Virus Genes (2012) 45:606–609
DOI 10.1007/s11262-012-0791-0

# First detection and complete genome sequence of *Deformed wing virus* in Chilean honeybees

**Gonzalo P. Barriga · Nicolás Cifuentes-Muñoz · Paulina A. Rivera · Matías Gutierrez · Amir Shmaryahu · Pablo D. T. Valenzuela · Esteban A. Engel**

Received: 19 April 2012 / Accepted: 16 July 2012 / Published online: 27 July 2012


**Abstract** *Deformed wing virus* (DWV) is one of the most common viruses affecting honey bee specimens. Although the presence of DWV has been reported in many countries, there is no data of the current situation in Chile. In this report, we detected the presence of DWV in apiaries from two different locations in central Chile. Furthermore, the genome of a Chilean DWV isolate was completely sequenced. This is the first report of the presence of a honey bee virus in Chile.

**Keywords** *Apis mellifera* · *Deformed wing virus* · DWV · Chile · Genome · Pyrosequencing

## Introduction

*Deformed wing virus* (DWV), a member of the *Iflaviridae* family, is one of the many known viruses that infect the honey bee (*Apis mellifera*) [1, 2]. The occurrence of deformity in bees has been associated with the presence of DWV, which is transmitted through the ectoparasite *Varroa destructor* during pupae stages [3]. The viral genome, excluding the poly-A tail, consists of a positive-sense ssRNA of 10,140 nucleotides (nt) in length, flanked at the 5′ and 3′ ends by nontranslated regions of 1,144 and 317 nt, respectively [4]. This unique ORF encodes a polyprotein of 328 kDa, in which the structural proteins VP1 (44 kDa), VP2 (32 kDa), and VP3 (28 kDa) are mapped in the N-terminal domain, while the nonstructural proteins RNA helicase, chymotrypsin-like 3C protease and an RNA-dependent RNA polymerase are mapped at the C-terminal domain [4].

Since the initial report of the presence of DWV in infected bees in Japan [5], many authors have reported the presence of the virus in several countries [1], including recent detections in Uruguay and Brazil [6, 7]. Despite the fact that Chile produces and export honeybees-related products, the current sanitary status of the country in terms of viral diseases is unknown. In this report, we recognized typical symptoms of wing deformity in honeybees collected from Chilean apiaries and confirmed infection with DWV. Furthermore, the complete genome sequence of a Chilean isolate was obtained. To our knowledge, this is the first work describing the presence of a virus in Chilean honeybees.

## Materials and methods

### RNA extraction and RT-PCR

Fifty-eight worker bee samples free of the *Varroa* mite were obtained from hives located in two different apiaries from

G. P. Barriga (✉) · N. Cifuentes-Muñoz · P. A. Rivera · M. Gutierrez · A. Shmaryahu · P. D. T. Valenzuela · E. A. Engel (✉)
Fundación Ciencia & Vida, Avenue Zañartu 1482, 7780272 Ñuñoa, Santiago, Chile
e-mail: gonzalo.barriga@gmail.com

E. A. Engel
e-mail: eaengeli@yahoo.com

G. P. Barriga · N. Cifuentes-Muñoz · P. D. T. Valenzuela · E. A. Engel
Facultad de Ciencias Biológicas, Universidad Andrés Bello, República 252, 8370134 Santiago, Chile

*Present Address:*
E. A. Engel
Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA

Valparaíso and Metropolitan regions of Chile. We selected bees with apparently healthy conditions (as control) and bees showing impaired ability to fly and/or deformity of the wings, as inspected visually. Samples were frozen in liquid nitrogen and macerated to extract total RNA [8]. Reverse transcription was then performed starting from 200 ng of total RNA, as previously described [9]. A fragment of the DWV capsid gene was amplified using specific primers 5-TAATAGATGCCGTGATGGTA-3 forward and 5-CTT ACTACTGGTGCGGGA-3 reverse, using the following conditions: 40 cycles of 94 °C × 1 min, 60 °C × 30 s, 72 °C × 1 min, with a final extension of 10 min at 72 °C.

Sample preparation for pyrosequencing and in silico analysis

Total nucleic acid of a DWV positive honeybee sample was randomly amplified as described previously [9]. Sequencing was performed on a FLX genome sequencer (Roche) according to manufacturer's protocol. Sequence filtering and clustering were performed as described elsewhere [10]. The phylogenetic tree was constructed using the Phylowin program with the maximum likelihood method [11] and a bootstrap of 500 replicates. The phylogenetic tree was visualized using SeaView 4 [12].

## Results and discussion

The beekeeping market has been growing in Chile in the last years due to the increasing export of honey and bees nucleus derived from *Apis mellifera*. There are no previous reports on the presence of honeybees infected with DWV or any other virus in Chile. As a first approach to determine the presence of DWV in Chile, we collected field samples from two different locations in the Valparaíso and Metropolitan regions, respectively. By visual inspection, worker honeybees with apparent symptoms of DWV infection were observed in both locations (Fig. 1a). Total RNA was extracted from fifty-eight samples and subsequent RT-PCR was performed to amplify a fragment of the viral capsid gene. We detected in eighteen bee samples the expected amplicon of ~510 pb (Fig. 1b, lanes 1–3 as an example). It is noteworthy that in some cases, we detected the amplicon in apparently healthy samples. Nevertheless, most of bees with apparently healthy conditions (no wings deformity or flying impairment) gave no positive amplification by RT-PCR (Fig. 1b, lanes 4–6 as an example). To corroborate that we were amplifying DWV sequences, the 510 pb amplicon from ten samples was further sequenced (GenBank accession numbers JX185661, JX185662, JX185663, JX185664, JX185665, JX185666, JX185667, JX185668, JX185669, JX185670) and compared with other

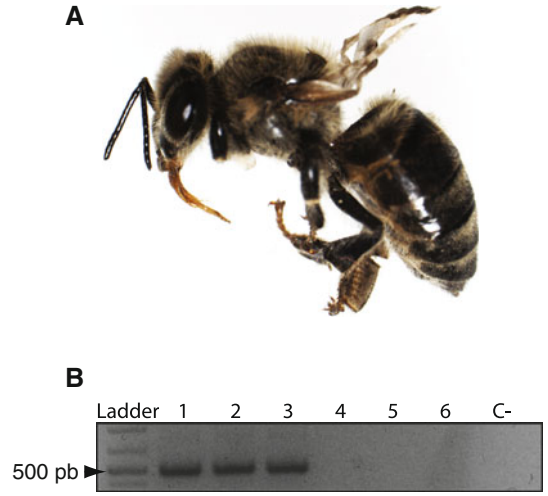


**Fig. 1** **a** Photograph corresponding to one of the honeybees collected from Chilean apiaries showing apparent symptoms of DWV infection such as wing deformity. **b** Agarose gel showing RT-PCR products of 510 pb for DWV virus capsid gene. *Lanes 1–3* bee samples with DWV symptoms. *Lanes 4–6* healthy bee samples. *C-* No cDNA negative control

known viral sequences of DWV from the GenBank database, obtaining in all cases 98 % of nucleotide identity with European DWV isolates (data not shown). This result confirmed for the first time, the presence of DWV in infected Chilean honeybees.

In order to obtain additional genetic information and to fully corroborate the nature of the DWV Chilean isolate, we fully sequenced the genome of one of the diseased honeybee samples. A sequence of 10,171 nt was obtained, showing a nucleotide identity of 98 % when compared with European isolates of DWV. The remaining nucleotidic differences of 2 % between DWV genomes were not confined to a specific genomic region and the substitutions were rather evenly distributed through the genomic sequence. We next determined, using the ORF Finder tool from the NCBI page, the presence of a single ORF encoding the DWV polyprotein and identified all the expected gene products previously described by Lanzi et al. [4].

Nine complete—or nearly complete—genome sequences of DWV isolates from different geographic locations, including the Chilean isolate, were subsequently used to construct a phylogenetic tree. The different DWV isolates segregated into three monophyletic groups, two of them supported by strong bootstrap values, arbitrarily named by us a and b; these groups were completely separated from the *Israel acute paralysis virus* (IAPV) genome sequence, which was used as an external node (Fig. 2). As shown in the tree, the Chilean isolate segregated into the monophyletic group a, within a sub-node shared with European DWV isolates from UK and Italy (Fig. 2). Supported by the bootstrap value obtained, it is clear that the Chilean isolate

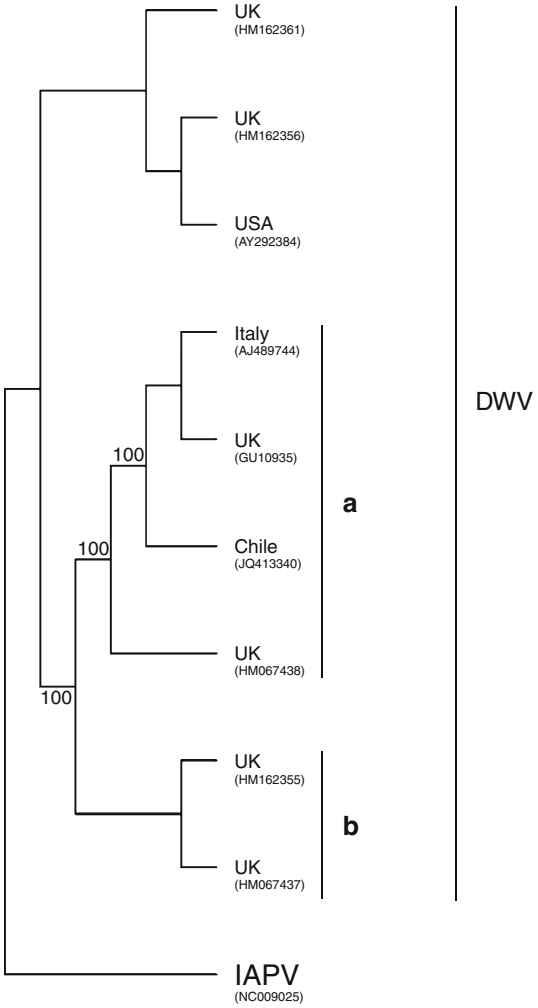


**Fig. 2** The complete genome sequence of the Chilean DWV isolate together with other complete—or nearly complete—genome sequences available at the GenBank database (9 in total) were used to construct a phylogenetic tree. Complete genome sequence of an IAPV isolate was used as outgroup. The phylogram was constructed using the maximum likelihood criteria. Only bootstrap values 70 % or above are indicated, resulting from 500 replicates. GenBank accession numbers of all sequences are indicated within brackets

is grouped different within this group, and therefore we can infer that it represents a new variant of the virus. We observed no correlation between the geographic location and distribution into one monophyletic group of the tree. This result can be explained by the high degree of conservation of nucleotide sequences—over 98 %—between different isolates, independent of their geographic origin and indicates a recent global distribution of the virus, as previously suggested [13]. Overall, the results confirmed the presence of DWV in Chile.

Since several years ago, there has been an important worldwide concern about viruses affecting honeybees [2]. There is no control for this kind of pathogens in Chile and conclusively the extent of infection and economic impact in Chilean hives remains completely unknown. In this report, we present new data regarding Chilean beekeepers and the presence of a pathogen that causes major colony losses [14]. For the accomplishment of this report, honeybees from two different apiaries were screened. An important observation was the presence—verified by visual inspection—of *Varroa destructor* in only one of the two locations. Since *Varroa destructor* has been associated with horizontal transmission of the virus by infection of the pupae [3], we suggest this route of infection at least in one of the apiaries. Nevertheless, vertical transmission [15] as a route of infection cannot be discarded in the second, or both, apiaries. An analysis of DWV in queens will be necessary to answer this hypothesis in future work. The possibility of apiaries getting infected by other wild species such as migratory honeybees [16] or even bumblebees [17] should also be considered.

Since multiple viruses can co-infect honeybees [1, 2], it is likely that DWV is not only the viral species present in Chilean apiaries. We are currently screening additional viruses in honeybee samples, pupae, queens, and workers. Furthermore, taking into account that the presence of DWV and *Varroa destructor* are considered predictive markers for the honey bee colony collapse [18], our findings represent crucial information to improve our knowledge about bee viruses and to identify control mechanisms.

**Acknowledgments** This study was partially supported by the CONICYT CCTE/PFB-16 Program. We would like to acknowledge Professor David Wang (Washington University in St. Louis, School of Medicine) for assistance with the pyrosequencing of DWV genome.

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