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NOTES AND COMMENTS

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A new variant of slow bee paralysis virus revealed by transcriptome analysis

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ABSTRACT

Using NGS data from an RNA-seq library, we reveal a novel variant of slow bee paralysis virus (SBPV) in a pooled sample of adult honey bees (*Apis mellifera*) collected in southwest Germany. We provide its sequence (NCBI Accession No. PP100271) and demonstrate that it is infective for adult honey bees by feeding.

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SBPV; *Apis mellifera*; +ss RNA virus; *Iflaviridae*; transmission; NGS

Slow bee paralysis virus (SBPV) is a pathogen of adult honey bees (*Apis mellifera*) causing paralysis of the front legs and early mortality (Bailey & Woods, 1974; de Miranda et al., 2010). Its virion structure has been described in fine resolution (Kalynych et al., 2016) and Kalynych et al. (2017) provide a plausible mechanism of genome release from its virion. Colony collapse has been associated with infection by SBPV following the arrival in Great Britain of *Varroa destructor* (Carreck et al., 2010), which transmits SBPV. Though geographically widespread, SBPV is often found at low (<5%) prevalence in honey bee populations (de Miranda et al., 2010; Doublet et al., 2024; McMahon et al., 2015). It is, though, found widely in bumble bees (*Bombus* spp.), where it can reach high prevalence (35% in *Bombus hortorum*; McMahon et al., 2015), presumably transmitted directly by the faecal-oral route.

Screening of honey bees for virus is typically undertaken using PCR-based techniques such as MLPA (multiplex-ligation probe dependent amplification: de Smet et al., 2012) or real-time quantitative PCR (qPCR) (de Miranda et al., 2013). Though offering great sensitivity, these methods only detect viral variants containing sequences complementary to those of the PCR primers. For SBPV, the widely employed PCR primers of de Miranda et al. (2010) amplify SBPV's hitherto two known major variants: Rothamsted (NCBI Reference Sequence NC_014137/Accession No. EU035616) and Harpenden (Accession No. GU938761), as well as a third accession (Accession No. KY243931, Kalynych et al., 2016), which differs from the Rothamsted variant by only 0.14% (ca. 13 bases).

Next Generation Sequencing (NGS) based transcriptome analysis (RNA-seq) overcomes the specificity-limitation of MLPA and qPCR by sequencing essentially all RNA molecules in an RNA extract, revealing hitherto unknown viruses and their variants (e.g. Li et al., 2023). We here use RNA-seq data to reveal a third major variant of SPBV in honey bees, which we term the Schwarzwald (SW2011) variant, and which MLPA (de Smet et al., 2012) and qPCR (de Miranda et al., 2010) did not detect.

RNA was extracted using standard methods (de Miranda et al., 2021; see Supplementary Material for details) from a pool of 30 adult workers collected from honey bee colony ts3, located near Kenzingen (48°11′30″N 7°46′6″E) in the Black Forest (German: Schwarzwald) of SW Germany, as part of an investigation into the correlates of overwinter colony decline (Natsopoulou et al., 2017). The sample was positive for DWV-B but gave no signal of SBPV or of other viruses using MLPA (Natsopoulou et al., 2017) or qPCR. RNA was commercially prepared for RNA-seq analysis (see Supplementary Material for details) and NGS sequenced on an Illumina platform (100 bp paired end reads), generating 44.5 million raw reads. After trimming and merging reads, and mapping them to the honey bee genome and transcriptome (version

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Table 1. Full RNA viral genomes assembled from the RNA-seq data, depth of read coverage (for SBPV, see Supplementary Figure S1), and % similarity to NCBI reference genomes.

Genome	Length (bases)	Read coverage	Genetic similarity to NCBI reference	Variant Name
DWV-A	10153	180	100% to NC_004830	A2011
DWV-B	10190	194514	99% to NC_006494	B2011
SBPV	9988	50853	84% to NC_014137	SW2011 (Schwarzwald)

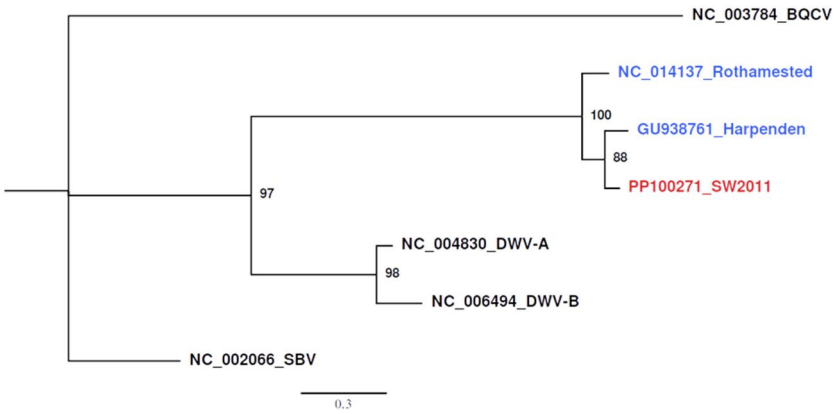

Figure 1. Maximum likelihood phylogenetic relationship of partial RdRp gene sequences (840 nucleotides) of the three major SBPV variants available in NCBI (in blue and red), including the newly described SW2011 (Schwarzwald, in red), as well as NCBI reference sequences for deformed wing virus (DWV), sacbrood virus (SBV) and black queen cell virus (BQCV), all in black, with 1,000 bootstrap replicate support. The scale shows the nucleotide substitution rate per site (0.3 equates to $0.3^{*}840 \approx 250$ nucleotides difference).

GCF_003254395.2_Amel_HAv3.1), we extracted 37.6 million non-host (primarily viral) reads (Supplementary Table S1), of which 29 million (77%) mapped to DWV-B (NC_006494), 7 million (18%) to SBPV (NC_014137) and ca. 35000 (<1%) to DWV-A (NC_004830) of the NCBI nucleotide collection. Viral reads could be assembled *de novo* into full-length genomes of DWV-B, SBPV and DWV-A (Table 1), demonstrating that our sample contained a mix of primarily DWV-B, some SBPV and a very small quantity of DWV-A.

The Schwarzwald variant (SW2011) is distinctly different from both hitherto known major SBPV variants, but more similar to the Harpenden variant (GU938761) than to the Rothamsted variant (NC_014137) (90% vs 84%; Figure 1). Further details of bioinformatic analyses and of genetic variation within the Schwarzwald variant are given in the Supplementary Material (Figure S2); its genome is available in NCBI (Accession No. PP100271).

We generated an SBPV inoculum from the pooled RNA of colony ts3, fed it to virus-free bees, and found that it replicated in 50% of them (Supplementary Table S2), evidenced using the generic SBPV PCR primers of de Miranda et al. (2010) and updated for the Schwarzwald (SW2011) variant of SBPV (5′-3′F: GTGCTTTAGTTCAATTACCATTG; R: ATTATGGGACGTGA GAATATAC). This demonstrates that SBPV variant Schwarzwald (SW2011) is indeed a viral variant capable of infecting adult honey bees.

Though the qPCR primers of de Miranda et al. (2010) have been updated to amplify all three SBPV variants (de Miranda et al., 2021; its Supplementary Table S1), there are likely other variants of this and other viruses that may continue to go undetected, for which RNA-seq would be necessary to ensure their detection. Mining of publicly available transcriptome datasets is also likely to bring additional viral variants to our attention.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Appendix
Bioinformatic commands

All bioinformatic analyses were undertaken in Ubuntu Linux. Figure preparation was undertaken in R (v. 4.2.2; R

Core Team, 2022). The following bioinformatic software was used for analysis, with commands shown in italics.

1. Trimming and merging raw reads was undertaken using fastp version 0.23.1 (Chen et al., 2018).

fastp -c –detect_adapter_for_pe -i mate1.fastq.gz -I mate2.fastq.gz –merge -o mate1.fastq -O mate2.fastq –merged_out merged –unpaired1 unpaired –unpaired2 unpaired -l 100 -z 1 -j out.json

cat mate1.fastq mate2.fastq merged unpaired > filtered.fastq

2. Mapping NGS reads to the honey bee genome was undertaken using hisat2 version 2.2.1 (Kim et al., 2019).

hisat2 -x GCF_003254395.2_Amel_HAv3.1 -k 1 -q -U filtered.fastq –no-spliced-alignment -p $CPU -S output.sam

awk '$3 == "*" {print "@"$1"\n"$10"\n+\n"$11}' output.sam > nonhost.fastq

3. Alignments were undertaken using hisat2 version 2.2.1 (Kim et al., 2019) with default settings.

hisat2 -x BeeViruses -k 1 -q -U nonhost.fastq –no-spliced-alignment -p $CPU -S BeeViruses.sam

4. Viral genome assembly was undertaken using rnaviralspades version 3.15.3 (Antipov et al., 2020).

rnaviralspades.py -s nonhost.fastq -t $CPU -o $LIB

5. Variant calling were undertaken using samtools version 1.12 (Danecek et al., 2021) and varscan version 2.4.4 (Koboldt et al., 2009).

samtools sort -@ $CPU -O bam -o InoculumB2013_SBPV.bam output2.sam

samtools index InoculumB2013_SBPV.bam

samtools mpileup -a -d 0 -f SBPV2013.fas InoculumB2013_SBPV.bam > mpileup.txt

varscan pileup2snp mpileup.txt –min-coverage 300 –min-var-freq 0.01 –p-value 0.01 > varscan.vcf

6. Depth coverage of the genome was calculated using samtools version 1.12.

samtools sort -@ 16 -O bam -o InoculumB2013_SBPV_sorted.bam InoculumB2013_SBPV.bam

samtools depth -a -H -o InoculumB2013_SBPV.cov -m 0 InoculumB2013_SBPV_sorted.bam

7. Haplotype calling was undertaken using quasirecomb version 1.2 (Töpfer et al., 2013).

quasirecomb -XX:+UseParallelGC -Xms2g -Xmx30g -XX:+UseNUMA -XX:NewRatio = 9 -i InoculumB2013_SBPV.bam -K 1-3 -noRecomb -o haplo_quasirecomb -conservative

7. Multiple alignment was undertaken using MAFFT version 7.487 (Katoh & Standley, 2013).

ginsi phylo.fas > phylo.aln

8. The phylogenetic tree (Figure 1 of the main text) was estimated using a maximum likelihood approach in IQ-TREE version 2.2.6 (Nguyen et al., 2015).

iqtree -s rdrp_7virus.aln -m MF

iqtree -s rdrp_7virus.aln -m HKY+F + R2

9. Tree visualization was undertaken using Figtree version 1.4.4. (http://tree.bio.ed.ac.uk/software/figtree/).