

Expressed sequences in Three Leafhoppers Vectors of Pierce's Disease



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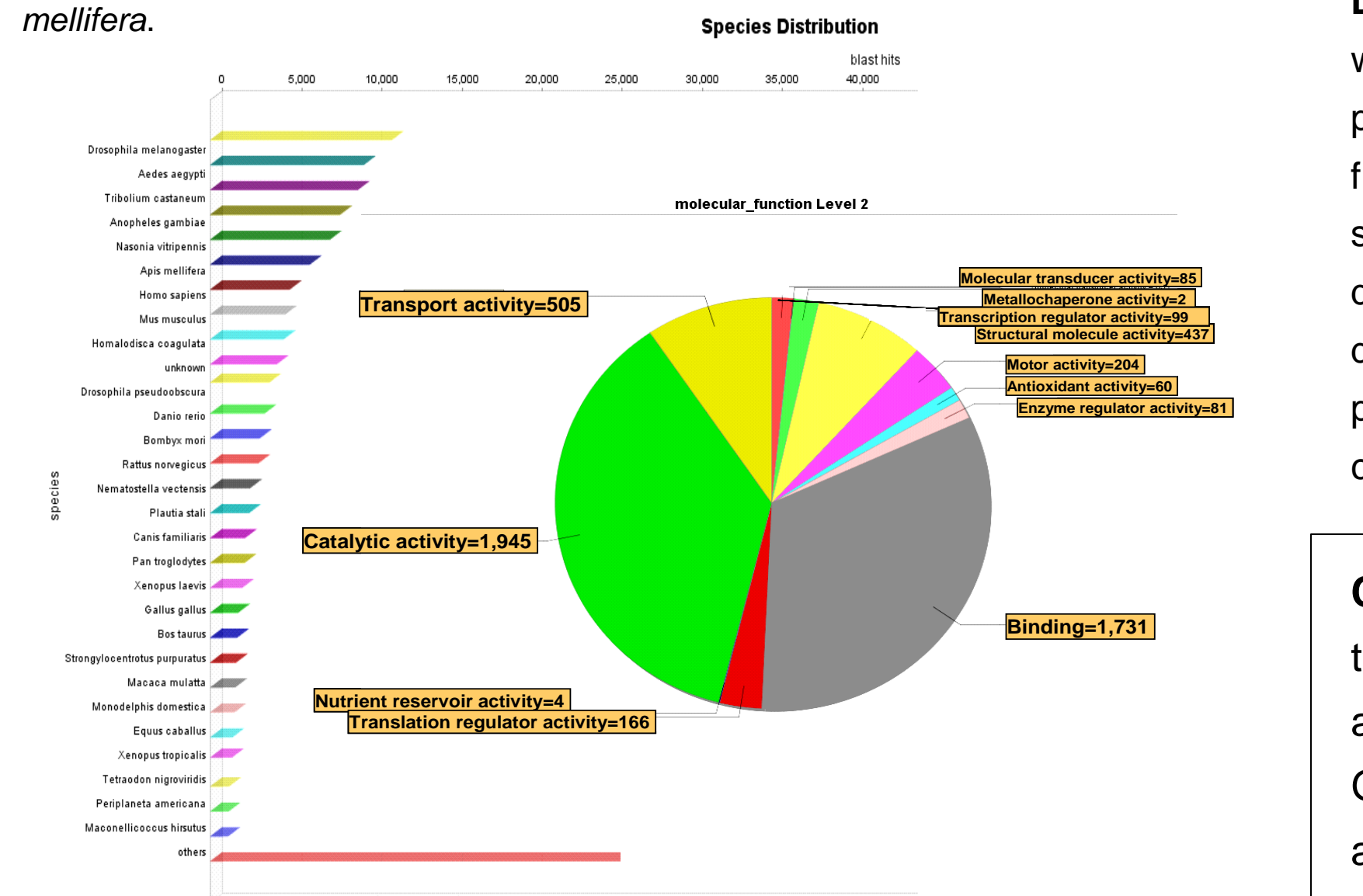


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ABSTRACT- Leafhoppers are considered the second most important vector of agricultural diseases. We examined the gene expression across three leafhopper leafhoppers, *Homalodisca vitripennis*, *Graphocephala atropunctata*, and *Oncometopia nigricans*, which are vectors of the plant-infecting bacterium, *Xylella fastidiosa*, which causes Pierce's disease of grapes. The use of genomic data is providing new information on the biology and relatedness of these and other leafhoppers. Using a genomics approach has also advanced the understanding of leafhopper immunity, pathology, and development. As new developments in genomics and RNAi methodologies emerge, researchers will be able to use this genetic information to design highly specific and effective management tools to reduce either leafhopper populations, and/or leafhopper-transmitted diseases. The importance of these leafhoppers as the vectors of Pierce's disease, the abundance of ESTs produced for each, and their differences in host plant preferences, provide an excellent opportunity to conduct comparative examination of these leafhopper's. Several cDNA libraries which had been made from adult GWSS, BGSS, and BWSS, plus nymphs, and tissues, provided a resource totaling ~50,000 ESTs. When assembled we obtained ~5,000 specific transcripts for each species for comparison. This is approximately one-third of all predicted active genes available, as other insect genomes have demonstrated ~15,000 total genes. These were used for analyses between these species as well as for larger analysis to known genomes. Further analyses were conducted *in silico* using software programs available online Internet Resources, NCBI, EXPASY, and others to compare assembled data, predict proteins and compare them to the broader scope of insect genomes. Many other genes of interest which have various functions in leafhopper biology and physiology have also been identified but are not reported herein. The EST sequences reported in this study have been deposited in GenBank's dbEST. (see references: Hunter 2005, 2006, 2007).

Fig. 1. Composite figure showing distribution of *Homalodisca vitripennis* transcripts across other species (along left), with the top 6 species homologies being in these insects whose genomes have been completed: *Drosophila melanogaster*, *Aedes aegyptii*, *Tribolium castaneum*, *Anopheles gambiae*, *Nasonia vitripennis*, and *Apis mellifera*.



Many of the discoveries made in other insects, such as *Drosophila*, and Honey Bee, can be applied when the same genetic transcripts can be identified. For example we increased our understanding of the roles and pathways of heat shock proteins in leafhoppers by examining the data completed in Locusts, Flies, and Nematodes. The same is true for digestive enzymes. The increasing application of transcriptional data is leading the way in the development of new strategies to reduce plant diseases and their insect vectors. Application of RNAi against a wide range of insect species from spruce budworm to whiteflies are viewed as the future in insect pest control, and many new methods which incorporate the use of native endophytic bacteria and/or viruses as the mechanism for delivery or expression of dsRNA within plants are being widely evaluated. The main advantages of applying genomic data in this manner to solve agricultural problems is that the plants are not 'transformed', thus the quality of the crop is not altered, saving time, money, and reducing the effort needed to find solutions to many emerging devastating agricultural problems.



Accession EC #	BLAST DeKegg Ids	GO TYPE	GO1	GO2	GO3	HC_ID	E-value (HG)	GA_ID	E-value (GA)	ON_ID	-value (ON)
Q7QE45	AgCP7442	Molecular Function	motor activity	structural molec	structural	WHMg 2758	0	WHGA067-5	5.4089E-102	WHON042_E12	1.1E-134
Q89GF8	Hypothetic Citrate cycle (TCA cycl	Molecular Function	aconitate hydratase	mitochondrion	tricarboxyl	WHMg 2485	0	WHGA2354	7.01792E-63	WHON0097	0
Q8P916	Putative cytoplasmic actin A3a1	Molecular Function	motor activity	structural molec	structural	WHMg 1531	0	WHGA2663	4.55082E-67	WHON0017	0
Q8P119	Putative elongation factor 1-alpha	Molecular Function	translation elongatio	GTP binding	cytoplasmic	WHMg 1396	0	WHGA0038	0	WHON0147	0
Q8P915	Putative muscle actin	Molecular Function	motor activity	structural molec	structural	WHMg 1395	0	WHGA2643	1.92228E-73	WHON0021	0
Q7PM43	ENRANG/Ubiquinone biosynthes	Molecular Function	iron ion binding	electron transport	mitochondrion	WHMg 2058	0	WHGA1900	8.2489E-107	WHON1268	0
Q7PPE7	ENRANG/Glycolysis / Gluconog	Molecular Function	magnesium ion bind	pyruvate kinase	glycolysis	WHMg 2042	0	WHGA2158	1.54504E-62	WHON0828	2.1E-138
Q8P916	Putative activated protein kinase	Molecular Function	receptor activity	kinase activity	receptor activity	WHMg 2042	0	WHGA006-4	1.6435E-120	WHON1478	1.98E-48
Q8P916	Putative cytoplasmic actin A3a1	Molecular Function	motor activity	structural molec	structural	WHMg 1531	0	WHGA2663	4.55082E-67	WHON0017	0
Q8P916	Putative delta-9 desaturase 1	Molecular Function	stearoyl-CoA 9-desat	iron ion binding	endoplasmic	WHMg 2025	0	WHGA0206	0	WHON0283	0
Q8P919	Putative elongation factor 1-alpha	Molecular Function	translation elongatio	GTP binding	cytoplasmic	WHMg 1396	0	WHGA0038	0	WHON0147	0
Q8P919	Putative iron Glycolysis / Gluconog	Molecular Function	motor activity	structural molec	structural	WHMg 2017	0	WHGA1213	0	WHON1126	0
Q8P916	Putative muscle actin	Molecular Function	motor activity	structural molec	structural	WHMg 1395	0	WHGA2643	1.92228E-73	WHON0021	0
Q8P911	Putative rhodopsin	Molecular Function	rhodopsin-like recep	receptor activity	G-protein	WHMg 1020	0	WHGA05-149	3.1982E-122	WHON0281	0
Q8P913	Glyceraldehyde-3-phosphate	Molecular Function	glyceraldehyde-3-ph	glucose metabol	glycolysis	WHMg 1552	4.3383E-168	WHGA0261	1.9645E-169	WHON0243	4.9E-173
Q8P912	Putative activated protein kinase	Molecular Function	receptor activity	kinase activity	receptor activity	WHMg 2008	4.6847E-166	WHGA2745	9.75547E-72	WHON1478	1.98E-48
Q8P910	Putative iron Glycolysis / Gluconog	Molecular Function	receptor activity	kinase activity	receptor activity	WHMg 2297	2.3954E-147	WHGA1213	0	WHON13_H01	2.59E-19
Q17083	Vitellogenin precursor	Molecular Function	lipid transporter acti	lipid transport	nutrient re	WHMg 2041	3.3648E-143	WHGA019-70	2.47424E-39	WHON0059	1.1E-105
Q9VFF0	CG3731-PA	Molecular Function	metalloendopeptid	proteolysis	metalloendopeptid	WHMg 2297	5.2528E-142	WHGA085-15	4.12314E-65	WHON036_H10	5.27E-30
Q7PFA8	AgCP12715	Molecular Function	catalytic activity	GTP binding	tricarboxyl	WHMg 1365	8.5228E-136	WHGA2684	2.53721E-45	WHON007_F09	2.43E-73
Q7PFA3	ENRANG/0000024398	Molecular Function	electron transporter	iron ion binding	tricarboxyl	WHMg 2462	1.1902E-129	WHGA1354	1.55312E-69	WHON030_F08	2.05E-64
Q7PPE7	ENRANG/0000021580	Molecular Function	magnesium ion bind	pyruvate kinase	glycolysis	WHMg 2668	7.9039E-108	WHGA2158	1.54504E-62	WHON0828	2.1E-138
Q7PPE7	ENRANG/Glycolysis / Gluconog	Molecular Function	magnesium ion bind	pyruvate kinase	glycolysis	WHMg 2668	2.4039E-107	WHGA2158	1.54504E-62	WHON0828	2.1E-138
Q9VFF0	CG3731-PA	Molecular Function	metalloendopeptid	proteolysis	metalloendopeptid	WHMg 2297	6.9007E-106	WHGA085-15	4.12314E-65	WHON036_H10	5.27E-30
Q9VFF0	CG1970-PA	Molecular Function	electron transporter	mitochondrion	electron tr	WHMg 031_H08	6.3715E-104	WHGA2174	1.50812E-85	WHON032_H08	4.28E-56
Q17083	Vitellogenin precursor	Molecular Function	lipid transporter acti	lipid transport	nutrient re	WHMg 2041	4.2689E-103	WHGA1453	4.34174E-57	WHON0059	1.1E-105
Q8P912	Putative ferritin GF2	Molecular Function	binding	iron ion transport	ion ho	Contig_1413	3.50913E-95	WHGA0709	7.98921E-91	WHON0085	2.56E-52
Q7P2476	AgCP2476	Molecular Function	malic enzyme activit	malate metabol	oxidoreduct	WHMg 041_F10	3.3499E-95	WHGA1635	5.71834E-84	WHON034_G07	5.86E-53
Q8P912	Putative ferritin GF2	Molecular Function	binding	iron ion transport	ion ho	WHMg 1739	4.47344E-95	WHGA0709	7.98921E-91	WHON0085	2.56E-52

Digestive Enzymes: Aminopeptidases, several cathepsin L-like cysteine proteases, and other proteases have been identified in these leafhoppers which are also in other piercing-sucking feeding insects (Foissac et al., 2002, Wright et al., 2006, Zhu et al., 2003). In aphids, a cathepsin B protease has been shown to be constitutively expressed in all aphid individuals, suggesting gene duplication and evolution of a novel biological function of cathepsin B in the aphid lineage (Houseman and Downe 1983). Cathepsin B proteases were also identified in these leafhoppers and may show similar duplication. Cleavage of food proteins into peptides and amino acids is an important process for which an array of proteases of different substrate specificity and enzymatic activities are produced in the alimentary tract and are involved in protein digestion Annotation of these data advances current understanding of leafhopper biological pathways while providing clues to the genetic basis of such processes in insect-pathogen, and insect-plant interactions (Fig. 2, 3). The availability of genomic data for these leafhoppers continues to increase, thus uses of the current data provides a solid foundation for future studies in leafhopper functional genomics.

CONCLUSIONS - The information gained from this study provides the first investigation using comparative genomics of the transcriptomes from three leafhopper vectors of Pierce's disease of grapes: *H. vitripennis*, *G. atropunctata*, and *O. nigricans*. Amino acid sequence comparisons BLASTX, BLASTP with other known proteins relies on conserved motifs of specific domain(s), NCBI GenBank. *In silico* comparative analyses continues to be a valuable tool to identify proteins and their functional domains as widely accepted method to address many biological questions.

Fig. 2. Sequence Distribution: Molecular Functions. Categories had to have at least 50 members. Represents EST's from three cDNA libraries, Adults, 5th instar, and Midgut. *Homalodisca vitripennis*, (Blast2GO analysis). Highest Categories in descending order: Ribosome structure= 296, Calcium ion binding=218, ATPase activity=154, Actin binding= 134, Microfilament motor activity=180, Endopeptidase activity=114, Oxidoreductase activity= 109, Protein Kinase activity= 107.

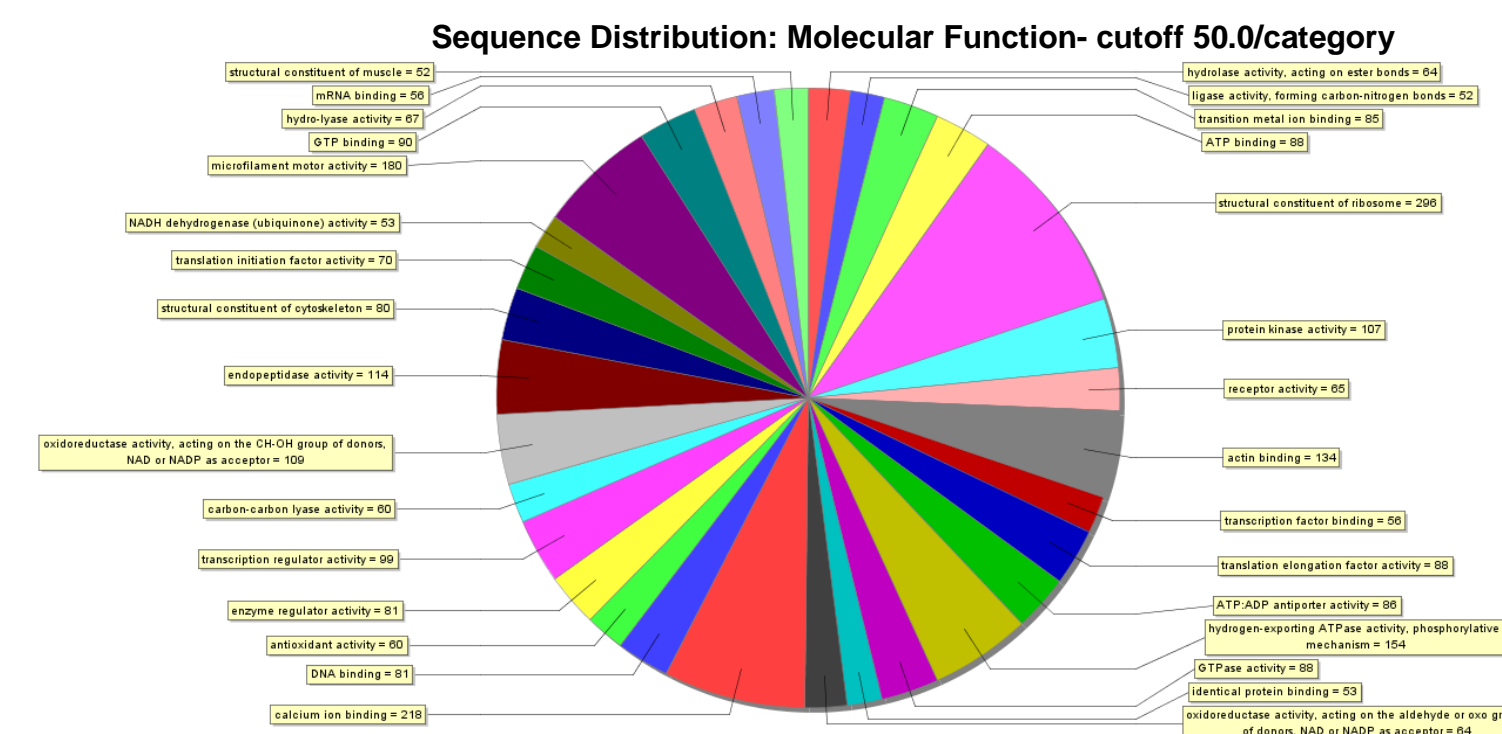
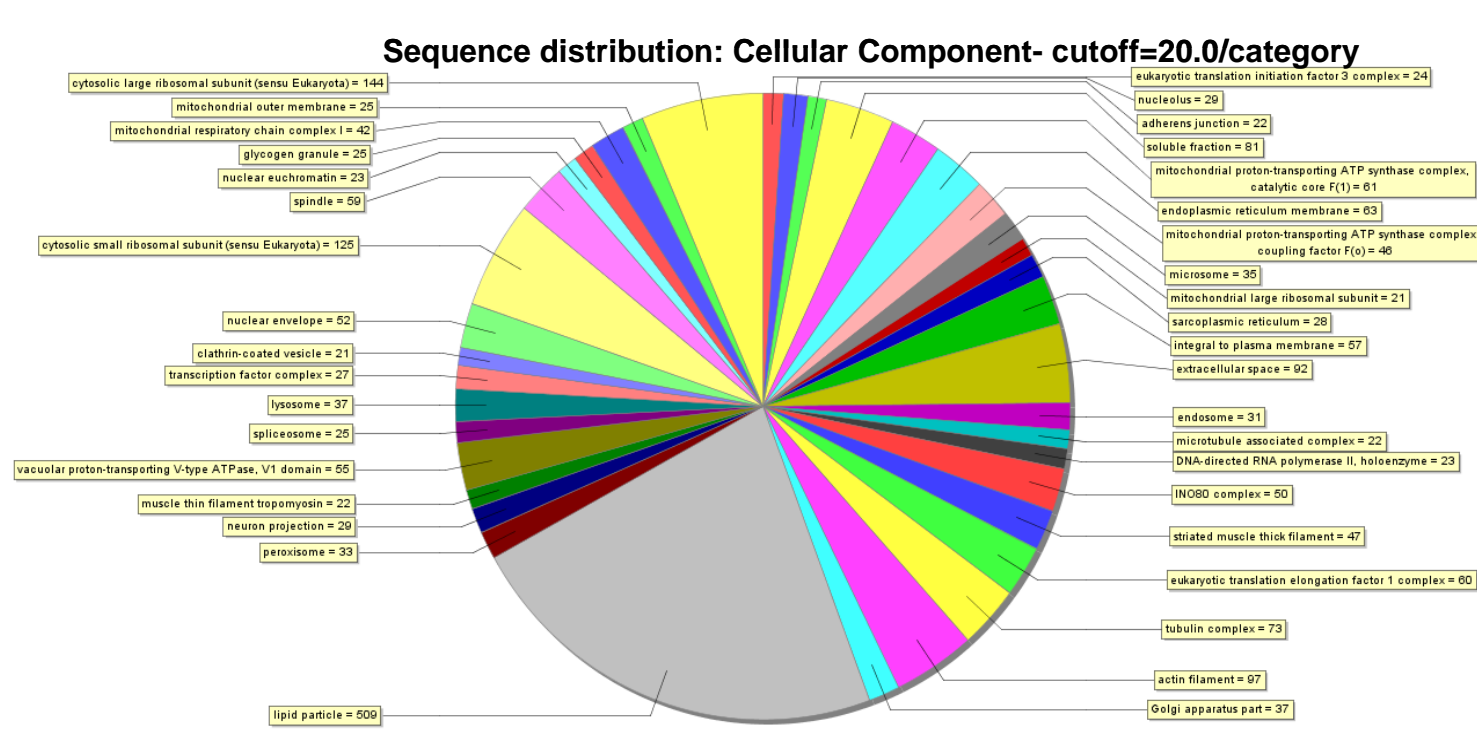


Fig. 3. Sequence Distribution: Cellular Component. Categories had to have at least 20 members. Represents EST's from three cDNA libraries, Adults, 5th instar, and Midgut. *Homalodisca vitripennis*, (Blast2GO analysis).



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