Vacuolar-type ATP synthase Gene Report

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Introduction:

Vacuolar-type ATP synthase (V-ATPase) is a highly conserved eukaryotic enzyme (Nelson *et al*. 2000). Originally found in the vacuole membrane, V-ATPase is now known to function in almost every cell's plasma membrane and endomembrane system (Maxson and Grinstein 2014, Hilario and Gogarten 1998). V-ATPase works to regulate the acidity of organelles, such as vacuoles, the Golgi apparatus, and coated vesicles, by translocating protons across their membranes and powering the secondary transport of ions. Structurally, V-ATPase has a noncatalytic transmembrane domain, the V0 rotor, and a catalytic cytoplasmic domain, the V1 stator. V-ATPase hydrolyzes ATP into ADP, thus acting opposite of the commonly known F-ATPase (Nelson *et al*. 2000). In insects, typically 13 protein subunits are required to build a single V-ATPase (Badillo-Vargas *et al.* 2014). The V0 domain consists of subunits a-e and V1 consists of subunits A-H (Beyenbach and Wieczorek 2006). There is also a critical accessory S subunit that helps assemble the enzyme (Miles *et al.* 2017). Unlike insects, mammals need 14 subunits to build V-ATPase, and they use two copies of accessory S, a protein that does not exist in fungi (Beyenbach and Wieczorek 2006, Miles *et al.* 2017, Forgac 2007). Only fungi have the integral proteolipid subunit c', which is replaced with an additional subunit V0-c in eukaryotes (Chavez *et al.* 2006, Maxson and Grinstein 2014). In insects, V-ATPase occurs highly throughout epithelial cells and are especially important in the digestive tract, helping to control an insect's nutrient uptake and solute transport (Dow *et al.* 1997). Many studies have demonstrated the lethality of silencing individual V-ATPase subunits across several phyla, including insects (Nelson *et al*. 2000). In addition, the prolific and fundamental nature of this enzyme makes V-ATPase an attractive potential target for RNAi gene silencing in *Diaphorina citri* (Asian citrus psyllid) towards the future pest control and subsequent prevention of the spread of Citrus greening disease.

Methods:

The *D. citri* Vacuolar ATP synthase genes were annotated following the guidelines for community annotation described previously (Hosmani et al 2019). Vacuolar ATP synthase insect orthologs from *Acyrthosiphon pisum* (pea aphid) were obtained from the KEGG database. Additional ortholog subunits occurring in non-insect eukaryotes, like *Homo sapiens,* were obtained from HUGO and the non-redundant NCBI Reference Sequence database. V-ATPase protein sequences were used to query the predicted protein set from the *Diaphorina citri* MCOT transcriptome via BLASTp. Reciprocal BLASTp analysis was performed to validate the *D. citri* MCOT significant hits using the NCBI non-redundant protein database. *D. citri* V-ATPase subunit genes were identified in the genome (version 1.91) by searching for the identified mapped MCOT models in the WebApollo system hosted at Boyce Thompson Institute. Multiple alignments of the predicted *D. citri* MCOT proteins, other gene model sequences, and insect V-ATPase orthologs were performed using Muscle (http://www.ebi.ac.uk/ Tools/msa/muscle/). Further analysis using RNASeq reads, Illumina DNAseq reads, StringTie models, and PacBio IsoSeq models were used to manually annotate the final V-ATPase gene models. Manually annotated V-ATPase gene models were then integrated into the v2.0 Official Gene Set. V-ATPase genes were verified in WebApollo through analysis using Augustus models,

Mikado transcriptome, SwissProt proteins, and SNAP prediction models. In the v2.0 *D. citri* genome, a paralog of subunit D necessary for the catalytic domain was discovered and will become part of the future v2.1 genome. Reciprocal BLASTp of manually annotated v2.0 V-ATPase genes were performed at NCBI comparing the Insecta taxid. Insect orthologs from *Acyrthosiphon pisum*, *Bemisia tabaci* (whitefly), *Aedes aegypti* (Yellow Fever mosquito), *Apis mellifera* (honey bee), *Tribolium castaneum* (red flour beetle) and *Drosophila melanogaster* (fruit fly) were obtained by reciprocal BLASTp analysis of the non-redundant protein database at NCBI. A neighbor-joining phylogenetic tree using Poisson correction method and 1000 replicate bootstrap test was constructed using full-length protein sequences in MEGA7 for the transmembrane complex, the catalytic complex, and the accessory S subunit, respectively.

Results and Conclusions:

All 13 subunits required to build a single Vacuolar ATP synthase enzyme were annotated in *Diaphorina citri,* the Asian citrus psyllid (ACP). There were no additional subunits found in *D. citri*, as there are in metazoans. Although insects are known to have the 13 orthologous proteins, there is a variation in the gene copy number amongst different species. A selection of insects from the orders of Hemiptera, Coleoptera, Diptera, and Hymenoptera were obtained for gene copy comparison in tables 1, 2, and 3. The transmembrane domain subunits V0-a, V0-b, V0-e; the catalytic domain subunits V1-A, V1-C, V1-D, V1-G; and the accessory S subunit all show variation in copy number. Other than V1-D and V1-G, the three Hemipterans (*D. citri*, *A. pisum*, and *B. tabaci*) do maintain the same paralog number for those respective genes, as compared to the other orders. This variation in copy number is interesting in contrast to the single copy genes V0-c, V0-d, V1-B, V1-E, V1-F, and V1-H that maintain only one gene copy across all Insecta orders found in tables 1, 2, and 3. Various V-ATPase subunits have been studied in plants, animals, fungi, and insects, and certain genes have been highlighted for their functional versatility in serving cell needs; for example, yeast and mammals have numerous gene copies and isoforms of the transmembrane proteolipid subunit a to serve various functions involving vacuoles, the Golgi, neurons, osteoclasts, and epididymal cells (Maxson and Grinstein 2014). *D. citri* has two copies of subunit a, like *A. pisum* and *B. tabaci*, whereas *D. melanogaster* has 5 copies of this gene. In *D. citri*, paralogs of the subunits V0-a, V1-D, and V1-G were found, and they maintain differences in their amino acid sequences.

	V0-a	V0-b	V0-c	V0-d	V0-e
Diaphorina citri	2	1	1	1	1
Acyrthosiphon pisum	2	1	1	1	1
Tribolium castaneum	2	2	1	1	2
Drosophila melanogaster	5	2	1	1	4
Aedes aegypti	3	1	1	1	2
Apis mellifera	2	1	1	1	2
Bemisia tabaci	2	1	1	1	1

Table 1: Gene copy comparison of V-ATPase transmembrane genes in D. citri and orthologous insect genes.

	V1-A	V1-B	V1-C	V1-D	V1-E	V1-F	V1-G	V1-H
Diaphorina citri	1	1	1	2	1	1	2	1
Acyrthosiphon pisum	1	1	1	2	1	1	2	1
Tribolium castaneum	1	1	1	3	1	1	2	1
Drosophila melanogaster	2	1	1	3	1	1	1	1
Aedes aegypti	1	1	2	2	1	1	3	1
Apis mellifera	1	1	1	2	1	1	1	1
Bemisia tabaci	1	1	1	1	1	1	1	1

Table 2: Gene copy comparison of V-ATPase catalytic genes in D. citri and orthologous insect genes.

	Accessory S
Diaphorina citri	1
Acyrthosiphon pisum	1
Tribolium castaneum	2
Drosophila melanogaster	2
Aedes aegypti	2
Apis mellifera	1
Bemisia tabaci	1

Table 3: Gene copy comparison of V-ATPase Accessory S gene in D. citri and orthologous insect genes.

Tables 4, 5, and 6 show results of protein BLAST analysis comparing the same insects as found above. Other than the accessory S protein, all subunits share relatively high identity, 54-94%, amongst individual pairwise alignments with each *D. citri* sequence. In contrast, the sequence identities 24-33% of accessory S protein show the highest gene divergence when comparing *D. citri* to other insects, see table 6. For the transmembrane domain subunits in table 4, proteolipid V0-c maintains some of the highest percentages of sequence identity, reflecting the importance of the protein function to form the c-ring that rotates and ultimately translocates protons across various membranes (Maxson and Grinstein 2014). This is supported in table 1, in which a single gene copy for V0-c is maintained across different orders of insects. For the catalytic domain subunits in table 5, V1-A and V1-B maintain the highest percentages of sequence identity, consistent with the importance of their function of containing the ATP binding sites at the V1-A/ V1-B protein interface. Apart from *Drosophila*, subunits V1-A and V1-B also maintain single copies of these two genes across different orders of insects, supporting their conserved nature as compared to other subunits in this enzyme.

D. citri		*A. pisum*	*T. castaneum*	*D. melanogaster*	*A. aegypti*	*A. mellifera*	*B. tabaci*
V0-a1	Accession	XP_008187043.1	XP_968579.1	NP_650722.1	XP_021706364.1	XP_006565533.1	XP_018903507.1
	Bit score	1332	1128	1151	1217	1166	1312
	QC	99%	100%	99%	99%	99%	100%
	Identity	75%	67%	66%	70%	69%	73%
V0-a2	Accession	XP_008183003.1	XP_008200805.1	NP_001163768.1	XP_021693137.1	XP_016769523.1	XP_018913654.1
	Bit score	720	689	654	728	742	692
	QC	100%	100%	100%	100%	100%	100%
	Identity	61%	60%	60%	62%	62%	60%

V0-b	Accession	NP_001155679.1	XP_975026.1	NP_652010.1	XP_001662256.1	XP_392599.1	XP_018909463.1
	Bit score	294	260	270	275	300	283
	QC	99%	99%	99%	99%	99%	99%
	Identity	74%	70%	73%	74%	76%	73%
V0-c	Accession	NP_001155531.1	XP_967959.1	NP_476801.1	XP_001654757.1	NP_001011570.1	XP_018897791.1
	Bit score	268	258	271	267	259	250
	QC	98%	98%	99%	98%	98%	99%
	Identity	92%	90%	92%	93%	89%	87%
V0-d	Accession	NP_001191854.1	XP_974905.1	NP_570080.1	XP_001661299.1	XP_393438.2	XP_018903442.1
	Bit score	656	671	661	677	679	669
	QC	99%	100%	99%	100%	100%	100%
	Identity	90%	92%	90%	92%	93%	92%
V0-e	Accession	XP_003242132.1	XP_971898.1	NP_001097499.1	XP_011493566.1	XP_624787.1	XP_018909271.1
	Bit score	127	123	106	133	127	119
	QC	96%	94%	96%	100%	94%	96%
	Identity	70%	73%	59%	71%	71%	71%

Table 4: Bit score, query coverage, and identity results from protein BLAST analysis of annotated D. citri V-ATPase transmembrane subunit genes to their presumptive orthologs.

D. citri		*A. pisum*	*T. castaneum*	*D. melanogaster*	*A. aegypti*	*A. mellifera*	*B. tabaci*
V1-A	Accession	XP_008179407.1	XP_976188.1	NP_652004.2	XP_021709029.1	XP_623495.1	XP_018897790.1
	Bit score	1176	1167	1162	1168	1174	1187
	QC	99%	99%	99%	100%	99%	99%
	Identity	92%	90%	90%	91%	91%	92%
V1-B	Accession	XP_003246082.1	XP_967844.1	NP_476908.1	XP_001651458.1	XP_624112.1	XP_018896879.1
	Bit score	966	966	966	968	955	976
	QC	98%	98%	98%	98%	98%	99%
	Identity	94%	93%	94%	94%	93%	94%
V1-C	Accession	XP_001946227.1	XP_008195426.1	NP_477266.1	XP_021695404.1	XP_006562159.1	XP_018915661.1
	Bit score	702	671	645	671	648	653
	QC	99%	98%	98%	99%	99%	99%
	Identity	87%	82%	79%	82%	80%	83%
V1-D1	Accession	NP_001119691.1	XP_975872.1	NP_651987.1	XP_001660426.1	XP_394769.2	XP_018904914.1
	Bit score	246	244	257	247	238	253
	QC	93%	86%	86%	86%	84%	88%
	Identity	54%	56%	58%	56%	56%	57%
V1-D2	Accession	NP_001119691.1	XP_975872.1	NP_651987.1	XP_001660426.1	XP_394769.2	XP_018904914.1
	Bit score	420	426	408	401	404	423
	QC	100%	100%	100%	100%	100%	100%
	Identity	87%	87%	83%	81%	87%	86%
V1-E	Accession	NP_001155650.1	XP_970621.1	NP_524237.1	XP_001655825.1	XP_625098.1	XP_018901912.1
	Bit score	301	341	325	335	336	336
	QC	99%	100%	100%	100%	100%	100%
	Identity	73%	73%	71%	71%	73%	72%
V1-F	Accession	NP_001119690.1	XP_975016.1	NP_476969.1	XP_001655376.1	XP_624852.1	XP_018905603.1
	Bit score	223	216	217	216	230	227
	QC	98%	98%	98%	100%	98%	98%
	Identity	85%	83%	81%	82%	89%	89%
V1-G	Accession	NP_001119628.1	XP_973974.1	NP_477437.1	XP_001652605.1	XP_624346.1	XP_018908074.1
	Bit score	184	173	154	171	180	188
	QC	99%	98%	96%	96%	98%	100
	Identity	80%	74%	66%	74%	77%	81
V1-H	Accession	XP_001949116.3	XP_966693.3	NP_523585.2	XP_001652018.1	XP_003251675.1	XP_018904009.1
	Bit score	763	745	679	719	741	779
	QC	98%	100%	96%	98%	98%	99%
	Identity	75%	75%	69%	71%	76%	78%

Table 5: Bit score, query coverage, and identity results from protein BLAST analysis of annotated D. citri V-ATPase catalytic subunit genes to their presumptive orthologs.

D. citri		*A. pisum*	*T. castaneum*	*D. melanogaster*	*A. aegypti*	*A. mellifera*	*B. tabaci*
Acc-S	Accession	NP_001162140.1	XP_974187.2	NP_610470.1	XP_001658652.1	XP_001121483.2	XP_018899028.1
	Bit score	136	114	77.4	92.4	90.1	177
	QC	83%	100%	96%	95%	93%	97%
	Identity	31%	27%	25%	24%	27%	33%

Table 6: Bit score, query coverage, and identity results from protein BLAST analysis of annotated D. citri V-ATPase accessory S subunit gene to its presumptive orthologs.

Figures 1, 2, and 3 depict phylogenetic analyses for the transmembrane and catalytic domains of V-ATPase, respectively, and one for the accessory S protein. The individual V-ATPase subunits form clades, regardless of insect species. These clades also have the highest bootstrap values. This confirms previous research that describes the enzyme as ancient and highly conserved. The evolution of V-ATPase has been analyzed for gene duplication and divergence from other ATP synthases, like F- and A-ATPase, which occur across the three domains of life (Hilario and Gogarten 1998). Figures 1, 2, and 3 all agree and suggest that the V-ATPase enzyme utilized in these particular insects existed in their common ancestor before they diverged into their respective species. The proteolipid subunit V0-c has the shortest branch lengths in figure 1, consistent with tables 1 and 4. Subunit c, which is required to form the critical c-ring rotor of V-ATPase, appears to have diverged the least when comparing the other transmembrane domain subunits and in other insect species. In contrast, subunit V0-e has diverged the most in figure 1's phylogenetic tree. This is consistent with the variable gene copy number observed across different orders of insects and the lower percentages of protein sequence identity seen in *D. citri* pairwise alignments, tables 1 and 4, respectively. In addition, the function of subunit V0-e is still unknown for the transmembrane domain subunits (Beyenbach and Wieczorek 2006).

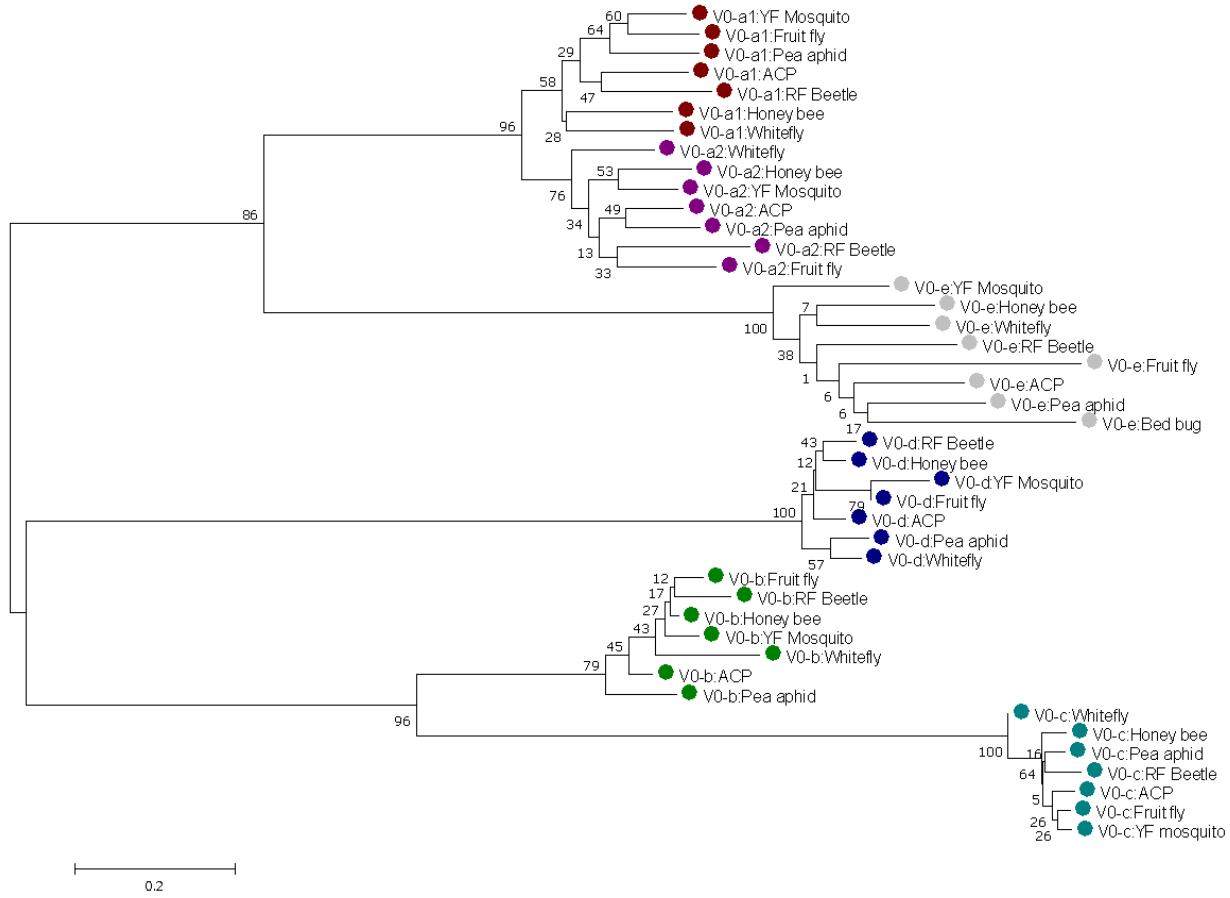

Figure 1: Phylogenetic tree of representative insect V-ATPase transmembrane orthologs.

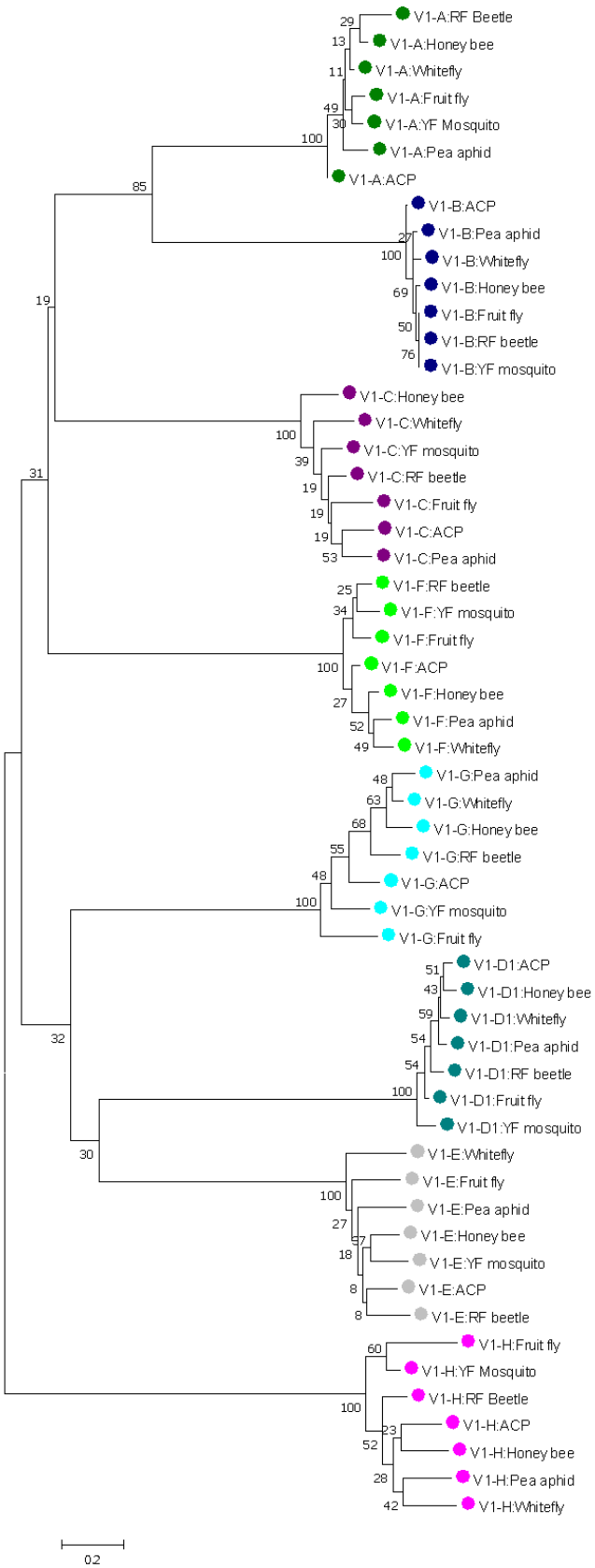

Figure 2: Phylogenetic tree of representative insect V-ATPase catalytic orthologs.

Figure 3 shows the evolutionary relatedness of the *D. citri* accessory S protein. It is a relatively new protein critically associated with the assembly of V-ATPase and is still being studied (Miles *et al*. 2017). For this select group of insect species, accessory S groups and forms a clade with the other hemipteran protein sequences. The accessory S subunit is a variable gene when comparing V-ATPase across the domains of life, a paralog variability also seen among different orders of insects, see table 3 (Miles *et al*. 2017, Forgac 2007). Accessory S has diverged the most of all the V-ATPase subunits in *D. citri* compared to other insects. This is seen in the figure 3 phylogenetic tree and is denoted with longer branch lengths. This divergence is also supported in the values of the pairwise alignments found in table 6, in which the protein shares very little sequence identity across the query lengths, respectively.

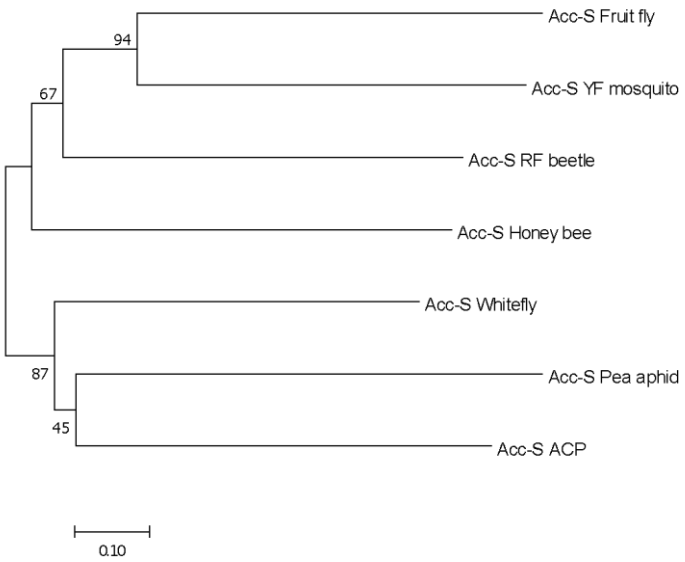

Figure 3: Phylogenetic tree of representative insect V-ATPase accessory S orthologs.

References:

Badillo-Vargas IE, Rotenberg D, Schneweis BA, Whitfield AE. 2015. RNA interference tools for the western flower thrips, *Frankliniella occidentalis*. J Insect Physiol. 76:36-46.

Beyenbach KW and Wieczorek H. 2006. The V-type H+ ATPase: molecular structure and function, physiological roles and regulation. J Experim Bio. 209:577-589.

Chavez C, Bowman EJ, Reidling JC, Haw KH, Bowman BJ. 2006. Analysis of strains with mutations in six genes encoding subunits of the V-ATPase: eukaryotes differ in the composition of the V0 sector of the enzyme. J Biol Chem. 281(37):27052-62.

Dow JAT, Davies S, Guo Y, Graham S, Finbow ME, Kaiser K. 1997. Molecular genetic analysis of V-ATPase function in *Drosophila melanogaster*. J Experim Bio. 200:237-245.

Forgac M. 2007. Vacuolar ATPases: rotary proton pumps in physiology and pathophysiology. Nat Rev Mol Cell Biol. 11:917-29.

Hilario E and Gogarten JP. 1998. The prokaryote-to-eukaryote transition reflected in the evolution of the V/F/A-ATPase catalytic and proteolipid subunits. J Mol Evo. 46:703–715.

Hosmani PS, Shippy T, Miller S, Benoit JB, Munoz-Torres M, Flores-Gonzalez M, et al. (2019) A quick guide for student-driven community genome annotation. PLoS Comput Biol 15(4): e1006682. https://doi.org/10.1371/journal.pcbi.1006682

Maxson ME and Grinstein S. 2014. The vacuolar-type H+-ATPase at a glance – more than a proton

pump. J Cell Sci. 127:4987–4993.

Miles AL, Burr SP, Grice GL, Nathan JA. 2017. The vacuolar-ATPase complex and assembly factors, TMEM199 and CCDC115, control HIF1alpha prolyl hydroxylation by regulating cellular iron levels. eLIFE [Internet]. [cited 2018 Jan 18]; 6:e22693. Available from: https://elifesciences.org/articles/22693 DOI: 10.7554/eLife.22693

Nelson N, Perzov N, Cohen A, Hagai K, Padler V, Nelson H. 2000. The cellular biology of proton-motive force generation by V-ATPases. J Exp Bio. 203:89-95.