Supplementary Materials: Aphis glycines virus 2, a Novel Insect Virus with a Unique Genome Structure

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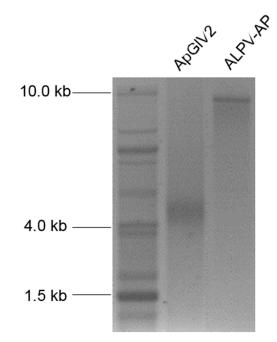


Figure S1. Gel electrophoresis (1% native agarose) of 2 µg RNA extracted from purified ApGIV2 and ALPV-AP virions. While the ALPV-AP RNA runs at the expected size of ~10 kb, the ApGIV2 RNA runs as a smear indicative of RNA degradation. Samples visualized with ethidium bromide staining.

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Peptide 4 (sequencing coverage 3.69%)
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MTRRTKKRANTTKTMEKALVTAMERLTTKRRVPRKKGCARVAEGEMVLRREEMLVDVTLSANKTDSTGSVVLALANFPWLKTVAGSFERYKWKRLNIHWRAAGGFNKGGLIAVGMDWSNQLSSAYTRQTLTSASERQKVLSLTPHMSLPISSTSINKTLGLPIKMLNSRNWYDAAKTDDEGAVGAIRYSAKCDSDTVERFIGEIWVDYEVVLQGTRAQLPPPPPPPPKHTLLYSFTEMRYVITRGQGATNNVNSRNSSGISQRVVDNDFTLSTDSFSDTVELLDGRTYRLIPTVQTETEDDAPLDPDFEGLLTNVVSPPEQYSNTAHLKFYPLRDILKFRLTDFQLNLHLALEVEVNYPTDMEALAILWDFSRCLVSPQVRTRRESVKSNKALFHCVAAGLYRYVPATRPYFKVSVEWFNTGISSALAYTMKTQIYAFGLFGDSRTQEGFGQIAPSRAIKKRPVVKGLTQFHRSKAMLLGY
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Peptide 5 (Sequencing coverage 11.98%)

MTRRTKKRAN TTKTMEK<mark>ALV</mark> TAMERLTTKR RVPRKKGCAR VAEGEMVLRR EEMLVDVTLS ANKTDSTGSV VLALANFPWL KTVAGSFERY KWKRLNIHWR AAGGFNKGGL IAVGMDWSNQ LSSAYTRQTL TSASERQKVL SLTPHMSLPI SSTSINKTLG LPIKMLNSRN WYDAAKTDDE GAVGAIRYSA KCDSDTVERF IGEIWVDYEV VLQGTRAQLP PPPPPPKH TLLYYSFTEM RYVITRGQGA TNNVNSRNSS GISQRVVDND FTLSTDSFSD TVELLDGRTY RLIPTVQTET EDDAPLDPDF EGLLTNVVSP PEQYSNTAHL KFYPLRDILK FRLTDFQLNL HLALEVEVNY PTDMEALAIL WDFSRCLVSP QVRTRRESVK SNKALFHCVA AGLYRYVPAT RPYFKVSVEW FNTGISSALA YTMKTQIYAF GLFGDSRTQE GFGQIAPSRA IKKRPVVKGL TQFHRSKAML Peptide 6 (Sequencing coverage 17.05%)

MTRRTKKRAN	TTKTMEKALV	TAMERLTTKR	RVPRKKGCAR	VAEGEMVLRR	EEMLVDVTLS
ANKTDSTGSV	VLALANFPWL	KTVAGSFERY	KWKRLNIHWR	AAGGFNKGGL	IAVGMDWSNQ
LSSAYTRQTL	TSASERQK <mark>VL</mark>	SLTPHMSLPI	SSTSINK <mark>TLG</mark>	<mark>LPIK</mark> MLNSRN	WYDAAK <mark>TDDE</mark>
<mark>GAVGAIR</mark> YSA	KCDSDTVERF	IGEIWVDYEV	VLQGTRAQLP	PPPPPPPKH	TLLYYSFTEM
RYVITRGQGA	TNNVNSRNSS	GISQRVVDND	FTLSTDSFSD	TVELLDGRTY	RLIPTVQTET
EDDAPLDPDF	EGLLTNVVSP	PEQYSNTAHL	KFYPLRDILK	FRLTDFQLNL	HLALEVEVNY
PTDMEALAIL	WDFSRCLVSP	QVRTRRESVK	SNKALFHCVA	AGLYRYVPAT	RPYFKVSVEW
FNTGISSALA	YTMKTQIYAF	GLFGDSRTQE	GFGQIAPSRA	IKKRPVVKGL	TQFHRSKAML

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Peptide 7 (Sequencing coverage 20.28%)
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MTRRTKKRAN	TTKTMEKALV	TAMERLTTKR	RVPRKKGCAR	VAEGEMVLRR	EEMLVDVTLS
			KWKRLNIHWR		
LSSAYTRQTL	TSASERQK <mark>VL</mark>	SLTPHMSLPI	SSTSINK <mark>TLG</mark>	LPIKMLNSR <mark>N</mark>	WYDAAK <mark>TDDE</mark>
<mark>GAVGAIR</mark> YSA	KCDSDTVERF	IGEIWVDYEV	VLQGTRAQLP	PPPPPPPKH	TLLYYSFTEM
RYVITRGQGA	TNNVNSRNSS	GISQRVVDND	FTLSTDSFSD	TVELLDGRTY	RLIPTVQTET
EDDAPLDPDF	EGLLTNVVSP	PEQYSNTAHL	KFYPLRDILK	FRLTDFQLNL	HLALEVEVNY
			SNKALFHCVA		
FNTGISSALA	YTMKTQIYAF	GLFGDSRTQE	GFGQIAPSRA	IKKRPVVKGL	TQFHRSKAML

Figure S2. Peptide sequences derived from ApGIV2 structural proteins. Peptide numbers correspond to protein bands in Figure 3B; Colors represent sequences of individual peptide fragments. Proteins were identified from these peptide sequences with high confidence based on Mascot search A2 values.

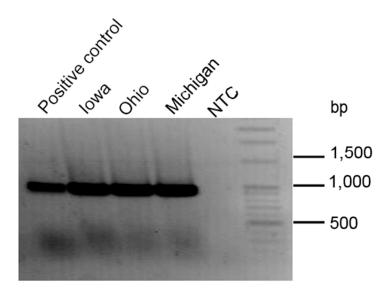


Figure S3. Detection of ApGIV2 RdRp sequence from field collected *A. glycines*. The 929 bp RT-PCR product was detected in field collected *A. glycines* from Iowa, Ohio and Michigan, USA. The positive control and *A. glycines* samples amplified the expected PCR products. No amplification was detected in the no template control sample (NTC).

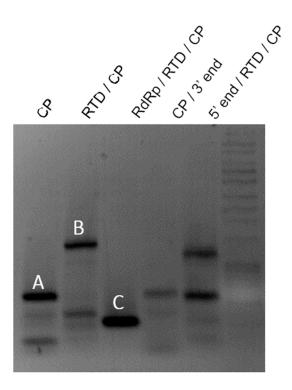


Figure S4. ApGIV2 sequences were not detected in the *A. glycines* genome. Primers spanning the entire ApGIV2 genome were used to test for the presence of ApGIV2 sequence in the *A. glycines* genome. A representative agarose gel with amplified PCR products is shown. Labels indicate region of ApGIV2 genome spanned by the PCR primers (Table 1). CP, capsid protein; RTD, read through domain; RdRp, RNA-dependent RNA polymerase. The sequenced PCR product from A and B did not have any significant matches to ApGIV2 or the NCBI database. PCR product C hit a pea aphid prestin-like transcript variant 5. Primers used: RdRp/CP (ApGIV2 R/C—F and ApGIV2 R/C—R to amplify 2976–3845 nt; 869 bp product): RTD/CP (ApGIV2 C/T—F and ApGIV2 C/T—R to amplify 3885–4445 nt; 561 bp product): RdRp/RTD/CP (ApGIV2 R/C—F and ApGIV2 C/T—R to amplify 2976–4445 nt; 1469 bp product): CP/3'-end (ApGIV2 C/T—F and ApGIV2 3'—R to amplify 3885–4405 nt; 617 bp product): S'END/RTD/CP (ApGIV2 5'—F and ApGIV2 C/T—R to amplify 160–4445 nt; 4285 bp product).