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Identification of a Novel Virus in Wasp-*Microplitis similis* Bracovirus: a Possible New Species of Polydnaviridae

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ABSTRACT

Polydnaviruses, double-stranded DNA viruses with segmented genomes, have evolved as obligate endosymbionts of parasitoid, and plays a very important role in the life cycle of parasitoids. The PDV symbiotic with different endoparasites are often different. Here we completed the the six protein tyrosine phosphatases (PTPs) and six ankyrins sequence of a PDV which from *Microplitis similis*, by sequencing and compared the gene features of this PDV to those of other polydnaviruses. Phylogenetic analysis indicated that it was closely related to *Microplitis demolitor* BV (MdBV) and *Microplitis mediator* BV (MmBV), and this result was supported by nucleotide multiple sequence alignment. So a new species of PDV-MsBV was identified and characterized in *Microplitis similis*. It provides a new material for the co-evolution of biology. At the same time, the structure and function of these 12 proteins were predicted and analyzed. The results showed a new perspective on the study of PTPs and ankyrins protein family in PDVs, and provide the materials and foundations for analysizing the function of MsBV.

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Introduction

Endoparasitoid wasps (Hymenoptera: Br- -aconidae) are one of the important natural enemies, which have been considered as potentially useful agents for biological control of insect pests. As a typical wasp, *Microplitis similis* that has been recorded in Changsha City, Hunan Province, China [1], has a typically lifestyle with its adult lay an egg into the host *Spodoptera exigua* larvae and development and growth in vivo, until a cocoon forms in vitro.

This lifestyle emphasizes the relationships between hosts and parasites, being accompanied by the complex immune and physiological interactions [2]. To overcome host defenses, the wasps have evolved an obligate association with the viruses belonging to the family *Polydnaviridae*, which can suppress the immune responses and disrupt the development of the host to create an environment favorable for the development of the parasitoid [3]. The PDVs was formally recognized as *Polydnaviridae* in 1995 because all isolates share a common life cycle and possess several traits found in other viruses. The PDVs do not replicate in the wasp's host and the replication only occurs in female wasps in the ovaries called the calyx. Virus particles are stored in the lumen of the oviducts with the resulting suspension of virus and protein called calyx fluid. When the females lay the eggs into the host larvae, the quantity of calyx fluid from her venom gland also was injected together.

Viral DNAs enter the nuclei of cells with transcription in the apparent absence of replication occurring over the period required for the wasp's progeny to complete development, so the transmiss- -ion of PDVs relies on the survival of parasitoids and parasitoid survival depends on infection of the host by the virus [4, 5].

Several bracoviruses (BVs) genomes have been sequenced. The viral genome sizes range from 200 kbps in *Microplitis demolitor* bracovirus (MdBV) to 600 kbp in *Cotesia vestalis* bracovirus (CvBV). In BVs, over 20 different protein families have been identified, *Cotesia congregata* bracovirus (CcBV), CvBV, *Glyptapan- -teles indiensis* bracovirus (GiBV), *Glyptapanteles flavicoxis* bracovirus (GfBV) and MdBV has 16, 17, 16, 15 and 7 protein families, respectively [6-8]. The six protein families are conserved in the multiple protein families, including Vank proteins, Tyrosine phosphatases proteins (PTPs), HP proteins, Ben proteins, Egf-like proteins, and Mucin-like proteins. The method of phylogenetic analysis using the conserved protein family is reliable [9, 10]. Here, the primers of PTPs and ankyrins genes were designed, through PCR amplification and homology comparison analysis to to determine whether there exist PDV- -*Microplitis similis* bracovirus (MsBV) in *Microplitis similis*. Simultaneously,   the

structure and function of the proteins encoded by those genes have been predicted. Our research had been to provide the materials and foundations for analysizing the function of MsBV.

Materials and methods

1. Insects rearing and DNA extraction

The wasp *M. similis* and its host *S. exigua* were maintained as previously described [1]. Viral DNA was extracted from *M. similis* female using TaKaRa MiniBEST Universal Genomic DNA Extraction Kit Ver.5.0 (TaKaRa, Biotechnology, Dalian) according to the manufacturer instructions.

2. PCR amplification and sequence analyses

All samples were first tested by nested--PCR with one pairs of outer primers and inner primers to confirm PDV. The PTPs and ankyrins region of the MsBV were amplified using the primer pairs (Table 1). PCR products were separated through 1% agarose gel, and then been sequenced. Putative open reading frames (ORFs) were predicted by ORF finders (https://www.ncbi.nlm.nih.gov/orffinder/). Homologous analy--ses were constructed by NCBI's BLASTN and BLASTP (www.ncbi.nlm.nih.gov/BLAST/) (Table 2).

3. Phylogenetic analyses

The phylogenetic analysis were conducted based on the sequences PTPs and ankyrins amino acid. The evolutionary history was inferred using the Neighbor-Joining method in same software with 1000 Bootstrap replicates by Using the Geneious 8.04 software.

4. Structure analysis and function predicted of protein

The DNA sequences were translated into acid amino sequences that were analyzed using Expert Protein Analysis System (ExPASy) Tools (http://expasy.org/tools/) for the basic physical and chemical properties of the protein. Then, the protein sequences were submitted to the I-TASSER website (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) for the secondary and tertiary structure prediction [11].

Results

1. Homologous segments identification

Two clear bands in expected size were observed through the first round of PCR amplification with the outer primers (data not shown). Furthermore, another 2 bands in predicted size also were showed based on the second round of PCR amplification with the inner primers, of were observed (Fig. 1). The sequences the PCR products revealed high identity with MdBV clone comp102069_c1_seq5 genomic sequence (Accession: KX223706.1).

Homologous segments from other PDVs were retrieved by BLASTN analysis using MsBV segments as query, to determine the relationship between MsBV and other PDVs segments. Based on a high degree of similarity (Table 2), a

one-to-one correspondence between MsBV and MdBV was proposed. MsBV segments have high identities with those of MdBV. The segment MsBV ank-1 shares 95% query coverage with segment C-1 of MdBV (AY875681.1), indicating that these two segments are closely related.

Table 1:   Primers used in this study

| Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Length (bp)
Outer P	GATGGGTGTTTTAGATCTTCTAGTTC	TCAGAAAAACTTTCTTCGCAGT	516
Inner P	CAGCGAGCAATAAAGATGAT	GAACGCCGTCTGAGTGTAGT	188
MsBVPTP-1	ATGAGTCAATGCAAATTCAGGA	CTAGTTATCTTTGATAAGAAGA	978
MsBVPTP-2	ATGGATTTGAGGAAATAAAT	CTATTTCTTTGACTTCTGTCAT	636
MsBVPTP-3	ATGCCCATCGAAGATAAGAG	TCAACACACGTTAACTGTATCA	894
MsBVPTP-4	TATCATGGATCTTATTATAAGAATG	TTACAATTAACCATTCCAGTGA	1154
MsBVPTP-5	AAGGGGGGATTATCCTACTT	GGGGGGACTGGGATGAGTTC	1092
MsBVPTP-6	ATGCAGGGACCTATGAA	CTAGAAGACTCTTTGAAATCTTC	398
MsBVank-1	ATGGAGAGGCAGTCTAGTACTA	TTAATCTTATCGGTTACATTCTT	616
MsBVank-2	ATGTGGTCACTGCAAGATTCTAT	CTAATGAGCTGATTCCCCCA	547
MsBVank-3	ATGATGAACAGAACACTTTCCT	TCACAACAGTCCCGCACTTA	440
MsBVank-4	ATGTTAGTAAATTCTGGATTCA	AAGCATCAGAACACATTTTCAC	579
MsBVank-5	ATGGCGGAGAATGGACATTTTC	TTAAATTGATATGCCTTCAATA	531
MsBVank-6	TTTATTTTTACAGATGTATATG	AATTGATATTTATTGAAACCAC	527
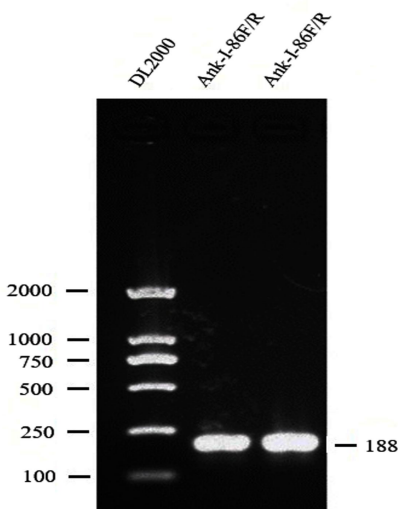
Figure 1:   PCR products with inner primers of nested PCR by 1% agarose gel

Table 2: Similar segments of MsBV in other PDVs by BLASTN search. (Data acquisition JUN[27th].2017)

| MsBV segment (Accession) | Similar segments in other PDVs, query coverage (QC) and max identities (MI) | | | | | |
| | MdBV | | | MmBV | | |
	Accession	QC	MI	Accession	QC	MI
PTP-1(MG953195)	AY875685.1	100	93	KX223702.1	91	88
PTP-2(MG953196)	AY875685.1	99	92	KX223732.1	99	88
PTP-3(MG953197)	AY875686.1	100	92	KX223706.1	99	88
PTP-4(MG953198)	AY875689.1	98	92	KX223703.1	95	87
PTP-5(MG953199)	AY875689.1	96	87	KX223696.1	70	89
PTP-6(MG953200)	AY875689.1	100	94	KX223696.1	100	91
Ank-1(MG953189)	AY875681.1	99	95	KX223748.1	82	93
				KX223714.1	34	89
Ank-2(MG953190)	AY875682.1	100	94	KX223748.1	100	91
Ank-3(MG953191)	AY875684.1	100	93	KX223742.1	100	87
Ank-4(MG953192)	AY875684.1	100	95	KX223731.1	100	90
Ank-5(MG953193)	AY875690.1	100	93	KX223731.1	100	88
Ank-6(MG953194)	AY875685.1	100	91	NO		

2. Phylogenetic relationships

We conducted two types of phylogenetic analyses, to examine the probable genetic relationships among the virus strains: (i) a PTP phylogeny based on all known PTPs from seven BVs (MsBV, MmBV, CvBV, CcBV, MdBV, GiBV, and GfBV) and one ichnovirus (IV) *Glypta fumiferanae* ichnovirus (GfIV); and (ii) an ankyrins phylogeny based on all known ankyrins proteins from seven BVs (MsBV, MdBV, CvBV, CcBV, MdBV, GiBV and GfBV) and four IVs (GfIV, *Campolitis sonorensis* ichnovirus (CsIV), *Hyposoter fugitivus* ichnovirus (HfIV) and *Tranosema rostrale* ichnovirus (TrIV). (Fig. 2).

Phylogenetic analysis of all PTPs revealed that MsBV was classified onto the same branch as the MdBV strain, and next to the branch containing the MmBV strain, form a monophyletic group I. This mono--phyletic group dispersed into all lineages except for the GfIV lineage. This result proposes that the two wasp groups acquired the viruses independently. PTPs from corresponding segments of MsBV and MdBV always clustered into the same group, further indicating that these two BVs are closely related.

Phylogenetic analysis of the ankyrins showed that all ankyrins from BVs did not form a lineage separated from the IV ankyrins (Fig. 3). MsBV, MdBV and MmBV formation of another monophyletic group II that MdBV was classified into the same branch as the MmBV strain, and next to the branch containing the MsBV strain, in addition to the formation of the above mentioned monophyletic group structure. This two monophyletic group mainly distributed in BVs, in addition MsBV--slgp1 and MdBV-slgp1/sngp (2, 5, 6) distributed in IVs.

3. PTPs and ankyrins protein sequence analysis

The six PTPs are obtained in here consists of 100~400 amino acids, with a relative molecular weight of 15~40 kDa and the theoretical protein isoelectric point of 8.74, 6.31, 8.82, 9.80, 8.41 and 9.05, respectively. The secondary structure of five PTPs (PTP-1, PTP-2, PTP-3, PTP-4 and PTP-5) are mainly composed of Helix and Coil, but the PTP-6 is mainly composed of Strand and Coil (Table 3).

The six ankyrins obtained in here consists of 90~200 amino acids, with a relative molecular weight of 10~20 kDa and a theoretical protein isoelectric point of 5.08, 7.61, 7.01, 4.56, 9.64 and 9.33, respectively. The secondary structure of six ankyrins are mainly composed of helix and coil (Table 3).
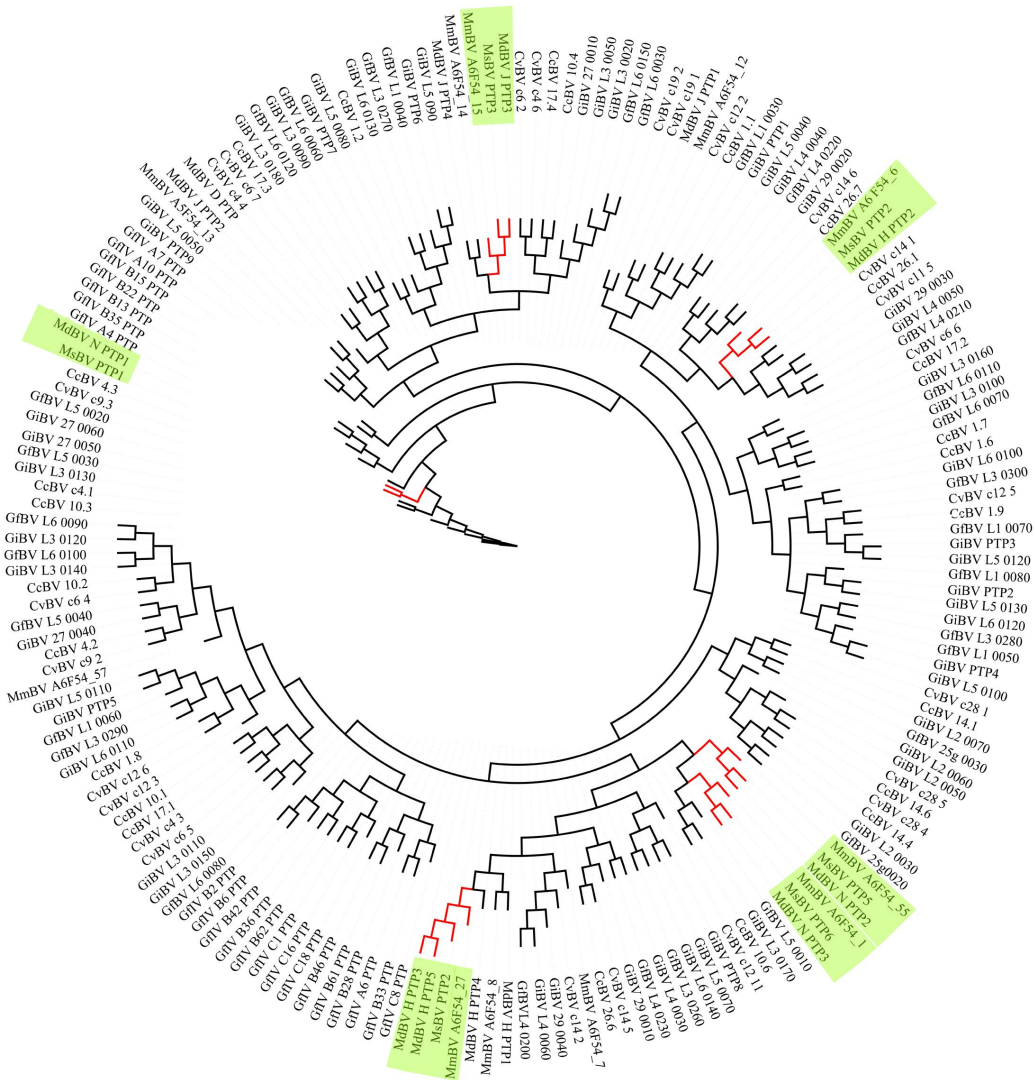
Figure 2: Phylogenetic analysis of PTPs from the seven BV and one IV genomes.
The evolutionary history was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Phylogenetic analyses were conducted in geneious 8.1.4
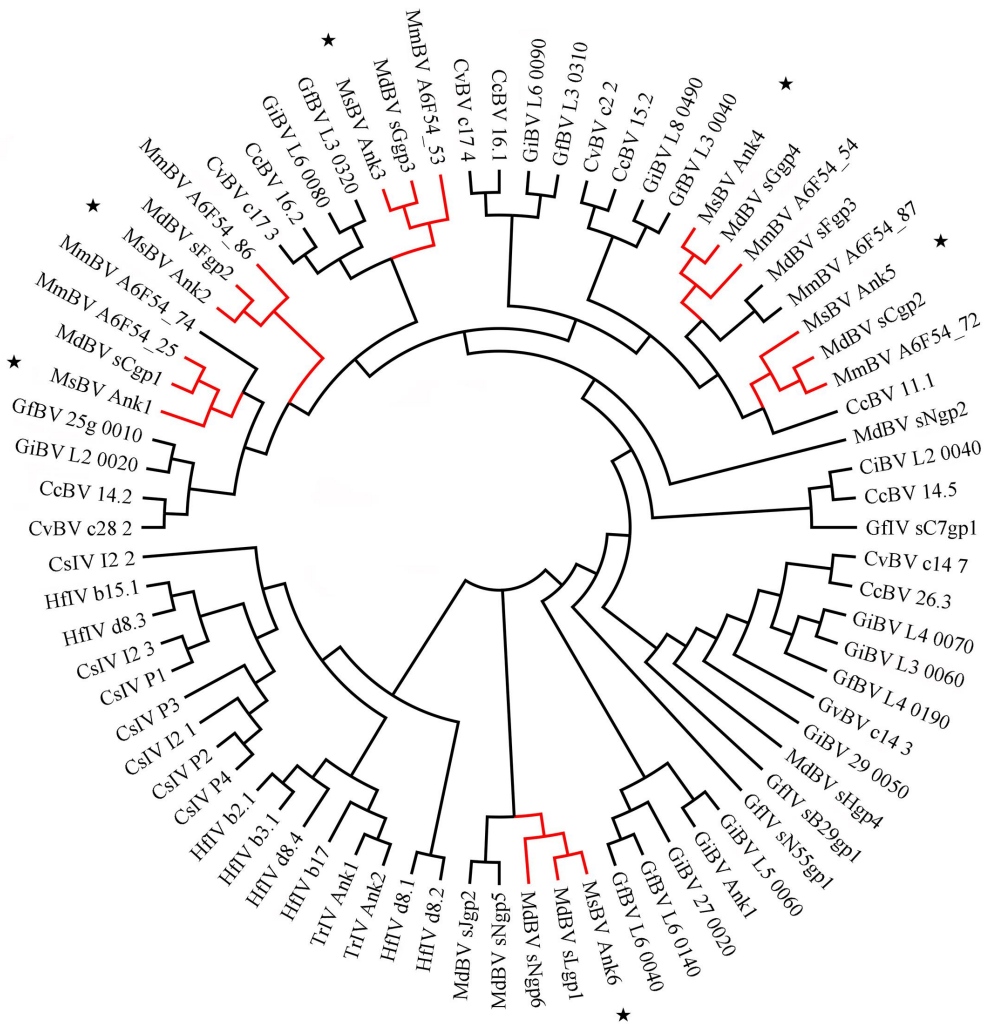
Figure 3: Phylogenetic analysis of ankyrins from the seven BV and four IV genomes. The evolutionary history was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Phylogenetic analyses were conducted in geneious 8.1.4

Table 3: The information of PTPs and ankyrins protein sequence

MsBV	Length (aa)	Molecular Weight (k Da)	Isoelectric point	Helix accounts (%)	Coil accounts (%)	Strand accounts (%)
PTP-1	325	37.82	8.74	40.2	41.5	18.3
PTP-2	186	21.74	6.31	37.9	38.9	23.2
PTP-3	297	34.41	8.82	40.1	42.1	17.8
PTP-4	285	33.24	9.80	33.7	46.3	20.0
PTP-5	228	26.58	8.41	29.8	47.4	22.8
PTP-6	132	15.88	9.05	25.8	44.7	29.5
Ank-1	174	19.96	5.08	50.6	49.4	0
Ank-2	152	17.54	7.61	44.7	53.9	0.4
Ank-3	128	14.53	7.01	44.5	55.5	0
Ank-4	169	19.82	4.56	43.2	56.8	0
Ank-5	176	19.27	9.64	47.2	52.3	0.5
Ank-6	121	13.51	9.33	51.2	38.8	10

4. Tertiary structure prediction of PTPs and ankyrins protein

The acid amino sequences of six PTPs and ankyrins are predicted, respectively. Their tertiary structures have a high degree of confidence (Fig. 4). The C-score is greater than -1.5 that indicates that the predicted protein folding is more appropriate. These main consists of α helices and β sheet. The tertiary structure of PTPs protein are more complicated, and the tertiary structure of ankyrins protein has typical characteristics: the L-type structure is formed on the spatial structure, and the β hairpin extends beyond the two α helices, almost perpendicular to the plane formed by the α helices. Through the β hairpin to form a continuous anti-parallel β film between the adjacent ankyrins model, and several pairs of α helices shoulder side by side, forming a tight and stretch spiral plane.
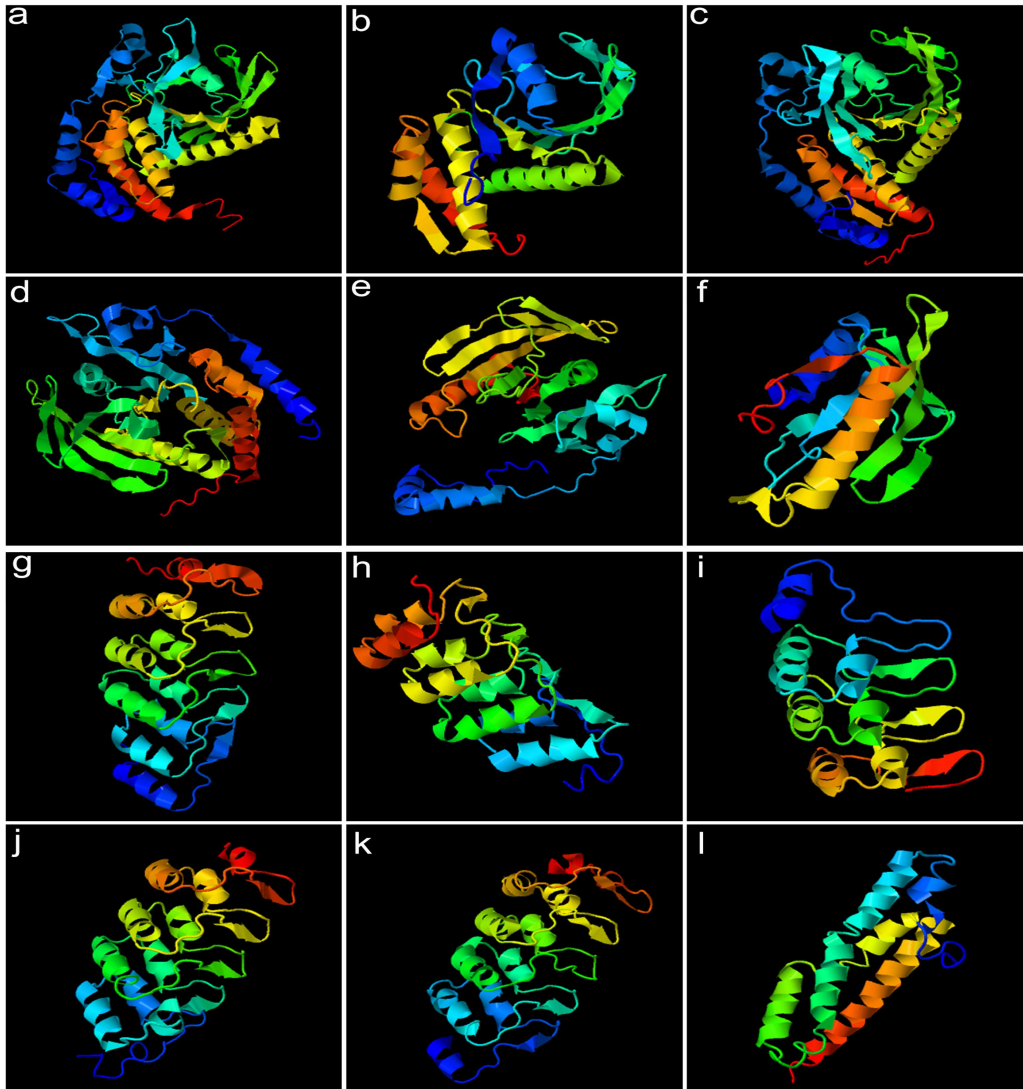
Figure 4: The predicted 3D structure of PTPs and ankyrins.

C-score is typically in the range of [-5, 2], where a C-score of a higher value signifies a model with a higher confidence and vice-versa. TM-score and RMSD are estimated based on C-score and protein length following the correlation observed these qualities. PTPs: A-F , Ank: G-L The C-score, estimated TM-score and RMSD of a-f: 1.03, 0.85±0.08，4.2±2.8A; 0.99, 0.85±0.08, 3.5±2.4A; 1.19, 0.88±0.07, 3.8±2.6A; 0.45, 0.66±0.13; 7.7±4.3A; 0.99, 0.85±0.08, 3.6±2.5A; 0.70, 0.81±0.09, 3.1±2.2A; g-l: 0.51, 0.78±0.10, 4.0±2.7A; 0.58, 0.79±0.09, 3.6±2.5A; 0.87, 0.83±0.08, 2.8±2.0A; 0.45, 0.77±0.10, 4.1±2.8A; 0.19, 0.74±0.11, 4.7±3.1A; 0.80, 0.82±0.28, 2.3±1.8A

5. Function prediction of PTPs and ankyrins protein

Protein ligands and ligand binding sites were further predicted based on the predicted structure of the PTPs and ankyrins protein. PTP-1 and PTP-3 have the same ligand binding sites: 57, 58 and 59. PTP-3 and PTP-5 have the same ligand binding sites: 60. The predicted results of PTP-2 and PTP-6 differ greatly from those predicted by other proteins (Table 4).

Ank-1, Ank-2, Ank-3, Ank-4 and Ank-5 have the same ligand binding sites: 67. The predicted results of Ank-6 differ greatly from those predicted by other proteins (Table 4). Part of the protein and ligand binding diagram shown in Fig. 5.

Discussion

In terms of PDVs, viruses infecting multicellular organisms exhibit a continuum of co-evolutionary interactions with their hosts [12]. PDVs are used by many parasitoid wasps to facilitate development of their progeny in the body of immune competent insect hosts, which are typically Lepidopteran larvae [13]. The generation of PDV is the result of co-evolution of parasitoid wasps and their hosts. PDVs are a special group of DNA viruses because of their segmented double-stranded DNA genomes and the obligately mutualistic relationship with their hymenopteran hosts. But the PDV genomes are very difficult to be fully sequenced because the genome is segmented and internal sequence homologies occur between segments. The number of genomic segments is hard to estimate. Different segments of similar size make it difficult to determine whether a given band is a high abundance segment or a mixture of segments [14]. Although the genetic structure of PDVs is complex, there six gene families are more conservative, and play an important function. In this study, we performed partial genes (ptp genes and ank genes) sequencing of MsBV, and predicted the structure and function of the proteins encoded by these genes. Simultaneously, we used this two conserved protein family to carry out the phylogenetic analysis for MsBV. A new species of PDV-MsBV was identified and characterized. Homology analysis showed that MsBV with MdBV and MmBV have very high homology. Phylogenetic analysis shows that MsBV with MdBV and MmBV are clustered together, indicating that MsBV is a PDV with a closer genetic relationship with MdBV and MmBV.

In order to analyze the function of PTPs and ankyrins protein were obtained, we made a preliminary prediction and analysis of these protein structure. The secondary structure of PTPs is contains Helix, Coil and Strand, leading to the complexity of its tertiary structure. The secondary structure of ankyrins is mainly composed of Helix and Coil, resulting in

Table 4: The predicted binding ligand of PTPs and ankyrins.

Protein	C-score	Cluster Size	Ligand name	Ligand binding sites
PTP-1	0.90	270	PEPTIDE (Fig.5 a)	56,57,58,59,129,235,236,237,238,239,240,241,279
	0.27	58	214	34,38,39,56,57,58,59,235,236,237,238,239,240,241,271,275,276,279
	0.03	7	PTR	34,58,59,239,271,275,276,279
PTP-2	0.95	318	PEPTIDE (Fig.5 b)	144,145,146,147,148,149,150,185,188
	0.08	17	BGD	44,45,46,144,145,146,148,149,150,188
	0.04	14	NXY	37,100,103,107,108,109,115,118,150,153,154,191,192,195
PTP-3	0.85	272	335 (Fig.5 c)	57,58,59,60,226,227,228,29,230,231,232,266,267,270
	0.03	8	BPM	33,59,60,262,266,267,270
	0.03	13	NXW	22,120,181,184,190,196,199,232,235,236,273,274,276,277
PTP-4	0.59	197	073 (Fig.5 d)	39,40,41,42,216,217,218,219,220,221,222,256,257,260
	0.05	13	NXW	5,108,168,171,176,177,183,186,222,225,226,263,264,266,267
	0.02	6	PEPTIDE	34,37,38,39,40,41,218,260
PTP-5	0.22	21	1BO (Fig.5 e)	60,61
	0.14	14	1NNYYA00	37,38,39,40,41,59,60,61
	0.14	14	PEPTIDE	58,59,60,61,62,135,136
PTP-6	0.47	47	2CMCA00 (Fig.5 f)	37,129,130
	0.11	10	1BO	28,32,37,130
	0.04	6	NXY	27,91,94,98,99,100,106,109,130
Ank-1	0.40	32	PEPTIDE (Fig.5 g)	56,58,63,66,67,68,95,100,103,104,106, 125,127,129,134,140,160
	0.23	13	NUCLEIC ACIDS	21,22,23,25,30,33,54,56,66,90,93,95,100,103,104,125,127,162
	0.05	5	PEPTIDE	23,25,30,33,34,35,58,63,67,68,72,90,93,95,100,104
Ank-2	0.26	18	PEPTIDE (Fig.5 h)	16,18,23,26,27,29,56,61,64,65,67,88,91,93,98,102,104,124,125
	0.14	18	PEPTIDE	16,18,23,26,27,28,56,61,64,65,67,88,91,93,98,104,125
	0.15	11	PEPTIDE	54,56,61,64,65,93,98,101,102,104,123,125,127,132,138
Ank-3	0.27	18	PEPTIDE (Fig.5 i)	20,22,27,30,31,59,64,67,68,70,89,91,93,98,104
	0.12	10	PEPTIDE	22,27,30,31,59,64,67,89,91,93,97,98,101,122,124,126
	0.06	3	2RFMA00	69,103,104,105,106,107
Ank-4	0.39	31	PEPTIDE (Fig.5 j)	25,28,29,58,63,66,67,95,100,103,125, 127,129,133,134,137,158,160,162
	0.14	11	PEPTIDE	25,28,58,63,66,90,92,95,100,103,127,129,134
	0.10	6	BU2	105,139,140,141,142,143
Ank 5	0.36	23	PEPTIDE (Fig.5 k)	56,58,63,66,67,69,95,100,103,104,106, 125,127,129,134,140,162
	0.13	10	PEPTIDE	21,26,29,58,63,66,90,93,95,100,103,127,1299,134
	0.06	6	PEPTIDE	1,21,26,29,30,32,54,56,58,63,67,72,91
Ank-6	0.22	29	HEM (Fig.5 l)	79,80,83,100,104,107,108,110
	0.04	5	ZN	98,101
	0.03	4	FE	105,108

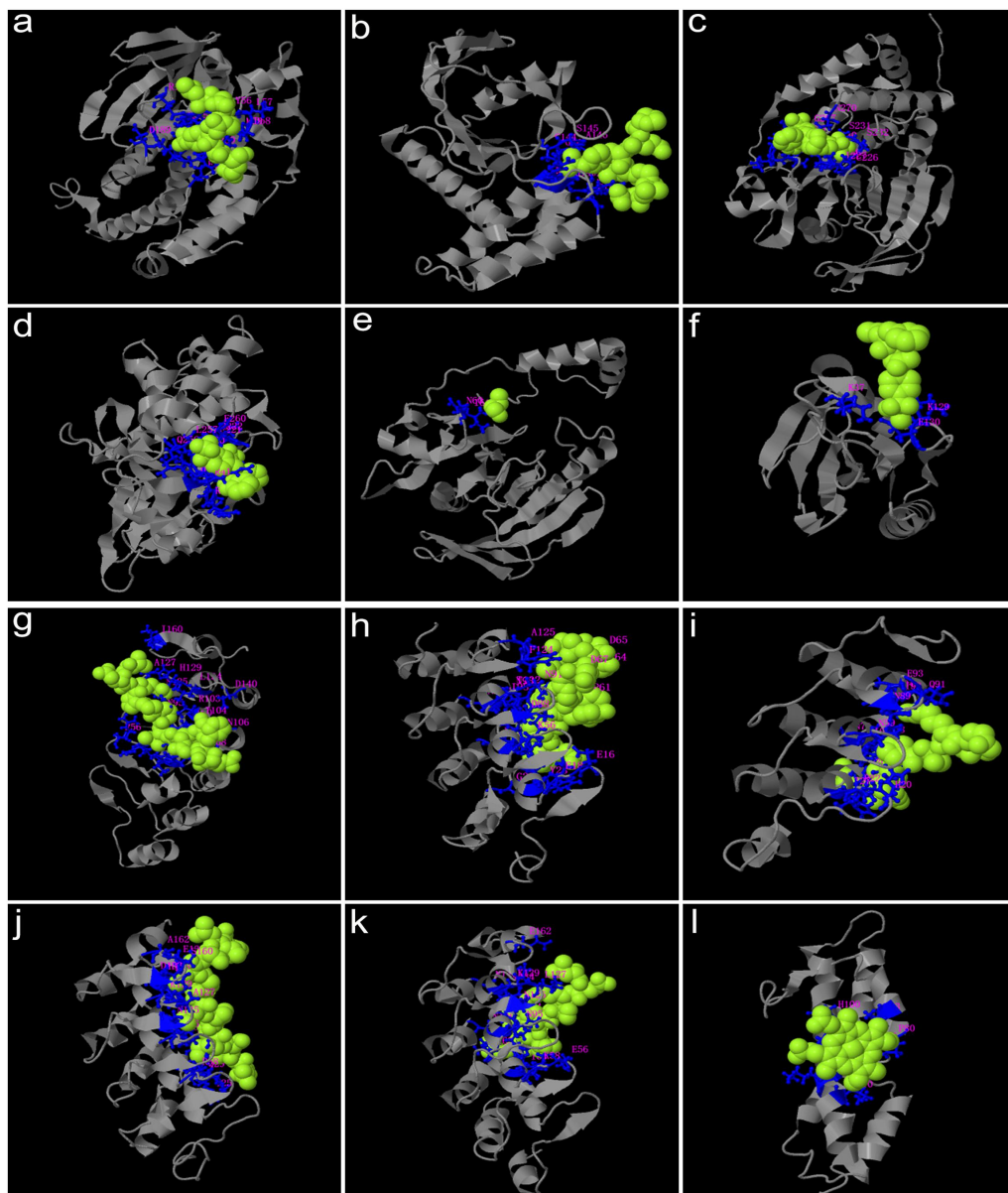Note: The name of the ligand is reference the I-TASSER website.

Figure 5: PTPs and ankyrins binding with predicted ligands.

a: PTP-1 bound with PEPTIDE; b: PTP-2 bound with PEPTIDE; c: PTP-3 bound with 335; d: PTP-4 bound with 73; e: PTP-5 bound with 1BO; f: PTP-6 bound with 2CMCA00. g: Ank-1 bound with PEPTIDE; h: Ank-2 bound with PEPTIDE; i: Ank-3 bound with PEPTIDE; j: Ank-4 bound with PEPTIDE; k: Ank-5 bound with PEPTIDE; l: Ank-6 bound with HEM. The name of the ligand is reference the I-TASSER website

its special tertiary structure. Analysis of the tertiary structure of ankyrins proteins suggests that it has a typical structural characteristic of ankyrins, the L-type structure is formed on the spatial structure, and the β hairpin (the short arm of "L") extends beyond the two α helices (the long arm of "L"), almost perpendicular to the plane formed by the α helices. Through the β hairpin to form a continuous anti-parallel β film between the adjacent ankyrins model, and several pairs of α helices shoulder side by side, relying on the role of hydrophobic mutual accumulation, forming a tight and stretch spiral plane, side of the hydrophobic, the other side of the hydrophilic. From a spatial point of view, the domain of ankyrins are like a curved hand: the β sheet forms the finger, and the helix plane forms the palm part, the finger and the palm are perpendicular to each other, thereby forming a hydrophobic deep groove inside the palm of the hand [15-17]. Ankyrins-repeat motif to be defined as a β-hairpin-helix-loop-helix ($\beta_2\alpha_2$) structure [17]. These six ankyrins protein all have this typical structure.

Previous studies have shown that PTPs are known to play a key role in the control of signal transduction pathways by dephosphorylating tyrosine residues on regulatory proteins [18]. Initial data suggest that PDV PTP, a member of the PTPs gene family expressed by GiBV, could be involved in the suppression of host haemocyte activity, although the phenotype

of immune suppression is not character--ized in this system [19]. PTPs have been directly implicated in both positive and negative regulation of intracellular signalling enzymes via dephosphorylation of associated tyrosine residues [20] and were speculated to be associated with suppression of encapsulation in the GiBV system [19]. In general, PTPs are known to regulate enzymes involved in cellular signaling, and, interestingly, have been recently linked to the regulation of the actin cytoskeleton and production of deleterious effects similar to those produced by PDV infection [20, 21]. The function and structure of proteins are closely related. The complex structure of the protein led to its diversity of functions. Vankyrin genes contain ankyrin repeats and show significant identity with a *Drosophila* transcription factor inhibitor, it is speculated that ankyrins interfere with signaling cascades associated with specific host transcription factors. In our study, we analysis the function of PTPs proteins suggested that it could bind to a variety of ligands, which 073 was a cytochrome P450 inhibitor. Ankyrins proteins could bind to a variety of peptide and Hemoglobin B. Its functional or structural properties are still not clearly understood.

In our research, MsBV were reported and studied, and enriches the members of the PDV, in addition to studying the

proteins of its two conserved protein families. Simultaneously, our investigations revealed that the biological function of the domain of PTPs and ankyrins is to mediate the interaction between proteins and proteins, whereas the structural properties of PTPs and ankyrins provide a basis for their complex functions, so further study the domain of PTPs and ankyrins for finding PTPs and ankyrins play the role of in PDVs and the interaction between protein and protein are of great importance.

Acknowledgments

Author contributions

Conceived and designed the experiments: LH, HY, GHH. Performed the experiments: LH, YYOY, JP, SQL. Analysed the data: LH. Wrote the paper: LH, GHH. Read and approved the manuscript: LH, HY, YYOY, JP, SQL, GHH.

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