Short Communication

BROAD GEOGRAPHIC AND HOST DISTRIBUTION OF *APIS MELLIFERA* FILAMENTOUS VIRUS IN SOUTH AMERICAN NATIVE BEES

Silvina Quintana[1,2,3]*
Gregorio Fernandez de Landa[1,2]
Pablo Revainera[1,2]
Facundo Meroi[1]
Leonardo Porrini[1,2]
Vanesa Di Geronimo[3]
Constanza Brasesco[1,2]
Santiago Plischuk[2,4]
Martín J. Eguaras[1,2]
Matias Maggi[1,2]

[1]Centro de Investigación en Abejas Sociales (CIAS). Instituto de Investigaciones en Producción Sanidad y Ambiente (IIPROSAM). Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Comisión de Investigaciones Científicas de la provincia de Buenos Aires (CIC). Facultad de Ciencias Exactas y Naturales. Universidad Nacional de Mar del Plata, Argentina
[2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
[3]Laboratorio de Biología Molecular, Instituto de Análisis Fares Taie. Mar del Plata, Argentina
[4]Centro de Estudios Parasitológicos y de Vectores (CEPAVE) (CCT La Plata-CONICET). La Plata, Argentina.

Received: 09 April 2019; accepted: 07 October 2019
*corresponding author: silquintana@hotmail.com

Abstract

***Apis mellifera* filamentous virus (*Am*FV) is a large double stranded DNA virus of honey bees and its prevalence and relationship with other parasites is poorly known. Samples consisted of fifty-one adult bees belonging to eight native species collected using entomological nets in six provinces of Argentina, from 2009 to 2018. Total genomic DNA was extracted from individual bees and a 551 bp fragment of the Bro-N gene of *Am*FV was amplified by qPCR. In the present work we have reported for the first time both the presence and the wide geographic distribution of *Am*FV in Argentinian species of native bees. This is the first report of the presence of this virus associated with *Xylocopa atamisquensis, X. augusti, X. frontalis, X. spendidula, Bombus pauloensis* and *Peponapis fervens*. Detecting pathogens that could threaten native bee health is of outmost importance to generate both conservation and management strategies.**

Keywords: Argentina, DNA virus, qPCR

INTRODUCTION

Bees are the most important pollinators in both agricultural and natural environments. Managed colonies, mainly from *Apis mellifera*, are known to host multiple pathogens that can spread to wild bees (Graystock et al., 2016). Alger demonstrated in 2019 the spillover for two RNA viruses from managed honey bees to wild bumble bees in Vermont (USA). These host-jumps could be a driver of population declines (Meeus et al., 2011). The *A. mellifera* filamentous Virus (*Am*FV) is a double stranded DNA virus, pathogenic to *A. mellifera* (Hartmann et al., 2015). Presence of this virus has been reported in honey bees from USA, Europe and China (Allen & Ball, 1996; Gauthier et al., 2015; Hou et al., 2017). It has also been found in other four bee species of the

genera *Osmia* and *Andrena* (Ravoet et al., 2014; Tehel, Brown & Paxton, 2016). The complete *Am*FV genome was described in 2015 (Gauthier et al., 2015). The purpose of this study was to detect the presence of AmFV DNA by qPCR in native bee samples from several localities of Argentina.

MATERIAL AND METHODS

Samples consisted of fifty one female adult bees belonging to eight native species collected in six provinces of Argentina while foraging (Fig. 1). Once the species were identified, total genomic DNA was individually extracted using a High Pure PCR Template Preparation kit (Roche Diagnostics). DNA amplification of a 134 bp PCR product (5'-AGATGGGGGCATTCGTATTG-3', 5'-ATCTGATCGCCTTCGAACCT-3') was performed as internal control (Nunes-Silva et al., 2016). *Am*FV DNA was detected using primers which amplify a 551 bp fragment of the Bro-N gene of *Am*FV (Gauthier et al., 2015). The PCR program consisted of 2' at 95°C and forty cycles of 95°C 20", 52°C 20" and 72°C 30". All qPCR reactions were carried out using EvaGreen as an intercalating fluorescent dye and showed a linear standard curve (R^2 = 0.997).

Amplified DNA fragments were purified and sequenced. Sequence alignment was performed with ClustalX 2.0 and a dendrogram (Fig. 2) was carried out on MEGA v.7 (Larkin et al., 2007; Kumar, Stecher & Tamura, 2016) using the neighbor-joining method with 1000 bootstraps. Sequences associated with *A. mellifera* from China (accession MF092817.1), Switzerland (accession JF304814.1) and Argentina (obtained by our research group from Buenos Aires and Santa Fe) were included besides those obtained here.



Fig. 1. Localities in Argentina where native bees were collected.

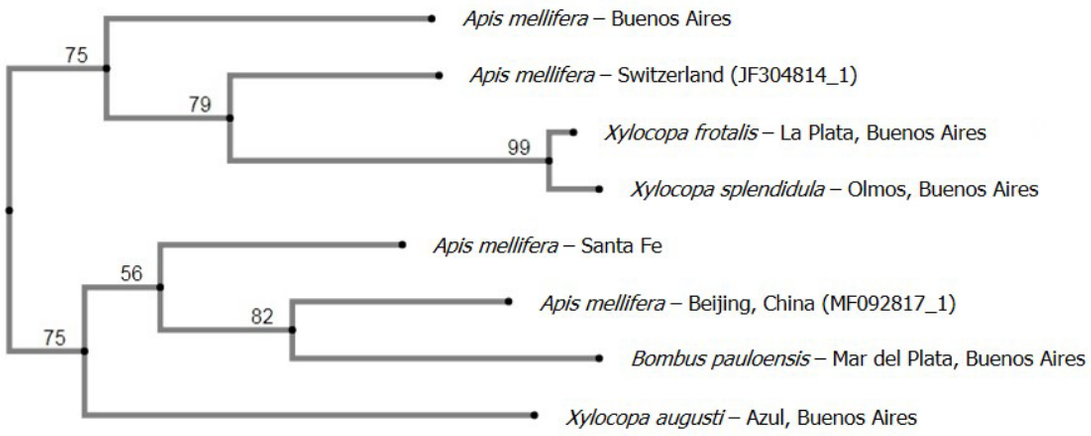


Fig. 2. Dendogram constructed by neighbor-joining method using Bro-N gene sequences of *Am*FV in honey bees from Argentina, China and Switzerland and *Am*FV sequences in native bees analyzed in this work.

RESULTS AND DISCUSSION

*Am*FV was detected in thirty-three bees (67%) belonging to *Xylocopa atamisquensis, X. augusti, X. frontalis, X. splendidula, Bombus pauloensis* and *Peponapis fervens*, from the six surveyed provinces. It was neither detected in *X. nigrocincta* nor *Halictillus amplilobus* (Tab. 1, Supp. Mat.).

Sequences of *Am*FV found in *A. mellifera, X. frontalis, X. splendidula, X. augusti* and *B. pauloensis* from Argentina showed high homology among themselves, which was in concordance with previous reports that indicated that its genome could be conserved (Gauthier et al., 2015).

Ravoet et al. (2014) and Tehel, Brown & Paxton (2016) isolated *Am*FV from *Andrena vaga, A. ventralis* (Andrenidae), *Osmia bicornis* and *O. cornuta* (Megachillidae). The association with *Xylocopa*, *Bombus* and *Peponapis* shows that other Apidae species are capable of infecting also. Although this virus is present in all these bee genera, the virulence for each one is still unknown. Both field surveys and genetic expression studies could provide more information about the threat that *Am*FV represents. Considering the role of native pollinators on agriculture and biodiversity maintenance as well as the global decline of their populations, these findings highlight the importance of monitoring bee pathogens, extending the knowledge about their stressors and providing new data for the conservation of native species.

ACKNOWLEDGEMENTS

This research was supported by FONCyT (PICT 2015-1045 granted to SQ and PICT 2016-0289 granted to SP), the Institute of Analysis Fares Taie, CONICET, and the UNMdP. Thanks to M. Pocco, J. Barneche, and M. Haramboure for their help with fieldwork.

REFERENCES

Alger, S. A., Burnham, P. A., Boncristiani, H. F., & Brody, A. K. (2019). RNA virus spillover from managed honeybees (*Apis mellifera*) to wild bumblebees (*Bombus* spp.). *PloS One*, *14*(6), e0217822. https://doi.org/10.1371/journal.pone.0217822

Allen, M., & Ball, B. (1996). The incidence and world distribution of honey bee viruses. *Bee World, 77*, 141-162.

Gauthier, L., Cornman, S., Hartmann, U., Cousserans, F., Evans, J.D., de Miranda, J.R., Neumann, P. (2015). The *Apis mellifera* filamentous virus genome. *Viruses, 7*, 3798-3815. https://doi.org/10.3390/v7072798

Graystock, P., Blane, E.J., McFrederick, Q.S., Goulson, D., Hughes, W.O. (2016). Do managed bees drive parasite spread and emergence in wild bees? *International Journal of Parasitology: Parasites and Wildlife, 5*(1), 64-75. https://doi.org/10.1016/j.ijppaw.2015.10.001

Hartmann, U., Forsgren, E., Charriere, J.D., Neumann, P., Gauthier, L. (2015). Dynamics of *Apis mellifera* filamentous virus (*Am*FV) infections in honey bees and relationships with other parasites. *Viruses, 7,* 2654-2667. https://doi.org/10.3390/v7052654

Hou, C., Li, B., Deng, S., Chu, Y., Diao, Q. (2017). Diagnosis and distribution of the *Apis mellifera* filamentous virus (*Am*FV) in honey bees (*Apis mellifera*) in China. *Insectes Sociaux, 64*, 597-603. https://doi.org/10.1007/s00040-017-0569-4

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biolology Evolution, 33*(7), 1870-1874. https://doi.org/10.1093/molbev/msw054

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Thompson, J.D. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics, 23*, 2947- 2948. https://doi.org/10.1093/bioinformatics/btm404

Meeus, I., Brown, M.J.F., De Graaf, D.C., & Smagghe, G. (2011). Effects of invasive parasites on bumblebee declines. *Conservation Biology, 25*, 662-671. https://doi.org/10.1111/j.1523-1739.2011.01707.x

Nunes-Silva, P., Piot, N., Meeus, I., Blochtein, B., Smag-

ghe, G. (2016). Absence of Leishmaniinae and Nosematidae in stingless bees. *Scientific Reports, 6*, 32547. https://doi.org/10.1038/srep32547

Ravoet, J., De Smet, L., Meeus, I., Smagghe, G., Wenseleers, T., de Graaf, D.C. (2014). Widespread occurrence of honey bee pathogens in solitary bees. *Journal of Invertebrate Pathology, 122*, 55-58. https://doi.org/10.1016/j.jip.2014.08.007

Tehel, A., Brown, M.J., & Paxton, R.J. (2016). Impact of managed honey bee viruses on wild bees. *Current Opinion in Virology, 19*, 16-22. https://doi.org/10.1016/j.coviro.2016.06.006

SUPPLEMENTARY MATERIAL

Table 1.

Table summary with information on specimens, sample sites, sampling dates, and result of *Am*FV molecular detection in the different species of native bees collected in Argentina. (seq) indicates that positive detection was confirmed by sequencing

Province	Locality	Sampling Date	Species	AmFV qPCR detection
Buenos Aires	Azul	2016 (Summer)	*X. augusti*	Positive (seq)
	Gorina	2016 (Summer)	*X. splendidula*	Positive
	La Plata	2010 (Summer)	*X. splendidula*	Negative
		2010 (Spring)	*X. frontalis*	Positive (seq)
		2011 (Spring)	*X. frontalis*	Negative
		2016 (Autumn)	*X. frontalis*	Positive
	Mar del Plata	2018 (Summer)	*B. pauloensis*	Negative
			B. pauloensis	Negative
			B. pauloensis	Positive
			B. pauloensis	Negative
			B. pauloensis	Positive
			B. pauloensis	Positive (seq)
			B. pauloensis	Positive
			B. pauloensis	Positive
			B. pauloensis	Positive
			B. pauloensis	Positive
			B. pauloensis	Positive (seq)
			B. pauloensis	Positive
			H. amplilobus	Negative
			X. augusti	Positive (seq)
			P. fervens	Positive (seq)
			P. fervens	Positive
	Olmos	2014 (Autumn)	*X. splendidula*	Positive (seq)
	Pearson	2014 (Summer)	*X. augusti*	Positive
		2016 (Winter)	*X. augusti*	Negative
	Punta Lara	2016 (Summer)	*X. splendidula*	Negative
	Villa Elisa	2017 (Summer)	*X. augusti*	Negative
	Villa Ventana	2015 (Summer)	*X. augusti*	Positive
		2016 (Summer)	*X. augusti*	Positive
			X. augusti	Negative
Chubut	Gaiman	2016 (Summer)	*X. splendidula*	Negative
			X. splendidula	Positive
Corrientes	Colonia Pellegrini	2009 (Spring)	*X. augusti*	Positive

Formosa	Ibarreta	2012 (Summer)	*X. atamisquensis*	Negative
			X. atamisquensis	Positive
	Las lomitas	2015 (Summer)	*X. atamisquensis*	Positive
			X. atamisquensis	Positive
	Palo Santo	2012 (Summer)	*X. nigrocincta*	Negative
			X. nigrocincta	Negative
Misiones	Puerto Iguazú	2015 (Summer)	*X. nigrocincta*	Negative
Río Negro	Allen	2011 (Spring)	*X. augusti*	Positive
	Belisle	2014 (Summer)	*X. splendidula*	Negative
			X. atamisquensis	Positive
	General Conesa	2018 (Summer)	*X. atamisquensis*	Positive
			X. atamisquensis	Negative
	General Roca	2014 (Summer)	*X. atamisquensis*	Positive
		2016 (Summer)	*X. augusti*	Positive
			X. augusti	Positive
			X. augusti	Positive
	Sierra Grande	2018 (Summer)	*X. atamisquensis*	Negative
	Villa Regina	2014 (Summer)	*X. augusti*	Positive