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Original Article

A new densovirus from the tropical fire ant with widespread distribution in Taiwan

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Abstract

Densovirinae viruses target invertebrates such as insects and crustaceans. While analyzing sequencing data from the tropical fire ant (*Solenopsis geminata* (Fabricius, 1804)), we discovered contaminating sequences suspected to be viral. Subsequent sequence assembly and comparisons confirmed the presence of a new virus, which we call Solenopsis geminata densovirus1 (SgDNV1). This virus is a single-stranded DNA type virus consisting of a 5289 nucleotide genome, featuring hairpin loops and inverted terminal repeats at both ends. The genome has five open reading frames that are predicted to encode three non-structural protein genes and two viral particle protein genes. Using a PCR assay, we found this virus widely distributed across central and southern Taiwan. In the laboratory, SgDNV1 also appears able to cross-infect the red imported fire ant (*Solenopsis invicta* Buren, 1972). Additionally, SgDNV1 sequence can be found in bat metagenomic samples from southern China. As densoviruses are not known to infect bats, this implies that prey species in this region are infected as well. Thus, our study coupled with data mining of public data helps shed light on the distribution, and possibly the ecology, of this densovirus.

Key words: ant-associated virus, Parvoviridae, Solenopsis geminata, Hymenoptera, Formicidae, geographic occurrence.

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Introduction

The tropical fire ant, *Solenopsis geminata* (Fabricius, 1804), is native to North, Central, and South America and has become a global invasive pest, mostly in the tropics and subtropics. This ant has become established in many countries in the Asia-Pacific region (Wetterer 2011, General & Alpert 2012, Gotzek & al. 2015, Nipitwattanaphon & al. 2020, Xu & al. 2022, Siddiqui & al. 2024), with some of these invasion events likely stemming from the Spanish fleets in the 1500s (Gotzek & al. 2015). Although less notorious than its congener, the red imported fire ant *Solenopsis invicta* Buren, 1972 (Tschinkel 2006), it is also a well-known pest species due to its tendency to collect and spread non-native plant seeds, aggressive behavior, and venomous stings that negatively affect native animals, livestock, and humans (Lai & al. 2009, Chen & al. 2014, Wauters & al. 2014, Lai & al. 2015, 2018, Srisong & al. 2018).

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The use of natural enemies has often been proposed for the control of invasive species. One possibility is viruses. Viruses are increasingly recognized as widespread associates of ants, and much of the current knowledge on ant viruses has come from studies of invasive ants, especially the red imported fire ant, *Solenopsis invicta* (see Baty & al. 2020). This has spawned a large search for *S. invicta* viruses, leading to the discovery and characterization of 20 viruses (Valles & al. 2025). Thus far, only one densovirus, Solenopsis invicta densovirus (SiDNV; GenBank KC991097; currently classified by the International Committee on Taxonomy of Viruses (ICTV) within the species *Scindoambidensovirus hymenopteran1*), has been reported for *S. invicta* (see Valles & al. 2013). Additionally, surveys of native and introduced populations indicate that SiDNV is restricted to native Argentine populations of *S. invicta* and absent from introduced populations in the USA (Valles & al. 2025), suggesting that it may be a candidate natural enemy and a potential biological control agent for *S. invicta* for the invasive populations. By contrast, so far only three viruses have been studied in *Solenopsis geminata*. One of these, SINV-1, can infect and replicate in *S. geminata* (see Valles & al. 2007, Allen & al. 2010, Valles & al. 2013). However, while the other two, SINV-3 and SINV-4, can be detected in the field or after laboratory exposure, active replication has not been observed, possibly indicating that *S. geminata* is not their true host (Porter & al. 2013, Valles & al. 2022).

During high-throughput chromosome conformation capture (Hi-C) sequencing for *Solenopsis geminata*, we discovered numerous repetitive fragments that were not obviously ant sequences, leading us to suspect contamination. Initial BLAST analyses (Altschul & al. 1990, Camacho & al. 2009) suggested that these sequences were from a novel densovirus (Parvoviridae). Here, we report the assembly and annotation of this viral genome. We also surveyed *S. geminata* colonies from multiple locations in Taiwan and detected this virus in colonies from several sites. Additionally, PCR screening with virus-specific primers suggests possible infection of *Solenopsis invicta* colonies in the laboratory. Finally, to learn more about the distribution and biology of this virus, we looked for the presence of this virus sequence in public databases.

Material and methods

Collection and rearing of fire ants

The focal tropical fire ant (*Solenopsis geminata*) colony, in which the virus was first discovered, was collected in 2014 at Wuqi Port, Taichung, Taiwan. Colonies were reared in the laboratory at about 27 – 30 °C, given water ad libitum in test tubes, and fed with a combination of crickets, cockroaches, bird seeds, and honey. Ant samples from additional colonies of *S. geminata* were collected from Wuri, Wuqi, Providence University, Dounan, National Cheng Kung University, Hualien, and Kanding in Taiwan. Red imported fire ant (*Solenopsis invicta*) colonies were collected in Taipei and New Taipei City, Taiwan. Additional sample details are in Table S1 (appendix, as digital supplementary material to this article, at the journal's web pages). Species identifications were based on worker morphology as described by Lin & Wu (2003) and Terayama (2009).

Sequencing, assembly, and annotation of the virus genome

The virus genome was assembled from Hi-C sequencing reads that were used to scaffold the *Solenopsis geminata* genome (which will be published elsewhere). To generate the Hi-C data, first, 24 virgin queens were washed with 1 × phosphate-buffered saline 3 times, transferred to a petri dish, chopped into tiny pieces with a sterile razor blade, pooled, and fixed with 1% formaldehyde. Next, the Hi-C sequencing library was prepared using the Dovetail™ Hi-C Library Preparation Kit (Dovetail Genomics, Scotts Valley, CA, USA) following the manufacturer's protocol and then sequenced (paired-end, 2 × 151 bp) on an Illumina Nextseq 2000, with both procedures performed by the NGS High Throughput Genomics Core, Academia Sinica, Taipei, Taiwan. The potential taxonomic sources of a subset of the Hi-C sequences were determined by BLAST comparisons (Altschul & al. 1990, Camacho & al. 2009) against the National Center for Biotechnology Information (NCBI) nucleotide database (Sayers & al. 2025) and also the *S. geminata* genome.

Because the viral sequences were already highly abundant in the library, assembling the virus genomes from a subset of the data was simpler. Thus, only the first 100,000 paired-end reads (i.e., 200,000 reads out of a total of 191,396,530 reads) were used. Adapter sequences and low-quality bases were trimmed using Trimmomatic (v0.38) (Bolger & al. 2014). Read qualities were assessed both before and after trimming with FastQC (v 0.12.0) (Andrews 2010), with no obvious problems being highlighted for the post-trimmed sequences. Post-trimmed sequences were assembled using SPAdes (v3.15.2) (Bankevich & al. 2012). Four candidate viral contigs (ID: NODE_2, NODE_115, NODE_5111, and NODE_5113) were identified by BLASTN comparisons of the assembled sequences with Solenopsis invicta densovirus (Taxonomy ID: 1414671).

These were then assembled manually into the final complete genome as follows. In an initial quality check, the single-end raw sequence reads were mapped to the four contigs, which revealed that three nodes (NODE_2, NODE_5111, and NODE_5113) appeared to be correctly assembled based on relatively uniform coverage and no abnormal breaks in sequence-read mapping alignment. In contrast, NODE_115 showed a sharp break at position 314; reads that aligned to the "left" stopped aligning to the right of this position and vice versa for the reads aligning to the "right" (Fig. S1). Thus, NODE_115 was an incorrect join of unlinked sequences and was therefore split at the misjoin into NODE_115a (1 - 313 nt) and NODE_115b (314 - 644 nt). Next, overlaps among the five contigs were identified through a series of BLASTN comparisons. Several perfect 77 nt overlaps were identified that joined four contigs,

in order from left to right, NODE_5113, NODE_115a (1 - 313 nt), NODE_2, and NODE_115b (314 - 644 nt) (Fig. S2). The reason for the coincidentally equal-sized 77 nt overlaps was that SPAdes assembler uses 77-mers as the maximum default k-mer during assembly. The final contig NODE_5111 was joined to the right end of NODE_115b (314 - 644 nt) based on only 4 nt of overlap. This manual join was initially made based on NODE_5111 having secondary structure suggestive of it being the inverted terminal repeat on the right end of the assembly. Subsequently, the 4-nt join was confirmed by mapping the raw reads to the final assembly.

To check the assembly quality, all raw Hi-C reads were aligned back to the viral genome using BWA-mem (v 0.7.18-r1243-dirty) by single-end mapping (Li 2013). Then, the output sam files were converted to bam format using Samtools (v 1.1.0) (Li & al. 2009). Finally, to evaluate coverage continuity and to identify potential assembly breakpoints in the viral genome assembly, the mapped reads and read depth were inspected in the Integrative Genomics Viewer (IGV; v2.12.3) (Thorvaldsdóttir & al. 2013).

The virus genome was annotated using Viral Annotation DefineR (VADR; v1.4.1), which uses hidden Markov models, covariance models, and protein-homology searches to classify focal sequences against curated viral genome models (Schäffer & al. 2020). For this study, custom VADR models were created from 32 NCBI RefSeq sequences assigned to the subfamily Densovirinae (accessed 22 March 2022) by running the v-build.pl script with default parameters on each sequence. Afterwards, the output files were concatenated to create a "VADR model library". Next, the v-annotate.pl script was run with default parameters comparing the assembled virus sequence with the model library to identify and annotate candidate coding sequences. The VADR annotation pipeline also output 12 alert messages, which were addressed through manual adjustments of the start or stop positions or disregarded (Tab. S2 and Note S1 for details). Finally, the secondary structure (folding) of the single-stranded DNA was predicted using the RNAfold website (Gruber & al. 2008).

Phylogenetic analysis

To determine the phylogenetic position of this virus within densoviruses, a phylogenetic analysis based on amino-acid sequences was conducted on non-structural protein 1 (NS1), a predicted multifunctional protein with roles in DNA replication and viral packaging. This was done using the ViCTree pipeline, which can automatically retrieve sequences from NCBI, filter sequences, align them, and then create a maximum likelihood phylogeny (Modha & al. 2018). The pipeline was run locally with a modified script to keep the intermediate files as follows: victree_ns1_ictv.sh -p SgDNV_NS1.faa -s txid40120_seeds.fa -t 40120 -T 12 -L 100 -v 50 -D 1.0 -b 100 -k 85 -r 1. The parameter “p” is the focal protein sequence, which was NS1; "s" is a seedset, which was the precompiled Densovirinae seed set distributed with ViCTree (txid40120_seeds.fa, Hughes 2016), which contains 21 NS1 protein sequences selected to represent the known diversity of the subfamily; "t" is the Taxa ID, which for Densovirinae was 40120; “T” is the thread number; "L" is BLAST hit length of 100 amino acids; "v" is query coverage set at 50%; "D" is identity threshold for deduplication in the CD-HIT substep, which was 100%; "b" is the number of bootstrap replicates for RAxML; "k" is the species cutoff percent identity which was 85% following ICTV criteria for different species; and "r" means to use trimAl before RAxML. Maximum-likelihood phylogenetic inference was performed using RAxML under the ViCTree default model, PROTGAMMAJTT, with 100 bootstrap replicates. Finally, trees were visualized using Interactive Tree of Life (iTOL) v7 (Letunic & Bork 2024) at the website (https://itol.embl.de/).

Primer design and PCR for confirming viral sequences

Ant DNA was extracted from single individuals using the LabPrep DNA VacEZor Kit (TAIGEN Bioscience Corporation, Taipei, Taiwan) following the manufacturer's instructions. Primers were designed using Primer3 (Untergasser & al. 2012), and their sequences are in Table S3. PCR mixtures consisted of 1 µL of sample DNA, 0.5 µL of 10 µM forward primer, 0.5 µL of 10 µM reverse primer, 10 µL of master mix (2 × SuperRed PCR Master Mix, BIOTOOLS, New Taipei City, Taiwan), and 8 µL of double-distilled water, totaling 20 µL. PCR reactions were done in a SuperCycler Thermal Cycler SC300G (Kyratec Pty Ltd., Brisbane, Queensland, Australia) with the following settings: 94 °C for 4 min; 35 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 50 s; a final extension at 72 °C for 10 min; and then a hold at 10 °C. PCR results were examined using standard agarose gel electrophoresis. In some cases, PCR products were Sanger sequenced via a service provider (Genomics BioSci & Tech., New Taipei City, Taiwan).

Data availability

Sequence data for this study are available at NCBI under BioProject PRJNA1455847. The accession number for the SgDNV1 genome sequence is GenBank PZ321489 and that for the raw Hi-C data for *Solenopsis geminata* (first 100,000 reads) is SRR38199624.

Results

Initial discovery of viral sequences

While analyzing Hi-C data for scaffolding the genome of the tropical fire ant *Solenopsis geminata* (C.-Y. Dai, ME. Chen, T. Chang & J. Wang, unpubl.), we noticed poor coverage of the genome from the dataset. Approximately 65% of the sequence reads did not map to the *S. geminata* genome. To determine the origin of these non-mapping sequences, we used BLAST to screen 30 random, non-mapping read pairs (i.e., 60 reads) against the NCBI nucleotide database. Of these, one or both of the reads for 16 pairs (53%) showed approximately 75 - 85% nucleotide identity to Solenopsis invicta densovirus (Taxonomy ID: 1414671).

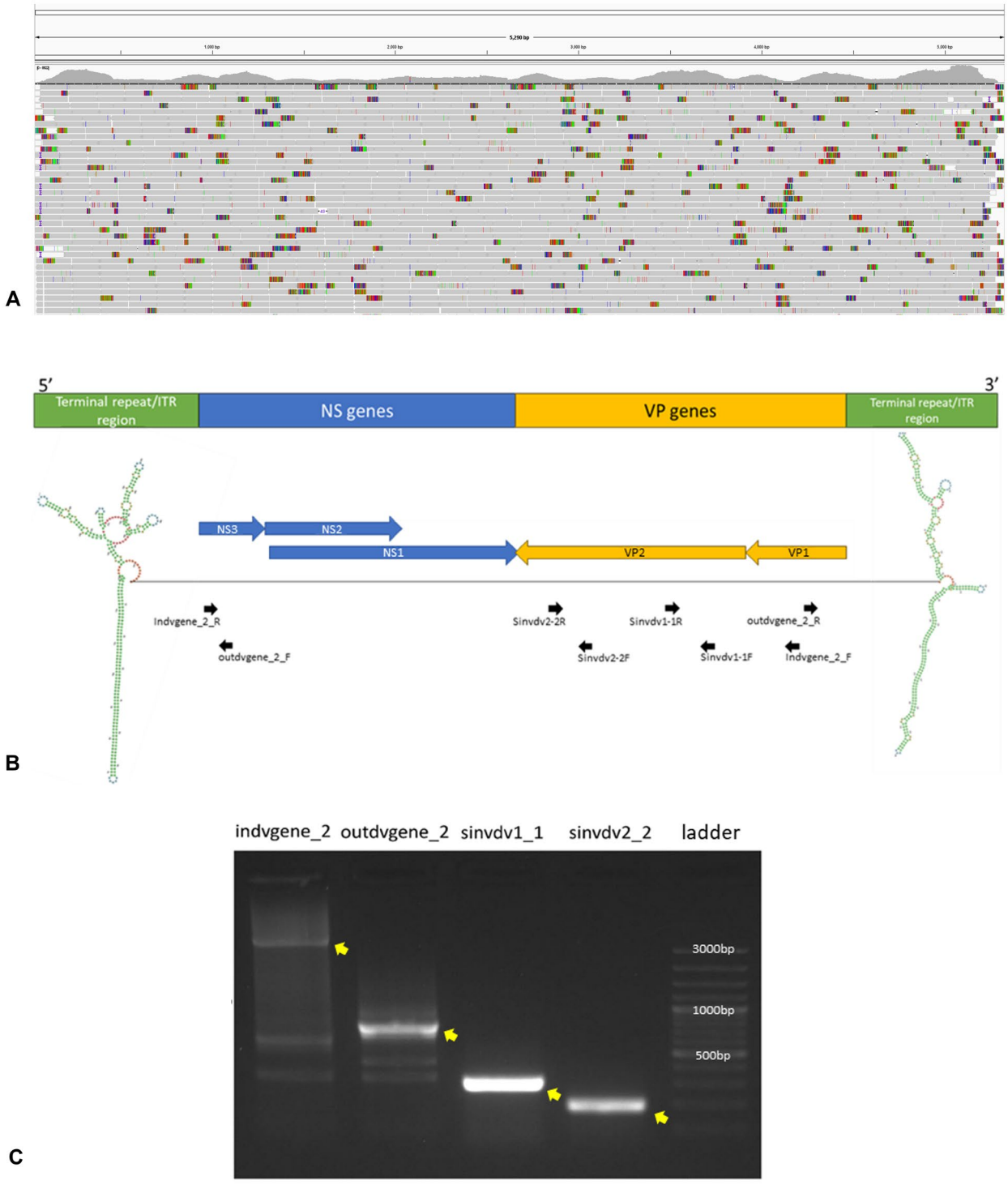

Fig. 1: Assembly validation, genome organization, and PCR confirmation of the putative SgDNV1 genome. (A) After assembly of the viral genome, the Hi-C reads used for assembly were mapped back to the reconstructed genome using BWA-MEM (Li 2013) in single-end mode to examine coverage continuity and to assess whether any obvious coverage gap was present. (B) Genome organization of the putative virus based on Viral Annotation DefineR-guided annotation (Schäffer & al. 2020) and manual assembly. (C) PCR validation of the assembled genome. Primer pair indvgene_2 was expected to generate a 4180 bp amplicon, and the expected product was obtained despite the presence of some non-specific bands. Primer pair outdvgene_2, designed in the outward orientation, yielded an unexpected product; however, subsequent examination of the sequence indicated that this result was an artifact and did not support an alternative circular genome structure. Primer pairs sinvdv1_1 and sinvdv2_2 were used to confirm viral gene regions and produced the expected amplicons. ITR, inverted terminal repeat; NS, non-structural protein; VP, viral protein.

Combining the non-mapping fraction (~ 65%) with the virus-like fraction (53%), we estimated that ~ 34% of total reads may be from a putative densovirus. This was a lower bound because BLAST screening may miss less conserved regions.

Assembly of the putative *Solenopsis geminata* densovirus genome

The large percentage of viral reads permitted us to assemble the genome of the putative densovirus. Because densovirus genomes are typically 4 - 6 kb (Cotmore & al. 2014, 2019), we used only the first 100,000 sequence read pairs for assembly with SPAdes (Bankevich & al. 2012). This assembly produced 5147 contigs covering 1,540,985 bp (N50 = 270 bp). BLAST searches revealed that most contigs were short and matched the host *S. geminata* genome, as expected.

We identified a 4440 nt contig (ID: NODE_2) that showed strong similarity to Solenopsis invicta densovirus (SiDNV; BLAST E-value = 0.0; 75.8% nucleotide identity over 73% query coverage). The depth of coverage of this contig was 1224 ×. Three additional shorter contigs (NODE_115, 644 nt; NODE_5111, 222 nt; NODE_5113, 218 nt) also had BLAST matches to SiDNV (or other densoviruses; all E-values < 3e-24), and all had depth of coverage > 2000 ×, compatible with this virus, as opposed to spurious contigs or another virus. Quality control examination of NODE_115 revealed that it should be split (see Material and methods). Then, based on overlaps between the contigs (details in Material and methods), we manually merged these contigs to yield a final putative viral genome of 5289 nt (Fig. 1). This genome is predicted to have hairpin structures at both ends, forming inverted terminal repeats (ITRs), which are the repeats located at both ends of the viral genome in opposite orientations.

Assembly validation

To verify the genome assembly, we conducted bioinformatic and molecular validation assays. For the bioinformatic validation, we first mapped all read pairs onto the assembled viral genome and inspected the alignments in the IGV (Thorvaldsdóttir & al. 2013). A correct assembly should have uniform and continuous read coverage across the whole genome, while assemblies containing incorrect joins and sequence artifacts should display sharp changes in coverage and alignment breaks. We found relatively even and continuous coverage across the whole genome with read depths ranging from 1.5 k to 6 k (Fig. 1A), consistent with a correct assembly. Furthermore, inspection of the IGV plots showed no vertical, colored lines (i.e., read mappings were gray), indicating sequence agreement between remapping and the assembly.

For the molecular validation, we designed two PCR assays. One set of PCR primers was predicted to amplify 4180 bp, corresponding to ~ 79% of the genome (Fig. 1C). Our PCR tests produced bands of the expected size, indicating a correct assembly over this region. Another set of primers were designed to check if the viral genome was likely linear or circular. Because the two termini of the putative genome had high sequence identity, and based only on the sequence, rather than being a linear genome, the ends could assemble into a circular genome. Thus, we designed "outward" directed primers, which would amplify a product if the genome were circular. This PCR assay produced a ~ 700 bp product, which was unexpected because the consensus is that densovirus genomes are linear. However, subsequent inspection suggested that this PCR product was likely a chimeric artifact generated near the ends before the terminal hairpins (Potapov & Ong 2017, Labadie & al. 2021) (see also Fig. S3). Taken together, these data are most consistent with a linear genome.

Annotation and identification of viral genes using VADR

We annotated the assembled genome using VADR (v1.4.1) (Schäffer & al. 2020), which can identify viral protein coding sequences (CDSs). Typical densovirus genomes encode three non-structural (NS) proteins that are important for viral replication and two viral proteins (VP) that form the viral capsid. VADR identified one non-structural gene, NS2, as a CDS and detected additional candidate genes similar to NS1, NS3, VP1, and VP2. Careful examination of these four genes revealed that the automatic annotation needed minor manual adjustments of the precise start and stop positions for three of them (see Material and methods). After the corrections, we found that the predicted CDSs for NS1 and NS2 overlap but in different frames, while NS3 is immediately upstream of NS2, like in several other densoviruses when NS3 is present (Abd-Alla & al. 2004, Pham Hanh & al. 2013). In contrast, VP2 is immediately downstream of VP1 for this virus, unlike several well-characterized densoviruses where these two proteins overlap (Croizier & al. 2000, Cotmore & al. 2019). Thus, this virus shares the general overall gene content and structure of previously identified densoviruses.

Phylogenetic analysis and confirmation of a new species

A new virus species needs to meet the International Committee on Taxonomy of Viruses (ICTV) criteria of > 15% amino-acid divergence at NS1 with the nearest species, while isolates with > 85% amino-acid identity are considered the same species (Cotmore & al. 2019). To determine if there were other closely related sequences and how our virus genome is positioned phylogenetically, we used the ViCTree pipeline to automatically download all 2731 potential NS1 amino-acid sequences within the Densovirinae and filter these sequences for a minimum length of 100 amino acids and query coverage of 50% to yield 761 sequences. Removing nearly identical sequences reduced the set of sequences to 618 that were used to generate maximum-likelihood phylogenies (see Material and methods). This analysis placed our virus in a strongly supported clade (bootstrap = 100) with a putative Ambidensovirus

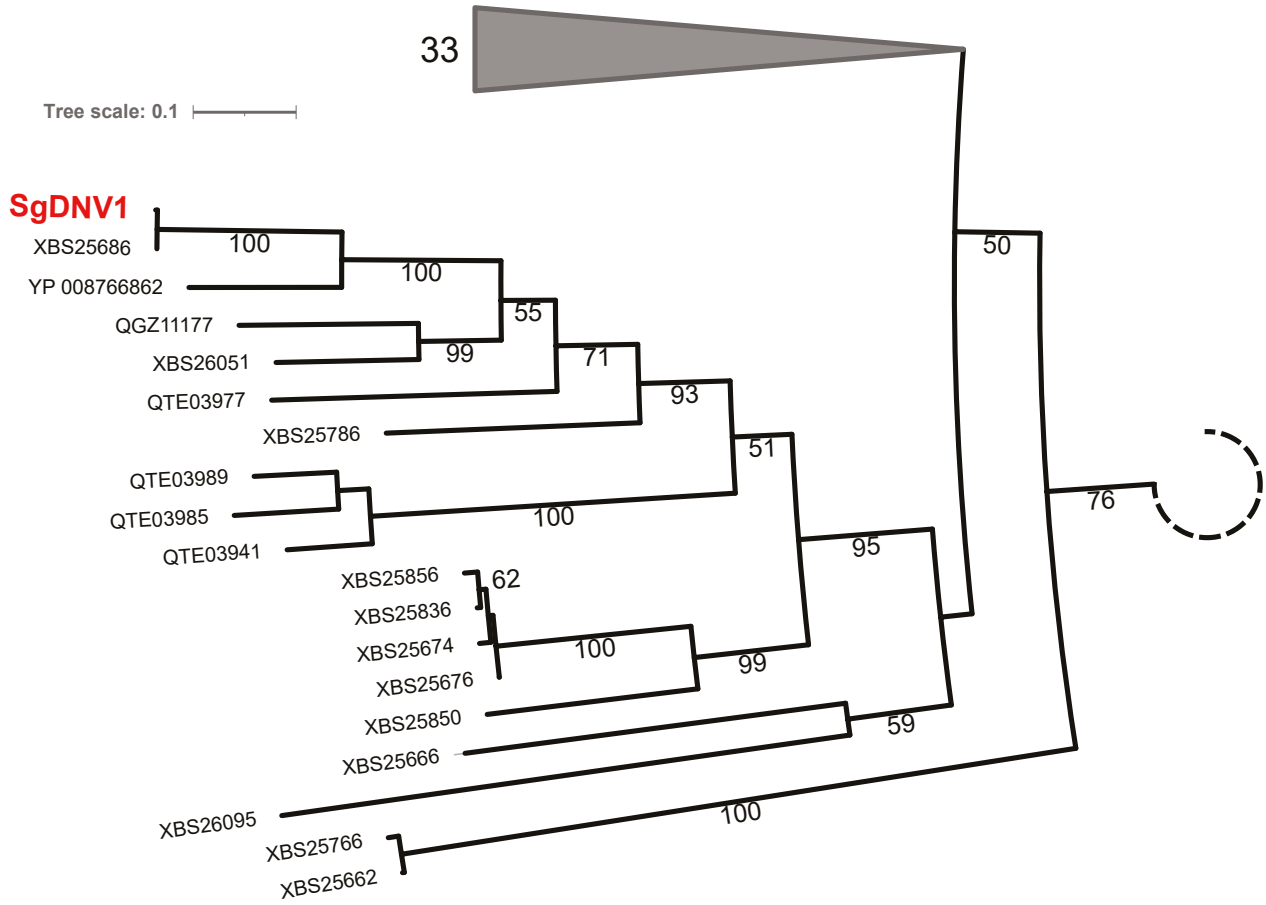

Fig. 2: NS1-based maximum-likelihood phylogeny of Densovirinae showing the placement of SgDNV1. Zoom in of a subclade containing SgDNV1. A ViCTree analysis was performed using 618 NS1 protein sequences retrieved from Densovirinae (see Material and methods). Maximum-likelihood phylogenetic inference was conducted with RAxML under the default PROTGAMMAJTT model with 100 bootstrap replicates. SgDNV1 formed a strongly supported clade (bootstrap = 100; 99.8% amino-acid identity) with XBS25686.1, which is a metagenome-derived NS1 sequence without an assigned species name from bat swab datasets (Wang & al. 2024). This clade was clearly distinct from the closest named virus, Solenopsis invicta densovirus (SiDNV; YP_008766862), which shares 75.3% NS1 amino-acid identity with SgDNV1. The gray triangle indicates a collapsed clade with 33 sequences, and the dashed partial circle represents the rest of the midpoint-rooted phylogeny. The complete maximum-likelihood phylogenic tree is in Figure S4. NS, non-structural protein.

NS1 (XBS25686.1; 99.82% amino-acid identity) (Fig. 2, and full phylogenetic tree in Fig. S4); this NS1 was predicted from a partial viral genome assembly of 3377 nt (NCBI accession PP793366.1, contains both NS1 and VP1). Because this phylogenetic analysis first collapsed nearly identical sequences, we conducted a BLAST homology search on the full dataset and found two additional putative Ambidensovirus NS1 sequences, XBS25694.1 and XBS26024.1, both with amino-acid identity > 99%. These two sequences were also predicted from partial genome assemblies of 3377 nt (PP793370.1, contains both NS1 and VP1) and 1614 nt (PP793589.1, contains only NS1), respectively. All three unnamed viruses came from a metagenomic analysis of bat oral and anal swab samples (Wang & al. 2024), and their NS1 sequences were distinct from the closest named SiDNV (75.33% amino-acid identity). Thus, this analysis revealed an undescribed species-level lineage under the ICTV standard. Because densoviruses have not been shown to use bats or other mammals as hosts, we have named this virus Solenopsis geminata densovirus1 (hereafter SgDNV1).

Virus distribution in Taiwan

The original virus genome sequence assembled from Hi-C data was obtained from a lab colony, Kang1, of *Solenopsis geminata* that was collected from Taichung, but this colony had died by the time of this survey. To screen for the virus in other *S. geminata* colonies in the lab and across samples from Taiwan, we designed two PCR-primer sets targeting genomic regions that showed low similarity to SiDNV, the closest available reference sequence (Fig. 1C). Primer set 1 (sinvdv1_1_F/R) generates a 304 bp product, and primer set 2 (SgDV3_F/R) generates a 573 bp product. As a positive control for our subsequent surveys, we used the DNA extracted from the original Hi-C colony (Kang1). Although we did not rigorously examine the health of

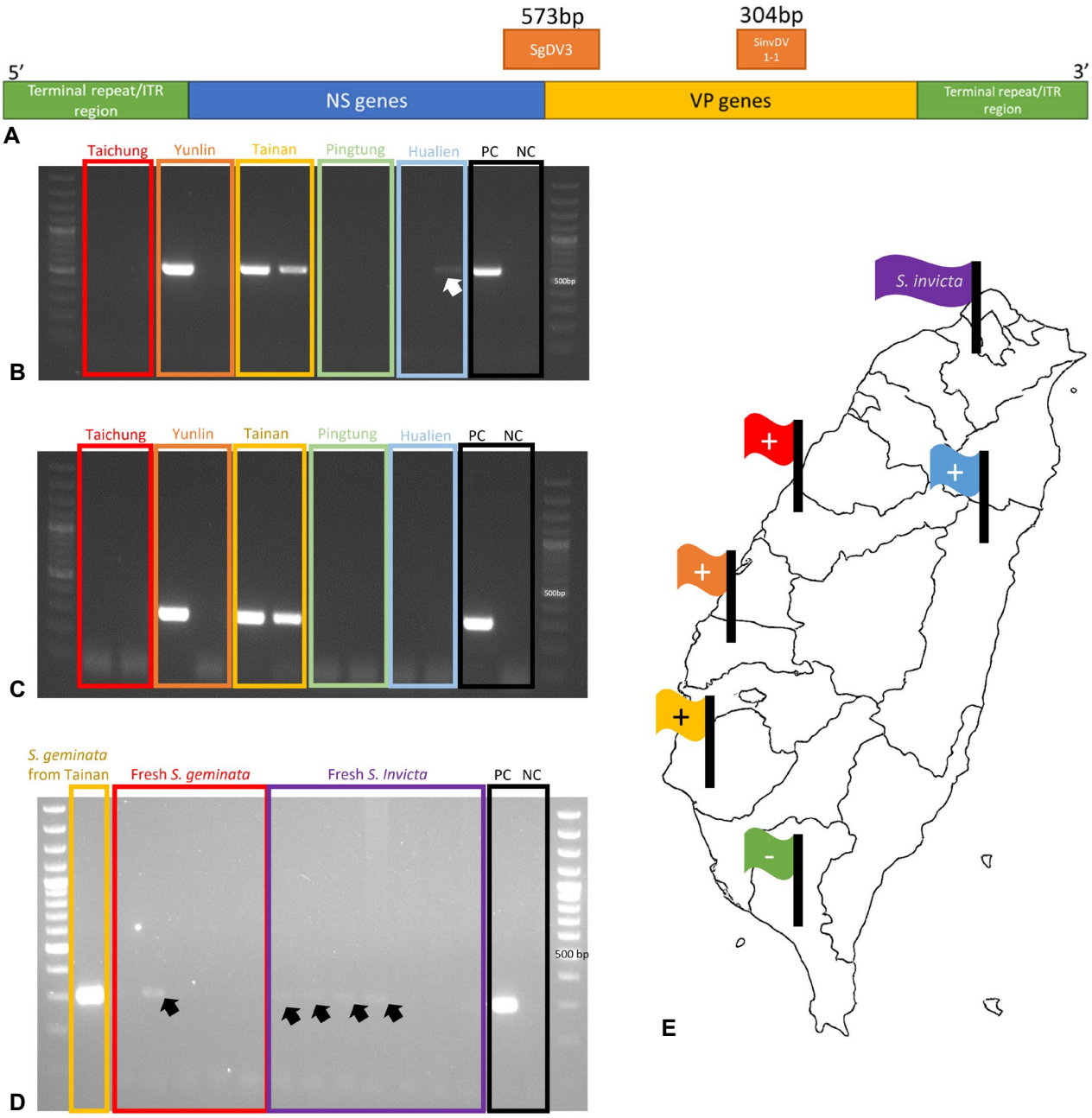

Fig. 3: Detection of SgDNV1 in *Solenopsis geminata* populations from Taiwan and in a co-reared *Solenopsis invicta* population. (A) Schematic of the two PCR primer sets used for virus detection. In addition to sinvdv1-1, which amplifies an expected 304 bp product, a new primer set, SgDV3, was designed to amplify a 573 bp fragment spanning the region between the NS and VP genes. (B) Screening of Taiwanese *S. geminata* populations using the SgDV3 primer set. Positive signals were detected in samples from Yunlin, Tainan, and Hualien. Although the two colonies from Taichung were negative in this gel image, other Taichung colonies were positive including the positive-control colony (Tab. S1). The arrow highlights a weak PCR amplification band. (C) Screening of Taiwanese *S. geminata* populations using the sinvdv1-1 primer set. Samples are the same as in Panel B. Positive signals were detected in samples from Yunlin and Tainan. As in panel B, the two colonies from Taichung were negative in this gel image, while other colonies from Taichung were positive (Tab. S1). In contrast to SgDV3, sinvdv1-1 did not detect the virus in the Hualien sample. (D) Detection using sinvdv1-1 in freshly collected *S. geminata* samples from Taichung and in *S. invicta* maintained in the same rearing room. Viral signals were detected in one fresh *S. geminata* sample from Taichung and four of the co-reared *S. invicta* samples. Arrows highlight weaker PCR amplification bands. (E) Geographic distribution of the tested colonies in Taiwan. Based on the current samples, viral detection was observed in all surveyed regions except Pingtung. Flags mark approximate positions of sampling sites; purple flag for *S. invicta* is indicated; plus sign flags are *S. geminata* with viral signal; minus sign flags are *S. geminata* without viral signal. PC: positive control, DNA from the original infect colony from Taichung. NC: negative control, water. ITR, inverted terminal repeat; NS, non-structural protein; VP, viral protein.

Kang1, this colony reached very high worker numbers (> 10,000) and lived for more than 2 years in the lab.

We screened both newly collected individuals (as of 2022; Providence University, Taichung) and archival samples collected across Taiwan between 2015 and 2017 (Wuri and Wuqi in Taichung; Dounan, Yunlin; National Cheng Kung University, Tainan; Hualien; and Kanding, Pingtung). We tested 5 fresh individuals (1 or 4 from 2 colonies) and 17 archival individuals (1 to 3 from each of 11 colonies, Tab. S1). Viral signals were detected in 8 of 13 (62%) colonies, with 1 / 3 from Wuri, 2 / 2 from Wuqi, and 1 / 2 from Providence University in Taichung; 1 / 2 from Dounan in Yunlin; 2 / 2 from National Cheng Kung University in Tainan; 0 / 1 from Kanding in Pingtung; and 1 / 1 from Hualien (Fig. 3, Tab. S1). Positive samples included both workers and males; however, no virgin or mated queen was tested. In 2 colonies (1104 and Hua), both positive and negative individuals were found (Tab. S1). The high detection rate implies that many of the colonies are naturally infected in the wild. At the same time, many colonies do not appear to be infected, at least by our simple PCR assay.

Detection of the virus in *Solenopsis invicta*

Because *Solenopsis geminata* colonies were maintained in the same rearing room with *S. invicta* colonies, we also screened 7 lab *S. invicta* colonies that had been in the lab for more than 6 months but less than 1 year. We detected the virus in 4 of the colonies (Fig. 3D), suggesting that the virus could be transmitted across species. The PCR band intensities were weak, and although this is not a quantitative measure, this may suggest that infection load in *S. invicta* may not be very high. Nevertheless, whether the *S. invicta* detections reflect transmission in the laboratory setting or infection acquired in the field remains to be determined.

Discussion

In this study, we extracted viral sequence reads from a Hi-C dataset of the tropical fire ant, *Solenopsis geminata*, and assembled them into a linear viral genome. After validation of the genome by read re-mapping and PCR confirmation, we found that the genome is 5289 nt long, encodes five genes, and has hairpin loops and ITRs at both ends. Gene annotation and phylogenetic analyses revealed that the virus belongs to an undescribed species-level lineage, which we name Solenopsis geminata densovirus1 (SgDNV1). Superficially, this virus does not appear to have an obvious deleterious effect on the health of the colonies, although more detailed studies would be needed to see if there might be subtle effects.

SgDNV1 appears prevalent across Taiwan

The identification of a new virus prompted us to screen *Solenopsis geminata* samples from several locations in Taiwan and collected across multiple years (2015 - 2022), including both newly collected material and archived specimens (Fig. 3). Despite the modest sampling intensity per site, SgDNV1 was detected in 8 of 13 colonies which corresponded to 6 of 7 sampling sites. Notably, detection was sometimes heterogeneous even within the same county / city (i.e., 3 positive sites out of 7 total in Taichung and 1 of 2 in Yunlin). No data from Northern Taiwan were available due to a lack of samples. This is because *S. geminata* is rarely reported from the northern regions, probably because the winters are too cold. The 8 positive samples were found in ants collected in 2015, 2016, and 2022, and adding the original sample found by Hi-C sequencing includes 2014. While our data are restricted to the regions and time points sampled here, the detection of the SgDNV1 is not confined to a single locality or a single collection period and suggests that infection is common in Taiwan. Larger and more systematic sampling would be required to test if there are fine-scale spatial patterns of virus infection.

Evidence for potential cross-species detection

The detection of SgDNV1 in *Solenopsis invicta* colonies maintained in the same rearing room with *Solenopsis geminata* raises the possibility of cross-species infection. This finding fits with fire ant virome studies, where it is often difficult to determine the true host or hosts. Reports of viruses detected in *S. geminata* are known only for three RNA viruses, particularly SINV-1, SINV-3, and SINV-4. The main host of SINV-1 appears to be *S. invicta*, and detections in congeneric fire ants such as *S. geminata* are frequently interpreted as spillover or transfer-host events, especially in sympatric regions of the two ants (Valles & al. 2007, Allen & al. 2010, Valles & al. 2013). In contrast, SINV-3 shows strong host restriction within South American fire ant populations, with experimental and field evidence indicating limited or absence of infection in *S. geminata* (Porter & al. 2013). Similarly, SINV-4 RNA can be detected in multiple ant species including *S. geminata*, however, evidence for active replication, such as detection of the replicative strand, is more limited and appears to be clearly supported only in *S. invicta* (see Valles & al. 2022).

For the virus discovered in this study, SgDNV1, PCR-based detection alone cannot distinguish productive infection from passive carryover or laboratory-associated transfer, and replication-based evidence is generally needed to confirm that an ant species is a true host rather than an incidental carrier (Baty & al. 2020). We therefore interpret the SgDNV1 signal in *Solenopsis invicta* as evidence of potential cross-species exposure rather than definitive transmission. Furthermore, in all 4 cases, the amplification signal was weak, which likely indicates low template abundance. Low abundance may indicate poor replicative ability in *S. invicta*, although it remains to be determined whether this is from an inability of the virus to infect *S. invicta* or a stronger immune response of *S. invicta*. A caveat of these detections is that they were based on PCR assays. It is possible that the positive results were from another closely related virus. Thus, sequence confirmation of the *S. invicta* PCR amplicons, quantitative assays of viral infection, and field sampling of *S. invicta* populations would be useful to determine whether these

detections reflect laboratory-associated transfer, transient exposure, or natural infection.

Public metagenomic records may help reveal *Solenopsis geminata* and SgDNV1 biology

Three highly similar NS1 records (XBS25686.1, XBS25694.1, and XBS26024.1; > 99% amino-acid identity to each other and to SgDNV1) were identified in a large-scale bat swab metagenomic dataset (Wang & al. 2024). However, because swab-based metagenomes also contain sequences derived from the diet or environmental exposure, these records alone do not establish bats as the natural host. Moreover, bats and other mammals have not been confirmed as hosts for other densoviruses (El-Far & al. 2004, Cotmore & al. 2019, Lim & al. 2026). Rather, these viral sequences may indicate that some of the bat prey, which are presumably mostly insects, was infected with SgDNV1. Notably, all three samples were from bats collected in Guangdong, China, where *Solenopsis geminata* has become established as an invasive pest (Siddiqui & al. 2024). This geographic coincidence may suggest an interesting scenario in which these bats recently ate *S. geminata*. Because bats usually eat insects in the air rather than off the ground, these sequence records may suggest that captured *S. geminata* were males and / or queens undergoing mating flights on or just before the date of bat capture. However, while we have found that SgDNV1 can be detected in males, queen infection has not yet been demonstrated. Nonetheless, these observations raise the speculative possibility that metagenomics of bats and other insectivores may yield useful data about ant mating patterns through their viruses.

These bat metagenomic samples from Guangdong, China, were collected hundreds of kilometers from our sampling sites in Taiwan. Assuming SgDNV1 originated in the native range with *Solenopsis geminata* in the Americas, SgDNV1 may be globally widespread. Overall, the presence of these highly similar sequences in databases indicates that SgDNV1 is not restricted to Taiwan and highlights the under-characterized diversity of densoviruses.

Conclusions

In sum, we have identified a new densovirus, Solenopsis geminata densovirus1 (SgDNV1), which is commonly found in *Solenopsis geminata* colonies in Taiwan. This virus can be detected in another fire ant, *Solenopsis invicta*, in the laboratory, but whether that represents infection still needs to be resolved. SgDNV1 has also been detected in bat metagenomic samples in China, hundreds of kilometers from our sampling sites in Taiwan, indicating that SgDNV1 may be broadly distributed. Finally, our study coupled with the detection in bats helps shed light on the biology of both densoviruses and ants.

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Declaration on use of generative artificial intelligence tools

The authors declare that they did not utilize generative artificial intelligence tools in any part of the composition of this manuscript except that the artificial-intelligence tool ChatGPT (OpenAI, San Francisco, CA; multiple models, including GPT-4o, GPT-5.1, GPT-5.2 Instant and Thinking, and GPT-5.4) was used for limited editorial assistance on existing text, including grammar correction, spelling and formatting checks. The authors take full responsibility of the content of the manuscript in every detail.

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