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SHORT NOTE

Molecular Characterization of Acute Bee Paralysis Virus (ABPV) and Black Queen Cell Virus (BQCV) in Honeybees (*Apis mellifera* L. (Hymenoptera: Apidae)) from the Campinas Region

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Abstract

The occurrence of Colony Collapse Disorder (CCD) has prompted extensive research on the role of viruses, including Deformed Wing Virus (DWV), Acute Bee Paralysis Virus (ABPV), and Black Queen Cell Virus (BQCV), in honeybee health. This study investigated the presence and the genetic characteristics of DWV, ABPV, and BQCV in *Apis mellifera* colonies in a Brazilian apiary in Jaguariúna, SP, Brazil. A total of 11 apparently healthy colonies were sampled, and adult honeybees were submitted to a multiplex PCR. Results showed that six studied colonies tested positive for ABPV in at least one sampling, while one tested positive for BQCV. DWV was not detected, nor were co-infections observed in the sampled colonies. Although the infected colonies did not exhibit clinical signs of disease, the fluctuating presence of ABPV and BQCV suggests temporal variations in viral dynamics, possibly influenced by environmental and nutritional factors. The absence of DWV detection may be attributed to low infestation levels of the *Varroa destructor* mite in the sampled apiary. Phylogenetic analysis revealed close genetic relationships between the obtained ABPV and BQCV sequences and strains from South America. These findings contribute to the limited knowledge of viral epidemiology in Brazilian honeybee populations.

The study of viruses in bees has been driven in recent decades by the occurrence of Colony Collapse Disorder (CCD) in *Apis mellifera* L. (vanEngelsdorp et al., 2009). Some virus species, such as Deformed Wing Virus (DWV), Acute Bee Paralysis Virus (ABPV), and Black Queen Cell Virus (BQCV), are widely distributed worldwide and have been implicated in CCD cases (Hristov et al., 2020; Alonso-Prados et al., 2021; Flores et al., 2021).

DWV, ABPV, and BQCV are single-stranded positive-sense RNA viruses, with DWV belonging to the family Iflaviridae and ABPV and BQCV belonging to the family Dicistroviridae. Transmission of these agents can occur through environmental factors, fecal-oral routes, and vertically. The importance of vector transmission, specifically through the *Varroa destructor* mite, is significant for DWV and ABPV but is not well established for BQCV (Beaurepaire et al., 2020).

These three viral agents have been reported in colonies without any alterations in many countries (Ullah et al., 2021). When clinical disease manifests, signs of DWV infection primarily include deformed or vestigial wings and reduced lifespan of bees (Martin & Brettell, 2019). ABPV causes tremors, inability to fly, crawling on the ground, and subsequent death (de Miranda et al., 2010). BQCV leads to the darkening of cells, larvae, and queen pupae, which become brown or blackened and die (Amiri et al., 2017).

In Brazil, the presence of DWV, ABPV, and BQCV has been detected in *A. mellifera* (Message et al., 1996; Teixeira et al., 2008; Peixoto et al., 2021) as well as in other species

(Ueira-Vieira et al., 2015; Souza et al., 2019; Guimarães-Cestaro et al., 2020; Teixeira et al., 2020). However, the volume of information regarding their distribution, prevalence, and effects on bee populations is still extremely scarce. Therefore, this study aimed to verify the presence of DWV, ABPV, and BQCV in *A. mellifera* colonies in an apiary located in Jaguariúna, SP, Brazil (-22.730222, -47.042498) to contribute to the understanding of the epidemiology of these viral agents in Brazil.

Sampling was conducted as part of an experiment aiming to control the parasite *Varroa* sp using biological agents (unpublished data). Sampling 1 (March 11th, 2022) was performed before the first treatment, and subsequent samplings (April 1st and 25th, 2022) were conducted after treatment. The *Varroa* sp infestation was not controlled by the tested biological agents, leading to the discontinuation of the experiment. No negative effects of the tested product on the bees were observed.

Adult honeybees, apparently healthy, were collected from 11 colonies. Samples from each colony were individually stored in Falcon tubes with 70% ethanol and refrigerated until processed at the Laboratory of Entomology and Phytopathology (LEF) at Embrapa Meio Ambiente in Jaguariúna, SP.

For total RNA extraction, the abdomens of 30 honeybees from each sampled colony were removed and macerated. RNA was extracted using the Total RNA Purification Kit (Norgen Biotek Corp.), following the manufacturer's instructions. The extracted RNA was evaluated using the NanoDrop2000 spectrophotometer (Thermo Scientific), and cDNA synthesis was performed using the GoScript Reverse Transcription Mix with Random Primers (Promega), according to the manufacturer's instructions. The presence of DNA and absence of inhibitors in the cDNA samples were confirmed through a conventional PCR targeting the endogenous rp49 gene using the GoTaq Hot Start Green G2 Master Mix (Promega), the primers described by Ueno et al. (2009), and the following reaction conditions: 95 °C for 5 minutes; 95 °C for 30 seconds, 60 °C for 30 seconds, and 72 °C for 30 seconds (35 cycles); and 72 °C for 7 minutes.

The cDNA samples were subjected to multiplex PCR following the protocol by Teixeira et al. (2008), using the GoTaq Hot Start Green G2 Master Mix (Promega). Primer pairs for DWV and ABPV were designed by the authors above, along with the multiplex reaction conditions, resulting in amplicons of 129 bp and 500 bp, respectively. For BQCV, the primers described by Benjeddou et al. (2001) were used, generating amplicons of 700 bp. Positive controls consisted of mini-genes amplified with the primers mentioned above, inserted into pGEM-T Easy Vector plasmids (Promega), and replicated in *E. coli* DH5α bacteria. The mini-genes were synthesized based on the positive sequences described by Teixeira et al. (2008) – EU292210, EU292211, and EU292212 for ABPV, BQCV, and DWV, respectively – but with an inverted internal nucleotide sequence, allowing differentiation between synthetic controls and newly obtained sequences through Sanger sequencing. PCR products were subjected to horizontal electrophoresis on a 1.5% agarose gel supplemented with 1 µL of SYBR Safe DNA Gel Stain (Thermo Fisher Scientific) per 10 mL of gel, using TBE 1X pH 8.0 running buffer (44.58 M Tris-base; 0.44 M boric acid; 12.49 mM EDTA) at 100 V/50 mA. Gel was visualized under ultraviolet (UV) light using an ImageQuant LAS500 photodocumentor (GE).

Positive samples were sent to the Institute of Biotechnology (IBTEC) at UNESP in Botucatu, SP, for purification using magnetic beads following the protocol by Jolivet & Foley (2015). Purified samples were quantified using the NanoDrop2000 spectrophotometer (Thermo Scientific). Sanger sequencing of the samples was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific), according to the manufacturer's

Table 1. Results obtained in the investigation of DWV, ABPV, and BQCV in 11 colonies from an apiary in Jaguariúna-SP, through multiplex PCR.

Colony	Sampling 1	Sampling 2	Sampling 3
2	(-)	nc	(-)
3	ABPV730 ON661266*	ABPV	(-)
4	(-)	nc	(-)
5	(-)	(-)	(-)
7	(-)	ABPV740 OP113959*	ABPV
8	ABPV734	ABPV	(-)
10	ABPV735 OP086041*	ABPV	(-)
11	ABPV736	ABPV743 OP125542*	(-)
12	BQCV737 OP561792*	(-)	(-)
13	nc	ABPV745 OP561791*	(-)
15	nc	(-)	(-)

nc = not collected; (-) = negative sample; ABPV = positive samples not sequenced; ABPV734 and ABPV736 = positive samples sequenced but not deposited in Genbank; * = respective accession numbers in Genbank.

instructions. Sequencing was conducted on an ABI 3500 DNA sequencer (Applied Biosystems). The construction of consensus sequences, alignment with similar sequences, and the generation of identity tables and phylogenetic trees were performed using Geneious Prime 2019.1.3 software (https://www.geneious.com). Similar sequences were identified using the NCBI Web BLAST (Johnson et al., 2008) database.

Out of the 11 studied colonies, 6 (54.5%) tested positive for ABPV in at least one sampling, and 1 (9%) tested positive for BQCV. Results are shown in Table 1 and Fig 1. The percentage identity among ABPV sequences ranged from 96.5% to 100%, as ABPV730 and ABPV734 were identical, as well as ABPV735 and ABPV736. The seven ABPV sequences were aligned with 14 additional sequences obtained from *A. mellifera* in Brazil and other countries, showing percentage identities ranging from 82.1% to 97.1%. BQCV737 sequence was aligned with 23 other sequences from *A. mellifera*, resulting in identities ranging from 94.7% to 99.7%. Complete percentage identity tables for ABPV and BQCV can be found in Table 2 and 3. Fig 2 and 3 show the phylogenetic trees for ABPV and BQCV sequences.

The first report of honeybee virus occurrence in Brazil was conducted by Message et al. (1996) using the double immunodiffusion serological method. The presence of "APV" (Acute Paralysis Virus, also known as ABPV) and BQCV, among other viruses, was detected in samples of adult bees associated with bee mortality. Subsequently, the first molecular genetic evidence of the presence of viruses in samples of Brazilian honeybees was described by Teixeira et al. (2008). This analysis was part of a study aimed at determining the causes of the decline of the *A. mellifera* colonies in the southeastern region of Brazil at that time. By developing and standardizing a multiplex RT-PCR, the one used in the present study, the authors found that 27.1%, 37%, and 20.3% of colony samples tested positive for ABPV, BQCV, and DWV, respectively, in bees from 10 apiaries in Altinópolis, SP. Co-infections with two or three viruses were also observed. Peixoto et al. (2021) detected virus positivity in samples of apparently healthy *A. mellifera* from 27 cities in 15 Brazilian states, with ABPV, BQCV, and DWV showing positivity rates of 76.2%, 17.5%, and 41.3%, respectively, along with co-infections. ABPV was the most prevalent virus, observed in all 15 states sampled, while BQCV had the lowest occurrence, detected in seven out of 15 states. DWV was reported in 11 states. In the present study, the higher occurrence of ABPV, about 54.5% of positive colonies, corroborates the results of Message et al. (1996) and Peixoto et al. (2021). As in the case of the latter, the sampled colonies were also apparently healthy. However, unlike previous research where colonies were sampled only once, the same colonies were sampled at two (colonies 2, 4, 13, and 15) or three (colonies 3, 5, 7, 8, 10, 11, and 12) different time points over 45 days. The different design allowed observation that five colonies were consistently negative for ABPV, while the other six colonies showed variable results: colony 12 was positive only in the first sampling, colony 13 only in the second sampling, colonies 3, 8, 10, and 11 were positive in both samplings and colony 7 was positive only in the third sampling, being the only positive colony in that sampling. These results may reflect variations in infection levels, which became undetectable by the methodology used, probably due to various factors such as environmental and nutritional changes. The absence of DWV detection differs from previous studies and may be attributed to the minimal infestation of *Varroa* sp in the sampled apiary (unpublished data). Cases of co-infection, as reported in the literature, were not observed.

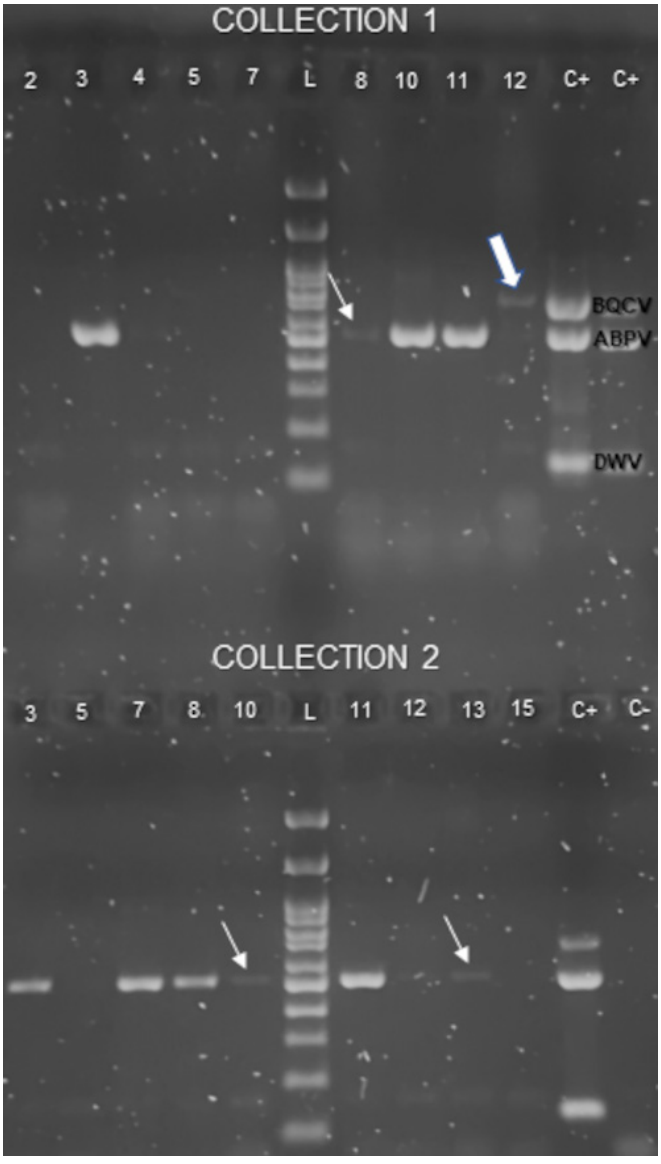

Fig 1. Agarose gel (1.5%) electrophoresis, stained with SYBR Safe DNA Gel Stain, and visualized under ultraviolet (UV) light using a photodocumentation system, showing the results obtained in the multiplex PCR for the samples from colonies studied in samplings 1 and 2. L = 100 bp DNA ladder (Neobio); C+ = positive control sample formed by a pool with the 3 mini-genes of each amplified target (DWV with 129 bp, ABPV with 500 bp, and BQCV with 700 bp); C- = negative control; narrow arrows = weak positive result for ABPV; broad arrow = positive result for BQCV.

Table 2. Percentage identity matrix between seven ABPV sequences obtained in this study and 14 other sequences from Brazil and other countries, conducted using Geneious Prime® 2022.2.2.

	ABPV730 ON661266	ABPV734	ABPV735 OP086041	ABPV736	ABPV740 OP113959	ABPV743 OP125542	ABPV745 OP561791	Brazil EU292210	Brazil MN809961	Uruguay AY763414	Chile KF011920	England AF150629	France AY230512	Germany AY053367	Poland AF486073	Poland AY053371	Hungary AY053372	Hungary AY053374	Hungary AF486072	Morocco MT863271	China MZ821781
ABPV730 ON661266		100.0	96.9	96.9	97.6	99.6	99.1	96.9	95.8	96.2	94.9	84.5	83.9	83.7	84.3	84.8	84.8	84.8	84.8	84.5	84.5
ABPV734	100.0		96.9	96.9	97.6	99.6	99.1	96.9	95.8	96.2	94.9	84.5	83.9	83.7	84.3	84.8	84.8	84.8	84.8	84.5	84.5
ABPV735 OP086041	96.9	96.9		100.0	98.0	96.9	96.5	96.5	95.4	95.8	94.9	83.2	82.1	82.8	83.0	83.0	82.6	82.6	82.6	82.3	83.7
ABPV736	96.9	96.9	100.0		98.0	96.9	96.5	96.5	95.4	95.8	94.9	83.2	82.1	82.8	83.0	83.0	82.6	82.6	82.6	82.3	83.7
ABPV740 OP113959	97.6	97.6	98.0	98.0		97.6	97.1	97.1	95.6	96.5	95.6	84.3	83.7	83.0	83.2	84.5	84.1	84.1	84.1	83.9	84.8
ABPV743 OP125542	99.6	99.6	96.9	96.9	97.6		98.7	96.5	95.8	95.8	94.5	84.5	83.9	83.7	84.3	84.8	84.8	84.8	84.8	84.5	84.5
ABPV745 OP561791	99.1	99.1	96.5	96.5	97.1	98.7		96.0	95.8	96.2	94.9	83.7	83.0	82.8	83.4	83.9	84.8	84.8	84.8	84.5	83.7
Brazil EU292210	96.9	96.9	96.5	96.5	97.1	96.5	96.0		96.2	97.1	97.1	84.5	83.9	83.2	83.9	84.3	83.9	83.9	83.9	83.7	84.5
Brazil MN809961	95.8	95.8	95.4	95.4	95.6	95.8	95.8	96.2		96.0	94.7	82.3	81.7	81.5	82.8	82.6	83.0	83.0	83.0	82.8	82.8
Uruguay AY763414	96.2	96.2	95.8	95.8	96.5	95.8	96.2	97.1	96.0		97.8	83.9	83.2	83.0	84.1	83.7	84.3	84.3	84.3	84.1	83.9
Chile KF011920	94.9	94.9	94.9	94.9	95.6	94.5	94.9	97.1	94.7	97.8		83.9	83.4	83.0	83.7	83.7	84.1	84.1	84.1	83.9	84.1
England AF150629	84.5	84.5	83.2	83.2	84.3	84.5	83.7	84.5	82.3	83.9	83.9		96.7	94.9	94.7	95.6	94.3	94.5	94.3	94.0	94.7
France AY230512	83.9	83.9	82.1	82.1	83.7	83.9	83.0	83.9	81.7	83.2	83.4	96.7		94.3	93.2	95.4	94.5	94.7	94.9	94.7	94.9
Germany AY053367	83.7	83.7	82.8	82.8	83.0	83.7	82.8	83.2	81.5	83.0	83.0	94.9	94.3		98.5	95.8	94.5	94.7	94.9	94.7	94.9
Poland AF486073	84.3	84.3	83.0	83.0	83.2	84.3	83.4	83.9	82.8	84.1	83.7	94.7	93.2	98.5		95.4	94.3	94.5	94.3	94.0	94.3
Poland AY053371	84.8	84.8	83.0	83.0	84.5	84.8	83.9	84.3	82.6	83.7	83.7	95.6	95.4	95.8	95.4		97.8	98.0	97.8	97.6	97.4
Hungary AY053372	84.8	84.8	82.6	82.6	84.1	84.8	84.8	83.9	83.0	84.3	84.1	94.3	94.5	94.5	94.3	97.8		99.3	99.1	98.9	96.5
Hungary AY053374	84.8	84.8	82.6	82.6	84.1	84.8	84.8	83.9	83.0	84.3	84.1	94.5	94.7	94.7	94.5	98.0	99.3		99.3	99.1	96.7
Hungary AF486072	84.8	84.8	82.6	82.6	84.1	84.8	84.8	83.9	83.0	84.3	84.1	94.3	94.9	94.9	94.3	97.8	99.1	99.3		99.8	96.5
Morocco MT863271	84.5	84.5	82.3	82.3	83.9	84.5	84.5	83.7	82.8	84.1	83.9	94.0	94.7	94.7	94.0	97.6	98.9	99.1	99.8		96.2
China MZ821781	84.5	84.5	83.7	83.7	84.8	84.5	83.7	84.5	82.8	83.9	84.1	94.7	94.9	94.9	94.3	97.4	96.5	96.7	96.5	96.2	

Message et al. (1996) reported some serological differences between the "APV" from Brazil and the same virus isolated from samples in England through identity and titration tests. Teixeira et al. (2008) found that the ABPV sequence (EU292210) was substantially closer to the one found in Uruguay than to those from North America or Europe. The phylogenetic tree of ABPV (Fig 2) clearly shows the separation between South American and European sequences, with the Chinese and Moroccan sequences also falling into the European group, while the first Brazilian sequence obtained (Teixeira et al., 2008) is closely related to the Uruguayan and Chilean sequences. The ABPV sequences obtained from the apiary of Jaguariúna, SP, in the present study, are placed within the branch of South American sequences but form a separate group, indicating greater genetic similarity among themselves, with percentage identities ranging from 96.5% to 100% (Table 2). ABPV730 and ABPV734 are identical sequences, despite coming from different colonies, as well as ABPV735 and ABPV736.

On the other hand, ABPV736 and ABPV743 are different sequences obtained from a single colony, indicating that different viral types can co-infect a population at a given time. This higher variability observed for ABPV, whether due to the higher prevalence of the virus in colonies or some possible peculiarities of the viral RNA polymerase, provides opportunities for future studies on ABPV lineages and variants. The BQCV737 sequence shows higher identity with the one obtained by Teixeira et al. (2008), 99.7% (Table 3), and the percentage identities among all aligned BQCV sequences are higher than those observed for ABPV, with no geographical separation observed in the phylogenetic tree (Fig 3).

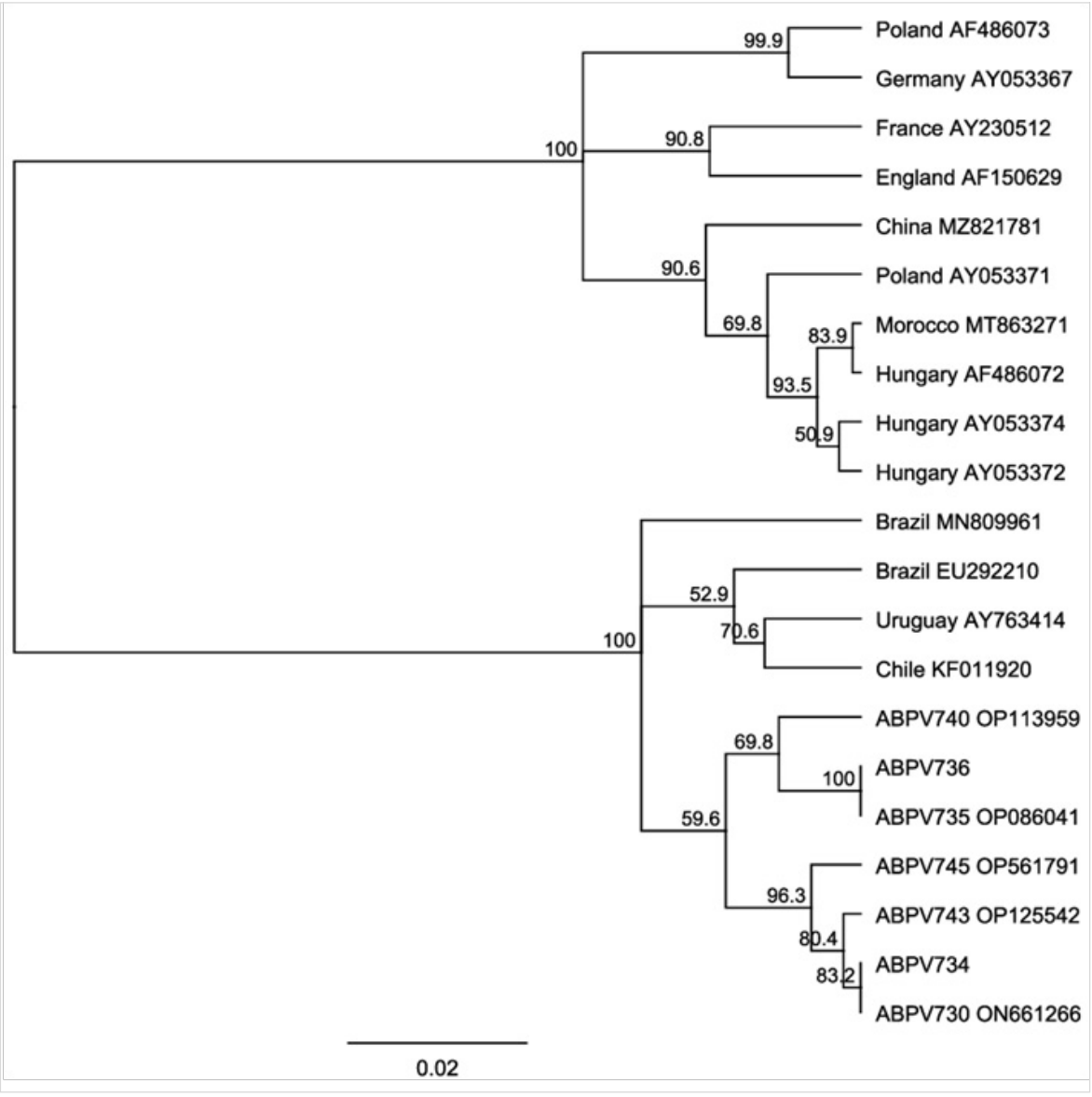

Fig 2. Phylogenetic tree of seven ABPV sequences obtained in this study, along with 14 sequences from Brazil and other countries (ABPV734 and ABPV736 were sequenced but not deposited in Genbank). Sequences belong to the capsid protein gene, and the analysis was performed using Geneious Prime® 2022.2.2, employing the Unweighted Pair Group Method using Arithmetic averages (UPGMA) distance method.

Table 3. Percentage identity matrix between the BQCV sequence obtained in this study and 23 sequences from Brazil and other countries, conducted using Geneious Prime® 2022.2.2.

	BQCV737 OP561792	Brazil EU292211	Uruguay DQ364629	USA HQ655487	USA HQ655458	USA AY626246	UK GU903462	Italy MT416539	Slovenia MH899954	Czech Rep. OL803818	Czech Rep. KY243932	Lithuania KP223794	South Africa AF183905	Turkey KX273080	Thailand KP730019	Thailand KP730018	South Korea EF639830	China MZ821813	China MZ821807	China MZ821805	P. N. Guinea MT482476	P. N. Guinea MT482475	Australia KY465684	Australia MF004373
BQCV737 OP561792		99.7	94.7	96.1	96.3	96.0	98.1	96.0	96.6	96.3	96.1	96.3	96.1	96.1	95.8	96.0	95.8	95.8	96.0	95.7	95.7	96.3	95.8	96.8
Brazil EU292211	99.7		95.0	96.3	96.5	96.1	98.4	96.1	96.8	96.5	96.3	96.5	96.3	96.5	96.0	96.1	96.0	96.0	96.1	95.8	95.8	96.5	96.0	97.1
Uruguay DQ364629	94.7	95.0		97.1	96.9	96.9	94.9	95.0	95.3	94.7	96.9	95.2	96.1	95.2	96.9	96.8	96.9	96.9	97.1	96.8	96.0	97.1	96.9	94.9
USA HQ655487	96.1	96.3	97.1		99.2	98.7	96.1	96.8	97.9	97.3	99.0	96.9	98.1	96.0	99.0	98.9	99.0	99.0	99.0	99.0	98.1	99.2	99.0	96.6
USA HQ655458	96.3	96.5	96.9	99.2		99.0	96.0	96.6	97.7	97.1	98.9	97.1	97.9	96.1	98.9	98.7	98.9	98.9	98.9	98.6	97.9	99.0	98.9	96.1
USA AY626246	96.0	96.1	96.9	98.7	99.0		95.8	96.5	97.3	96.6	98.7	96.8	97.3	95.5	99.0	98.9	99.0	98.7	99.0	98.7	97.7	98.9	98.7	95.7
UK GU903462	98.1	98.4	94.9	96.1	96.0	95.8		96.0	96.9	96.5	96.1	96.3	95.7	95.7	96.1	96.0	96.1	95.8	96.3	96.0	96.0	96.6	96.1	97.6
Italy MT416539	96.0	96.1	95.0	96.8	96.6	96.5	96.0		97.9	97.4	96.5	96.6	96.0	95.8	96.1	96.3	96.1	96.1	96.1	96.1	96.0	96.6	96.1	95.7
Slovenia MH899954	96.6	96.8	95.3	97.9	97.7	97.3	96.9	97.9		99.4	97.6	96.6	96.9	95.5	97.3	97.1	97.3	97.3	97.3	97.3	97.1	97.7	97.3	97.1
Czech Rep. OL803818	96.3	96.5	94.7	97.3	97.1	96.6	96.5	97.4	99.4		96.9	96.1	96.9	95.0	96.6	96.5	96.6	96.9	96.6	96.6	96.5	97.1	96.6	96.8
Czech Rep. KY243932	96.1	96.3	96.9	99.0	98.9	98.7	96.1	96.5	97.6	96.9		96.9	97.4	95.7	99.0	98.9	99.0	99.4	99.0	98.7	98.4	99.2	99.7	96.6
Lithuania KP223794	96.3	96.5	95.2	96.9	97.1	96.8	96.3	96.6	96.6	96.1	96.9		96.1	96.8	96.9	96.8	96.9	96.6	96.9	96.6	96.9	97.4	96.9	95.3
South Africa AF183905	96.1	96.3	96.1	98.1	97.9	97.3	95.7	96.0	96.9	96.9	97.4	96.1		96.1	97.4	97.3	97.4	97.7	97.6	97.6	96.8	97.6	97.4	96.6
Turkey KX273080	96.1	96.5	95.2	96.0	96.1	95.5	95.7	95.8	95.5	95.0	95.7	96.8	96.1		96.0	96.1	96.0	95.7	96.1	96.0	95.5	96.5	96.0	95.0
Thailand KP730019	95.8	96.0	96.9	99.0	98.9	99.0	96.1	96.1	97.3	96.6	99.0	96.9	97.4	96.0		99.5	99.7	99.0	99.7	99.4	98.4	99.5	99.4	96.0
Thailand KP730018	96.0	96.1	96.8	98.9	98.7	98.9	96.0	96.3	97.1	96.5	98.9	96.8	97.3	96.1	99.5		99.5	98.9	99.5	99.2	98.2	99.4	99.2	95.8
South Korea EF639830	95.8	96.0	96.9	99.0	98.9	99.0	96.1	96.1	97.3	96.6	99.0	96.9	97.4	96.0	99.7	99.5		99.0	99.7	99.4	98.4	99.5	99.4	96.0
China MZ821813	95.8	96.0	96.9	99.0	98.9	98.7	95.8	96.1	97.3	96.9	99.4	96.6	97.7	95.7	99.0	98.9	99.0		99.0	98.7	98.1	99.2	99.4	96.6
China MZ821807	96.0	96.1	97.1	99.0	98.9	99.0	96.3	96.1	97.3	96.6	99.0	96.9	97.6	96.1	99.7	99.5	99.7	99.0		99.7	98.4	99.5	99.4	96.1
China MZ821805	95.7	95.8	96.8	99.0	98.6	98.7	96.0	96.1	97.3	96.6	98.7	96.6	97.6	96.0	99.4	99.2	99.4	98.7	99.7		98.1	99.2	99.0	96.1
P.N. Guinea MT482476	95.7	95.8	96.0	98.1	97.9	97.7	96.0	96.0	97.1	96.5	98.4	96.9	96.8	95.5	98.4	98.2	98.4	98.1	98.4	98.1		98.9	98.7	95.5
P.N. Guinea MT482475	96.3	96.5	97.1	99.2	99.0	98.9	96.6	96.6	97.7	97.1	99.2	97.4	97.6	96.5	99.5	99.4	99.5	99.2	99.5	99.2	98.9		99.5	96.1
Australia KY465684	95.8	96.0	96.9	99.0	98.9	98.7	96.1	96.1	97.3	96.6	99.7	96.9	97.4	96.0	99.4	99.2	99.4	99.4	99.4	99.0	98.7	99.5		96.3
Australia MF004373	96.8	97.1	94.9	96.6	96.1	95.7	97.6	95.7	97.1	96.8	96.6	95.3	96.6	95.0	96.0	95.8	96.0	96.6	96.1	96.1	95.5	96.1	96.3	

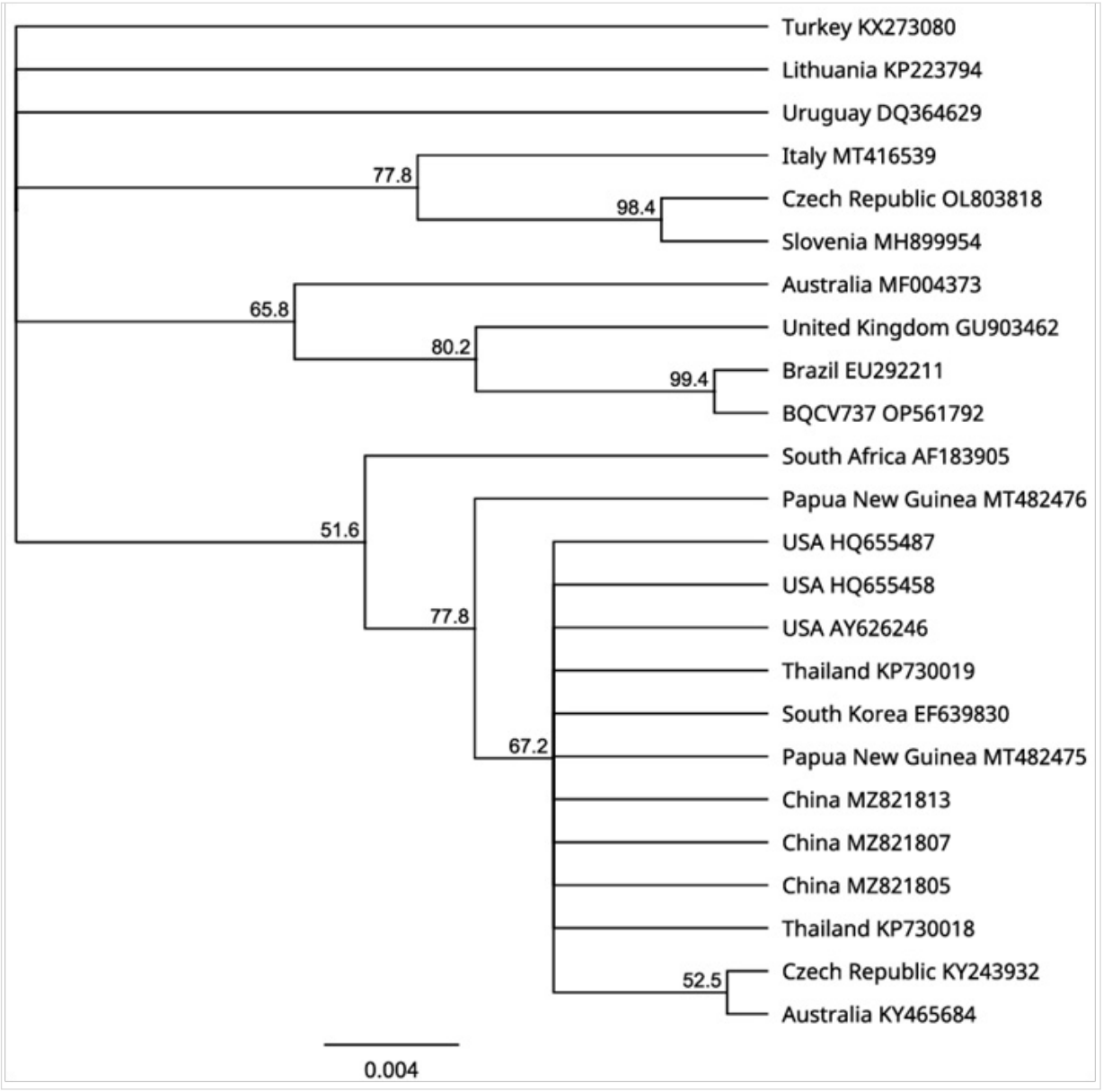

Fig 3. Phylogenetic tree of the BQCV sequence obtained in this study, along with 23 sequences from Brazil and other countries. Sequences belong to the capsid protein gene, and the analysis was performed using Geneious Prime® 2022.2.2, employing the Unweighted Pair Group Method using Arithmetic averages (UPGMA) distance method.

Determining the prevalence of viral agents in bee populations is crucial to distinguish endemic viruses that do not cause clinical manifestations from those potentially responsible for observable diseases. In conclusion, ABPV and BQCV are present in this apiary in Jaguariúna, SP, and detectable virus levels vary over time. Additionally, honeybees from positive colonies do not show evidence of disease despite the infection. Sequences obtained here and deposited in an international database represent a valuable contribution to the limited knowledge regarding viruses that infect honeybees in Brazil.

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Authors' Contributions

MFN: conceptualization, methodology, formal analysis, investigation, writing-original draft.
CDM: conceptualization, methodology, formal analysis, writing-original draft.
MGBF: investigation, resources.
DP: investigation, resources.
SSP: writing-original draft.
JPAJ: conceptualization, methodology, funding acquisition.
CM: resources, supervision, project administration.

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