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Original Article

Genome-wide Identification of the *Chitinase* and *Nonenzymatic Chitinase-Like* (*Idgf*) Genes in *Drosophila suzukii* (Diptera: Drosophilidae)

Tuğberk Tunçyürek [1], Uğur Sari [2], Hanife Yandayan Genç [3] ✉

[1]School of Graduate Studies, Department of Agricultural Biotechnology, Çanakkale Onsekiz Mart University, Çanakkale, TÜRKİYE
[2,3] Department of Agricultural Biotechnology, Faculty of Agriculture, Çanakkale Onsekiz Mart University, Çanakkale, TÜRKİYE

[1]https://orcid.org/0009-0002-4478-5834, [2]https://orcid.org/0000-0001-7564-997X, [3]https://orcid.org/0000-0001-8180-4307

✉: hgenc@comu.edu.tr

ABSTRACT

Drosophila suzukii (Spotted-winged *drosophila*) is an invasive fruit pest in Türkiye. *Chitinases* (*Cht*) are glycoside hydrolase 18 family enzymes that degrade chitin, while *imaginal disc growth factors* (*Idgf*) are non-enzymatic chitinase-like proteins vital for insect development. However, *chitinase* and *chitinase-like* genes have not yet been identified in the *D. suzukii* genome, and limited information is available about them. In this study, a comprehensive genome-wide analysis of the *Cht* and *Idgf* genes in *D. suzukii* was performed using a bioinformatics approach. Fifteen genes (11 *Cht* and 4 *Idgf*) were identified in the *D. suzukii* genome, and their sequences, domain structures, and phylogenetic relationships were characterized. Gene structure analysis revealed considerable diversity in exon-intron organization and gene lengths. Fifteen conserved protein motifs were analyzed, and motifs 1, 6, and 8 were detected in all members and others unique to subsets of genes, highlighting both shared and specialized functional regions. Cis-regulatory elements analysis uncovered 543 cis-regulatory elements (including core promoters, stress-responsive elements, enhancers/silencers, and insulators), suggesting complex regulation of these genes. A protein–protein interaction network analysis grouped the Cht/Idgf proteins into three clusters, with DsCht2, DsCht5-2, and DsCht7 emerging as central nodes in chitin metabolism pathways. 3D structural models were predicted for all proteins, and it was determined that the model confidence scores for the proteins varied. In addition, it was confirmed that the catalytic domain features were conserved for these proteins. Finally, RNA-Seq expression profiles showed tissue-specific expression patterns, notably high expression of *DsIdgf4* in antennae and *DsCht7* in multiple tissues, implying roles in sensory function and chitin metabolism. These findings provide the first comprehensive overview of the chitinase and chitinase-like gene family in *D. suzukii*, laying a foundation for further functional characterization studies and potential pest management strategies targeting chitin metabolic pathways.

Key words: *Drosophila suzukii*, Chitinases, Imaginal Disc Growth Factors, Genome Wide Analysis, RNA-Seq Tissue-Specific Expression

Drosophila suzukii 'de (Diptera: Drosophilidae) *Kitinaz* ve *Nonenzimatik Kitinaz Benzeri* (*Idgf*) Genlerinin Genom Çapında Tanımlanması

ÖZ

Drosophila suzukii (Benekli kanatlı *Drosophila*), Türkiye'de istilacı bir meyve zararlısıdır. Kitinazlar (*Cht*), kitini parçalayan glikozid hidrolaz 18 ailesi enzimleridir, hayali disk büyüme faktörleri (*Idgf*), böceklerin gelişimi için hayati öneme sahip enzimatik olmayan kitinaz benzeri proteinlerdir. Ancak, *D. suzukii* genomunda kitinaz ve kitinaz benzeri genler henüz tanımlanmamıştır ve bunlar hakkında sınırlı bilgi mevcuttur. Bu çalışmada, biyoinformatik bir yaklaşım kullanılarak *D. suzukii*'deki *Cht* ve *Idgf* genlerinin kapsamlı bir genom çapında analizi gerçekleştirilmiştir. *D. suzukii* genomunda 15 gen (11 *Cht* ve 4 *Idgf*) tanımlanmış ve bunların dizileri, domain yapıları ve filogenetik ilişkileri karakterize edilmiştir. Gen yapısı analizi, ekson-intron organizasyonunda ve gen uzunluklarında önemli çeşitlilik olduğunu ortaya koymuştur. On beş korunmuş protein motifi analiz edildi ve motif 1, 6 ve 8 tüm üyelerde, diğerleri ise gen alt kümelerinde benzersiz olarak tespit edildi, bu da hem ortak hem de

özel fonksiyonel bölgeleri ortaya çıkardı. Cis-düzenleyici elemanlar analizi, 543 cis-düzenleyici eleman (çekirdek promotörler, strese duyarlı elemanlar, güçlendiriciler/susturucular ve yalıtkanlar dahil) ortaya çıkardı, bu da bu genlerin karmaşık bir şekilde düzenlendiğini göstermiştir. Bir protein-protein etkileşim ağı analizi, Cht/Idgf proteinlerini üç küme halinde gruplandırdı ve DsCht2, DsCht5-2 ve DsCht7, kitin metabolizma yolaklarında merkezi düğümler olarak ortaya çıktı. Tüm proteinler için 3D yapısal modeller tahmin edilmiş ve proteinlere ait model güvenilirlik puanlarının değişiklik gösterdiği saptanmıştır. Bununla beraber bu proteinler için katalitik alan özelliklerinin korunduğu doğrulanmıştır. Son olarak, RNA-Seq ekspresyon profilleri doku spesifik ekspresyon paternleri gösterdi. Özellikle *DsIdgf4*'ün antenlerde ve *DsCht7*'nin çoklu dokularda yüksek ekspresyonu, duyusal fonksiyon ve kitin metabolizmasında rol oynadığını düşündürmektedir. Bu bulgular, *D. suzukii*'deki *kitinaz* ve *kitinaz benzeri* gen ailesinin ilk kapsamlı genel görünümünü sunarak kitin metabolik yolaklarını hedef alan daha ileri fonksiyonel karakterizasyon çalışmaları ve potansiyel zararlı yönetimi stratejileri için bir temel oluşturmaktadır.

Anahtar kelimeler: *Drosophila suzukii*, Kitinazlar, Imaginal Disk Büyüme Faktörleri, Genom Çapında Analiz, RNA-Seq Doku Spesifik Ekspresyon

1. INTRODUCTION

Chitin, a β-1,4-linked linear homopolymer of N-acetylglucosamine, is one of the most abundant glycosaminoglycans in nature. This is due to the fact that it plays a critical structural role in many organisms including arthropods, fungi, and various microorganisms (Cohen, 2010; Merzendorfer, 2006; Rudall and Kenchington, 1973). In insects, chitin is an essential component of vital structures such as exoskeletons, trachea and the peritrophic matrix (PM), as well as salivary glands, eggshells, and muscle attachment points (Kramer and Koga, 1986; Kramer et al., 1988; Merzendorfer and Zimoch, 2003a, Preprint; Vincent and Wegst, 2004). The presence of this tough yet non-rigid polysaccharide enables insects to protect themselves against environmental damage while maintaining their internal physiological balance. However, chitin is still somewhat inflexible, requiring its regular breakdown and reorganization during processes such as molting and metamorphosis, which are essential for insect growth and development (Nakabachi et al., 2010). Chitin degradation in insects is carried out by a complex network of enzymes, primarily chitinases (Chts) and their associated chitinase-like proteins, the imaginal disc growth factors (Idgfs) (Merzendorfer and Zimoch, 2003, Rabadiya and Behr, 2024). Chitinases belong to glycoside hydrolase family 18 (GH18) and function as endo-β-N-acetylglucosaminases by hydrolyzing the β-1,4 glycoside bonds in the chitin (Karlsson and Stenlid, 2009). They thus play key roles in multiple physiological processes such as molting, PM reorganization, wing expansion, and abdominal contraction (Arakane and Muthukrishnan, 2010). Insects have a wide range of Cht and chitinase-like proteins that have been phylogenetically grouped based on their functional specializations and structures (Liu et al., 2020). For example, group I and II chitinases are involved in skin digestion, while group III proteins regulate post-hatching events (Zhu et al., 2004). Despite their critical nature, many chitinase-like proteins have domains that are not enzymatically active; nevertheless, these are of functional importance in chitin binding and structural integrity (Karlsson and Stenlid, 2009). Idgfs are within the subclass of chitinase-like proteins that lack hydrolytic activity yet play vital roles in insect development (Kawamura et al., 1999). Idgfs are also involved in processes such as cell proliferation, tissue differentiation and immunity (Bryant, 2001). In *Drosophila melanogaster*, the Idgf family has been shown to play important roles in regulating larval and adult development (Adams et al., 2000). However, the mechanisms that explain the molecular functions of these proteins remain poorly understood, especially in other insect species (Dukariya and Kumar, 2020).

Drosophila suzukii, also known as spotted wing *drosophila*, with chromosome number 2n=8 (Drosopoulou et al., 2019). It is an invasive pest species that causes an important economic loss in worldwide due to its ability to damage a wide range of soft fruits (Asplen et al., 2015). While chemical control is still the dominant strategy for managing *D. suzukii* populations, increasing insecticide resistance in recent years has highlighted the need for alternative approaches to be developed (Bruck et al., 2011; Shawer, 2021).

In this study, genome-wide bioinformatic analysis of Cht and Idgf proteins in *D. suzukii* was performed. Through sequence homology, domain architecture analysis and phylogenetic classification, the *Cht* and *Idgf* gene families in this species were identified. The work also examined the transcriptomic profile of these genes to understand their potential physiological roles in developmental processes to offer a comprehensive basis for understanding the functional dynamics of chitin-related proteins in *D. suzukii*. This is intented to pave the way for the development of innovative pest management strategies targeting these vital proteins.

2. MATERIALS AND METHODS

2.1 Identification and Characterization of *Cht* and *Idgf* Genes

To identify putative *Chts* and *Idgf* genes in *D. suzukii*, the Pfam ID (PF00704) of this gene family was queried against the Ensembl Metazoa database (https:metazoa.ensembl.org). Protein sequences of putative genes were downloaded from the Ensembl Metazoa database. Furthermore, putative *D. suzukii* Chts and Idgf protein sequences were analyzed using BLAST against the *D. suzukii* genome (http://spottedwingflybase.org) to confirm gene IDs. The catalytic domain of glycosyl hydrolases family 18 (GH18s), which is specific for Chts and Idgf proteins, was verified using the HMMER (https://www.ebi.ac.uk/Tools/hmmer/search/phmmer) web tool. Subsequently, the protein sequences of *D. melanogaster* and *D. suzukii* Chts and Idgf protein sequences were aligned using EMBL-EBI Pairwise Sequence Alignment (https://www.ebi.ac.uk/jdispatcher/psa) and the identity of the protein sequences was calculated. The genes with the highest percentage identity were numbered. The isoelectric point (pI) and molecular weight (MW) of Cht and Idgf proteins were determined using the ExPASy web tool (Gasteiger et al., 2003). The subcellular localization of these proteins was predicted using WoLF PSORT (https://wolfpsort.hgc.jp) (Chou and Shen, 2010). In addition, the peptide sequences of the *Cht* and *Idgf* genes were evaluated with TMHMM 2.0 web-based software to detect the presence of transmembrane helical regions (Krogh et al., 2001).

2.2 Phylogenetic Analysis

To construct a phylogenetic tree, protein sequences of *Cht* and *Idgf* genes of *D. suzukii* were compared with the protein sequences of different insect orders which selected as *Acromyrmex echinatior* (*Ae*, one sequence), *Agrius convolvuli* (*Ac*, 1), *Anopheles gambiae* (*Ag*, 17), *Apis mellifera* (*Am*, 3), *Bactrocera dorsalis* (*Bd*, 12), *Bemisia tabaci* (*Bt*, 12), *Bombyx mori* (*Bm*, 1), *Camponotus floridanus* (*Cf*, 1), *Danaus plexippus* (*Dp*, 2), *Drosophila melanogaster* (*Dm*, 16), *Harpegnathos saltator* (*Hs*, 2), *Nilaparvata lugens* (*Nl*, 6), *Ostrinia furnacalis* (*Of*, 1), *Papilio xuthus* (*Px*, 1), *Spodoptera litura* (*Sl*, 1) and *Tribolium castaneum* (*Tc*, 24) containing the GH 18 domain were downloaded from the NCBI database (https://www.ncbi.nlm.nih.gov/) (Supplementary Material). These species were selected to represent different insect orders (24 from Coleoptera, 18 from Hemiptera, 7 from Lepidoptera, 7 from Hymenoptera and 60 from Diptera) because they provide comprehensive information on the evolutionary processes and diversity of *Cht* and *Idgf* genes and are considered as model organisms. Since preliminary evaluations showed that including more species strengthens the interpretability of the results, sequences from a total of seventeen species were included in the analysis. A total of 116 Cht and Idgf protein sequences were analyzed using MacVector software using the T-Coffee algorithm for multiple sequence alignment and subjected to phylogenetic tree construction with the Neighbor-Joining algorithm and 1000 bootstrap replicates to assess the clades. Analysis results were visualized with the iTOL (https://itol.embl.de) web-based tool.

2.3 Analysis of Gene Structure and Conserved Motifs of DsCht and DsIdgf Members

Conserved motifs in the amino acid sequences of the *Cht* and *Idgf* gene family were examined using the Multiple Expectation Maximization for Motif Elicitation (MEME) Suite tool (https://meme-suite.org/meme/index.html) (Bailey et al., 2015). Additionally, the schematic gene structure of *Cht* and *Idgf* genes was visualized using Gene Structure Display Server software (https://gsds.gao-lab.org/index.php) (Hu et al., 2015).

2.4 Cis-Regulatory Element Analysis

For the analysis of cis-regulatory elements in the promoter regions of the *Cht* and *Idgf* genes, an upstream 2000 bp region was selected and these elements were predicted using Analysis of DNA Sequences for TRANSFAC Signals (http://gene-regulation.com) and Juven-Gershon lab tools (https://www.juven-gershonlab.org/) web-based tool (Wingender et al., 2000).

2.5 PPI (Protein-Protein Interaction) and Three-Dimensional (3D) Structure Analysis of Cht and Idgf Proteins

Protein-protein interaction (PPI) networks of Cht and Idgf proteins were predicted using the STRING database (Jensen et al., 2009). The three-dimensional structures of Cht and Idgf proteins were modeled with the I-TASSER algorithm (Yang & Zhang, 2015). Furthermore, the modeling results were visualized in detail with the RCSB Protein Data Bank 3D Viewer software (Berman et al., 2000).

2.6 Transcriptome Profiling of *Cht* and *Idgf* Genes

Transcriptome data for whole body, female and male antenna tissues of *D. suzukii* were obtained from Ahn et al. (2020). These data were analyzed as normalized average reads per base for each *Cht* and *Idgf* gene and the results were visualized with TBtools software (Chen et al., 2024).

3. RESULTS

3.1 Identification of *Cht* and *Idgf* Genes

The results revealed a total of fifteen relevant genes in *D. suzukii*, eleven encoding *Cht* and four encoding *Idgf* (Table 1). The amino acid sequences of *DsCht* and *DsIdgf* genes were then aligned with the amino acid sequences of *DmCht* and *DmIdgf* genes and the identity ratios between the genes were analyzed. The lowest identity ratio (40.5%) was found between *DsCht10* and *DmCht10* genes, while the highest identity ratio (98.6%) was found between *DsIdgf4* and *DmIdgf4* genes (Table 1). Sequence analysis showed that the genomic lengths of *DsCht* and *DsIdgf* genes ranged from 1,186 bp (DsCht9) to 14,879 bp (DsCht6). Furthermore, the lengths of the peptide sequences ranged from 370 amino acids (DsCht9) to 4723 amino acids (DsCht6) (Table 1). The number of exons in the coding sequences (CDS) ranged from 2 to 24, while the total CDS length varied from 1113 bp (DsCht9) to 14,172 bp (DsCht6) (Table 1). The molecular weights of DsCht and DsIdgf proteins ranged from 41 kDa (DsCht9) to 175.5 kDa (DsCht10), while their isoelectric points (pI) were determined between 4.50 (DsCht4) and 8.51 (DsCht12) (Table 1).

Table 1. Some features of *D. suzukii DsCht* and *DsIdgf* genes.

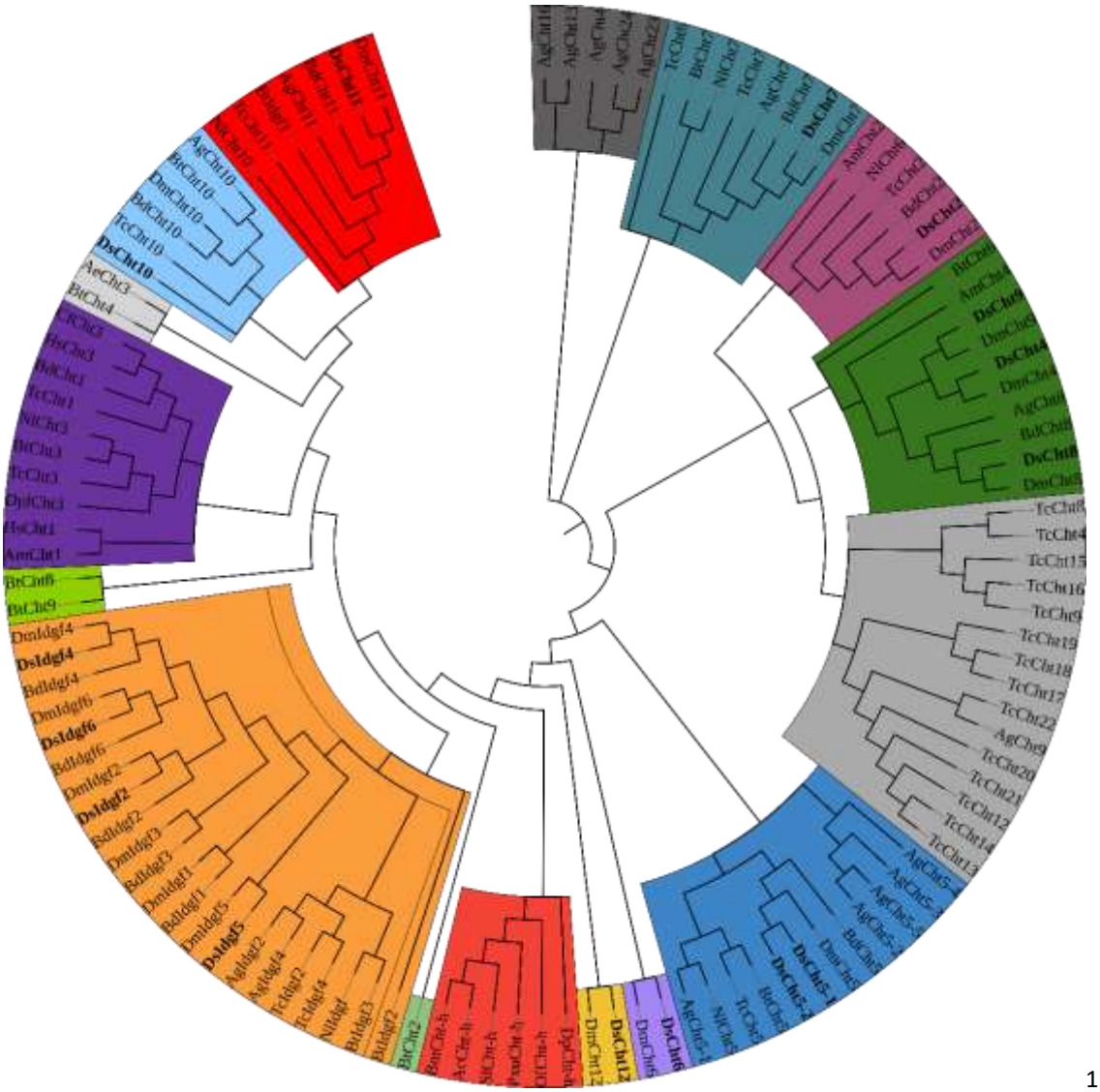
Gene Name	Gene ID	Genomic Sequence (bp)	CDS (bp)	Peptide sequences (aa)	Subcellular Localization	MW (kDA)	pI	Identity with Chitinase protein of *Drosophila melanogaster* (%)
DsCht2	LOC108013251	1743	1455	484	Endoplasmic Reticulum	53,8	5,48	92,6
DsCht4	LOC108008481	1475	1410	469	Plasma	50,7	4,50	86,2
DsCht5-1	LOC108015649	2010	1779	592	Endoplasmic Reticulum	67	6,19	89,3
DsCht5-2	LOC118877614	2051	1779	592	Endoplasmic Reticulum	66,9	6,23	88,8
DsCht6	LOC108015177	14879	14172	4723	Extracellular	51,7	5,23	82,9
DsCht7	LOC108007102	3930	3102	1033	Endoplasmic Reticulum	115,7	5,88	96
DsCht8	LOC108009767	1466	1431	476	Endoplasmic Reticulum	52,6	5,06	87,8
DsCht9	LOC108008564	1186	1113	370	Extracellular	41	4,59	93,2
DsCht10	LOC108011048	4740	4683	1560	Plasma	175,5	7,34	40,5
DsCht11	LOC108016893	1620	1299	432	Plasma	48,8	6,27	81,1
DsCht12	LOC108009768	1670	1413	470	Extracellular	54,2	8,51	76,2
DsIdgf2	LOC108020907	1454	1323	440	Extracellular	49,3	6,47	89,8
DsIdgf4	LOC108015400	1573	1329	442	Endoplasmic Reticulum	48,5	7,65	98,6
DsIdgf5	LOC108017795	1529	1332	443	Extracellular	49,5	6,40	89,2
DsIdgf6	LOC108009387	1552	1365	454	Endoplasmic Reticulum	50,6	7,99	94,3

Abbreviations: MW, molecular weight; pI: isoelectric point; CDS, coding sequences.

3.2 Phylogenetic Analysis

To predict the functions of Cht and Idgf proteins across different species and better understand their evolutionary relationships, a phylogenetic tree was constructed using 15 Cht and Idgf protein sequences from *D. suzukii* and 101 Cht and Idgf protein sequences from 16 different insect species. The phylogenetic tree divided the Cht and Idgf protein sequences from *D. suzukii* and other selected species into three main groups and 14 distinct subgroups based on sequence similarity of their GH18 catalytic domains (Figure 1). DsCht proteins are particularly similar to Cht proteins from *Drosophila* species, suggesting that chitinase proteins are highly conserved in evolutionary terms and there is high similarity between homologs across *Drosophila* species. DsIdgf proteins, on the other hand, constitute a distinct subgroup within the Idgf family, appearing to be specific to

Drosophila species. The close phylogenetic relationship of these proteins to their homologs in *D. melanogaster*, for example, suggests both evolutionary and functional conservation. However, a clear divergence was observed when compared with Idgf proteins from more distant evolutionary groups (Figure 1).



1

Figure 1. Phylogenetic relationships among Cht and Idgf proteins. *D. suzukii (Ds) (shown in bold)* and selected species; *Acromyrmex echinatior (Ae), Agrius convolvuli (Ac), Anopheles gambiae (Ag), Apis mellifera (Am), Bactrocera dorsalis (Bd), Bemisia tabaci (Bt), Bombyx mori (Bm), Camponotus floridanus (Cf), Danaus plexippus (Dp), Drosophila melanogaster (Dm) Harpegnathos saltator (Hs), Nilaparvata lugens (Nl), Ostrinia furnacalis (Of), Papilio xuthus (Px), Spodoptera litura (Sl) and Tribolium castanium (Tc).*

3.3 Gene Structure and Conserved Motif Analysis

The exon and intron structures of *DsCht* and *DsIdgf* genes were analyzed using Gene Structure Display Server (GSDS) software (Figure 2). According to the analysis results: *DsCht2* and *DsCht12* genes 4 exons and 3 introns; *DsCht4* gene 5 exons and 4 introns; *DsCht5-1*, *DsCht5-2* and *DsCht7* genes 6 exons and 5 introns; *DsCht6* gene had 14 exons and 13 introns; *DsCht8*, *DsCht9*, *DsIdgf2*, *DsIdgf4*, *DsIdgf5* and *DsIdgf6* genes had 3 exons and 2 introns (Figure 2). However, *DsCht10* contains 7 exons and 6 introns and *DsCht11* gene contains 2 exons and 1 intron (Figure 2). The longest genomic sequence was found by *DsCht6* with 14,879 bp, while the shortest genomic sequence was determined by *DsCht9* with 1186 bp (Figure 2). These findings clearly reveal the structural diversity and organizational differences of the genes.

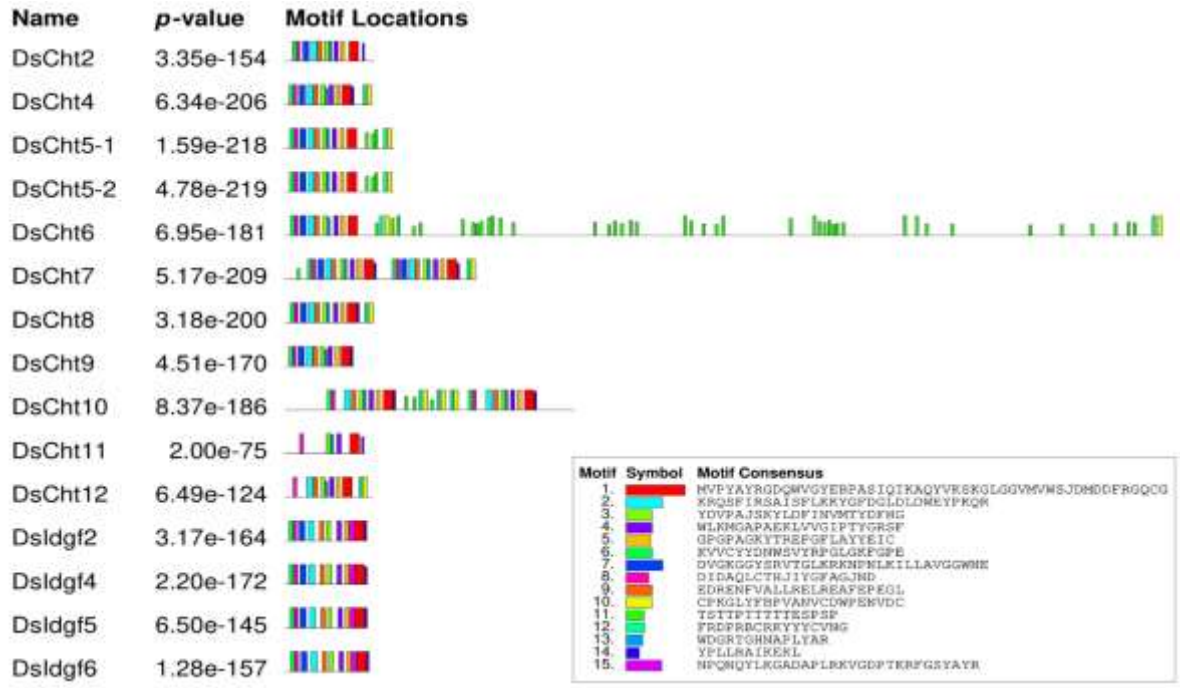


Figure 2. Gene structure of the Chitinase (*Cht* and *Idgf*) family in *D. suzukii*. UTR, untranslated region; CDS, coding sequence.

Motif analysis was performed based on the parameters set initially. The maximum number of motifs was determined to be 15 in this analysis and the optimum amino acid length of the motifs was set to be between 5 and 50 (Figure 3a). Within the results, a total of 15 conserved motifs were identified in the *Cht* and *Idgf* gene families, with the lengths of these motifs found to vary from 11 to 50 amino acids (Figure 3b). These results clearly demonstrate the evolutionarily conserved functional regions of Cht and Idgf proteins and the structural similarities in these gene families. On motif analysis, Motifs 1, 6, and 8 were detected in all members of both Cht and Idgf protein families. In contrast, Motif 15 was only identified in Idgf2, Idgf4, Idgf5 and Idgf6 members (Figure 3a). Notably, Motif 14 was found in all members except *Cht5-1, Cht5-2, Cht6* and *Cht12* genes, suggesting that the motif may be specific to certain functional properties of these proteins.

The motif analysis carried out for Idgf and chitinase Cht proteins in *D. suzukii* thus identified evolutionarily conserved functional regions in these proteins. Those sequences that show particularly strong conservation, such as Motif 1, are thought to play an important role in chitin metabolism. Furthermore, the strong presence of Motif 11 in DsCht10 may allow this protein to have more contact with the chitin substrate, potentially creating a stronger binding capacity.

a)



b)

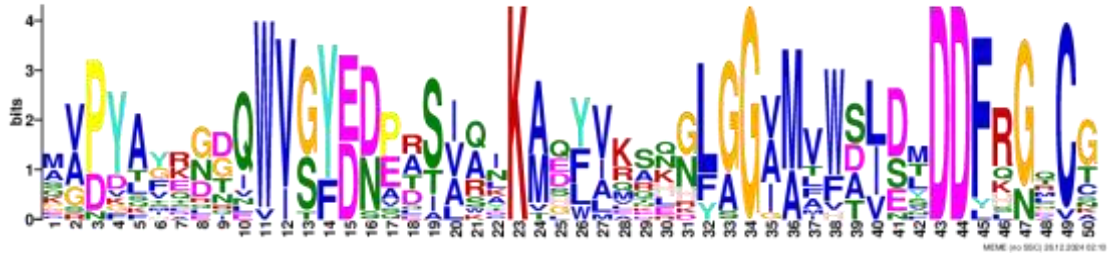


Figure 3. (a) Distribution of conserved motifs detected in *DsCht* and *DsIdgf* genes, (b) Motif 1 was common to all *DsCht* and *DsIdgf* genes.

3.4 Analysis of Cis-Regulatory Elements of *Cht* and *Idgf* Genes

Analysis of cis-regulatory elements in the 2000 bp upstream promoter regions of *Cht* and *Idgf* genes was performed using TRANSFAC Signals and the Juven-Gershon tool (Figure 4). As a result of this analysis, cis-regulatory elements were separated based on the coexistence of cis-regulatory elements and transcription binding factors. The results revealed that the *Cht* and *Idgf* gene families contain a total of 543 cis-regulatory elements, which are divided into five different types: basic promoter elements, response elements, enhancer elements, enhancer/silencer elements, and those factors associated with insulator elements. Of these, the basic promoter element Initiator (Inr) appeared a total of 311 times. HSF1 (Heat Shock Transcription Factor 1) was seen in response elements (16), the A5CE-2 element was seen in enhancer elements (total 10), DEP2 was present in enhancer/silencing elements (total 4), and the BEAF-32A motif was seen in insulator elements (total 202) (Figure 4).

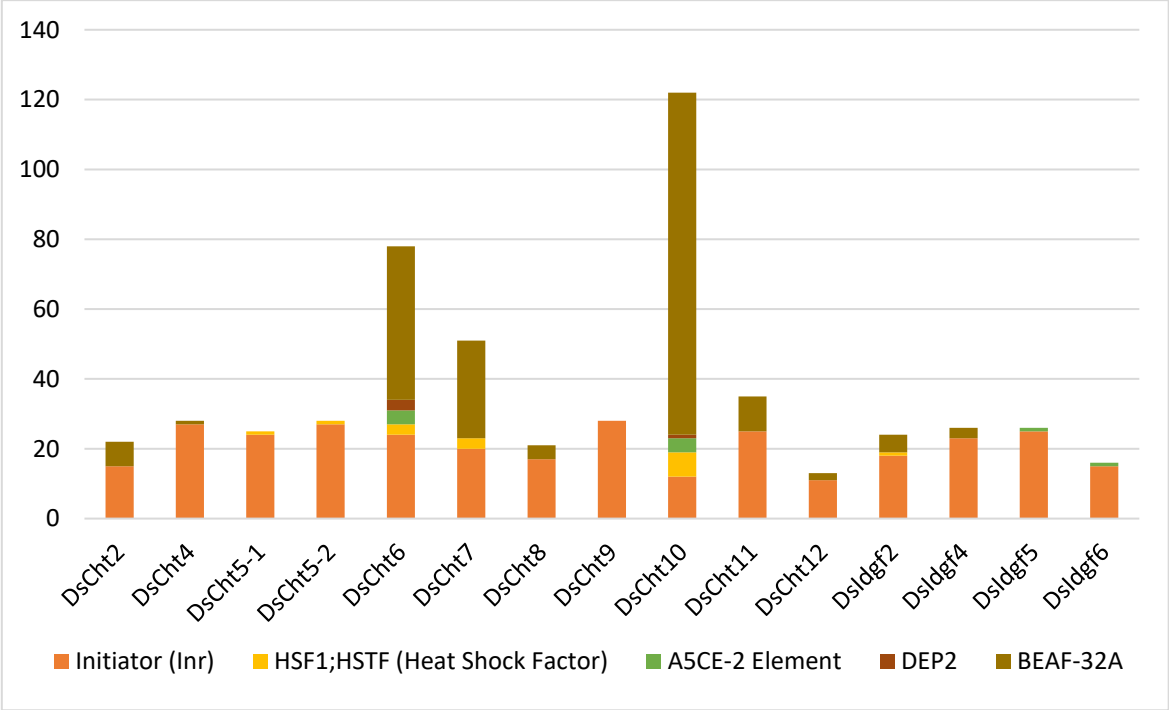


Figure 4. Cis-regulatory elements in the 2000 bp upstream promoter region of *DsCht* and *DsIdgf* genes.

3.5 Protein-protein Interaction Prediction Analysis

The protein-protein interaction (PPI) network created using the STRING database revealed the potential roles of Idgf and Cht proteins in *D. suzukii* in biological processes, as well as their interactions with one another. Especially DsCht2, DsCht5-2, and DsCht7 proteins emerged as central proteins in the clusters. These proteins are thought to play a key role in the regulation of aminoglycan metabolism. Cluster 2 (Green Bubble), chitin catabolism proteins exhibit strong interactions, indicating that they cooperate in chitin degradation processes. Notably, XP_016936682.2 (Hexosaminidase 2) plays an important role in this cluster, while the XP_036673225.2 (N-acetylglucosamine kinase) protein functions as a bridge between the red and green clusters. In cluster 3 (Blue

Bubble), however, DsCht12 and DsIdgf2 proteins did not show any interaction with other proteins in the network. This suggests that these proteins may have unique or independent functions.

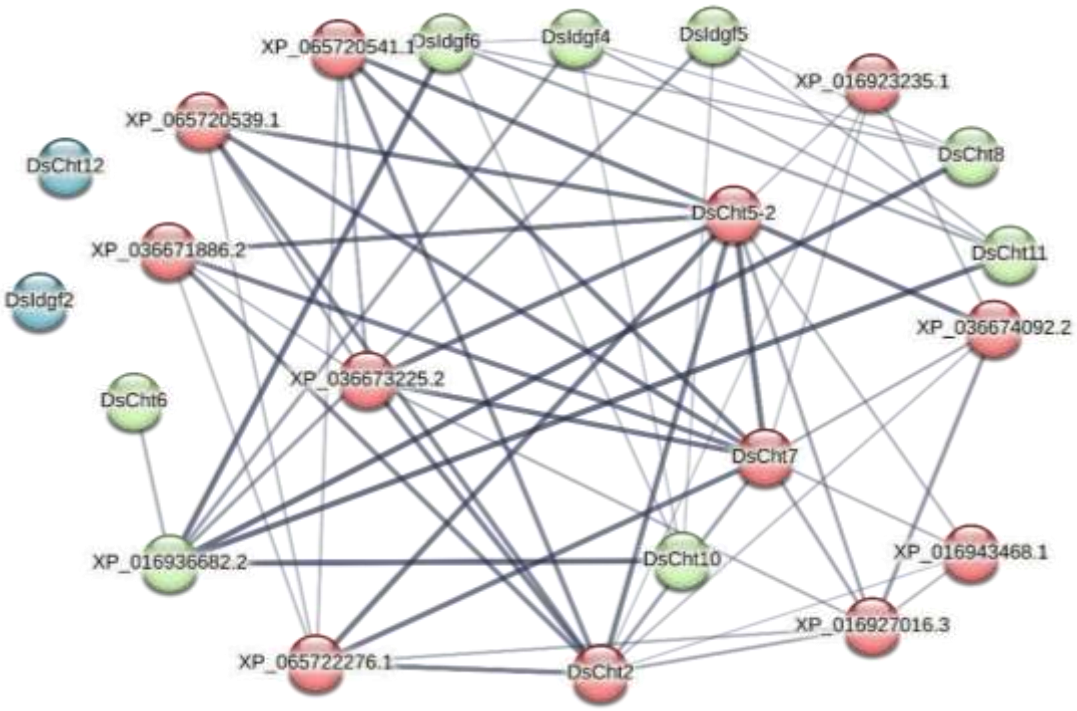


Figure 5. Protein-protein interaction (PPI) network of DsCht and DsIdgf proteins. The lines (gray) indicate the shared physical complex, and as their thickness increases, so does the interaction's reliability. The PPI network was created using the K-means Clustering algorithm from the STRING database to investigate the possible role of DsCht and DsIdgf proteins in detail during potential interactions with other proteins. According to the clustering analysis, the network is divided into three clusters: Cluster 1 (red balloon), Cluster 2 (green balloon), and Cluster 3 (blue balloon).

3.6 3D Model Structures of *D. suzukii* Cht and Idgf Proteins

The 3D model structures of Cht (chitinase) and Idgf (imaginal disc growth factor) proteins of *D. suzukii* were predicted using the I-TASSER tool and then visualized with the PDB 3D Viewer (Figure 6). C-score was used to assess the reliability of the models (Roy et al., 2010). The C-score is a confidence score that evaluates the quality of the predicted models and usually takes values in the range [-5, 2]; higher scores indicate that the model is more reliable (Yang et al., 2014). Furthermore, the TM-score was used to measure the structural similarity among the predicted model and native protein structures that takes a value between 0 and 1, with a value of 1 indicating a complete fit among the two structures (Zhang, 2008). In addition, root-mean-square deviation (RMSD) values were also analyzed to verify model quality and ranged from 0 to 30 Å (Roy et al., 2010). The results of the analysis revealed that the C-scores of *D. suzukii* Cht and Idgf proteins ranged from 0.03 to -1.91 (Table 2).

Among the proteins analyzed, DsIdgf5 has the highest C-score, reflecting the highest reliability among the predicted models. In contrast, DsCht10 has the lowest C-score, suggesting a lower reliability. The lengths of the conserved chitinase and Idgf domains in these proteins range from 321 amino acids (DsCht12) to 398 amino acids (DsIdgf6) (Table 2).

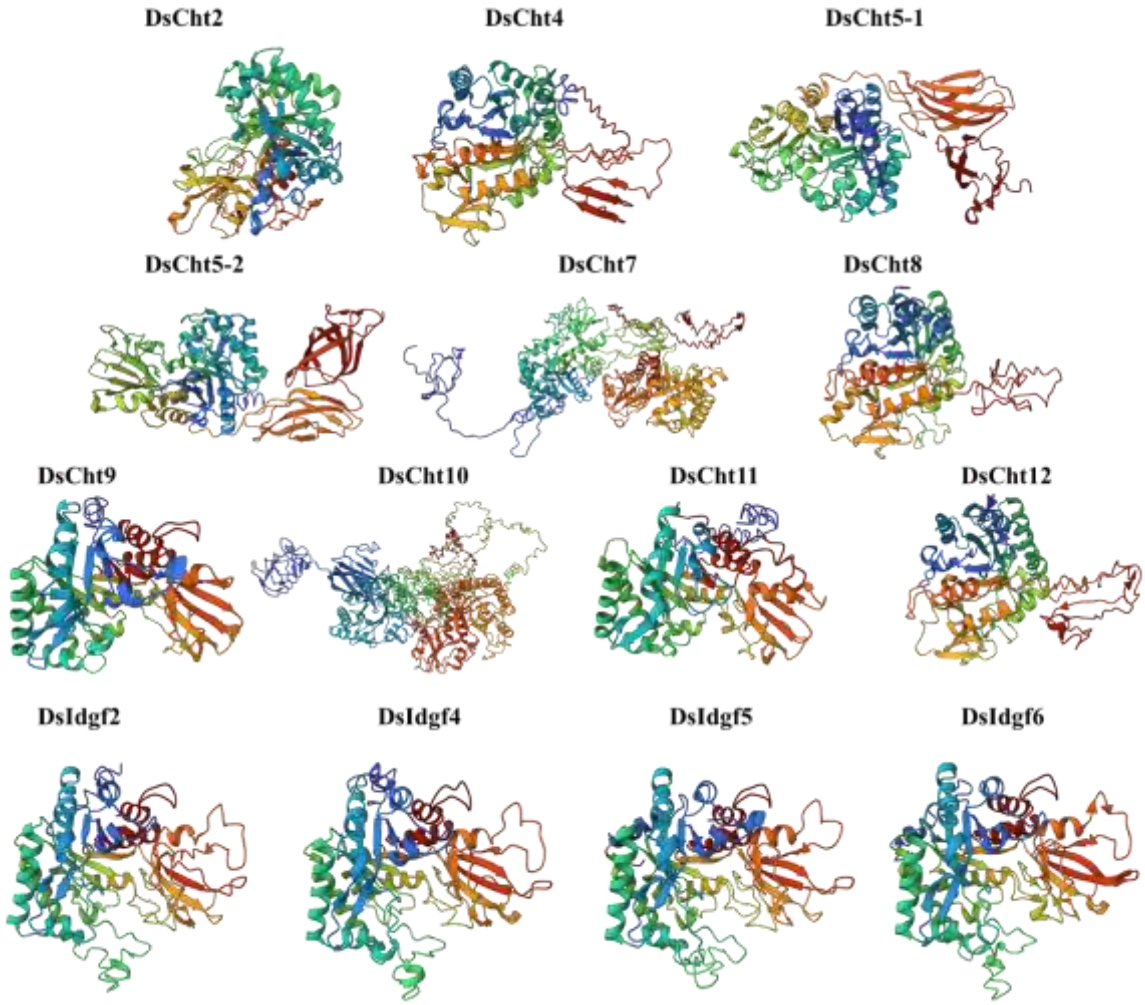


Figure 6. Three-dimensional structure modeling of eleven DsCht and four DsIdgf proteins in *D. suzukii*.

Table 2. Quantitative results obtained from I-TASSER modeling of DsCht and DsIdgf proteins and sequence position information of the glycoside hydrolase 18 domain region in *D. suzukii*. RMSD, root-mean-square deviation; GH18, glycoside hydrolase 18.

Protein	C-score	TM-score	RMSD (Å)	Peptide sequence	GH18 domain (start to and)
DsCht2	-1.91	0.49±0.15	11.8±4.5Å	484	43-389
DsCht4	-1.54	0.53±0.15	10.8±4.6Å	469	26-351 / 416-467
DsCht5-1	-1.11	0.58±0.14	10.3±4.6Å	592	28-379 / 528-573
DsCht5-2	-0.99	0.59±0.14	10.0±4.6Å	592	28-379 / 528-573
DsCht7	-1.8	0.50±0.15	13.5±4.0Å	1033	124-468 / 580-918
DsCht8	-1.5	0.53±0.15	10.7±4.6Å	476	30-379
DsCht9	0.01	0.71±0.11	6.6±4.0Å	370	23-346
DsCht10	-2.44	0.43±0.14	16.3±3.0Å	1560	229-574 / 989-1333
DsCht11	-1.85	0.49±0.15	11.4±4.5Å	432	64-394
DsCht12	-0.78	0.61±0.14	9.0±4.6Å	470	34-355
DsIdgf2	-0.35	0.67±0.13	7.8±4.4Å	440	24-419
DsIdgf4	-0.4	0.66±0.13	7.9±4.4Å	442	27-420
DsIdgf5	0.03	0.72±0.11	7.0±4.1Å	443	30-422
DsIdgf6	-0.1	0.70±0.12	7.3±4.2Å	454	33-431

3.7 RNA-Seq gene expression profiles of *DsCht and DsIdgf* genes

High-throughput gene expression data was retrieved from the study by Ahn et al. (2020) and whole body, female antenna and male antenna tissues were selected to determine the expression levels of tissue-specific *Cht* and *Idgf* genes. Fifteen Ds*Cht* and Ds*Idgf* genes expression profile (FKPM) were visualized as heat mapping using TBtools (Figure 7). Based on the resulting expression patterns, the *DsIdgf4* gene exhibited high

levels of expression in all tissues, particularly antennae (Figure 7). In addition, *DsIdgf4, DsCht12*, and *DsIdgf6* genes showed higher expression in whole body in contrast to other tissue samples.

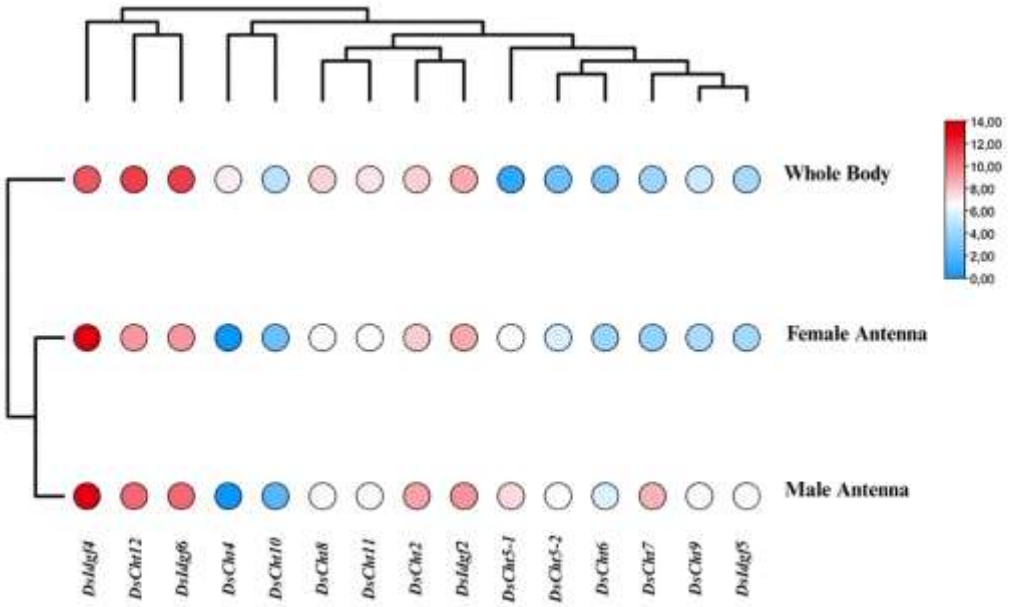


Figure 7. RNA-Seq gene expression profiles of *DsCht* and *DsIdgf* genes in different tissues.

4. DISCUSSION

This study presents the first comprehensive characterization of the *chitinase (Cht)* and *imaginal disc growth factor (Idgf)* gene families in *D. suzukii*. A total of 15 GH18-family genes (11 putative *chitinases* and four *Idgfs*) were thereby identified from the *D. suzukii* genome.

The size of the *D. suzukii* chitinase family (15 genes) is within the range observed in other insects, though specific numbers vary significantly across taxa. For instance, the hemipteran pests tend to have around 10 to 14 chitinase family genes, with *Bemisia tabaci* having 14 chitinase family genes (10 *Cht*, 3 *Idgf*, and 1 endo-β-N-acetylglucosaminidase [ENGase]) (Peng et al., 2021), and *Diaphorina citri* being reported to have roughly a dozen (Wu et al., 2022). In *B. dorsalis*, Liu et al. (2018) found 12 Cht-related genes (seven *Cht* and five *Idgf*), slightly fewer than the 11+4 identified in *D. suzukii* in this work. In contrast, *Leptinotarsa decemlineata* harbors a markedly expanded chitinase family, with 19 chitinase genes (*LdChts*) identified (Chen et al., 2024), along with at least several *Idgfs* (four *Idgf* paralogs were noted, namely *LdIdgf1–4*). Likewise, *Aedes albopictus* possesses 14 chitinase-related genes (12 *Cht* and two *Idgf*) (An et al., 2023). Such differences in gene numbers likely reflect lineage-specific gene duplication or loss events; gene duplication tends to produce additional family members that are diversified in function, whereas some lineages (e.g. certain flies) have lost certain paralogs, such that *D. suzukii* has only four IDGFs whereas *D. melanogaster* has six. Despite these quantitative differences, however, nearly all insects studied so far share a core set of chitinase groups, underscoring the presence of conserved evolutionary framework for this gene family. While Shippy et al. (2022) noted an exceptional case in *D. citri*, identifying a novel chitinase-like gene putatively acquired via horizontal gene transfer, no analogue to this unique event was evident in the *D. suzukii* data, where all genes fell into known insect clades.

Phylogenetic analysis classified the *D. suzukii* Cht/Idgf proteins into known insect chitinase groups. Notably, *D. suzukii* appears to be lacking certain clades, consistent with other dipterans, such as *Bactrocera dorsalis* (Liu et al., 2018). Overall, the *D. suzukii* Cht data shows good alignment with the conserved GH18 clades identified in insects, confirming the phylogenetic consistency of these gene families across species. However, this study fills a gap in the literature by adding *D. suzukii* to the list of insects associated with chitinase/Idgf complements, thereby enriching comparative genomic data on chitin metabolism genes.

The domain architectures and conserved motifs of the *D. suzukii* Cht proteins support their putative functions in chitin metabolism. Several DsChts (including DsCht10) emerged as notable for their “motif richness” and multi-domain structures, containing multiple chitin-binding domains in addition to the catalytic domain. Such features are typical of Group I chitinases, which are often large, multi-domain enzymes thought to act on bulky chitin substrates. In *B. dorsalis*, similarly, four of the seven chitinases (BdCht5, 7, 8, 10) possess chitin-binding domain (Liu et al., 2018), reinforcing the idea that only a subset of chitinases in each species retain these auxiliary

domains for substrate binding. Conservation of the four signature motifs (KxxxGGW, FDGXDLDWEYP, MxYDxxG, and GxxxWxxDxDD) was observed in most *D. suzukii* Chts, parallel to findings in other species (e.g., 16 of seventeen *Ae. albopictus* Cht/Idgf proteins contained all four motifs, with one enzyme lacking Motif IV) (An et al., 2023). The catalytic glutamate in Motif II is preserved in all DsChts, whereas in the DsIdgfs this residue is naturally substituted, consistent with IDGFs having lost their prior enzymatic activity. In contrast, the retention of these motifs and catalytic residues in DsChts implies functional enzymatic roles. Indeed, in *Ae. Albopictus,* the *chitinase* gene (*AaCht10*) that retained all key motifs (including the active-site Glu and a chitin-binding domain) was shown to be essential for molting (An et al., 2023).

To visualize the enzymes, 3D structure models for representative DsChts were generated; these models revealed the characteristic TIM-barrel folds of GH18 chitinases with well-aligned active sites. The structural features were also like known crystal structures of insect chitinases, with the first such structure, OfChtI from *Ostrinia furnacalis*, showing a groove-shaped active cleft in a classical GH18 fold (Chen et al., 2014). This structural conservation across species suggests that DsCht enzymes may tend to operate via the same mechanisms as other insect chitinases, allowing the development of confidence that functional insights from model insects may be usefully extrapolated to *D. suzukii*.

The upstream regulatory sequences of the *D. suzukii Cht* genes were also examined to develop insight into their control mechanisms. Promoter analysis revealed numerous putative binding sites for molting hormones and developmental transcription factors. Several DsCht promoters contain consensus ecdysone response elements, suggesting that the 20-hydroxyecdysone (20E) hormone may directly regulate these genes on molting. This is consistent with experimental evidence from other insects: for example, in the pea aphid *Myzus persicae*, 20E treatment upregulated the transcription of nine chitinase genes prior to molting (Zhang et al., 2024). Some potential binding sites for Broad-Complex and Kruppel-like factors, which are known regulators of cuticle genes during molts, were also identified, as were sites for juvenile hormone (JH) signaling components. The presence of these elements indicates that *D. suzukii* may well employ the same hormonal regulatory cascades seen in other insects to time the expression of its chitinase data to suit each molt. Such coordination ensures chitinases are expressed when and where needed, whether at high rates in epidermal cells before ecdysis to facilitate old cuticle degradation, or constitutively in midgut cells for continuous peritrophic membrane turnover. This insight into regulatory functions aligns with the observed expression profiles and reinforces the idea that the chitinase gene network in *D. suzukii* is tightly integrated into the insect's developmental program.

The protein–protein interaction (PPI) network analysis suggested that these enzymes potentially interact with other components of the chitin metabolic pathway. For instance, several DsChts were predicted to connect with chitin synthase (the enzyme that polymerizes chitin), with cuticle structural proteins, and with each other, thereby forming a network to effectively underpin cuticle formation and degradation. While these interactions were developed using computational predictions, they make biological sense because chitinases often work with chitin deacetylases and cuticle protein assemblies during cuticle reorganization (Yu et al., 2019). The developed PPI network also hinted at links between Idgfs and signaling pathways such as insulin/IGF signaling in a manner consistent with reports that Idgfs (like *Drosophila* Idgf2) can modulate insulin pathway activity in growth control (Partridge et al., 2011).

Expression profiling of the *D. suzukii Cht/Idgf* genes highlights their roles in development and specific tissue function across patterns broadly consistent with previous reports. Many of the *DsCht* genes are highly expressed during molting stages or in chitin-rich organs, reflecting their involvement in cuticle digestion and remodeling. This included elevated transcript levels of several *chitinases* during the larva-to-pupa transition, which mirrors findings in *L. decemlineata* that show most *LdChts* peaking around the larval-pupal molt (Chen et al., 2024). Likewise, *Aedes albopictus chitinases* also demonstrate stage-specific peaks: eight *AaCht* genes are predominantly expressed in early stages (egg and pupa), whereas others such as *AaCht10* were expressed across multiple life stages (An et al., 2023).

D. suzukii may also possess an intestinal-specific chitinase like those found in other insects, which function in the peritrophic matrix of the midgut. In *L. decemlineata*, a subset of nine *chitinase* genes is expressed exclusively in the digestive system (Chen et al., 2024), likely to manage chitin in the intestinal lining; given that the peritrophic membrane must be periodically renewed in both larvae and adults, it would not be surprising to find similar specialization in *D. suzukii*. Intriguingly, however, one of the most distinctive expression patterns determined here was for DsIdgf4, which was highly enriched in the antennae. Idgfs in other species are typically associated with general growth and development, being commonly found in hemolymph or imaginal discs, and such antenna-specific expression is unusual. This novel finding suggests that DsIdgf4 might have a specialized role in chemosensory organ development or function in *D. suzukii*, making it tempting to speculate that DsIdgf4 could be involved in antennal structural integrity or in supporting the olfactory tissues, perhaps by means of interaction with the cuticular matrix of sensilla. To be known, such localization of an Idgf has not been reported

previously, highlighting a potential species-specific adaptation in *D. suzukii*. Nonetheless, *Idgfs* are known to play diverse roles across species: for example, *Idgf4* in *B. dorsalis* is abundantly expressed in late pupae and adults, and RNAi silencing of *BdIdgf4* causes high mortality and deformities, including malformed wings (Gu et al., 2019). Similarly, Chen et al. (2023) demonstrated that *L. decemlineata LdIdgf2* and *LdIdgf4* are crucial for successful metamorphosis, with knockdown impeding larval–prepupal and pupal transitions. These observations from other insects suggest that *Idgf* genes, while non-enzymatic, are functionally critical, and the discovery of an antenna Idgf in *D. suzukii* therefore opens up new questions about Idgf functional diversification, such as whether *DsIdgf4* might contribute to antennal development or even stress responses in this species, based on the fact that Idgfs have been implicated in thermal stress and immunity in other contexts. Functional studies in other insects have shown that disrupting chitinase genes has deleterious effects. In *L. decemlineata*, the silencing of multiple *chitinase* genes via RNAi caused severe molting defects; notably, knocking down even a single gene, *LdCht10*, was enough to arrest molting and kill larvae (Chen et al., 2024). Likewise, in *Ae. albopictus*, RNAi targeting of *AaCht10* resulted in a ~65% reduction in adult emergence due to lethal failures in the pupal-adult molt (An et al., 2023). Even partial suppression of *chitinase* genes can lead to malformed exoskeletons or wings as seen in beetles and flies, for example *B. dorsalis* adults displayed wing deformities when *BdIdgf4* was silenced (Liu et al., 2018).

5. CONCLUSION

This study significantly extends knowledge of the *chitinase* and *chitinase-like Idgf* gene families in *D. suzukii* by documenting the gene inventory, evolutionary relationships, structural features, expression profiles, and regulatory elements of these genes. These findings were compared with similar work in multiple other insects (Hemiptera, Coleoptera, and Diptera species), with results that reveal a strong phylogenetic and functional coherence across studies, reinforcing the idea that insect chitinases constitute a conserved toolkit for molting and cuticle dynamics. At the same time, species-specific nuances, such as the unique antennal Idgf expression and the exact gene count in *D. suzukii*, have emerged that add new pieces to the puzzle of how different insects adapt the *chitinase* gene family to their individual life histories.

Importantly, the findings have several practical implications, paving the way for functional studies (e.g., RNAi trials) in *D. suzukii* based on evidence that *chitinase/Idgf* genes could be leveraged as molecular targets in next-generation pest control methods. Such translational avenues, built on a foundation of comparative genomics and physiology, exemplify how developing understanding of the basic biology of pest species can help inform and improve strategies to manage them.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data availability statement

RNA sequencing data analyzed in this study have been retrieved from the NCBI can be accessed by PRJNA577281 BioProject ID.

Author Contributions

The authors declare that they have contributed equally to the article.

Supplementary Data:
c2a8/79d2/0e68/68f1feffdd1f69.18872922.xlsx

ORCID

Tuğberk TUNÇYÜREK https://orcid.org/0009-0002-4478-5834
Uğur SARI https://orcid.org/0000-0001-7564-997X
Hanife YANDAYAN GENÇ https://orcid.org/0000-0001-8180-4307

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