Molecular analysis of capsid protein of Homalodisca coagulata Virus-1, a new leafhopper-infecting virus from the glassy-winged sharpshooter, Homalodisca coagulata

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Source: Journal of Insect Science, 6(28) : 1-10

Published By: Entomological Society of America

URL: https://doi.org/10.1673/2006_06_28.1

Molecular analysis of capsid protein of *Homalodisca coagulata Virus-1*, a new leafhopper-infecting virus from the glassy-winged sharpshooter, *Homalodisca coagulata*

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Abstract

A new virus that infects and causes increased mortality in leafhoppers was isolated from the glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae). The virus, named *Homalodisca coagulata virus -1*, HoCV-1, was associated with increased mortality of cultured 5th instar *H. coagulata*. To identify the presence of *H. coagulata* viral pathogens, cDNA expression libraries were made from adult and nymphs. Analysis using reverse transcriptase PCR demonstrated that the virus was present in midgut tissues. As the viral capsid proteins are commonly used in classification of newly discovered viruses, the capsid proteins (CP) of the virus discovered in *H. coagulata* was examined. The order of the polyprotein subunits of HoCV-1 capsid proteins was determined to be CP2, CP4, CP3, and CP1. The CP4/CP3 (AFGL/GKPK) cleavage boundary site was clearly identified when the sequences were aligned. The putative CP3/CP1 (ADVQ/SAFA) cleavage site and the putative CP2/CP4 (VTMQ/EQSA) cleavage site of HoCV-1, respectively, were located in the same region as that of the other viruses. After alignment, the CP3/CP1 cleavage sites and CP2/CP4 cleavage sites of the viruses analyzed fell within 50 amino acids of one another. As with the *cricket paralysis virus*, HoCV-1 was found to be mainly comprised of β-sandwiches in CP1-3 with a jelly roll topological motif. CP4 of HoCV-1 appeared to be mainly α-helical in structure. CP1-4 domains are most homologous to insect picorna-like virus coat proteins as was demonstrated by the Results of the BLASTP and PSI-BLAST tests, and is strongly supported by the structural modeling. While sequence homology between the *cricket paralysis virus* and HoCV-1 was low, the global structure of the proteins was conserved. Sequence identities were analyzed by *in silico* comparison to known genes in the public database, NCBI. Phylogenetic analysis performed using the optimized protein alignment generated a phylogram containing 5 clades. Clade 1 consisted of *Drosophila C virus*, Clade 2 consisted of *cricket paralysis virus*, Clade 3 of *Triatoma virus*, *Plautia stali intestine virus*, *Himetobi P virus*, *black queen cell virus*, and HoCV-1. Clade 4 encompassed *acute bee paralysis virus* and *Kashmir bee virus*, and Clade 5 consisted of *Rhopalosiphum padi virus*. Analysis of the capsid protein of this new leafhopper virus provided significant evidence that it is related to other ssRNA insect viruses within the Family, *Dicistroviridae*. The HoCV-1, capsid protein sequence has been deposited in GenBank, Accession number: DQ308403.

Keywords: Cicadellidae, Dicistroviridae, Hemiptera, HoCV-1, Insect, Picorna, ssRNA, *H. Vitripennis*
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Received: 3.12.2005 | Accepted: 12.4.2006 | Published: 18.10.2006

ISSN: 1536-2442 | Volume 2006, Number 28

Cite this paper as:
Hunter WB, Katsar CS, Chaparro JX. 2006. Molecular analysis of capsid protein of *Homalodisca coagulata Virus-1*, a new leafhopper-infecting virus from the glassy-winged sharpshooter, *Homalodisca coagulata*. *Journal of Insect Science* 6:28, available online: insectscience.org/6.28

Introduction

Few viral pathogens are known from leafhoppers. The glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae) is the primary vector of Pierce's disease of grapes, which is caused by the bacterial plant pathogen, *Xylella fastidiosa*. There are numerous plant diseases caused by the *Xylella* bacteria which are known as 'Scorch' diseases due to the dry leaf appearance in susceptible plants. In grapes, susceptible plants have reduced yields and may die after infection thus causing severe economic losses. Research to examine the interactions between *H. coagulata* and *Xylella* requires mass rearing of large numbers of *H. coagulata* for use in transmission experiments. Attempts to mass rear *H. coagulata*, resulted in an observable increase in mortality during the 5th instar stage (personal observations) making it difficult to produce adult *H. coagulata* for research needs. Mortality in cultured insects is often due to the presence of undetected insect pathogens. Insect cell cultures have been used to detect insect pathogens (Hunter *et al.* 2001, Funk *et al.* 2001). Our approach was to identify the presence of *H. coagulata* viral pathogens, using cDNA expression libraries, which provide a overview of the transcripts in adults and nymphs. As viral capsid protein (CP) sequences, are commonly used in classification of newly discovered viruses we chose to elucidate the CP of the virus discovered in *H. coagulata*. Sequence identities were analyzed by comparison to known genes in the public database, NCBI. Viral sequences which were identified were then validated by amplification and bidirectional sequencing. A new viral pathogen of the *H. coagulata* was identified and shown to be significantly related to insect viruses within the Family, *Dicistroviridae*. The virus herein was named: *Homalodisca coagulata virus–1*, HoCV-1.

Materials and Methods

cDNA library construction

A whole body, adult cDNA library was made from 160 adult *H. coagulata,* and a 5th instar cDNA library was made from 140 nymphs collected from citrus groves near Riverside, California. Insects were collected into liquid nitrogen and total RNA subsequently extracted using the guanidinium salt-phenol-chloroform procedure as described by Strommer (1993). Contaminating DNA was removed using RQ1 RNase-free DNase (Promega, http://www.promega.com) and poly(A)+ RNA was purified using a MicroPoly(A)Pure™ kit (Ambion, Inc., www.ambion.com) following the manufacturer's instructions. A directional cDNA library was constructed in Lambda Uni-ZAP® XR Vector using Stratagene's ZAP-cDNA Synthesis kit (Stratagene, http://www.stratagene.com). The resulting DNA was packaged into lambda particles using Gigapack® III Gold Packaging Extract (Stratagene). Mass excision of the amplified library was carried out using Ex-Assist® helper phage (Stratagene). An aliquot of the excised, amplified library was used for infecting XL1-Blue MRF' cells and subsequently plated on LB agar containing 100 µg/ml ampicillin. Bacterial clones containing excised pBluescript SK(+) phagemids were recovered by random colony selection. pBluescript SK(+) phagemids were grown overnight at 37°C and 240 rpm in 96-deep well culture plates containing 1.7 ml of LB broth, supplemented with 100 µg/ml ampicillin. Archived stocks were prepared from the cell cultures using 75 µl of a LB-amp, glycerol mixture and 75 µl of cells. These archived stocks are held at the U.S. Horticultural Research Laboratory where they are kept in an ultra low temperature freezer (–80° C). Plasmid DNA was extracted using the Qiagen 9600 liquid handling robot and the QIAprep 96 Turbo miniprep kit according to the recommended protocol (QIAGEN, http://www.qiagen.com). Bidirectional sequencing of HoCV-1 clones were completed using T3 and T7 primers, sequencing reactions were performed using the ABI PRISM® BigDye™ Primer Cycle Sequencing Kit (Applied Biosystems, http://www.appliedbiosystems.com) along with a universal T3 primer. Sequencing reaction products were precipitated with 70% isopropanol, resuspended in 15 µl of sterile water and loaded onto an ABI 3730xl DNA Analyzer (Applied Biosystems). Two other *H. coagulata* cDNA libraries (salivary gland and midgut) were also examined for virus sequences.

Sample preparation and rt-PCR analysis

Midgut and salivary gland tissues from approximately 40 *H. coagulata* adults, collected near Riverside, California, were tested for presence of HoCV-1, using reverse transcriptase PCR (rtPCR), with the amplicons sequenced and compared using Blast analyses, NCBI database to identify homologous viral sequences. Upon arrival at the laboratory, *H. coagulata* individuals were removed from RNA*later*® and washed in 1X phosphate buffered saline (PBS), pH 7.0 at 4°C. Tissues from whole insects were dissected in cold 1X PBS, pH 7.0. Samples were ground directly in Buffer RLT (Qiagen) using sterile plastic pestles. RNA extractions were performed using the RNeasy® Mini Kit (Qiagen) following the manufacturer's instructions. Total RNA was eluted in RNase-free water and quantified by UV spectrophotometry. An equal concentration (500 ng) of total RNA was retrotranscribed for each sample reaction by first combining RNA, 1 µl dNTPs (10mM), and 1 µl oligo$(dT)_{17}$ primer (2.0 µg/µl) for a reaction volume of 13 µl. The mixture was incubated at 65°C for 5 min after which, 4 µl 5x buffer, 2 µl dithiothreitol (DTT), 1 µl RNasin® ribonuclease inhibitor (40 U/µl) (Promega), and 1 µl SuperScript™ III (200 U/µl) (Invitrogen) were added. The reaction mixture was then incubated at 55°C for 1 hr and subsequently terminated at 65°C for 10 min. Primers were designed using Primer3 (Rozen and Skaletsky 2000) GenBank® accession number DQ308403 (*Homalodisca coagulata virus-1* capsid protein). Amplification was performed using 22.5 µl Platinum® PCR Supermix (Invitrogen), 1 µl of the forward (5′-TCC GAG TTC TCA GCC AAA CT-3′) and reverse (5′-CGG CAT ATC GAA ATG AGG TT-3′) primers combined (10 µM) each, and 1.5 µl cDNA. The reaction mixture was subjected to an initial denaturation at 95°C for 2 min followed by 35 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 1 min, and concluded with a final DNA extension at 72°C for 5 min. Validation of five amplicons by sequencing were completed, after which samples were considered positive when a visible amplicon (443 nucleotides) was present after separation on a 1 % agarose (TAE) gel stained with ethidium-bromide, EtBr (0.5 µg/ml).

Computer analysis of HoCV-1 nucleic acid and deduced protein sequences

Base confidence scores were designated using TraceTuner® (Paracel, http://www.paracel.com). Low-quality bases (confidence score <20) were trimmed from both ends of sequences. All quality trimming, vector trimming and sequence fragment alignments were executed using Sequencher® software (Gene Codes Corp., http://www.genecodes.com). Amplicons were sequenced and compared by Blast analyses for adults and midgut and salivary gland tissues that tested positive for HoCV-1 by rtPCR to identify homologous viral sequences.

The potential status of the of the HoCV-1 capsid protein was determined based on Blast homology searches using the National Center for Biotechnology Information Blast server (http://www.ncbi.nlm.nih.gov) with the sequence comparisons made to protein databases (BLASTX, TBLASTX, BLASTP). Contig assembly parameters were set using a minimum overlap of 50 bases and 90% identity match. Multiple alignments were performed with CLUSTAL X, version 1.83 (Thompson *et al.* 1997) using the following sequences (with their respective GenBank® accession numbers): ABPV (NC_002548), BQCV (NC_003784), CrPV (NC_003924), DCV (NC_001834), HiPV (NC_003782), KBV (NC_004807), PSIV (NC_003779), RhPV (NC_001874), TrV (NC_003783). Protein molecular weights were approximated using suite 2 of the Stothard method for sequence manipulation (Stothard 2000). Phylogenetic trees were constructed using the capsid protein, CP, sequences via the neighbor-joining method using PAUP* version 4.0 (Swofford 2003). For each tree, confidence levels were estimated using the bootstrap resampling procedure (2000 replications).

Theoretical modeling of the HoCV-1 capsid proteins

The theoretical structures of HoCV-1, CP1-4 were determined using CrPV, protein database, PDB, entries 1b35a, 1b35b, 1b35c and 1b35d respectively. The theoretical structures of HoCV-1 CP 1–4 were numbered according to the standard convention, with amino acids numbered starting from 001 in each protein.

Computer analysis of EST sequences

Vector and quality trimming, base quality scores generated with TraceTuner® (Paracel, http://www.paracel.com) and sequence fragment alignments were executed using Sequencher® software (Gene Codes, http://www.genecodes.com). Sequence corresponding to vector contaminants was removed from the dataset. To estimate the number of genes represented in the library and the redundancy of specific genes, ESTs were assembled into contigs using Sequencher®. Contig assembly parameters that were set using a minimum overlap of 50 bases and 95% identity match.

Standard DNA and protein sequence analyses performed used BLASTn and BLASTp, respectively (Altschul et al. 1990*1997*). The National Center for Biotechnology Information (NCBI) biosynthesis databases were searched via the Entrez Search and Retrieval system (http://www.ncbi.nlm.nih.gov/ gquery/gquery.fcgi). The theoretical molecular weight and pI value of the predicted protein was calculated by Compute PI/MW on the ExPASy server (http://au.expasy.org). Compute pI/MW calculates the molecular weight of an input sequence by adding the average isotopic mass of each amino acid plus one water molecule.

Structural modeling was performed using the Robetta automated server (http://robetta.bakerlab.org). Robetta is a full-chain protein structure prediction server and permits both initial and comparative models of protein domains. In addition, Robetta performs domain parsing and 3-D modeling included fragment library generation. The programs utilize the Ginzu domain parsing and fold detection method and the Rosetta fragment insertion method (Bonneau *et al. 20*02, Chivian et al. 2003, Kim *et al. 20*04, Simons et al. 1997). Protein chains were scanned to identify homologs using PDB-BLAST, FFAS03, 3D-Jury, and the Pfam-A protein family database. PSI-BLAST multiple sequence alignment then assigns regions of increased likelihood of including an adjoining domain using sequence clusters (Bowers et al. 2000; Rohl and Baker 2002). Graphic rendering of the predicted 3-D structures were done using the PDB file created by Robetta and Protein Explorer (http://www.molvis.sdsc.edu/protexpl/frntdoor.htm).

Phylogenetic analyses

Multiple sequence alignments of predicted HoCV-1 capsid protein amino acid sequences (1–4) were performed using CLUSTAL X version 1.81 algorithm (Thompson et al. 1997). Guide trees were generated using neighbor-joining and Bayesian methods. Neighbor-joining analyses were performed using PAUP* version 4.0β10 (Swofford 2003). Neighbor joining estimates of the phylogenies were obtained by estimating distance parameters in PAUP* (Swofford 2003). Neighbor-joining bootstrap analyses of 2000 replicates were performed on each data set using a heuristic search to identify the most optimal tree. Analyses were unrooted.

Results

A total of 8,600 random EST sequences were generated from *H. coagulata*. Viral sequences were initially discovered through analysis of the cDNA library generated from whole bodies of adults. Approximately 5.8% (500) of the clones were homologous to viral sequences as indicated by BLASTX Results. Analysis using rtPCR showed that the virus was in the midgut tissues, but not in salivary glands (Fig. 1). Blast analysis of the viral capsid protein indicated that the amino acid sequence had homology to the capsid proteins of insect picorna-like viruses, within the Family *Dicistroviridae*. The capsid protein sequence of HoCV-1 was submitted into GenBank (accession number: DQ308403), and was used to perform TBLASTX search of the NCBI database. Sequences with significant amino acid similarity were downloaded for comparison to the HoCV-1 sequence. Comparisons were made to capsid protein sequences of: *cricket paralysis virus* (CrPV) [NC_003924], *Drosophila C virus* (DCV) [NC_001834], *Triatoma virus* (TrV) [NC_003783], *Himetobi P virus* (HiPV) [NC_003782], *Plautia stali intestine virus* (PSIV) [NC_003779], *black queen cell virus* (NC_003784), *acute bee paralysis virus* (ABPV) (NC_002548), *Kashmir bee virus* (KBV) [NC_004807], and *Rhopalosiphum padi virus* (RhPV) [NC_001874] (Table 1).

The HoCV-1 capsid protein had a predicted molecular weight of 97.94 kD and a pI of 5.69 (http://us.expasy.org/cgibin/pi_tool). The comparison of the predicted amino acid sequence of putative HoCV-1 capsid protein identified several clusters of conserved amino acids (Fig. 2) separated by large regions of low, or no homology, or gaps. Amino acid identity between CP1-4 of CrPV and HoCV-1 ranged from less than 20% for CP1 and CP4, to 35% for CP2 (Table 2).

The phylogenetic analysis performed using the optimized protein alignment generated a phylogram containing 5 clades (Fig. 3). Clade 1 consisted of *Drosophila C virus*, Clade 2 consisted of *cricket paralysis virus*, Clade 3 of *Triatoma virus*, *Plautia stali intestine virus*, *Himetobi P virus*, *black queen cell virus*, and HoCV-1. Clade 4 encompassed *Acute bee paralysis virus* and *Kashmir bee virus*, and Clade 5 consisted of *Rhopalosiphum padi virus*. When the four capsid polyprotein units were analyzed individually HoCV-1 always fell within the same clade (Figure 3)

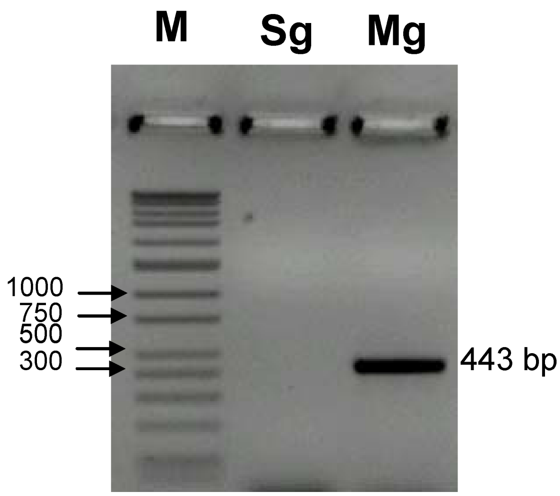

Figure 1. Gel of detected virus from salivary gland (Sg) and midgut (Mg) tissues of *Homalodisca coagulata* adults tested for presence of HoCV-1, using rtPCR. Both types of tissues from individual insects were dissected and analyzed in a pairwise fashion. Only midgut tissues were shown to test positively for virus presence. M = ladder wide-range DNA marker (16 fragments 50-10,000 bp), Sg = salivary glands, Mg = midgut tissue. Amplified fragment ~ 443 bp, was validated by sequencing of the amplified product and Blast analysis.

Table 1.

Organism	NCBI Accession number	Function	E-Value
HiPV	NP_620561	Capsid Protein Precursor	3.00E-78
TrV	AAF00473	Capsid Protein Precursor	6.00E-76
PSIV	BAA22088	Capsid Protein Precursor	2.00E-70
BQCV	NP_620565	Structural Polyprotein	2.00E-65
DCV	AAC58808	Capsid Polyprotein	2.00E-51
RhPV	AAC95510	Structural Polyprotein	3.00E-50
ABPV	NP_066242	Capsid Protein	2.00E-29
KBV	NP_851404	Structural Polyprotein	1.00E-28
CrPV chain B	1B35 (B)	Chain B, CrPV Coat Protein	1.00E-24
CrPV chain C	1B35 (C)	Chain C, CrPV Coat Protein	5.00E-14

Table 2.

Capsid Protein	% Amino Acid Identity
VP1	<21
VP2	35
VP3	27
VP4	<21

Table 3.

CP2/CP4	Virus	Amino Acid Position	Cleavage Site
	DCV	275	IFAQ/VASE
	CrPV	290	ISAQ/AASE
	RhPV	233	SIAQ/VGEE
	TrV	252	IVAQ/AAKE
	PSIV	256	LILQ/SGET
	HiPV	258	AREQ/VNLN
	BQCV	233	MLAQ/AGLK
	ABPV	320	VTMQ/INSK
	HoCV-1		VTMQ/EQSA
CP4/CP3			
	DCV	340	MLGF/SKPT
	CrPV	348	LFGF/SKPT
	RhPV	295	AFGF/SKPQ
	TrV	309	ALGF/SKPL
	PSIV	312	AFGF/SKPQ
	HiPV	326	IPGF/KKPD
	BQCV	308	LFGF/SKPL
	ABPV	403	AIGF/WSKP
	HoCV-1		AFGL/GKPK
CP3/CP1			
	DCV	631	IVAQ/VMGE
	CrPV	646	IVAQ/VMGE
	RhPV	555	SIAQ/VGTD
	TrV	593	PIAQ/VGFA
	PSIV	580	LTLQ/SGDT
	HiPV	613	AQEQ/ANFA
	BQCV	575	MVAQ/SNSG
	ABPV	703	ASMQ/INLA
	HoCV-1		ADVQ/SAFA

as *Triatoma virus*, *Plautia stali intestine virus*, *Himetobi P virus*, and *black queen cell virus*.

The amino acid sequence alignment was scanned for putative cleavage sites of the capsid polyprotein (Table 3). Viruses in this group typically have three cleavage sites (van Munster *et al.* 2002). The order of the polyprotein subunits is CP2, CP4, CP3, CP1. The CP4/CP3 (AFGL/GKPK) cleavage boundary site was clearly aligned with all the aligned viruses. The putative CP3/CP1 (ADVQ/SAFA) site and the putative CP2/CP4 (VTMQ/EQSA) cleavage site of HoCV-1, respectively, were located in the same region as that of the other viruses. After alignment, the CP3/CP1 cleavage sites and CP2/CP4 cleavage sites of all viruses analyzed fell within 50 amino acids of one another (Figure 2). Putative cleavage sites remain to be validated by n-terminus sequencing. As with CrPV, HoCV-1 was found to be mainly comprised of β-sandwiches in structure in CP1-3 with a jelly roll topological motif (http://pdbbeta.rcsb.org/pdb/explore.do?structureId=1b35) (Bonneau et al. 2002). There were few secondary structures found in CP4 of both CrPV and HoCV-1. CP4 of HoCV-1 appeared to be mainly α-helical in structure. CP1-4 domains are most homologous to insect picorna-like virus capsid proteins as demonstrated by the BLASTP and PSI-BLAST Results, as well as by the structural modeling. While sequence homology between CrPV and HoCV-1 was low (Table 2, Figure 2) the global structure of the proteins was conserved (Figure 4).

Discussion

Analysis of the HoCV-1 capsid protein demonstrated that the virus is taxonomically related to members within the Family *Dicistroviridae*. The newly created family *Dicistroviridae* contains a single genus, *Cripavirus*. The type species of the genus *Cripavirus* is the *cricket paralysis virus* (CrPV) (Christian *et al.*

```
DCV     --------NNIENEDRK---ITSEQ-KEIVHFSSEGVTPSTTAVPDIVSLSTDYLSMTTRE-DRIHTIKDFLSRPIIIQTGLWSSATTAETQ---------------LYT
CrPV    -------NSHIENEDKR---LMSEQ-KEIVHFVSEGITPSTTALPDIVNLSTNYLDMTTRE-DRIHSIKDFLSGPIIIATNLWSSSDPVEKQ---------------LYT
TrV     --------GEINESIE-----FRN---QITTFVHDNPIITE-QLIGDSPQPSGDVRSVSD--ARTHSIIDFLERPQFIGSFLWNTSDIENKE---------------IFS
PSIV    --------NGVEYQ---------EQ---IVSFSDDAMKIDE-CLISCAPQTMNESRPASD--FREHTIVDFLERPRVVATHIWSTADARNTN---------------LVD
HiPV    --NPSVSAGRIDESVE-----F-TQ--EITHFADNAPVIDS-SIAGETNLKPSLVTDFHD--NRQHSVISFLQRPQLIKTVEWAPG-TAQGSL--------------LTT
ABPV    TNTSNVHNTQLASTS--EENSVETE--QITTFHD--VETPN-RINTPMAQDTSSARSMDD----THSIIQFLQRPVLIDHIEVIAGSTADDNKPLNRYVLNRQNPQPFVK
KBV     -------NTKLASTS--AENAIEKE--QITTFHD--VETPN-RIDTPMAQDTSSARSMDD----THSIIQFLQRPVLIDNIEIVAGTTADNNTALSRYVLDRTNPQKYIK
BQCV    --------EQINENYE-NKQQLVE-QTEITTFENDLIVLED-GPQMEEPLPYAFHGQHTD--NRQHTVVNFLQRPQVIFDSSWASDLPRNKQ---------------FMD
RhPV    --------ENTTTKI------QQQ----ILSFSSEGESPSSSTVLAPLKLQDPILDCAR--DGKTHTVNSFLERPINFRTATWSNQPAGERLFS----------------
HoCV1   ------INTNIDGET--SENTFEE-KREITHFTEDDRVLTD-AVTEITSLPLSLLQYGDE--PREHSVISFLQRPEKIATVTWTTAQTKTTN----------------LV
                                    *  *                                 *    **  *

DCV     -ANFPEVFISNTMYQEKLRGFVGLRATLVIKVQVNSQPFQQGRLMLQYYPYAQYMPN-----RVSLVNSTLQGRSGCPRTDLDLSVGTEVEMRIPYVSPHVYYNLITGQG
CrPV    -ANFPEVLISNAMYQDKLKGFVGLRATLVVKVQVNSQPFQQGRLMLQYIPYAQYMPN-----RVTLINETLQGRSGCPTTDLELSVGTEVEMRIPYVSPHLYYNLITGQG
TrV     -LKLPDALMSP-MIREKLSGFTSFSASTVFHIQVNAHPFQCGRLVLAAVPVPDILPLH----RLNMLSFDVSNVITLPHVQLDISKETEVLLKIPYVSPFVQYDLVTKFT
PSIV    -LEIPKALLDN-MNLNKFDGFSSFSATVEFKLQINSQPFQAGLLIMGALPSKDLIGS-----RNTDVKVAVDKSLYIPHTLFDISKTSEITLSVPYVSPFPQYNLVLEPI
HiPV    -IDIPDDLMTS-MVYDKLDGFATFKADTIFRVQVNAQPFQCGRLVMAYIPMPDSLST-----RTAELTRAIDRIIALPHVQLDISEQSEVTLRVPYISPYSAYNLIEGRY
ABPV    SWTLPSVVLSAGGKGQKLANFKYLRCDVKVKIVLNANPFIAGRLYLAYSPYDDRVDP-----ARSILNTSRAGVTGYPGIEIDFQLDNSVEMTIPYASFQEAYDLVTGTE
KBV     QWTLPSTVLKAGGKAQKLANFKYLRCDVQVKIVLNANPFIAGRLYLAYSPYDDKVAP-----ERRIIYTSRAGVTGYPGVELDFQLDNSVEMTIPYASFQEAYDLVSGNE
BQCV    SIMIPDDIISFPMFAEKLKGFSSLRATAVITVQFQTQPFQAGRVMLGSFPLPTLNPT-----RVKFATNHVSRLMLLNHVQCDIAKETEVSLRIPFVSPYNSYDLVSKRF
RhPV    -FNYPSNVVTNPMYSRKLQNFLGLRADLVVRVQVNAQPFHAGRLMLSWTPFLDYLGTNRKYYYTDPSSTFLTSVSGNPRVEIDLSTTTEATMTIPFVSPFLYYNLVTGSG
HoCV1   SLPIPSSVLTT-MYREKLRGFGLLRADIVFKLQFNSQPFQAGRLIATYIPVPAYLLQ-----RTRMARASLTRLTSLPNVIIDISKQTECNITLPYVSSFTHYDLTSGGG
            *           *   *                **  *                                                    *  *    * *

DCV     SFGAIYLVVYSQLRDQVTGTGSVEYTVWAHLEDVDVQYPTGANI---------FTGSSPNFAS-LGQKMSDGKFTEKDLRDIWTSKAYNKQPDK---------IFAQVAS
CrPV    SFGSIYVVVYSQLHDQVSGTGSIEYTVWAHLEDVDVQYPTGANI---------FTGNSPNYLS-IAERIATGDFTETELWIHKTYLKR---PAR---------IYAQAAK
TrV     PWAAFLAHVYAPLNTPSAAS--LQVNVFAHFEDIKLGFP--------------------------------------------------------------IVAQAGK
PSIV    NWSNFFIKVYSPLVSKQTDQ--LDLVLWARFKDIKLGYPTV-------------L-PVKTPTTD-----------------------------------------LILQSGE
HiPV    RWGRVVVAVYSPLNQVSQPN--LKVNIFGYYDNVTLGYPTLGTI---------ALSPVA---V------------------------------------------AREQVNL
ABPV    DFVKLYLFTITPILSPTSTSASSKVDLSVYMWLDNISLVIPTYRVNTSIVPNVGTVVQTVQNMTT-RDSETIRKAMVALRKNNKSTYDYIVQALSSAVPEVKNVTMQINS
KBV     DFVQLYLFTIAPVLGPSAESANSKVDLSVYMWLDNISLVIPTYRLN-PNLPTGQTLTRIVQNS----DSDKLKEALKIAKSKNPSGYKYIMGVLEQYNP----SVKQVSM
BQCV    PWAKVVGLVYSPL-TTTIP-----VDYIVYGHFEDVELGCPTSG-------------------------------------------------------------MLAQAGL
RhPV    DIGTFQLIVYSPLVDLVSGGN---IDYTIWVNMTNVRTEFPTG--------------------------MPT---------------------------------SIAQVGE
HoCV1   DWGLFDLWVYSPLSSASSQT----INISIRAYLDNVRLGAPTQQSLVTAEKMLKANVQT-RDLSRGTSSCGSISARAQGGKQTAGSGDGSFGSLLNKGTKS--VTMQEQS

DCV     EITQLKE-------------------------------SGTISSGIGQ-VSEGLSTMSKIPILGNMFTKPAWISAQVSNIFKMLGFSKPTVQGLPCESKLRGQVRMANFD
CrPV    ELKQLET-------------------------------NNSPSTALGQ-ISEGLTTLSHIPVLGNIFSTPAWISAKAADLAKLFGFSKPTVQGKIGECKLRGQGRMANFD
TrV     E---------TSA-------MRKGKEQLGP--ISGLTNTVTGVVGGVADAVKGFFPSIGKYADPLVGIG--N---GLTGLLSALGFSKPLTTIPPTIVVQRPSQYALSAQ
PSIV    TSGP--VSKVAATIVDVAEGVGKGVS-----------------------------NYIPTVKP-FVNGMTTVGRGIQSLIA-AFGFSKPQKLDNQNQVVVRPGFNLANVD
HiPV    NSEADMLRIAESRNF-PTKIAASINGVIQKGSDILG-------------------NVLPQTKS-FTNPVAKISDAAFDIISMIPGFKKPDKTNHGETVLFRPTQYFGNVD
ABPV    KKNNSNKMATPVKEKTKNIPKPKTENPKIGPISELATGVNK-VANGIERIPVIGEMAKPVTSTIKWVADKIGSVA-------AIGFWSKPRNLEQVNLYQNVPGWGYSLY
KBV     Q----IATP----------NKSKST—KPTSENPKIGPISEVASGVKTAANGIERIPVLGEIAKPVTAAVKWFADIVGGVA--AIGFWSKPRNQNQVMPYQNVPGWGYSLY
BQCV    KVQPPTNSDSRQRAKESKGKKDYSKTGAAFKQSVSKLGEHLP---FLKSGANW-VSENILQPA---------DEIIGPILS-LFGFSKPLL-PITN-PTVLRPANTFAIT
RhPV    EGSTQQKQGFVTRQSEAYSTIMEPLT-KIPG-----------------------VGQ--LIGYAKSGV----D-ALHAVAS-THGWSKPLNPADMQLFKQAPSKFMCNS-
HoCV1   AG--------------------TISRVGHSIREGLVRGLNVVGEFIPGLSEITDTANSVAMGVLNTLA---------------AFGLGKPKNLDKIAPRTLHAFSDFAQA-

DCV     GADTSHKLLSGTSPDEMDLSHVLSIPNFWDRFTWNTTDATSSILWDN----YVTP--MKIKPY---SSTILDR---------FRCTHMGFVANTHGYWCGSIVYTFKFVK
CrPV    GMDMSHKMALSSIETKEGLAGTSLDEM-DLSRVLSIPNYWDRFTWKTSDVTNTVLWDN---YVSP--FKVKPY---SATITDR---------FRCTHMGYVANAFTYWRG
TrV     NEIETKSGFNNADGVDQGLPLSLKYGNEVILKTPFAGTSSDEVALEYVLKIPNYFSRFKYSSTSLPKQVLWTS----PVHP--QIIRNH---VTVVDAP---------GQ
PSIV    GVDTGVPMSAFAGNALPLMSSLGGSDA-DELSFKYLLQQPNYIDSFSYSSTITSPTTIWST---HLCP--FFLS-----KTDIQGH---------PQPTLLYYLSNFFL-
HiPV    GVEHSHKLGYHAMNRIDFQPDFAGSKM-DEMSFDYVKRIPNYIDSFSYSNSNVYGDTLWST---AVSP--CYRSAD---YTTTNGARNFS----FPTPTSLTYAIGPFS-
ABPV    KGIDNSVPLAFDPNNELGDLRDVFPSGVDEMAIGYVCGNPAVKHVLSWNTTDKVQAPISNGDDWGGVIP--VGMPCYSKIIRTTENDTTRT-NTEIMDPAPCEYVCNMFS
KBV     KGIDMSVPLAYDPNNELGDLRDVFPSAVDEMAIGYVCGNPAIKHVLTWSTTDVVQNPISNGDDWGGVIP--VGMPCYSKTIRAVKGATSTS-KTEVMDPAPCEYVANLFS
BQCV    DTNDMSHSLALSNDTNVPFVKALDGSGLDEMSFDYLKKIPQFIQSKFFTTTKPQEVLFQT----KVMP--HYFVPAGDVTVAMDKDKTRT----IWQPSHLAYITSMFK
RhPV    DGSDMATNLGLTSQNEIEHLQSLFRTDSDEMSIDYVARTYNYVSRFNWIKGNGPGTVLYSH----VVSPT-SWFTEVG--ITGLSIP-------------HLYFAASNFV
HoCV1   TGVDNGHILSLHGDNKVTVLPGFAGSNTDELSMTYLMQTLQYYDTHTISTTTAVGTQIAAY----RVTPFRFDLDLAKTAQSFVSGS---PLIN-FGQPNLQWYIGSNFK

DCV     TQFHSGRLRISFIPFYYN-----------TTISAG-VPDVSRTQKVIVDLRTSTEVSFTIPYVSSRPWMYC--IRPEASWLGTDN---ALMYNAVTGIVRVEVLNQLVAA
CrPV    SIVYTFKFVKTQYHSGRLRISFIPYYYN-----------TTISTG-TPDVSR TQKIVVDLRTSTEVSFTVPYIASRPWLYC--IRPESSWLSKDNKDGALMYNCVSGIV
TrV     PTLLAYATGFFKYWRGGLVYTFRFVKTNYHSGRVQITFHPFVGYDD---------VMDSDGKIVRDEYVYRVVVDLRDQTEATLVVPFTSLTPYKVCADVFNSANRPKYN
PSIV    YWRGSLKFTFRFVKTNYHSGRLELVFSPFS------------QTQSSDFVNRSAYAYKVVMDLREQTEFSVVIPYVNTRNYSYCD-MRTTG--PPIDA---TVTNP--NT
HiPV    LWRGSIVYTFRAVKTEYHSGRIEFSFNPFIN--L---------DMYNTNKTTRSEYVYKVILDLRTQTEISFTVPYAGTTPFKR----IRPEI--NPLSSSGISVDD---
ABPV    YWRATMCYRIAIVKTAFHTGRLGIFFGPGKIPITTTKDNISPDLTQLDGIKAPSDNNYKYILDLTNDTEITIRVPFVSNK--MF----MKSTGIYGGNSENNWDFSES--
KBV     YWRATMCYRITVVKTAFHTGRLEIFFEPGSIPTVRTADNLGPDQTQLNGTIAPSDNNYKYILDLTNDTEVTIKVPYVSNK--MF----MKTVGIYGAHDEDNWNFDES--
BQCV    YWTGSLVYTFKFVKTDYHSGRVEISFHPFS--------------DYTAN--SYSDYTYRIIVDLREKSEFSVTIPFISPVPYKR----ISRPD-WDKPYS---KYAHAS-
RhPV    LWRGGMNIKLKFVKTKFHSGRVRILYVPGFFG-----------GTLPTN--FETDANYSTVVDLRSDTDVEFNVPYVATVPWLH----VNSTP-WVTAFSQIHACRS---
HoCV1   YWRGDIVMHLALVKTDYHSVRLKIVYDPMAQS------------AAAVT-YDASEYCYSIVVDFRDKTDIYVRLPFISATPWKL---VPPST-YTGYTPPPVNQQEGLS-
```

Figure 2. Multiple alignment of predicted amino acid sequence of putative capsid proteins of *Homalodisca coagulata* virus-1 (HoCV-1) with the homologous proteins of *Drosophila C* virus (DCV) (Johnson and Christian 1998), *cricket paralysis* virus (CrPV) (Koonin and Gorbalenya 1992), *Triatoma* virus (TrV) (Czibener *et al.* 2000), *Plautia stali* virus (PSIV) (Sasaki *et al.* 1998), *Himetobi* P virus (HiPV) (Nakashima *et al.* 1999), *acute bee paralysis* virus (ABPV) (Govan *et al.* 2000), *Kashmir bee virus* (KBV)(De Miranda *et al.* 2004), *black queen cell* virus (BQCV) (Leat *et al.* 2000), *Rhopalosiphum padi* virus (RhPV) (Moon *et al.* 1998). Predicted cleavage sites are highlighted in yellow, areas of identity in grey shading, (*) denotes conserved amino acids.

```
DCV    NNVFQ-SIDTIVEVSGGPDLTFAAPMA-PSYVPYSGGFTLADDAAAKKQREEEYDNNIPQTISNRGKREVEDAR-----------IVAQVMGEDLAIQRN-DAQHGVHPM
CrPV   RVEVLNQLVAAQNVFSEIDVICEVSGGPDLEFAGPTCPSYVPYAGDLTLADTRKIEAERTQEYSNNEDNRITTQCSR--------IVAQVMGEDQQIPRN-EAQHGVHPI
TrV    YEPRDFKVYDN--TTDQFFTGTLCVSALTPLVSSSAVVSSTIDVLVEVKASDDFEVAVPNT-PLWLPVD-SLTERPSLDGV----PIAQVGFASAGTR-----------
PSIV   VIAHASPGMIAINALTPLQLASELLPTSIDCVVEVSGGDDFELQAPIN-EGWVGFD-SAS---------------SSQ-------LTLQSGDTFGSTGIR----------
HiPV   --FNVFATGVLGVRALTPLVLGSTVVPSTIQILVEMKGGPDFEVECPNS-TGWMPIH-SIT--PAATGRDTVDSELVST------AQEQANFASTGQH-----------
ABPV   --FTGFLCIRPITKFMCPETVSN-NVSIVV-WKWAEDVVVVEPKP-LLSGPTQVFQ---P---P--V--TSADSINTID------ASMQINLANKADENVVTFFDSDDAE
KBV    -------FTGFLCIRPITKLMAPDTVSQ-KVSIVV-WKWAEDVVVVEPKP-LTSGPTQVYN--P-----P-A---VARDLVKQIDVSMQINLSNKTDENTISFFDSGDPE
BQCV   --------TGTLVLKALTSLKATNTVVSNSVEILIEVNAGDDFNVIAPIE-NIFFPFS-LS--p------------GRKG-----MVAQSNSG---------------TE
RhPV   -----------IVVEVLNELVNTSTVSD-TIEVLVEVCAAEDIEFAIPIV-PSLTPRAAPQGS-----SRRTY--DLIT------SIAQVGTDT--------------GD
HoCV1  -----TYSGYVAVFVDNILQASSAVVSQSIEMVSEFCAASNLDMGFPHGGQNWIPISTVLN--P-----------GDPIQ-----ADVQSAFAADGILK-----------

DCV    TIDTHKIDSNWSPEAHCIGEKIMSIRQLIKRFGMALNSLNLIS-DAPNTLIA-PFS-VQHP-----TPVVAPAEP------MS-----------LFEYYYFIYGFWRGGM
CrPV   SIDTHRISNNWSPQAMCIGEKIVSIRQLIKRFGIFGDANTLQA-DGSSFVVA-PFT-VTSPT-----KTLTSTRN------YT-----------QFDYYYYLYAFWRGSM
TrV    --DIRSSYV-EGKFIPQDITGMSRNHELDEQPSQECIGERILSFSELIKRNSWRYVSDEKSL-IYPAYAFD-NPA-AMYTAADKLPVWTLTPRS-------G--------
PSIV   ---D--SR-VNTV-DNKVDFQSVTGNNRSLDVDTSHAEHCMGERMVSMRPLLKRPSYAFTSTGNLF-TYIDILR--LNSIFTDDTGSYLVDFGATTKD-------NPI---
HiPV   --DIRSDYL-EDKIEIKDITGISSNISLNTEKSLSCVGESFGNFRDLIKRFGW-FKNQSVAF-TNTKILSG-IPI-VNYTSSIAGTGLTLTADG-------TSIGAMYAF
ABPV   ERNMEALLKGSGEQIMNLRSLLRTFRTISENWNLPP-NTKTAITD-LTD-------------VADKEG------RD-----------YMSYLSYIYRFYRGGRRYKFFN-
KBV    RMNNEALMRGCGEQIVNLRPLLRTFRTINDNWSLAA-NTKTPITD-LTN-------------TADAEG------RD-----------YMSYLSFLYRFYRGGRRYKFFN-
BQCV   QQNP-RGSSLLTDPESITKSDPYNPNISLLISGEVFTNFRNLIKRVN-FRKATTLNG-KRISDTFD-INSLIEAPR--LDIAQYVDTETKEAK--YG--FSYFWSAPTT-
RhPV   TPLEVSREEPTTFNDVPLQPTTTTFNASMLMMGEKVTSFRQLIKRFSAITPPTQNRYWEFTQPFW--INT-NRLEG--VTQEGSSDIDG---------I-----------
HoCV1  ---TRTNMQ-ENTLDIKNITGMAPR-PLHDNITSYTTGEEVYSLRMLMKRFNWIAS---VPSGQASIALPNTVKT-IDAAAPVSNPNQIVDIRTG---PPYAN---NTVS

DCV    RFKLQAVRTNSAETS-VK--TDTTWTVNLWNSVQDSFNSLINVFSTTDYPIKSTG-----------ALPAGTSGFGNSMTYIDPEVEGFMEFEIPYYNISHI------SP
CrPV   RIKMVAETQDGTGTPRKK--TNFTWFVRMFNSLQDSFNSLISTSSS--------AVTTTVLPSGTINMGPST-------QVIDPTVEGLIEVEVPYYNISHI------TP
TrV    FPTLLVAD---QP------KPLVEVALFTMQDQGYIIKANDYST--------------------DFCSSN-------IYENFVTKGIAEVQTPYYSRVNT------SVVS
PSIV   -D------KGQVVPNLYYAYISGLTTSSNTYMS------------------YP-FSVE--------QYN---AKSLCEFNYPYYNSFKF------SAVA-----------
HiPV   YRGGIRLKIVPG ------------HDLPAGEMVQGALIDNSQNTNVPQP---------------------LALQSL------QYELS-DKRLYEFSWPYYCPTYL----
ABPV   --------ACNLLSRIVQMYAFYRGGINIKVAP--- --T-TA---LKQSQTCYVRSF-LIPRYYT--ADNTNN---------------------DGPS--------HIT
KBV    -G---------STPLTMVSSMYAFFRGGFRAKVYI--- --T-TP---LKQSQTCYVRSF-LIPRNYT--ADEINT---------------------DGPS--------H
BQCV   ---VD------FVRATVSPQQTYGSDVAPTTHISTP---------------------LAIE--------QIP---IKGVAEFQIPYYAPCLS------SSFR--------
RhPV   ------------ASPLVVALKKAPNSLYSGVRVIDTNGT----------------WTYPDYKGA--------EVFMTPNEGIHELSIPYYSSYPV------TLTT----Y
HoCV1  DCALVDVVGALFAFRAGGFRWLNIVAEMYALYRGGVRVKVVTEKG GSE--LIS------AYLVPFGPYNTYGIPPSTFTNLISN---------------------TSVY

DCV    AT---TYVRGT-------ESPIT--INSVLRGHLPPQIVAVAP------------------------------------------------------------------Q
CrPV   AV---TIDDGT---------PS---MEDYLKGHSPPCLLTFSPRDSISA---------------------------------------------TNHHITASFMRAPG
TrV    APVLYNAGNIS-------PLMPNVMYKITSNSSNILLGHSA-------------------------------------------------------------------A
PSIV   ---------------------TNQ--------------------------------------------------------------------------------------
HiPV   --TTYPSGSLNYISDLV-----NPTTYAR-ITTISEYATAYAMAAAD------------------ ------------------------------------------D

ABPV   YPVLNPVHEVEVPYYCQYRK------LPVA-----STTD---------KGYDASLMYYSNVGTNQIVARAG--------------------------------------N
KBV    ITYPVINPVHEVEVPFYSQYRK------IPIA---STSD---------KGYDSSLMYYTNVGTQQIVARAG--------------------------------------N
BQCV   ---------------------ANS-------------------------------------------------------------------------------------
RhPV   NSTDSDVL--DARNGFNRVIARFHSDTSAYVYR-AA-------------------------------------------------------------------------G
HoCV1  --------ELDSRQVKGSAEFATPFYHPCYTQVNSNFSYFT-----EGGEPD-LYFHFTQP-SWFASLYAFYRGSMRYKIAPLSNWDSQTVTVVSRSNPGSEMNIAKSAG

DCV    GTIATTDVVN------------------------------   866
CrPV   DDFSFMYLLEV-----------------------------   878
TrV    DDFRFGFLLGAPLAISATALRDNFTGSSATVSL-------   821
PSIV   ----------------------------------------   737
HiPV   --FDCGFYLGA-----------------------------   816
ABPV   DDFTFGWLIGT-----------------------------   888
KBV    DDFTFGWMIGT-----------------------------   864
BQCV   ----------------------------------------   758
RhPV   DDFSFGFLLGAP----------------------------   776
HoCV1  DDLNFGFLLGVPDCLPSQIVAGLLSRPSSQPNLPNSTPIS   899
```

Figure 2 (con't).

2000). Other species within this genus are: *Drosophila C virus* (DCV), *Plautia stali intestine virus* (PSIV), *Himetobi P virus* (HPV), *Triatoma virus* (TrV), *black queen cell virus* (BQCV), and *Rhopalosiphum padi virus* (RhPV). Tentative species within this genus include: *acute bee paralysis virus* (ABPV), and four other candidate species: *aphid lethal paralysis virus* (ALPV), *Kashmir bee virus* (KBV), *cloudy wing virus* (CWV) and *Taura syndrome virus* (TSV) (Mayo 2002). The use of viral capsid proteins have been shown to be a suitable target for phylogenetic studies in other insect viruses (Liljas *et al.* 2002, Tidona *et al.* 1998). As more insect-infecting viruses are sequenced and their genomic organization analyzed new virus relationships will be identified within the insect picorna-like viruses. Five clades were identified here: 1) DCV; 2) CrPV, 3) TrV, PSIV, HiPV, BQCV and HoCV-1; 4) ABPV, and KBV; and 5) RPV (Fig. 3). HoCV-1 had the highest matching BLASTP score (Table 1.) with *Himetobi P virus*, HiPV, which was discovered in planthoppers (Toriyama, *et al.* 1992). Planthoppers are a taxonomically closely related insect group to

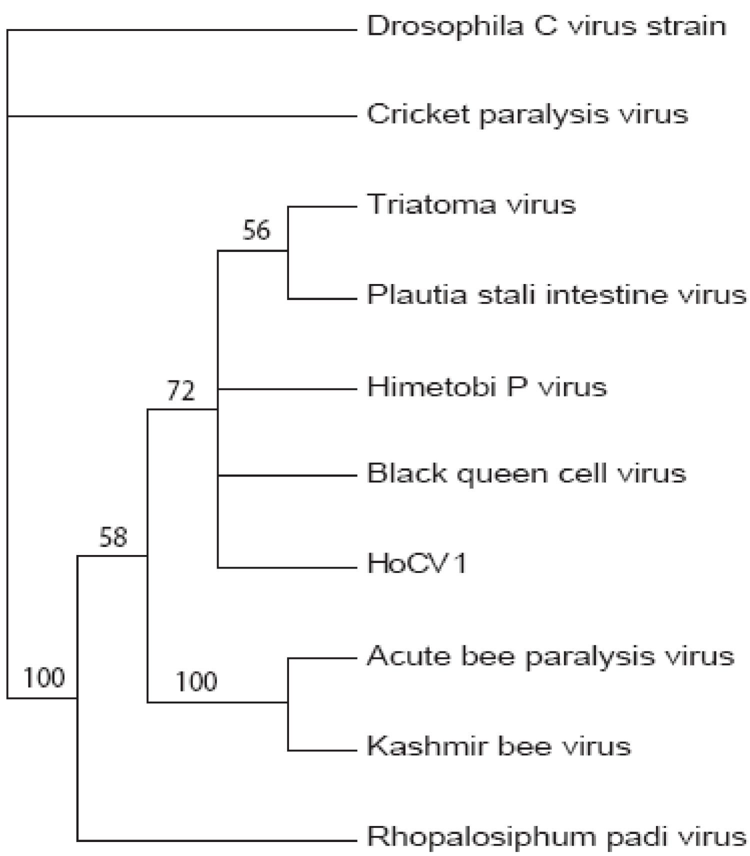

Figure 3. Neighbor-joining analyses of *Homalodisca coagulata virus-1*, HoCV-1 capsid protein amino acid sequence with selected Cripavirus members: *Drosophila C virus* (DCV) (Johnson and Christian 1998), *cricket paralysis virus* (CrPV) (Koonin and Gorbalenya 1992), *Triatoma virus* (TrV) (Czibener *et al.* 2000), *Plautia stali virus* (PSIV) (Sasaki *et al.* 1998), *Himetobi P virus* (HiPV) (Nakashima *et al.* 1999), *black queen cell virus* (BQCV) (Leat *et al.* 2000), *acute bee paralysis virus* (ABPV) (Govan *et al.* 2000), *Kashmir bee virus* (KBV)(De Miranda *et al.* 2004), *Rhopalosiphum padi* virus (RhPV) (Moon *et al.* 1998). Phylogenetic trees were constructed via the neighbor-joining method using PAUP* version 4.0 (Swofford 2003). For each tree, confidence levels were estimated using the bootstrap resampling procedure (2000 replications).

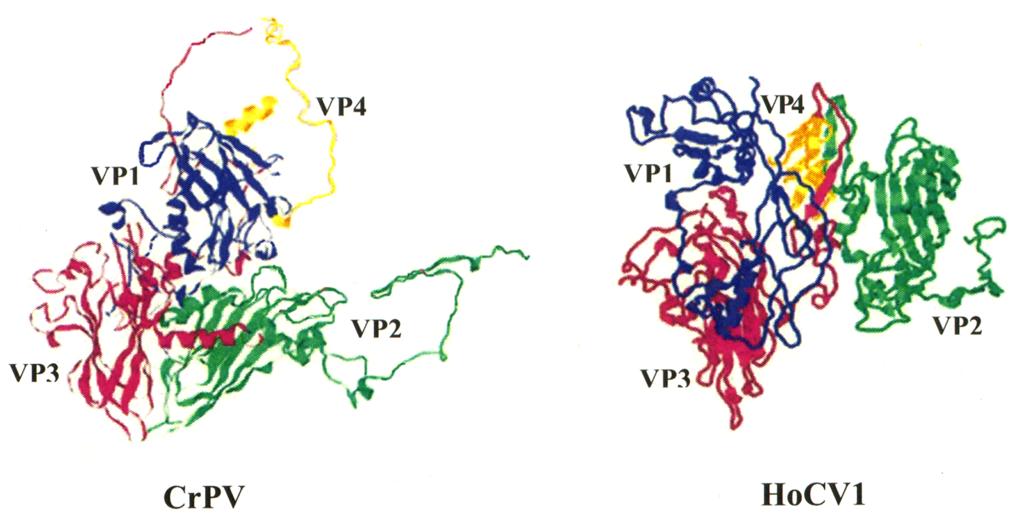

Figure 4. Capsid protein structural prediction and model comparison of *Homalodisca coagulata virus-1*, HoCV-1 to the *Cripavirus* type member, *cricket paralysis virus*, CrPV, Family *Dicistroviridae*.

leafhoppers within the Hemiptera. The virus was detected primarily in midgut tissues of *H. coagulata*, and this may be the primary site of infection and replication at least in leafhoppers.

Invading insects are constantly exposed to native pathogens as they move into new environmental niches. In this case, a leafhopper-infecting virus, HoCV-1, was identified that infects *H. coagulata*. Future research will need to focus on whether HoCV-1 can be used to reduce *H. coagulata* populations as a biological control agent, either by augmentation and release, or through other means such as bioengineering. Insect pathogens reduce populations naturally in the wild and if more individuals can be exposed to viral infections then the virus may ultimately be useful as a biological control measure (Hunter-Fujita *et al.* 1998). Many crops of economic importance such as grapes, almonds, pecans, and other woody tree crops, especially in California, and throughout the Southeastern part of the United States would benefit if leafhopper populations could be safely reduced. Further studies need to evaluate the mode(s) of virus transmission and stability in *H. coagulata* populations under field conditions. The largest obstacle with all viral pathogens is the development of a mass propagation system. Although cell cultures of *H. coagulata* have been established (Kamita *et al.* 2005) the ability for mass production of leafhopper viruses for commercial applications still remain unknown. The potential usefulness of HoCV-1 as a biological control agent against the *H. coagulata* and in reducing the spread of Pierce's Disease, awaits the answers to these questions.

Notes

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Acknowledgements

We gratefully thank Dr. Phat Dang, Genomics Lab, USDA, ARS, U.S. Horticultural Research Lab, Ft. Pierce, FL, for sequencing and technical assistance, Laura Hunnicutt, Biological Technician, USDA, ARS, U.S. Horticultural Research Lab, Ft. Pierce, FL for bioinformatics and technical assistance, Dr. Gary Puterka and Mike Reinke, USDA, ARS, Research Entomologist, Stillwater, OK, USA, for samples of *H. coagulata* adults and nymphs, Dr. Heather Costa, University of Riverside, CA, for samples of adult *H. coagulata*.

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