Utilizing a chromosomal-length genome assembly to annotate the Wnt signaling pathway in the Asian citrus psyllid, *Diaphorina citri*

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ABSTRACT

The Asian citrus psyllid, *Diaphorina citri*, is an insect vector that transmits *Candidatus Liberibacter asiaticus*, the causal agent of the Huanglongbing (HLB), or citrus greening disease. This disease has devastated Florida's citrus industry, and threatens California's industry as well as other citrus producing regions around the world. To find novel solutions to the disease, a better understanding of the vector is needed. The *D. citri* genome has been used to identify and characterize genes involved in Wnt signaling pathways. Wnt signaling is utilized for many important biological processes in metazoans, such as patterning and tissue generation. Curation based on RNA sequencing data and sequence homology confirms 24 Wnt signaling genes within the *D. citri* genome, including homologs for beta-catenin, Frizzled receptors, and seven Wnt-ligands. Through phylogenetic analysis, we classify *D. citri* Wnt ligands as *Wg/Wnt1*, *Wnt5*, *Wnt6*, *Wnt7*, *Wnt10*, *Wnt11*, and *WntA*. The *D. citri* version 3.0 genome with chromosomal length scaffolds reveals a conserved *Wnt1-Wnt6-Wnt10* gene cluster with a gene configuration like that in *Drosophila melanogaster*. These findings provide greater insight into the evolutionary history of *D. citri* and Wnt signaling in this important hemipteran vector. Manual annotation was essential for identifying high quality gene models. These gene models can be used to develop molecular systems, such as CRISPR and RNAi, which target and control psyllid populations to manage the spread of HLB. Manual annotation of Wnt signaling pathways was done as part of a collaborative community annotation project.

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DATA DESCRIPTION

Introduction

Diaphorina citri (NCBI:txid121845) is the insect vector of Huanglongbing (HLB), or citrus greening disease, which has devastated global citrus production [1, 2]. HLB management is heavily based on controlling the spread of *D. citri*. To better understand the insect's

biology, the *D. citri* genome has been manually annotated to curate accurate gene model predictions. Accurate gene models can be used to develop novel insect control systems that utilize molecular therapeutics such as CRISPR (clustered regularly interspaced short palindromic repeats) and RNA interference (RNAi) to control the spread of *D. citri* [3, 4]. These molecular therapeutics would be gene-specific, thus would reduce reliance on broad-spectrum insecticides that have given rise to resistant *D. citri* populations [5–7].

Context

Here, we report *D. citri* genes involved in both canonical and noncanonical Wnt signaling. Wnt signaling is important for many biological processes in metazoans, such as patterning, cell polarity, tissue generation, and stem cell maintenance [8–10]. In the model insects *Drosophila melanogaster* and *Tribolium castaneum*, knockout and knockdown of Wnt ligands and other Wnt signaling components have detrimental effects on embryo development and adult homeostasis [11–16]. Wnt signaling components could therefore be effective knockout targets to limit the spread of *D. citri*, thus reducing HLB incidence. We curated a comprehensive repertoire of Wnt signaling genes in *D. citri*. Twenty-four gene models corresponding to canonical and noncanonical Wnt signaling genes have been annotated, including seven Wnt ligands, three *frizzled* homologs, *arrow*, *armadillo/beta-catenin*, and receptor tyrosine kinases *ROR* and *doughnut*. We were unable to find *Wnt8/D*, *Wnt9*, and *Wnt16* as well as *Wnt2-4*, which have been lost in insects. The mechanisms of Wnt signaling appear to be mostly conserved and comparable to those found in *D. melanogaster* (Table 1). A model for canonical Wnt signaling in *D. citri* based on curated genes is shown (Figure 1). This is an important first step towards understanding critical biological processes that might be targeted to control the spread of *D. citri*, and may provide broader insights into the mechanisms of Wnt signaling in this important hemipteran vector.

METHODS

We used the psyllid genome curation workflow used for community annotation (Figure 2) [17].

To summarize, orthologous protein sequences for Wnt pathway genes were collected from the National Center for Biotechnology Information (NCBI) (RRID:SCR_006472) protein database [18] and used to BLAST (RRID:SCR_004870) search the *D. citri* MCOT transcriptome database [19]. The MCOT transcriptome is a transcriptome assembly utilizing Maker (RRID:SCR_005309), Cufflinks (RRID:SCR_014597), Oases (RRID:SCR_011896), and Trinity (RRID:SCR_013048) pipelines to provide a comprehensive set of predicted gene models. High-scoring MCOT models (accessions available in Table 2) were then searched on the NCBI protein database using NCBI BLAST to confirm the viability of the predicted MCOT models. The high-scoring MCOT models that had promising NCBI search results were used to search the *D. citri* genome. Genome regions containing computationally predicted gene models with high sequence identity to the query sequence from the MCOT transcriptome were investigated within JBrowse (RRID:SCR_001004). Gene models were modified using the Apollo (RRID:SCR_001936) gene annotation platform, based on mapped DNA-Seq, RNA-Seq, Iso-Seq, orthologous proteins, and other lines of evidence to edit and confirm manual annotations and gene structure. The gene models were analyzed with NCBI BLAST to assess

Figure 1. Theoretical model of canonical Wnt signaling cascade in *D. citri* based on curated genes. (1) Wnt is secreted. (2) Wnt concentration gradient forms. (3) Wnt binds to Frizzled and releases Armadillo. (4) Armadillo migrates into the nucleus, associates with transcription factor Pangolin, and regulates gene expression. (5) Armadillo is degraded in the absence of Wnt.

Table 1. Summary of gene copy numbers in various model insect species, including *Diaphorina citri*. Wnt pathway ortholog numbers in five different insect species. *Drosophila melanogaster*, *Apis mellifera*, *Tribolium castaneum*, and *Acyrtosiphon pisum* copy numbers were determined using Flybase, OrthoDB, NCBI Genbank, Uniprot, and several other publications [15, 20–22]. *Diaphorina citri* numbers represent the number of manually annotated genes in the *D. citri* v3.0 genome.

Gene	<i>Drosophila melanogaster</i>	<i>Apis mellifera</i>	<i>Tribolium castaneum</i>	<i>Acyrtosiphon pisum</i>	<i>Diaphorina citri</i> v3
<i>Wnt1</i>	1	1	1	1	1
<i>Wnt5</i>	1	1	1	1	1
<i>Wnt6</i>	1	1	1	0	1
<i>Wnt7</i>	1	1	1	1	1
<i>Wnt8/D</i>	1	0	1	0	0
<i>Wnt9</i>	1	0	1	0	0
<i>Wnt10</i>	1	1	1	0	1
<i>Wnt11</i>	0	1	1	1	1
<i>Wnt16</i>	0	0	0	1	0
<i>WntA</i>	0	1	1	1	1
<i>Pangolin</i>	1	1	1	1	1
<i>Armadillo</i>	1	1	2	2	1
<i>Wntless</i>	1	1	1	1	1
<i>Porcupine</i>	1	1	1	1	1
<i>Derailed</i>	2	1	0	1	1
<i>Doughnut</i>	1	1	1	1	1
<i>Arrow</i>	1	1	1	1	1
<i>Frizzled</i>	4	2	3	2	3
<i>ROR</i>	2	2	3	2	2
<i>Dishevelled</i>	1	1	1	1	1
<i>Shaggy</i>	1	1	1	2	1
<i>Axin</i>	1	1	1	1	1
<i>ck1-gamma</i>	1	1	1	1	1
<i>Apc</i>	2	1	1	1	1

their completeness. MUSCLE (RRID:SCR_011812) multiple sequence alignments of the *D. citri* gene model sequences and orthologous sequences were created through MEGA7

Figure 2. Protocol for psyllid genome curation [17]. <https://www.protocols.io/widgets/doi?uri=dx.doi.org/10.17504/protocols.io.bniimcce>

(RRID:SCR_000667) [23]. Neighbor-joining trees were constructed using MEGA7 with p-distance for determining branch length and 1000 bootstrapping replications to measure the precision of branch placement. In special cases, phylogenetic analysis in conjunction with NCBI BLAST scores was used to properly name and characterize the manually annotated gene models.

RNA-seq data from whole body adults and nymphs raised on *C. medica* and *C. sinensis* are available from NCBI BioProject PRJNA609978. We used proteins from *Drosophila melanogaster* (Dm) [24], *Tribolium castaneum* [25], *Bombyx mori* [26], *Apis mellifera* [27], *Nasonia vitripennis* [28], *Acyrtosiphon pisum* [29], *Nilaparvata lugens* [30, 31], *Sipha flava* [32], *Halyomorpha halys* [33], *Cimex lectularius* [34], *Aedes aegypti* [35], *Anopheles gambiae* [36], *Branchiostoma floridae* [37], *Penaeus vannamei* [38], *Folsomia candida* [39], *Spodoptera litura* [40], *Homo sapiens* [32] and *Oncopeltus fasciatus* [34, 41].

Data validation and quality control

The loss of Wnt ligand genes is more common in insects than in other metazoans [20], which leads to a highly variable array of *Wnt* genes and Wnt signaling components from species to species [15, 21, 22, 42]. We performed a phylogenetic analysis to characterize the *D. citri* Wnt repertoire (Figure 3). The ortholog sequences used for this analysis were collected from the NCBI protein database [18]; see the 'Availability of Supporting Data and Materials' section for accession numbers. Seven *D. citri* Wnts were identified and classified as *Wnt1* (also known as *wingless*), *Wnt5*, *Wnt6*, *Wnt7*, *Wnt10*, *Wnt11*, and *WntA* (Figures 3 and 4). In comparison, seven *Wnt* genes have been identified in *D. melanogaster*, nine in *T. castaneum*, and six in *Acyrtosiphon pisum* [22, 42]. The collection of *Wnt* genes found in *D. citri* is like that found in other insects, and no *Wnt* subfamilies have been identified as being unique to *D. citri*. Contrary to previous reports [43], *D. citri* does appear to possess a *Wnt6* gene.

Wnt1, *Wnt6*, and *Wnt10* typically occur close together in a highly conserved gene cluster [44, 45]. The chromosomal length genome assembly in v3.0 suggests that this cluster is also conserved in *D. citri*, located at a position between 26.4 Mb (megabases) and 26.6 Mb on scaffold 4 (i.e. chromosome 4) [46]. In comparison, the *Wnt1-6-10* cluster is located at a position between 7.30 Mb and 7.38 Mb on chromosome 2L of *D. melanogaster*, and between 5.50 Mb and 5.53 Mb on linkage group 5 in *T. castaneum*. The only gene from this cluster

Figure 3. Neighbor-joining tree of Wnt protein sequences. Phylogenetic analysis was performed to categorize the seven *D. citri* Wnt genes (signified by dots). Wnt families are distinguished by clades and are color coded. Bootstrap values are based on 1000 replicates and values under 25 are removed. Ortholog sequences were collected from NCBI protein database [18]. Analysis was performed using MEGA7 [23].

Figure 4. Wnt genes in six insects. A colored box indicates the presence of a Wnt subfamily (1–11, 16, and A) in that insect, while a white box indicates the loss of a subfamily. For example, all six species have Wnt1 and Wnt5, none have Wnt2–4, and only *A. pisum* has Wnt16. Homologs of Wnt8 in *T. castaneum* and *D. melanogaster* are also referred to as WntD.

present in *A. pisum* is Wnt1, which is located on the X chromosome. The close phylogenetic relationship of Wnt1, Wnt6, and Wnt10 in *D. citri* (Figure 3) supports the hypothesis that this cluster is the result of an ancient duplication event, one that may predate the

Figure 5. *Wnt1-6-10* cluster comparison. Organization of the *Wnt1-6-10* cluster in *D. citri* is similar to that of *D. melanogaster* and differs from what may be a basal arthropod gene arrangement seen in *A. gambiae*, *T. castaneum*, *A. mellifera*, and *D. pulex*. Gene lengths are not to scale.

divergence of cnidarians and bilaterians [45]. The orientation of these clustered *D. citri* *Wnt* genes is like that found in *D. melanogaster* and differs from what may be a basal arthropodal organization of *Wnts* found in species of Coleoptera, Hymenoptera, and Cladocera (Figure 5). *Wnt9* is also associated with this gene cluster when present in the genome. However, as with *A. pisum*, *Wnt9* was not found in the *D. citri* genome and appears to have been lost during evolution. A second *Wnt* cluster, *Wnt5* and *Wnt7*, is also common among non-insect metazoans. This cluster is not seen in *D. citri*; however, *D. citri* *Wnt5* and *7* are located relatively close to one another (within 220 Kb [kilobase pairs]) on scaffold 13 (i.e. chromosome 13).

The mechanisms that act to conserve these *Wnt* gene clusters are not well understood. In the basal metazoan *Nematostella vectensis*, clustered *Wnt* genes do not exhibit similar expression patterns or *Hox*-like collinearity [44], and may not share regulatory elements. Whole body transcript expression data from egg, nymph, and adult stages [47] obtained from the Citrus Greening Expression Network (CGEN) [48] shows varying levels of expression among the clustered genes in different life stages of *D. citri* (Figure 6). However, it appears that *Wnt1* and *10* are similarly upregulated during embryonic psyllid development and downregulated during the adult stage. Similar transcript levels of *Wnt1* and *6* are seen in the nymphal stage. This suggests there may be shared regulation dependent upon life stage. Furthermore, ordering within the clusters is subject to rearrangement (Figure 5) [42, 44]. This may indicate that gene directionality is not a factor in conserving this cluster. Our annotation findings support the hypothesis that the *Wnt1-6-10* cluster is being preserved through either natural selection or an unknown mechanism. A better understanding of the regulatory hierarchy controlling *Wnt* expression might shed light on the significance of *Wnt* gene associations in the genome. Future characterization of the coding and noncoding regions surrounding these *D. citri* *Wnts* (e.g., tandem repeat analysis) could also provide more insight into the mechanisms causing *Wnt* duplication events.

The organization of the genomic reference sequence into chromosomal length scaffolds was essential for revealing *D. citri* gene clustering. Because of their shorter scaffold lengths, previous genome assemblies were often unsupportive in confirming the proximity of genes.

Figure 6. Transcript levels of clustered *Wnt* transcripts during different *D. citri* life stages. Whole body transcript expression data from egg, nymph, and adult stages [47] were collected from CGEN [48]. The psyllids were raised on *Citrus macrophylla* and were not infected with *Candidatus Liberibacter asiaticus*. Expression values shown in transcripts per million (TPM).

Genome v2.0 assembly errors had likely misrepresented the location of *Wnt10*, making it appear to be separated from *Wnt1* and *Wnt6*. A complete *Wnt1-6-10* cluster was found in the improved chromosome length assembly v3.0. Thus, the quality of the reference genome should be considered when performing phylogenetic studies.

Orthologs for *Wnt2*, *Wnt3*, *Wnt4*, *Wnt8/D*, *Wnt9*, and *Wnt16* were not located in the *D. citri* genome. The close identity of certain *Wnt* subfamilies makes it difficult to distinguish between them; however, the loss of *Wnt2–4* is expected because they are absent in all insects [20]. *Apis mellifera* and the hemipteran *A. pisum* have been reported to lack *Wnt8/D*. Perhaps this *Wnt* subfamily has been lost in the divergence from other insect groups [22]. Additionally, *Wnt16* was not found in *D. citri* v3.0. This finding contrasts with the gene predictions of other hemipteran genomes available at NCBI, namely *A. pisum*, *Sipha flava*, and *Nilaparvata lugens* (Figure 3). Based on whole body RNA expression data collected from CGEN, *Wnt6* has the highest average transcript levels of all the *Wnt* genes in both nymph and adult psyllids (Figure 7). The relatively high number of transcripts suggests that *Wnt6* is important during both metamorphosis and adult stage homeostasis, and may be a good knockout target for molecular therapeutics. Transcript expression of *Wnt6* in adults is mainly concentrated in the legs and thorax, averaging 102 transcripts per million (TPM) and 272 TPM, respectively. This is considerably higher than all other *Wnt* genes in these tissues, which only average between 0.26 and 3.00 TPM. It is unclear if other *Wnts* can be upregulated to compensate for the loss of *Wnt6*. Perhaps targeting multiple *Wnt* genes, or the mechanisms by which Wnt is secreted (i.e. Porcupine and Wntless), would be more disruptive to *D. citri* physiology.

Several receptors and co-receptors associated with canonical and noncanonical signaling have been identified (Table 2). Three paralogs for the Wnt receptor encoding *frizzled* have

Figure 7. Transcript levels of *D. citri* Wnt repertoire in both nymph and adult psyllids from whole body RNA extractions. Green bars indicate the average transcript levels for Wnt in nymph samples [47], and gray bars represent the average transcript levels for Wnt in adult samples. Averages are based on six nymph samples and six adult samples. Expression levels shown in transcripts per million (TPM). Standard deviation of samples is shown by error bars. RNA-seq data was collected from CGEN [48].

been found in *D. citri*. We classified and numerically designated *D. citri*'s three *frizzled* genes based on how their encoded protein sequences form clades with *D. melanogaster* orthologs (Figure 8). Our analysis showed that *D. citri*, and other hemipterans such as *Halymorpha halys* and *N. lugens*, possess a Frizzled protein like that of *D. melanogaster*'s Frizzled 3. Some hemipteran Frizzled orthologs form a distinct clade separate from the Dipteran sequences (Figure 8). The hemipteran clade suggests that these genes might belong to a different subfamily of Frizzled, maybe one specific to Hemiptera. However, this ortholog has not been reported in the *A. pisum* genome [22].

Orthologs for both *ROR1* and *ROR2* have been identified. Interestingly, *ROR1* has two isoforms, the first of which contains an immunoglobulin (IG) domain that is lacking from isoform 2 (Figure 9). *ROR1* isoform 2 (Dcitr05g14430.1.2) appears to average higher transcript levels in *D. citri* egg, nymph, and adult tissues than *ROR1* isoform 1 (Dcitr05g14430.1.1) based on CGEN data (Figure 10). Many transcripts for isoform 2 were detected in the psyllid egg (Figure 10). This suggests that expression of isoform 2 may be important in the early developmental stages of *D. citri*.

Conclusion

Controlling the spread of *D. citri* is an important strategy for reducing the spread of HLB. With this study, we hope to provide a greater insight into *D. citri* biology, as well as accurate gene models that can be used in future research and applications. We have curated a comprehensive repertoire of Wnt signaling genes in *D. citri*. In total, 24 gene models

Table 2. Evidence supporting gene annotation. Manually annotated Wnt pathway genes in *Diaphorina citri*. There are 24 gene models in total. Each gene model has been assigned an identifier, and the evidence used to validate or modify the structure of the gene model has been listed. MCOT transcriptome identifiers that best support the manual annotation are also listed. The table is marked with an 'X' when supporting evidence of *de novo* transcriptome, Iso-Seq, RNA-Seq and ortholog support is present. MCOT: comprehensive transcriptome based on genome MAKER, Cufflinks, Oases, and Trinity transcript predictions; MAKER: gene predictions; *De novo* transcriptome: an independent transcriptome using Iso-Seq long-reads and RNA-Seq data; Iso-Seq transcripts: full-length transcripts generated with Pacific Biosciences technology; RNA-Seq: reads mapped to genome are also used as supporting evidence for splice junctions; Ortholog evidence: proteins from related hemipteran species and *Drosophila melanogaster*.

Gene	OGS Identifier	MCOT	<i>de novo</i> transcriptome	Iso-Seq	RNA-Seq	Ortholog
<i>Wnt1</i>	Dcitr04g11660.1.1	MCOT05703.0.CO	X		X	X
<i>Wnt5</i>	Dcitr13g03650.1.1	MCOT16538.0.CO	X		X	
<i>Wnt6</i>	Dcitr04g11650.1.1	MCOT21516.1.CO	X	X	X	
<i>Wnt7</i> [†]	Dcitr13g03730.1.1	MCOT12704.0.CO	X		X	X
<i>Wnt10</i>	Dcitr04g11640.1.1	MCOT09136.0.MO	X		X	X
<i>Wnt11</i>	Dcitr09g05250.1.1	MCOT15024.0.CT	X		X	
<i>WntA</i>	Dcitr13g02920.1.1	MCOT02236.1.CT	X		X	X
<i>Pangolin</i> [†]	Dcitr06g15680.1.1	MCOT15454.2.CC	X		X	
<i>Armadillo</i>	Dcitr10g09220.1.1	MCOT18153.0.CT		X	X	X
<i>Wntless</i>	Dcitr01g07340.1.1	MCOT02320.0.CC	X	X	X	X
<i>Porcupine</i>	Dcitr13g04750.1.1	MCOT19771.0.CO	X	X	X	
<i>Derailed</i>	Dcitr01g12220.1.1	MCOT04433.1.CO	X	X	X	
<i>Doughnut</i>	Dcitr01g07650.1.1	MCOT18207.0.CT	X	X	X	X
<i>Arrow</i>	Dcitr11g02670.1.1	MCOT01906.1.CO	X	X	X	X
<i>Frizzled</i>	Dcitr04g04630.1.1	MCOT11925.0.MO	X		X	
<i>Frizzled 2</i>	Dcitr10g03570.1.1	MCOT07682.0.MO	X	X	X	
<i>Frizzled 3</i>	Dcitr01g12100.1.1	MCOT03353.0.CC	X	X		
<i>ROR1</i>	Dcitr05g14430.1.1	MCOT18375.0.CT	X	X	X	X
	Dcitr05g14430.1.2	MCOT01992.1.CT				
<i>ROR2</i>	Dcitr08g10450.1.1	MCOT22482.0.CC	X	X	X	X
<i>Dishevelled</i>	Dcitr01g03830.1.1	MCOT11762.0.MO	X		X	X
<i>Shaggy</i>	Dcitr03g15060.1.1	MCOT06728.0.CT	X	X	X	X
<i>Axin</i>	Dcitr07g09620.1.1	MCOT05716.1.CT	X		X	
<i>ck1-gamma</i>	Dcitr11g04200.1.1	MCOT05782.2.CO	X	X	X	X
<i>Apc-like</i>	Dcitr07g12790.1.1	MCOT14853.2.CO	X		X	

[†]Gene is manually annotated as a partial model in Genome v3.0. A complete representation of the gene and protein sequence can be determined with MCOT transcriptome data.

corresponding to canonical and noncanonical Wnt signaling have been annotated. The mechanisms of Wnt signaling appear to be mostly conserved and comparable to those found in *D. melanogaster* and other insects. These findings provide a greater insight into the evolutionary history of *D. citri* and Wnt signaling in this important hemipteran vector. Manual annotation and an improved genome assembly with chromosomal length scaffold were essential for identifying high quality gene models.

REUSE POTENTIAL

The manually curated genes will be included in the Citrus Greening Expression Network (CGEN) [48] as a part of the Official Gene Set version 3. This visualization tool is useful for understanding psyllid biology and comparative analysis because it contains public transcriptomics data for *Diaphorina citri* from various tissues, life stages, CLas infection levels and citrus hosts. Future work could utilize these gene models in developing CRISPR and RNAi systems that target and disrupt critical biological processes in *D. citri*, thus controlling the spread of HLB. This work was done as part of a collaborative community annotation project [49].

Figure 8. Neighbor-joining tree of insect Frizzled protein sequences. Proteins grouped in the Frizzled 1 subfamily are highlighted in green, Frizzled 2 in orange, Frizzled 3 in blue, and Frizzled 4 in magenta. Circles indicate the *D. citri* sequences. Ortholog sequences were collected from the NCBI protein database [18]. Some NCBI sequences (such as XP_006568530.1, XP_008188372.2, and XP_022194032.1) may have numeric labels derived from computational predictions that do not reflect sequence or functional similarity. Analysis was performed using MEGA7 [23].

Figure 9. Domain comparison of *ROR1* isoforms. The immunoglobulin domain (IG_like) is present in isoform 1. Other shared domains include a cysteine-rich frizzled domain (CRD_FZ), a Kringle domain (KR), and a protein kinase catalytic domain (PKc_like). Domains were calculated and visualized using the NCBI Conserved Domain Architecture Retrieval Tool (CDART).

DATA AVAILABILITY

Our annotation and gene curation workflow is described by Shippy *et al.* [17]. The *Diaphorina citri* genome assembly, official gene sets, and transcriptome data are accessible on the Citrus Greening website [50]. All accessions for genes used for phylogenetic analysis are provided within this report (Tables 2, 3, Figure 8). We have included the Newick and Multiple Sequence Alignment files used to construct the Wnt neighbor-joining phylogenetic tree and other data is available in the *GigaScience* GigaDB repository [51].

Figure 10. Expression of *ROR1* isoforms in egg, nymph and adult *D. citri*. Pink bars indicate the average transcript levels for isoform 1 (Dcitr05g14430.1.1), and orange bars indicate the average transcript levels for isoform 2 (Dcitr05g14430.1.2). Note: only one egg sample was used for comparison. Egg transcripts from the whole egg (one sample), nymph transcripts from the whole body (six samples), and adult transcripts from the whole body, abdomen, and thorax (14 samples) are shown. Expression values shown in transcripts per million (TPM). Data labels note the average TPM. Standard deviation of samples, when available, is shown by error bars. RNA-seq data was collected from CGEN [48].

EDITOR'S NOTE

This article is one of a series of Data Releases crediting the outputs of a student-focused and community-driven manual annotation project, curating gene models and – if required – correcting assembly anomalies, for the *Diaphorina citri* genome project [46].

DECLARATIONS

LIST OF ABBREVIATIONS

CGEN: Citrus Greening Expression Network; HLB: Huanglongbing; MCOT: Maker, Cufflinks, Oases, and Trinity; NCBI: National Center for Biotechnology Information

ETHICAL APPROVAL

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

Table 3. Accessions for Wnt phylogenetic tree.

NCBI accession	Species	NCBI protein name	Referred to in Figure 3 as
XP_002609873.1	<i>Branchiostoma floridae</i>	Hypothetical protein BRAFLDRAFT_60204	WntA
XP_024085687.1	<i>Cimex lectularius</i>	Wnt-8b-like	Wnt8
XP_014257242.2	<i>Cimex lectularius</i>	Wnt-7b isoform X1	Wnt7
NP_476972.2	<i>Drosophila melanogaster</i>	Wnt oncogene analog 4 isoform A	Wnt9
NP_476924.1	<i>Drosophila melanogaster</i>	Wnt oncogene analog 5 isoform A	Wnt5
NP_476810.1	<i>Drosophila melanogaster</i>	Wnt oncogene analog 2 isoform A	Wnt7
NP_609109.3	<i>Drosophila melanogaster</i>	Wnt oncogene analog 10	Wnt10
NP_609108.3	<i>Drosophila melanogaster</i>	Wnt oncogene analog 6 isoform B	Wnt6
NP_523502.1	<i>Drosophila melanogaster</i>	Wingless	Wnt1
NP_650272.1	<i>Drosophila melanogaster</i>	wnt inhibitor of dorsal	Wnt8/D
ALO81632.1	<i>Penaeus vannamei</i>	Wnt-16	Wnt16
OXA45577.1	<i>Folsomia candida</i>	Wnt-16	Wnt16
XP_025422997.1	<i>Sipha flava</i>	Wnt-16-like	Wnt16
XP_022821085.1	<i>Spodoptera litura</i>	Wnt-4-like	Wnt9
XP_015835609.1	<i>Tribolium castaneum</i>	Wnt-4	Wnt9
XP_008196351.1	<i>Tribolium castaneum</i>	Wnt-7b isoform X1	Wnt7
XP_008195370.1	<i>Tribolium castaneum</i>	Wnt-1	WntA
XP_015835988.1	<i>Tribolium castaneum</i>	Wnt-11b-1 isoform X1	Wnt11
XP_008193179.1	<i>Tribolium castaneum</i>	Wnt-10a isoform X1	Wnt10
NP_001164137.1	<i>Tribolium castaneum</i>	Wnt6 protein precursor	Wnt6
NP_001107822.1	<i>Tribolium castaneum</i>	wingless precursor	Wnt1
XP_974684.1	<i>Tribolium castaneum</i>	Wnt-5b	Wnt5
XP_971439.1	<i>Tribolium castaneum</i>	Wnt-8a isoform X1	Wnt8
XP_021702998.1	<i>Aedes aegypti</i>	Wnt-4	WntA
XP_557821.3	<i>Anopheles gambiae</i>	AGAP008678-PA	WntA
XP_006561993.1	<i>Apis mellifera</i>	Wnt-5b isoform X1	Wnt5
XP_006557287.1	<i>Apis mellifera</i>	Wnt-7b isoform X1	Wnt7
XP_006567803.2	<i>Apis mellifera</i>	Wnt-11b	Wnt11
XP_016771882.1	<i>Apis mellifera</i>	Wnt-6 isoform X1	Wnt6
XP_026300091.1	<i>Apis mellifera</i>	Wnt-1	Wnt1
XP_396944.4	<i>Apis mellifera</i>	Wnt-10b	Wnt10
XP_001949667.2	<i>Acyrtosiphon pisum</i>	Wnt-5b	Wnt5
XP_016664156.1	<i>Acyrtosiphon pisum</i>	Wnt-16	Wnt16
XP_001948541.2	<i>Acyrtosiphon pisum</i>	Wnt-2	Wnt7
XP_001947400.1	<i>Acyrtosiphon pisum</i>	Wnt-1	WntA
XP_001944637.3	<i>Acyrtosiphon pisum</i>	Wnt-11b-like isoform X1	Wnt11
XP_001945295.1	<i>Acyrtosiphon pisum</i>	Wnt-1	Wnt1
XP_022184533.1	<i>Nilaparyata lugens</i>	Wnt-16-like	Wnt16
XP_022188550.1	<i>Nilaparyata lugens</i>	Wnt-7b	Wnt7
BAB62039.1	<i>Homo sapiens</i>	WNT5B	Wnt5B
NP_003382.1	<i>Homo sapiens</i>	Wnt-2 precursor	Wnt2
NP_057171.2	<i>Homo sapiens</i>	Wnt-16 isoform 2	Wnt16
NP_004616.2	<i>Homo sapiens</i>	Wnt-7a precursor	Wnt7a
NP_478679.1	<i>Homo sapiens</i>	Wnt-7b precursor	Wnt7b
NP_004617.2	<i>Homo sapiens</i>	Wnt-11 precursor	Wnt11
NP_003386.1	<i>Homo sapiens</i>	Wnt-9a precursor	Wnt9a
NP_003387.1	<i>Homo sapiens</i>	Wnt-9b isoform 1 precursor	Wnt9b
NP_110388.2	<i>Homo sapiens</i>	Wnt-4 precursor	Wnt4
NP_079492.2	<i>Homo sapiens</i>	Wnt-10a precursor	Wnt10a
NP_003385.2	<i>Homo sapiens</i>	Wnt-10b precursor	Wnt10b
NP_006513.1	<i>Homo sapiens</i>	Wnt-6 precursor	Wnt6
NP_005421.1	<i>Homo sapiens</i>	proto-oncogene Wnt-1 precursor	Wnt1
NP_001287867.1	<i>Homo sapiens</i>	Wnt-8a isoform 1 precursor	Wnt8

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

WBH, SJB, TD and LAM conceptualized the study; TD, SS, TDS and SJB supervised the study; SJB, TD, SS, and LAM contributed to project administration; CV, MR, and RN conducted investigation; PH, MF-G, and SS contributed to software development; SS, TDS, PH, and MF-G developed methodology; SJB, TD, WBH, and LAM acquired funding; CV prepared and wrote the original draft; TD, SS, TDS, and SJB reviewed and edited the draft.

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