

For *S. nodosum*, a cluster called SND_clu01m showed a 40% similarity to the SVEP. Although these two salivary proteins showed less than 50% structural identity, their molecular nature, which reflects secondary and tertiary proteins, may show more similarity, as in the case of SVEP and maxadilan (Cupp et al., 1988). This SND_clu01m protein may be one of the candidates for therapeutic use in animal and human medicine, which includes treatment for increasing blood supply to wound tissues.

Surprisingly, one cluster, SND_clu02m, encoding a protein that was similar to odorant-binding proteins (OBPs) of *R. palmarum*, a palm beetle, was found in this study. OBPs belong to a moderately sized multigene family that has been identified among the neopterous insects, or about 98% of all known insect species (Vogt et al., 1999; Vogt, 2003, 2005). The first OBPs characterized belong to a Lipidopteran subgroup that is subdivided into Pheromone Binding Proteins and General Odorant Binding Proteins (Vogt and Riddiford, 1981; Vogt et al., 1991a, b; 2002). These proteins are small (~6 – 15 kDa), water soluble and bind odorants that reside within the sensillum lumen, where they pass to transport odors through the aqueous lumen from pores in the sensillum wall to odor receptors in the membranes of sensory neurons (Kaisling, 2001; Leal, 2003; Plettner, 2003; Vogt et al., 1985; Vogt, 2005). Thirteen OBPs have been identified in a male antennal cDNA library from *M. sexta* (Robertson et al., 1999; Vogt et al., 2002); and more than 50 OBP genes have been identified from each genome of *D. melanogaster* (McKenna et al., 1994; Pikielny et al., 1994; Kim et al., 1998; Galindo and Smith, 2001; Graham and Davies, 2002; Hekmat-Scafe et al., 2002; Vogt et al., 2002) and *An. gambiae* (Robertson et al., 2001; Biessmann et al., 2002; Vogt, 2002; Xu et al., 2003).

Comparisons between a number of OBPs revealed common structural components: the relatively small (14 - 20 kDa) polypeptides contain a single peptide, a hydrophobic domain exists between residues 40 and 60, and six conserved cysteines (Vogt, 2005). As the list identified, members of the OBP gene family have grown, and it has become evident that not all members associate with olfactory organs. In an expression study of 34 *D. melanogaster* OBP genes (based on putative promoters driving GFP expression in transgenic flies), 22 genes were expressed in either olfactory or gustatory tissues (9 in both), and 5 were expressed broadly in non-chemosensory regions; the expression of 7 genes went undetected (Galindo and Smith, 2001). In an immunohistological study of 5 *D. melanogaster* OBPs, 4 were localized within the lumen of

olfactory sensilla, but one associated with non-chemosensory structures and regions (Park et al., 2000; Shanbhag et al., 2001). An OBP from the beetle, *Tenebrio molitor*, was located in the hemolymph (Graham et al., 2001). Thus, members of the OBP family may have diverse chemosensory and non-chemosensory functions depending on the OBP in question (Picimbon, 2003).

In this study, the SND_clu02m shared significant sequence similarity with the 6 conserved cysteine residues of the OBP structural molecules. As the cDNA was isolated from the salivary gland cDNA library (not the antennal or maxillary palp cDNA libraries), its tissues, gender and developmental expression patterns should be investigated using immunohistological study methods. Expression data would imply the function of this gene product.

Adult black flies and other insects commonly obtain energy for developing their longevity by flying to and from nectars and plant tissues (Schlein and Jacobson, 1999; Hamiton and Naiem, 2000). These nectars and plant tissues contain various amounts of sucrose and starch that are the main products of photosynthesis. Starch is specifically hydrolyzed by alpha-amylase to maltose, which is then cleaved to glucose by alpha-glucosidase or maltase (Dixon and Webb, 1979). Alpha-amylase and alpha-glucosidase activities have been detected in the salivary glands of many insects, for example, *L. longipalpis* sand fly (Gontijo et al., 1998; Charlab et al., 1999; Ribeiro et al., 2000) and *Ae. aegypti* mosquito (James et al., 1989; Marinotti and James, 1990; Grossman and James, 1993; Grossman et al., 1997). It is not surprising that five cDNA fragments encoding partial alpha-amylase (SND_clu03m) were isolated from the female salivary gland cDNA library of *S. nodosum*. Although they are not full-length cDNAs, they can be used to produce probes for screening the library in order to obtain the full-length genes. Further investigation on enzyme activities and expression pattern in this simuliid species should be performed. In *Drosophila*, the amylase gene family has been extensively studied, as there are many amylase isozymes involved in the evolution of the fly species (Zhang et al., 2003; Goto et al., 2005). This gene should be investigated more in other simuliid species for further study on the adaptation of sugar feeding, because different black fly species live at different altitudes (Takaoka et al., 2003) where various and different plants are found.

In the secreted protein category, other four clusters, SND_clu04m, SND_clu05m, SND_clu06m and SND_clu01s, which encode partial proteins with similarity to the salivary

protein of *Cx. pipiens quinquefasciatus*, putative salivary mucin of *Ae. aegypti*, chymotrypsin-like of *Macaca mulatta* and venom allergen of *Ae. aegypti*, respectively, were sequenced. However, the full-length sequences of these genes are needed for further study on their function and molecular evolution.

For the cellular protein category, 5 clusters encoded partial ribosomal proteins, which were involved in protein synthesis and secretion. Other clusters encoded protein similar to lectin precursor, protein transporter, elongation factor 1 beta, cell surface protein precursor and beta-galactosidase precursor. These proteins were involved in general cellular metabolism. In *An. darlingi*, cellular protein category clusters (also called housekeeping category clusters) represented ~35% of the salivary gland transcriptome. These housekeeping gene products included ribosomal proteins, Golgi vesicular membrane trafficking protein, mitochondrial enzymes, enzymes from the glycolysis, glucose-6-P and Krebs cycle pathways. Moreover, enzymes associated with transcription/translation factors, signal/transport transduction pathways, and protein folding are also included.

Although the emphasis on insect salivary gland research has regarded proteins associated with sugar digestion or vertebrate hemostasis, it is possible that these novel molecules may play a role elsewhere, as in preventing mast cell degranulation. This is a notion reinforced by presence of the adenosine deaminase and purine hydrolase as well as a previously reported inhibitory activity of *Aedes* salivary homogenates on TNF production by mast cells (Bissonette et al., 1993). A role in angiogenesis, as well as bacterial or antifungal activity, are other possible functions of salivary protein.

In this study, the majority of proteins of cellular proteins are abundantly expressed in the salivary glands of *S. nodosum* (34.38%). It is clear that our knowledge on the proteome and transcriptome of the blood-feeding blackfly remains limited and there is a lack of functional experimental analysis of blackfly salivary proteins. Information obtained in this study surely contribute to a better understanding of the blackfly saliva complexity. Molecular information on different blackfly salivary proteins is essential to establish their function in the blackfly feeding process, the parasite development cycle, and immunomodulation at the vector-host interphase. High-throughput protein expression or DNA vaccine construction could help in expressing the candidate proteins or creating immunoreagents that can block or adsorb the activity to confirm the

identity of interesting proteins. Combining these approaches would permit very fast identification of novel biologically active compounds in the salivary glands of *S. nodosum* and a better understanding of the evolutionary path which this black fly took toward adaptation to sugar and blood diet and/or parasite-host interaction.

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APPENDIX

I. Sequence clusters of salivary gland cDNAs from female *S. nodosum* coding for secreted proteins (Secreted protein category) : The first translated amino acid is underlined. The termination codon (TGA or TAG or TAA) is listed in bold and underlined. The concensus polyadenylation signal sequence (AATAAA) is listed in bold.

GROUP 1

Cluster SND_clu01m
Clone SND155, SND164

>SND155 778 bp

GACTCATCACACATTTTTCACAAAAGGTTTGGCTTCAAAATTTGTTTTTTAGAAATGTTGGATTTTAAAGAGTTTTTCGTTCAA
AAGTTCCAGTATTTGTAGCGACGACAAAGTCCAATAATTGAACATACTACCTTATAAACAAAAAAGAGTCCATTTCGGACGCC
TGATATCGATCATCTATCTTTTCAAAGTAGAGGAGTAAGTGTCAACATGCGTGCCTTCATCATTCTCGTCGTTCTGAGCTCAA
CAACGTTTAGTGAACAACAGCCGATAGTCCAATTGCCGACGACCAATGCATCACCATCCAGAAAGACAATCTGGTGATGGGCGAA
CTGAAGATGGCGAATATGCCACCTATGTGTACATGGCCACCAAAAGCGGACAGTACAACGACCAATGGATTCTTCAGAGTGTGGG
CAACGGTTTCTACACACTGAAGAATAAAAAATTCGGGGCGTACACTGGGATTGGATTCTTACGACTACTTCAAACTACCAGTGATG
CGGTGGGACAAATGAGCCATTTCCAGTTTGTACCTCAAGGTGGCGGCTATGAAATCATCAACAAAGTGGCCAAACGTATAACATCC
ATGGGTGCTGAAACCATGGCCAAATTTGACTAGTGTGTTCAATATTTCAAAGTCGAGAGATGTTAAGAAAATTTGTTGAAAATTAAT
AATTCATTTTACCCTACTTTACCCAAAATAAAAAATAACGGACGCACTAGGGGGTGTGTGATAACTTTTCCATTTTCTGTAATAAA
AAAA

>SND155 148 aa

M R A F I I L V V L S S T T F S A T T A D S P I A D Q Q C I T I Q K D N L V M G E L K
D G E Y A T Y V Y M A T K S G Q Y N D Q W I L Q S V G N G F Y T L K N K N S G R T L G
L D S Y D Y F K T T S D A V G Q M S H F Q F V P Q G G G Y E I I N K V A K R I T S M G
A E T M A K L T S D V Q Y F K V E R C

Signal peptide : M R A F I I L V V L S S T T F S A T T A

Sequences producing significant alignments:	Score (Bits)	E Value
gb AAC26163.1 erythema protein SVEP [Simulium vittatum]	103	2e-21
ref XP_818651.1 lipophosphoglycan biosynthetic protein [Tryp...]	41.2	0.019
ref ZP_01459501.1 alpha-glucosidase [Stigmatella aurantiaca ...]	40.8	0.022

>SND164 794 bp

GAGCTGCATGACAAAGTCATCGGGCATTATCTGAACATAAAACACTATCAATAAGTTGGAGTCATTACCAGAATGTTGGATTTTAA
AGAGTTTTCGTTCAAAAGTTCCAGTATTTGTAGCGACGACAAAGTCCAATAATTGAACATACTACCTTATAAACAAAAAAGAGTCCATTTCGGACGCC
GTTCCATTTCGGACGCCCTGATATCGATCATCTATCTTTTCAAAGTAGAGGAGTAAGTGTCAACATGCGTGCCTTCATCATTCTC
GTCGTTCTGAGCTCAACAACGTTTAGTGAACAACAGCCGATAGTCCAATTGCCGACGACCAATGCATCACCATCCAGAAAGACAA
TCTGGTGATGGGCGAAGTGAAGATGGCGAATATGCCACCTATGTGTACATGGCCACCAAAAGCGGACAGTACAACGACCAATGGA
TTCTTCAGAGTGTGGGCAACGTTTCTACACACTGAAGAATAAAAAATTCGGGGCGTACACTGGGATTGGATTCTTACGACTACTTC
AAACTACCAGTGATGCGGTGGGACAAATGAGCCATTTCCAGTTTGTACCTCAAGGTGGCGGCTATGAAATCATCAACAAAGTGGC
CAACCGTATAACATCCATGGGTGCTGAAACCATGGCCAAATTTGACTAGTGTGTTCAATATTTCAAAGTCGAGAGATGTTAAGAAA
ATTGTTGAAAATTAATAATTTTACCTACTTTACCCAAAATAAAAAATAACGGACGCACTAGGGGGTGTGTGATAACTTTTCT
CCATTTTCTGTAATAAAAAA

>SND155 148 aa

M R A F I I L V V L S S T T F S A T T A D S P I A D Q Q C I T I Q K D N L V M G E L K
D G E Y A T Y V Y M A T K S G Q Y N D Q W I L Q S V G N G F Y T L K N K N S G R T L G
L D S Y D Y F K T T S D A V G Q M S H F Q F V P Q G G G Y E I I N K V A K R I T S M G
A E T M A K L T S D V Q Y F K V E R C

Signal peptide : M R A F I I L V V L S S T T F S A T T A

Sequences producing significant alignments:	Score (Bits)	E Value
gb AAC26163.1 erythema protein SVEP [Simulium vittatum]	103	2e-21
ref XP_818651.1 lipophosphoglycan biosynthetic protein [Tryp...]	41.2	0.019
ref ZP_01459501.1 alpha-glucosidase [Stigmatella aurantiaca ...]	40.8	0.022

GROUP 2

Cluster SND_clu02m
 Clone SND153, SND219, SND226, SND702, SND704

>SND153 774 bp

ACTGCTTGACATTTCAGCTCGGCAAAATAGGCCAGCTCGTCTGCCACTGCAGCTCGGGCATCTTGTGGCCGCATCAAAACCCCTC
 AAGTTCCCGGTGGCCACTTTACTGCGCAGCTTGTCTGTCTGTCGATAAAGAGCTGCTTCTGATCGGCAGTTAAGCGAATAAGGGT
 GGCATCTTCTTAATGTGCGCACCTGACGCTGTTTATCTTCTTGGGCCCTTGCAGCACACTTGAAGTGACGGCACACAAACAAA
 CTGTGTGAAAAAAGCCGGTAACCAAGCGGCAACATAAAAAATTGGAAGTTCAATGATTCGGAAGAGGTGAAATGCTACTTCAAGT
 GTCTGTTAATCAGTGAAAAATATTATGGATAAAAAATGGTCAAACTTCAATGGCACAAACATTTTCAAAATTTTCAACACCCAAAGAG
 CTGAAAGGTAATGCGGACAGATGCTTCACATACGCCAGATCTGAAATGCCACACTTGTGCCGGGTGCTATACAATTTTCAAATG
 TAACCTTGTATGCAATCCCGAAGGTGTTAAGAAATCAATGATCGAATATTTTTCGAAAAATCCAAAAAGCCAAAAATGGTGGCA
 AAAAGAAGGGCAAGAAAGTAAGGTTTAAAAATAAGTCCGAAGACAAAGAGACACTGAAGCAATAAAGCCAGTCTATAAGCTTTT
 CCCAAAAATATAGCTGACGTTTTTGTGAATTCCTCAAAAGTTATCTTTTGTAAATAAATTTGTTTTTACAGTAAAAA

>SND153 148 aa

M S H L T L F I L L L G L A S T L A S D G T Q P N C V K K A G K Q A A N I K N W K F N
 D S E V K C Y F K C L L I S E N I M D K N G Q N F N G T T F F K F F N T Q E L K G N
 A D R C L T Y A Q I L N A H T C A G S Y T I F K C N L D A N P E G V K K S M I E Y F S
 K K S K K P K N G G K K K G K K S K V

Signal peptide : M S H L T L F I L L L G L A S T L A

Sequences producing significant alignments:

	Score (Bits)	E Value
gb AAD31875.1 AF139912.1 odorant-binding protein RpalOBP2 [Rhync	44.3	0.002
gb AAD31883.1 AF141865.1 odorant-binding protein RpalOBP2' [Rhyn	43.9	0.003
gb AAC63437.1 pheromone binding protein [Anomala osakana]	36.6	0.45
gb AAO64978.1 odorant-binding protein RpalOBP4 [Rhynchophorus p	36.2	0.52
ref NP_001035316.1 odorant binding protein 11 [Apis mellifer...	36.2	0.58

>SND219 813 bp

AGGCCACGGTGTGTTGGCAGCGATCGTGCAGACATTTTACACTGCTTGACATTTCAGCTCGGCAAAATAGGCCAGCTCGTCTGCCAC
 TGCAGCTCGGGCATCTTGTGGCCGCATCAAAACCCCTCAAGTTCCCGGTGGCCACTTTACTGCGCAGCTTGTCTGTCTGCTCGAT
 AAAGAGCTGCTTCTGATCGGCAGTTAAGCGAATAAGGGTGGCATCTTCTTAATGTGCGACCTGACGCTGTTTATCCTTCTTGGG
 CCTTGCCAGCACACTTGAAGTGACGGCACACAACTGTGTGAAAAAAGCCGGTAACCAAGCGGCAACATAAAAAATTGGA
 AGTTCAATGATTCGGAAGAGGTGAATGCTACTTCAAGTGTCTGTTAATCAGTGAAATATTATGGATAAAAAATGGTCAAACTTC
 AATGGCACAAACATTTTCAATTTTCAACACCCAAAGAGCTGAAAGGTAATGCGGACAGATGCTTCACATACGCCAGATCCTGAA
 TGCCACACTTGTGCCGGGTGCTATACAATTTTCAAATGTAACCTTGTATGCAATCCCGAAGGTGTTAAGAAATCAATGATCGAAT
 ATTTTTCGAAAAATCCAAAAAGCCAAAAATGGTGGCAAAAGAGGGCAAGAAAGTAAGGTTTAAAAATAAGTCCGAAGACAA
 AGAGACATGAAGCAATAAAGCCAGTCTATAAGCTTTTCCCAAAATATAGCTGACGTTTTTGTGAATTCCTCAAAAGTTATCTTT
 TGTAAATAAATTTTGTAAACAGTAAAAA

>SND219 148 aa

M S H L T L F I L L L G L A S T L A S D G T Q P N C V K K A G K Q A A N I K N W K F N
 D S E V K C Y F K C L L I S E N I M D K N G Q N F N G T T F F K F F N T Q E L K G N
 A D R C L T Y A Q I L N A H T C A G S Y T I F K C N L D A N P E G V K K S M I E Y F S
 K K S K K P K N G G K K K G K K S K V

Signal peptide : M S H L T L F I L L L G L A S T L A

Sequences producing significant alignments:

	Score (Bits)	E Value
gb AAD31875.1 AF139912.1 odorant-binding protein RpalOBP2 [Rhync	44.3	0.002
gb AAD31883.1 AF141865.1 odorant-binding protein RpalOBP2' [Rhyn	43.9	0.003
gb AAC63437.1 pheromone binding protein [Anomala osakana]	36.6	0.45
gb AAO64978.1 odorant-binding protein RpalOBP4 [Rhynchophorus p	36.2	0.52
ref NP_001035316.1 odorant binding protein 11 [Apis mellifer...	36.2	0.58

>SND226 794 bp

GCGATCGTGCAGACATTTTACACTGCTTGACATTTCAGCTCGGCAAAATAGGCCAGCTCGTCTGCCACTGCAGCTCGGGCATCTTGT
 TGGCCGCATCAAAACCCCTCAAGTTCCCGGTGGCCACTTTACTGCGCAGCTTGTCTGTCTGTCGATAAAGAGCTGCTTCTGATCG
 GCAGTTAAGCGAATAAGGGTGGCATCTTCTTAATGTGCGACCTGACGCTGTTTATCCTTCTTGGGCCCTTGCAGCACACTTGA
 AGTGACGGCACACAACTGTGTGAAAAAAGCCGGTAACCAAGCGGCAACATAAAAAATTGGAAGTTCAATGATTCGGAAGA
 GGTGAATGCTACTTCAAGTGTCTGTTAATCAGTGAAATATTATGGATAAAAAATGGTCAAACTTCAATGGCACAAACATTTTCA
 AATTTTCAACACCAAGAGCTGAAAGGTAATGCGGACAGATGCTTCACATACGCCAGATCCTGAATGCCACACTTGTGCCGGG
 TCGTATACAATTTTCAAATGTAACCTTGTATGCAATCCCGAAGGTGTTAAGAAATCAATGATCGAATATTTTTCGAAAAATCCAA
 AAGCCAAAAATGGTGGCAAAAGAGGGCAAGAAAGTAAGGTTTAAAAATAAGTCCGAAGACAAAGAGACACTGAAGCAATAA

AGCCAGTCTATAAGCTTTTCCCAAATATAGCTGACGTTTTTGTGAATTCCTTTGTTAAATAAATTGTTTT
TAACAGTAAAAA

>SND226 148 aa

M S H L T L F I L L G L A S T L A S D G T Q P N C V K K A G K Q A A N I K N W K F N
D S E E V K C Y F K C L L I S E N I M D K N G Q N F N G T T F F K F F N T Q E L K G N
A D R C L T Y A Q I L N A H T C A G S Y T I F K C N L D A N P E G V K K S M I E Y F S
K K S K K P K N G G K K K G K K S K V

Signal peptide : M S H L T L F I L L G L A S T L A

Sequences producing significant alignments:	Score (Bits)	E Value
gb AAD31875.1 AF139912.1 odorant-binding protein RpalOBP2 [Rhync	44.3	0.002
gb AAD31883.1 AF141865.1 odorant-binding protein RpalOBP2' [Rhyn	43.9	0.003
gb AAC63437.1 pheromone binding protein [Anomala osakana]	36.6	0.45
gb AAC64978.1 odorant-binding protein RpalOBP4 [Rhynchophorus p	36.2	0.52
ref NP_001035316.1 odorant binding protein 11 [Apis mellifer...	36.2	0.58

GROUP 3

Cluster SND_clu03m
Clone SND513, SND517, SND526, SND528, SND542

>SND513, SND517, SND526, SND528, SND542 771 bp

CACCGATTGGAGAACATTATGAACTGTACGGAACCCAGAACGACCGGTGCTCAAGTGCCGTTCAACTTTGTCCTTTTGGAGCA
ACACTAACATCAACAGCACTGGCCGTGACTTCTTCAACTGGCCAGGAGTGGTGTAGACGCCATGCCGAGGGCAACAGCCAAAT
TGGGTGCTCGGCAATCAGCAACAACGAATGTCCAGCCGGTGGGTGTGGGCGGTCAAGATGTCCTGATGATTTTCTTTCAGAC
AATGCCCTGGCATTGGCGTCAACTACTACGGTGAGGAATCGAAATGGTGGACACTTACATTCCGTGGAATGAGACTCTTGATACGG
CCGCTGCTCGGTCAACCCAGAGATTTTCAACTCAATCTCGGTGACCCGGTCCGAACACCAATGCAGTGGGACGACTCGAAATTC
GCTGGCTTTTCAACCGGTGACCGCAGTGGTGGCGGTGGGCTGACTATCGTACGAACAATGTGAAAAACAGCTCGCCACTCC
ACGAAGTCACCTGAATATGTTCAAGAAGCTTTGGAACCTTCGCAACACGAGGCGGTGCTCCAGGACGGCACCTTCGAGGGCCACG
CTTGAACGATGATGTGATCGTCTATCGGCGCATGGTGAAGGTGTCAAGGCCTACTATGTCGATTGAATTTTGGCAAGAACGAG
CATGAAGTTGATGTCGAAGCGGTGTTCCAGAGGCACCTCCAAGGTTGAAGTGGTGTGTCGTCGTTGAACACGAAGGTTGA

>SND513, SND517, SND526, SND528, SND542 256 aa

T D L E N I M K L Y G T P E R P G A Q V P F N F V L L S N T N I N S T G R D F F N L A
K E W L D A M P E G N T A N W V L G N H D N K R M S S R L G V G R Q D V L M I F L Q T
M P G I A V N Y Y G E E I E M V D T Y I P W N E T L D T A A C R S N P E I F N S I S R
D P V R T P M Q W D D S K F A G F S T G D R T W L F V A V D Y R T N N V K N Q L A T P
R S H L N M F K K L L E L R K H E A V L Q D G T F E G H A L N D D V I V Y R R M V E G
V K A Y Y V V L N F G K N E H E V D V Q A V F P E A P P R L E V V V S S L N T K V

Sequences producing significant alignments:	Score (Bits)	E Value
ref XP_320938.3 ENSANGP00000017682 [Anopheles gambiae str. P...	265	2e-69
gb EAT48295.1 alpha-amylase [Aedes aegypti]	263	5e-69
gb EAT48296.1 alpha-amylase [Aedes aegypti]	261	4e-68

GROUP 4

Cluster SND_clu04m
Clones SND296, SND305, SND327, SND345, SND348, SND357, SND705

>SND296 772 bp

TGACGACGAAGAAGAACAGGTAGACTCAGATCAAGTACCGGATCTCGAGACAAAGACTCGCTTGGCACAAATCAAGAATTGGAAA
ACACAGATTTCAGAGATCAGGATGAAGATGAAGAAGATGGTGGATCTAGCGAGGATGATGATGGAAGTCATGTCTCAGGCGAAGAA
GACGGAGATCAAGATCTGAAGACGCTGATGATGGTGTGGAGCAGTCGATCAAGATGGCCGAGGCGAGTTGCCGAAGGTGATCA
TGCAGATGGCGCTGCCGGAAGAGGTGGAAAGAGGCCAAGAAAGGAAAGGAAAGGCTAAAGCTGATGAGGGTGGCGCTTCCCATG
GATCTGACGGCTATGGAGGTACGATGGATATGGCGATGAAGAATCTGCCGTTTACACGTACCGAGCGGTGATGCAAAATTCGGAC
GGAATACAAATAAATGCCCGAGGACCTCTCAACGGTATGGCTGACCAACGGGTGTCATGCATTAACACGATGTCATTGGCATAGT
TGTGCACATTCAACACTTCAGTAGGATCGAGCACTGCTTCGGCAATCTGGCTGAGATTGTTCAATTATATGCAGGTGACGCTGTTG
AGCGATACCAATCTGCCAGGGCACTTGCATCGATACAGTTTCTAATGATTTTACTAATGAATGCATGATCTAACAAATCGATTA
AATCCATGTCTACAGGAGGGTTCTACTGAATGTATGCTTAGATGCTCAATTGAATAAAGTGCTTCTAAATCGAAAAA

>SND296 238 aa

D D E E E Q V D S D Q V P D T R D K D S L G T N Q E L E N T D S E D Q D E D E E D G G
 S S E D D D G S H V S G E E D G D Q R S E D A D D G V G A V D Q D G R G A V A E G D H
 A D G A A G K G G K K A K K G K G K A K A D E G A A S H G S D G Y G G Y D G Y G D E E
 S A V H T Y Q R V M Q I L D G I Q I N A P G P L N G R L T N G L H A L K H D V I G I V
 V H I Q H F S R I E H C F G N L A E I V Q L Y A G D A V E R Y Q Y C Q G T C I D T V S
 N D F T N E M H D L T N R L N P C L Q G G F Y

Sequences producing significant alignments:		Score (Bits)	E Value
gb AAR18428.1	salivary protein [Culex pipiens quinquefasciatus]	45.4	0.002
gb AAU06523.1	putative secreted salivary protein [Culicoides so]	37.7	0.51
gb AAR18452.1	putative salivary protein [Culex pipiens quinquef]	37.4	0.67
gb AAU06524.1	putative secreted salivary protein [Culicoides so]	37.0	0.88
gb ABA43056.1	29 kDa salivary protein SP08 [Phlebotomus pernici]	34.3	5.7

>SND305 650 bp

GAAGATGGTGGATCTAGCGAGGATGATGATGGAGGTCTGTCTCAGGTGAAATGGAGATCATAGATCTGAAGACGCTGATGATGG
 TGTGGAGCAGTCGATCAAGATGGCCGAGGCGCAGTTGCCGAAGGTGATCATGCAGATGGCGCTGCCGGAAGGTGGAAAGAAAGG
 CCAAGAAAGGAAAGGAAAGGCTAAACTGATGAGGGTCCCGCTTCCATGGATCTGACGGCTATGGAGGTTACGATGGCTACGGC
 GATGAAGAATCTGCCGTTCCACAGTACCGGGTGATGCAAAATCTGGACGGAATACAAATAAATGCCCGAGGACCTCAACCGG
 TAGGCTGACCAACGGGTTGCATGCACCTAAACACGATGTCATTGGCATAGTTGTGCACATTCAACACTTCAGTAGGATCGAGCACT
 GCTTCGGCAATCTGGCTGAGATTGTTCAATTGTATGCAGGCGAGCGCTGTTGAGCGATACCACTACTGCCAGGGCACTTGCATCGAT
 TCGCTTTCTAATGATTTTACTAATGAAATGCATGATCTAACAAATCGATTAAATCCGTGTCTACAAGGAGGGTTCTACTGAATGCA
 TGCTTATATGCTCAATTGAATAAATGTTTCTAATCGAAAAA

>SND305 198 aa

E D G G S S E D D D G G H V S G E N G D H R S E D A D D G V G A V D Q D G R G A V A E
 G D H A D G A A G K G G K K A K K G K G K A K T D E G A A S H G S D G Y G G Y D G Y G
 D E E S A V H T Y Q R V M Q I L D G I Q I N A P G P L N G R L T N G L H A L K H D V I
 G I V V H I Q H F S R I E H C F G N L A E I V Q L Y A G D A V E R Y Q Y C Q G T C I D
 V V S N D F T N E M H D L T N R L N P C L Q G G F Y

Sequences producing significant alignments:		Score (Bits)	E Value
gb AAR18428.1	salivary protein [Culex pipiens quinquefasciatus]	45.4	0.002
gb AAU06523.1	putative secreted salivary protein [Culicoides so]	37.7	0.51
gb AAR18452.1	putative salivary protein [Culex pipiens quinquef]	37.4	0.67
gb AAU06524.1	putative secreted salivary protein [Culicoides so]	37.0	0.88
gb ABA43056.1	29 kDa salivary protein SP08 [Phlebotomus pernici]	34.3	5.7

>SND327 773 bp

TTGACGACGAAGAAGACAGGTAGACTCAGATCAAGTACCGGATCTCGAGACAAAGACTCGCTTGGCACAATCAAGAATTGGAA
 AACACAGATTCAGAAGATCAGGATGAAGATGAAGAAGATGGTGGATCTAGCGAGGATGATGATGGAAGTCATGCTCAGGCGAAGA
 AGACGGAGATCAAGATCTGAAGACGCTGATGATGGTGTGGAGCAGTCGATCAAGATGGCCGAGGCGCAGTTGCCGAAGGTGATC
 ATGCAGATGGCGCTGCCGGAAGGTGGAAGAAGGCAAGAAAGGAAAGGCTAAAGCTGATGAGGGTGGCGCTTCCCAT
 GGATCTGACGGCTATGGAGGTTACGATGGATATGGCGATGAAGAATCTGCCGTTACACGTACCGCGGGTGATGCAAAATCTGGA
 CGGAATACAAATAAATGCCCGAGGACCTCTCAACGGTAGGCTGACCAACGGGTTGCATGCACCTAAACACGATGTCATTGGCATAG
 TTGTGCACATTCAACACTTCAGTAGGATCGAGCACTGCTTCGGCAATCTGGCTGAGATTGTTCAATTATATGAGGTTGACGCTGTT
 GAGCGATACCAATACTGCCAGGCGACTTGCATCGATACAGTTTCTAATGATTTTACTAATGAAATGCATGATCTAACAAATCGATT
 AATCCATGCTCTACAAGGAGGGTTCTACTGAATGTATGCTTAGATGCTCAATTGAATAAAGTGCTTCTAATCGAAAAA

>SND327 238 aa

D D E E E Q V D S D Q V P D T R D K D S L G T N Q E L E N T D S E D Q D E D E E D G G
 S S E D D D G S H V S G E E D G D Q R S E D A D D G V G A V D Q D G R G A V A E G D H
 A D G A A G K G G K K A K K G K G K A K A D E G A A S H G S D G Y G G Y D G Y G D E E
 S A V H T Y Q R V M Q I L D G I Q I N A P G P L N G R L T N G L H A L K H D V I G I V
 V H I Q H F S R I E H C F G N L A E I V Q L Y A G D A V E R Y Q Y C Q G T C I D T V S
 N D F T N E M H D L T N R L N P C L Q G G F Y

Sequences producing significant alignments:		Score (Bits)	E Value
gb AAR18428.1	salivary protein [Culex pipiens quinquefasciatus]	46.2	0.002
gb AAU06523.1	putative secreted salivary protein [Culicoides so]	38.9	0.32
gb AAU06524.1	putative secreted salivary protein [Culicoides so]	38.1	0.54
gb AAR18452.1	putative salivary protein [Culex pipiens quinquef]	37.7	0.71

SN436 584 bp
CCAGATGTTGTTGACAACATTTTCAATGTCTGCTTGTGATTTTTCTCTGTCGACGCCCTACCAACCGCCAACATTACGGAC
AAACCCATGATGTTGATGACAAACACCCGACTATTACGACAGGAAGCATACGATTACTTGGCCAAACGAGGCGGGTCTGTACCA
AAGAAGCCGCGCGGTGTTTGTATGTGGCGGTGGCAGGCCGGAAGAACGGGCAAGCGCGAAAGTTGAAAAGCAGGAAAG
TTGGAAGAAGCCGGAAACCTGTGCAAGCAAGGCAAGAAGAACAAAGAATGGAAAGAGGGTTGGAAGGACAGAAAGGGCAG
AAAGGCCAAAAGGGTCAGATAAAAAAGTTGGCCGTAAAAATCCAGCACGGCGTAAAGTAAAAATCTAATAAAGTTTATGTAGA
TGTTTCTGAAATAAACCGTGATAGTGCGAGTGCAAGTGATCAAGATCAAGACCATCTCCAAAAAACAGTAAAGAGGATCTCC
TTGCTTGGCCACTATTTTGGAAATTTTGTGAAAAATCAAGAAACTACTTATAGTCAAAAAA

>SND277, SND285, SND294, SND310, SND321, SND329, SND340, SND349, SND367, SND373, SND436 132 aa
 K M L L T T F F N V C L L I F L V D A A Y Q P P T F S D K P H D G D D K T P O Y Y D Q
 K D Y D Y L A N E A G L Y Q K K P P G G L I V G G G Q A G K T G Q A G K V G K A G K V
 G K A G K P G A K Q G K K K P K N G K K G V K G Q K G Q K G Q K G Q N K K V G R K N P
 S T A

Sequences producing significant alignments:		Score (Bits)	E Value
gb ABF18029.1	putative salivary mucin [Aedes aegypti]	46.6	9e-04
gb EAT42064.1	conserved hypothetical protein [Aedes aegypti]	45.8	0.001
gb ABF18030.1	putative salivary mucin 2 [Aedes aegypti]	45.8	0.001
gb AAU06502.1	unknown salivary protein [Culicoides sonorensis]	44.7	0.003
gb AAV90640.1	putative salivary mucin [Aedes albopictus]	40.4	0.061
gb AAR18436.1	putative salivary mucin [Culex pipiens quinquefasciatus]	33.5	7.5

GROUP 6

Cluster SND_clu06m
 Clones SND182, SND186, SND192, SND244

>SND182, SND186, SND192 459 bp
 CGACAAGGATATGGACCGAGGTTTGACAGTGGCCGAAGGAAATATGCGAAGGAACAAAGTGTATTAATTCATGACCTTCAACA
 ATTACGGGTACCCAGACGAGGCTCGAGTTTGTGCTTGAGTATCGAGCAAAACGCGAGACTGACGAAGAGCACTGTGCCCCAGGA
 GCACATAACTTGGAAAGCTTCTGTTTAAATGTCAATGGCCAGACCAAGAGGACCCCTTACTTAGCGTGCATGCTGCCTAGG
 AACAGGGATAGTCGAAACAAAGATAGCCCCACACAGCTGATCGGCGTCGTCAAAAGTCCAAACAAGAACTGCAATTCACGATAG
 ACGAACCGGACACCAACAGCTACGTTTACGTTCTCAAGCATGGATTGAGCAAACTATCCAGCATTAATAAATTAATTCATATTTG
 GCTTTAAAAA

>SND182, SND186, SND192 136 aa
 D K D M D R G L T V A E G K Y A K E Q S V Y K F M T F N N Y G Y P D E A S S F V L E Y
 R A K R E T D E H C A P G A H N L E S F C F K M S N G P D Q E D P Y L A C N A G L G
 T G I V E N K D S P T Q L I G V V T K S N K N C N S T I D E P D T N S Y V Y V F K A W
 I E Q T I Q H

Sequences producing significant alignments:		Score (Bits)	E Value
ref XP_001089922.1	PREDICTED: similar to chymotrypsin-like [Mac	36.2	0.57
ref NP_446461.1	chymotrypsin-like [Rattus norvegicus] >dbj B...	35.8	0.81
dbj BAB24967.1	unnamed protein product [Mus musculus]	35.0	1.2
dbj BAB22549.1	unnamed protein product [Mus musculus]	35.0	1.2
ref NP_075671.1	chymotrypsin-like [Mus musculus] >dbj BAB202...	35.0	1.2

>SND244 801 bp
 CGGACTCGTATTATGTCTGCTCCCAAGAAATTCGGACAACAGCAGGAGGCAAAAGTACTGAAAAAATTGACGACACCATCACCT
 ACAACATCTTCGACCCCGTTCCCAATGAACACTTCCATTGGATGGGCTATGTTGTGCGGAACCAAGGCCGTTTATTACGTGACC
 TTTGTTAAGGCAAAAGTCTGTGCTCATCAGCCGGGAGTTGCGTGGCAAGAGATACCAAGACGCTTTGGAATGTTCCAACT
 GCGATTGGCAGCCACAGATGGCTCGACAATCGGAAGAATATCGAGTGTGAGCGTTCCGTTGGAGTCCGAGAATAGATTCTCTAC
 TGATGAGAATCATTTGGCGACAAGGATATGGACCGAGGTTTGACAGTGGCCGAAGGAAATATGCGAAGGAACAAAGTGTATATAA
 TTCATGACCTTCAACAATTACGGGTACCCAGACGAGGCTTCGAGTTTGTGCTTGAGTATCGAGCAAAACGCGAGACTGACGAAGA
 GCACTGTGCCCCAGGAGCACATACTTGGAAAGCTTCTGTTTAAATGTCAATGGCCAGACCAAGAGGACCCCTTACTTAGCGT
 GCAATGCTGGCCTAGGAACAGGGATAGTCGAAACAAAGATAGCCCCACACAGCTGATCGGCGTCGTCAAAAGTCCAAACAAGAAC
 TGCAATTCACGATAGACGAACCGGACACCAACAGCTACGTTTACGTTCTCAAGCATGGATTGAGCAAACTATCCAGCATTAATAA
 ATAAATTCATATTTGGCTTTAAAAA

>SND244 256 aa
 G L V L C L L P R N F G Q Q A G G K S T E K I D D T I T Y N I F A P R S N E H F H W M
 G M L L R N Q R P V C Y V T F V K A K S L W S I T A G S C V R K R Y Q R R F G M F Q V
 R F G S H R W L D N R K E Y R V S A F R W S P R N R F L L M R I I G D K D M D R G L T
 V A E G K Y A K E Q S V Y K F M T F N N Y G Y P D E A S S F V L E Y R A K R E T D E E
 H C A P G A H N L E S F C F K M S N G P D Q E D P Y L A C N A G L G T G I V E N K D S
 P T Q L I G V V T K S N K N C N S T I D E P D T N S Y V Y V F K A W I E Q T I Q H

Sequences producing significant alignments:		Score (Bits)	E Value
ref XP_001089922.1	PREDICTED: similar to chymotrypsin-like [Mac	35.8	2.6
gb AAF91345.1	chymotrypsin-like serine protease precursor [G...	35.4	3.1
sp Q4TTV7 GPL GLOAU	Lectizyme precursor (Proteolytic lectin) ...	35.4	3.2

GROUP

7

Cluster SNO_clu1s
Clone SNO085

>SNO085 360 bp

CACCCAAATAGTCAGCGACCGTACCCATCGAGTGGGCTGCGCCATTTCCTCGCTACCCCGAATGGAGTCAAAAGTACCAATGAAGT
TGAAGACCATGCTGTTGGCTGCGATTATTCGTTTCACGAACATTTTCAAGGAGCCAGTCTTCGAGACGGGAAAGACGGCAAGTGT
TGTAACAAACGGCAACCCACCATGTGTTCAAGGATTGTGCAAGGAAACGGAACCGATTACGCCCTATGAGAATTTCTTCGCTGGTG
GTTGCCCAATATCAGAATCGCGGTGAATGTAGACACAGATGGATTGAGGAATAAAGTTTTCCGTTAATGAATATTTAGGG
CAAGAAAAAAAAAAAA

>SNO085 94 aa

T Q I V S D R T H R V G C A I S R Y P E W S Q K Y Q M N L K T M L L A C D Y S F T N I
F K E P V F E T G K T A S A C K N G N H H V F K G L C K E T E P I T P Y E N F F A W W
L P K Y Q N R G

Sequences producing significant alignments:		Score	E
		(Bits)	Value
gb EAT46094.1	venom allergen [Aedes aegypti]	66.2	6e-10
gb EAT46342.1	venom allergen [Aedes aegypti]	65.5	1e-09
gb AAP68776.1	antigen 5-related 2 salivary protein [Anopheles d	65.1	1e-09
ref XP_316457.2	ENSANGP00000025115 [Anopheles gambiae str. P...	63.9	3e-09
gb EAT42985.1	venom allergen [Aedes aegypti]	63.2	5e-09
gb AAV90675.1	salivary antigen-5 related protein AG5-1 [Aedes a	63.2	5e-09
ref XP_316454.2	ENSANGP00000020471 [Anopheles gambiae str. P...	62.8	7e-09
gb EAA06321.3	AGAP000356-PA [Anopheles gambiae str. PEST]	61.6	1e-08
ref XP_314254.3	ENSANGP00000018746 [Anopheles gambiae str. PEST	61.6	1e-08
gb AAL68780.1 AF457550.1	antigen 5-related 3 protein [Anopheles	61.6	1e-08
gb AAD32191.1 AF132511.1	antigen 5-related protein [Lutzomyia lo	61.6	1e-08

II. Sequence clusters of salivary gland cDNAs from female *S. nodosum* coding for cellular proteins (Cellular protein category) : The first translated amino acid is underlined. The termination codon (TGA or TAG or TAA) is listed in bold and underlined. The concensus polyadenylation signal sequence (AATAAA) is listed in bold.

GROUP 1

Cluster SND_clu07m
Clone SND063, SND064, SND713, SND716

>SND063, SND064, SND713, SND716 767 bp
TTTTGATATTTCGTACCGTTACAGCGTTAAATGTCTCATCGTAAGTTTTCAGCACCTCGTCATGGTTTCGATGGCCTTCTACCCCA
AGAAGCGTGGCCCAACGCCATCGTGGTAAGGTTAAGGCCTTCCCAAGGATGACCCATCCAAACCGGTCCATTGACCGCATTTCATG
TCCTACAAGGCTGGCATGACCCACATTGTTTCGTGAGGCGGATCGTCCCGGCTCAAAGATCAACAGAAGGAGATCGTCGAAGCGGT
CACCATTGTTGGACACGCCACCAATTGTCATTGTCGGCGCCGTTGGTTACATTGAAACACCCCATGGACCACGTGCTCTCACCATTG
TGTGGGCTCAACATTGTTCCGAAGAGTGGCGTTCGTCGTTTCTACAGAAGTGGTACTGCGCCAAAAGAGGCGCTTCAACAGGCG
TCAAGAAGTGGACCGATGAGCTGGGCAAGAAGACATCGAGGACACATTCAAGAAAATGATCCGCTACTGCAAGCACATCCGTGT
GTTGGCCCATTCGCAAAATCCGCTTGATCAAGCAAGGCCAGAAGAGGCTCACCTGATGGAGATCCAATTGAATGGTGGCACCATTG
AGGACAAGATCAACTGGTGAAGGAGCACTTGGAAAAGCCCATTCCTGTCGGAGGTGTTTGGCCAGGACGAGATGATCGATTGT
ATTGCGTCAACCAAGGTAAGGTTTCAAGGTTGTCACAGCCGTTGGCACACAAAAGATTGCGCGCAAGACACACA

>SND063, SND064, SND713, SND716 245 aa
M S H R K F S A P R H G S M A F Y P K K R A Q R H R G K V K A F P K D D P S K P V H L
T A F M S Y K A G M T H I V R E A D R P G S K I N K K E I V E A V T I L D T P P I V I
V G A V G Y I E T P H G P R A L T N V W A Q H L S E E C R R R F Y K N W Y C A K K K A
F T K A S K K W T D E L G K K N I E D T F K K M I R Y C K H I R V L A H S Q I R L I K
Q G Q K K A H L M E I Q L N G G T I E D K I N W V K E H L E K P I P V S E V F A Q D E
M I D C I A V T K G K G F K G V T S R W H T K E L P R K T H

Sequences producing significant alignments: Score E (Bits) Value

Score	E		Score	E
			(Bits)	Value
Sequences producing significant alignments:				
gb EAA08849.4	AGAP003556-PA [Anopheles gambiae str. PEST]	443	6e-123	
gb ABF18250.1	ribosomal protein L3 [Aedes aegypti]	441	3e-122	
gb AAR96131.1	RH62603p [Drosophila melanogaster]	421	2e-116	
ref NP_524316.1	Ribosomal protein L3 CG4863-PA, isoform A [D...	421	2e-116	
ref XP_001359937.1	GA18487-PA [Drosophila pseudoobscura] >gb...	419	7e-116	
ref XP_313303.3	ENSANGP00000011028 [Anopheles gambiae str. PEST]	416	1e-114	
ref XP_971875.1	PREDICTED: similar to CG4863-PA, isoform A [...]	416	1e-114	
ref NP_001037126.1	ribosomal protein L3 [Bombyx mori] >gb AA...	414	3e-114	

Cluster SND_clu08m
Clone SND161, SND188, SND231, SND234, SND717, SND718

>SND161, SND188, SND231, SND234, SND717, SND718 350 bp
CAATTGAACGATTTTGAACGTTTCCGTGTGCGACTCGCCAAAGCGGAACGTAACAGATTGTACACCTGTATTCAAGAAATTGA
AGAGTTCCGGCTATCAGTCCGGCTGTTCTTCGGCAAGCCAGCTAAAGGAACAGTTTCATGGCCCCAGGTGCGAACATTTGATCCT
ACACTGAGAAAGAGAAGGTGAAAAAGATAAGAGGTCAAGAAGCGGAAAAAGCCCGCCAAAAGGCAAGAAATAAATAACTGA
TAAATTAATCTGTATCAGCATTTCCGAAGAAAAAGAAACAATTTGTTTTCAAAAATAAACATCCATTACTGAAGAGAAAAA
AAAAAA

>SND161, SND188, SND231, SND234, SND717, SND718 82 aa
N L N D F E R F R V R L A K R E R N Q I V T P V F K K L K S S A I T S G L F F G K P A
K G T V S W P Q V R T F D P T L R K K K V K K D K K V K K A K K P A P K G K K

Sequences producing significant alignments:	Score	E
	(Bits)	Value
gb EAT36458.1	60S ribosomal protein L14 [Aedes aegypti]	53.1
gb ABF18086.1	60S ribosomal protein L14 [Aedes aegypti]	51.6
gb AA33361.1	RH72463p [Drosophila melanogaster]	34.3
gb AAV37013.1	GM06787p [Drosophila melanogaster]	33.9

Cluster SND_clu09m
Clone SND359, SND731, SND721, SND724

>SND013, SND076 SND078 474 bp

TGATGGGCTGCTCTGCGTGATGGCGGGCCCTCCACCACCGCGGGTGTGGTGGTCCACGCCACCCCGTCTGAACATGGTCAC
GGACATGGACATGGCCATGGTCATGGACACGGACATGGTCACCATCCGCACCCACCCACCATCTCTCCACCACCCAGAGAACCC
AAAACCAGAGGAGCCCAACCAGAGGAGCCCAACCCGAAGAGCGGCTCCAGAAGAACCCGCCCCAGAGAACCTGCGCCGGAGG
AGCCCGCCCCCGAAGAGCGGACACAGAGGAACCATCCCAACCCGAAGAGCGCTGAGCAACAGAACCACTGAGCAGCCCGAGCAA
CCTGAGCAGCCGAGCAACCCGAAGATGGATCAGACCAATCCAGCAAGGCCAATGATGCACTGAAAGTCGCCCTTTCTGTTAAA
ATAAACTGTTGTACATTAATTTAGTTGACGTTCAAA

>SND013, SND076 SND078 132 aa

M G L L C V M A A P P P P P G A G G P R P P R P E H G H G H G H G H G H G H G H G H
P H H P H H P P P P P E E P K P E E P K P E E P K P E E P A P E E P A P E E P A P E E
P A P E E P A P E E P S Q P E Q P E Q P E Q P E Q P E Q P E Q P E Q P E D G S D Q S Q
Q G Q

Sequences producing significant alignments:	Score (Bits)	E Value
ref NP_786369.1 cell surface protein precursor [Lactobacillu...	77.8	2e-13
ref YP_815393.1 hypothetical protein LGAS_1663 [Lactobacillu...	72.4	8e-12
ref YP_038951.1 conserved hypothetical protein, possible cel...	62.4	8e-09
ref NP_344663.1 pneumococcal surface protein A [Streptococcu...	62.0	9e-09
ref YP_086232.1 cell surface protein [Bacillus cereus E33L] ...	62.0	1e-08

>SND127, SND129 508 bp

GGAAATCTAAGACTACTTTTCGAAGCGAAAATGAAAACCGCAATTATGATGGCTGTGGCCTTGATGGGCTGCTCTGCGTGATGGC
GGCGCTCCACCACCGCGGGTGTGGTGGTCCACGCCACCCCGTCTGAACACGGTCAAGGACATGGACATGGCCATGGTCATG
GACACGGACATGGTCACCATCCGCACCATCCACCATCTCTCTCCGCCACCGAAGAACCAAAACAGAGGAGCCAGACCCGAA
GAGCCAGCTCCAGAAGAACCCGCCCCAGAGGAACCTGCGCCGGAGGAGCCCGCCCCGAAGAGCGGCGACCCAGAGGAACCATCCCA
ACCCGAACCACTGAGCAGCCCGAGCAACCTGAGCAGCCCGAGCAACCCGAAGATGGATCAGACCAATCCAGCAAGGCCAATGAT
GCACTGAAAGTCGCCCTTTCTGTTAAAATAAACTGTTGTACATTAATTTAGTTGACGTTCAAAAAA

>SND127, SND129 142 aa

G I L R L L F E A K M K T A I M M A V A L M G L L C V M A A P P P P P G A G G P R P P
R P E H G H G H G H G H G H G H G H G H H P H H P H H P P P P P E E P K P E E P R P E
E P A P E E P A P E E P A P E E P A P E E P S Q P E Q P E Q P E Q P E Q P E Q P E
Q P E D G S D Q S Q Q G Q

Sequences producing significant alignments:	Score (Bits)	E Value
ref NP_786369.1 cell surface protein precursor [Lactobacillu...	73.2	5e-12
ref YP_815393.1 hypothetical protein LGAS_1663 [Lactobacillu...	67.4	2e-10
ref NP_344663.1 pneumococcal surface protein A [Streptococcu...	65.5	1e-09
ref XP_873006.2 PREDICTED: similar to chromosome 9 open read...	60.5	3e-08
dbj BAC53723.1 Piccolo [Mus musculus]	55.5	9e-07

>SND530, SND539 531 bp

GGAAATCTAAGACTACTTTTCGAAGCGAAAATGAAAACCGCAATTATGATGGCTGTGGCCTTGATGGGCTGCTCTGCTGTGATGGC
GGCGCTCCACCACCGCGGGTGTGGTGGTCCACGCCACCCCGTCTGAACATGGTCAAGGACATGGACATGGCCATGGTCATG
GACACGGACATGGTCACCATCCGCACCATCCACCATCTCTCTCCGCCACCGAAGAACCAAAACAGAGGAGCCCAACAGAG
GAGCCCAACCCGAAGAGCGGCTCCAGAAGAACCCGCCCCAGAGGAACCTGCGCCGGAGGAGCCCGCCCCGAAGAGCCGGCACC
AGAGGAACCATCCCAACCCGAAGAGCGCTGAGCAACCCGAACCAACCTGAGCAACCTGAGCAGCCCGAGCAACCCGAAGATGGATCAG
ACCAATCCAGCAAGGCCAATGATGCACTGAAAGTCACCCCTTTCTGTTAAAATAAACTGTTGTACATTAATTTAGTTG
AACGTTCAAAAAA

>SND530, SND539 150 aa

G I L R L L F E A K M K T A I M I A V A L M G L L C V M A A P P P P P G A G G P R P P
R P E H G H G H G H G H G H G H G H G H H P H H P H H P P P P P E E P K P E E P R P E
E P K P E E P A P E E P A P E E P A P E E P S Q P E Q P E Q P E Q P E Q P E Q P E
P E Q P E Q P E Q P E D G S D Q S Q Q G Q

Sequences producing significant alignments:	Score (Bits)	E Value
ref NP_786369.1 cell surface protein precursor [Lactobacillu...	77.4	2e-13
ref YP_815393.1 hypothetical protein LGAS_1663 [Lactobacillu...	74.3	2e-12
ref NP_344663.1 pneumococcal surface protein A [Streptococcu...	65.1	1e-09
ref YP_038951.1 conserved hypothetical protein, possible cel...	64.7	2e-09

GROUP 6

Cluster SND_clu02s
Clone SND504

>SND504 605 bp

CGACCACAATGGCTTAATTCCTTCATTCAACATCAATCTTTTTGTCAGTGATCACTCAACTCCAATTCGTCGTTGGCAGTGGAAAGT
 TTTGGAACAGCTTGCGTCGCTTTGATAAGCCGCGACAAAGACCAATCAGCTAAGGAATACCTGTATCAGAACCTAAACGACAAATG
 GGTGTATTATGGAGCTGGCCTCGCCACCAAACCGAATGGGATGTAGTAGAGGATGGTCAGCTATTGAAGAATCGACAGTCTAAAC
 TTTGTCTAGCTGCATTGGCCTATAAATCGGGTGCTATCAAACTATGCTGGTGTGCAAACTTGCAACAAAAACAAACAAACCAA
 AAGTGGACACTGGTTTCAGATGGCAAGGTCAAGAATGTTGGAACAAACCGATATTTACAGCAGTCTGAGTCCAACCGCAGTGTTC
 TCTGGGCGACAACGGTGACAGATTCAAATTGTTGATTGTAATAGTTGACAATGTCCAACTTCTAAAATGACAAGCTGCAAAAC
 TGCACCTGACTGCTTCAGTATTGAATTGGCAATGACTAAATCACAGATCCGATTCCAAATATACCAGCTTAAATAAATCAAAAAA
 AAA

>SND504 152 aa

FFISTSIFFAVITQLQFVVGS GSFGTACVALISRDKDQSAKEY
 LYQNLNDKWVYYGAGLATKTEWDVVEDGQLLKNRQSKLCLAAAL
 AYKSGAIKHYAGVETCNKNKQNKQKWTLVSDGKVKNVGTNRYLQ
 QSESNSRVHLGDNGDRFQIVDC

Sequences producing significant alignments:	Score (Bits)	E Value
gb ABLO6969.1 beta-agarase precursor [Vibrio sp. V134]	38.5	0.26
ref XP_425982.2 PREDICTED: similar to mannose receptor C1 [Gall	37.7	0.43
gb AAK82459.1 type 2 ribosome-inactivating protein cinnamomi...	37.0	0.74

III. Sequence clusters of salivary gland cDNAs from female *S. nodosum* coding for probably hypothetical proteins (Conserved unknown protein category): The first translated amino acid is underlined. The termination codon (TGA or TAG or TAA) is listed in bold and underlined. The concensus polyadenylation signal sequence (AATAAA) is listed in bold.

GROUP 1

Cluster SND_clu15m
Clone SND003, SND004, SND092, SND093

>SND003, SND004, SND092, SND093 579 bp
CAAACCTTGTAAATTGCCCCACGACAGTGCAGAAACACGGATAAGTACAGAAATGGCAGGACAAAATTTAGCCTACAACGCCACACA
GTGAGTCCTTCAAACCTACCGCCCAATTTTGAAGAGATGATGCGAGCCTTCTACGATGAATACAAGGACGCCAATATGTCCTAC
ATCAATGCATACCGAAGTCATGACGAGGCAAGAAAATCGGCCACTTTACCCAAATAGTCAGCGACCGTACCAATCGAGTGGGCTG
CGCCATTGCCCGCTACACCGAATGGAGTCAAAAGTACCAATGAAGTGAAGACCATGCTGTTGGCCTGCGATTATTCGTTTCACGA
ACATTTTCAAGGAGCCAGTCTACGAGACGGGAAGACGGCTAGTGTCTGTAACAAACGGCAACCCACCATGTGTACAAAGGATTGTGC
AAGGAAACGGAACCGATTACGCCCTATGAAAATTCTTCGCCTGGTGGTTGCCCAATATCAGAAATCGCGGTTGAAATGTAGACAC
AGATGGATTGGAAGAATAAAGTTTTCGTTGTTGAAACTTAGGAGCAAGAAAAA

>SND003, SND004, SND092, SND093 167 aa
K T C K F A H D K C R N T D K Y R M A G Q N L A Y N A H S E S F K P T A Q F L K E M M
R A F Y D E Y K D A N M S Y I N A Y R S H D E G K K I G H F T Q I V S D R T N R V G C
A I A R Y T E W S Q K Y Q M N L K T M L L A C D Y S E T N I F K E P V Y E T G K T A S
A C K N G N H H V Y K G L C K E T E P I T P Y E N F F A W W L P K Y Q N R G

Sequences producing significant alignments:	Score (Bits)	E Value
gi 54643131 gb EAL31875.1 GA21762-PA [Drosophila pseudoobscura]	131	8e-30
gi 21711633 gb AAM75007.1 GH04057p [Drosophila melanogaster]	130	2e-29
gi 24641974 ref NP_572957.1 CG9400-PA [Drosophila melanogast...]	130	3e-29

GROUP 2

Cluster SND_clu16m
Clone SND021, SND031, SND053, SND058

>SND021, SND031, SND053, SND058 481 bp
TTTAAGACTGTATTGATGACTCAGAATTCGGAATTTGAAATTTTAAGGGTGCTCCTATATAAGGTTACAAATGGAAGTCAAGAAA
ATTTAGCCTAGACTGGTCTGACGATCCTTGCTCTTGAAACCATGAGACCCAGCTCAGGGATATTACCTGAAACCTGGAGTAACT
ATTAAAAAGCAGGATCAAACTTGGATTTCAGTATGATGTTAAGCCCTAGAAATGGAAAATATTTCTTCAGTAATTTGGTACT
CTAAACACGACGACTGGACCTGTTCTTAACGTTTCGACCGGAAGACGGCCAGATATTTGGAATTAGCCAGAAAACAACCGGATT
TTTATTGCAAAAGCGGAATCATTTCACCAAGTTGGAACGTTGATTGATAGGGGACCTATGGATACACAGAGCTCTTTTACAC
AACITTTCTAGATCCATTGCAATAAACAATTGAATTTCAAAAAA

>SND021, SND031, SND053, SND058 70 aa
A L E L E N I F F S N F G T L N T T T G P V L N V S T G R R P D I L E I S Q K T T G F
F I A K A G I I S P K L E T L I D R G T Y G Y T E L F

Sequences producing significant alignments:	Score (Bits)	E Value
emb CAG02640.1 unnamed protein product [Tetraodon nigroviridis]	35.0	1.5

GROUP 3

Cluster SND_clu17m
Clone SND022, SND026, SND733, SND735

>SND022, SND026, SND733, SND735 282 bp
GTCCTGAAGCTGTACAAAAAGACAGAAATGTGATACCTTTCATTGATACCGTCGATGTCACGCCAATGCCAATGATGGCCCTCTCG
GGTGCTGGGATCACACACAAAGGGACGAAAATTTGGTGGCTACCTGACACCGATTGCTTTAACATTGTGAGATTATTATAGCGG
GTCGTGTTGCCACGGGAACAGTTTTCCTTGAGAATTTAGATTTCCTATCGTTGTTCTGCTAAATTTGGTTCCTAATAAATAC
AATCAGAATCTATTTAAAAA

>SND022, SND026, SND733, SND735 69 aa
 L K L Y K K D R N V I P F I D T V D V T P M P M M A L S G A G I T H K Q H E N C G G Y
 L T P V A L T L S D Y Y T R S V G H A E Q F S L R I

Sequences producing significant alignments:	Score (Bits)	E Value
<u>gi 66772013 gb AA55318.1</u> IP1260lp [Drosophila melanogaster]	43.1	0.003
<u>gi 24580947 ref NP_722735.1</u> CG31664-PA [Drosophila melanogaster]	43.1	0.003
<u>gi 66771991 gb AA55307.1</u> IP1250lp [Drosophila melanogaster]	43.1	0.003
<u>gi 54643658 gb EAL32401.1</u> GA15301-PA [Drosophila pseudoobscura]	41.2	0.012

GROUP 4

Cluster SND_clu18m
 Clone SND044, SND051

>SND044, SND051 531 bp
 CAGTGGCGTGGCGGTTTGTATGTCCCTTCTACTTTGCGGACGAGGGTCCGAGCCACGCCGCCAACTCTTGTACATTGTGCTCAA
 CTAAATTAATAAACCAATTCATTCACACATATTTTCTTACTTTAATTTTATTCTTGTATAAACCTTTTCAGCCAAATGGGTCG
 ATTGAAAAACGTTGAGACAAAAGCACAAATATAAACAAAATGCATTTCAAAAACCATATTTTCATTGTAAACTTAGTGCAGAA
 TTTAAAGAAAATCAAAAAGAAAGCATTCCAAAAAAATGTAGACGAAGAATCACTCACATGGCCCTACGACATGCTTGGGGAC
 CAGCTCGGTTTATCAGAGAGAAAAATATGAACCCCTATTAAAAAAATAAAAATAAAACTTCACTGAAATGTTTTTAAATGGAA
 GCTAAAGAAATATTTCAAAAATATTGTAAACGTGAATGATTTATTATATAATAAAAAATATGTAAAAACGGCCTTTGGTGAAT
 GAAATCAAAAAA

>SND044, SND051 80 aa
 S G V A V L L C P F H F A D E G P S H A A N S C T L C S T K L K N H S F T H I F F T L
 I F Y S C I N L F S Q N G S I E K T L R Q K H N Y K Q K C I S K T I F S L

Sequences producing significant alignments:	Score (Bits)	E Value
<u>gi 54639011 gb EAL28413.1</u> GA18044-PA [Drosophila pseudoobscura]	26.9	2.5 -1
<u>gi 89294174 gb EAA92162.1</u> hypothetical protein THERM_008035...	32.7	7.2 -2

GROUP 5

Cluster SND_clu3s
 Clone SND168

>SND168 364 bp
 CAAATGTCCGACATTTTCGACAAACAAAGTGATAATTGGCCTCAAATTCACAAAGTACGAGTCCAAATTGATAACCCCTACAAATCAA
 ATAGGCACCCCTTCAACCCCTACGGTTATGTGGACGAAACCCAGGCTGCATGGCGCGAACTGGACCCGATGCTGTTGCCGACCACT
 ATTGGGACTTTCCCGGCTACCATCATGTGCGGCGAATGTTCGAGCCTTGCGAATGAAGAAGTTAGAAGTGCCCGAAAATACGGTG
 ATGACCGGCATCAAGTTTCAAGGATGAAGTGCCCGACATTTGATACGGTACACATATTTCAACTTTACCGCGGTGTTCT
 GGATCCATAAATATTTTCT

>SND168 117 aa
 Q M S D I F D N K V I I G L K F T K Y E S K L I T L Q I K E A P L Q P Y G Y V D E T Q
 A A W R E L D P M L L P T N Y W D F P G Y H I M S A N V R A L R M K K L E V P E N T V
 M T G I K F I V K D E V P D I S I R Y T Y F N F T S G V L D P

Sequences producing significant alignments:	Score (Bits)	E Value
<u>ref XP_969643.1</u> PREDICTED: similar to CG2206-PB, isoform B [...]	62.0	9e-09
<u>ref XP_969492.1</u> PREDICTED: similar to CG2206-PA, isoform A [...]	57.8	2e-07
<u>gb AA55307.1</u> IP1250lp [Drosophila melanogaster]	57.4	2e-07
<u>ref NP_722735.1</u> CG31664-PA [Drosophila melanogaster] >gb AAF...	56.6	4e-07
<u>gb AA55318.1</u> IP1260lp [Drosophila melanogaster]	56.6	4e-07

GROUP 6

Cluster SND_clu4s
 Clone SND175

>SND175 712 bp

TAATTTTCTAGCTGCTGTAGTITTTGCCGTTGTTGTAGCAGATGAACGAGAAGGAATGAAATACAGCGA¹TCGAATTGGTGTGC
CAAAAGGATAGAGAAGAAAGTAATGGACCGATGGCTGCTGTCGTCGCGAAAGTTCTGGTGTGGGTACCGCAGAGAAGTCGGTGA
AAGTTTGGAGAAAGTACTATAGCTGTTCCGAAITGGCCCGCGAAGAAATTTATGCCAGAAAGCGAAATGATAGAGAGTGT
TCCTGAAAGGCGAATCATTTGAAGAGACTTCTGATGAGTATGAGGAGGTTTACGGCATCAATTTGACAAAGAGAAAT²GAATCAGCG
TCTTAGCTTTAGAAACATTTTCTTATATTTCTGAAGAAATCGGAATAAACTTGGTTTGGTCAATGACAAATGTAAAAAAATAT
AAACTCTAGATAAAATTAACCGAGCGACATAGGGGTGGCGATAATTTCCCATATGCTTACTAGGATTTGAAAAAATATGGA
GTATCAAAATTTCTCTATAGACCAAAATTTCTCTGTTGAAGTTCGTTAAGGGATCGAAAAATTAARCTCAGTAGCATTTATCCGG
AGGATTAAGAAATTTAAAAATTTGTCTTCTAGTAGGACCACCTTTGCATGGGAATTTTAAAGAGTTTCAAAATTTCTAAAAAT
ATATATTTTATGACGACCTTAAAT

>SND175 111 aa

[illegible]

	Score	E
Sequences producing significant alignments:	(Bits)	Value
ref XP_341506.3 PREDICTED: similar to FYVE, RhoGEF and PH do...	32.3	9.4

GROUP 7

```
Cluster      SND_clu5s
Clone        SND176
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>SND176 529 bp

CTAATTTCAGTTTGATACACTAGTGGTGGTTGTGCGATTACAGATTTTTAGCAGTGTTTATTCCTCCATTATTTGGGAACATGCCAGAT
TGGAAAAGATAGAAAATTTTTCATCAACACTATATAATTACTTGCACACTATACACGCAAGTAGATTGGATATTGCAAAAGTTTACGAAA
AAAATGGTAACCCAGGGAGCCAGGGGGTAGATGCCACATGAAACCCCGATTTCAGAAAGGAAATCTTTTGGATGGGGTGAAATA
ACTTTGAAAAAATCTCCCAACCTGGAAATTTGATTTTTTGTGTTAACTTTAGGAACAATTGGACGGAAGGCATTTTGGGATACATGG
TAAAGCTGTCAACTATCACATCTTTTTTACAAGAAATATTACGAATCCAATAGAATTTAACTGAACTTAGACTGTGTATTTTT
TTTGAATAATTAATTTTAAATTTGCAAAAATAAAAAGTTTAGAATAGCAAATTCAAAAAATAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAA

>SND176 134 aa

L I Q I L I L V V V A I Q I F S S V Y S P F I W E H A Q I G K D R K F F I N T Y N Y C
 N Y T R K V L D I A K V Y E K N G N P G T Q G V D V H I N P D F K K G K L F G W G E I
 T L K N S Q P G N L I F C V N F R N N W T E G I F G I H G K A V N Y H I F F Y K N I Y
 E S N S R I

Sequences producing significant alignments:	Score (Bits)	E Value
<u>gb AAX33317.1</u> secondary cell wall-related glycosyltransferas...	33.5	3.6
<u>ref XP_650433.1</u> hypothetical protein 220.t00001 [Entamoeba h...	32.7	6.8
<u>ref XP_653666.1</u> hypothetical protein 76.t00030 [Entamoeba hi...	32.7	6.9

GROUP	8
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Cluster      SND_clu6s
Clone        SND246
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>SND246 407 bp

TGC~~A~~AACATGAGGTTGATTGCAATCAGTGTTTTTTCCTGCTTTGCCCTCTGTGATGCCAACGCGCTCGACGATGCGCTGCCAACAAAC
 CAATGGAGGAAAGGTATCTGTAGGGCGCTGTTCAAGCGATTTTACTACGACTCGGTGACAAAAAGGTGTCGGAAGTTTACTACGGC
 GGGTCGAGGGCAACGACAACAATTTGAAACGAGAGTGGCATGTATGAAAAAGTGATGCGCGGTATGTCAGCTGATCAAAACAAG
 AAAACGCCAAAAATAAAAAATAAAACAACCGATCGACGAGTAAAGAAATAAAATTTTGTCTAATCTCGTCAGTATCAG
 AATACATGAATGGAATACAAATTTTGAGATAAAGTTCAGATAAGAAATCAAAAAAATAAA

>SND246 100 aa

ANMR LIAISVFFLLCLCHANALDDACQQPMEEGICRALFKRFY
YDSVTKRISCRKFY YGGCEGNDNNFETR VACMKKCM P DVQLIKTR
KRKNKNKNKNOPVQQ

	Score	E
	(Bits)	Value
Sequences producing significant alignments:		
ref XP_534613.2 PREDICTED: similar to alpha 3 type VI collag...	70.9	2e-11
tpe CAI77244.1 TPA: collagen, type VI, alpha 3 [Canis familiari	70.9	2e-11

gb|AAR24535.1| Kunitz protease inhibitor 2 (Bitis gabonica) 70.9 2e-11

GROUP 9

Cluster SND_clu7s
Clone SND266

>SND266 554 bp

TTTTTTTTTGTGCGAGTCAATAAAAAATCAAAACAAATGGCTTGGCTCTCGATAATTGGCAAGGATAACTCGCCTATTACATTGC
CACCCAAGACCTGGACAAGGAGCTGGAGTTATCTACCGCTGCCATGCCGCTTGGATGTGATCGACGAGAAATGCAACCAAAAC
AAGCGGCTGTGCGATCGAGGGACCTGTTTTGGGTCAATTGTATTCTACCGAATGTATCGAATATATGGCTACGTGACGAATACC
AAAGTGAAATGCATCATTTGTCATCGACAATAGTAATGCGGCGAGTCCGCGAGAGAATGAAATTCGAACATGTTCCGGAACCTCCA
CCTACTGCTATCGGAAGCAATTTGCAATCCATTTTACATGCCGCGGATCTCTTGGTTTCGAAATCTTTTGACAAGCGTGTTCGAT
CTATCATGAACGGTGGTACAGTCTAAACTATTAAATTAATAATTATATTCTACCGAATCATTGATAATTGTTAACTTTA
TTATTTTAGAATAAAAAAGGTTATACCTTAAAAA

>SND266 139 aa

M A C V S I I G K D N S P I Y I A T Q D L D K E L S Y R V H A A L D V I D E K C N
Q K Q A A V D S R D L F L G Q L Y S T E L Y R I Y G Y V T N T K V K C I I V I D N S N
A A A P R E N E I R T M F R N L H L L L S E A I C N P F Y M P G D L L V S K S F D K R
V R S I M N G G T V

Sequences producing significant alignments:	Score (Bits)	E Value
gb EAT36697.1 conserved hypothetical protein [Aedes aegypti]...	182	5e-45
ref XP_316566.1 ENSANGP00000010040 [Anopheles gambiae str. P...]	178	6e-44
ref NP_610662.1 CG9067-PA [Drosophila melanogaster] >gb AAF5...	175	8e-43
ref XP_001361253.1 GA21516-PA [Drosophila pseudoobscura] >gb...	157	2e-37

GROUP 10

Cluster SND_clu19m
Clone SND005, SND292, SND297, SND356, SND364

>SND005 496 nt

CACAAATCTAACTTTAACCATGAAAGTGATCATCTTCTTAGTCGCTGTTGTCGTCGGTGTAAATGCCTGGGAGCAAGGTCGCCAT
GGGCTGGAGCCGCTACTTGAAGAGATCTGGAAGCGGTAAAGAGGTGAAGTGAATGGCCTGTGCCAAAAGGAACAGGATCTAAC
TGCTCTGCTCTCGTGCATGTATTTTCTGGGATACAGAAAATGAGAAGAAATCAAGACTATTTAGACTTGTACAACCTCTGCAAAAA
CTCATCCGAGGGAGAGGATGAGTGCAGAACGAAACGAAATCTGAGGGACTGCTTTTGGCAGACAAGACCTATGAAAGACATCGG
ATGATTCGAGGAGTGTACAGCATCGGTTCCGGCATAGATAAGAAGACAAAATGTGTGAAAATTCCTGTTTTCTAGCAATTTTT
CAAAACAAAATTGTAACATCATCGTGCACAAATAAATGCTATTTCCGTTCAATGAAAAA

>SND005 126 aa

I N L T L T M K V I I F L V A V V V G V N A W E Q G R P W A G A V L E K D L E A V R G
E V E L A C A K K E P G S N C L V S C D V F F W D T E N E K N Q D Y L D L Y N F C K N
S S E G E D E C E R N E N L R D C F L A D K T Y E K T S D D S E E L Y S I G S A

Sequences producing significant alignments:	Score (Bits)	E Value
ref XP_001369831.1 PREDICTED: hypothetical protein (Monodelphis	37.7	0.26
ref XP_969600.1 PREDICTED: similar to Peroxidase precursor (...)	35.8	0.97

>SND292, SND297, SND356 539 bp

AGACCATTAATCTAACTTTAACCATGAAAGTGATCATCTTCTTAGTCGCTGTTGTCGTCGGTGTCAATGCCTGGGAGCAAGGTCGC
CCATGGGCGGAGCCGCTACTTGAAGAGATCTGGAAGCGGTAAAGAGGTGAAGTGAATGGCCTGTGCCAAAAGGAACAGGATC
TAATGCTCGTCTCTTGGATGTTTTTTTTTGGGATACAGAAAATGAGAAGAAATCAAGACTATTTAGACTTGTACAACCTCTGCA
AAACTCATCCGAGGGAGAGGATGAGTGCAGAACGAAACGAAATCTGAGGGACTGCTTTTGGCAGACAAGACCTATGAAAGACA
TCGGATGATTCGAGGAGTGTACAGCATCGGTTCCGGCATAGATAAGAAGACAAAATGTGTGAAAATTCCTGTTTTCTAGCAATA
TTTTCAAAACAAAATTGTAACATCATCGTGCACAAATAAAGGCTATTTCCGTTCAATGGACGATATTGTTCCGCTATTGTTCTGTTA
CTGTCACGTATATAAAAAA

>SND292, SND297, SND356 127 aa

T I N L T L T M K V I I F L V A V V V G V N A W E Q G R P W A G A V L E K D L E A V R
G E V E L A C A K K E P G S N C L V S C D V F F W D T E N E K N Q D Y L D L Y N F C K
N S S E G E D E C E R N E N L R D C F L A D K T Y E K T S D D S E E L Y S I G S A

Sequences producing significant alignments:	Score (Bits)	E Value
ref XP_001369831.1 PREDICTED: hypothetical protein [Monodelphis]	37.7	0.22
ref XP_969600.1 PREDICTED: similar to Peroxidase precursor (...)	35.8	0.85
ref XP_789710.2 PREDICTED: hypothetical protein [Strongyloce...]	35.0	1.5

>SND364 567 bp
 AGACAATCCACAAATCTAATCTTAACCATGAAAGTGATCATTTTCTTAGTCGCTGTTGTCGTCGGTGTCAATGCCTGGGAGCAAGG
 TCGCCCATGGCCCGGAGCCGCTACTTGAAAAAGATCTGGAAGCGGTAAGAGGCGAAGTCGAATTGGCATGTGCCAAAAAGGAGCCAG
 GATCAAACTGCCTCGTCTCGTGCATGTATTTTCTGGGATACAGAAAATGAAAAGAATCAAGACTATTAGACTGTACAACTTC
 TGCAAAACTCATCCGAGGGAGAGGATGAGTGCAGAACGAAACGAAAATCTGAGGGACTGCTTTTGGCAGACAAAGACCTATGAAAA
 GACATTAGATGATTCGAGGAGTGTACAGCATCGGTTCCGCTAGATAAGAAGACGAAATGTGTGAAAATTCCTGTTTTTCCAAT
 ATTTTCAAAAACAAATTTGAACATCATCGCTGACAATTAAATGCTATTTCGGTTCAATGGACGATATTGTTCCGCAATTGTTTCAAT
 ATCTGCACGTATATAAAATAAATTTCTTCCCATTCACGCGGCGGACGAGAA

>SND364 129 aa
 R Q S T N L T L T M K V I I F L V A V V V G V N A W E Q G R P W A G A V L E K D L E A
 V R G E V E L A C A K K E P G S N C L V S C D V F F W D T E N E K N Q D Y L D L Y N F
 C K N S S E G E D E C E R N E N L R D C F L A D K T Y E K T L D D S E E L Y S I G S A

Sequences producing significant alignments:	Score (Bits)	E Value
ref XP_001369831.1 PREDICTED: hypothetical protein [Monodelphis]	40.4	0.032
ref YP_364846.1 putative secreted protein [Xanthomonas campe...]	38.1	0.15

GROUP 11

Cluster SND_clu20m
 Clone SND363, SND401, SND441, SND443, SND447, SND457, SND470, SND711

>SND363, SND401, SND441, SND443, SND447, SND457, SND470, SND711 441 bp
 TGTTTATAGCGATTGTGGGGGTACCGTTGAAGGTACTACCGTACACGAAGCCAGATATGTCGAATTGGCCTGTGACAAAGTAACG
 GACAGCGACTGCTTAATCAACTGTAATCTGCTGTTCCCAACCATCTTATATCCGAGATGACAAAGCATTGTAGCTACCAAGAGAAATA
 CACGAAATGTGTCAGGAGTGTGTCAGGAGAAATTTGCAATCGGAACCAACAGATCAGAGACTGTTTGTGAAAGATGAAGGGGATG
 TCACGAACTCGAAGATGACGAAATGCGCTGTCACCATCTACTGGCACCAGAGTTGGAAGTTTCAAGCTTTTCCCTTTGGTCTCA
 GGAGGGTTGCCACAATATAGCTTTAAGTGGTGGCGGTTTGTCTATTGTGAAAATCAAGGTTCAATAAATTTTAACTACAAAAAGT
 GGAAAAA

>SND363, SND401, SND441, SND443, SND447, SND457, SND470, SND711 107 aa
 F I A I V G V T V E G T T V H E A R Y V E L A C D K V T D S D C L I N C N L L F Q P S
 Y I R D D K H L S Y Q E K Y T K C V Q E S S G E F C N R N Q Q I R D C F V K D E G D V
 N E L E D D E M A S Y T I Y W H Q K L E V

Sequences producing significant alignments:	Score (Bits)	E Value
ref XP_780578.2 PREDICTED: similar to stromal cell derived f...	33.9	3.3

GROUP 12

Cluster SND_clu8s
 Clone SND393

>SND393 428 bp
 CACCGTTAAAGGTACCAACCGTACACGAAGCCAGATATGTCGAATTGGCCTGTGACAAAGTAACGGACAGCGACTGCTTAATCAACT
 GTAATCTGCTGTTCCCAACCATCTTATATCCGAGATGACAAGCATTTGAGCTACCAAGAGAAGTACACGAAATGTGTCCAGGAGTCG
 GGAGAATTTTGAATCGAAACCAACAGATCAGAGACTGTTTGTGAAAGATGAAGGGGATGTCAACGAACTCGAAGATGACGAAAT
 GCGCTGCTACACCATCTACTGGCACCAGAAAGTTGGAAGTTTGAAGCTTTTCTTTGGTCTCAGGAGGGTTGCCACAATAGCTTTAA
 GTGGTGTGCGGTTTGTGACAGGGGAAATCAAGGTTCAATAAATTTTACTTAAAAAAGTGAAAAA

>SND393 99 aa
 T V K G T T V H E A R Y V E L A C D K V T D S D C L I N C N L L F Q P S Y I R D D K H
 L S Y Q E K Y T K C V Q E S S G E F C N R N Q Q I R D C F V K D E G D V N E L E D D E M
 A S Y T I Y W H Q K L E V

Sequences producing significant alignments:	Score (Bits)	E Value
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emb|CAF95522.1| unnamed protein product [Tetraodon nigroviridis] 36.6 0.65
 ref|XP_001350583.1| hypothetical protein PFL0875w [Plasmodium...] 34.3 2.5
 ref|XP_001488789.1| PREDICTED: similar to EPH receptor A2 [Equus] 33.5 4.2

GROUP 13

Cluster SND_clu21m
 Clone SND415, SND418, SND495

>SND415, SND495 551 bp
 AACGAACGCTTTCAAGCTTTTATTTCTAATTTTGTGGTCAACGGCATTCTCGGAGCTGACCTCCAAACCAAAAGTGAAAAATCAA
 AAGGACTGACGAAGAAAACCAATCGAGTTTCGAAAGCTGCCGAGTGACCAACAACCAACAGCCACGGTTTACCAGCAACT
 GTGGACCCCGCCGCTCCCAACCCAGCTAAAACCCAGCAAGTCCGGTGAAGAAACCAAAAGCCTCAAGAGAAGAAAACAAAGAAATCGAC
 CGCAAGAAAGCAGAACCCACACCAAGCATTGACAACTACCGTCAACAGCAAAACCAAAAGCTGACGACATTTACCAAAAAC
 CAGTGGTCCCGAAGTACCCAAAGCCTTGAAACAGAAAAACCGGCTGGCAAGCAGAAACAAAGCAGCTACTAAAAAATTGACAGAA
 GAAAGCGAAATGAAAAAAGAAACATGCTGATTACCTTCAATTTACGATCGCTTCACAATTTGTGAAAAATAAAAAGACAAAA
 AAAAAACAATTTCCCCCGAAACTTAAAAA

>SND415, SND495 147 aa
 T N A F K L F I L I L L V N G I L G A D P P T K S G K S K G L T K K T K S S S K A A A
 V T T T T Q Q P T V L P A T V D P A V P T T L K P A S P V K K P K A S K K K T K K S T
 A K K A E P N T K H I D N Y R Q P A N P K A D D I Y Q K P V V P E L P K A L K Q K K P
 A G K Q N K A A T K K L T R E K R N

Sequences producing significant alignments:	Score (Bits)	E Value
gb EAT34172.1 uncoordinated protein [Aedes aegypti]	42.0	0.010
emb CAE66724.1 Hypothetical protein CBG12070 [Caenorhabditis br]	33.1	4.4
ref NP_001020985.1 UNCoordinated family member (unc-89) [Cae...]	32.7	6.1
ref NP_001020990.1 UNCoordinated family member (unc-89) [Cae...]	32.3	8.5

>SND418 406 bp
 ACCACCGTACACGAAGCCAGATATGTGCAATTGGCCTGTGACAAAGTAACGGACAGCGACTGCTTAATCAACTGTAATCTGCTGTT
 CCAACCATCTTATATCCGAGATGAAAAACATTGAGCTACCAAGAGAGTACACGAAATGTGTCCAGGAGTCCGCGGAGAAATTT
 GCAATCGAAACCAACAGATCAGAGACTGTTTTGTGAAAGATGAAGGGGATGTCAACGAACCTCAAGATGACGAAATGGCGTCGTAC
 ACCATCTACTGGCACCAGAGTTGGAGTTTGAAGCTTTCCTTTGGTCTCAGGAGGGTTGCCACAAATATAGCTTTAAGTGGTGGC
 GGTTTTGTGCTTGTGAAATCAAGGTTCAATAAATTTTAACTACAAAAAGTGAAAAA

>SND418 96 aa
 T T V H E A R Y V E L A C D K V T D S D C L I N C N L L F Q P S Y I R D E K H L S Y Q
 E K Y T K C V Q E S S G E F C N R N Q Q I R D C F V K D E G D V N E L Q D D E M A S Y
 T I Y W H Q K L E V

Sequences producing significant alignments:	Score (Bits)	E Value
gb EAT34172.1 uncoordinated protein [Aedes aegypti]	42.0	0.010
emb CAE66724.1 Hypothetical protein CBG12070 [Caenorhabditis br]	33.1	4.4
ref NP_001020985.1 UNCoordinated family member (unc-89) [Cae...]	32.7	6.1
ref NP_001020990.1 UNCoordinated family member (unc-89) [Cae...]	32.3	8.5

GROUP 14

Cluster SND_clu9s
 Clone SND438

>SND438 563 bp
 CGTCATGGCCGGTGCAATTTTCATTGCCTTTGGGCTCTTCCATCCGTTTAAGATCTGCTTAGGAATAGCGATGATGGTGGCGGCA
 CCATGGCCGGCTTTAAATTTGGCCGATGAGGATAAGAAACGGGTAGCGGGTGGCTGCACCCGGTCCCAACAAGTACGTCGTGGGA
 GGAATCGCATTCGGTTGTTATTTCTTCTCTACCTTATGGACTGTGTCGTTATCGTTACTTTATCCATTCTGCTGCCATTCTGTGC
 TACATTCGTCATTCTTCTGTTGAGACTGCGTAATATCAAAAACAAAGTGCCCAACATCGCTTCGACCTGAACAAGTCCACACCAA
 TGGCCATGTTTTTGGACTCGATGGGCTGTTGCCAGAAACATTTGAACACCGGTAGAGCGGGTCATAATTCACATCAAGCTTTGC
 ATTCAGCACTGGTTGTGGTAACCTTCAATAAATTAACGTTCAAAAGAGCTGAAAGCAAAAATCTCCTTACATACATATAACCA
 ATCTCAATTTGAACGCAAAAAGTTTAAAAAACCAGCACACTG

>SND438 129 aa

V M A G A I F I A F G L F H P F K I C L G I A M M V G G T M A A F K L A D E D K K R V
 A G A A P G P N K Y V V L G G I A F G C Y F F L Y L M D C V V I V T L I L L P F C A
 T F V H S S L R L R N I K N K V A N I A S T L N K S T P M A M F L D S M G L L P E T F

	Score (Bits)	E Value
Sequences producing significant alignments:		
ref XP_314002.3 ENSANGP00000010049 [Anopheles gambiae str. P...]	117	2e-25
gb EAT46897.1 pral protein [Aedes aegypti] >gb EAT46898.1 p...	116	4e-25
ref XP_968412.1 PREDICTED: similar to ADP-ribosylation-like ...	79.3	6e-14
ref XP_393613.1 PREDICTED: similar to ADP-ribosylation facto...	63.9	2e-09
gb EDL91426.1 ADP-ribosylation factor-like 6 interacting pro...	55.1	1e-06

GROUP 15

Cluster SND_clu22m
 Clone SND509, SND518

>SND509, SND518 480 bp
 AGTTTCATCAAAATAACCATGCGAGCCTTTTCGATGAGCACAAAGACGCAACATGACCTACATTCGAAAGTATCAAAATCAGGAT
 AAAGGCAAGCAAAATTGGTCACTTCACCCAGCTGGTCAGCGATCGCACCAATCGCGTGGGTGCGCCATCTCGCGTTACACCGAGTG
 GAGTCCAGAGTATCAGGCGAATTTGAAACTTTGCTGCTGGCCTGCGACTACTCTTACACAAACATTTGGCAGGAGGCGTGTACC
 AAGAGGGACCAATGGCTAGCGAATGTCAAAGGGGAAGGCATCACGTGTACGAAGGTCTATGCAAGGCAACAGAACCGATAAATCCC
 TATGAAATTTCTTTCCGTGGTGGTGGCAATGTACCAAAATCTATTGAAAGTTCAGAGCAAAATAAACACACGACGTTTCTTAATG
 AGTCAGTCCAATAAACAGAAACGTGTGAGCACGGTGTGTCATAGAAAAA

>SND509, SND518 135 aa
 F I K I T M R A F F D E H K D A N M T Y I R K Y Q N H D K G K Q I G H F T Q L V S D R
 T N R V G C A I S R Y T E W S P E Y Q A N L K T L L A C D Y S Y T N I W Q E A S Y Q
 E G P M A S E C Q K G R H H V Y E G L C K A T E P I N P Y E N F F P W W L P M Y Q N L
 L K V Q S K

	Score (Bits)	E Value
Sequences producing significant alignments:		
gb AAM75007.1 GH04057p [Drosophila melanogaster]	93.6	3e-18
ref NP_572957.1 CG9400-PA [Drosophila melanogaster] >gb AAF4...	93.6	3e-18
ref XP_001354820.1 GA21762-PA [Drosophila pseudoobscura] >gb...	92.0	9e-18
ref NP_727412.2 CG32679-PA [Drosophila melanogaster] >gb AAN...	91.3	1e-17

IV. Sequence clusters of salivary gland cDNAs from female *S. nodosum* coding for unknown proteins. : (Unknown protein category)

GROUP 1

Cluster SND_clu23m
Clones SND020, SND025, SND028, SND033, SND048

>SND020, SND025, SND028, SND033, SND048 353 bp
CGGCGAGCAGGCAAACTGAACTAAGAGCTACAGAATGAAATTGATTGTGCTGTTGCTGCTGGTGGTGGGACTAGCCATGG
GCGAGGTGGCCAATCCAGTTCCAGATGCGGACACGCCAAGCCATCAAGAACCGGAAACCTATAACTCAACAGCTGACGTTGTTGTT
GAGGCTGTTCTTTTCGAGGGGGGAACATGTCGTACATACCAGACAACGTGGCCAATTTTCGCAAAATAACCCACTTTTGATTTCGGTTA
GGTCAACTTTGCTCTATTCCACATTTGGCCATATCAATTACAAATCACATGTTGAAATCTAAATAAAGGACTGTATGGTTTAAATTT
TTTCTTGT

>SND020, SND025, SND028, SND033, SND048 78 aa
G E Q A N K L E T K S Y R M K L I V L L L L V V G L A M G E V A N P V P D A D T P S H
Q E P E T Y N S T A D V V V E A V L S E G E H V V I P D N V A N F R K

No significant similarity found.

GROUP 2

Cluster SND_clu10s
Clone SND172

>SND172 293 bp
AAGTTGTTGTGGTTCTTCTGTTCTGTTCTTCTCATTTGGTATTGTCTCAGGTGCACGTTGTAAATGGTTTGCRAACCTAGACCA
AAAGGTCAACCACATGTCAAGAGAGATGTAAATATCATAGTAATGGCCATAGTCGTCCTAGAGGTACCCAGCAATATATGTACC
GAAAGTAAAGACGGCACAGGATGGATCAAGTGCACCGGACGGATACTAATCAAGACGATTGTACGTAATTTTCTCGAATAAATCGA
CTATGGTCAGGTAAAAA

>SND172 59 aa
K L L W F F F C S Y S S L V L S Q V H V V K W F A N L D Q K V N H N V R E N V N I I V
M A I V V L E V P S K Y M Y R K

No significant similarity found.

GROUP 3

Cluster SND_clu11s
Clone SND298

>SND298 354 bp
GTTTGAAGTGGTGAAGGGCAATGAAGTGGATTGGGCACATTTGGACGATTTACTGGACGATGATGCTGCTAAACAAAAGGTGAAA
GAATGCCGAGAGGCCAACAGGTCAATCCAGAGGACTGTGAATCGGTTTCATAGGATGTATAAATGTGTGAGTAAGCAACATGCAGA
GGCCTTAAGGGGAGCGTTCCGGAAACAATTCACTCAATAAAGGTGGAAATGAATACACAAGGTGGGCGAAAACACGTTACAGACGT
TTTATGAAAGTGGATTTTTAGTATTTTAACGTGCAAAARCAACAGGTTAAATGTCATCAAAATAATAAAATTATATTTCAATTGT
AAAAA

>SND298 69 aa
L E V V K G N E V D W A H L D D L L D D D A A K Q K V K E C R E A N Q V N P E D C E S
V H R M Y K C V S K Q H A E A L R G A F R K Q F S Q

No significant similarity found.

GROUP 4

Cluster SND_clu12s
Clone SND308

>SND308 544 bp

GGAATTGTAAGACTACTTTTTCGAAGCGAAAAATGAAAACCGCAATTATGATGGCTGTGGCCTTGATGGGCCTGCTCTGCGTGATGG
CGGCGCCTCCACCACCGCCGGGTGCTGGTGGTCCACGCCACCGCGTCTTGAACATGGTCACGGACATGGACATGGCCATGGTCAT
GGACACGGACATGGTCACCATCCGCACCACCCACACCATCTCTCCACCACCAGAGAACCACCAAAACCAGAGGAGCCCAACCCGA
AGAGCGGCTCCAGAAGAACCAGCCGCCCCAGAGGAGCCGCCCCAGAGGAACCTGCGCCGGAGGAGCCCGCCCCGAGAGCCGGCAC
CAGAGGAACCATCCCAACCCGAACAGCCTGAGCAACCAGAACACCTGAGCAGCCGAGCAACCTGAGCAGCCGAGCAACCCGAA
GATGGATCAGACCAATCCCAACAAGGCCAATGATGCACTGAAAGTCGCCCTTTTCTGTTAAGATAAACTGTTGTCACAATAAATT
GCTTTAGTTGAACGTTCAAAAAAAAAA

>SND308 45 aa

G I V R L L F R S E N E N R N Y D G C G L D G P A L R D G G A S T T A G C W W S T P T
A S

No significant similarity found.

GROUP 5

Cluster SND_clu13s
Clone SND398

>SND398 266 bp

GGAAGCATAATTTTTAGAGGAATCTCGACAAGATTGCAACGCTAAAAAACCAATTTGTCTCAACTGATAGCCTTTAAACATATA
GGAACCTTAAGAATAACCTAACATATCGATCTGTAAATTATAATGCAATTTCTGTAGATAAGTGTAACCGTCCAATATTTTTT
CGACTTTATTCTTCGACGCCAACGTTGAACGACCATGTTTTTTTGAGAATAAACTCATATTTTAAAAATAAAAAAAAAAAAA
AAAAAAA

>SND398 21 aa

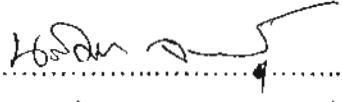
K H N F L E E S R Q D C N A K K T I C L N

No significant similarity found.

ผลที่ได้รับจากโครงการ (output)

ผลที่ได้รับจากโครงการ (output):

1. มีผลงานวิจัยที่ได้ตีพิมพ์เผยแพร่แล้วในวารสารระดับนานาชาติ 1 เรื่อง ชื่อ "Morphology and electrophoretic protein profiles of female salivary glands in four Oriental black fly species (Diptera: Simuliidae)" ในวารสารชื่อ "Journal of Vector Ecology 2006; 31(2): 406-411" (Impact factor 2006 = 0.879) (เอกสารแนบหมายเลข 1)
2. ได้นำเสนอผลงานเรื่อง "Morphology and electrophoretic protein profiles of female salivary glands in four Oriental black fly species (Diptera: Simuliidae)" ในงานประชุมวิชาการ Joint International Tropical Medicine Meeting 2006 (29 Nov – 1 Dec), Bangkok, Thailand. (เอกสารแนบหมายเลข 2)
3. ผลงานวิจัยส่วนที่เหลือ ซึ่งเป็นข้อมูลพื้นฐานทางด้านอนุชีววิทยา สำหรับการนำไปใช้ศึกษาบทบาทที่เป็นไปได้ของผลผลิตจากต่อมน้ำลายของ *S. nodosum* นั้น จะนำไปเป็นข้อมูลที่จะใช้ขอทุนสนับสนุนทำงานวิจัยต่อยอดในเชิงลึกต่อไป

ลงนาม..... 

(ผู้ช่วยศาสตราจารย์ดร.นริศรา จริยะพันธุ์)

(หัวหน้าโครงการ)

วันที่ 19 กันยายน 2550

ภาคผนวก

เอกสารแนบหมายเลข 1

ผลงานวิจัยที่ได้ตีพิมพ์เผยแพร่แล้วในวารสารระดับนานาชาติ 1 เรื่อง ชื่อ "Morphology and electrophoretic protein profiles of female salivary glands in four Oriental black fly species (Diptera: Simuliidae)" ในวารสารชื่อ "Journal of Vector Ecology 2006; 31(2): 406-411"

Morphology and electrophoretic protein profiles of female salivary glands in four Oriental black fly species (Diptera: Simuliidae)

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ABSTRACT: Saliva of female flies is responsible for localized hypersensitivity reactions and life-threatening systemic hemorrhagic syndromes in humans and animals. In this study, morphology and electrophoretic protein profiles of female salivary glands of Oriental black flies in the subgenus *Simulium* Latreille s. str., *Simulium* (*Simulium*) *nigrogilvum*, *S. (S.) rufibasis*, *S. (S.) nodosum*, and subgenus *Gomphostilbia* Enderlein, *S. (Gomphostilbia)* *asakoe* were analyzed. The paired female salivary glands of the four simuliid species were morphologically similar and situated on either side of the esophagus. Each gland is composed of two main parts, a secretory arm and a reservoir. In each species, the size of the gland correlated with salivary gland protein contents. SDS-PAGE analysis revealed differences of electrophoretic protein profiles and specifically major protein bands of the female salivary glands in each species, suggesting that protein profiles might be useful for construction of an additional tool to distinguish these black fly species. The information obtained from this study is an initial step for further research on salivary proteins that are involved in vertebrate hemostatic response. *Journal of Vector Ecology* 31 (2): 406-411, 2006.

Keyword Index: *Simulium*, black fly, salivary gland, protein, morphology.

INTRODUCTION

Adult female black flies (Diptera: Simuliidae) have been well known as pests to humans and animals, and they are also vectors of some parasites and pathogens, like *Onchocerca* spp. in humans and cattle and *Leucocytozoon* spp. in birds (Mead et al. 1997, Stallings et al. 2002). Saliva of female flies is responsible for localized hypersensitivity reactions (Frese and Thiel 1974, Farkas 1984, Hoffman 1987) and life-threatening systemic hemorrhagic syndromes in humans (Pinheiro et al. 1974). The fly bites also cause severe symptoms or even death through a hypersensitization in animals, which is induced by zootoxins according to the biting density of the fly and the animal's physical and immunological condition (Farkas 1984).

In the Oriental region, several man-biting simuliid species have been identified, e.g., *Simulium* (*Simulium*) *indicum* Becher, which is widely distributed at the southern foot of the Himalayas, from Pakistan in the west, through India and Nepal, to Myanmar and southern China in the east (Lewis 1974, Datta 1992); *S. (S.) asishi* Datta, *S. (S.) himalayense* Puri, *S. (S.) nodosum* Puri, and *S. (Gomphostilbia)* *tenistylum* Datta in India (Datta 1992); and *S. (S.) japonicum* Matsumura in the Ryukyu Islands, Japan (Takaoka 1977). In Thailand, at least 56 black-fly species have been identified (Takaoka and Choochote 2004a, 2005a, b, c, d, e, f, g, h, i). Some of them have been studied in many areas, for example, population cytogenetic studies (Kuvangkadilok et al. 1998, 1999a, b, c, 2000), taxonomic

studies (Takaoka and Kuvangkadilok 1999, Kuvangkadilok and Takaoka 2000, Takaoka 2001, Takaoka and Choochote 2002, Otsuka et al. 2003, Takaoka and Choochote 2004a, b), and natural transmission of zoonotic filariasis (Takaoka et al. 2003, Fukuda et al. 2003). Recently, five simuliid species, i.e., *S. (G.) asakoe*, *S. (S.) nigrogilvum*, *S. (S.) rufibasis*, *S. (S.) chamlongi*, and *S. (S.) nodosum*, have been proven as man-biting species in Thailand (Choochote et al. 2005). Choochote et al. (2005) also reported the seasonal abundance and daily flying activity of these adult black flies at four different altitudes in Doi Inthanon National Park, northern Thailand. However, little information is available on the morphology and electrophoretic protein profiles of the female salivary glands of these black flies species.

Studies on the salivary glands of black flies are of interest since Bennett (1963) described and illustrated female salivary glands of 14 simuliid species from Algonquin Park, Ontario, and suggested that these organs may be useful in the identification of some simuliid species. The saliva components in mosquitoes and sand flies vary considerably between species and even between populations of different geographical origin (e.g., Peng et al. 1998, Moreira et al. 2001, Lanzaro et al. 1999). Our investigation concentrated only on adult females because they feed on blood, whereas males feed only on sugar meals. Therefore, the general morphology and electrophoretic protein profiles of the female salivary glands of *S. (S.) nigrogilvum*, *S. (S.) nodosum*, *S. (S.) rufibasis*, and *S. (G.) asakoe* were analyzed in this study. Also, the salivary

gland protein contents were determined. Our initial finding revealed differences in electrophoretic protein profiles and specifically major protein bands of the salivary glands in the four simuliid species.

MATERIALS AND METHODS

Black flies

Black flies were collected from Doi Inthanon National Park, Chiang Mai province, northern Thailand, by human-baited traps. The collection times were according to the peak biting periods for each species (Choochote et al. 2005). Female black flies were caught by an insect net while landing or flying around human-baited traps. They were kept in a paper cup covered by a net. A pad of cotton wool soaked with 5% sucrose solution was placed on top of a cup and stored in a humid chamber. The flies were then transported to the insectarium at the Department of Parasitology, Faculty of Medicine, Chiang Mai University for dissection of their salivary glands. Identification of the

black fly species followed the standard keys of Takaoka and Choochote (2004b).

Salivary gland dissection

The salivary glands of the adult black flies in each species, i.e., *S. (S.) nigrogilvum*, *S. (S.) nodosum*, *S. (S.) rufibasis*, and *S. (G.) asakoe* were dissected individually using fine entomological needles under a stereoscopic microscope in phosphate-buffered saline (PBS; 10mM sodium phosphate, 145 mM sodium chloride, pH 7.2). Each pair of glands was stored separately in a microcentrifuge tube with a small volume of PBS at -80°C for later analysis.

Light microscopy

The salivary glands of the female black flies were dissected in PBS and allowed to settle onto slides without drying out. Photographs of the glands were taken using a camera (Vanox-S AH2 Olympus, Tokyo, Japan) attached to a light microscope.

Protein quantification

The protein content of each salivary gland pair of each species was determined using a Micro BCA Protein Assay Kit (Pierce, Rockford, IL) according to the manufacturer's instruction. The protein concentration was determined based on a bovine serum albumin (BSA) standard curve.

SDS-PAGE and silver staining

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to standard techniques (Hames 1990) and the proteins were silver-stained using a GelCode® Color Silver Stain Kit (Pierce, Rockford, IL) according to the manufacturer's instructions.

RESULTS

The salivary glands of the female black flies in the subgenus *Simulium* Latreille s. str., *S. (S.) nigrogilvum*, *S. (S.) rufibasis*, *S. (S.) nodosum*, and subgenus *Gomphostilbia* Enderlein, *S. (G.) asakoe* were morphologically similar. Each female black fly had a pair of salivary glands (Figure 1) located on the dorsal part of the prothorax. Using the terminology of Gosbee et al. (1969), each gland consisted of a cylindrical U-shaped secretory lobe and a more or less spherical reservoir (Figure 2). Salivary ducts arising from each reservoir united to form a common salivary duct that passed to the hypopharynx. The secretory lobe was about 90-120 μm wide at its proximal end, while the reservoir was about 120-200 μm at its widest diameter (Figure 2). The average length of the secretory lobes of *S. (S.) nigrogilvum*, *S. (S.) rufibasis*, and *S. (S.) nodosum* was approximately 800, 600, and 500 μm , respectively, whereas *S. (G.) asakoe* had a shorter lobe at approximately 400 μm long (Figure 2). The size of the salivary glands varied considerably. The salivary glands from large species of flies, i.e., *S. (S.) nigrogilvum*, *S. (S.) rufibasis*, and *S. (S.)*

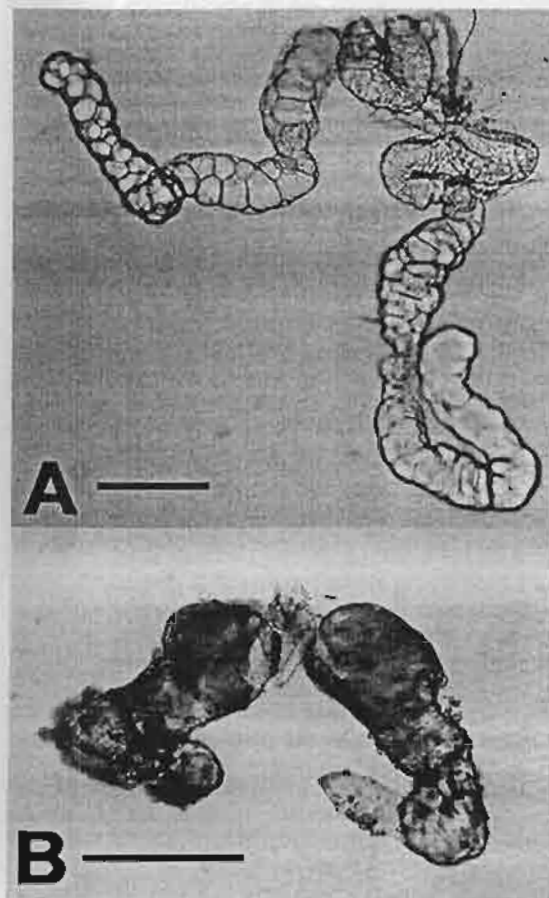


Figure 1. Salivary gland pair of female black flies. (A) *S. (S.) nigrogilvum*; (B) *S. (G.) asakoe*. Bars = 100 μm .

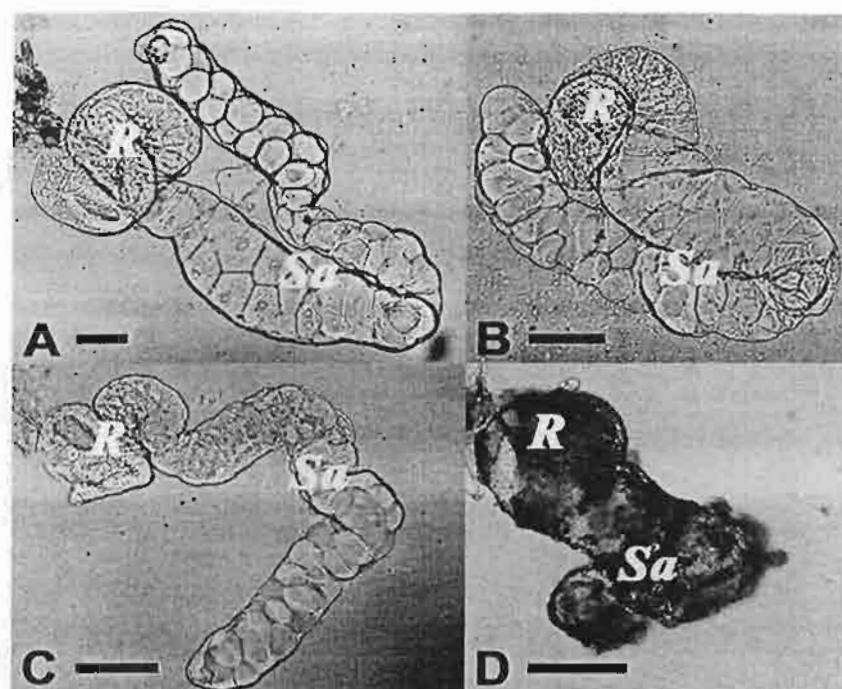


Figure 2. Salivary glands of female black flies. (A) *S. (S.) nigrogilvum*; (B) *S. (S.) rufibasis*; (C) *S. (S.) nodosum*; (D) *S. (G.) asakoe*. R, reservoir; Sa, cylindrical U-shaped secretory arm. Bars = 50 μ m.

nodosum, consecutively, were larger than those of the small species, *S. (G.) asakoe*. However, glands from a large specimen of a species were bigger than those from a small individual of the same species. It was noted from Figures 1 and 2 that only the salivary glands of *S. (G.) asakoe* were surrounded by a red-brown fine membranous sheath.

The protein contents of the whole salivary glands of each species were quantified. The protein content of each female gland pair of *S. (S.) nigrogilvum*, *S. (S.) rufibasis*, *S. (S.) nodosum*, and *S. (G.) asakoe* was 1.15 ± 0.14 , 0.75 ± 0.05 , 0.68 ± 0.14 , and 0.51 ± 0.11 μ g (mean $\pm$ SD, $n = 10$), respectively.

To investigate electrophoretic protein profiles of their female salivary glands, total proteins in the glands of individual flies from each species were examined in silver-stained SDS-polyacrylamide gels (Figure 3). The salivary gland protein profiles of each species differed from one another. At least 9, 8, 7, and 6 major protein and several minor protein bands were detected in the female glands of *S. (S.) nigrogilvum*, *S. (S.) rufibasis*, *S. (S.) nodosum*, and *S. (G.) asakoe*, respectively. Polymorphisms of salivary gland protein profiles of each sample in the same species were also observed.

DISCUSSION

Salivary glands are of interest in hematophagous insects because they produce a wide array of secreted compounds that are delivered with the saliva and help blood-feeding by affecting the host hemostatic response. The saliva produced

by the insect salivary glands is the vehicle that carries pathogens and it may also enhance or facilitate infectivity during the blood meal (Ribeiro and Francischetti 2003). In addition, the saliva contains immunogens that cause human IgE and lymphocyte-mediated hypersensitivities (Arlian 2002).

Numerous studies have been made on the salivary glands of mosquitoes and other flies, but few on black flies (Ribeiro and Francischetti 2003). In this study, morphology and electrophoretic protein profiles of female salivary glands of Oriental black flies in the subgenus *Simulium* Latreille s. str., *S. (S.) nigrogilvum*, *S. (S.) rufibasis*, *S. (S.) nodosum*, and subgenus *Gomphostilbia* Enderlein, *S. (G.) asakoe* were reported for the first time. The results of this study revealed that the morphology of the female salivary glands of the four Oriental black flies was similar and followed the same pattern as described for *S. vittatum* (Gosbee et al. 1969) and *S. nigroparvum* (Cox 1938).

The size of the salivary glands varied considerably. In this study, the glands from large species of black flies, i.e., *S. (S.) nigrogilvum*, *S. (S.) nodosum*, and *S. (S.) rufibasis*, were larger than those from the small species, *S. (G.) asakoe*. Glands from a large specimen of a species were sometimes bigger than those from a small individual of the same species. Hence, the size of the salivary gland is not a useful criterion for distinguishing species. These results correlated with the study of Bennett (1963).

Bennett (1963) also reported that the coloration of a fine membranous sheath surrounding the salivary glands of *S. quebecensis* was strikingly different from that of *S. talipes*.

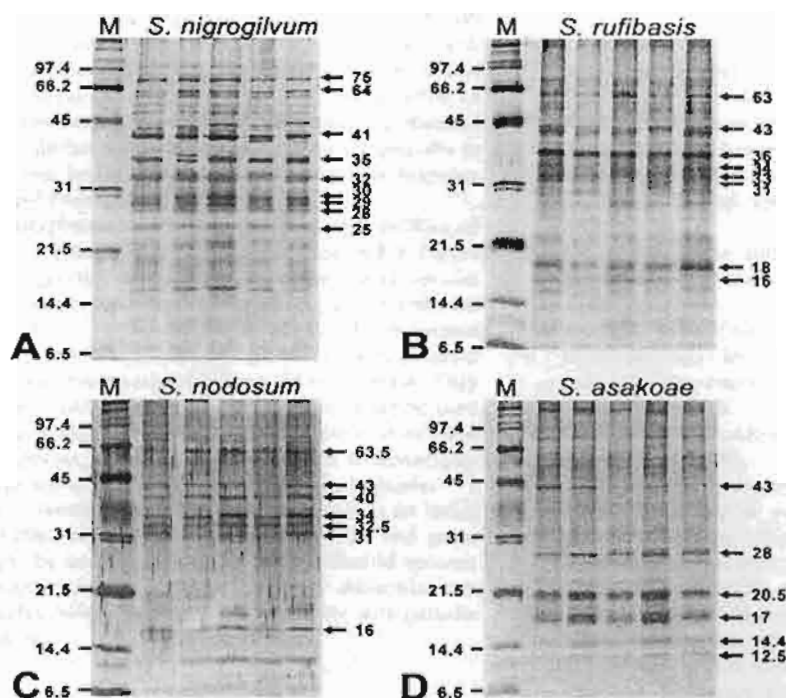


Figure 3. SDS-PAGE analysis of salivary gland (SG) proteins of female black flies. SG proteins of individual flies from each species were loaded into each lane and then separated on 15% SDS-polyacrylamide gels and stained with silver. (A) *S. (S.) nigrogilvum*; (B) *S. (S.) rufibasis*; (C) *S. (S.) nodosum*; (D) *S. (G.) asakoe*. Arrows indicate major protein bands detected in samples of each species. Molecular mass markers (M) are on the left.

It was, therefore, simple to differentiate between the two species. In this study, *S. (G.) asakoe* had a characteristic red-brown coloration around its salivary glands, which was not found in the salivary glands of the other three species. This result leads to the question as to whether or not this characteristic color is a specific feature of the salivary glands of *S. (G.) asakoe*. Investigation of the salivary gland morphology of simuliids in members of the subgenus *Gomphostilbia* should be performed to clarify this issue.

Our study also revealed that the average amount of protein content per gland pair correlated with the size of the salivary glands in each species. Because salivary components of many hematophagous arthropods are introduced into vertebrate hosts during feeding, depletion in total salivary gland protein content of the four simuliid species after blood feeding should be determined and compared.

An analysis of salivary gland proteins of *S. vittatum* and three New World simuliid species, i.e., *S. argus*, *S. ochraceum*, and *S. metallicum*, using SDS-PAGE was reported by Cross et al. (1993). After the analysis on SDS silver-stained gels, salivary gland profiles of *S. metallicum*, *S. ochraceum*, *S. vittatum*, and *S. argus* comprised individually separated proteins of 11, 12, 10, and 20 bands, respectively. Comparing the protein profiles

of salivary gland extracts revealed that those of the two zoophilic species (*S. vittatum* and *S. argus*) were similar and emphasized the close relationship between these two species. However, these profiles differed markedly both qualitatively and quantitatively from those observed from salivary gland extracts of the two anthropophilic species (*S. metallicum* and *S. ochraceum*). Furthermore, salivary glands extracted from the anthropophilic species contained higher levels of total soluble protein than those from the zoophilic species. The study suggested that differences in saliva composition may represent modifications of this part of the more anthropophilic species because of evolutionary adaptation to feeding on hosts.

In this study, the four Oriental black fly species, which were reported as anthropophilic (Choochote et al. 2005), had a small number of major proteins detected in the salivary glands of each species. In addition, some molecular masses of the major protein bands detected in female salivary gland samples of each species were different, suggesting salivary gland-specific proteins in different species. These results may support the hypothesis of evolutionary adaptation to feeding because different black fly species live at different altitudes and/or regions where various vertebrate hosts and plants are found (Takaoka et al. 2003, Choochote et al. 2005). However, the amount and profile of salivary

gland proteins of these black flies should be compared with other simuliid species from different regions of the world. Moreover, further studies concerning the biochemical and pharmacological activities of individual components of salivary materials in these female black flies are required before any further conclusions can be drawn, especially in the molecules involved in vertebrate hemostatic response (Ribeiro and Francischetti 2003).

Polymorphisms of salivary gland protein profiles of samples in the same species were also observed in Figure 3. It is suggested that each species may have species complex status as found in *Lutzomyia longipalpis* complex (Lanzaro et al. 1999). Soares et al. (2000) examined intraspecific variability in the genus *Rhodnius* using starch gel electrophoresis of salivary heme proteins. They reported that salivary heme protein profiles could be used to distinguish eight *Rhodnius* species. In the case of these simuliid species, therefore, it is of interest to investigate polymorphism of nitrophorins in their salivary glands.

The information obtained from this study is an initial step to further identify salivary gland proteins and genes that might be used to distinguish these simuliid species, and to support further studies on salivary molecules that may involve blood feeding, host immunity, and parasite transmission.

Acknowledgments

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เอกสารแนบหมายเลข 2

ผลงานนำเสนอเรื่อง “Morphology and electrophoretic protein profiles of female salivary glands in four Oriental black fly species (Diptera: Simuliidae)” ในงานประชุมวิชาการ Joint International Tropical Medicine Meeting 2006 (29 Nov – 1 Dec), Bangkok, Thailand.

MORPHOLOGY AND ELECTROPHORETIC PROTEIN PROFILES OF FEMALE SALIVARY GLANDS IN FOUR ORIENTAL BLACK FLY SPECIES (DIPTERA: SIMULIIDAE)

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INTRODUCTION

Studies on the salivary glands of black flies are of interest since Bennett (1963) described and illustrated female salivary glands of 14 simuliid species from Algonquin Park, Ontario, and suggested that these organs may be useful in the identification of some simuliid species. In addition, saliva of female flies is responsible for localized hypersensitivity reactions and life-threatening systemic hemorrhagic syndromes in humans and animals. Therefore, the general morphology and electrophoretic protein profiles of the female salivary glands of *N. (S.) nigrogilvum*, *N. (S.) nodosum*, *N. (S.) rufibasis*, and *N. (G.) asakoe* were analyzed in this study. Also, the salivary gland protein contents were determined.

RESULTS AND DISCUSSION

The salivary glands of the female black flies in the subgenus *Simulium* Latreille s. str., *N. (S.) nigrogilvum*, *N. (S.) rufibasis*, *N. (S.) nodosum* and subgenus *Gomphostilbia* Enderlein, *N. (G.) asakoe* were morphologically similar. Each female black fly had a pair of salivary glands (Figure 1) located on the dorsal part of the prothorax. Using the terminology of Gosbee et al. (1969), each gland consisted of a cylindrical U-shaped secretory lobe and a more or less spherical reservoir (Figure 2). Salivary ducts arising from each reservoir united to form a common salivary duct that passed to the hypopharynx. The secretory lobe was about 90-120 µm wide at its proximal end, while the reservoir was about 120-200 µm at its widest diameter (Figure 2).



Figure 1. Salivary gland pair of female black flies: (A) *N. (S.) nigrogilvum*, (B) *N. (G.) asakoe*. Bars = 100 µm.

The average length of the secretory lobes of *N. (S.) nigrogilvum*, *N. (S.) rufibasis* and *N. (S.) nodosum* was approximately 800, 600 and 500 µm, respectively, whereas *N. (G.) asakoe* had a shorter lobe at approximately 400 µm long (Figure 2). The size of the salivary glands varied considerably. The salivary glands from large species of flies, i.e., *N. (S.) nigrogilvum*, *N. (S.) rufibasis* and *N. (S.) nodosum*, consecutively, were larger than those of the small species, *N. (G.) asakoe*. However, glands from a large specimen of a species were bigger than those from a small individual of the same species. Hence, the size of the salivary gland is not a useful criterion for distinguishing species. These results correlated with the study of Bennett (1963).

Bennett (1963) also reported that the coloration of a fine membranous sheath surrounding the salivary glands of *N. quebecensis* was strikingly different from that of *N. talpae*. It was, therefore, simple to differentiate between the two species. It was noted from Figure 1 and 2 that only the salivary glands of *N. (G.) asakoe* were surrounded by a red-brown fine membranous sheath. This result leads to the question as to whether or not this characteristic color is a specific feature of the salivary glands of *N. (G.) asakoe*. Investigation of the salivary gland morphology of simuliids in members of the subgenus *Gomphostilbia* should be performed to elucidate this issue.



Figure 2. Salivary glands of female black flies: (A) *N. (S.) nigrogilvum*, (B) *N. (S.) rufibasis*, (C) *N. (S.) nodosum*, (D) *N. (G.) asakoe*. R, reservoir; Sa, cylindrical U-shaped secretory arm. Bars = 50 µm.

The protein contents of the whole salivary glands of each species were quantified. The protein content of each female gland pair of *N. (S.) nigrogilvum*, *N. (S.) rufibasis*, *N. (S.) nodosum* and *N. (G.) asakoe* was 1.15 ± 0.14 , 0.75 ± 0.05 , 0.68 ± 0.14 and 0.51 ± 0.11 µg (mean $\pm$ SD, n = 10), respectively.

To investigate electrophoretic protein profiles of their female salivary glands, total proteins in the glands of individual flies from each species were examined in silver stained SDS-polyacrylamide gels (Figure 3). The salivary gland protein profiles of each species differed from one another. At least 9, 8, 7 and 6 major protein and several minor protein bands were detected in the female glands of *N. (S.) nigrogilvum*, *N. (S.) rufibasis*, *N. (S.) nodosum* and *N. (G.) asakoe*, respectively. Some molecular masses of the major protein bands detected in female salivary gland samples of each species were different, suggesting salivary gland-specific proteins in different species. These results may support the hypothesis of evolutionary adaptation to feeding because different black fly species live at different altitudes and/or regions where various vertebrate hosts and plants are found (Takaoka et al. 2003, Choochote et al. 2005). However, the amount and profile of salivary gland proteins of these black flies should be compared with other simuliid species from different regions of the World. Moreover, further studies concerning the biochemical and pharmacological activities of individual components of salivary materials in these female black flies are required before any further conclusions can be drawn, especially in the molecules involved in vertebrate hemostatic response (Ribeiro and Francischetti 2003).

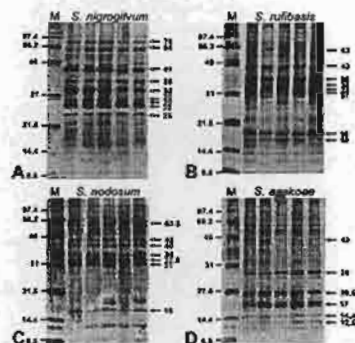


Figure 3. SDS-PAGE analysis of salivary gland (SG) proteins of female black flies. SG proteins of individual flies from each species were loaded into each lane and then separated on 15% SDS-polyacrylamide gels and stained with silver. (A) *N. (S.) nigrogilvum*, (B) *N. (S.) rufibasis*, (C) *N. (S.) nodosum*, (D) *N. (G.) asakoe*. Arrows indicate major protein bands detected in samples of each species. Molecular mass markers (M) are on the left.

Polymorphisms of salivary gland protein profiles of samples in the same species were also observed in Figure 3. It is suggested that each species may have species complex status as found in *Lutzomyia longipalpis* complex (Lanzaro et al. 1999). Soares et al. (2000) examined intraspecific variability in the genus *Rhodnius* using starch gel electrophoresis of salivary heme proteins. They reported that salivary heme protein profiles could be used to distinguish 8 *Rhodnius* species. In the case of these simuliid species, therefore, it is of interest to investigate polymorphism of nitrophorins in their salivary glands.

As black fly bites cause localized hypersensitivity reactions, this information would lead to further study on species-specific antigens in the salivary glands of these pests. In addition, the information obtained from this study would be an initial step for further identification of salivary gland proteins and genes that might be used to distinguish these simuliid species, and also studies on salivary molecules that may involve blood feeding, host immunity and parasite transmission.

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