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SEARCHING FOR PATHOGENIC VIRUSES IN STINGLESS BEES AFFECTED BY A SEASONAL DISEASE

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In Southern Brazil the stingless bee *Melipona quadrifasciata* is no longer found in the wild and beekeepers report annual losses of their colonies due to a seasonal and acute disease of unknown causes. The symptoms are similar to those caused by certain viral infections in other bees, such as those associated with Colony Collapse Disorder (CCD). Therefore, the aim of this work is to investigate whether the annual colony collapse of *M. quadrifasciata* is related to a viral infection. For this, samples of *M. quadrifasciata* workers from symptomatic (unhealthy) and asymptomatic (healthy) colonies were collected during the outbreak periods (late summer) of 2016 and 2017. Pools of 5 (2016 sampling) and 25 (2017 sampling) workers were subjected to filtration and ultracentrifugation for the isolation of viral particles, followed by the extraction of DNA and RNA, cDNA synthesis, and, eventually, random amplification. DNA and cDNA libraries were prepared using a Nextera XT DNA sample preparation kit and sequenced using an Illumina MiSeq instrument (2x150 and 2x250 paired-end reads, insert size of 300pb and 500pb, respectively). The quality of the sequencing was inspected (FastQC) followed by the removal of reads (Sickle) with a phred quality score lower than 30. The filtered reads were then assembled into contigs using SPAdes v.3.10.1 and metaSPAdes with different parameters. Assembly statistics were generated (Quast 4.5) for comparison and choice of the best assembly based on the size and quantity of contigs. Contigs were locally blasted (e-value 1e-5) against a non-reduntant viral database extracted from the total non-redundant database (nr, NCBI) and taxonomic classification was confirmed by using blastX (e-value 1e-5) against the total nr. Alignment of reads against contigs was done with Bowtie 1.2.0 to extract with Samtools 1.3.1 the information about coverage. Four assemblies from two successive years were obtained: assembly (1) has 10399 contigs and N50 of 696pb (unhealthy bees; 2016; obtained from mixed DNA and cDNA); (2) has 2625 contigs and N50 of 1443pb (healthy bees; 2016; obtained from mixed DNA and cDNA); (3) has 16856 contigs and N50 1122pb (unhealthy bees; 2017; DNA only); and (4) has 10226 contigs and N50 872pb (unhealthy bees; 2017; cDNA only). The viromes of assemblies 1-4 contain 3, 1, 240 and 311 viral contigs respectively, with average coverages per base of 5.41, 3.67, 35.69, and 72.13x. Viromes (3) and (4), obtained with a larger sampling in 2017, contain three contigs similar to pathogenic bee viruses. Assembly (3) contains a 220bp sequence similar (46.5% identity; 1.91e-12 e-value) to the Apis mellifera Filamentous Virus (AmFV) with coverage of 1.4x. Assembly (4) also contains a 209pb sequence similar to AmFV (96% identity; 1.38e-21 e-value) with coverage of 1.4x and a 330pb sequence similar (37.8% identity, 4.26e-17 e-value) to Acute Bee Paralysis Virus (ABPV), with coverage of 3.3x. Both viruses, for which we find similar sequences in our assemblies, are known to cause symptoms, such as inability to fly, trembling and crawling, which are also observed in unhealthy *M. quadrifasciata* workers. Moreover, ABPV is known to cause the death of a worker in a few days with less than 100 viral particles, which could explain its low coverage. Whether or not these viruses, or other unknown viruses, are at the root of the collapse of *M. quadrifasciata* colonies is a matter of further investigation of our research group.