

Coding-complete genome sequence of a divergent black queen cell virus strain identified from honeybees (*Apis mellifera*) in Romania

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ABSTRACT We report the 8,451-nt coding-complete genome sequence of a divergent black queen cell virus strain (BQCV-Ro). This strain was identified via retrospective analyses of metagenomic virome data of *Apis mellifera* samples from Europe. The genome assembly is supported by extensive read mapping, with 100% horizontal coverage and a mean sequencing depth of ~38,171×. and shares 94.3% identity with the BQCV Yeongdeok isolate.

KEYWORDS black queen cell virus, *Apis mellifera*, viral metagenomics

Black queen cell virus (BQCV), a member of the genus *Triatovirus* (family *Dicistroviridae*), is a major viral pathogen of the honeybee (*Apis mellifera*) (1). While often asymptomatic in adult bees, it causes mortality in queen larvae at elevated titers and is characterized by the darkening of the queen cell walls (2). Here, we describe a divergent BQCV strain (BQCV-Ro) identified through the re-analysis of a metagenomic data set of honeybees originally collected in the framework of the EU-funded B-GOOD project (3).

The sample was derived from an apparently healthy Romanian honeybee hive located at latitude 46.7598 and longitude 23.574, collected on 17 July 2020. The guts of 10 worker bees were dissected, and their rectums were pooled before the sample was processed following the NetoVIR protocol for the enrichment of both DNA and RNA viral particles (4). In short, the sample was homogenized (MINILYS, Bertin Technologies) at 3,000 rpm for 1 min with 2.8 mm zirconium oxide beads (Precellys), centrifuged at 17,000 × *g* for 3 min, passed through a 0.8 μm PES filter, and treated with nuclease to remove host and environmental nucleic acids. RNA and DNA were extracted using the QIAamp Viral RNA Mini Kit (QIAGEN) without carrier RNA. The extract underwent reverse transcription and random amplification (WTA2, Merck), and the resulting cDNA was processed into Illumina sequencing libraries using the Nextera XT kit (Illumina) before sequencing on the NovaSeq 6000 (Illumina) platform using 150 bp paired-end reads. Raw paired-end reads were quality-filtered and adapter-excised via Trimmomatic v.0.39 (5). Host-derived sequences were computationally subtracted by aligning reads to the *Apis mellifera* reference genome ([GCF_003254395.2](https://www.ncbi.nlm.nih.gov/assembly/GCF_003254395.2)) using Bowtie2 v.2.5.1 (6). *De novo* assembly of the remaining reads was performed using metaSPAdes v3.15.3. Taxonomic classification of the obtained contigs was achieved using DIAMOND v2.0.9 against the NCBI non-redundant (nr) database (downloaded on 17 March 2023), supplemented by BLASTn searches against the NCBI nucleotide (nt) database (downloaded on 17 March 2023). Unless otherwise specified, default parameters were used for all software tools. Strain BQCV-Ro was identified in a honeybee colony in Romania during the summer and possesses a coding-complete genome of 8,451 nucleotides with a GC content of 40.2%, consistent with the 34–45% range characteristic of *Dicistroviridae* members (7). Read mapping revealed 100% horizontal coverage with a mean depth

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of approximately 38,171×. Open reading frames (ORFs) were predicted *de novo* using NCBI ORFfinder (standard genetic code; minimum length 300 nt), identifying two major ORFs separated by a 316 nt intergenic region. BLASTp analysis against the NCBI nr database showed that ORF1 (4,887 nt) shared 98.4% amino acid identity (100% coverage) with a black queen cell virus non-structural polyprotein (accession [WIL00273.1](#)), while ORF2 (2,454 nt) showed 98.7% identity (100% coverage) to a structural polyprotein (accession [ABS82440.1](#)). A BLASTn analysis of the complete sequence demonstrated 94.3% nucleotide identity (100% query coverage) to the BQCV Yeongdeok strain from the Republic of Korea (GenBank accession [OR496416.1](#)). The characterization of this sequence expands the documented genetic landscape of *Apis* pathogens in Eastern Europe and underscores the efficacy of mining extant metagenomic data sets for the identification of circulating viral strains.

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DATA AVAILABILITY

The black queen cell virus genome sequence has been deposited in GenBank under accession number [PX926923](#). The raw sequencing reads have been deposited in the NCBI Sequence Read Archive (SRA) under accession number [SRR34920129](#), as part of BioProject [PRJNA1302169](#) (BioSample [SAMN50464891](#)).

ETHICS APPROVAL

This study involved only invertebrates (*Apis mellifera*) and was therefore exempt from institutional ethical review under applicable guidelines.

REFERENCES

1. Avci O, Oz ME, Dogan M. 2022. Silent threat in honey bee colonies: infection dynamics and molecular epidemiological assessment of black queen cell virus in Turkey. *Arch Virol* 167:1499–1508. <https://doi.org/10.1007/s00705-022-05458-y>
2. Spurny R, Přidal A, Pálková L, Kiem HKT, de Miranda JR, Plevka P. 2017. Virion structure of black queen cell virus, a common honeybee pathogen. *J Virol* 91:02100–02116. <https://doi.org/10.1128/JVI.02100-16>
3. van Dooremalen C, Ulgezen ZN, Dall'Olio R, Godeau U, Duan X, Sousa JP, Schäfer MO, Beaurepaire A, van Gennip P, Schoonman M, et al. 2024. Bridging the gap between field experiments and machine learning: the EC H2020 B-GOOD project as a case study towards automated predictive health monitoring of honey bee colonies. *Insects* 15:76. <https://doi.org/10.3390/insects15010076>
4. Conceição-Neto N, Zeller M, Lefrère H, De Bruyn P, Beller L, Deboutte W, Yinda CK, Lavigne R, Maes P, Van Ranst M, et al. 2015. Modular approach to customise sample preparation procedures for viral metagenomics: a reproducible protocol for virome analysis. *Sci Rep* 5:16532. <https://doi.org/10.1038/srep16532>
5. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>
6. Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. *Nat Methods* 9:357–359. <https://doi.org/10.1038/nmeth.1923>
7. International Committee on Taxonomy of Viruses (ICTV). 2023. Dicistroviridae. ICTV Report. <https://ictv.global/report/chapter/dicistroviridae/dicistroviridae>.