

1 Single-stranded RNA viruses infecting the invasive Argentine ant, *Linepithema humile*

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## 18 Supplementary methods

19 Primer design and sequencing of extended contigs  
20 Primers (Supplementary Table 2) were designed using NCBI's Primer-BLAST  
21 (Ye *et al.* 2012). The organism in the Primer-BLAST pair specificity settings was  
22 Hymenoptera (taxid: 7399) and the maximum product size was 1000 bases. All  
23 other settings were the defaults. For the *Linepithema humile virus 1* (LHUV-1)  
24 genome discovery the IVA extended LHUV-1 contig was used as the template.  
25 The IVA extended n1905 contig was used as the template for the 5' end of the  
26 genome and the Kashmir bee virus (KBV) genome (NCBI GenBank accession  
27 AY275710.1) as the template for the 3' end. For LHUV-1 genome extension,  
28 cDNA from Argentine ants previously found to have a high viral titer of LHUV-1  
29 were used. The same was done for n1905 genome extension but with Argentine  
30 ants with high loads of replicating KBV. The PCR reactions contained 2  $\mu\text{L}$  of  
31 template DNA, 12.5  $\mu\text{L}$  of 2x MyTaq™ Red Mix (Bioline), 8.5  $\mu\text{L}$  molecular grade  
32 water and 1  $\mu\text{L}$  of each the forward and reverse primers (10  $\mu\text{M}$ ) for a total  
33 volume of 25  $\mu\text{L}$ . The PCR thermal cycling conditions consisted of an initial  
34 denaturation at 95°C for 120 s, followed by 40 cycles of denaturation at 95°C for  
35 20 s, annealing temperature was 57°C, 60°C, 63°C depending on the primers  
36 used (Supplementary Table 2) for 10 s and 72°C for 30 s, with a final elongation  
37 at 72°C for 5 min. The PCR products were visualized with agarose gel (1.5%)  
38 electrophoresis. PCR products were sent to Massey Genome Services (Massey  
39 University, Palmerston North) for both forward and reverse Sanger sequencing.  
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52 **Supplementary Table S5:** Alignment used to build the phylogenetic trees presented in Figures 4 and 5 (Microsoft Excel  
53 spreadsheet).

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