

# Prevalence and phylogenetic analysis of honey bee viruses in the Biobío Region of Chile and their association with other honey bee pathogens

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Different episodes of mortalities of honey bee (*Apis mellifera* L.) colonies have been associated with the presence of honey bee pathogens. Since the Biobío Region has among the highest number of apiaries in Chile, the aim of the present study was to identify viruses in the Region affecting honey bees, evaluate their relation to other pathogens, and conduct a phylogenetic analysis. Pupae and adult bees were collected from 60 apiaries of *Apis mellifera* L. in the Biobío Region over 2 yr. RNA viruses were detected by reverse transcription PCR (RT-PCR), and *Acarapis woodi*, *Nosema* spp., and *Varroa destructor* via PCR. Three viruses were detected: *Acute bee paralysis virus* (ABPV), *Black queen cell virus* (BQCV) and *Deformed wing virus* (DWV) in 2%, 10%, and 42% of the apiaries, respectively. No statistical correlation was observed between the presence of the different viruses, *V. destructor*, *A. woodi*, and the two *Nosema* species, and the bee development stages. One year after the first sampling, DWV and BQCV were detected mainly in foraging adult bee samples. Three percent of the apiaries were infected with *N. apis* and 18% with *N. ceranae*, 5% were positive for *V. destructor*, while *A. woodi* was not detected. PCR products were sequenced and compared to the Genbank database. Chilean sequences of ABPV, BQCV, and DWV showed high percentages of similarity to other isolates in South America.

**Key words:** *Acute bee paralysis virus*, *Apis mellifera*, *Black queen cell virus*, *Deformed wing virus*, *Nosema ceranae*.

## INTRODUCTION

Pollinators are a key component of global biodiversity, providing vital ecosystem services to crops and wild plants (Potts et al., 2010). Klein et al. (2007) found that fruit, vegetable and seed production from 87 of the leading global food crops are dependent on pollinators. The honey bee (*Apis mellifera* L.) is the most important natural pollinator for a wide variety of crops and flowering plants (Potts et al., 2010).

There are around 500 000 commercial hives in Chile, which are distributed from the Antofagasta Region to the Aysén del General Carlos Ibáñez del Campo Region (Laval et al., 2010). Considering just the pollination requirements from October to November, the demand in Central Chile

ranges from 758 312 to 947 890 colonies (average of 8-10 colonies ha<sup>-1</sup>), which is twice the number of existing ones, given that the total area requiring pollinators is close to 177 000 ha (Estay, 2012). Moreover, in 2007 the demand for hives for pollination was 1 202 694 for almond, cherry, European plum, Japanese plum, apple, pear, avocado, kiwi, blueberry, and raspberry production (de la Cuadra, 2010).

However, colonies of *A. mellifera* are suffering significant losses worldwide. Between 1961 and 2007, honey bee populations in Europe and North America declined by 26% and 49%, respectively (vanEngelsdorp and Meixner, 2010). Different episodes of mortality and reductions in honey production associated with viral infections have been reported worldwide (Chen et al., 2005; Chen and Siede, 2007; Bacandritsos et al., 2010; Gumusova et al., 2010; Genersch and Aubert, 2010; Ai et al., 2012). In effect, there is increasing concern about the effect of viral infections on honey bee populations, largely due to the potential relation between viral diseases and honey bee mortality (Cox Foster et al., 2007; Maori et al., 2009; Hunter et al., 2010).

Although there is no official information about the mortality of colonies in Chile, beekeepers have observed an increase in the percentage of winter losses in recent years. The symptoms and causes are not clear. Allen and Ball (1996) reported that at least 18 viruses infect *A.*

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*mellifera*, which can be present in all bee development stages: egg, larva, pupae, and adult (Chen et al., 2006). Among these, seven viruses are the most common: *Black queen cell virus* (BQCV); *Deformed wing virus* (DWV); *Kashmir bee virus* (KBV); *Sacbrood bee virus* (SBV); *Acute bee paralysis virus* (ABPV); *Chronic bee paralysis virus* (CBPV) and *Israeli acute paralysis virus* (IAPV) (Tentcheva et al., 2004; Yooa et al., 2012) and their complete genome sequences have been reported (Ghosh et al., 1999; Govan et al., 2000; Leat et al., 2000; Chen et al., 2004a; Fujiyuki et al., 2004; Maori et al., 2007).

In South America the presence of honey bee viruses (ABPV, CBPV, BQCV, SBV, and DWV) has been reported in Argentina, Uruguay, and Brazil (Antúnez et al., 2005; 2006; Teixeira et al., 2008; Reynaldi et al., 2010). Rodríguez et al. (2012) made the first report of the presence in Chile of BQCV, SBV, and DWV in apiaries at Curepto, Maule Region. The most prevalent virus in colonies with high levels of mortality was BQCV. According to field observations, the affected hives presented large numbers of dead bees at the hive entrances. As well shiny black alopecic bees were observed dragging themselves on the ground, and queen bees showing similar symptoms and then disappearing from the colonies. In addition, Barriga et al. (2012) detected DWV in two places in the Chilean Metropolitan Region.

Since the Biobío Region has among the highest number of apiaries in the country, a number of samples were collected from colonies from different locations in this region in order to identify viruses affecting the honey bee *A. mellifera*, and evaluate the presence of other pathogens such as: mites *Varroa destructor* and *Acarapis woodi*, and the microsporidia *Nosema apis* and *Nosema ceranae*.

## MATERIALS AND METHODS

### Samplings from apiaries

This study was conducted during the springs and summers of 2010 and 2011. The first part (2010) was carried out in 20 districts (36°46'22" S, 73°3'47" W) of the Biobío Region, in longitudinal strips in the Andean foothills, the coastal area, and the central valley. Three apiaries were randomly selected in each of the 20 district for sampling, making a total of 60 apiary samplings. Three colonies were sampled from each apiary, from which 30 pupae

were collected from the honeycombs and 30 foraging bees from the hive entrance (Yue and Genersch, 2005). The pupae or foraging bees from the same apiary formed part of a composite sample that was stored at -80 °C until analyzed.

A second sampling was carried out in 2011 only in apiaries where BQCV had been detected from the previous sampling. Larvae, pupae, nurse bees, foragers, young queen larvae (when present), and *V. destructor* mites were collected from three hives from each apiary. Samples were analyzed for the presence of the viruses *N. apis*, *N. ceranae*, *A. woodi*, and *V. destructor*.

### RNA and DNA extraction

Ten bees or 20 mites from each apiary sample were placed in a sterile porcelain mortar and ground in liquid nitrogen. RNA was extracted from 0.171 mg tissue using the SV Total RNA Isolation System Kit (Promega, Madison, Wisconsin, USA), following the manufacturer's protocol. Total RNA was used to detect viruses.

The remaining tissue (ca. 20 mg) was processed for DNA extraction using the Wizard® SV Genomic DNA Purific (Promega, USA). The DNA was used to detect *N. apis*, *N. ceranae*, *A. woodi*, and *V. destructor* via polymerase chain reaction (PCR).

### RT-PCR to detect viruses

The RNA samples were tested for the presence of four viruses: BQCV, DWV, SBV, and ABPV, through reverse transcription PCR (RT-PCR) using the primers listed in Table 1.

Reverse transcription of the samples was conducted with M-MLV Reverse Transcriptase (Invitrogen, Foster City, California, USA) following the manufacturer's protocol. The amplifications were performed in a thermal cycler (BIORAD, My Gene TM Series Peltier Thermal Cycler, San Francisco, CA, USA). Obtained cDNA was then used for PCR. The reaction mix contained 0.2 mM of dNTPs, 1 μM of each primer, 1x buffer, 1.5 mM MgCl<sub>2</sub>, 1 U of Taq polymerase (TM Invitrogen, USA) and 1 μL of cDNA (undiluted), to a total final volume of 30 μL. For ABPV, BQCV, and SBV, denaturation and activation of the polymerase were performed at 95 °C for 2 min, followed by 40 PCR cycles: 30 s at 95 °C, 1 min at 55 °C, and 2 min at 68 °C. Reactions were completed by a final elongation step at 68 °C for 7 min.

**Table 1. Primers used for the detection of different bee viruses.**

Pathogen	Primers (5'→3')	Target sequence	Size Pb	Source
ABPV	F TTATGTGTCCAGAGACTGTATCCA	Capsid protein gene	900	Benjeddou et al., 2001
	R GCTCCTATGCTCGGTTTTTCGGT			
BQCV	F TGGTCAGCTCCCACTACCTTAAAC	Nonstructural polyprotein (orf1) structural polyprotein (orf2) gene	700	Benjeddou et al., 2001
	R GCAACAAGAAGAAACGTAACCCAG			
DWV	F TTTGCAAGATGCTGTAIGTGG	Polyprotein gene	395	Tentcheva et al., 2004
	R GTCGTGCAGCTCGATAGGAT			
SBV	F GCTGAGGTAGGATCTTTGCGT	Helicase, protease and polymerase gene	824	Chen et al., 2004a; 2004b
	R TCATCATCTTACCATCCGA			

ABPV: *Acute bee paralysis virus*; BQCV: *Black queen cell virus*; DWV: *Deformed wing virus*; SBV: *Sacbrood bee virus*.  
F: Forward; R: Reverse.

For DWV, denaturation and activation of the polymerase was performed at 95 °C for 2 min followed by 30 PCR cycles: 30 s at 95 °C, 1 min at 52 °C, and 1 min at 72 °C; and a final elongation step at 72 °C for 7 min.

Positive controls (cDNA) were provided by the Department of Microbiology of the Institute for Biological Research Clemente Estable, Montevideo, Uruguay.

#### **PCR to detect *Nosema* sp., *A. woodi* and *V. destructor***

The PCR reaction mix to detection *N. apis* and *N. ceranae* followed the protocol described by Martín-Hernández et al. (2007). A positive control was included with all PCRs, which consisted of DNA from *N. ceranae* and *N. apis* obtained from the Regional Apicultural Center, Marchamalo, Spain.

The PCR reactions for *A. woodi* were according to Evans et al. (2007). A positive control sample of DNA extracted from bees infested with *A. woodi* was provided by the Asociación Gremial de Apicultores Biobío Region (BIOMIELA.G.) and was included with all the PCRs. The positive control was analyzed according to the procedure of the World Organization for Animal Health (OIE, 2008). The PCR reaction to detect *V. destructor* was performed according to Evans and Lopez (2002). A positive control sample of DNA extracted from bees infested with varroa was included with all PCR reactions. The primers used to detect *A. woodi* and *V. destructor* were synthesized by Invitrogen according to Evans et al. (2007) and Evans and Lopez (2002), respectively.

#### **Analysis of amplified products**

The PCR and RT-PCR products were analyzed by electrophoresis in a 1% Tris-acetate-EDTA buffer agarose gel and stained with ethidium bromide. Amplified products were photographed in a UV light transilluminator (Vilber Lourmat Modelo TFX-20.M, EEC) and their length was determined with a 100-bp DNA ladder (Invitrogen, USA).

#### **Nucleotide sequencing and phylogenetic analysis**

ABPV, BQCV, and DWV amplicons were sequenced using the primer pairs described above (Table 1) by Macrogen (Seoul, Korea). The nucleotide sequences were identified using the Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA). Multiple nucleotide alignment was carried with the program BioEdit version 7.0.0 and using published ABPV, BQCV, and DWV sequences as references (Hall, 1999). A phylogenetic tree was constructed with Mega 5 software (Tamura et al., 2011) using the neighbor-joining (NJ) method with a bootstrap value of 1000 to assess robustness.

#### **Statistical analysis**

A statistical analysis of frequency for each virus was performed to evaluate correlations among the presence of different viruses, *V. destructor*, *A. woodi*, *N. apis*,

and *N. ceranae*, and different bee developmental stages. Each data set was summarized in contingency tables and analyzed using the Pearson chi square test.

## **RESULTS**

#### **Virus detection**

Of the 60 apiaries sampled in 2010, 2% was positive for ABPV, 10% for BQCV, and 42% for DWV. SBV was not detected (Table 2). Only 7% of the apiaries were co-infected by BQCV and DWV. There was no co-infection between ABPV and other viruses. DWV and BQCV were detected in both adult bees and pupae while ABPV was only detected in adult bees.

Three percent of tested apiaries were positive for *N. apis* and 18% for *N. ceranae*. Five of the positive samples for *N. ceranae* were co-infected with DWV (8% of the total), and one of these also presented BQCV (2% of the total). Only 5% of the apiaries were positive for *V. destructor*, two samples had co-infection with DWV. *Acarapis woodi* was not detected. No statistical correlation was found between the presence of the different viruses, *V. destructor*, *A. woodi*, and the two *Nosema* species and the bee developmental stage ( $p = 0.124$ ).

To verify the identity of detected amplicons, PCR products were sequenced and compared to the Genbank database. The sequences obtained shared a high percentage of similarity to published sequences for ABPV (98% sequence similarity to AY763414.1), BQCV (98% to EF517520.1), DWV (100% to HM067437.1), *N. apis* (100% to JQ639306.1), *N. ceranae* (100% to JQ639307.1), *V. destructor* (100% to AJ493124.2), and *A. woodi* (100% to GO916565.1). BQCV was detected in six apiaries in the first year (2010), but in the second year (2011) it was possible to sample only four of these apiaries, in three of which BQCV was found. It was detected mainly in foraging bees, although in one case it was also found in nurse bees and queen larvae. DWV was detected in all the samples and was present in different bee developmental stages. All the analyzed samples of *V. destructor* were infected by DWV.

*Nosema ceranae*-positive samples were found in two apiaries, in one of which microsporidia was detected in larvae, pupae, nurse bees, foragers, queen larvae, and *V. destructor*, while in the other apiary *N. ceranae* was only detected in foraging bees. *Nosema apis* was only detected in the samples from one apiary, in pupae and forager bee samples. Samples from this apiary were negative to the other pathogens. ABPV, SBV, and *A. woodi* were not detected in any of the sampled apiaries (Table 3).

#### **Nucleotide sequencing and phylogenetic analysis**

Phylogenetic analysis was carried out by sequencing fragments 905-bp of the gene coding for a capsid protein of ABPV (KF011920), 300-bp of a gene coding a structural protein of BQVC (KF011921), and 395-bp

**Table 2. Detection of ABPV, SBV, DWV, BQCV, *Nosema apis*, *N. ceranae*, *Acarapis woodi*, and *Varroa destructor* in apiaries in different locations in the Biobío Region, during the spring-summer of 2010.**

Geographic areas	Location	Geographic coordinates	Stage	ABPV	BQCV	DWV	SBV	<i>N. apis</i>	<i>N. ceranae</i>	<i>A. woodi</i>	<i>V. destructor</i>
Coastal area	Cobquecura	S 36°09'	Pupal	---	---	+++	---	---	-+-	---	---
		W 72°48'	Adult	---	---	+++	---	---	---	---	---
	Coelemu	S 36°25'	Pupal	---	---	-+-	---	---	---	---	---
		W 72°39'	Adult	---	---	---	---	---	---	---	---
	Tomé	S 36°36'	Pupal	---	---	---	---	---	---	---	---
		W 72°49'	Adult	---	---	---	---	-+-	---	---	---
	Penco	S 36°44'	Pupal	---	---	++-	---	---	++-	---	---
		W 72°57'	Adult	---	---	++-	---	---	++-	---	---
	Lebu	S 37°36'	Pupal	---	---	++-	---	---	---	---	---
		W 73°32'	Adult	---	-+-	-+-	---	---	-+-	---	---
Cañete	S 37°47'	Pupal	---	---	++-	---	---	-+-	---	---	
	W 73°23'	Adult	---	---	++-	---	---	-+-	---	---	
Contulmo	S 38°00'	Pupal	---	---	-+-	---	---	---	---	---	
	W 73°13'	Adult	---	---	---	---	-+-	-++	---	---	
Concepción	S 37°59'	Pupal	---	---	---	---	---	---	---	---	
	W 73°14'	Adult	---	---	---	---	---	---	---	---	
Central valley	San Carlos	S 36°26'	Pupal	---	---	---	---	---	---	---	---
		W 71°58'	Adult	---	---	---	---	---	---	---	---
	Bulnes	S 36°44'	Pupal	---	---	++-	---	---	---	---	-+-
		W 72°18'	Adult	---	---	-++	---	---	---	---	---
	Quillón	S 36°29'	Pupal	---	---	---	---	---	-+-	---	---
		W 72°17'	Adult	---	-+-	---	---	---	+++	---	---
	Coihueco	S 36°35'	Pupal	---	-+-	-+-	---	---	---	---	---
		W 72°05'	Adult	---	---	-+-	---	---	---	---	---
	Chillán Viejo	S 36°36'	Pupal	---	+-	-+-	---	---	---	---	---
		W 72°06'	Adult	---	---	---	---	---	---	---	---
Chillán	S 36°34'	Pupal	---	---	---	---	---	---	---	---	
	W 73°03'	Adult	---	---	---	---	---	---	---	---	
Andean range	San Fabián de Alico	S 36°35'	Pupal	---	---	-+-	---	---	---	---	---
		W 71°30'	Adult	---	---	---	---	---	---	---	---
	El Carmen	S 36°46'	Pupal	---	---	---	---	---	---	---	---
		W 71°30'	Adult	---	---	---	---	---	---	---	---
	Pinto	S 36°46'	Pupal	---	---	+++	---	---	---	---	-
		W 71°49'	Adult	-+-	+-	-+-	---	---	---	---	-+-
	Alto Bío Bío	S 37°39'	Pupal	---	---	---	---	---	---	---	---
		W 71°44'	Adult	---	---	-+-	---	---	---	---	---
	Quilaco	S 37°38'	Pupal	---	---	---	---	---	---	---	---
		W 71°43'	Adult	---	---	+++	---	---	---	---	---
Santa Bárbara	S 37°36'	Pupal	---	-+-	++-	---	---	---	---	---	
	W 71°47'	Adult	---	-+-	-+-	---	---	---	---	---	

+: Positive; -: Negative.

ABPV: *Acute bee paralysis virus*; BQCV: *Black queen cell virus*; DWV: *Deformed wing virus*; SBV: *Sacbrood bee virus*.

of a gene coding polyprotein of DWV (KC175443.1 and ID1606171), to describe the probable genetic relationship between Chilean viruses and those published in Genbank. In most cases, bootstrap support was high (> 70%), indicating that clusters were statistically valid.

The phylogenetic trees based on protein coding sequences are shown in Figures 1, 2, and 3, for ABPV,

BQCV, and DWV, respectively. The ABPV isolates originating from Hungary, Poland, Germany, and Austria are clustered in one branch and separated from the South Africa and US isolates, although they share the same origin. The sequences from Brazil, Uruguay, and Chile, clustered in a branch that diverged from the cluster of European, South Africa and US isolates (Figure 1). The

**Table 3. Detection of ABPV, SBV, DWV, BQCV, *Nosema apis*, *N. ceranae*, *Acarapis woodi* and *Varroa destructor*, in apiaries in different locations in the Biobío Region, in the spring of 2011.**

Geographic areas	Location	Geographic coordinates	ABPV	BQCV	DWV	SBV	<i>N. apis</i>	<i>N. ceranae</i>	<i>A. woodi</i>
Lebu	S 37°36' W 73°32'	Larvae	-	-	+	-	-	+	
		Pupae	-	-	-	-	-	+	
		Nurse bees	-	+	+	-	-	+	
		Forager bees	-	+	+	-	-	+	-
		Queen larvae	-	+	+	-	-	+	
		<i>V. destructor</i>	-	-	+	-	-	+	
Santa Bárbara	S 37°36' W 71°47'	Larvae	-	-	+	-	-	-	
		Pupae	-	-	+	-	+	-	
		Nurse bees	-	-	+	-	-	-	
		Forager bees	-	-	+	-	+	-	
		Queen larvae*	-	-	-	-	-	-	
		<i>V. destructor</i> *	-	-	-	-	-	-	-
Chillán Viejo	S 36°36' W 72°06'	Larvae	-	-	-	-	-	-	
		Pupae	-	-	-	-	-	-	
		Nurse bees	-	-	-	-	-	-	
		Forager bees	-	+	-	-	-	+	
		Queen larvae*	-	-	-	-	-	-	
		<i>V. destructor</i>	-	-	+	-	-	-	
El Carmen	S 36°46' W 71°30'	Larvae	-	-	-	-	-	-	
		Pupae	-	-	+	-	-	-	
		Nurse bees	-	-	+	-	-	-	
		Forager bees	-	+	+	-	-	-	
		Queen larvae*	-	-	-	-	-	-	
		<i>V. destructor</i>	-	-	+	-	-	-	

+: Positive; -: Negative; \*: not collected.

ABPV: Acute bee paralysis virus; BQCV: Black queen cell virus; DWV: Deformed wing virus; SBV: Sacbrood bee virus.

BQCV sequences from South Korea, the USA, and Uruguay are clustered in one branch, while sequences from Chile and Brazil form a distinct branch from other isolates showing a high divergence from the first group (Figure 2).

Chilean sequences of DWV are closely related to isolates from the UK, the USA, Spain, Japan, and Uruguay, forming a sole cluster, with at least two sub-clusters, one with three UK isolates and closely related isolates from the USA, Italy, and Spain. The other sub-cluster consists of Chilean, Uruguayan, and UK isolates, while isolates from Israel and the Netherlands form a distinct branch separated from other isolates.

## DISCUSSION

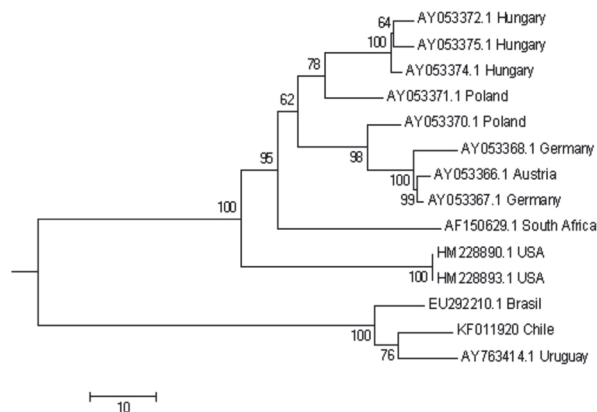
The Biobío Region has the largest number of beehives in Chile, with about 69 597 hives, which is 15% of the hives in the country (Laval et al., 2010). In the present work, we analyzed 180 hives located in 60 apiaries, equivalent to 0.26% of the hives in the region. The first sample was collected in spring-summer 2010 at different locations and geographic areas with distinct rainfall and thermal conditions. According to the beekeepers, the main problem is *V. destructor*, which in most cases is controlled in autumn using acaricide Amitraz (*N,N'*-[(methylimino)dimethylidene]di-2,4-xylylidine). In some cases, beekeepers reported the loss of part of the bee population, but no defined symptoms were detected.

From the collected samples three viruses were detected: DWV, BQCV, and ABPV. High numbers of

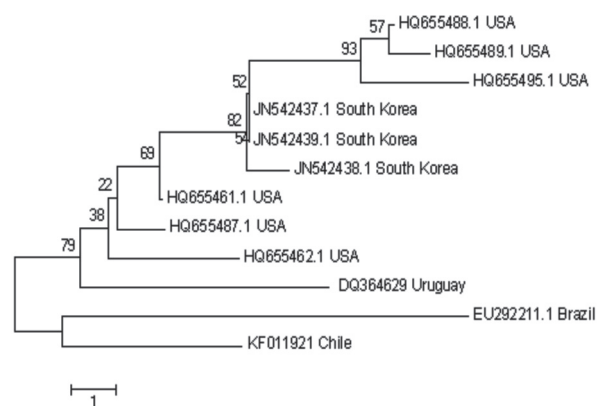
samples infected with DWV were detected (42% of apiaries), even when the number of positive samples to *V. destructor*, the main vector for this virus (de Miranda and Genersch, 2010), was low. The infestation value of *V. destructor* is probably underestimated, given that the analysis was performed on a small number of bees (30). As well, that spring was not the best season to perform the analysis, since colonies had been treated with acaricide in autumn. As well, obtained results could indicate that there are other transmission pathways of the viruses, such as horizontal transmission through contaminated food (pollen and honey) or vertical transmission from mother to brood (Chen et al., 2005; 2006).

The Chilean DWV sequence found in this study was highly similar to sequences from the USA, Spain, and Japan. Barriga et al. (2012) detected DWV in two sites in the Metropolitan Region of Chile and sequenced the complete genome of the virus. The authors indicated that the Chilean DWV strain is closely related to strains in England and Italy. The two studies agree that the virus has affected apiaries in particular sites and suggest expanding nationwide surveys. Phylogenetic studies by Berenyi et al. (2007) with DWV sequences of different geographic origins (Germany, Austria, Poland, Hungary, Slovenia, Nepal, Sri Lanka, the United Arab Emirates, Canada, and New Zealand) showed that certain regions of the DWV genome can be highly conserved, independent of the geographic origin of the bee sample, finding high genetic similarity and little genetic distance between them.

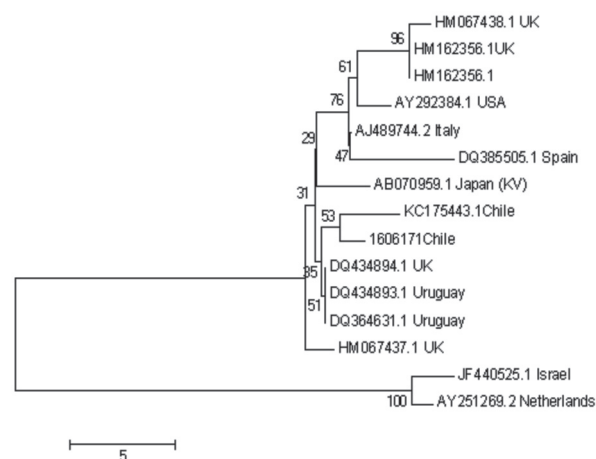
The most common and prevalent viruses affecting *A. mellifera* in the world are DWV and BQCV (Zhang et al., 2012). Earlier reports detected 82% of BQCV infection



**Figure 1.** Neighbor-joining phylogenetic tree based on a fragment of 905-bp of the gene that encodes for a structural protein of the *acute bee paralysis virus* (ABPV). Bootstrap values are shown above the branches.



**Figure 2.** Neighbor-joining phylogenetic tree based on a fragment of 300-bp that encodes for a non-structural polyprotein of the *Black queen cell virus* (BQCV). Bootstrap values are shown above the branches.



**Figure 3.** Neighbor-joining phylogenetic tree based on a fragment of 395-bp of the gene that encodes for a polyprotein of *Deformed wing virus* (DWV). Bootstrap values are shown above the branches.

in colonies from apiaries with high bee mortality rates at Curepto, Maule Region, Chile, located approximately 300 km from the studied area (Rodríguez et al., 2012). In contrast, only 10% of samples in this work were positive for BQCV. The Chilean sequence of this virus was genetically distant to available sequences from other countries. When aligning the sequence to Brazilian and Uruguayan sequences, 96% identities were found. The phylogenetic proximity to the Brazilian sequence may explain the origin of this virus in Chile, although we do not know of any commercial exchange of biological material with this country.

The present work makes the first report of the presence of ABPV in Chile, although its prevalence in the analyzed samples is low (2%). This virus has been reported in other South America countries, such as Argentina, Brazil, and Uruguay (Antúnez et al., 2005; Teixeira et al., 2008; Reynaldi et al., 2010). The Chilean isolate nucleotide sequence had a high percentage of similarity (in the Capsid protein gene) to Brazilian and Uruguayan isolates (97% and 93%, respectively) (there is no published data on the ABPV sequence from Argentina), confirming that the ABPV capsid polyprotein gene is a relatively conserved genomic region (Bakonyi et al., 2002). The proximity of Brazil and Uruguay to Chile suggests they are origin of the virus in Chile.

*Nosema ceranae* has also been detected in the present work, in 18% of analyzed apiaries. Although it has been described as an adult bee parasite (Higes et al., 2008; Forsgren and Fries, 2010), this microsporidia was also found in pupae, probably due to spore contamination from nurse bees, which have been described as carriers of this parasite (Meana et al., 2010) or through feeding with contaminated pollen (Higes et al., 2008). Other studies carried out in the spring of 2010 and summer of 2011 in the same region revealed that *N. ceranae* is widely distributed in the region (Martínez et al., 2012). However, in the present study we only detected *N. ceranae* in 18% of the analyzed apiaries. A possible reason is that the authors of the other studies sampled a larger number of beehives (N = 240) in a larger number of communities (N = 26) in the Biobío Region.

One year after the first collection, BQCV prevailed in the hives. This virus was detected mainly in forager bee samples and at one apiary among queen larvae and nurse bees. BQCV was originally detected among dead queen larvae and pupae, and different reports show that the disease is associated with *N. apis* infestation (Bailey et al., 1983; Allen and Ball, 1996). However, co-infection between BQCV and *N. apis* was not detected in the apiaries studied in this work. We detected *N. ceranae* in co-infection with BQCV and DWV in only one of the apiaries. Numerous viral diseases have been reported to be associated with *V. destructor* infestation (Ball and Allen, 1988; Allen and Ball, 1996; Martin et al., 1998; Tentcheva et al., 2004). In this work we analyzed the presence of

DWV, BQCV, SBV, and ABPV in *V. destructor*, but we only detected DWV in mite samples. We also detected the presence of *N. ceranae* in *V. destructor*. It is possible that the mite transports *N. ceranae* spores in its body. However, with only one positive sample, more analysis is needed to prove this hypothesis.

There are several reports globally on viral bee infections and numerous studies related to their pathogenesis, genetic variability, and phylogeny. However, knowledge about viral bee pathogens in Chile is still limited and incipient. Beekeeping in the studied area focuses not only on honey production, but also pollination, and queen bee and nucleus colony production. Therefore there is permanent movement of biological material, which can accelerate the spread and transmission of diseases.

This study arose from the need for information about the health of beehives and the presence of unreported pathogens, as well as the need to develop appropriate detection techniques. It is expected that the information generated will be useful not only in explaining bee mortality when there are no visible symptoms or pathogens, but also in developing new techniques to manage bee health in Chilean apiaries.

## CONCLUSIONS

Three viruses were detected in the Biobío Region, Chile: *Acute bee paralysis virus*, *Black queen cell virus*, and *Deformed wing virus* in 2%, 10%, and 42% of the apiaries, respectively. Three percent of the apiaries were infected with *Nosema apis* and 18% with *N. ceranae*, only 5% were positive for *Varroa destructor*, and *Acarapis woodi* was not detected. No statistical correlation was found between the presence of different viruses, *V. destructor*, *A. woodi*, and the two *Nosema* species, and the bee developmental stage.

PCR products were sequenced and compared to the Genbank database. Chilean sequences of ABPV, BQCV, and DWV showed high percentage of sequence similarity to other isolates in South America.

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