

# GENOME-WIDE IDENTIFICATION AND CHARACTERIZATION OF CHITINASE GENES IN COFFEE BERRY BORER (*Hypothenemus hampei*) AND ITS POTENTIAL AS MOLECULAR TARGETS FOR PEST MANAGEMENT

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## INTRODUCTION



- Chitinases (*CHTs*) degrade chitin, a key structural component of the insect cuticle and peritrophic matrix. In insects, *CHTs* are essential for molting, growth, digestion, and immune defense.
- The coffee berry borer (CBB, *Hypothenemus hampei*) is one of the most destructive pests of coffee, causing severe global yield losses.
- CHT and CHT-like gene family in CBB has not been systematically characterized. Hence, a genome-wide analysis of CBB chitinases (*HhCHTs*) can identify key developmental enzymes and potential molecular targets for pest management, including RNAi-based strategies.

## METHODS



Identification *HhCHTs* using BLASTp and domain search



Domain and motif analysis and subcellular localization



Phylogenetic analysis and physicochemical characterization



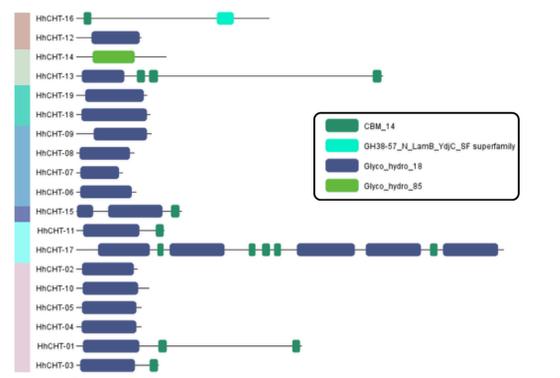
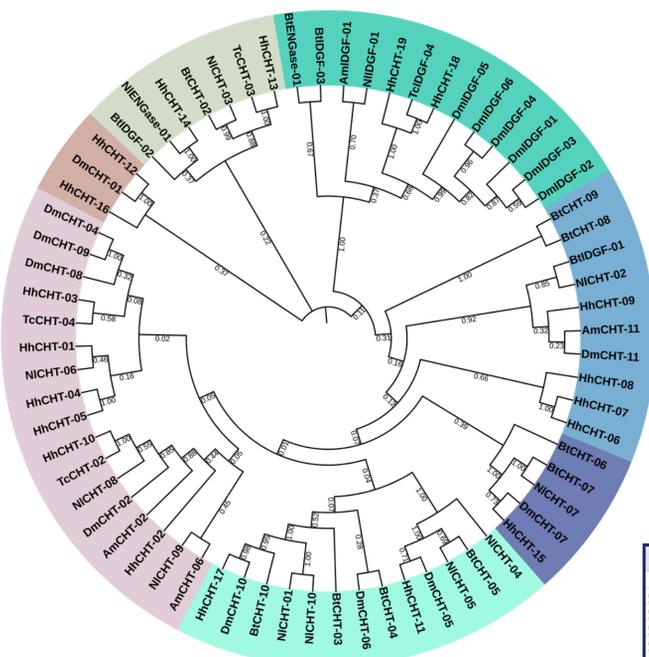
Protein-protein interaction & protein structure modeling

## RESULTS AND DISCUSSIONS

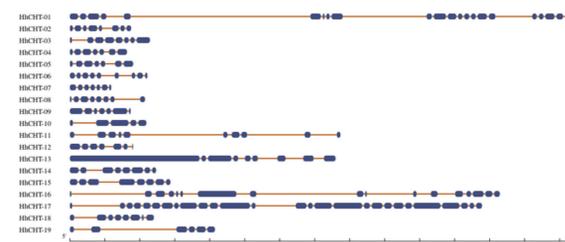
**Table 1.** A total of 19 *CHT* and *CHT-like* genes were identified in CBB, showing significant structural and physicochemical diversity. Variations in protein length and molecular weight suggested extensive domain changes. Most proteins were expected to be moderately stable, soluble, and suited for neutral to acidic environments.

Property	Average	Minimum	Maximum	Standard Deviation
Protein Length (amino acid)	730	287	2665	613.46
Theoretical pI	5.97	3.7	8.8	1.42
Molecular Weight (kDa)	82.20	31.60	303.89	69.26
Instability Index	35.69	21.33	53.78	8.3
Aliphatic Index	80.21	58.28	94.41	10.57
GRAVY	-0.34	-0.9	0.006	0.22

**Figure 1.** Phylogenetic analysis grouped the *HhCHTs* into seven clusters, together with chitinases from other insect taxa. Dm - *D. melanogaster*; Nl - *N. lugens*; Bt - *B. tabaci*; Tc - *T. castaneum*; Am - *A. mellifera*; ENGase - Endo- $\beta$ -N-acetylglucosaminidase; IDGF - imaginal disc growth factors.

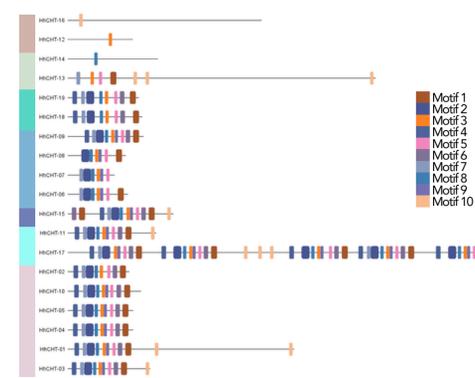


**Figure 3.** Gene structure analysis showed variability in exon-intron number, suggesting evolutionary adaptability and potential functional specialization.



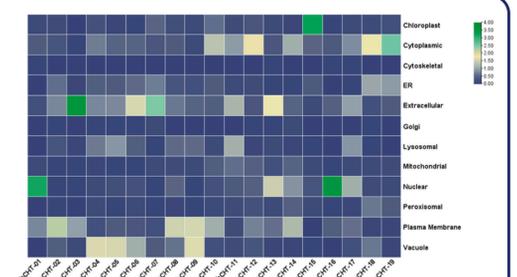
**Figure 5.** STRING 12.0 revealed a highly interconnected network, with several *HhCHTs* interacting closely with  $\beta$ -hexosaminidases and proteins containing GH20 or GH18 domains. Gene Ontology enrichment analysis further associated these interacting proteins with chitin metabolic and catabolic processes, cuticle organization, and chitin-binding and hydrolase activities, highlighting the coordinated role of *HhCHTs* in chitin metabolism.

GO-term	description	GO-term	description
GO:0006022	Aminoglycan metabolic process	GO:0004568	Chitinase activity
GO:0006032	Chitin catabolic process	GO:0008061	Chitin binding
GO:1901136	Carbohydrate derivative catabolic process	GO:0004553	Hydrolase activity, hydrolyzing O-glycosyl compounds
GO:0005975	Carbohydrate metabolic process	GO:0004563	beta-N-acetylhexosaminidase activity
GO:0018990	Ecdysis, chitin-based cuticle	GO:0102148	N-acetyl-beta-D-galactosaminidase activity

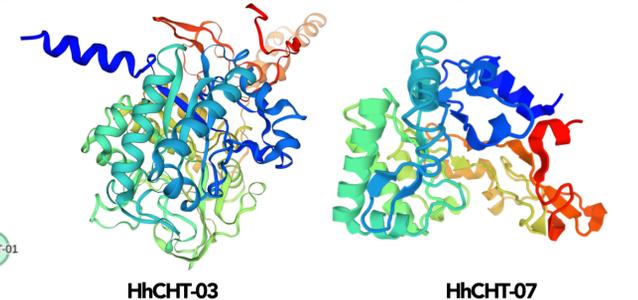


**Figure 2.** Domain and motif analyses revealed conservation of the GH18 catalytic domain among *HhCHTs*. In contrast, *HhCHT-16* and *-14* contained only CBM14 and GH85 domains, classifying both as chitinase-like proteins. Notably, *HhCHT-17* possessed multiple copies of both CBM14 and GH18 domains, suggesting a complex multidomain architecture.

**Figure 4.** CELLO v.2.5 predicted that *HhCHTs* are distributed across multiple cellular compartments. Specifically, *HhCHT-03*, *-06*, *-07*, *-11*, and *-13* are extracellular, which imply their roles in cuticle formation or remodeling.



**Figure 6.** *HhCHT-03* and *-07* were used as representatives for protein structure prediction in I-TASSER. Model quality was assessed using C-score, TM-score, and RMSD. The obtained C-score (-1.70 and 0.06) and TM-score (0.51-0.72) indicate a reliable global topology, whereas the estimated RMSD suggests moderate local deviations, primarily in flexible regions.



- HhCHT-03**
  - C-score = -1.70
  - Estimated TM-score = 0.51±0.15
  - Estimated RMSD = 11.4±4.5Å
- HhCHT-07**
  - C-score = 0.06
  - Estimated TM-score = 0.72±0.11
  - Estimated RMSD = 6.0±3.7Å

## CONCLUSION AND FUTURE WORKS

- This study presents the first comprehensive genome-wide characterization of the chitinase and chitinase-like gene family in the coffee berry borer (*Hypothenemus hampei*).
- Integrated analyses of gene structure, conserved motifs, domain architecture, phylogeny, subcellular localization, protein-protein interactions, and structure prediction highlight the functional diversity and evolutionary conservation of *HhCHTs*, underscoring their critical roles in chitin metabolism and cuticle-associated processes.

- Future work will focus on experimental validation of selected *HhCHTs* through RT-qPCR to assess spatiotemporal expression patterns, as well as functional characterization using gene silencing approaches.
- These efforts will provide deeper insights into the biological roles of *HhCHTs* and support the development of targeted, RNA interference-based molecular strategies for sustainable management of the coffee berry borer.

**Data Availability & References**  
The data and references that support the findings of this study are openly available in figshare: [doi:10.6084/m9.figshare.31287832](https://doi.org/10.6084/m9.figshare.31287832).