



# Transcriptome analysis of shrimps

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# Two major farmed species

- **Black Tiger Shrimp**  
*Penaeus monodon*



- **Pacific White Shrimp**  
*Litopenaeus vannamei*





# Nucleotide sequences of shrimps submitted in GenBank

31 August, 2013

Species	Nucleotide: DNA and RNA sequences	GSS: genome survey sequences	EST: expressed sequence tag	Total
<i>Litopenaeus vannamei</i>	76,444	229	162,933	<b>239,606</b>
<i>Penaeus monodon</i>	67,083	21,124	39,908	<b>128,115</b>
<i>Fenneropenaeus chinensis</i>	510	19	10,512	<b>11,041</b>
<i>Marsupenaeus japonicus</i>	1,013	258	3,783	<b>5,054</b>
<i>Litopenaeus setiferus</i>	225	-	1,059	<b>1,284</b>
<b>Total</b>	<b>145,275</b>	<b>21,630</b>	<b>218,195</b>	<b>385,100</b>

# *Penaeus monodon* Transcriptome

12,182 unique ESTs



109,679 unique  
sequences from  
RNA Seq

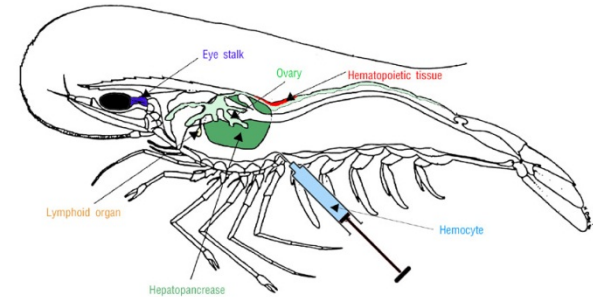
clustering



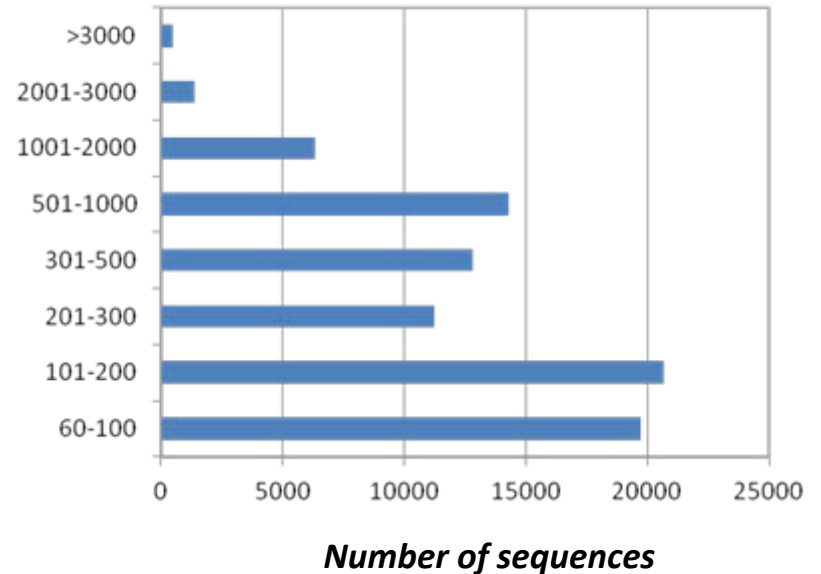
88,131  
unique sequences

16,521  
known genes

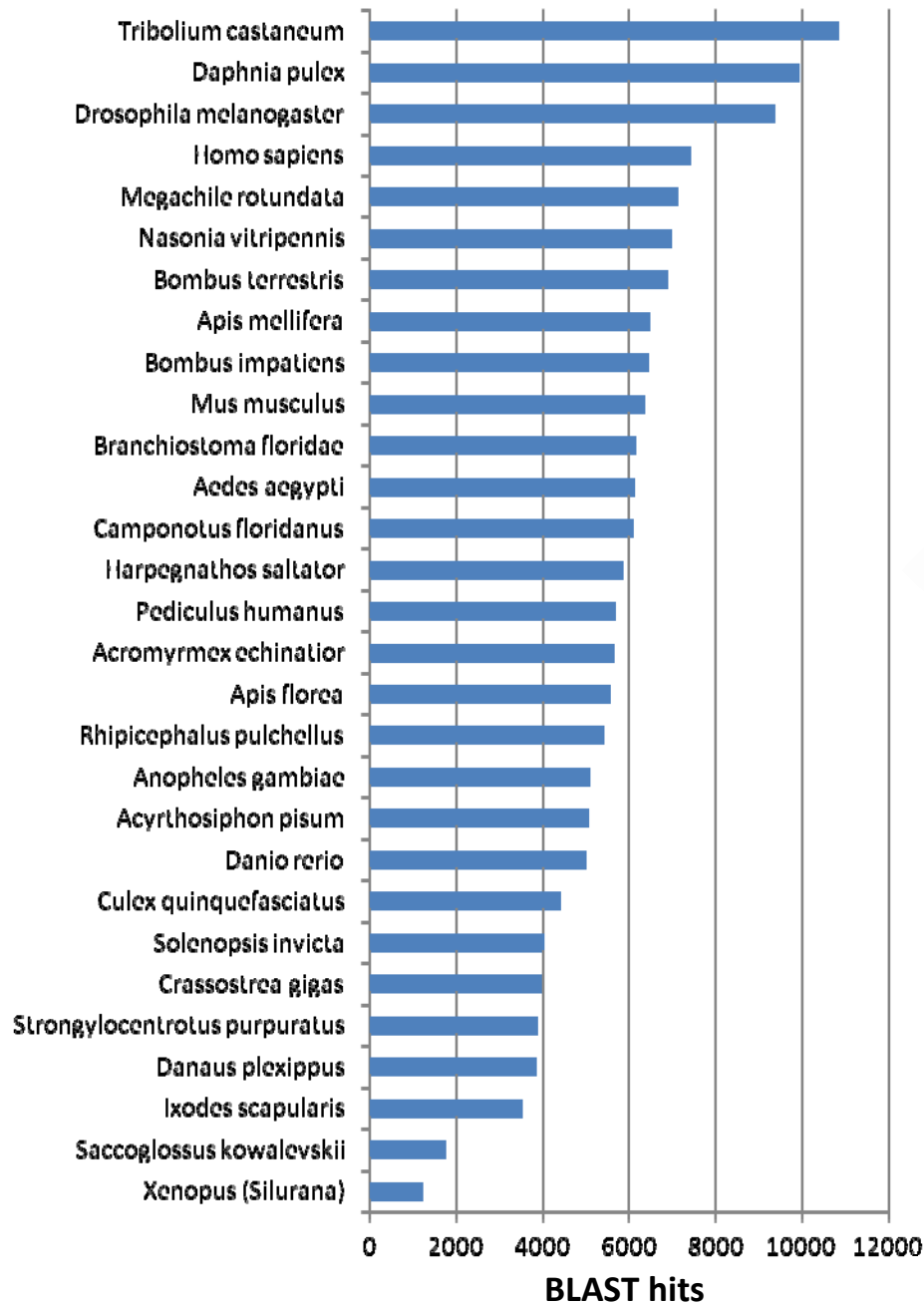
71,880  
unknown genes



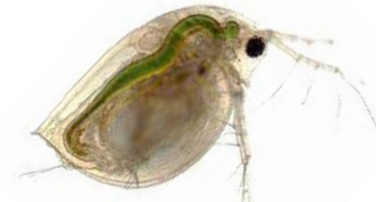
Sequence length (bp)



# Species distribution



*Tribolium castaneum*



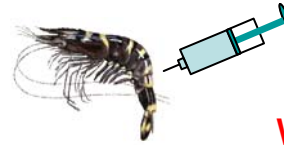
*Daphnia pulex*



*Drosophila melanogaster*

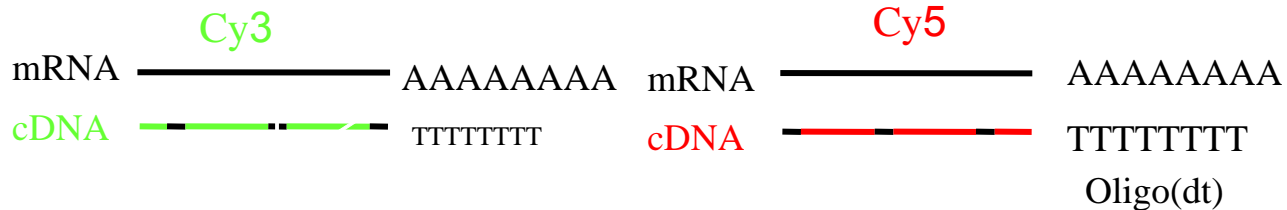
# Microarray analysis of black tiger shrimp hemocyte after pathogen infections

Control shrimp:  
Diluent medium

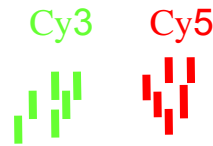


Experimental shrimp:  
WSSV, YHV, *V. harveyi*

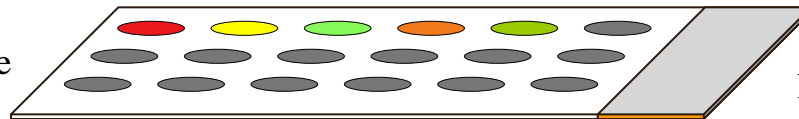
Sampling at 6, 24 and 48 hours interval after injection



cDNA synthesis and labelling with fluorescence



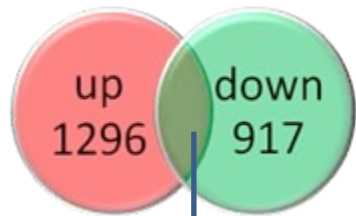
Shrimp cDNA microarray slide



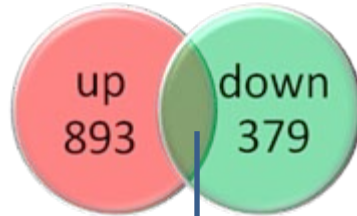
hybridizations

Scanning

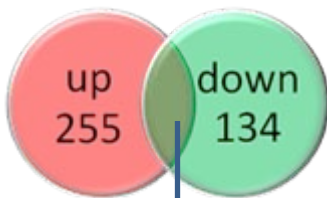
# Expression profiling of genes responded to pathogen infections



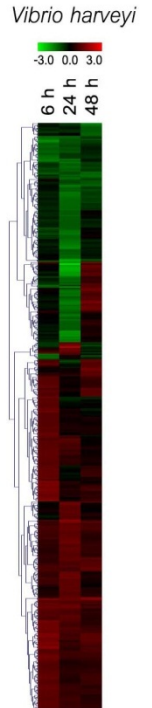
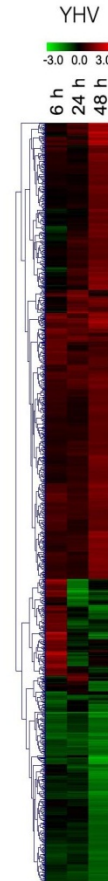
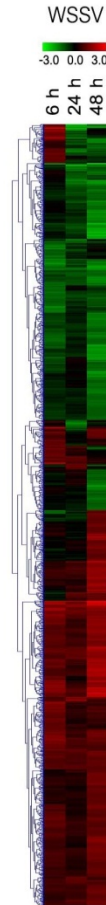
WSSV  
2274 responsive genes  
Known gene: 41%



YHV  
1327 responsive genes  
Known gene: 41%



*V. harveyi*  
410 responsive genes  
Known gene: 37%



# Pathogen-induced genes : WSSV-YHV



## Defense & homeostasis

### AMP

ALF  
Penaeidin  
Crustin

High induction at 6 hpi

### PRP

c-type lectin  
c-type lectin receptor  
Chitin binding lectin  
Ficolin  
Thrombospondin

High induction at both early and late infection stages

### Proteinase & their inhibitor

Serine protease  
Cysteine protease  
Zinc metalloprotease  
Meprin A metalloprotease  
Legumain  
EB module family protein  
Kazal type proteinase inhibitor  
Protease inhibitor  
Serine protease inhibitor  
Alpha 2 macroglobulin

### ProPO

Prophenoloxidase activating factor III  
Serpine peptidase inhibitor  
Pacifastin  
Serpine 12

High induction at both early and late infection stages

### Chaperone

Heat shock  
Chaperonin  
Dnak protein

High induction at late infection stages

### Detoxification

SOD  
Glutathione s transferase  
Metallothionine

### Clotting protein

Transglutaminase  
Coagulation factor VIII

### Others

Laccase  
Agglutinin  
Thymosin  
Mucin  
Hemomucin  
PMAV  
Single whey acidic protein  
Syntenin  
γ-interferon

High induction at 6 hpi





# RNA-Seq

The use of next-generation sequencing technology to compare the transcriptome of shrimp resistant and susceptible to Taura syndrome virus (TSV)

## ❖ **Shrimp samples**

obtained from SyAqua Siam Co., Ltd

TSV-Resistant lines : R1, R2, R3 and R4

TSV-Susceptible lines: S1, S2, S3, and S4



## ★ Sequencing of short expressed reads from *L.vannamei* hemocyte

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	<b>TSV-resistant shrimp</b>	<b>TSV-susceptible shrimp</b>
<b>No. of reads</b>	<b>201,064,238</b>	<b>177,287,468</b>
<b>Average read length (bp)</b>	<b>100</b>	<b>100</b>
<b>Number of reads after trimming</b>	<b>193,608,168</b>	<b>171,229,468</b>
<b>Percentage retained</b>	<b>96.29%</b>	<b>96.58%</b>
<b>Average read length after trimming (bp)</b>	<b>90.9</b>	<b>90.9</b>

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- **A total of 378 million reads were generated.**
- **Low-quality sequences (quality scores <20) and short reads sequences less than 30 bp were removed.**
- **A total of 364 million remaining high-quality sequences (96.4%) were preserved and carried forward for assembly and analysis.**



## Differentially expressed genes in hemocyte of TSV-resistant and susceptible *L. vannamei*

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	No. of differentially expressed contigs*
Up-regulated	877
Down-regulated	497
Total	1,374

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- From 61,637 unique genes, 1,374 exhibited significant differentially expressed profile.
- BLAST search against NCBI database (E-value  $\leq 1e-5$ ,) revealed 698 known genes (50%)

\* TSV- susceptible shrimps were used as the control group.



## Differentially expressed genes blasted against artemia genome

From 1374 contigs;

184 of the 1374 seq show homology with threshold value E-10

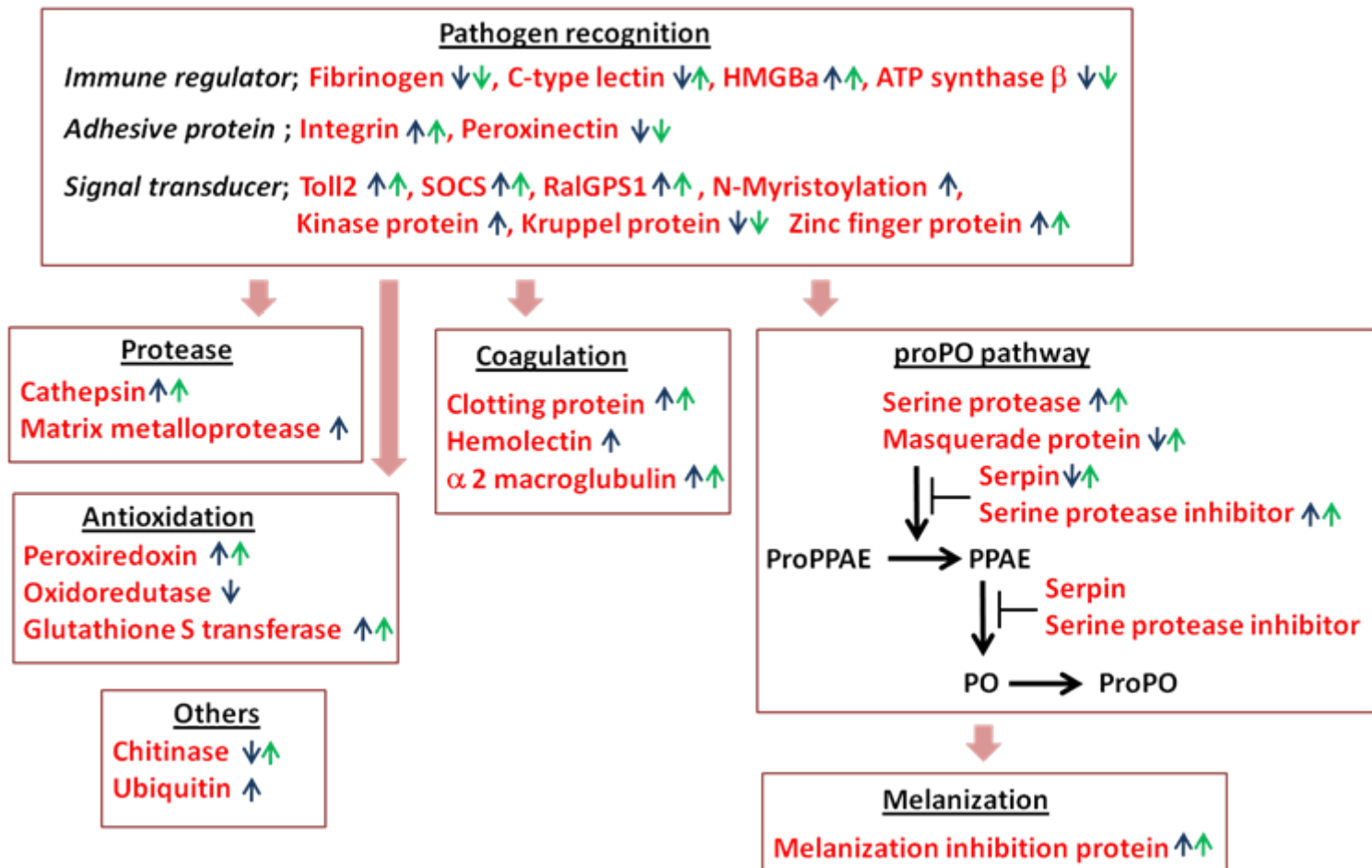
142 of the 1374 seq show homology with threshold value E-15

109 of the 1374 seq show homology with threshold value E-20

ID	Accession NO.	Description	Fold Change	size (bp)	Max ident	E value
2558302	EFX74517.1	hypothetical protein DAPPUDRAFT_307174 [Daphnia pulex]	7.82	817	28%	5.00E-06
2710986	EFX75090.1	hypothetical protein DAPPUDRAFT_306915 [Daphnia pulex]	5.35	3307	30%	1.00E-07
1851079	EFX68432.1	hypothetical protein DAPPUDRAFT_114543 [Daphnia pulex]	2.98	661	38%	0.092
2733200	EFX74605.1	Shn zinc finger protein [Daphnia pulex]	1.72	2045	67%	1.00E-86
247657	EFX87746.1	hypothetical protein DAPPUDRAFT_22032 [Daphnia pulex]	1.56	291	45%	5.00E-14
2665131	EFX70702.1	hypothetical protein DAPPUDRAFT_30436 [Daphnia pulex]	-1.69	400	35%	7.00E-04
2733427	EFX78111.1	Septin-4-like protein [Daphnia pulex] putative MCM3, Minichromosome maintenance complex	-1.84	1385	28%	5.00E-39
2318977	EFX79105.1	component 3 [Daphnia pulex]	-4.09	2788	66%	0
2111954	EFX67816.1	hypothetical protein DAPPUDRAFT_330617 [Daphnia pulex]	-4.56	642	46%	0.02

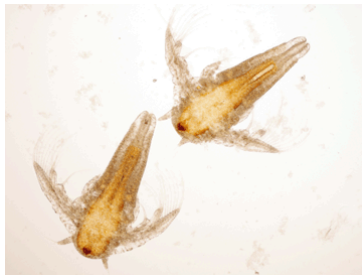


# Putative immune pathway involved in resistance to TSV in *L. vannamei*



# Proposed collaborative work

- To search for gene homology between shrimp and artemia
- To identify some pathways that might be shared by the two species
- To study gene function using artemia as a model organism





**Thank you**