Musca domestica L. (Diptera: Muscidae)'de Katyon ATPaz Gen Ailesinin Genom Çapında Analizi

Genome-Wide Analysis of the *Cation ATPase* Gene Family in *Musca domestica* L. (Diptera: Muscidae)

Özet

Musca domestica L., patojenleri bulaştırarak ve önemli ekonomik kayıplara neden olarak insan ve hayvan sağlığını tehdit eden önemli bir zararlıdır. Geleneksel insektisitlere karşı yaygın direnç, vektör kontrolü için yeni moleküler hedeflerin keşfedilmesini zorunlu kılmaktadır. Hücresel homeostaz, sinir impulsu iletimi ve osmoregülasyon için temel olan Katyon ATPaz gen ailesi, umut verici bir hedefi temsil etmektedir. Bu çalışma, *M. domestica*, *Aedes albopictus ve Drosophila melanogaster* genomlarında 36 Katyon ATPaz genini tanımlayan genom çapında bir analiz sunmaktadır. Gen yapıları, korunmuş motifler ve fizikokimyasal özellikler kapsamlı bir şekilde karakterize edilmiştir. 13 *M. domestica* Katyon ATPaz proteini, 927 ile 1602 kalıntı arasında değişen amino asit uzunlukları ve 102.4 ile 179.8 kDa arasında değişen moleküler ağırlıklar sergilemiştir. Teorik izoelektrik noktaları 5.30 ile 6.11 arasında değişirken, alifatik indeks değerleri (84.72–101.78) yüksek alifatik içeriği ortaya koymuştur.

Karşılaştırmalı filogenetik analizler, evrimsel geçmişlerini ve yapısal farklılıklarını aydınlatmıştır. Dahası, 3B modelleme, etkileşim ağları ve Gen Ontolojisi (GO) analizleri ile işlevsel özellikleri değerlendirilmiştir. *In silico* anotasyonlar, inorganik katyonların transmembran taşınmasının yanı sıra kalsiyum ve metal iyonu homeostazındaki kritik rollere işaret etmektedir. Domain analizleri, sitozolik kalsiyum regülasyonuna aktif katılımı gösteren P-tipi ATPaz domainlerinin varlığını doğrulamıştır. *M. domestica*'daki Katyon ATPaz ailesinin ilk kapsamlı analizini sunan bu araştırma, yenilikçi vektör kontrol stratejilerinin geliştirilmesi için temel bir çerçeve oluşturmaktadır.

Anahtar kelimeler: *Musca domestica*, Katyon ATPaz, Gen ailesi, Biyoinformatik araçlar.

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Abstract

Musca domestica L. is a significant pest threatening human and animal health by transmitting pathogens and causing substantial economic losses. Widespread resistance to traditional insecticides necessitates the discovery of novel molecular targets for vector control. The *Cation ATP*ase gene family, essential for fundamental physiological processes such as cellular homeostasis, nerve impulse transmission, and osmoregulation, represents a promising target. This study presents a comprehensive genome-wide analysis identifying 36 *Cation ATP*ase genes across the genomes of *M. domestica, Aedes albopictus,* and *Drosophila melanogaster.* Gene structures, conserved motifs, and physicochemical properties were thoroughly characterized. The 13 *M. domestica* Cation ATPase proteins exhibited amino acid lengths ranging from 927 to 1602 residues and molecular weights ranging from 102.4 to 179.8 kDa. Their theoretical isoelectric points ranged from 5.30 to 6.11, while their aliphatic index values (84.72–101.78) revealed high aliphatic content. Comparative phylogenetic analyses elucidated their evolutionary history and structural divergence. Furthermore, 3D modeling, protein-protein interaction networks, and Gene Ontology analyses evaluated functional properties. *In silico* annotations indicate critical roles in calcium and metal ion homeostasis, alongside the transmembrane transport of inorganic cations. Domain analyses confirmed the presence of P-type ATPase domains, indicating active participation in cytosolic calcium regulation. Providing the first comprehensive analysis of the *Cation ATP*ase family in *M. domestica*, this research establishes a baseline framework for developing innovative vector control strategies.

Keywords: *Musca domestica, Cation ATP*ase, Gene family, Bioinformatic tools.

1.Introduction

Musca domestica L. (Diptera: Muscidae), a synanthropic species, is globally recognized as a major vector threatening both human and animal health. Due to its feeding and breeding habits as well as its high ecological adaptability, it serves as a primary mechanical vector for more than 100 pathogens [1,2]. In addition to public health concerns, the stress and disturbance caused by high fly populations in intensive livestock facilities are associated with suppressed feed intake in animals, ultimately leading to significant economic losses in milk and meat production [3]. For decades, the management of *M. domestica* populations has relied heavily on neurotoxic chemical insecticides, including organophosphates, carbamates, pyrethroids, and neonicotinoids. However, the emergence of widespread and high-level resistance in *M. domestica* populations has been driven by the intensive, repetitive, and often improper application of these agents [4]. Molecular investigations reveal that resistance is primarily associated with target site insensitivity, overexpression of metabolic detoxification enzymes such as cytochrome P450s (CYPs), glutathione S-transferases (GSTs), and esterases), and reduced cuticular penetration (cuticle thickening) [5,6]. The growing resistance crisis to current insecticide classes has necessitated a strategic shift towards the exploration of novel molecular targets in insect physiology that remain underexploited. In the pursuit of novel targets, the *Cation ATP*ase gene family, specifically Na+/K+ -ATPase and Ca2+ -ATPase, emerges as a promising candidate. These enzymes are recognized as integral components of cellular homeostasis, neural transmission, and osmoregulation [7]. In insects, Na+/K+ -ATPase plays a pivotal role not only within the nervous system but also in fluid secretion and nutrient absorption. Recent toxicological studies have demonstrated that plant-derived bioactive compounds and certain next-generation insecticides exert their toxic effects

by targeting and inhibiting these enzymes [8,9]. Furthermore, adaptive modulations in the expression levels of ion transport genes have been proposed to facilitate the survival of *M. domestica* under environmental or chemical stress [10]. Therefore, elucidating the structure and expression profiles of *Cation ATP*ase genes is crucial for both understanding resistance mechanisms and developing sustainable control strategies directed at this gene family.

In the present study, a comprehensive genome-wide analysis was conducted to identify a total of 36 distinct *Cation ATP*ase genes within the genomes of *M. domestica*, *Aedes albopictus Skuse,* and *Drosophila melanogaster* Meigen. The gene structures, along with the conserved motifs and physicochemical properties of their encoded proteins, were subjected to a detailed examination. Phylogenetic trees of the Cation ATPase proteins across the analyzed species were reconstructed to analyze their evolutionary relationships and orthology. Furthermore, three-dimensional structural modeling, protein-protein interaction networks, and Gene Ontology enrichment analyses were performed to predict their functional characteristics. This research presents the first systematic characterization of the *Cation ATP*ase family in *M. domestica* and provides fundamental data for the development of novel vector control strategies.

2. Materials and Methods

2.1. Identification of Cation ATPase Genes

To initiate the identification process, the Pfam accession numbers corresponding to the *Cation ATP*ase gene family (PF00689 and PF00690) were determined via the InterPro database (https://www.ebi.ac.uk/interpro) [11]. Subsequently, the coding DNA sequences (CDS) and protein sequences for *Aedes albopictus (Aalb)* [12], *M. domestica (Mdom) [13],* and *D. melanogaster (Dmen)* [14] were retrieved from the InsectBase 2.0 database (https://v2.insect-genome.com) [15]. To validate the identity of the retrieved sequences, the presence of *Cation ATP*ase domains within the respective protein sequences was verified using the SMART database (https://smart.embl.de) [16].

2.2. Sequence Alignment and Phylogenetic Analyses

The Cation ATPase protein sequences from the studied species were aligned using the ClustalW algorithm (https://www.genome.jp/tools-bin/clustalw) [17]. To enhance alignment precision, the BLOSUM weight matrix was applied using the 'slow/accurate' configuration. Subsequently, phylogenetic reconstruction was performed using an approximate Maximum Likelihood (ML) approach via FastTree within the ETE3 (v3.1.3) pipeline, and nodal reliability was assessed using SH-like local support values. Tree topology was visualized usingN the iTOL platform (https://itol.embl.de) [18].

2.3. Characteristics of Cation ATPase Proteins

Physicochemical properties, including amino acid sequence length, molecular weight (MW), theoretical isoelectric point (pI), instability index, aliphatic index, and Grand Average of Hydropathicity (GRAVY) scores, were computed using the ProtParam tool (https://web.expasy.org/protparam) [19] for the Cation ATPase proteins identified in the studied species.

2.4. Structure of Cation ATPase Genes

To elucidate the genomic architecture of the Cation ATPase genes, their exon-intron arrangements were visualized using GSDS v2.0 (https://gsds.gao-lab.org) [20]. The analysis was conducted following the methodology detailed in our previous study [21].

2.5. Identification of Conserved Motifs

Conserved motifs within the Cation ATPase protein sequences of the examined species were identified using the MEME Suite v5.5.9 (https://meme-suite.org/meme/tools/meme) [22]. The analysis was performed using the parameters detailed in our previous study [21].

2.6. 3D Structure, Protein-Protein Interactions, and Gene Ontology Analysis of Cation ATPase Proteins

The three-dimensional (3D) structural models of the *M. domestica* Cation ATPase proteins were predicted using the Phyre2 web server (https://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) [23]. To ensure model reliability, predictions with confidence scores and sequence coverage below 70% were excluded from further structural analyses.

Subsequently, protein-protein interaction (PPI) networks were constructed using the STRING database (https://string-db.org/) [24]. Functional enrichment analyses based on Gene Ontology (GO) terms were conducted using the STRING platform.

3. Results and Discussion

3.1. Identification of Cation ATPase Genes

A total of 36 *Cation ATP*ase genes were identified across three dipteran species, distributed as follows: 13 genes in *M. domestica*, 11 in *Ae*. albopictus, and 12 in *D. melanogaster*. Their characteristic domain architectures were verified using the SMART database.

3.2. Sequence Alignment and Phylogenetic Analyses

Phylogenetic analysis clustered the identified Cation ATPase proteins into two major clades (Figure 1). This division corresponds to differences in protein domain architecture. The first major clade consists of Cation ATPase proteins that contain the Hydrolase_3 domain, whereas the second clade comprises proteins that lack this domain. This domain-based segregation is strongly supported by the high SH-like local support values on the principal branches, indicating a robust phylogenetic topology. These findings underscore the impact of domain architecture in the evolutionary divergence and functional specialization of ATPase proteins [25,26]. Examination of the tree topology indicates that the diversification of the *Cation ATP*ase gene family in dipteran species has been largely shaped by lineage-specific expansion. In particular, the clustering of multiple paralogs from the same species, supported by strong SH-like local support values, indicates that species-specific gene duplication events have played a significant role in driving this expansion [27, 28]. This evolutionary pattern is consistent with findings across insect genomes, where gene families involved in ion transport and cellular homeostasis commonly undergo lineage-specific expansion and diversification [26, 29].

The phylogenetic relationships among Cation ATPase proteins were evaluated by comparative analysis of *M. domestica* sequences together with homologs from *D. melanogaster* and *A. albopictus*. The circular phylogenetic tree presented in Figure 1 shows that *M. domestica* Cation ATPase proteins form distinct clustering patterns with homologous sequences from other dipteran species. Percent identity values among *M. domestica* paralogs ranged from 50.8% to 100%, whereas comparisons with *D. melanogaster* and *A. albopictus* sequences ranged from 31.5% to 80.8%. The high support values observed in several branches indicate conserved phylogenetic relationships among specific Cation ATPase members across species. In contrast, the more distinct placement of Dmen-N-CATPase-01 suggests a higher degree of sequence divergence relative to the other homologs. These findings indicate that the *M. domestica* Cation ATPase family includes both conserved homologous members and proteins showing copy- or species-level divergence.

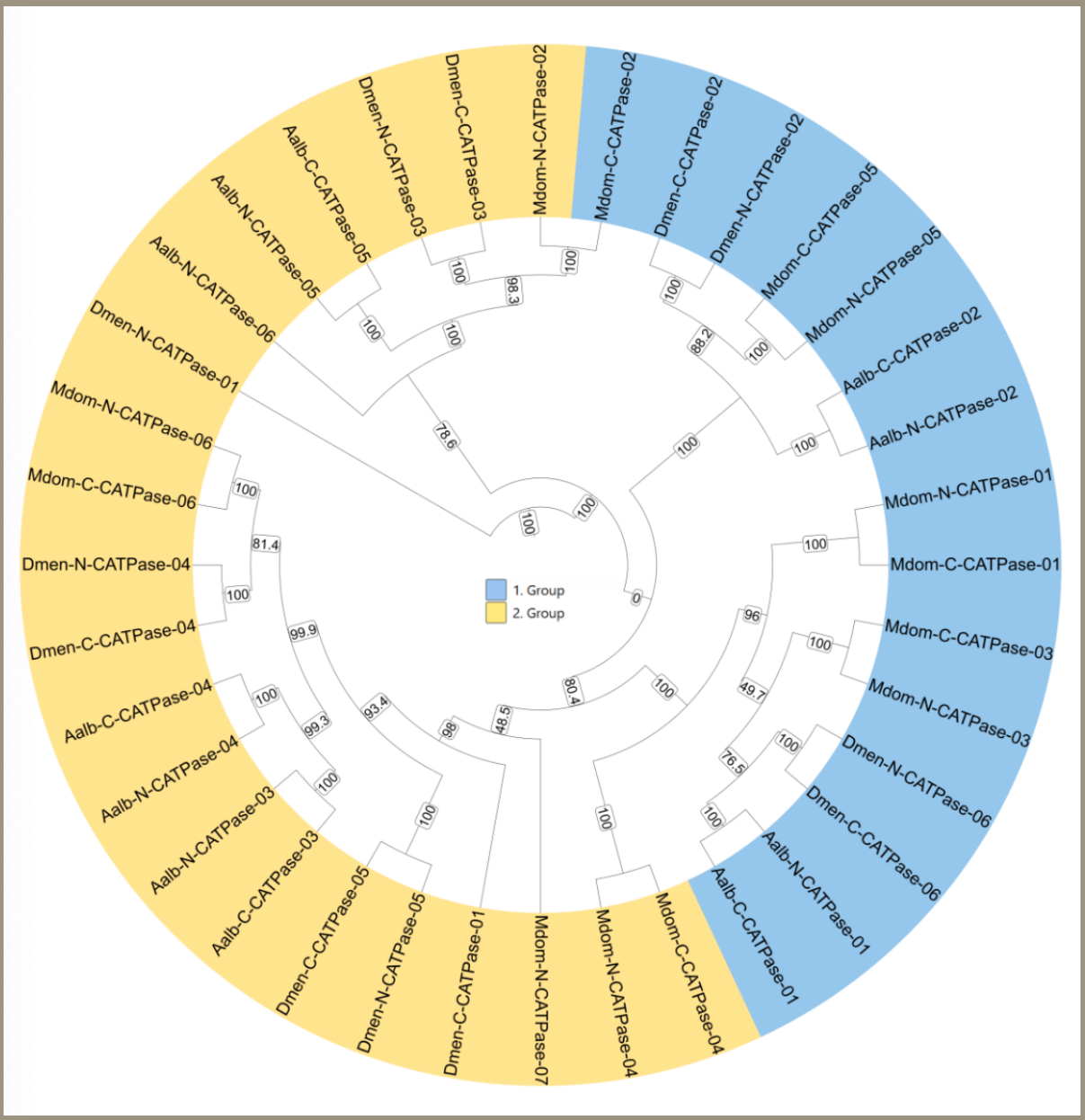

Figure 1. Phylogenetic tree illustrating the relationships among *Cation ATP*ase family genes.

3.3. Characteristics of Cation ATPase Proteins

To characterize the physicochemical properties of the 13 Cation ATPase proteins identified in *M. domestica,* a comprehensive analysis of their biophysical parameters was conducted (Table 1). These proteins exhibited amino acid lengths ranging from 927 to 1602 residues, with molecular weights varying between 102.4 kDa and 179.8 kDa. This broad distribution indicates a considerable size variation among the Cation ATPase proteins of *M.domestica*, which is commonly observed in large ion-transporting protein families [26]. The theoretical isoelectric point (pI) values ranged from 5.30 to 6.11, indicating that all proteins possess a net acidic character. This acidic pI profile is consistent with the established localization of Cation ATPase proteins within cytosolic or membrane-associated environments, where their activity is likely regulated by protein–protein interactions, rather than direct interactions with negatively charged nucleic acids [25]. *In silico* stability analysis predicted 15 cation ATPase proteins to be stable, whereas only

Mdom-N-CATPase-07 was classified as unstable, exhibiting a relatively high instability index of 45.52. This observation indicates that Mdom-N-CATPase-07 may possess a more dynamic structural conformation, potentially facilitating a specialized role in regulatory processes [27]. The aliphatic index values ranged from 84.72 to 101.78, indicating a relatively high aliphatic content for most proteins, which indicates enhanced thermostability. High aliphatic index values are generally associated with increased structural stability across a wide temperature range, which may confer an adaptive advantage to poikilothermic organisms such as *M. domestica* exposed to fluctuating environmental temperatures [30]. Furthermore, the GRAVY values were predominantly close to zero or negative, indicating that the Cation ATPase proteins exhibit an overall hydrophilic tendency, which likely reflects the presence of large cytosolic catalytic domains. This hydrophilic nature is consistent with their structural organization as integral membrane proteins possessing substantial cytosolic components, functioning in aqueous cellular environments and participating in ion transport and energy metabolism [26].

Table 1: Physicochemical properties of the Cation ATPase proteins in *M. domestica.*

Sequence Name	Number of Amino Acids	Molecular Weight (Da)	Molecular Weight (kDa)	Theoretical pI	Instability Index	Stability	Aliphatic Index	GRAVY
Mdom-C-CATPase-01	1055	116225.16	116,225	5.81	38.95	Stable	99.35	-0.005
Mdom-C-CATPase-02	927	102447.47	102,447	6.03	37.17	Stable	101.17	0.107
Mdom-C-CATPase-03	1031	112821.20	112,821	5.73	32.52	Stable	101.78	0.052
Mdom-C-CATPase-04	1025	112575.11	112,575	6.11	33.59	Stable	97.94	0.011
Mdom-C-CATPase-05	937	102587.05	102,587	5.30	34.84	Stable	94.92	0.005

Mdom-C-CATPase-06	1040	115444.81	115,445	5.34	34.84	Stable	93.31	-0.041
Mdom-N-CATPase-01	1055	116225.16	116,225	5.81	38.95	Stable	99.35	-0.005
Mdom-N-CATPase-02	927	102447.47	102,447	6.03	37.17	Stable	101.17	0.107
Mdom-N-CATPase-03	1031	112821.20	112,821	5.73	32.52	Stable	101.78	0.052
Mdom-N-CATPase-04	1025	112575.11	112,575	6.11	33.59	Stable	97.94	0.011
Mdom-N-CATPase-05	937	102587.05	102,587	5.30	34.84	Stable	94.92	0.005
Mdom-N-CATPase-06	1040	115444.81	115,445	5.34	34.84	Stable	93.31	-0.041
Mdom-N-CATPase-07	1602	179832.65	179,833	5.91	45.52	Unstable	84.72	-0.233

(pI: Theoretical isoelectric point, GRAVY: Grand Average of Hydropathy)

3.4. Structure of Cation ATPase Genes

To gain deeper insight into the structural evolution of the *Cation ATP*ase gene family, the exon–intron organization of 36 such genes identified in *M. domestica, Ae. albopictus,* and *D. melanogaster* was comparatively analyzed (Figure 2). The analysis revealed a strong correspondence between gene structure and their phylogenetic relationships. Overall, the *Cation ATP*ase genes exhibited considerable variation in exon–intron architecture, reflecting the structural diversification of this gene family during dipteran evolution. Despite this variability, genes within the same phylogenetic clades generally displayed highly similar exon–intron patterns, indicating a conserved structural framework. This concordance between phylogeny and gene organization indicates that the observed diversification was driven by lineage-specific duplication events and subsequent structural divergence, whereas structural conservation was maintained within specific clades. In *M. domestica*, several paralogous *Cation ATP*ase genes exhibited nearly identical exon counts, intron positions, and coding sequence lengths, strongly supporting recent gene duplications. Similar structural conservation was also evident in *Ae. albopictus* and *D. melanogaster*, where closely related orthologous genes maintained comparable gene architectures across species. Such conservation implies strong selective constraints and highlights the evolutionary stability of core structural features within the Cation ATPase family. Conversely, certain clades displayed increased structural complexity, characterized by increased exon counts and longer intronic regions. These features were particularly pronounced in

specific subclades, indicating that intron gain, exon duplication, or exon shuffling may have contributed to the structural expansion of these genes. These genomic modifications are widely recognized as key mechanisms driving functional diversification in large multigene families [31,32,33].

Additionally, variations in untranslated region (UTR) length and organization were observed among the analyzed genes. The presence of extended 5′ and 3′ UTRs in some Cation ATPase members may indicate species- or gene-specific regulatory adaptations, potentially influencing post-transcriptional regulation, such as mRNA stability or translational efficiency. Collectively, the exon–intron structure analysis indicates that the structural evolution of the *Cation ATP*ase gene family in Diptera has been shaped by a combination of gene duplication, structural conservation, and selective architectural diversification. These findings further support the notion that the Cation *ATP*ase family represents a structurally dynamic yet evolutionarily constrained group.

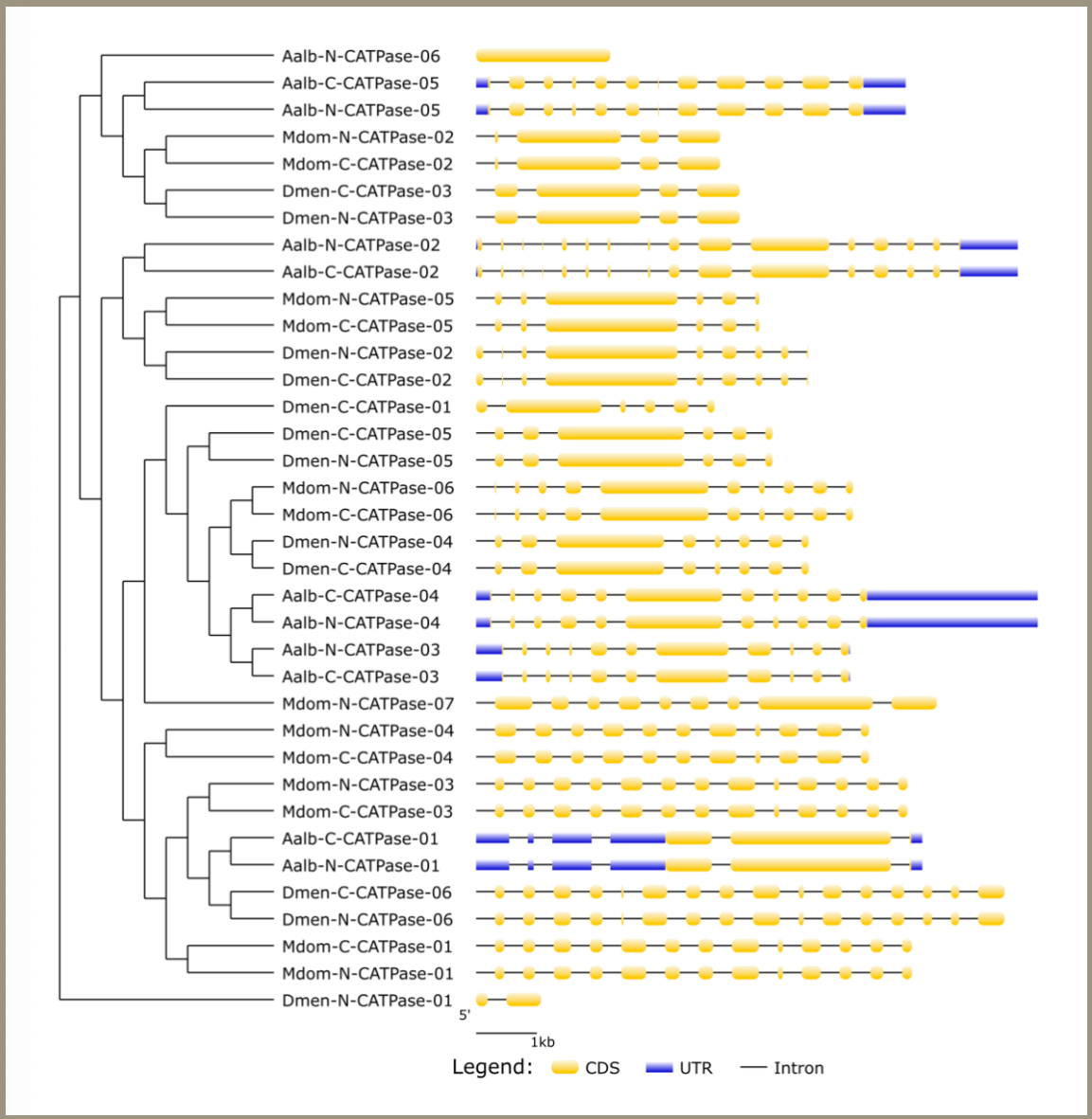

Figure 2. Exon–intron organizations of *Cation ATP*ase genes, visualized using GSDS.

3.5. Identification of Conserved Motifs

To identify conserved sequence patterns at the protein sequence level, a motif analysis of the 13 Cation ATPase proteins identified in *M. domestica* was performed using the MEME Suite (Figure 3). The analysis revealed a total of ten conserved motifs distributed across the analyzed proteins. All identified motifs exhibited highly significant *p*-values, indicating that they represent evolutionarily conserved features with potential functional significance. Furthermore, motif distribution exhibited considerable variation in both motif number and arrangement among Cation ATPase family members. While most proteins displayed multiple conserved motifs, others contained significantly fewer. Notably, Mdom-N-CATPase-07 contained only a single conserved motif; this severe truncation indicates it may represent an incomplete genomic assembly, a pseudogene, or a catalytically inactive variant. Truncated isoforms derived from full-length P-type pumps can exert a dominant-negative regulatory effect by forming heterodimers with active pumps [41,44]. In this context, the presence of only a single motif in Mdom-N-CATPase-07 raises the possibility that this protein, rather than functioning as a catalytic pump, may act as an auxiliary subunit modulating the membrane trafficking or activity of other Cation ATPases. This structural diversity may reflect the genetic basis of physiological adaptation mechanisms in *M. domestica*, although functional assays are required to link such variants to specific environmental stress or insecticide resistance. In contrast, several proteins, including Mdom-C-CATPase-01 through Mdom-C-CATPase-04 and Mdom-N-CATPase-01 through Mdom-N-CATPase-04, exhibited highly similar motif compositions and linear arrangements. This strong conservation of motif architecture supports a close evolutionary relationship and implies functional similarity.

Furthermore, the consistent positioning of most motifs highlights conserved functional regions within the Cation ATPase family. These conserved motifs are likely associated with essential domains responsible for ATP hydrolysis and ion translocation. Conversely, motifs restricted to specific paralogs may contribute to functional specialization, facilitating differential regulatory mechanisms or substrate specificities. Collectively, the motif analysis demonstrates that *M. domestica* Cation ATPase proteins possess both highly conserved core motifs and paralog-specific motif configurations, reflecting a dynamic evolutionary balance between core structural maintenance and functional diversification.

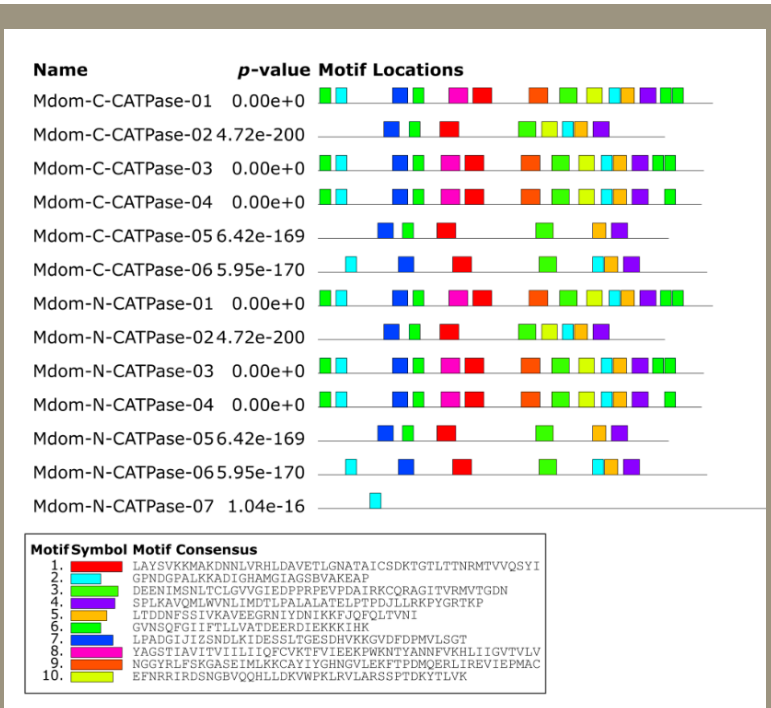

Figure 3. The conserved motifs of Cation ATPase proteins in *M. domestica*.

3.6. 3D Structure, Protein-Protein Interactions, and Gene Ontology Analysis of Cation ATPase Proteins

To place the Cation ATPase proteins of *M. domestica* within a functional cellular context, protein–protein interaction (PPI) networks were constructed using the STRING database for five representative members each from the Mdom-N-CATPase and Mdom-C-CATPase subgroups (Figure 4). This comparative analysis aimed to assess whether clade-specific structural variations are reflected in distinct interaction patterns and functional roles. The constructed interaction networks revealed that members of both the Mdom-N-CATPase and Mdom-C-CATPase subgroups form tightly interconnected clusters. Within each group, high-confidence interactions were observed, indicating a high degree of functional coherence and cooperative activity. The dense connectivity patterns indicate that proteins from both subgroups participate in shared or closely related biological processes. In the Mdom-N-CATPase network, all five analyzed paralogs exhibited extensive mutual interactions, forming a dense interaction module. A similar interaction architecture was observed in the Mdom-C-CATPase network, where all five analyzed paralogs also displayed high-confidence reciprocal interactions. The parallel organization of these two networks implies that both subgroups maintain conserved interaction interfaces within the Cation ATPase family [26,34,35]. The comparable network topology observed between the Mdom-N-CATPase and Mdom-C-CATPase subgroups indicates that clade-specific divergence has not disrupted the core interaction capacity of these proteins. Instead, the preservation of dense interaction networks supports the notion that members of both subgroups are functionally active and may act in concert to mediate protein complex formation or regulatory processes.

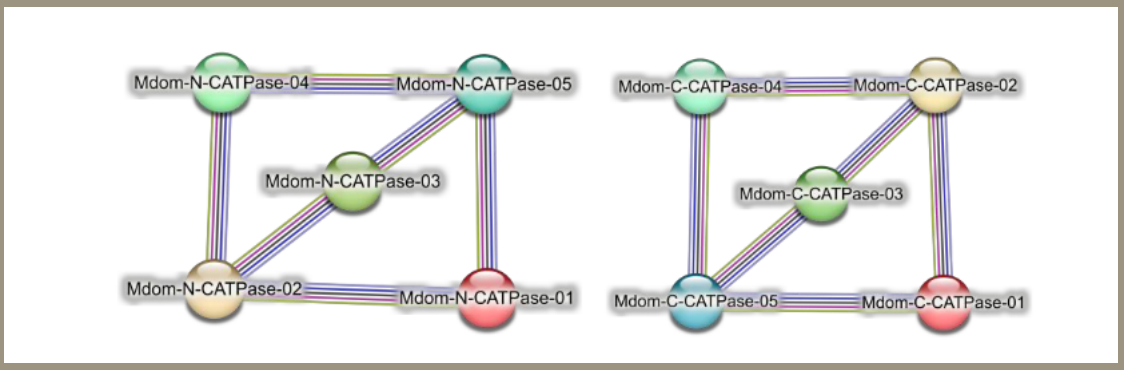

Figure 3. Protein-Protein Interactions of the Mdom-N-CATPase and Mdom-C-CATPase subgroups.

To investigate structural conservation, three-dimensional (3D) models of 13 representative proteins were generated using Phyre2. These structures exhibit a high degree of structural similarity, and the characteristic transmembrane helices and cytosolic domains are largely conserved (Figure 5).

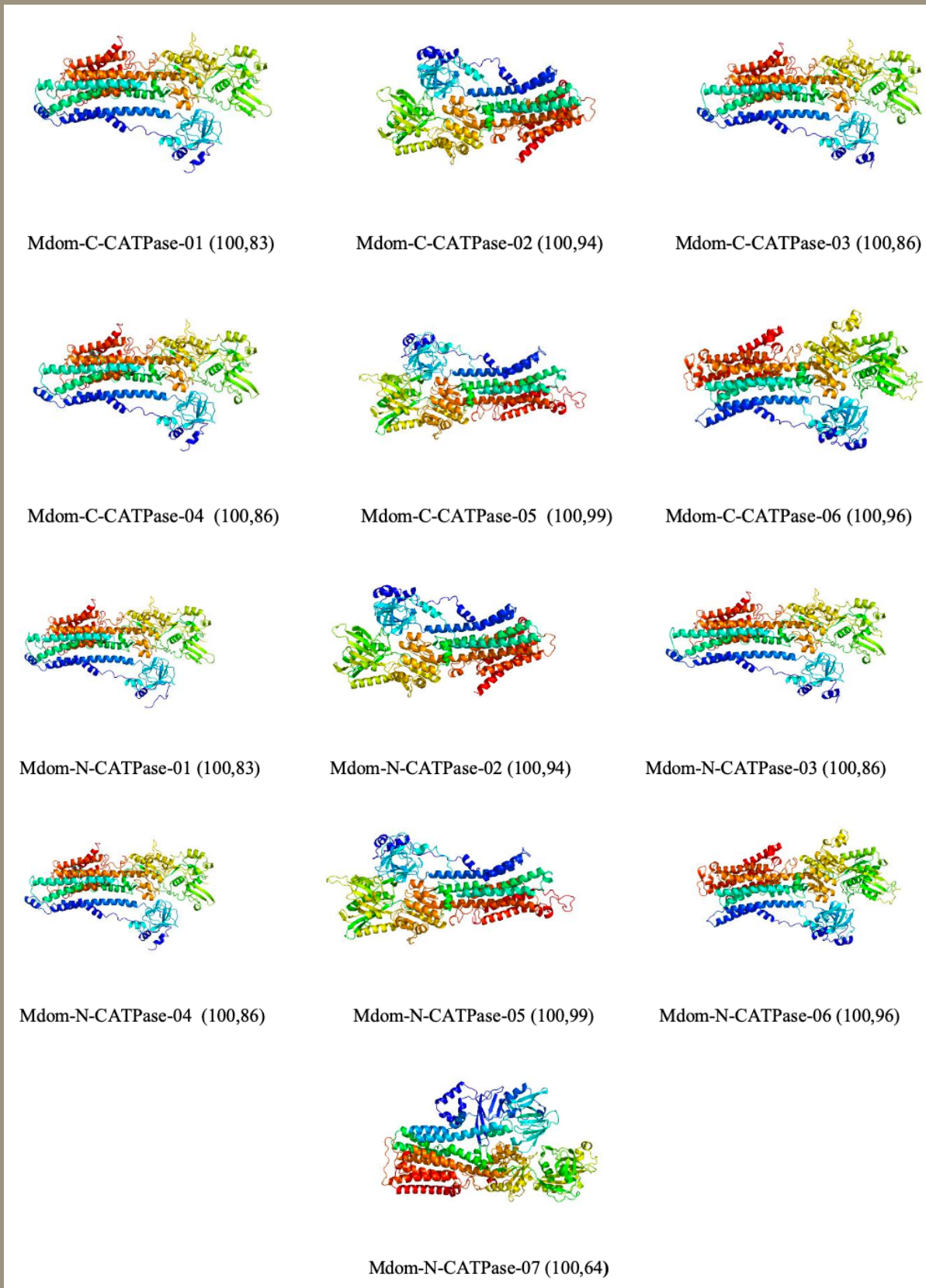

Figure 5. Three-dimensional structures of representative *M. domestica* Cation ATPase proteins. The values in parentheses represent the confidence and coverage, respectively.

Gene Ontology (GO) enrichment analysis of the Mdom-C-CATPase subgroup indicated that these proteins function as P-type cation pumps, demonstrating a particularly high enrichment for P-type ion transporter activity and ATPase-coupled cation transmembrane transport (Figure 6). P-type ATPases are ion pumps that drive ion transport by undergoing conformational changes via ATP-mediated phosphorylation during the transport cycle [35]. Furthermore, Biological Process annotations (Figure 7) indicate that, in addition to inorganic cation transmembrane transport, these proteins are involved in the maintenance of cellular calcium and metal ion homeostasis. These findings indicate that these proteins likely represent plasma membrane or sarco/endoplasmic reticulum calcium pumps, which is consistent with the canonical roles of these pumps in modulating intracellular calcium gradients [36, 37]. Furthermore, the significant enrichment for cytosolic calcium regulation highlights the active role of Mdom-C-CATPase subgroup members in intracellular calcium signaling. Given the critical role of calcium homeostasis in muscle contraction, nerve transmission, and the regulation of detoxification enzymes in *M. domestica* and other insect species [38], this subgroup constitutes a promising molecular target for the development of novel pump-inhibiting insecticides.

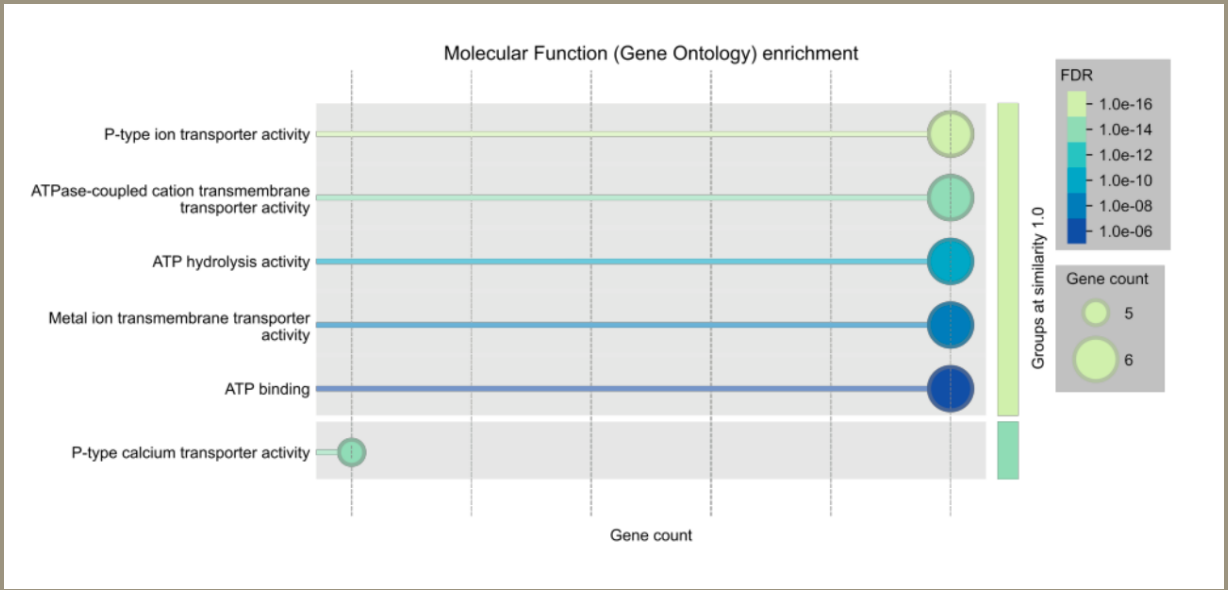

Figure 6. Gene Ontology Molecular Function analysis of the Mdom-C-CATPase subgroup in *M. domestica*.

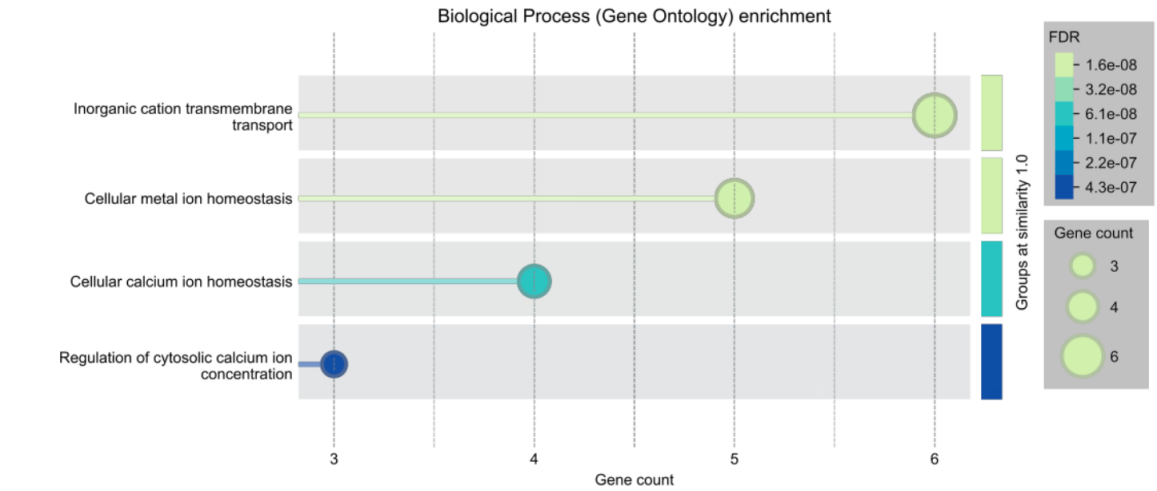

Figure 7. Gene Ontology Biological Process analysis of the Mdom-C-CATPase subgroup in *M. domestica.*

The Gene Ontology enrichment analysis of the Mdom-N-CATPase subgroup indicated functions associated with catalytic activity and ion transport. Within the Molecular Function category (Figure 8), this subgroup was significantly enriched in the terms 'P-type transmembrane transporter activity' (FDR < 1.0 x 10-19) and 'ATPase-coupled cation transmembrane transporter activity'. All seven analyzed members of the Mdom-N-CATPase subgroup were annotated with 'ATP hydrolysis' and 'ATP binding' terms. As observed in the Mdom-C-CATPase subgroup, five members of this subgroup were also predicted to exhibit 'P-type calcium transporter activity'. Notably, enrichment for the 'metal ion transmembrane transporter activity' term among six members of the Mdom-N-CATPase subgroup was highly significant. The Biological Process annotations presented in Figure 9 indicate that the Mdom-N-CATPase subgroup plays a significant functional role. The most significantly enriched process was 'cellular metal ion homeostasis' (FDR < 1.0 x 10-09), while other significantly enriched terms included 'inorganic cation transmembrane transport' and 'cellular calcium ion homeostasis'. In particular, their association with the regulation of cytosolic calcium ion concentration indicates that members of this subgroup function not only as primary transporters but also as key modulators of intracellular calcium signaling.

Functional enrichment analysis of the Mdom-N-CATPase subgroup in *M. domestica* revealed that these proteins harbor structural motifs essential for their catalytic activity and conformational transitions. The highly significant enrichment of P-type transmembrane transporter activity (FDR < 1.0 x 10-19) within the Molecular Function category for this subgroup aligns with the presence of the Actuator (A) domain, a characteristic structure of P-type ATPases [39]. The N-terminal regions of P-type pumps typically harbor the A domain, characterized by the conserved TGES motif. This motif facilitates the dephosphorylation of the phosphorylation (P) domain, a process essential for governing the E1-E2 conformational transitions. Consequently, the A domain plays a central role in coupling ATP hydrolysis to ion transport [40]. The significant enrichment of cellular metal ion homeostasis and inorganic cation transmembrane transport processes indicates that members of this subgroup function as general cation regulators in *M. domestica*, while the specific enrichment of the regulation of cytosolic calcium ion concentration highlights that certain paralogs have specialized as calcium pumps. In insects, transcripts encoding these pumps frequently undergo alternative splicing within their N-terminal regions and this structural variability may affect the pumps ion affinity

or transport rate in different tissues [41, 42]. Furthermore, the robust enrichment of ATP binding and ATP hydrolysis activities in the Mdom-N-CATPase subgroup is consistent with the canonical roles of the N-terminal and Actuator domains in driving the catalytic cycle, contrasting with the autoinhibitory functions often associated with C-terminal tail interactions
[36, 40, 43].

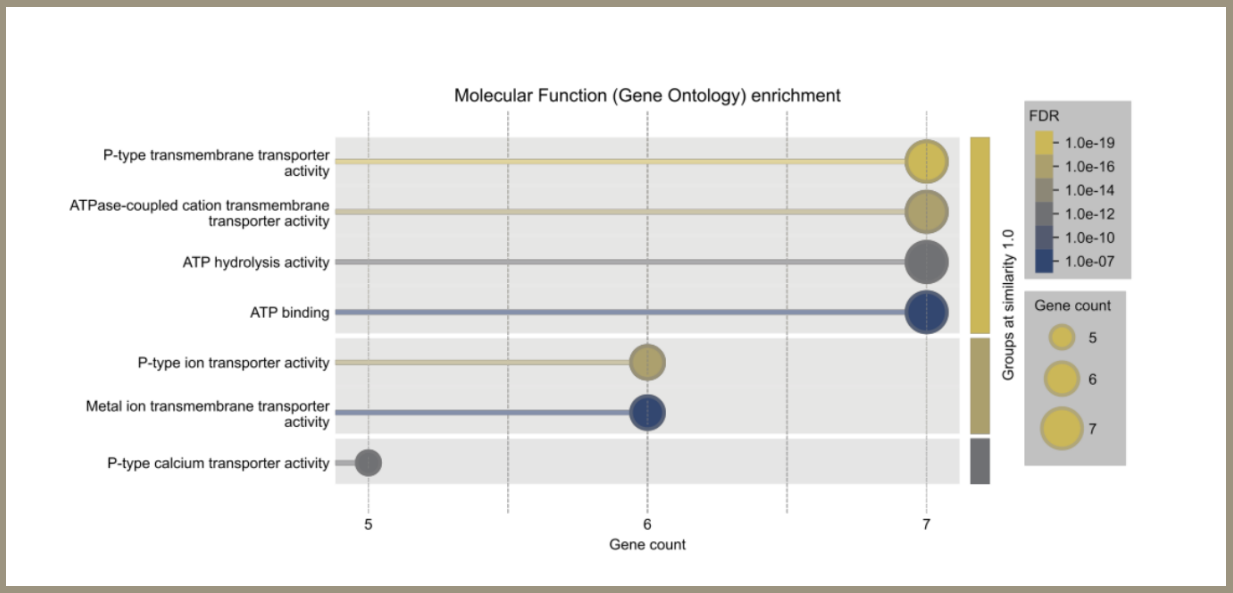

Figure 8. Gene Ontology Molecular Function analysis of the Mdom-N-CATPase subgroup in *M. domestica.*

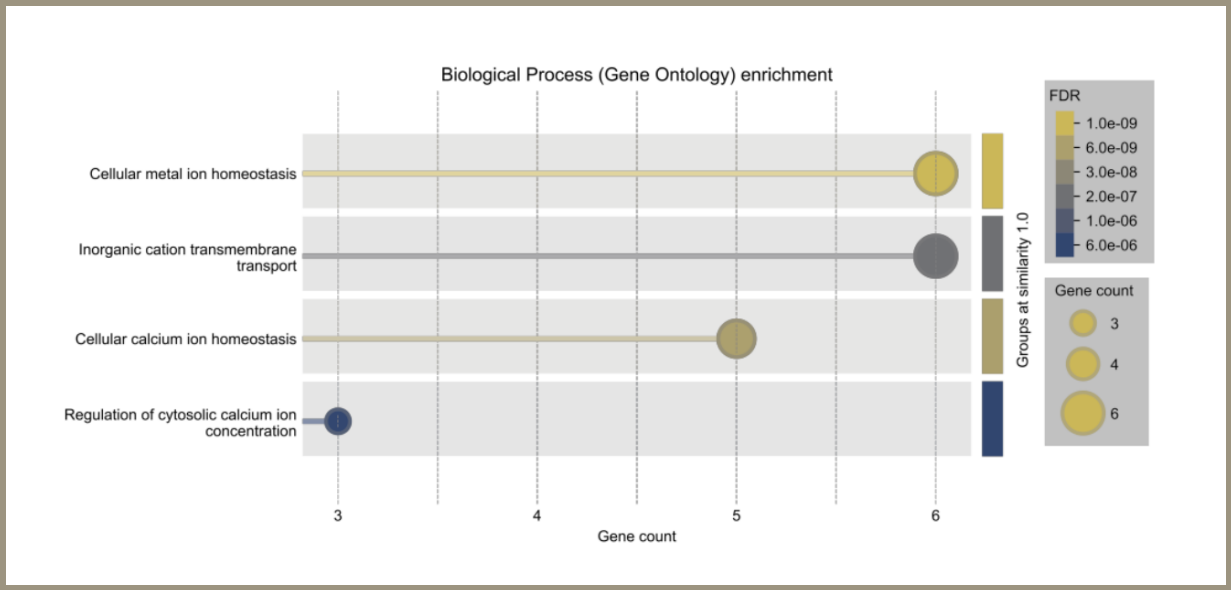

Figure 9. Gene Ontology Biological Process analysis of the Mdom-N-CATPase subgroup in *M. domestica.*

In conclusion, the comprehensive in silico characterization of the Cation ATPase family in Musca domestica, supported by 3D structural modeling and domain-level analyses, provides a computational basis for evaluating these proteins as candidate molecular targets in vector control studies. The structural features, conserved protein regions, and predicted interaction networks identified in this study may support the prioritization of plant-derived bioactive compounds and bio-engineered inhibitor candidates in structure-based virtual screening approaches. However, these findings should not be interpreted as direct evidence of insecticidal activity, but rather as a target-oriented preliminary framework that requires experimental validation. The integration of genomic, structural biology, pharmaceutical biotechnology, and phytopharmacological approaches may guide future efforts to develop more selective, sustainable, and environmentally compatible bio-insecticide strategies against *M. domestica.*

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