

## ARTICLE / INVESTIGACIÓN

# Identification of novel-vector control target proteins of *Aedes sp.*: A Systems Network Biology Approach

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**Abstract:** *Aedes* is an important vector for various viruses that cause dengue, chikungunya and Zika, which affect human health globally. Due to regular outbreaks of these diseases worldwide, there is a need to identify essential vector proteins that are critical for the survival of the vector, which may be targeted to control the spread of vector-borne disease (VBD). *In silico* computational methods involving comparative proteomics, analysis of orthologous proteins common amongst members of *Aedes* genus and protein-protein interaction (PPI) pathway were used to identify essential proteins that could act as novel therapeutic candidates. Twenty-three conserved proteins between *A. aegypti* and *A. albopictus* were identified from a BLASTP search with an e-value threshold of 0.005, and their PPI networks were constructed in the STRING database. The merged network was analyzed using various Cytoscape plugins viz. ClusterONE, Cytohubba and MCODE. Thirty-one hub proteins were identified from the system's network biology analysis, and detailed data and literature mining were carried out. Twelve novel vector-control target proteins of *A. aegypti*, having no human homologs, were determined in the present study that can effectively act as potential therapeutic candidates for drug design and vaccine development.

**Key words:** Vector-borne disease (VBD), Therapeutic candidates, Network Systems Biology, Protein-protein interactions (PPI), Computational biology.

## Introduction

Vector-borne diseases (VBD) are significant contributors to the global disease burden and account for more than 17% of all infectious diseases, causing more than 700000 deaths annually<sup>1</sup>. An important taxon of arthropods that affects human health globally by acting as a vector of arthropod-borne viruses are mosquitoes belonging to the *Aedes* genus. It's been over five decades that *Aedes*-borne diseases, such as dengue, Zika, chikungunya, and yellow fever, have emerged and re-emerged globally<sup>2,3</sup>. The global population at risk is expanding in concert with changes in the distribution of two key vectors: *Aedes aegypti* and *Aedes albopictus*. Their desiccation-resistant eggs facilitate the invasion of *Aedes* into new areas, and the distribution of these species is largely driven by both human movement and the presence of a suitable climate<sup>4</sup>.

Dengue virus has been reported to cause about 390 million human infections per year; the chikungunya virus spread worldwide in the early 2000s, with recent reports of the spread of Zika virus, while yellow fever has resurged in Africa and America. In most cases, vector control is the only means to cope with various foreign diseases.

The feeding preference of *Aedes* is only mammalian species, including birds, mammals and humans, which increases during the multiplication phase of their life cycle. The transmission of viruses from the mosquitoes to the organisms sharing the same niche is due to the bite of the infected vector, which releases the virus into the body of the recipient organism. *Aedes aegypti* was earlier found in forested territories, utilizing tree openings as natural surroundings as their habi-

tat<sup>5</sup>. Now, they have transformed and evolved along with the increasing urbanization and have found their habitat in leftover jars, tires, broken water-filled buckets and even in the tanks or ponds having stagnant water. They usually prefer areas where they can easily find their host and feed on them eventually<sup>6</sup>.

Another member of the *Aedes* genus is now listed as one of the top 100 invasive species by the Invasive Species Specialist Group<sup>7</sup> on account of being a suitable vector for causing human diseases chikungunya virus, dengue virus, and dirofilariasis is *A. albopictus*. Experimental evidence has shown that its preferred host is humans, but being an opportunistic feeder, it can depend upon other hosts such as domestic and wild animals, birds, reptiles, and amphibians for its food cycle<sup>8,9</sup>.

Vector-borne diseases (VBD) are a worldwide concern and a significant cause of human morbidity and mortality, causing immeasurable suffering and impeding economic development. To protect humans from these destructive diseases, controlling insect vectors is often the most suitable and sometimes the only option available. Many control strategies have been designed and followed since centuries ago, but despite such efforts and programs, mosquito-borne diseases are prospering throughout the world<sup>10</sup>.

The amalgamation of "omics" data with computational biology tools in network systems biology offers the advantage of speedy identification of critical vector proteins necessary for the survival of the vectors, which may play an essential role in controlling vector populations. *In silico* methods such as comparative and subtractive genomics can be effectively be used

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to identify proteins/genes of vectors that are non-homologous to their human hosts. These proteins/genes may then be targeted using drugs and vaccines by inhibiting critical vector pathways and preventing host-seeking and feeding behaviors.

The present study was undertaken to identify critical vector-control target proteins for the *Aedes* genus using a network systems biology approach. Such an approach offers the opportunity to ascertain potential targets for drug development and vaccine design that would facilitate the production of novel therapeutics specific against the disease-carrying vectors.

## Materials and methods

### Proteome data mining

The UniProt KB database (<https://www.uniprot.org>)<sup>11</sup> was used to extract proteins present in vector *Aedes aegypti* and for functional annotation of the essential proteins along with eggNOG (<http://eggnog5.embl.de>)<sup>12</sup>.

### BLAST

Basic alignment search tool<sup>13</sup> was used to identify non-human homologs proteins of *Aedes aegypti*. This was followed by the identification of conserved essential proteins amongst two central disease-causing species of the *Aedes* genus, namely *Aedes aegypti* and *Aedes albopictus*.

### Vector specific pathway analysis using KEGG

Complete form (KEGG)<sup>14</sup> was used to ascertain the metabolic pathways of non-homologous essential proteins that were identified in the previous step.

### Sub-cellular location prediction

To predict the subcellular location of the essential proteins, a consensus was drawn from the results obtained from UniProt<sup>11</sup>, Cello<sup>15</sup>, I-loc animal<sup>16</sup>, SherLoc2<sup>17</sup> and Wolf PSORT<sup>18</sup>.

### Host-vector protein-protein interactions

#### STRING

(Search Tool for the Retrieval of Interacting Genes/Proteins) (<https://string-db.org/>) (version 11.0) database was used to build protein-protein interaction networks of essential proteins of *Aedes aegypti* and their interacting partners. The networks obtained were analyzed using Cytoscape (version 3.8.0) and its various plugins such as ClusterONE, CytoHubba, MCODE<sup>19</sup>.

#### ClusterONE

(<http://apps.cytoscape.org/apps/clusterone>). ClusterONE (Clustering with Overlapping Neighborhood Expansion) was used to identify highly connected regions in the form of clusters that were based on weighted graphs with associated confidence values<sup>20</sup>.

#### CytoHubba

(<http://apps.cytoscape.org/apps/cytohubba>). CytoHubba with the scoring method of maximal clique centrality (MCC) was used to rank hub genes<sup>21</sup>.

#### MCODE

(<http://apps.cytoscape.org/apps/mcode>). MCODE (Molecular Complex Detection) was used to find highly connected regions called clusters based solely on connectivity data<sup>22</sup>.

## Results

### Proteome data mining for identification of non-human homologs

UniProt KB was used to retrieve proteins present in *Aedes aegypti*. To increase reliability and reduce redundancy in data mining, only the reviewed 253 protein data entries were selected. All the 253 protein sequences of *A. aegypti* were subjected to a BLASTP search against the host proteome of *Homo sapiens* with an e-value threshold of 0.005<sup>23</sup>. A total of 24 proteins of *A. aegypti* that showed "no significant similarity" to humans were shortlisted as non-human homologs (Table 1). After that, these proteins were analyzed using computational biology approaches to identify essential proteins of *Aedes* genus that may act as potential drug and vaccine targets.

### Identification of essential proteins in *Aedes aegypti*

The presence of orthologous proteins between two or more species indicates their essentiality for the survival of the genus. Orthologous proteins are known to retain equivalent functions in different organisms<sup>24</sup>. Due to the absence of any information about the essential proteins/genes of *A. aegypti* in DEG (Database of Essential Genes), various bioinformatics tools were used to identify the orthologues. Twenty-four proteins of *A. aegypti* (Table 1) were subjected to a manual BLASTP search with e-value threshold of 0.005, to identify conserved proteins against proteome of another *Aedes* genus member i.e. *A. albopictus*. Unique orthologues proteins were selected manually using a cut-off  $\geq 50\%$  identity between *A. aegypti* and *A. albopictus*. Twenty-three proteins (except TKSIA\_AEDAE) were identified as conserved essential proteins common between the *Aedes* species. These conserved proteins may be critical for the survival and pathogenicity of members of *Aedes* genus (Table 1).

### Vector specific pathway analysis and sub-localization prediction

The 23 proteins identified as conserved essential proteins were then categorized as "pathway dependent" or "pathway independent" using UniProt KB GO and KEGG pathway database, and their subcellular location was determined by drawing consensus amongst results obtained from UniProt, Cello, I-loc animal, SherLoc2 and Wolf PSORT. Out of the 23 proteins, 20 were found to be pathway independent, and 3 proteins, namely DEFA\_AEDAE, DEFB\_AEDAE and DEFC\_AEDAE were found pathway dependent, involved in innate immunity<sup>25</sup>. All these 23 proteins of *A. aegypti* that were absent in human hosts were further explored using a network systems biology approach to identify critical vector target proteins that can act as drug and vaccine targets to curb the spread of VBDs.

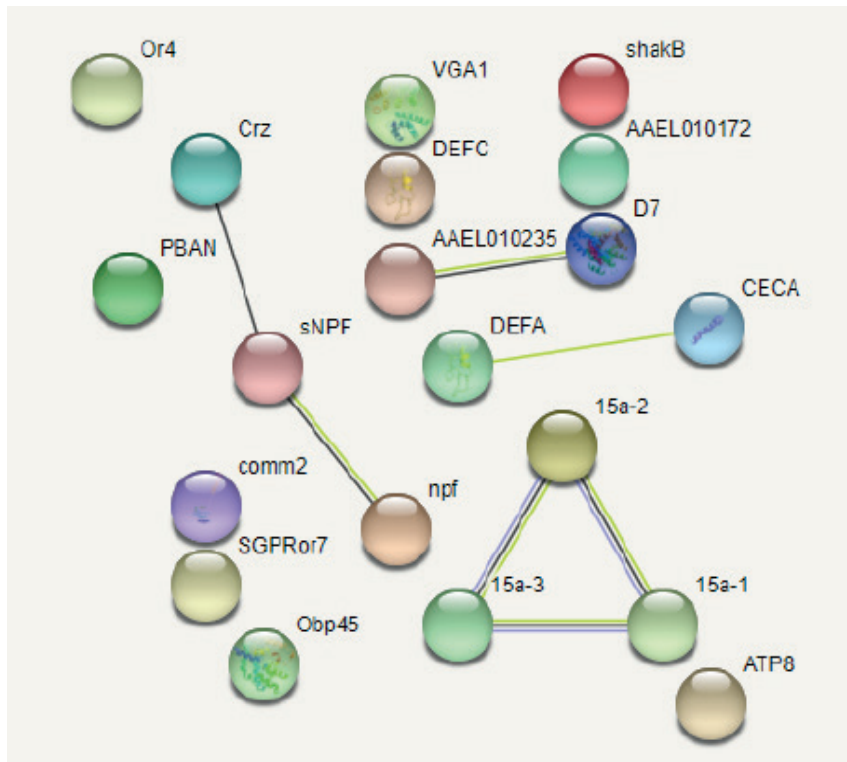
### Host-vector protein-protein interactions

#### Construction of gene interaction network

The interaction network for the 23 essential proteins of *Aedes aegypti* was constructed using the STRING database. On analysis, it was observed that HPEP\_AEDAE and BURS\_AEDAE were absent, while DEFA\_AEDAE and DEFB\_AEDAE were represented as the same protein in STRING results for *Aedes sp.* Therefore the resulting PPI network had 20 proteins that showed no significant interactions amongst themselves since these proteins were involved in different pathways of survival and pathogenesis of the vector (Figure 1). Three

Table 1. Essential proteins common in both <i>Aedes aegypti</i> and <i>Aedes albopictus</i>					
1	COMM2_AEDAE	9	ATP8_AEDAE	17	DEFC_AEDAE
2	V15A1_AEDAE	10	SNPF_AEDAE	18	CECA_AEDAE
3	OBP45_AEDAE	11	V15A3_AEDAE	19	VIT1_AEDAE
4	NPF_AEDAE	12	PBAN_AEDAE	20	DEFB_AEDAE
5	LCK_AEDAE	13	DEFA_AEDAE	21	ALL3_AEDAE
6	ALL2_AEDAE	14	OR4_AEDA	22	SHAKB_AEDAE
7	CORZ_AEDAE	15	V15A2_AEDAE	23	HPEP_AEDAE
8	BURS_AEDAE	16	ORCO_AEDAE	24	TKSIA_AEDAE*
* rejected because it was not common between the <i>Aedes aegypti</i> and <i>A. albopictus</i> species					

**Table 1.** Essential proteins common in both *Aedes aegypti* and *Aedes albopictus*.



**Figure 1.** Intra-species interactions amongst *Aedes* proteins constructed in the STRING database.

PPIs were observed, i.e., ALL2\_AEDAE (D7) and ALL3\_AEDAE (AAEL010235) that are salivary gland allergens and reported to be involved in blood-feeding of the human host<sup>26,27</sup>; CORZ\_AEDAE (crz) and NPF\_AEDAE with SNPF\_AEDAE, in which all are neuropeptides with CORZ being involved in heart contraction<sup>28</sup>; V15A1\_AEDAE (15a-1), V15A2\_AEDAE (15a-2) and V15A3\_AEDAE (15a-3) are proteins involved in oogenesis and therefore may act as an essential control point in the reproduction of *Aedes mosquito*<sup>29,30</sup>.

To better understand the interactions of the essential proteins with other proteins of *A. aegypti*, interaction networks of each of the 20 proteins were built individually in STRING and were imported and merged into a single network using Cytoscape software. The resulting merged network contained 96 edges (representing genes/proteins) and 243 nodes (representing interactions between genes/proteins) (Figure 2). This network was further analyzed using various plugins of Cytoscape to identify essential proteins of *A. aegypti*.

On analysis of the merged network with Cytoscape plugin ClusterONE, color-coded nodes were obtained in which red and grey nodes represent highly significant genes and outliers, respectively (Figure 3). Eleven clusters were obtained that had a significant P-value (<0.05) (Table 2), but only three top-ranked clusters having the most significant number of nodes and edges were further studied.

Another Cytoscape plugin MCODE was used to find clusters or highly connected regions in the protein-protein interaction network in which node colors represent the node score and range from black to red (lowest to highest, respectively), while white indicates a score of zero (Figure 4). Eleven clusters were also obtained from MCODE, out of which the first three clusters were considered as significant based on the higher number of nodes and edges (Table 3).

To further validate the results of genes identified as essential using Cytoscape plugins- ClusterONE and MCODE another plugin CytoHubba was used to analyse the merged



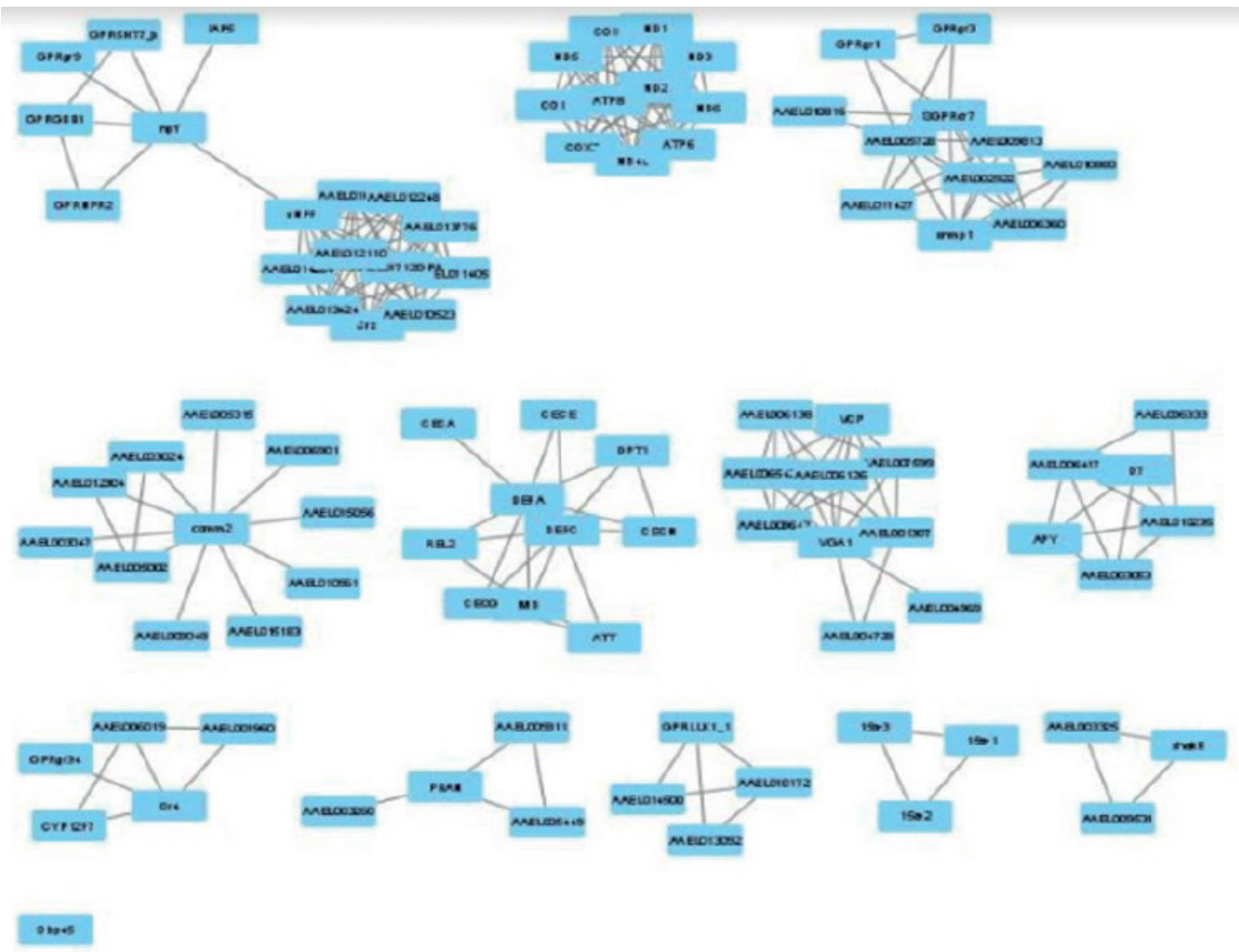


Figure 2. Merged PPI network having 96 nodes, 243 edges in Cytoscape.

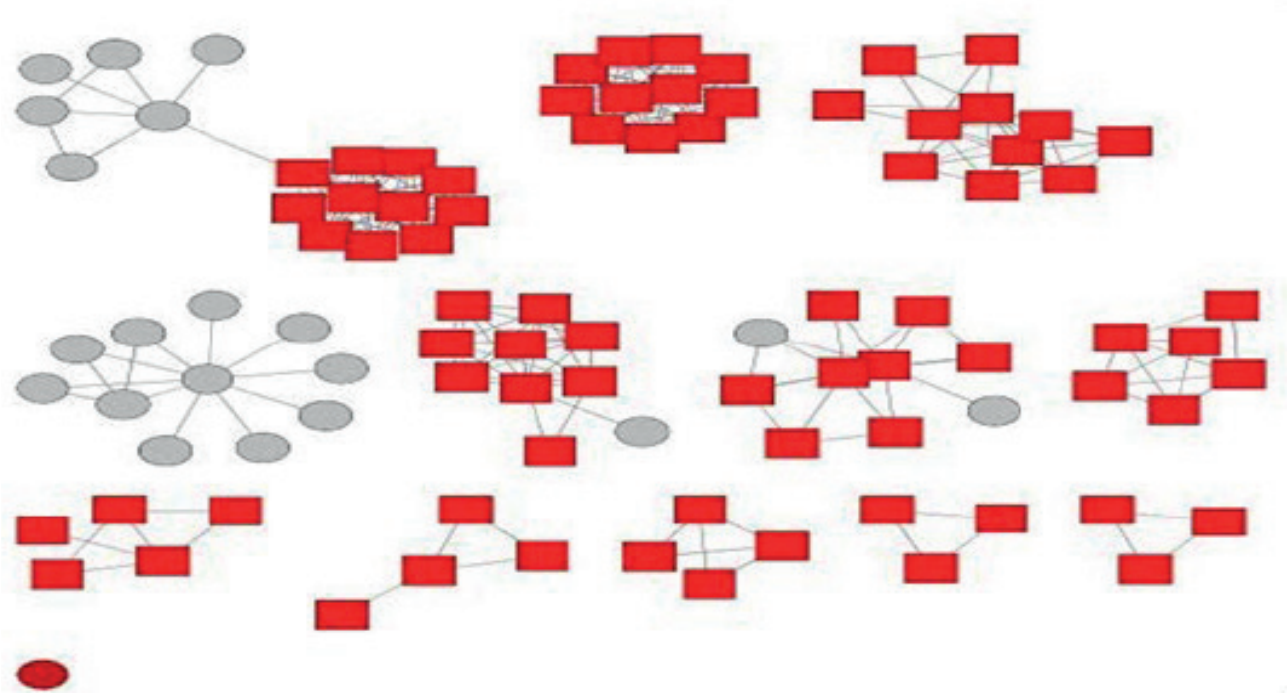


Figure 3. ClusterONE results were obtained by analyzing the interaction network.

PPI network (Figure 2) to identify the "Hubs" which represent the highly connected nodes. A hub is more likely to be essential because it has more interactions and, therefore, a higher likelihood to engage in an essential interaction<sup>31</sup>. The network obtained showed genes/proteins color-coded with highly significant genes in red (31 genes), orange and least significant in yellow (Figure 5).

The proteins present in the three top-ranked clusters obtained from ClusterONE and MCODE were identical, as were the 31 top-ranked genes obtained from CytoHubba, thereby validating our results obtained from different Cytoscape plugins that use different methods to identify hub proteins that can further be explored to act as putative target proteins for vector control (Table 4).

#### Data mining of shortlisted proteins

UniProtKB was used to retrieve information, such as target name, associated metabolic pathway, length, molecular weight, subcellular location etc., of the identified 31 essential genes of *Aedes* genus that code for non-homologous human proteins. Thereafter, a rigorous manual search in PDB was done to identify any structural homologs amongst the proteins of *Aedes* genus with its human host as unintended binding of the drug/vaccine, designed for *Aedes* to the structurally similar proteins of the human host could have devastating effects in the hosts, since structurally similar and related proteins may have similar binding sites with capabilities to recognize chemically similar ligands<sup>32</sup>.

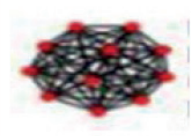

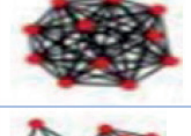





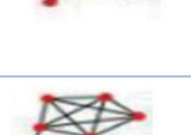
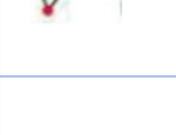
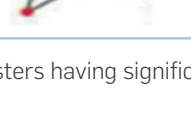
Out of 31 proteins of *Aedes* only 9 proteins did not have any structural homology with humans in PDB. These 09 proteins shortlisted three other proteins, namely BRUS\_AEDAE,

HPEP\_AEDAE (that had no entries in STRING database), and ALL3\_AEDAE (salivary gland allergen reported to have binary interactions with 2 human proteins, namely COA1 and CO2A3) were analyzed for their biological function in UniProt. Therefore, 12 proteins were shortlisted as novel vector-control target proteins of *A. aegypti* that had no human homologs and could effectively act as potential drug and vaccine targets (Table 5) to prevent the outbreak of VBDs by controlling the vector population of *Aedes*.

#### Literature mining of critical vector-control target proteins of *A. aegypti*

In the present study to identify critical vector-control target proteins for *Aedes* genus that can be used as potential targets for vaccine and drug development, we identified 12 proteins, out of which 5 proteins are neuropeptides. Two uncharacterized proteins, AAEL013776\_AEDAE and AAEL013424\_AEDAE, need to be further solved in structure and function to predict their importance in vector metabolic activities and survival.

Neuropeptides are one of the most diverse classes of signaling molecules and play a significant role in regulating a wide range of physiological processes in *Aedes* mosquitoes such as host-seeking behavior, blood-feeding, molting sex-specific processes, including reproduction<sup>33</sup>. Regulatory peptides act as neurochemicals, and hormones govern these processes in mosquitoes. These peptides are processed, stored, and released within the nervous system as neurotransmitters and from the midgut endocrine system and neurosecretory cells as circulating hormones. They exert their action by binding to membrane receptors, most often to G-protein coupled re-

Sr. No.	Clusters	No. of Nodes	P-value	Sr. No.	Clusters	No. of Nodes	P-value
1		11	2.705E-6	7		5	0.004
2		11	4.222E-6	8		4	0.010
3		11	1.231E-5	9		4	0.010
4		9	8.122E-5	10		3	0.023
5		8	3.882E-4	11		3	0.023
6		6	0.001				

**Table 2.** Eleven clusters having significant P-value (<0.05) obtained from ClusterONE.

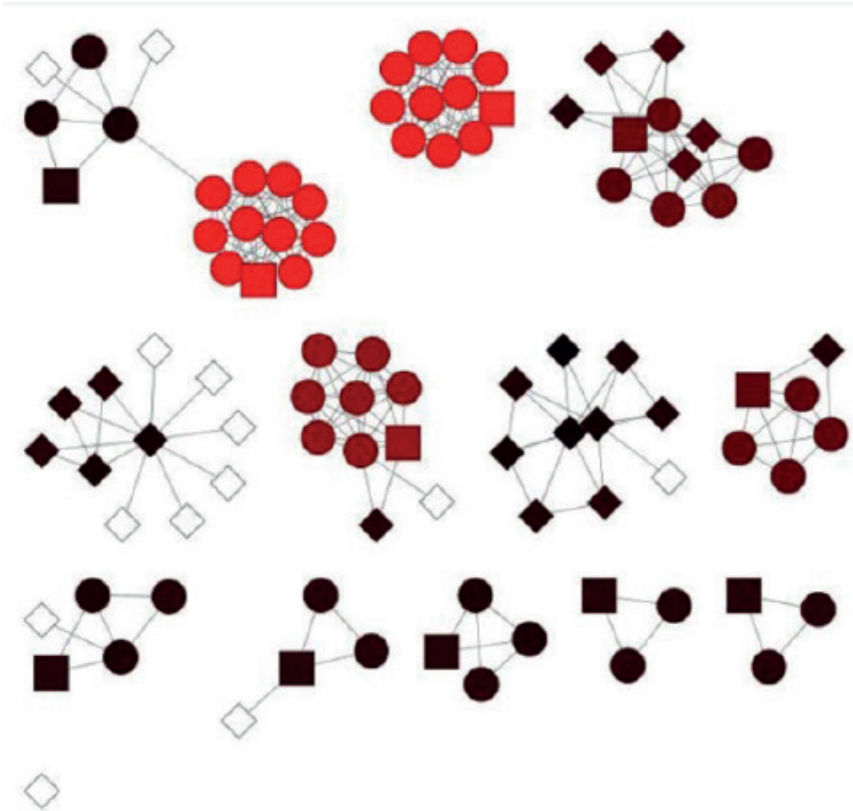
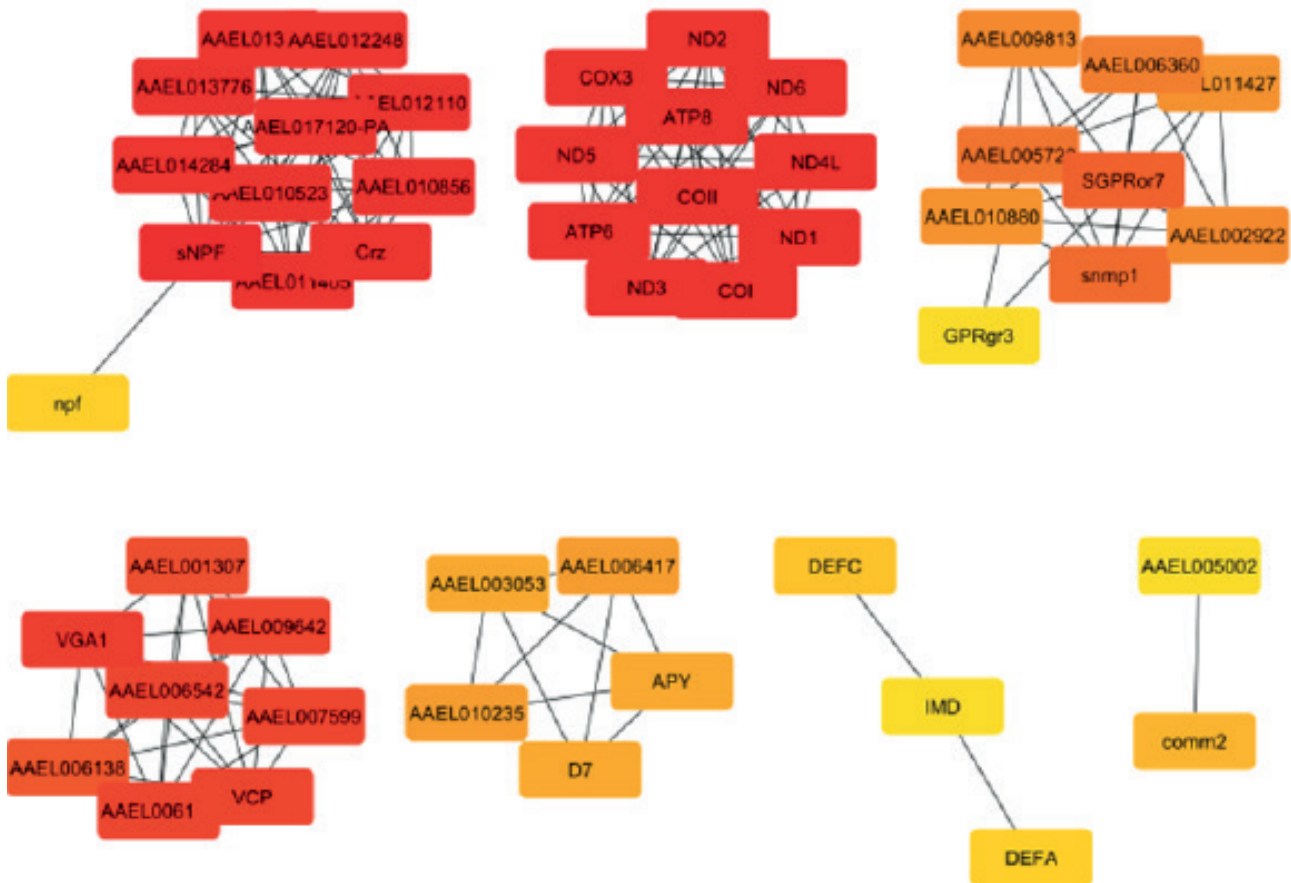


Figure 4. MCODE results obtained by analyzing the PPI network.

No.	Clusters	Nodes	Edges	S. No.	Clusters	Nodes	Edges
1		11	55	7		4	5
2		11	55	8		4	5
3		8	27	9		3	3
4		5	10	10		3	3
5		6	12	11		3	3
6		4	5				

Table 3. Eleven clusters were obtained from the interaction network.





**Figure 5.** Graphical view of ranked hub nodes obtained from Cytoscape plugin Cytohubba with color-coding highly essential (Red) - less essential (Yellow).

ceptors (GPCRs) and, to a lesser extent, to receptor tyrosine kinases<sup>34</sup>.

Host seeking is a fundamental aspect of mosquito and vertebrate host interactions, and the process is reported to be regulated by head peptide (HPEP\_AEDAE), a neuropeptide encoded by gene *HP-1* in blood-fed female *A. aegypti*, and also other sex-specific processes as it was found to be expressed in larvae and males. Ingestion of a blood meal has been reported to trigger egg maturation in *A. aegypti*, and after that, females show a marked decrease in responses to host emanations that initially stimulated host-seeking behavior<sup>35</sup>.

Short neuropeptide F (sNPF\_AEDAE), a member of the neuropeptide F/Y superfamily, was isolated from adult *A. aegypti*, and NPF-like immunostaining was observed in the brain and midgut of adults and larvae. The reported fluctuations in the concentrations of sNPF reflect changes in the regulatory role of this peptide in digestive processes and the reproductive cycle with a sudden drop once a blood meal was imbibed<sup>28</sup>.

Proteins such as odorant receptors (ORs) play critical roles in olfactory behaviors, including a co-receptor, designated ORCO\_AEDAE, expressed in most olfactory sensory neurons in both adults and larvae, and is highly conserved among the order Diptera to which the *Aedes* genus belongs. It plays a crucial role in forming a ligand-gated ion channel to generate signals to induce behavioral responses, including host-seeking and host selection<sup>36</sup>.

The arthropod saliva contains proteins delivered into the bite wound that can modulate the host's hemostatic and immune responses to facilitate blood meal intake and pathogen transmission. *Aegyptin* (ALL3\_AEDAE), an abundantly expressed salivary protein of *A. aegypti*, is a collagen-binding

protein that inhibits platelet aggregation and adhesion, thereby playing a significant and nonredundant role in successful feeding<sup>29,37</sup>. It has been reported to have interactions with the human collagen alpha-1 (I) and (III) chains i.e., COA1\_HUMAN & CO2A3\_HUMAN, respectively, thereby helping to transmit the pathogen in the vector saliva to the human host.

Gonadotropin-releasing hormone-related peptide Corazonin (CRZ) has been shown to have roles in pigmentation, ecdysis or act as a cardio stimulatory factor while its receptor-CRZR is expressed in the primary sex organs of adult male and female mosquitoes. This indicates a potential physiological role of CORZ\_AEDAE in regulating reproductive biology in *A. aegypti*<sup>37</sup>.

The gene encoding vitellogenin (Vg), a significant yolk protein precursor (YPP) in most oviparous animals, is expressed in extra ovarian tissues in a sex-, tissue- and stage-specific manner. Steroid hormones play a critical role in the regulation of Vg genes. In egg-laying vertebrates, estrogen triggers the transcription of the Vg<sup>38</sup>. Before blood-feeding, ovary maturation remains at a previtellogenic state of arrest, and the production of vitellogenin (VIT1\_AEDAE), the major yolk protein precursor, by the fat body is tightly repressed. After a blood meal, multiple regulatory factors act synergistically to trigger vitellogenin protein synthesis in the fat body. The vitellogenin proteins are then released into the hemolymph and taken up by the developing oocytes via receptor-mediated endocytosis. Therefore, stringent control of the expression of the Vg gene in mosquitoes is tied to the availability of a blood meal<sup>30</sup>.

Two other unreviewed proteins shortlisted in the present study, namely Q177I5\_AEDAE and Q177I2\_AEDAE have reported lipid transporter activity, and on the investigation of their

ClusterONE		CytoHubba		MCODE	
		RANK	Protein		
Cluster 1	ND1	1	sNPF	ND1	Cluster 1
	ND3	2	AAEL017120-PA	ND3	
	ND5	2	AAEL010856	ND2	
	ND6	2	AAEL014284	ND6	
	ATP6	2	AAEL012248	ATP6	
	ND4L	2	AAEL012110	ND4L	
	COX3	2	AAEL013424	COX3	
	COI	2	AAEL010523	COi	
	ATP8	2	AAEL013776	ND5	
	ND2	2	AAEL011405	COii	
	COII	2	Crz	ATP8	
		2	COii		
Cluster 2	AAEL012248	2	COi	AAEL010856	Cluster 2
	AAEL012110	2	ND1	AAEL012248	
	AAEL014284	2	ND2	AAEL013776	
	AAEL010856	2	COX3	AAEL011405	
	AAEL011405	2	ND5	AAEL010523	
	Crz	2	ND3	AAEL017120-PA	
	AAEL013776	2	ND4L	AAEL012110	
	AAEL010523	2	ATP6	AAEL013424	
	AAEL017120-P A	2	ATP8	AAEL014284	
	sNPF	2	ND6	Crz	
	AAEL013424	23	VGA1	sNPF	
		24	AAEL007599		
Cluster 3	AAEL010816	24	VCP	VCP	Cluster 3
	SGPRor7	24	AAEL009642	VGA1	
	GPRgr1	24	AAEL006542	AAEL007599	
	AAEL011427	24	AAEL006126	AAEL001307	
	AAEL002922	29	AAEL001307	AAEL006126	
	AAEL006360	30	AAEL006138	AAEL009642	
	Snmp1	31	SGPRor7	AAEL006542	
	AAEL009813			AAEL006138	
	AAEL005278				
	AAEL010880				
	GPRgr3				

**Table 4.** Top interacting Genes retrieved from Cytoscape plugins.



S. No.	Entry name	Non homologous protein target name	Uniprot id	Associated metabolic pathway (Uniprot/EggNOG)	Length	Molecular weight
1.	ATP8_AEDAE	ATP synthase protein 8	B0FWC9	• Energy production and conversion	53	6435
2.	AAEL013776_AEDAE	Uncharacterized protein	Q16I67	• Translation • ribosomal structure and biogenesis	140	15642
3.	AAEL013424_AEDAE	Uncharacterized protein	Q16J76	• Function unknown	173	18946
4.	CORZ_AEDAE	Pro-corazonin	Q17AN4	• Corazonin receptor binding • Neuropeptide signaling • Positive regulation of heart contraction	137	15559
5.	SNPF_AEDAE	Short neuropeptide F	A0SIX6	• Neuropeptide signaling • Regulation of multicellular organism growth • Regulation of response to food	215	24637
6.	VIT1_AEDAE	Vitellogenin-A1	Q16927	• Lipid transporter activity • Nutrient reservoir activity	2169	252892
7.	Q177I5_AEDAE	AAEL006126-PA	Q177I5	• Lipid transporter activity	2087	242048
8.	Q177I2_AEDAE	AAEL006138-PA	Q177I2	• Lipid transporter activity	2142	249436
9.	BURS_AEDAE	Bursicon	P85316	• Cuticle pigmentation • Chitin-based cuticle sclerotization • Signal transduction • Hormone activity	163	17719
10.	ALL3_AEDAE	30 kDa salivary gland allergen Aed a 3	AAEL010235	• Causes an allergic reaction in humans by interaction with COA1_HUMAN & CO2A3_HUMAN	273	29155
11.	HPEP_AEDAE	Head peptide	Q9GQV7	• Neuropeptide signaling pathway • Negative regulation of host-seeking behavior	128	14605
12.	ORCO_AEDAE	Odorant receptor coreceptor	Q178U6	• Detection of chemical stimulus involved in sensory perception of smell • Signal transduction • Eating behavior	478	54277

**Table 5.** Non-homologous proteins of *A. aegypti* with reference to humans as potential drug and vaccine targets from unique pathways.

families and domains using InterPro, Pfam and Prosite databases, they were found to be related to vitellogenin, thereby indicating that they might also be involved in the process of oogenesis.

Bursicon is a neuropeptide hormone consisting of two subunits, burs  $\alpha$  and burs  $\beta$ , members of the cystine-knot protein (CKP) family that usually form dimers (homo- or heterodimers) and are responsible for certain biological functions by binding to specific receptors. Zhang *et al.* (2017) report that the biological significance of bursicon extends beyond its classical developmental roles in cuticle tanning and wing expansion to mediating prophylactic immunity during molting periods. Bursicon (BURS\_AEDAE) homodimers were shown to induce expression of AMP genes *via* Relish2 in *A. aegypti* as prophylactic immunity to protect mosquitoes during the vulnerable stages of each molt<sup>39</sup>.

The  $F_1F_0$  ATP synthase is a large multi-subunit enzyme that uses the proton gradient generated by the respiratory chain to synthesize ATP.  $F_1$ -ATP synthase region is composed of five different subunits ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$ ) assembled as a complex of three alternating  $\alpha$  and  $\beta$  subunits together with a single unit of each of the other three subunits<sup>40</sup>. It was initially believed that  $F_1F_0$  ATP synthase was strictly confined to the mitochondrial inner membrane. But for a wide variety of normal cells, including endothelial cells, adipocytes, keratinocytes, and hepatic cells, active ATP synthase activity on the cell surface has been documented<sup>41</sup>. Fongsaran *et al.* (2014) reported that ATP8 plays a role in Chikungunya virus (CHIKV) infection of mosquito cells<sup>41</sup>, and there is evidence showing that the same protein is involved in the internalization of white spot syndrome virus (WSSV) to shrimp cells<sup>42</sup>, and possibly binding of dengue virus to insect cells<sup>43</sup>, suggests that this is a conserved virus-arthropod interaction. Therefore, by targeting ATP8\_AEDAE, the interaction of the pathogens and the mosquito vector can be disrupted and thereby, transmission to the human host may be effectively prevented.

Furthermore, targeting proteins involved in various physiological processes such as reproductive (CORZ\_AEDAE and VIT1\_AEDAE), immune (BURS\_AEDAE) and metabolic activities (ATP8\_AEDAE) can affect the survival of the vectors and thereby contain the spread of VBDs by controlling vector population.

Concomitantly, blood-feeding on humans also propagates disease transmission because the mosquito ingests the pathogen from an infected human and transmits it to new hosts during subsequent blood meals. The concentration of Head peptide and sNPF are higher in mosquitoes before blood feeding i.e., during the host-seeking phase and following blood meal, their concentrations fall<sup>44,45</sup>. Therefore, Head peptide (HPEP\_AEDAE), sNPF (SNPF\_AEDAE), ORCO (ORCO\_AEDAE) and ALL3 (ALL3\_AEDAE) may also prove useful targets for blocking host-seeking and biting behavior, thereby preventing the transmission of pathogens from vector to the human host and thus constitute an alternative strategy to curb the disease.

## Conclusions

The proteins identified in the present study can develop novel strategies to target critical vector components to successfully block the development of the vector or pathogen/parasite in its vector.

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## Informed Consent Statement

Not applicable.

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## Conflicts of Interest

The authors declare no conflict of interest.

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