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Source: Journal of Insect Science, 9(21) : 1-13

Published By: Entomological Society of America

URL: https://doi.org/10.1673/031.009.2101

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# Analysis and functional annotation of expressed sequence tags from the Asian longhorned beetle, *Anoplophora glabripennis*

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## Abstract

The Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), is one of the most economically and ecologically devastating forest insects to invade North America in recent years. Despite its substantial impact, limited effort has been expended to define the genetic and molecular make-up of this species. Considering the significant role played by late-stadia larvae in host tree decimation, a small-scale EST sequencing project was done using a cDNA library constructed from 5$^{th}$-instar *A. glabripennis*. The resultant dataset consisted of 599 high quality ESTs that, upon assembly, yielded 381 potentially unique transcripts. Each of these transcripts was catalogued as to putative molecular function, biological process, and associated cellular component according to the Gene Ontology classification system. Using this annotated dataset, a subset of assembled sequences was identified that are putatively associated with *A. glabripennis* development and metamorphosis. This work will contribute to understanding of the diverse molecular mechanisms that underlie coleopteran morphogenesis and enable the future development of novel control strategies for management of this insect pest.

**Keywords:** Coleoptera, development, EST, insect, morphogenesis, transcriptome
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**Received:** 3 September 2007 | **Accepted:** 8 March 2008 | **Published:** 20 May 2009
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**ISSN:** 1536-2442 | Vol. 9, Number 21

**Cite this paper as:**
Hunter WB, Smith MT, Hunnicutt LE. 2009. Analysis and functional annotation of expressed sequence tags from the Asian longhorned beetle, *Anoplophora glabripennis*. 13pp. *Journal of Insect Science* 9:21, available online: insectscience.org/9.21


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## Introduction

The Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), is a pest native to eastern China and Korea (Lingafelter and Hoebeke 2002). In 1996, this insect was introduced into the United States, presumably via wood packing materials used to import cargo from Asia (Smith 2003). Since its initial discovery in the state of New York, infestations have been detected in Illinois (Poland et al. 1998), New Jersey (Haack 2003), and the province of Ontario, Canada (CFIA 2005; Haack 2006). *A. glabripennis* grow and reproduce on an array of hardwoods including members of the genera *Acer* (maple), *Aesculus* (horsechestnut), *Betula* (birch), *Celtis* (hackberry), *Plantanus* (plant tree, sycamore), *Populus* (poplar), *Salix* (willow), and *Ulmus* (elm) (Sawyer 2003; Ric et al. 2006). Late-instar grubs are especially destructive, forging winding galleries into the heartwood of the tree. This feeding behavior causes branch dieback and, in cases of heavy or persistent infestations, can result in structural deterioration and often tree mortality (Haack et al. 1997).

Burgeoning globalized trade presents a serious challenge in that *A. glabripennis* now have the opportunity to infiltrate via multiple points of entry, mitigating the efficacy of the small number of quarantine facilities currently in place. Undetected, this devastating pest could disseminate throughout regions of North America where suitable host trees exist. Nowak et al. (2001) estimated that, if this occurs, up to 1.2 billion urban shade trees with a compensatory value of $669 billion could be lost. While substantial, these figures do not factor in collateral losses such as degraded aesthetics and lowered property values nor do they take into account the potential impact to both commercial and natural forest stands.

At present, eradication efforts center on the identification and removal of trees showing signs of *A. glabripennis* infestation. As of 2002, $110.9 million was expended by federal, state, and city governments in New York and Illinois as part of this program (Stewart 2002). To gauge the utility of systemic insecticides as a supplement to this effort, scientists from the USDA Forest Service performed field evaluations in which trees were treated with either imidacloprid or thiocloprid. While successful in reducing *A. glabripennis* populations, neither compound provided complete control (Poland et al. 2006).

Only recently has research begun to shift focus from chemical-based control strategies to the development of sustainable biocontrol alternatives including entomopathogenic fungi, rhabditoid nematode species, microsporidia, natural predators and parasitoids (Smith et al. 2002, and references therein; Hajek et al. 2006) as well as artificial lures and bait/trap tree systems (Li et al. 1999; Zhang et al. 2002). Furthermore, nominal effort has gone into the investigation of genome-based approaches for management of *A. glabripennis*. To facilitate this work, our laboratory conducted a small-scale EST sequencing project and posted preliminary data to the National Center for Biotechnology Information (NCBI) dbEST where it is freely accessible to the scientific community. Because of the significant role played by late-stadia larvae in host tree decimation, 5$^{th}$-instar *A. glabripennis* were selected as a base for the transcriptome survey described herein.

## Materials and Methods

### Insects

Fifth-instar *A. glabripennis* were obtained from a colony managed by Michael Smith at the USDA ARS Beneficial Insects Introduction Research Unit (Newark, DE). Insects were maintained as previously described by Dubois et al. (2002). Larvae were ground directly in guanidine-isothiocyanate buffer (1 larva per 20 ml buffer) and stored at −40°C prior to shipment.

### RNA extraction and library construction

Upon arrival at the USDA ARS U.S. Horticultural Research Laboratory (Ft. Pierce, FL), the majority of samples were transferred to an ultra-low temperature freezer (−80°C) for archival purposes and a single larva was subjected to further processing. Buffer RLT (Qiagen, www.qiagen.com) was added to the primary sample at 2.5X the original volume along with 150 µl β-mercaptoethanol. The sample was placed at −40°C for 10 min then incubated at 37°C for 20 min. Intact tissues were further homogenized with a QIAShredder® and total RNA extracted using an RNeasy® Maxi Kit (Qiagen) according to the manufacturer's instructions. The eluate was precipitated in 0.1 volumes 3M sodium acetate and 2.5 volumes absolute ethanol at −40°C overnight and the resultant pellet resuspended in 35 µl RNase-free water. Poly(A)+ RNA was purified using the MicroPoly(A)Pure Kit (Ambion, www.ambion.com). A primary library was constructed with Stratagene's ZAP-cDNA® Library Construction Kit (Stratagene, www.stratagene.com) and subsequently mass excised using ExAssist® Helper Phage (Stratagene). The library had a titer of 9.75 x $10^5$ colony forming units per ml with inserts averaging ~1,221 bp. Transformants were recovered by random colony selection and grown overnight at 32°C, 125 rpm in LB Broth supplemented with 100 mg/ml ampicillin.

### EST sequencing

Plasmid DNA was extracted using the Qiagen Liquid Handling Robot (Model 9600) in conjunction with the QIAprep 96 Turbo Miniprep Kit according to the recommended protocol. Single-pass sequencing was performed using the ABI PRISM® BigDye™ Primer Cycle Sequencing Kit (Applied Biosystems, www.appliedbiosystems.com) and a universal T3 primer. Reaction products were precipitated, resuspended in 15


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µl sterile water, and loaded onto an ABI 3730 DNA Analyzer (Applied Biosystems).

### Sequence analysis

Base calling was performed by TraceTuner™ (Paracel, www.paracel.com) and low-quality bases (quality score <20) were stripped from both ends of each EST. Quality trimming, vector trimming, and sequence fragment alignments were executed using Sequencher™ software (Gene Codes, www.genecodes.com). Sequencher contig assembly parameters were set using a minimum overlap of 50 bp and 90% identity. Contigs joined by vector sequence were flagged for possible miss-assembly and manually edited. The EST sequences reported in this study have been deposited in GenBank's dbEST under accession numbers DR108748-DR109303.

### Sequence annotation and Gene Ontology classification

Putative sequence identity was determined based on BLAST similarity searches using the NCBI BLAST server (www.ncbi.nlm.nih.gov) with comparisons made to both non-redundant nucleic acid and protein databases using BLASTN and BLASTX, respectively. Matches with an E-value ≤-10 were considered significant and were classified according to the Gene Ontology classification system. In the case of CG numbers (e.g., CG30437-PA), annotations were conferred using the associated CV term provided by FlyBase (www.flybase.org). All other sequences were associated with a molecular function, biological process, and cellular component based on searches to the Gene Ontology database (www.geneontology.org). Custom Perl scripts and Excel spreadsheets were used for BLAST parsing and table generation. The SignalP 3.0 Server was used to predict the presence and location of signal peptide cleavage sites (www.cbs.dtu.dk/services/SignalP/).

## Results and Discussion

### General overview

A single *A. glabripennis* larva was used for this study so that allelic variations within an individual (EST allele counts) could be distinguished from those that may exist across a population (population allele frequency). 5′-end one-pass sequencing of the cDNA library yielded 672 ESTs, of which 599 were designated as high quality (i.e., ≥200 bases with a TraceTuner™ score of 20 or better). ESTs ranged in size from 206 to 828 bases with an average length of 650 bases. Upon assembly, these sequences were condensed to form 47 contiguous sequences (contigs), leaving 334 as singletons. Contigs and singlets together culminated in 381 unique sequences that putatively represent distinct transcripts. Contigs ranged in size from 392 to 2,240 bases with an average length of 954 bases; whereas, singletons varied from 206 to 828 bases with an average length of 647 bases.

### Highly redundant transcripts

The calculated redundancy of the library was ~32% with nine contigs found to be highly redundant (i.e., containing ≥5 ESTs; Table 1) accounting for 24% of the total ESTs. Two of the contigs, representing 20 ESTs, had significant sequence similarity to mitochondrial genes and were subsequently discarded from the transcriptome survey. Nearly half of the highly redundant contigs had no significant similarity (E >−10) to any sequence listed within NCBI's nr database. These transcripts correspond to potentially novel genes specific to *A. glabripennis* and warrant further examination. The remaining three contiguous sequences returned significant matches to proteins previously identified in other coleopteran species. The most frequently represented of these transcripts, WHALB[0244], constituted 22 ESTs and matched most closely to a 56 kDa early-staged encapsulation-relating protein previously identified from *Tenebrio molitor* larvae. Upon assessment of alignment integrity, two sequence variants differing by 14 single nucleotide polymorphisms (SNPs) were resolved. Consequently, WHALB[0244] was

**Table 1.** Most abundantly represented transcripts in the *A. glabripennis* cDNA library

| Contig | ESTs | Accession No. | GenBank Descriptor [Source Organism] | E-value |
|---|---|---|---|---|
| [0243] | 51 | CAM36311 | hypothetical protein [*Thermobia domestica*] | 4.00E-09 |
| [0244] | 22 | BAA78480 | 56 kDa early-staged encapsulation-inducing protein [*Tenebrio molitor*] | 5.00E-25 |
| [0259] | 19 | AAM44045 | arylphorin-like hexamerin [*Apriona germari*] | 0 |
| [0241] | 15 | YP_659513 | cytochrome c oxidase subunit I [*Anoplophora glabripennis*] | 0 |
| [0255] | 12 | XP_381775 | hypothetical protein FG01599.1 [*Gibberella zeae* PH-1] | 6.00E-05 |
| [0250] | 9 | BAA78480 | 56 kDa early-staged encapsulation-inducing protein [*Tenebrio molitor*] | 4.00E-05 |
| [0275] | 6 | BAA78480 | 56 kDa early-staged encapsulation-inducing protein [*Tenebrio molitor*] | 0.002 |
| [0270] | 5 | YP_659517 | cytochrome c oxidase subunit III [*Anoplophora glabripennis*] | 5.00E-89 |
| [0278] | 5 | XP_973799 | PREDICTED: similar to CG6806-PA [*Tribolium castaneum*] | E-116 |

NOTE: items shaded in grey were treated as contaminating sequences and were removed prior to further annotation.


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dissolved and realigned to form two assembled sequences, WHALB[0244a] containing 10 ESTs and WHALB[0244b] containing 12 ESTs. Both variants possessed a single open reading frame (ORF) consisting of 456 amino acid residues, the first 15 of which are thought to encode a leader/signal peptide. Amino acid abundance analyses of the translated protein sequences revealed a preponderance of Gln (99 residues or 22%), Gly (46–47 residues or 10%), and Leu (45 residues or 10%) within the coding domain while Cys and His levels were negligible (<0.5%). Although comparable to the cDNA of *T. molitor* 56-kDa encapsulation-relating protein with respect to amino acid abundance and overall sequence similarity, significant distinctions were noted including an eight amino acid insertion shortly after the signal peptide and 11 deletions scattered along the length of the coding domain (Cho et al. 1999). This would seem to indicate that WHALB[0244a] and WHALB[0244b] represent novel proteins which may play a role in *A. glabripennis* cellular defense. As such, both coding domains have been deposited into GenBank under accession numbers EF583868 and EF583869. The second most highly redundant contig, WHALB[0259], contained 19 ESTs and appeared to span the coding region for an arylphorin-like hexameric storage protein, denoted AglHEX (accession number EF583870). AglHEX had an ORF of 2,151 nucleotides, encoding a protein precursor 717 amino acids in length. The N-terminal of this precursor most likely contains a cleavage site between $AYS_{17}/A_{18}V$, indicating a signal peptide for transmembrane transport. In addition, the following highly conserved larval storage protein (LSP) signature sequence patterns were noted: LSP signature-1 motif Y(F/Y/W)XED(L/I/V/M)$X_2$N$X_6$H$X_3$P) and LSP signature-2 motif T$X_2$RDPX(F/Y)(F/Y/W) with the corresponding sequences in AglHEX as YYLEDVGLNAFYYYYHLYYP$_{218–237}$ and TSMRDPVF$_{421–428}$ (Zhu et al. 2002). Contig WHALB[0278], comprised of five ESTs, showed greatest sequence similarity to a predicted protein from *Tribolium castaneum* annotated as similar to *Drosophila melanogaster* CG6806-PA. When queried to FlyBase, it was determined that this transcript also corresponded to a LSP [partial LSP-2; ~400 amino acids missing from the protein's N-terminal]. Of 307 in-frame residues, WHALB[0278] contained five Met (2%) and 48 aromatic amino acids (16%), a composition indicative of arylphorin-like storage proteins (Telfer and Kungel 1991).

## Functional classification of 5$^{th}$-instar *A. glabripennis* ESTs

A BLASTN search of the entire dataset revealed six contigs and five singletons with significant sequence similarity either to non-nuclear transcripts (e.g., rRNA genes and mitochondrial genes) or contaminating organismal transcripts (e.g., transcripts of plant, bacterial, or trematode origin). These assembled sequences, representing 34 ESTs, were removed from the dataset prior to further analysis.

A total of 258 sequences (29 contigs and 229 singletons; 58% ESTs) showed significant sequence similarity to known proteins. Four sequences (3 contigs and 1 singleton; 4% ESTs) had hits with E-values $\geq 10^{-150}$, 35 sequences (9 contigs and 26 singletons; 8% ESTs) had hits with E-values between $10^{-100}$ and $10^{-149}$, 114 sequences (11 contigs and 103 singletons; 22% ESTs) had hits with E-values between $10^{-50}$ and $10^{-99}$, 48 sequences (3 contigs and 45 singletons; 9% ESTs) had hits with E-values between $10^{-30}$ and $10^{-49}$, and 56 sequences (3 contigs and 53 singletons; 14% ESTs) had hits with E-values between $10^{-10}$ and $10^{-29}$. The remainder of the sequences (9 contigs and 85 singletons; 42% ESTs) failed to return meaningful matches (E $>-10$). The best match (i.e., hit with the lowest E-value; E $\leq -10$) most often corresponded to sequences derived from the Insecta with 225 ESTs (69%) showing greatest similarity to *T. castaneum*, followed by 8 ESTs (2%) for *Drosophila* spp., and 6 ESTs (2%) for *Apis mellifera*. Of the remaining ESTs, 63 (19%) showed greatest similarity to coleopteran species other than *T. castaneum*, 12 (4%) to non-coleopteran insect species, and 14 (4%) most closely resembled sequences derived from non-insect source material. Sequences with a significant hit were further characterized using controlled vocabularies using Gene Ontology. Overviews which include hierarchical listings of associated molecular functions, biological processes, and cellular components are provided in Tables 2, 3, and 4, respectively.

## Transcripts putatively associated with *A. glabripennis* development and metamorphosis

Table 5 highlights a subset of developmental and metamorphosis-related transcripts identified in the *A. glabripennis* library. A brief discussion illustrating the role(s) of several of these transcripts is offered below along with select references.

## Autophagic cell death

WHALB004-85 and WHALB007-57 encompassed the complete coding domains of a putative peptidyl-prolyl cis-trans isomerase (PPIase) and eukaryotic translation initiation factor. Using serial analysis of gene expression (SAGE), Gorski et al. (2003) substantiated the involvement of equivalent proteins (e.g., Dmel\cyp1 and Dmel\eIF-5A) in authophagic cell death. While generally considered as a defense mechanism, this process is believed to be imperative for organelle turnover and recycling during the transition from late instar/pre-pupa to pupa in holometabolous insects such as *A. glabripennis*.


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**Table 2.** Molecular function

| Gene Ontology (GO) Term[a] | Number of ESTs[b] | % of total ESTs represented | Number of contigs | Number of singlets |
|---|---|---|---|---|
| [p] Antioxidant activity | 2 | 0.36% | 0 | 2 |
| [p] Binding | | | | |
| [c] Amine binding | 1 | 0.18% | 0 | 1 |
| [c] Carbohydrate binding | 5 | 0.89% | 0 | 5 |
| [c] Cofactor binding | 2 | 0.36% | 0 | 2 |
| [c] Hormone binding | 2 | 0.36% | 1 | 0 |
| [c] Ion binding | 22 | 3.91% | 2 | 17 |
| [c] Isoprenoid binding | 3 | 0.53% | 0 | 3 |
| [c] Lipid binding | 5 | 0.89% | 0 | 5 |
| [c] Nucleic acid binding | 29 | 5.16% | 3 | 22 |
| [c] Nucleotide binding | 26 | 4.63% | 4 | 18 |
| [c] Odorant binding | 1 | 0.18% | 0 | 1 |
| [c] Oxygen binding | 2 | 0.36% | 1 | 0 |
| [c] Protein binding | 57 | 10.14% | 7 | 42 |
| [c] Tetrapyrrole binding | 5 | 0.89% | 0 | 5 |
| [c] Vitamin binding | 4 | 0.71% | 0 | 4 |
| [c] No further information provided | 2 | 0.36% | 1 | 0 |
| [p] Catalytic activity | | | | |
| [c] Deaminase activity | 3 | 0.53% | 1 | 1 |
| [c] Helicase activity | 1 | 0.18% | 0 | 1 |
| [c] Hydrolase activity | | | | |
| [i] Hydrolase activity, acting on acid anhydrides | 18 | 3.20% | 4 | 10 |
| [i] Hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds | 3 | 0.53% | 0 | 3 |
| [i] Hydrolase activity, acting on ester bonds | 9 | 1.60% | 1 | 7 |
| [i] Hydrolase activity, acting on ether bonds | 1 | 0.18% | 0 | 1 |
| [i] Hydrolase activity, acting on glycosyl bonds | 2 | 0.36% | 0 | 2 |
| [i] Peptidase activity | 39 | 6.94% | 3 | 13 |
| [c] Isomerase activity | 7 | 1.25% | 1 | 5 |
| [c] Ligase activity | 9 | 1.60% | 0 | 9 |
| [c] Lyase activity | 7 | 1.25% | 0 | 7 |
| [c] Oxidoreductase activity | 22 | 3.91% | 2 | 18 |
| [c] Small protein conjugating enzyme activity | 2 | 0.36% | 0 | 2 |
| [c] Transferase activity | 19 | 3.38% | 1 | 17 |
| [p] Chaperone regulator activity | 1 | 0.18% | 0 | 1 |
| [p] Enzyme regulator activity | | | | |
| [c] Enzyme activator activity | 2 | 0.36% | 0 | 2 |
| [c] Enzyme inhibitor activity | 12 | 2.14% | 3 | 6 |
| [c] GTPase regulator activity | 3 | 0.53% | 0 | 3 |
| [c] Kinase regulator activity | 3 | 0.53% | 0 | 3 |
| [c] Phosphatase regulator activity | 1 | 0.18% | 0 | 1 |
| [p] Motor activity | 1 | 0.18% | 0 | 1 |
| [p] Nutrient reservoir activity | 24 | 4.27% | 2 | 0 |
| [p] Signal transducer activity | 7 | 1.25% | 0 | 7 |
| [p] Structural molecule activity | | | | |


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**Table 2** (cont.)

| Gene Ontology (GO) Term[a] | Number of ESTs[b] | % of total ESTs represented | Number of contigs | Number of singlets |
|---|---|---|---|---|
| [c] Structural constituent of cuticle | 1 | 0.18% | 0 | 1 |
| [c] Structural constituent of cytoskeleton | 10 | 1.78% | 1 | 8 |
| [c] Structural constituent of muscle | 1 | 0.18% | 0 | 1 |
| [c] Structural constituent of peritrophic membrane (sensu Insecta) | 1 | 0.18% | 0 | 1 |
| [c] Structural constituent of ribosome | 26 | 4.63% | 4 | 17 |
| [c] No further information provided | 6 | 1.07% | 1 | 4 |
| [p] Transcription regulator activity | 7 | 1.25% | 0 | 7 |
| [p] Translation regulator activity | 7 | 1.25% | 2 | 3 |
| [p] Transporter activity | | | | |
| [c] Amine transporter activity | 1 | 0.18% | 0 | 1 |
| [c] Auxiliary transport protein activity | 1 | 0.18% | 0 | 1 |
| [c] Carbohydrate transporter activity | 1 | 0.18% | 0 | 1 |
| [c] Carrier activity | 9 | 1.60% | 1 | 7 |
| [c] Intracellular transporter activity | 1 | 0.18% | 0 | 1 |
| [c] Ion transporter activity | 2 | 0.36% | 0 | 2 |
| [c] Lipid transporter activity | 2 | 0.36% | 0 | 2 |
| [c] Neurotransmitter transporter activity | 1 | 0.18% | 0 | 1 |
| [c] Organic acid transporter activity | 1 | 0.18% | 0 | 1 |
| [c] Oxygen transporter activity | 25 | 4.45% | 2 | 1 |
| [c] Water transporter activity | 1 | 0.18% | 0 | 1 |
| [c] No further information provided | 5 | 0.89% | 0 | 5 |
| [p] Molecular function unknown | 89 | 15.84% | 4 | 61 |

[a]Classification is hierarchial: indented terms are children [c] of parent terms [p] listed above. All functional assignments of 5th-instar *A. glabripennis* ESTs described here are the "inferred from electronic annotation" (IEA) using the top 5 BLASTX hits with an E-value of ≤ -10 generated from NCBI's nr database. The definition term associated with each sequence was entered into both FlyBase and AmiGO where it was given a molecular function designation according to the Gene Ontology Consortium.

[b]Because a single EST can be associated with several GO terms, the total number of ESTs may be larger than the actual number of ESTs analyzed. However, no single EST was catalogued under the same GO term more than once.

## Bristle morphogenesis

Singletons WHALB002-36 and WHALB004-32 corresponded to *D. melanogaster* singed [CG32858-PA, isoform A] and darkener of apricot (Doa) [CG33553-PE, isoform E]. Although most often associated with neurosensory bristle development, these proteins are thought to be critical in an array of developmental processes including antennal morphogenesis, compound eye development, salivary gland autophagic cell death, and sex differentiation (Yun et al. 1994).

## Nervous system development

Analysis of the primary sequence of WHALB[0248] exposed what appears to be a "false contig" (i.e., product of two distinct transcripts erroneously conjoined through alignment of analogous sequence). The contig was subsequently dissolved and each EST compared to the nr database separately. Based on results of the query, WHALB007-9 was retained along with the BLASTX match definition listed in Table 5. Because WHALB007-9 and WHALB[0269] were assigned equivalent designations, it was necessary to ascertain whether these sequences could be assembled using less stringent parameters. However, superposition of the translated sequences to *D. melanogaster* CG4264-PA, isoform A isoform 1 revealed an 8 amino acid gap corresponding to Dmel $TQASIEID_{278–285}$ that failed to link the *A. glabripennis* sequences. While not contiguous, these assembled sequences represent transcripts that putatively encode heat shock protein cognate 4 (Hsc70-4), a protein which functions in nerve projection events such as axon guidance, axonal fasciculation, neurotransmitter secretion and synaptic vesicle transport.


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**Table 3.** Biological process

| Gene Ontology (GO) Term[a] | Number of ESTs[b] | % of total ESTs represented | Number of contigs | Number of singlets |
|---|---|---|---|---|
| [p] Cellular process | | | | |
| [c] Cell adhesion | 3 | 0.46% | 1 | 1 |
| [c] Cell communication | 36 | 5.53% | 3 | 30 |
| [c] Cell differentiation | | | | |
| [i] Cell fate commitment | 1 | 0.15% | 0 | 1 |
| [i] Neuron differentiation | 6 | 0.92% | 2 | 2 |
| [i] Oocyte differentiation | 6 | 0.92% | 1 | 4 |
| [i] Photoreceptor cell differentiation | 4 | 0.61% | 0 | 4 |
| [i] No further information provided | 1 | 0.15% | 0 | 1 |
| [c] Cellular physiological process | | | | |
| [i] Cell cycle | 7 | 1.08% | 0 | 7 |
| [i] Cell death | 4 | 0.61% | 0 | 4 |
| [i] Cell division | 2 | 0.31% | 0 | 2 |
| [i] Cell homeostasis | 13 | 2.00% | 2 | 8 |
| [i] Cell motility | 4 | 0.61% | 0 | 4 |
| [i] Cell organization and biogenesis | 21 | 3.23% | 4 | 13 |
| [i] Cell proliferation | 4 | 0.61% | 0 | 4 |
| [i] Cellular metabolism | | | | |
| [ii] Alkene metabolism | 1 | 0.15% | 0 | 1 |
| [ii] Amine metabolism | 12 | 1.84% | 1 | 10 |
| [ii] Cofactor metabolism | 5 | 0.77% | 0 | 5 |
| [ii] Generation of precursor metabolites and energy | 4 | 0.61% | 0 | 4 |
| [ii] Nucleobase, nucleoside, nucleotide and nucleic acid metabolism | 22 | 3.38% | 2 | 18 |
| [ii] Organic acid metabolism | 1 | 0.15% | 0 | 1 |
| [ii] Vitamin metabolism | 1 | 0.15% | 0 | 1 |
| [i] Cellularization | 3 | 0.46% | 0 | 3 |
| [i] Chromosome segregation | 4 | 0.61% | 0 | 4 |
| [i] Transport | 63 | 9.68% | 5 | 33 |
| [i] No further information provided | 4 | 0.61% | 1 | 2 |
| [p] Development | | | | |
| [c] Aging | 2 | 0.31% | 0 | 2 |
| [c] Appendage development | 3 | 0.46% | 0 | 3 |
| [c] Embryonic development | 9 | 1.38% | 2 | 5 |
| [c] Morphogenesis | 5 | 0.77% | 0 | 5 |
| [c] Organ development | 12 | 1.84% | 2 | 8 |
| [c] Pattern specification | 7 | 1.08% | 1 | 5 |
| [c] Pigmentation during development | 2 | 0.31% | 1 | 0 |
| [c] Post-embryonic development | 4 | 0.61% | 1 | 2 |
| [c] Sex differentiation | 1 | 0.15% | 0 | 1 |
| [c] System development | | | | |
| [i] Nervous system development | 13 | 2.00% | 3 | 7 |
| [c] Tissue development | 11 | 1.69% | 1 | 9 |
| [p] Growth | 1 | 0.15% | 0 | 1 |
| [p] Physiological process | | | | |


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**Table 3** (cont.)

| Gene Ontology (GO) Term[a] | Number of ESTs[b] | % of total ESTs represented | Number of contigs | Number of singlets |
|---|---|---|---|---|
| [c] Localization | 2 | 0.31% | 0 | 2 |
| [c] Metabolism | | | | |
| [i] Biosynthesis | | | | |
| [ii] Organismal biosynthesis | | | | |
| [iii] Cuticle biosynthesis | 4 | 0.61% | 0 | 4 |
| [i] Cellular metabolism | | | | |
| [ii] Aromatic compound metabolism | 2 | 0.31% | 1 | 0 |
| [ii] Hormone metabolism | 1 | 0.15% | 0 | 1 |
| [ii] One-carbon compound metabolism | 2 | 0.31% | 0 | 2 |
| [ii] Oxygen and reactive oxygen species metabolism | 2 | 0.31% | 0 | 2 |
| [ii] Pheromone metabolism | 1 | 0.15% | 0 | 1 |
| [ii] Phosphrous metabolism | 2 | 0.31% | 0 | 2 |
| [i] Macromolecule metabolism | | | | |
| [ii] Carbohydrate metabolism | 10 | 1.54% | 0 | 10 |
| [ii] Protein metabolism | 107 | 16.44% | 16 | 54 |
| [i] Primary metabolism | | | | |
| [ii] Lipid metabolism | 16 | 2.46% | 1 | 14 |
| [i] No further information provided | 5 | 0.77% | 1 | 3 |
| [c] Organismal physiological process | | | | |
| [i] Molting cycle | 1 | 0.15% | 0 | 1 |
| [i] Muscle contraction | 4 | 0.61% | 1 | 2 |
| [i] Organismal movement | 1 | 0.15% | 0 | 1 |
| [c] Regulation of physiological process | | | | |
| [i] Regulation of cellular physiological process | 30 | 4.61% | 4 | 22 |
| [p] Regulation of biological process | | | | |
| [c] Negative regulation of biological process | 1 | 0.15% | 0 | 1 |
| [c] Regulation of catalytic activity | 1 | 0.15% | 0 | 1 |
| [p] Reproduction | 13 | 2.00% | 0 | 13 |
| [p] Response to stimulus | | | | |
| [c] Behavior | 14 | 2.15% | 4 | 6 |
| [c] Response to abiotic stimulus | 8 | 1.23% | 1 | 6 |
| [c] Response to biotic stimulus | 14 | 2.15% | 2 | 10 |
| [c] Response to external stimulus | 1 | 0.15% | 0 | 1 |
| [c] Response to stress | 8 | 1.23% | 2 | 4 |
| [c] Sensory perception | 3 | 0.46% | 0 | 3 |
| [p] Biological process unknown | 96 | 14.75% | 4 | 68 |

[a]Classification is hierarchial: indented terms are children [c] of parent terms [p] listed above. All functional assignments of 5th-instar *A. glabripennis* ESTs described here are the "inferred from electronic annotation" (IEA) using the top 5 BLASTX hits with an E-value of $\leq$ -10 generated from NCBI's nr database. The definition term associated with each sequence was entered into both FlyBase and AmiGO where it was given a biological process designation according to the Gene Ontology Consortium.
[b]Because a single EST can be associated with several GO terms, the total number of ESTs may be larger than the actual number of ESTs analyzed. However, no single EST was catalogued under the same GO term more than once.


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**Table 4.** Cellular component

| Gene Ontology (GO) Term[a] | Number of ESTs[b] | % of total ESTs represented | Number of contigs | Number of singlets |
|---|---|---|---|---|
| [p] Extracellular matrix | 1 | 0.23% | 0 | 1 |
| [p] Extracellular region | 47 | 10.73% | 5 | 17 |
| [p] Cell | | | | |
| [c] Cell part | | | | |
| [i] Apical part of cell | 1 | 0.23% | 0 | 1 |
| [i] Cell projection | | | | |
| [ii] Flagellum | 2 | 0.46% | 0 | 2 |
| [ii] Neuron projection | 3 | 0.68% | 0 | 3 |
| [i] Cell soma | 1 | 0.23% | 0 | 1 |
| [i] Intracellular | | | | |
| [ii] Intracellular part | | | | |
| [iii] Cytoplasm | 30 | 6.85% | 4 | 22 |
| [iii] Cytoplasmic part | | | | |
| [iv] Cell cortex | 1 | 0.23% | 0 | 1 |
| [iv] Contractile fiber | 3 | 0.68% | 1 | 1 |
| [iv] Cytoplasmic vesicle | 6 | 1.37% | 0 | 6 |
| [iv] Cytosol | | | | |
| [v] Cytosolic part | | | | |
| [vi] Cytosolic large ribosomal subunit (sensu Eukaryota) | 14 | 3.20% | 2 | 9 |
| [vi] Cytosolic small ribosomal subunit (sensu Eukaryota) | 10 | 2.28% | 1 | 8 |
| [v] No further information provided | 10 | 2.28% | 3 | 4 |
| [iv] Eukaryotic 43S preinitiation complex | 2 | 0.46% | 0 | 2 |
| [iv] Fusome | 1 | 0.23% | 0 | 1 |
| [iv] Vacuole | 1 | 0.23% | 0 | 1 |
| [iii] Intracellular organelle | | | | |
| [iv] Intracellular membrane-bound organelle | | | | |
| [iv] Endoplasmic reticulum | 7 | 1.60% | 1 | 5 |
| [iv] Endosome | 4 | 0.91% | 0 | 4 |
| [iv] Golgi apparatus | 2 | 0.46% | 0 | 2 |
| [iv] Mitochondrion | 15 | 3.42% | 2 | 11 |
| [iv] Nucleus | 25 | 5.71% | 3 | 19 |
| [iv] Intracellular non-membrane-bound organelle | | | | |
| [iv] Chromosome | 4 | 0.91% | 0 | 4 |
| [iv] Cytoskeleton | 3 | 0.68% | 0 | 3 |
| [iv] Rhabdomere | 1 | 0.23% | 0 | 1 |
| [iv] Ribosome | 9 | 2.05% | 1 | 7 |
| [iii] Proteasome complex (sensu Eukaryota) | 4 | 0.91% | 0 | 4 |
| [iii] Proton-transporting ATP synthase complex | 2 | 0.46% | 0 | 2 |
| [iii] Respiratory chain complex I (sensu Eukaryota) | 2 | 0.46% | 0 | 2 |
| [iii] Respiratory chain complex III (sensu Eukaryota) | 1 | 0.23% | 0 | 1 |
| [ii] No further information provided | 8 | 1.83% | 1 | 6 |
| [i] Membrane | | | | |


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**Table 4** (cont.)

| Gene Ontology (GO) Term[a] | Number of ESTs[b] | % of total ESTs represented | Number of contigs | Number of singlets |
|---|---|---|---|---|
| [ii] Coated membrane | 1 | 0.23% | 0 | 1 |
| [ii] Membrane part | | | | |
| [iii] Intrinsic to membrane | | | | |
| [iv] Integral to membrane | 12 | 2.74% | 1 | 10 |
| [ii] Organelle membrane | | | | |
| [iii] Mitochodrial membrane | 3 | 0.68% | 1 | 1 |
| [ii] Plasma membrane | 8 | 1.83% | 0 | 8 |
| [ii] No further information provided | 4 | 0.91% | 0 | 4 |
| [p] Envelope | | | | |
| [c] Organelle envelope | | | | |
| [i] Mitochondrial envelope | 3 | 0.68% | 1 | 1 |
| [p] Macromolecular complex | | | | |
| [i] Protein complex | | | | |
| [ii] ATP-binding cassette (ABC) transporter complex | 1 | 0.23% | 0 | 1 |
| [ii] Cyclin-dependent protein kinase holoenzyme complex | 1 | 0.23% | 0 | 1 |
| [ii] Eukaryotic translation elongation factor 1 complex | 4 | 0.91% | 2 | 0 |
| [ii] Exosome (RNase complex) | 1 | 0.23% | 0 | 1 |
| [ii] Ferritin complex | 5 | 1.14% | 1 | 2 |
| [ii] Larval serum protein complex | 24 | 5.48% | 2 | 0 |
| [ii] Oligosaccharyl transferase complex | 2 | 0.46% | 1 | 0 |
| [ii] Protein serine/threonine phosphatase complex | 1 | 0.23% | 0 | 1 |
| [ii] Ubiquitin ligase complex | 1 | 0.23% | 0 | 1 |
| [ii] Unlocalized protein complex | 1 | 0.23% | 0 | 1 |
| [p] Cellular component unknown | 146 | 33.33% | 9 | 108 |

[a]Classification is hierarchial: indented terms are children [c] of parent terms [p] listed above. All functional assignments of 5th-instar *A. glabripennis* ESTs described here are the "inferred from electronic annotation" (IEA) using the top 5 BLASTX hits with an E-value of ≤ -10 generated from NCBI's nr database. The definition term associated with each sequence was entered into both FlyBase and AmiGO where it was given a cellular component designation according to the Gene Ontology Consortium.
[b]Because a single EST can be associated with several GO terms, the total number of ESTs may be larger than the actual number of ESTs analyzed. However, no single EST was catalogued under the same GO term more than once.

WHALB[0262] consisted of a single ORF containing the entire coding domain of a putative protein paralleling *D. melanogaster* ciboulot (cib). Like Dmel\cib, the translated sequence of the *A. glabripennis* coding domain is highly congruent, at least on an amino acid level, to β-thymosins (e.g., *Bombyx mori* thymosin isoform 2, 5.00E-40; accession no. ABF51487). In particular, an actin binding motif found in both β-thymosins and cib was identified as $KLKHTETQEK_{74-83}$ within the WHALB[0262] ORF (Nachmias 1993). However, as observed in Dmel\cib, Agla\cib may possess biochemical properties comparable to profilin rather than thymosin with binding to monomeric actin occurring exclusively at the barbed (or plus) end of the filament and enhanced actin-based motility observed *in vitro* (Loisel et al. 1999). This regulation of actin assembly is thought to be a key factor governing axonal outgrowth during the differentiation events that underlie brain metamorphosis (Boquet et al. 2000).

## Muscle development

WHALB[0331] and WHALB003-14 were catalogued under the transcript class "muscle development". Although annotated based on non-traceable author statements listed in either NCBI's GenBank or FlyBase, these assembled sequences clearly possess sequence similarity to commonly accepted muscle-associated proteins such as muscle protein 20-like protein and muscle LIM protein.

## Cuticle development and puparium formation

Transcripts that potentially code for proteins involved in cuticle biosynthesis were also identified within the *A.*


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**Table 5.** Transcripts putatively associated with A. glabripennis development and metamorphosis

| Assembled Sequence Identifier | BLASTX Match Defintion | Accession No. | E-value |
|---|---|---|---|
| Autophagic cell death | | | |
| WHALB004-85 | PREDICTED: similar to CG9916-PA isoform 1 [*Tribolium castaneum*] | XP_966308 | 1.00E-85 |
| WHALB007-57 | PREDICTED: similar to CG3186-PA, isoform A [*Tribolium castaneum*] | XP_974942 | 1.00E-87 |
| Bristle morphogenesis | | | |
| WHALB002-36 | PREDICTED: similar to CG33553-PE, isoform E [*Tribolium castaneum*] | XP_970939 | E-108 |
| WHALB004-32 | PREDICTED: similar to CG32858-PA, isoform A [*Tribolium castaneum*] | XP_972494 | 4.00E-89 |
| Cuticle development | | | |
| WHALB[0273] | PREDICTED: similar to CG8063-PA [*Tribolium castaneum*] | XP_969206 | E-139 |
| WHALB002-14 | vesicle coat complex COPII GTPase subunit SAR1 [*Aedes aegypti*] | ABF18297 | 7.00E-86 |
| WHALB007-12 | PREDICTED: similar to glucose dehydrogenase [*Tribolium castaneum*] | XP_968177 | 1.00E-65 |
| Imaginal disc morphogenesis | | | |
| WHALB002-13 | PREDICTED: similar to CG6235-PE, isoform E [*Tribolium castaneum*] | XP_970874 | 4.00E-94 |
| WHALB005-39 | PREDICTED: similar to CG2723-PA [*Tribolium castaneum*] | XP_967178 | 4.00E-42 |
| WHALB006-40 | effete CG7425-PA [*Drosophila melanogaster*] | NP_731941 | 5.00E-76 |
| WHALB007-95 | PREDICTED: similar to CG7734-PA, isoform A [*Tribolium castaneum*] | XP_971641 | 4.00E-21 |
| Muscle development | | | |
| WHALB[0331] | muscle protein 20-like protein [*Anoplophora glabripennis*] | AAY68367 | E-101 |
| WHALB003-14 | PREDICTED: similar to CG1019-PA, isoform A isoform 1 [*Tribolium castaneum*] | XP_967871 | 2.00E-69 |
| Nervous system development | | | |
| WHALB[0248][a] | PREDICTED: similar to CG4264-PA, isoform A isoform 1 [*Tribolium castaneum*] | XP_966611 | E-104 |
| WHALB[0262] | PREDICTED: similar to CG4944-PB, isoform B isoform 1 [*Tribolium castaneum*] | XP_968496 | 2.00E-51 |
| WHALB[0269] | PREDICTED: similar to CG4264-PA, isoform A isoform 1 [*Tribolium castaneum*] | XP_966611 | E-145 |
| WHALB001-27 | PREDICTED: similar to CG10652-PA, isoform A [*Tribolium castaneum*] | XP_970626 | 6.00E-59 |
| WHALB001-89 | PREDICTED: similar to CG3359-PB, isoform B [*Tribolium castaneum*] | XP_971500 | 2.00E-17 |
| WHALB002-4 | PREDICTED: similar to CG10339-PA [*Tribolium castaneum*] | XP_968892 | 2.00E-51 |
| WHALB006-83 | shade CG13478-PB, isoform B [*Drosophila melanogaster*] | NP_996074 | 5.00E-22 |
| WHALB007-31 | PREDICTED: similar to CG10339-PA [*Tribolium castaneum*] | XP_968892 | 7.00E-41 |
| Photoreceptor morphogenesis | | | |
| WHALB002-29 | PREDICTED: similar to CG5771-PB, isoform B [*Tribolium castaneum*] | XP_973251 | 4.00E-67 |
| WHALB003-62 | putative 14-3-3 protein [*Maconellicoccus hirsutus*] | ABM55627 | 3.00E-98 |
| WHALB005-67 | PREDICTED: similar to CG10701-PD, isoform D isoform 2 [*Tribolium castaneum*] | XP_976132 | E-109 |
| Pupation | | | |
| WHALB004-95 | PREDICTED: similar to CG8669-PA, isoform A isoform 2 [*Tribolium castaneum*] | XP_975896 | 6.00E-21 |

[a]WHALB[0248] represents a "false contig". Singleton WHALB007-9 was retained under this BLASTX match definition. The accession number remains the same; however, the E-value returned was 2.00E-104 upon removal of the second EST.

*glabripennis* library. For example, WHALB002-14 showed significant sequence similarity to the coat protein complex (COPII) small G protein Sar1. In 2005, Abrams and Andrew found that mutations of this gene in *Drosophila* resulted in a range of cuticle defects including reduced cuticle length and pigmentation as well as changes in ventral dentricle and dorsal hair morphology.

Two ESTs aligned to form WHALB[0273], a contiguous sequence with homology to *D. melanogaster* yellow-f2 [CG8063-PA]. This enzyme plays a major role in melanization reactions that may contribute to sclerotization/tanning of the late stadia or adult insect cuticle (Han et al. 2002). In addition, WHALB004-95 and WHALB007-12 returned matches to cryptocephal (crc) [CG8669-PA, isoform A isoform 2] and glucose dehydrogenase (Gld), respectively. Gene expression and deletion studies have shown that both Gld and crc act either in response to the late larval ecdysteroid pulse or in the regulation of ecdysone biosynthesis/secretion during the onset of pupariation (Andres et al. 1993; Hewes et al. 2000).

## Imaginal disc morphogenesis

WHALB002-13 closely resembled *D. melanogaster* twins (tws) [CG6235-PA, isoform A] with a 90% identity and 97% positives. This gene product was originally


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discovered via a P-element mutation that induced the formation of extra anlagen in the posterior compartment of the wing disc of *Drosophila* (Uemura et al. 1993). This phenomenon of precursor duplication illustrates the importance of phosphorylation and dephosphorlyation events in the regulation of tissue pattern specification not only in relation to imaginal disc morphogenesis, but also in regards to several other crucial developmental processes including maturation of the peripheral nervous system and determination of photoreceptor fate in the compound eye. In 2004, Bajpai et al. further demonstrated that Dmel*tws*$^{j11C8}$, which codes for the B/PR55 regulatory subunit of protein phosphatase 2A (PP2A), functions as a positive regulator of Wg/Wnt signaling. This signal transduction pathway was also linked to singletons WHALB001-37, WHALB004-34, and WHALB00-94, although their role(s) in insect development may involve alternate biological processes such as fatty-acid/retinoid binding and lipid transport.

### Photoreceptor morphogenesis

WHALB002-29 possessed sequence similarity to *D. melanogaster* Rab 11 [GG5771-PB, isoform B], a small GTPase implicated in a variety of trafficking events associated with photoreceptor terminal differentiation including colocalization with rhodopsin at the base of the rhabdomere, formation of multivesicular body (MVB) endosomal compartments, and development of specialized structures within Garland cells (Satoh et al. 2005). Likewise, WHALB005-67 returned a significant BLAST hit to Moesin, an integral component in *Drosophila* photoreceptor morphogenesis. Although the singleton represented only a partial coding domain, query of the translated sequence using RPS-BLST revealed a portion of the N-terminal FERM domain (FERM_C) confirming its placement within the Ezrin-Radixin-Moesin (ERM) family of proteins. While these proteins are broadly associated with actin-based scaffolding, gene disruption studies involving RNAi and loss-of-function mutations in *Drosophila* have suggested that Dmel\Moe, in particular, is essential for proper assembly of the apical membrane skeleton that supports the microvillar array of the rhabdomere (Karagiosis and Ready 2003).

## Conclusions

This study represents the first investigation regarding the transcriptome of *A. glabripennis*. The resultant sequence data has been made available to the public and has been catalogued according to a controlled vocabulary to facilitate use of the dataset in future studies. Further, several transcripts have been identified that are specific to *A. glabripennis* that may be involved in growth and morphogenesis. Collectively, these sequences provide a strong foundation for functional genomics studies that will enable the development of more biorational control measures to combat this invasive pest.

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## Acknowledgments

*A. glabripennis* larvae were kindly provided by J. Tropp (USDA-ARS-BIIR). The authors would also like to thank M. Hentz and J. Mozoruk for critical review of the final manuscript.

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