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Short communication

Complete genome sequence and characterization of a new iflavirus from the small brown planthopper (*Laodelphax striatellus*)

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ABSTRACT

A novel iflavirus, tentatively named laodelphax striatellus iflavirus 1 (LsIV1), was identified in *Laodelphax striatellus* by total RNA-sequencing, and its genome sequence was confirmed by Sanger sequencing. The complete genome consisted of 10,831 nucleotides with a polyA tail and included one open reading frame, encoding a 361.7-kD polyprotein. Conserved motifs for structural proteins, helicase, protease, and RNA-dependent RNA polymerase were identified by aligning the deduced amino acid sequence of LsIV1 with several other iflaviruses. The genome has the highest identity with another planthopper iflavirus, nilaparvata lugens honeydew virus-3 (39.7%), under the species demarcation threshold (90%). Results of the identities and phylogenetic analysis based on the deduced amino acid sequences of the complete polyprotein and helicase of LsIV1 and other iflaviruses, indicated it is a new species belonging to the family *Iflaviridae*. Furthermore, we did not observe any differences of biological characterizations like development and reproduction between viruliferous and virus-free SBPH. Meanwhile, we found that female could transmit LsIV1 with higher transmission efficiency.

The small brown planthopper (SBPH), *Laodelphax striatellus*, is one of the most serious insect pests of cereal crops and can seriously damage cereal plants especially when they are feeding at high density. Even at a much lower density, as an important vector of cereal-infecting viruses, SBPH infestations can lead to more significant yield losses when plants are infected by the transmitted viruses (Liu et al., 2018; Qin et al., 2018). In addition, SBPH can also host several insect viruses. Himetobi P virus (HiPV, genus *Triatovirus*, family *Dicistroviridae*, order *Picornavirales*) was identified in SBPH and two other planthopper species, white-backed planthopper (*Sogatella furcifera*) and brown planthopper (*Nilaparvata lugens*) (Toriyama et al., 1992). Complete genome sequences of two iflaviruses in SBPH have also been submitted to GenBank, i.e., laodelphax striatellus honeydew virus 1 (LsHV1) in Nanjing, China (GenBank Accession No. NC_023627) and laodelphax striatellus picorna-like virus 2 (LsPV2) in South Korea (GenBank Accession No. NC_025788).

Viruses in the family *Iflaviridae* possess small non-enveloped virions with monopartite, positive-stranded RNA genomes ranging from 9 to 11 kb. The viral genome contains one ORF encoding a large polyprotein, which is posttranslationally processed by the viral protease(s) into various precursor and mature proteins (Oers, 2010). To date, the family has only one genus, *Iflavirus*, containing 15 approved species and 20 unapproved members that have been deposited in the NCBI database with complete genomes (https://www.ncbi.nlm.nih.gov/genome/?term = iflaviridae), the type species is the infectious flacherie virus (IFV) sharing some features with other members of *Picornavirales*. All the iflaviruses were identified from arthropod hosts, primarily insects including both beneficial and pest insects (Gilmer and Ratti, 2017). The role of viral infection may be asymptomatic (nilaparvata lugens honeydew virus 1, NlHV-1) or cause loss of cocoons (IFV), death of larvae (sacbrood virus, SBV) and morphological deformities (deformed wing virus, DWV) (Genersch et al., 2006; Hashimoto and Kawase, 1983; Murakami et al., 2013; Shen et al., 2005). In the present study, we used RNA-sequencing (RNA-seq) to detect plant and insect viruses in *L. striatellus* collected from a rice field in Henan Province. A new iflavirus was identified and tentatively named laodelphax striatellus iflavirus 1 (LsIV1).

During a field investigation in June 2016 in Kaifeng, Henan Province, China, SBPH was observed at a high density on wheat and rice plants. Adult individuals were collected and subsequently

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maintained in insect-proof cages with rice seedlings (cv. Wuyujing 3) at 25°C with 16 h light/8 h dark in a growth incubator. Five months later, we randomly pooled 10 adult individuals from the colony for RNA-seq. Total RNA was extracted using TRIzol reagent according to the manufacturer's instructions (Invitrogen, USA). The rRNA was removed using a Ribo-Zero Magnetic kit (Epicentre, USA), and the RNA-seq library was constructed using TruSeq total RNA Sample Prep Kit (Illumina, USA). Subsequently, RNA-seq was performed on the HiSeq X Ten platform with PE150 bp (Illumina). A total of 32,766,442 clean reads were obtained after removing adaptor sequences and low-quality reads. After *de novo* assembly and blast analyses, two known viral contigs of rice stripe virus and HiPV, an unknown contig of 10,806 nt having high identity with iflaviruses, and four other unknown viruses (not discussed here) were reconstructed.

For confirming the validity of the unknown contig, seven specific primer pairs (Table S1) were designed by Primer Premier 5 (Premier, Canada) to amplify the respective overlapping fragments of 1,847, 1,510, 1,497, 1,487, 1,533, 1562 and 1949 nt through the contig sequence (Fig. S1). RT-PCR was carried out using the primordial total RNA and these primer pairs. Then the PCR products were cloned and sequenced as described (Zhang et al., 2017). In addition, the terminal sequences of the genome were determined using 5′ and 3′ rapid amplification of cDNA ends (RACE) kits according to the manufacturer's instructions (Invitrogen). The results of Sanger sequencing were assembled using the DNAMAN (v6) program (Lynnon Biosoft, USA). Finally, an additional 26-nt sequence at the 5′ terminal was obtained to form a complete genome of 10,831 nt excluding the polyA tail, and the genomic sequence was deposited in GenBank under accession number MG815140. We tentatively named the virus as laodelphax striatellus iflavirus 1 (LsIV1).

A large open reading frame (ORF, nt 1025˜10,543) within the viral genome was predicted by SnapGene (Ian, 2004), encoding a 361.7 kD polyprotein (Fig. 1A). The 5′ untranslated region (UTR) was long and contained 24 AUG triplets. In addition, several stem-loop structures within this region were generated on the secondary structure prediction RNAfold Webserver (http://rna.tbi.univie.ac.at/RNAfold/GBqKKw8zAI). All the results suggested that there is an internal ribosome entry site in the 5′ UTR to initiate the polyprotein translation (Murakami et al., 2014; Ongus et al., 2006; Wu et al., 2007). Alignment of the deduced amino acid sequence (Fig. 1B) of LsIV1 with that of other iflaviruses indicated that the putative polyprotein had a typical iflavirus genomic organization, i.e., structural proteins and nonstructural proteins locate at N-terminus and C-terminus respectively (Fig. 1A).

The leader (L) protein, upstream of the coat protein (CP), had been observed in most of the iflaviruses but not in other members of *Picornavirales* except the foot and mouth disease virus (FMDV). The start position of the CP region of LsIV1 was unknown; we predicted the Mr of the region from the beginning of ORF to the first consensus motif NXNXFQXG of the structural protein. The Mr was 43.1 kD, whereas those of other iflaviruses were 29.4˜49.4 kD (Murakami et al., 2013). IFV had the smallest mass of this region, 29.4 kD, while it still had a short L protein (Isawa et al., 1998), suggesting that LsIV1 may possess an L protein.

The conserved motifs WXGXLX3FXFX7GX5YXP and FXRG within the structural protein region, which had been reported in picornaviruses (Liljas et al., 2002), were not identical in LsIV1. But similar motifs, WRGX6DXVASXFX4LX3YIP and RXXRG, were found among LsIV1 and several iflaviruses (Fig. 1B). The cleavage site for VP3/VP4 in DWV was X/D in the sequence NX/DXP (Lanzi et al., 2006), which is also present in LsIV1 and conserved in several iflaviruses as sequence NR/DNP (Fig. 1B).

The nonstructural proteins of LsIV1 had conserved motifs that were identified as helicases, protease and RNA dependent RNA polymerase (RdRp) of viruses in the picorna-like viruses (Koonin and Dolja, 1993). The three conserved domains, Hel-A (GX2GXGKS), Hel-B (QX5DD), and Hel-C (KX5PX5CSN), in helicase sequences of picorna-like viruses were present in the LsIV1 helicase (Fig. 1B). But the Hel-C (namely KX5PX5SSN) in LsIV1, EoV and NlHV3, was a little different. The core motifs of the 3C-like protease of other picornaviruses, GXCG and GXHXXG, were detected in LsIV1 and other iflaviruses used in this alignment. Eight conserved domains in RdRp were found between amino acids 3116˜3418 of the deduced sequence of LsIV1.

The characterization of the genomic sequence of LsIV1 indicated it is a member of *Iflavirus*. The identities of the complete genomic nucleotide (nt) sequences and deduced amino acid (aa) sequences of complete polyprotein of LsIV1 with other iflaviruses were then calculated by the Needle program [http://weblab.cbi.pku.edu.cn/program.inputForm.do?program = needle(v6.0.1)]. The identities ranged from 42.0˜52.9% for the nt sequence and 18.7˜39.7% for the aa sequence, and LsIV1 had the highest identities with NlHV3 (Table S2).

Further, we constructed phylogenetic trees based on the deduced amino acid sequences of the complete polyprotein (Fig. 2A) and helicase-RdRp (Fig. 2B) of LsIV1 and other iflaviruses using the neighbor-joining method by MEGA 6.0, using human enterovirus C (type species of genus *Enterovirus*, family *Picornaviridae*) as an outgroup. All the iflaviruses clustered in a big clade, but a number of distinct subclades were present. Both of these two phylogenetic trees indicated that LsIV1 clustered in the same clade with antheraea pernyi iflavirus (ApIV), lymantria dispar iflavirus 1 (LdIV1), DWV, varroa destructor virus 1 (VDV1), NlHV3 and slow bee paralysis virus (SBPV) and had the closest phylogentic relationship with NlHV3. Subsequently, we pairwise-compared the aa sequence of the complete polyprotein of LsIV1 with that of other iflaviruses (Table 1) and found it had the highest identity with NlHV3 (39.7%), much lower than the species demarcation criterion (CP amino acid sequence identity is less than 90%). The identities among the group with ApIV, LdIV1, DWV and VDV1; the group with ectropis obliqua virus (EoV), perina nuda virus (PNV), spodoptera exigua iflavirus 1 (SeIV1) and diamondback moth iflavirus (DmIV); the group with lygus lineolaris virus 1 (LLV1) and SBV; the group with NlHV1, LsHV1 and LsPV2; and the group with NlHV3 and LsHV3 were much higher than with others, corresponding to the phylogenetic relationships, which suggested that different genera are present in the family *Iflaviridae*.

Another demarcation criterion for iflaviruses is the natural host range of each virus. Although SBPH is also infected by other iflaviruses (LsHV1 and LsPV2), the identities of amino acid sequence of the polyprotein with LsHV1 and with LsPV2 were much lower (not greater than 25.0%). Using RT-PCR by primer pair CP-F/R, we also tested the presence of LsIV1 in two other planthoppers, *N. lugens* and *S. furcifera*, and in a rice seedling on which *L. striatellus* had been reared for 2 weeks (Table S1). All the tests were negative for the virus. Therefore, we proposed that LsIV1 is a novel species in the family *Iflaviridae*.

A total of 11 colonies of *L. striatellus* samples were collected from 11 regions in 7 provinces of China in May 2017 to further investigate the incidence of LsIV1 in the field (Fig. 3). The planthoppers were maintained in our greenhouse for 1 month, then their progenies were tested for LsIV1 using RT-PCR. As a result, 5 colonies from 4 provinces (Zhengzhou and Kaifeng in Henan, Huaian in Jiangsu, Kunming in Yunan and Hefei in Anhui) were infected by LsIV3 (Fig. 3). Then, we obtained partial sequences of RdRp in different colonies, and found the identities among them all higher than 91%. To confirm the biological differences between LsIV1 colony and LsIV1-free colony, we chose Kaifeng group which has LsIV3 to propagate offspring by one male and one female. After several generations, we obtained almost 100% LsIV1 group and LsIV1-free group. Then one LsIV1 male mates with one LsIV1

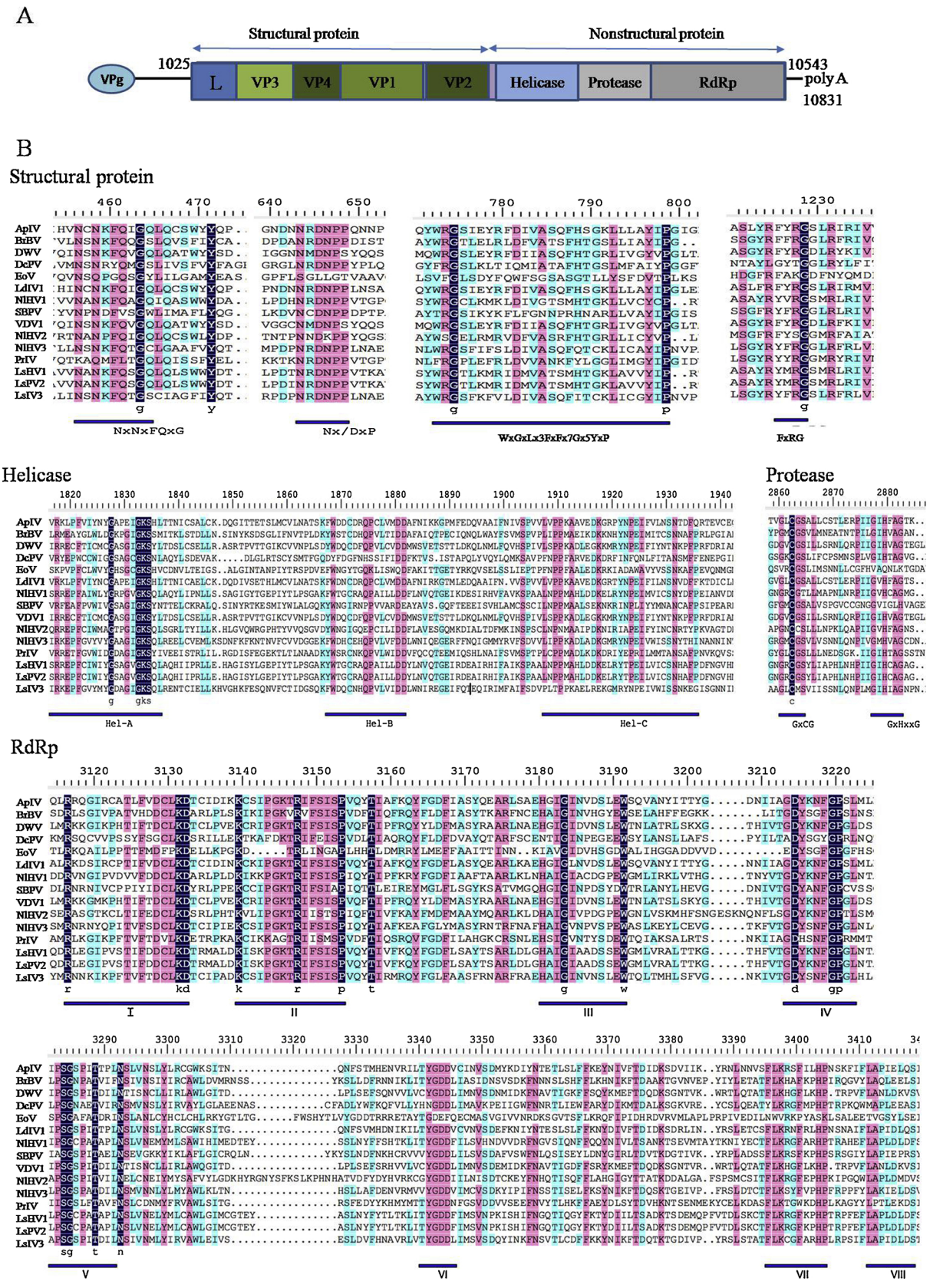

Fig. 1. (A) Schematic diagram of the genome organization of LsIV1. The genome encodes a single polyprotein (nt 1025~10,543) with a structural protein at the N-terminal and a nonstructural protein at the C-terminal. L: leader protein. (B) Deduced amino acid sequence alignment of LsIV1 and other iflaviruses. The numbers above the sequences indicated the location. Viral information is given in Table S2.

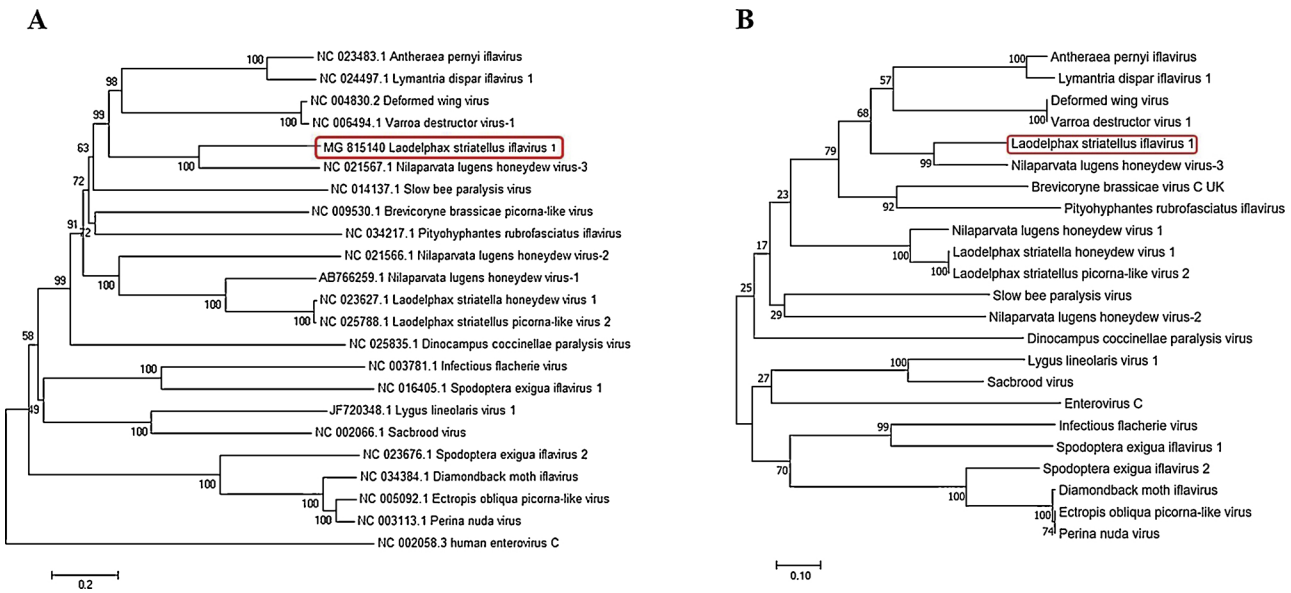

Fig. 2. (A) Phylogenetic tree based on the complete polyprotein sequences of iflaviruses using the neighbor-joining method by MEGA 6.0. Human enterovirus C was used as the outgroup. Bootstrap values (1000 replications) are shown. LsIV1 is boxed in red. (B) Phylogenetic tree based on the helicase sequences of RdRp using the neighbor-joining method by MEGA 6.0. Human enterovirus C was used as the outgroup. Bootstrap values (1000 replications) are shown. LsIV1 is boxed in red. (C) Distribution of the sampling regions in China. + indicates the samples are positive of LsIV1, - indicates the samples are negative.

female, meanwhile, one LsIV1-free male mates with one LsIV1-free female as control. Both of treatment group and control group contain at least 10 replicates. Biological characterizations like development and reproduction were observed all over the generation of SBPH. Finally, we found there is no significant difference between these two groups. The offsprings in average are 165 and 175 of LsIV1-free and LsIV1 groups when reared in incubator (Fig. 4A), and the time of every instar as 3.5˜4, 3, 3.3˜3.7, 4, 4˜4.6, 13˜13.7 days, respectively (Fig.4B). Meanwhile, we found the LsIV1-infected rate of offsprings about LsIV1♀ × LsIV1-free♂is much higher than that of LsIV1-free♀ × LsIV1♂(Table 3), 97.2% and 50% respectively (Table 2). Ovaries and eggs of LsIV1-infected SBPHs were dissected from whole bodies and then total RNA was isolated from them. RT-PCR result indicated that LsIV1 could detected in ovaries (Fig. 2S). All of these results may demonstrate that female has higher transmission efficiency than male because in addition to having a horizontal transmission, female could transmit LsIV1 by ovary to next generation. In conclusion, there is no obvious difference between these two groups and then we speculated LsIV1 could not influence the biological features like development and reproduction of SBPH.

Table 1
Pairwise comparisons of amino acid sequences of the complete polyprotein of LsIV1 and other iflaviruses.

Identity	BrBV	DWV	DcPV	EoV	IFV	LLV	LdIV1	NlHV1	PNV	SBV	SBPV	SeIV1	SeIV2	VDV1	NlHV2	NlHV3	DmIV	PrIV	LsHV1	LsPV2	LsIV1
ApIV	26.2	**30.0**	22.4	18.5	18.6	20.2	**70.6**	25.9	19.3	21.1	26.9	18.5	17.9	**30.0**	24.1	27.0	19.3	25.3	24.9	24.9	27.0
BrBV		26.5	23.4	19.6	19.5	19.8	25.9	25.0	21.5	2119	24.8	17.8	19.2	26.6	25.1	20.4	20.1	25.5	26.5	256.1	25.3
DWV			22.8	19.8	19.5	20.2	**31.0**	25.9	19.6	22.6	27.9	17.6	20.3	**95.2**	23.8	28.2	19.6	25.2	26.3	26.4	28.5
DcPV				18.4	18.4	19.4	22.9	22.6	19.3	20.4	21.9	18.1	18.8	22.6	21.7	22.8	17.9	21.3	23.1	23.0	23.9
EoV					19.1	18.6	19.0	20.1	**87.4**	19.0	19.1	16.9	**38.7**	19.8	19.1	19.4	**75.0**	19.5	18.5	18.4	19.5
IFV						19.7	17.8	18.7	18.6	21.1	20.4	28.9	18.2	19.6	18.5	17.9	18.9	19.4	18.6	18.4	19.5
LLV							20.4	20.6	17.8	**33.8**	21.0	19.6	17.5	20.5	20.6	19.9	18.6	19.1	20.2	19.8	20.5
LdIV1								25.7	19.3	21.8	26.7	18.5	19.1	**30.9**	24.2	27.3	19.2	24.1	24.6	24.9	27.5
NlHV1									19.7	20.2	25.2	18.0	18.3	24.9	28.4	24.5	18.9	23.6	54.1	**54.4**	**24.7**
PNV										19.4	20.0	17.6	**38.3**	20.0	19.3	18.3	**74.4**	19.1	18.8	18.3	19.7
SBV											21.9	20.5	18.1	22.8	20.2	19.1	19.1	20.7	20.3	20.1	21.2
SBPV												18.8	19.2	27.7	22.9	24.9	18.7	23.2	25.7	25.8	25.0
SeIV1													16.3	17.6	17.8	17.8	17.7	18.1	17.8	17.6	18.7
SeIV2														19.6	17.7	18.5	**40.8**	19.0	19.3	19.3	18.8
VDV1															23.5	28.1	19.4	25.1	26.2	26.1	29.0
NlHV2																24.1	19.2	22.9	28.2	28.0	23.7
NlHV3																	18.6	24.0	24.1	23.7	**39.7**
DmIV																		18.9	18.4	18.7	18.9
PrIV																			23.9	23.9	23.9
LsHV1																				**98.8**	25.0
LsPV2																					24.3

Viral information is given in Table S2. Identities greater than 30% are in Bold.

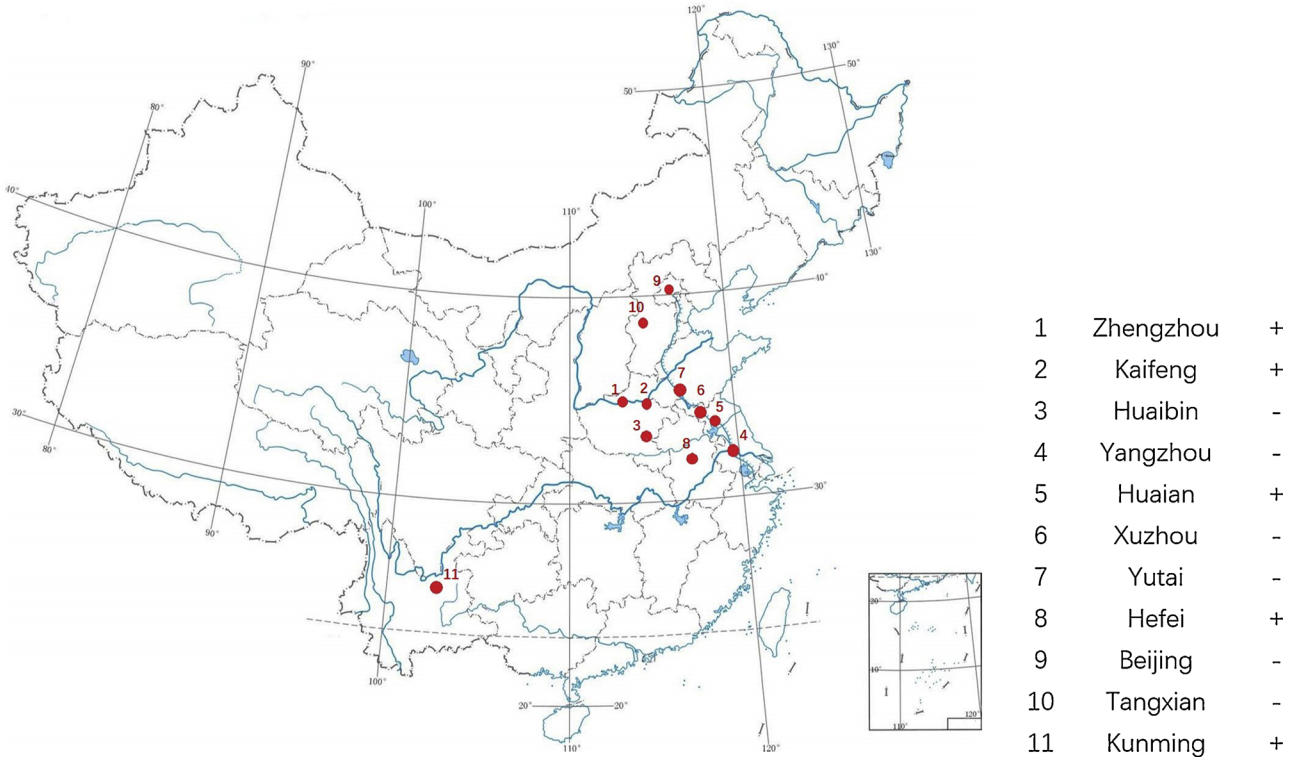

Fig. 3. Distribution of LsIV1 in some provinces of China. +: LsIV1 positive colony, -: LsIV1-free colony.

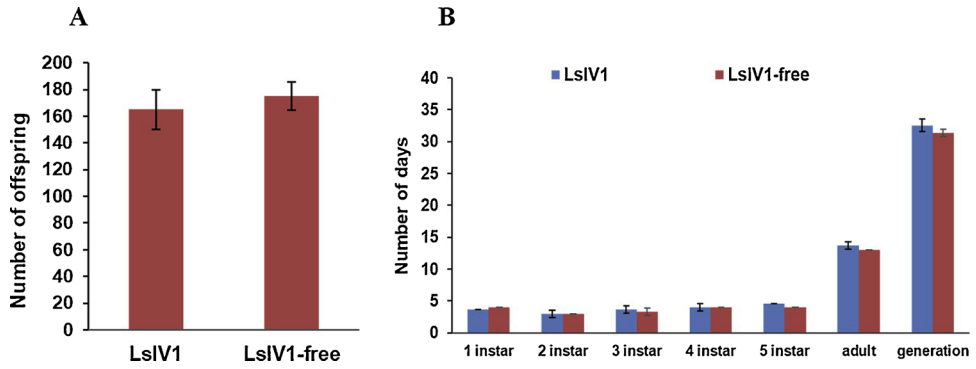

Fig. 4. (A) Numbers of offspring in LsIV1-colony and LsIV1-free colony. (B) Comparison of number of days during different SBPH's instars between LsIV1-colony and LsIV1-free colony.

Table 2
The LsIV1-infected rate (%) of offspring of four mating methods.

	V♀×N♂*	N♀×V♂	V♀×V♂	N♀×N♂
Female	95.8	50.0	95.8	0
Male	98.6	50.0	95.0	0
Total	97.2	50.0	95.45	0

* V♀: female with LsIV1, N♀: female without LsIV1, V♂: male with LsIV1, N♂: male without LsIV1.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.virusres.2019.197651.

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