequence is named with the in-house designation of the *S. vittatum-tribulatum* database; GenBank accession numbers will be provided as a supplement. Figure 1 is an example expression profile for one sequence, c149121_g1_i1|m.16749, a thrombin-like enzyme. It is not expressed in larvae, more highly expressed in nulliparous adult females than males and extremely highly expressed in parous females.

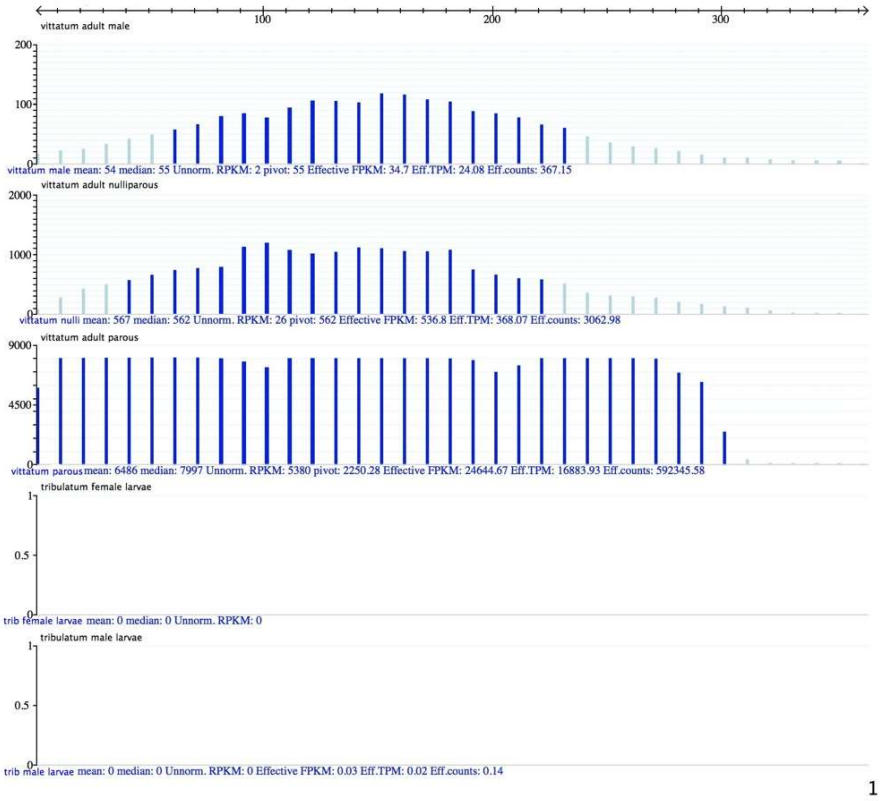


Figure 1. Expression levels of sequence c149121_g1_i1|m.16749, a thrombin-like protease. The X axis of each stage represents the length of the cDNA, the Y axis reflects the expression levels. The blue vertical bars represent the number of sequence reads mapping to a position along the length of the sequences. Note that the Y axis scale of the 5 stages differs. Here, parous females show greatly elevated expression

GO category: "negative regulation of blood coagulation" (50819)

Trypsin group

Sequence name: c149491_g1_i1|m.17165

All uniprot matches were discovered in mosquitos. The proteins are thought to assist in digesting blood meals, as the corresponding gene begins being expressed when a female mosquito begins host seeking.

mRNA sequence

>c149491_g1_i1|m.17165

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CCCTGGACGGTGAATTCCCGTTTGCCGTGTCGCTGCGTCGAGCCTCGT
CAGGTAGCCATACTTGCGGGGCCAGTATTTTGAGCCCACTGTGGGTATT
GACCGCGGCTCATTGTGTGGCGTCGCAGAATCCCGCTGACTTCGAGGT
GCAATATGGTGGTGTGCATATCAGCGAGGATGCGGTCAACGTCATTCA
GGTTGCTGCTGTGAAAATGCACGCCAAGTATTCGCCTCAAAATCAGTTT
GAGAACGACATTGCGGTGCTTAGGCTCAAGTCCCCCATCCCATTAAAC
AACTACACAGCCGCTGTTACGCTACCCGCTCGCATGCAAGACACACCC
GGCGGTTCCGACGCCACCCTGATCGGTTGGGGTTTAGACGAGTCTGG
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CCACGAGCAGTGTCGTCGGATCCACGGTTACATTCATGACTCAAACATC
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TAATTTGAAAAACATGATATTTGTGTCCAAAACCTCTCCTCGTTCTCCTT
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TTGCTGCAGCAAATATATAAGATCTCAAAATTTCACTTGTCT

Protein sequence

>c149491_g1_i1|m.17165

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AVKMHAKYSPQNQFENDIAVLRLKSPIPLNNYTAAVTLPARMQDTPGGS
DATLIGWGLDESGGDVQETLQKVDYFIPDHEQCRRIHGYIHDSNICAYY
PGGGKGQCSGDSGGPLLVDGEQVGVVSWSIKPCASAPYPGVLTRVSHF
IDWIANTTGLEL*

Effective FPKM

Vittatum adult male: 42.26

Vittatum adult nulliparous female: 32.06
Vittatum adult parous female: 149.09
Tribulatum female larvae: 0.17
Tribulatum male larvae: 0.12

Sequence name: c146679_g1_i1|m.13998
mRNA sequence
>c146679_g1_i1|m.13998
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CGCCGCACCCTACCAGGTCTCGCTGCAAACCGCCTTCGGTCACAACTG
TGGCGGGGCTATTGTCAGCGATAGGTGGGTCGTTACTGCCGCCCACTG
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CGATCGACTTGAAGTACGTCAATTACACCGAGTGCAAGAGGCTGCACC
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GGGGTCAGGGCGCGTGCAACGGCGACAGCGGTGGTCCTCTCACATAC
AAGGGCCAGCTAGTGGCACTCGTCAACTGGGGCATCCCATGCGCTCA
AGGCTTGCCAGACGCACACGCTCGTGTCTCCTACTACCACGACTGGAT
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Protein sequence
>c146679_g1_i1|m.13998
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RYNRPTFHNDIGLLRLKSPLVYSDVVKPIVYSVHELPNNATLTLTGWGRLS
GGGAVPNLLQTIDLKYVNYTECKRLHQGDESVDIGHVCTFNKRGQGAC
NGDSGGPLTYKGQLVALVNWGIPCAQGLPDAHARVSYYHDWIRTTIASN
S*
Effective FPKM

Vittatum adult male: 284.42
Vittatum adult nulliparous female: 48.55
Vittatum adult parous female: 389.95
Tribulatum female larvae: not expressed
Tribulatum male larvae: 0.36

Sequence name: c147142_g1_i1|m.14461
mRNA sequence
>c147142_g1_i1|m.14461
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Protein sequence
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IYKGELVGVANFVIGGCGTENPDGYAKVSYYIDWIH
Effective FPKM
Vittatum adult male: 25.12
Vittatum adult nulliparous female: 410.95
Vittatum adult parous female: 877.45
Tribulatum female larvae: 0.91
Tribulatum male larvae: 0.49

Sequence name: c148289_g1_i1|m.15646
mRNA sequence
>c148289_g1_i1|m.15646
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Protein sequence
>c148289_g1_i1|m.15646
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CEGDSGGPLVCRGILAGIASWSGDDMTCYSKTIPYEVYANVANPMILRW
IEAIIS*
Effective FPKM
Vittatum adult male: 37.18
Vittatum adult nulliparous female: not expressed
Vittatum adult parous female: not expressed
Tribulatum female larvae: not expressed
Tribulatum male larvae: not expressed

Kallikrein Group
Sequence name: c147712_g1_i1|m.14987

mRNA sequence

>c147712_g1_i1|m.14987

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CAGTTGAGGTACAACACGATGTTGAAGCTGTCACATCAGGAGTGTGCT
GACAAAACGGGCATCAACTATGATGGGTTGCTGTGTGTCAACAATTTGG
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CATCATACAAACCTGATGGATTTGCCAAAGTGTCCTTTTTCGTTGACTGG
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Protein sequence

>c147712_g1_i1|m.14987

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ALLRLATPLVFDALTNAVPLHKHEVPTNATIVISGFGRTDYNSRPSKQLRY
NTMLKLSHQECADKTGINYDGLLCVNNLEYNGICFGDSGGPALYNGELV
GVANFAISGCGSSYKPDGFAKVSFFVDWINEHTQKPELVYL*

Effective FPKM

Vittatum adult male: 6.71
Vittatum adult nulliparous female: 4.89
Vittatum adult parous female: 25.37
Tribulatum female larvae: not expressed
Tribulatum male larvae: not expressed

Sequence name: c148587_g1_i1|m.15989

mRNA sequence

>c148587_g1_i1|m.15989

CACAAACATGAAGTGCTTTCTTCTCTTGTTCCTGTGGCAACAAGCCTTT
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ATTTCCTTACATGGTTGCCATCATTCGATACTACGAATCATCGATCATCTG
TTCAGGATCGATCATTCACGTCAAGTGGATCCTGACGGCTGCGCATTGT
GAATACCCTGTTGGTCATTTCAGCGTCCGCGTTGGATCCTCGTATGCGG
GCCATGGTGGAGACTTGTATGACCCGGAAAAGTTCATACAACACCCCA
AATATTTCGATCAGAGAACATATTACGCAGACATTGCGCTGATCAAATTG
ATCCATCCAATCCGGCTGTCACGAACGATTCAAACTGTCAGGTTGGTTG
GGCGCCAGACGGAATTTCCGTTTGGAACTGGGTGCGAATTTGCTGGGT
TTGGCAAAACAATGGTGGCTTCGGAGGATGCCAAGGAGGATCGCTTGC

GGTTTTTGGGGATGCCAATATTACAGTTTGCCGATTGTCAGGTTTTTAGA
AGTTTCTGGCCGGCTACTATGCTGTGTGTTGGATTCAACAGTGTTCGGG
CGTTGGCTTGCAAGGGTGATTCAGGAGCACCGTTGGTTTGCAAAGGCG
TCATTTTTGGGGTTGCTACGATAGCCTACAGACAATGCCAGGCCGCTCC
GAATGGGTTCATCAGAATAGAACCGTTCTTAGGTTTCATAAGAAACGTA
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Protein sequence

>[c148587_g1_i1|m.15989]

TNMKCFLLLFLWQQAFGFDERVVGGPPVEIDRFPYMVAIIRYYESSIICSG
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QRTYYADIALIKLIHPIRLSRTIQTVRLVGRQTEFPFGTGCEFAGFGKTMVA
SEDAKEDRLRFLGMPILQFADCQVFRSFWPATMLCVGFNSVRALACKGD
SGAPLVCKGVIFGVATIAYRQCQAAPNGFIRIEPFLGFIRNVTGMNV*

Effective FPKM

Vittatum adult male: 18.58
Vittatum adult nulliparous female: not expressed
Vittatum adult parous female: not expressed
Tribulatum female larvae: not expressed
Tribulatum male larvae: not expressed

Cytochrome Group

Sequence name: c149976_g2_i1|m.17901

mRNA sequence

>[c149976_g2_i1|m.17901]

AACAGCACATGTCTTAACCAGATGAAGTGGAATTGCTCGGTTTTACAAT
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TGTTTTCTTCGAAACAAATCTGAGACTCTAAACTGCATCTGCAATCTGTT
TTTAAACTGTTTGCCGCCATTTTTGTTGTTGCTTCTACGTTCGGTCTATCG
TTGTCATCATCGTTAGGAGATTTTTAACAATTCTTTTTTTTCCCAAACTGC
ACAAATGCAAATTGCACTTCTCGAGACTGTAAAAACATGAAGTGTTCAA
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CAGTCTGTAGATTAATGAACGAGCAGTTTTATTTGTGTTGTTGTTGTTTT
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CTCCTGACAACTGTGGTGCTGATTTTTGGCGCCCATCAGCTGATTCGAG
GTGTCAGCTGGTACATGCGGCGAAGGAAATTTGTGAAGCTGGTCAACA
AGATCCCGGGTCCGAAGAGTTATCCATTTTTTGGGACCGTGTGGGATTT
GATGGGAACACCCAGGCAAGAAATCTTCAAAATCATAGCGAGTCGTGT

GGAAACCTTCGGTGGAATCTACCGCCTTTGGATCGGCACCCAGCCAGA
TGTTCGACTGGCCCGGGCCGAGTTTGTCGAGCCGATCTTGTGCAGCAG
CAAGCATTTGAAAAAGTCTCTTATTTACGATTTCCTCCGACCGTGGTTGG
GCGAGGGTTTGTTGGTGTCCGACGGTGAACATTGGCACAGACACCGC
AAAATTATTACGCCAACATTTCATTTTACCATTTTGGAAGGATTCTGCTCG
ATATTTTATGAAAACAGTGAAACGCTGGTAGATCGGGTGAAAAAATTTT
GCGGCACAGGAACTGGCTTCGATATCTATCCGTACATAACGAAAATGGC
TTTGGATATCATTTGCGAAACATCCTTGGGCACAAAAGTGAATGCCCAA
AGTGACACCGAAACCGGATATGTCAAGTCTTTGTACGATTTTATCGGAC
TGCTCATGGACCGTTTCTTCAAAGTGTGGCTCCACAACGACTTCATCTT
CGGCCTCACCGACCATGGCAAACAATTTGCCAACAGTCTCACCAGTAT
GCATGACTTTACCAAAAAGATAATCAAGGAGAAACAAGAAGAACGGCG
CGAAGCGAAAGCAACCAACCGAAACGAGGCTGTTGACGACAACGCCG
ACGACGATGATGGTAATTTTGGCAGAAAGAAGCGGCGAGCATTACTCG
ATGTTTTGCTTGAGTCAAACGAGAACGGCAATTTGATGACGGATACGAA
TATACGTGAGGAAGTTGATACGTTTATGTTTGAGGGTCACGACACAACA
ACTGCCGGCATAACCTGGTCCTTATTTTTGATTGGATTA

Protein sequence

>c149976_g2_i1|m.17901

MELFKLLLTTVVLIFGAHQLIRGVSWYMRRRKFVKLVNKIPGPKSYPFFGT
VWDLMGTPRQEIFKIIASRVETFGGIYRLWIGTQPDVRLARAEFVEPILCS
SKHLKKSLIYDFLRPWLGEGLLVSDGEHWHRHRKIITPTFHFTILEGFCSI
FYENSETLVDRVKKFCGTGTGFDIYPYITKMALDIICETSLGTKVNAQSDT
ETGYVKSLYDFIGLLMDRFFKVWLHNDFIFGLTDHGKQFANSLTSMHDFT
KKIIKEKQEERREAKATNRNEAVDDNADDDDGNFGRKKRRALLDVLLES
NENGNLMTDTNIREEVDTFMFEGHDTTTAGITWSLFLIGL

Effective FPKM

Vittatum adult male: 5.7
Vittatum adult nulliparous female: 11.49
Vittatum adult parous female: 124.57
Tribulatum female larvae: 63.49
Tribulatum male larvae: 46.2

Sequence name: c148999_g1_i1|m.16540

mRNA sequence

>c148999_g1_i1|m.16540

GATGAAGGAGATGACGTACGTGAATCAGTGTTTGAATGAGTCCATGCG
AAAGTACCCACCAGTTCCGGACTTGATACGAATTGTCACTGAAAATTAC
CGCGTTCCCAATTCCAAAGTTGTCCTCAACAAAGGCGTGAGTGTTTTCA
TCCCGGTGTATGCCATCCATCATGACCCCGAGATCTACGAGAATCCGCA
TGTGTTCGACCCAGACAGGTTCACGCCTGAGAATGAAGCCAAACGCCA

TTCATGCGCTTTCTTGCCATTTGGAGAGGGTCCCCGAAATTGCATCGGA
CTTCGATTCGGGTTGATGCAGGCTCGTGTTGGAATGGCCATGCTTTTGA
ACAGCTTCAGGTTTGAACTTTCCGATAAAAGTAGTGGTACGCTGCAATT
TGTAAAGAATTCCCCGGTTTTGTCGCCAGCCGGTGGGATTTGGTTGAA
GGCTGAGAGGCTCTAGAGTCTAGTAAACACAAAAAAAGGTTGTAAATA
GCAATAAAAAATAATGTTTCATTTATAGAAAACTGTATACCAAGGTTTGC
CTTAAAAAATATTTTTGATTTTTTACAAATTTTTGAAAAATGTTGCTTTGAT
CCAAAAAAATATTTTTATAAATTTACAATTGACCATAACTTTTGAACGGAA
ATCTTTGGAAAAACTTTGAACAGCATTTTGAAAAAGCCTTACCTTGAACA
ATAATTTTATTTTCAGATTGATTGTTTTAGTGAAGAATTTTTGTAGGGTGG
TTCAAAAACTATGAAAAAATTGGAAAGTTTCAAGTGACTTTATATTTTTTG
GTGTAATCGTCGAAATATTTTTAATAAATTTTAAATGATGCATTGAAGTAT
CGACAATATGAAATTTTTGAGTTATGCAGTTTTAAATCATACATTTTTCG

Protein sequence

>[c148999_g1_i1|m.16540]
MKEMTYVNQCLNESMRKYPPVPDLIRIVTENYRVPNSKVVLNKGVSVFIP
VYAIHHDPEIYENPHVFDPDRFTPENEAKRHSCAFLPFGEGPRNCIGLRF
GLMQARVGMAMLLNSFRFELSDKSSGTLQFVKNSPVLSPAGGIWLKAER
L*

Effective FPKM

Vittatum adult male: 21.27
Vittatum adult nulliparous female: 13.3
Vittatum adult parous female: 14.03
Tribulatum female larvae: 85.26
Tribulatum male larvae: 56.08

Sequence name: c147118_g2_i1|m.14436

mRNA sequence

>[c147118_g2_i1|m.14436]
GGAACTACAGAATCCGATGAGGACAACTCGCCGCAAATCTTCATCAAC
CAACTAATGAAGATTGCAAAGGGCAATGATGCCTTCAACGATGGCGAA
GTGATTGCTGAAACCATTACGGCTGTGCTGGCGGGCCACGAAACCTCG
GCCAACGCCATGTCACACACAATTTTGATGCTGGCCATGCATCCTGAGG
CCCAAGAAAAAGTGTTTGCCGAACTGAAGAGTGTGTTCAAAACCCAGG
ATGCTCCCATCACAGCTGAGGACTTGAAAGAACTGGTCTACTTGGAAC
AGGTGATCAAAGAAGCGATGAGGATGAACACAGTTGTGCCCCTGTATT
GCAGGCG

Protein sequence

>[c147118_g2_i1|m.14436]
GTTESDEDNSPQIFINQLMKIAKGNDAFNDGEVIAETITAVLAGHETSAN
AMSHTILMLAMHPEAQEKVFAELKSVFKTQDAPITAEDLKELVYLEQVIKE

AMRMNTVVPLYCR

Effective FPKM

Vittatum adult male: not expressed
Vittatum adult nulliparous female: not expressed
Vittatum adult parous female: not expressed
Tribulatum female larvae: 59.08
Tribulatum male larvae: 46

Snake Venom Group

The uniprot matches for sequences in this category were largely proteins discovered in snake venom, all of which appear to affect blood (coagulants, platelet aggregators, plasminogen activators, pesterase & amidase activity, etc). Interesting to note is that Thrombin-like enzyme elegaxobin-1 causes rabbit blood coagulation, but not bovine, which is one of the primary prey of this blackfly species.

Sequence name: c146901_g1_i1|m.14234

mRNA sequence

>c146901_g1_i1|m.14234

GTCAGCAGCCATCCCTACTGCTGAGCGCTGATCGAAACGATTCTAGCC
AAGAGCGAACAATCCACACAGGAGATTACGAATCATACACTGGCAACC
GCTCGCCGCTCAGAGTATACCTGGAATCAAACGCTGGCAAAGGATTGG
CCGTCCTCAACAAACTCCGAAGGGGGGTATCAATAGATGGCAACAAAT
GAATCGCAGACATTTTTTGTTGGGCCCTGGTTTGAAAAACGTCCAAAAT
ACTCCATAAAAGAACAATGCTAGTAATCAGATAACCCATTGTTTCTCAAA
ATGTTGAAGGAGTTGATTATAGCTTGTCTCGCTGTAGGCGCGGTGTTGG
CGGCGGTTCCCCAAAGAGGCGAGCCCGTTGCTGTCTTAAACAAAGACC
TGGAAGAGCGCATCATCAATGGTCAGGACGCTTCTCCCGGCCAGTTCC
CATGGGCCGGTCTCTTGATCGCTGGCGGTGCTATTTGCAGTTGTACACT
CGTCCGTGCTGACTGGGTGCTGACGGCCGGTCATTGTGTGGACCACG
TTGGTTCGGGCTCTGTGTGGTTTGGTGATCCACGCCGTGGAAGCGCCG
GGGAAGTGCACCGCAATTTCGTACAGGTCATTTTGCACCCTAACTACAA
CGGTGGAGGCAGTGATGACATCGCTATGTTGCGTCTACAATCGCCTTAC
CCGTTGGGCGGCAATATTCAGACCATCGCTTTGCCAGACCATGATGGT
CAGTGGTTCGAGAACCAATGGGGCTACATCACCGGCTATGGTGGAATC
GGAGGGGGTCAATTGCCGAGTGTGATGCAGTTTGCTGCTATGCGCGCC
CTACCCCGCAACGAGTGCAATGCACAATGGCCCGGCCAGGCTGGATTC
TTCATTTGTGCTTTTTCGGGAACTTCGTCGGCGTGCCAAGGTGATTCGG
GTGGCGGTTTCGTCATTCACGATAACGGTGCTTGGCGTGTGGTCGGTG
TAGCCTCAATGGTGTTCATGATCAATGGCGAGTGTGCCATTTCATTGTC
GAGCGGTTATGTTCGTGTGGCCTGGCACGCCGGCTGGATTCGGTGGG

TGATGGGATGATTGGTATGGAAATAAACTCAAGCAAAGTTATTGTTTCTT
TTGATTTCCCGTTTACGATACGAGAGGAGCGGTTGTGGTTACAAAGGTA
AATTCATGTTACAAACATCAAATGATTTTTTTATCGTTCCTCTTCTCTTACG
CCACGTGGGACATTGCAAACAAATACAAAAACTTAAAATCTG

Protein sequence

>c146901_g1_i1|m.14234

MLKELIIACLAVGAVLAAVPQRGEPVAVLNKDLEERIINGQDASPGQFPW
AGLLIAGGAICSCTLVRADWVLTAGHCVDHVGSGSVWFGDPRRGSAGE
VHRNFVQVILHPNYNGGGSDDIAMLRLQSPYPLGGNIQTIALPDHDGQW
FENQWGYITGYGGIGGGQLPSVMQFAAMRALPRNECNAQWPGQAGFFI
CAFSGTSSACQGDSGGGFVIHDNGAWRVVGVASMVFMINGECAISLSS
GYVRVAWHAGWIRWVMG*

Effective FPKM

Vittatum adult male: 3.65
Vittatum adult nulliparous female: not expressed
Vittatum adult parous female: not expressed
Tribulatum female larvae: 352.94
Tribulatum male larvae: 174.66

Sequence name: c149121_g1_i1|m.16749

mRNA sequence

>c149121_g1_i1|m.16749

GTTGGTGTTCGACGAGGCCACCAAACCCGTGTCGCTGTTCAAACGTCC
AGTGCCGACTGGCGCCAGGGTTATCATTTCGGGATTCGGTTGCACCGG
ATGGAATGAACCACCATCGAAGCATTTGAAGTACAATGAGATGATCACC
CTGTCGGACAAGGATTGTGCGGCTAAAACTGGCAATGATCATGCGGGC
ATCATATGTTTCAACGAGGTGGTGGAGAATGGTGCGTGCAAGGGGGAT
TCAGGAGGTCCTGCCATTTACAAAGGTGAATTGGTTGGGGTGGCGAAC
TTTGTCATTGGCGGGTGTGGTACAGAGAACCCGGATGGCTACGCCAAA
GTGTCGTACTATATTGATTGGATCCATAA

Protein sequence

>c149121_g1_i1|m.16749

LVFDEATKPVSLFKRPVPTGARVIISGFGCTGWNEPPSKHLKYNEMITLS
DKDCAAKTGNDHAGIICFNEVVENGACKGDSGGPAIYKGELVGVANFVI
GGCGTENPDGYAKVSYYIDWIH

Effective FPKM

Vittatum adult male: 34.7
Vittatum adult nulliparous female: 536.8
Vittatum adult parous female: 24,644.67
Tribulatum female larvae: not expressed
Tribulatum male larvae: not expressed

Sequence name: c118662_g1_i1|m.5190
mRNA sequence
>c118662_g1_i1|m.5190
TACCTTGAAGTGAGAACTAAAAATTTGTTGTTAAGCTGTGGTGGGTCGT
TGATCAACAAGAGATACGTACTGACGGCTGCCCACTGTGTGATCTCGA
AAAACCAGATCCCGGACCGCTACAAACCTGTCACCGTGCGGCTGGGC
GAATGGGACACTGACACCGAAACGGACTGTGACATTTCCCTCGACGGC
GACCAAGACTGTGCCGATTTTCCCGTTCAAAACATACCAATCGAAGCGA
CCATTCCGCACGCCAACTTCGACTTCAGCAACAGTCAAAACGACATTGC
GCTCATCCGTATGGCCCATGACTGTCAGTACAACGACATTGTCAAACCC
ATCTGTCTACCAGTGACCTCACAACTGCGTCAAACCAACCTGGACGGC
AACATGCTCATCGCGGCCGGTTGGGGCCGAACAGAAACCTCGAAGCC
GAGCAATCTCAAGCTGAAAGTAAGCTTGAAGGTGGTACCACTGCAAAC
ATGTCAGTCAATATTTCCCAGCCGGAGTTTGTGGACGAAACAGATGTGC
GCTGGCGATGTGGCGGGCAAGGATGTGTGCAACGGTGACTCGGGG
Protein sequence
>c118662_g1_i1|m.5190
YLEVRTKNLLLSCGGSLINKRYVLTAAHCVISKNQIPDRYKPVTVRLGEW
DTDTETDCDISLDGDQDCADFPVQNIPIEATIPHANFDFSNSQNDIALIR
MAHDCQYNDIVKPICLPVTSQLRQTNLDGNMLIAAGWGRTETSKPSNLK
LKVSLKVVPLQTCQSIFPSRSLWTKQMCAGDVAGKDVCNGDSG
Effective FPKM
Vittatum adult male: not expressed
Vittatum adult nulliparous female: 0.32
Vittatum adult parous female: 0.44
Tribulatum female larvae: 0.92
Tribulatum male larvae: 0.8

Outliers Group
Coagulation Factor IX
Sequence name: c148757_g1_i1|m.16258
mRNA sequence
>c148757_g1_i1|m.16258
ATGTCAGTTGCTATGGCAGTATAATTGACGAGTTCTTCATACTGACTTCA
GCGTCTTGCATCAAGAAATTTCCAAATGGTCCAGACACTGTGGTCTATC
AGGTGAAAAATTACGCAAAATCCTCGTTTGAAACTTTTGCCGTTGGAAA
CGTATCCATCCATCCCAAGTTTGACAATACATACAATGACATAGCTTTAG
TCCAACTAATAAACAAAATTCGATTTGATCAACCTGGACAGATAGCCTG
CATTGGATCAACAAATCAGTTGCCCGATCGATTGGAAGCCTCTGGTCAC
GGTTCCCATATTAATTTATACGAAAACAGTTAAAATCTACTAAATCTACTA

CTTATATACCTTTTTCGCGAAATTCAAATTTGTAATTTAGTATATTTCAGAG
TTTGGCAACCGACATTTGTCCATTAAATTAAACGTGATCGATGCTGAAAT
ATGTAATAGGATAATTCAAAAACGAACGACAACCCACCAAGTTGTTCAA
GATTCCCAACTTTGTGCAGCACTGCATCATTTTGCTATTCCGAATAGTTG
CGAGACGGACCTCGGAGGTCCTTTGCAACGCCAACTGTGGGATATGTC
TCGTTACTTTCCGTTTGTGTTTGGTGTCAATTCTATTGGAACTGAGTGTG
GTTTTGGCATCCCACTGGTCACCAGTCGAGTGGCGTCTTTCATTCCATG
GATCGACTCGATTGTGTATGCCAATCCTAAGCGTTTGAATCCGAGAGAT
CCAGCAATTGAAGAACATTTTCAAGAATGCATTCGTAAGAGCATGCTAC
ACCGAAAAGATGACAACAACAAAACAGACAATGAGTGGCAGGAAAATA
AGAACTTGAACCGACTGTCTTCGCTGGCTATAGTTCAACACTTTGCAAA
CGGGTCACAACGACTGACTTGGAGCTGTGACGCCGTCATCATCCATCA
CAAATTCATTGTTTCCACGGCCAGATGTGCGTCAGCAAGTAAACTATCC
AAAGTTCTAGTAGGTATCGCGACCGAACCCGTAGCTTTGGAAGTTGTG
AGGACACGCGTTCACCCAAGGTATGATCCTGCAACTGGTGGTAATGAT
GTGGCTTTAATTGAAACCACTACCTACATCAAATTCAATGTTAATGTGGC
ACCAGCATGCATGTGGTATAACCAGACCCACACGCCATTGTTCCTTTCG
AAGTTAATAATTCCTCAAGACGCATCGATGAACGCGTCAATTGTGTCAC
CCATTAAAATGTTTGCGATTACTAATATGGACTGTGCAGCAGACCATAGC
AATCTTCGAACCGACAACTTTTGTGCTCTACTGTCTGACAATTGTGATCC
CGTAGTCAGGCATGAAGCTGTCTCGGTTTGTTCACCAGAAGACTGTCC
ACAAAATCATGGAGAAACATTGGTGGCATTTGAGAGTCGCAACGGAAA
AGAGTCGCCGGTATTGGTTGGTTTGTCATCGCCGAAAAACTACTGTGAT
CTAGAGAAGGCGCTAGTTTTCACCAGAATTTCGGTGTTTCGAGATTGGA
TTGAGAATATTTTGAATTTTTCTGATGAATTTTTAGTGTTCAATGACTAGA
GTTTTGGATGAAGTTGACGTTGAAGACAGCTTACAACCCAATTTGTGAA
TTAAGTAAAAGAGCTAAATAGGAAAAAGGCGTTTCGTACTAG

Protein sequence

>[c148757_g1_i1|m.16258](c148757_g1_i1|m.16258)

MSRYFPFVFGVNSIGTECGFGIPLVTSRVASFIPWIDSIVYANPKRLNPRD
PAIEEHFQECIRKSMLHRKDDNNKTDNEWQENKNLNRLSSLAIVQHFAN
GSQRLTWSCDAVIIHHKFIVSTARCASASKLSKVLVGIATEPVALEVVRTR
VHPRYDPATGGNDVALIETTTYIKFNVNVAPACMWYNQTHTPLFLSKLIIP
QDASMNASIVSPIKMFAITNMDCAADHSNLRTDNFCAL
LSDNCDPVVRHEAVSVCSPEDCPQNHGETLVAFESRNGKESPVLVGLSS
PKNYCDLEKALVFTRISVFRDWIENILNFSDEFLVFND*

Effective FPKM

Vittatum adult male: 6.74
Vittatum adult nulliparous female: not expressed
Vittatum adult parous female: 5.53
Tribulatum female larvae: not expressed
Tribulatum male larvae: not expressed

Glia-derived nexin
Sequence name: c136679_g2_i1|m.8309
mRNA sequence
>c136679_g2_i1|m.8309
GTGTTCAACTCCGACAAGGCCGATTTACGCGGTTTGACCGAGACCCAGT
CCAAAGACCTGCACCTGTCCGACATCTTACAGATAAACACGTTCAGCAC
ATGCGGCGAAGGTAAGATCCAAGACCAACATCACGTCGAAATCTACCCA
GCACCTGCCCTCCGTCGCAACCTCAGCCTGGACACCTCCAGCGAACAG
ATGGTGTACGCCTCCTCCAACGTTGACTATCAGCGTGCCTTTGACGACCC
CATCTTCGACAACAAGTACATCGAGCTGCCGCTGTCGTTGCGACCACGA
CAGGCCCGCATCCCAGAGACGCCACGCCTGAAGTTCGACCGACCGTTCT
TGTATTTTGTGCGCCACAACCCAACGGGTATCATTCTGTTTATGGGCCG
Protein sequence
>c136679_g2_i1|m.8309
VFNSDKADLRGLTETQSKDLHLSDILQINTFSTCGEGKIQDQHHVEIYPAPA
LRRNLSLDTSSEQMVYASSNVDYQRAFDDPIFDNKYIELPLSLRPRQARIPE
TPRLKFDRPFLYFVRHNPTGIILFMG
Effective FPKM
Vittatum adult male: 5.13
Vittatum adult nulliparous female: 1.92
Vittatum adult parous female: not expressed
Tribulatum female larvae: 17.67
Tribulatum male larvae: 41.65

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