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ORIGINAL RESEARCH ARTICLE

Is *Acarapis woodi* mite currently infesting *Apis mellifera* colonies in Argentina?

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The honey bee tracheal mite *Acarapis woodi* (Rennie) (Acari: Tarsonemidae) is an internal obligate parasite of the respiratory system of adult honey bees (*Apis mellifera*), which may be one of the factors causing the loss of bee colonies and the decrease in the beekeeping industry. In Argentina, the last prevalence study of *A. woodi* was in 1998, and therefore there is no current data on the prevalence and impact of the mite in colonies of *A. mellifera* in this country. For this reason, the objective of this study was to determine the prevalence of *A. woodi* in honey bee colonies of apiaries belonging to different geographic regions of Argentina (Northwest, Chaco, Mesopotamic, Pampean and Patagonic) using two different methods of diagnosis. One method was traditional microscopy, which is based on direct visualization of *A. woodi* in the prothoracic tracheas of bees, and the other technique was molecular analysis using real-time PCR with specific primers to amplify CO1 gene of *A. woodi*. To perform the analysis of prevalence, bees were collected during winter, because during this season, populations of *A. woodi* are more abundant in bee colonies. The results were negative for both methods of diagnosis, with prevalence equal to 0%. Considering that in recent years it has been reported an increased mortality in bee colonies in Argentina, we note that acarapisosis is not currently impacting on the disappearance of *A. mellifera* colonies in Argentina.

¿Está actualmente *Acarapis woodi* infestando colonias de *Apis mellifera* en Argentina?

El ácaro traqueal de la abeja de miel *Acarapis woodi* (Rennie) (Acari: Tarsonemidae) es un parásito interno obligado del sistema respiratorio de las abejas adultas de la miel (*Apis mellifera*), el cual puede ser uno de los factores que causan la pérdida de colmenas de abejas y la disminución de la industria apícola. En Argentina, el último estudio de prevalencia de *A. woodi* fue en 1998, por lo que no hay datos actuales sobre la prevalencia e impacto de este ácaro en colonias de *Apis mellifera* en este país. Por esta razón, el objetivo de este estudio fue determinar la prevalencia de *A. woodi* en colmenas de abejas melíferas de apiarios pertenecientes a diferentes regiones geográficas de Argentina (Noroeste, Chaco, Mesopotamia, Pampeana y Patagonia) utilizando dos métodos diferentes de diagnóstico. Un método fue la microscopía tradicional, la cual se basa en la visualización directa de *A. woodi* en las tráqueas protorácicas de las abejas, y la otra técnica fue el análisis molecular utilizando PCR en tiempo real con cebadores específicos para amplificar el gen CO1 de *A. woodi*. Para realizar el análisis de la prevalencia, las abejas fueron recolectadas durante el invierno, ya que durante esta temporada, las poblaciones de *A. woodi* son más abundantes en las colonias de abejas. Los resultados fueron negativos para ambos métodos diagnóstico, con prevalencia igual al 0%. Considerando que en los últimos años se ha reportado un aumento de la mortalidad en las colmenas de abejas en Argentina, se observa que la acarapisosis no afecta actualmente a la desaparición de las colonias de *A. mellifera* en Argentina.

Keywords: *Acarapis woodi*; *Apis mellifera*; real-time PCR; dissection method classic; Argentina

Introduction

The honey bee tracheal mite *Acarapis woodi* is an internal obligate parasite of adult honey bees (*Apis mellifera*). This mite lives and reproduces primarily in the large prothoracic trachea of the bee, but can also be located on the head and abdominal and thoracic sacs (Garrido-Bailón et al., 2012). This organism feeds on the haemolymph of its host and it is a vector of several honey bee viruses (Bailey, 1975, 1985, 1999; Collison, 2001; Komeili & Ambrose, 1991; Sammataro et al., 2013). The pathogenic effects of *A. woodi* mites on individual bees depend on the number of parasites within the tracheae. Increased parasitic load causes the tracheal walls, which are normally white and translucent, to become opaque and discolored, with blotchy black areas that are thought to be due to melanin crusts (Giordani, 1965). Bee damage can be attributed to both mechanical injury and to physiological disorders resulting from the obstruction of the air ducts, lesions in the trachea walls and the depletion of haemolymph (Sammataro et al.,

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2013). At colony level, a heavy tracheal mite infestation causes diminished brood area, smaller bee populations, lesser winter clusters, increased honey consumption and lower honey yields (Eischen, 1987; Morse & Nowogrodzki, 1990; Otis &and Scott-Dupree, 1992; Royce & Rossignol, 1989). Mite populations are higher in winter or in early spring because of the reduced brood production of the bee queen. During this time, a high proportion of older, overwintering bees are present in the colonies, and the mites have a long time to reproduce. Infestation decreases in summer due to the dilution of mite populations as they enter the large population of emerging host bees (Sammataro et al., 2013). However, generally bees do not show symptoms that are reliable indicators of mite infestation. The traditional diagnostic methods are very time consuming and they are based on direct visualization of *A. woodi* or of the lesions in the tracheas of bees (Sammataro et al., 2013). Other methods as enzyme-linked immunosorbent assays (ELISA) (Fichter, 1988; Grant et al., 1993; Ragsdale & Furgala, 1987; Ragsdale & Kjer, 1989) or methods based on the visualization of guanine under ultraviolet light, the main end product of nitrogen metabolism in mites (Mozes-Koch & Gerson, 1997), are little used in routine diagnostic. In recent years, new molecular diagnostic techniques were developed to identify *A. woodi* using PCRs to amplify the CO1 gene (Evans et al., 2007; Garrido-Bailón et al., 2012; Kojima et al., 2011).

A. woodi was first discovered in 1919 in the UK (Rennie et al., 1921) and was later found in mainland Europe, Africa, Asia, South America, and North America (Ellis & Munn, 2005). Up to date, it is not known to occur in Australia, New Zealand or Scandinavia (Denmark, Cromroy, & Sanford, 2000; Hoy, 2011). A work by Kojima et al. (2011) reported that *A. woodi* was found in Asian honey bees, *Apis cerana japonica*, in Japan. In Argentina, the only precedent that exists on monitoring populations of *A. woodi* is the work of Eguaras et al. (1998). However, after this study there is no current data on the prevalence of *A. woodi* and its impact on the colonies of *A. mellifera* in Argentina. In recent years it has been reported in this country increased mortality in bee colonies (Maggi et al., 2013), but in neither case was evaluated the presence of trachea mites of genus *Acarapis*.

It is for this reason that the objective of this study was to determine the prevalence of *A. woodi* in bee colonies of apiaries belonging to different geographic regions of Argentina (Northwest, Chaco, Mesopotamia, Pampas and Patagonia Plain). It is intended by this study, to generate an actualization of data involving the presence, prevalence, and impact of the mite in bee colonies of Argentina.

Materials and methods

Location of study and sampling method

Apiaries that were used for the analysis of prevalence of *A. woodi* were located in the following provinces of Argentina: Buenos Aires (Pampas Plain region, 49 locations sampled), Chaco (Chaco region, a total of two locations sampled), Entre Rios (Mesopotamian region, one location sampled), Misiones (Mesopotamian region, a total of seven locations sampled), Rio Negro (Patagonia, one location sampled) and Salta (Northwest region, one location sampled) (Figure 1). Five colonies by apiary were sampled for *A. woodi* detection. Samples collected from Buenos Aires Province (Bs As) were significantly higher from that of the rest of the sampled provinces, because beekeeping in this province is the most intensive in the country, being the area with the highest production of honey, pollen and other products of the hive (Maggi et al., 2013).

Sampling took place during the month of June. Colonies from Bs As, Entre Rios, Rio Negro and Salta received the last acaricide treatment in March of the same year (three months before sampling). Samples collected from Chaco province, belong to honey bee populations where acaricide treatments had never been used (wild honey bee colonies). Bees were collected during winter, because during this season, populations of *A. woodi* are more abundant in bee colonies (Sammataro et al., 2013). Bee samples (60 bees per sample) were taken from inside the hive, using plastic cups directly from frames in the honey super or from the inner covers where older bees congregate. Then, samples of adult worker bees were transferred to wide-mouth jars properly labeled containing 70% ethanol (Sammataro et al., 2013). Parasitized honey bee samples by *A. woodi* used as positive controls, were provided by Dr Gabriel Sarlo from Chile.

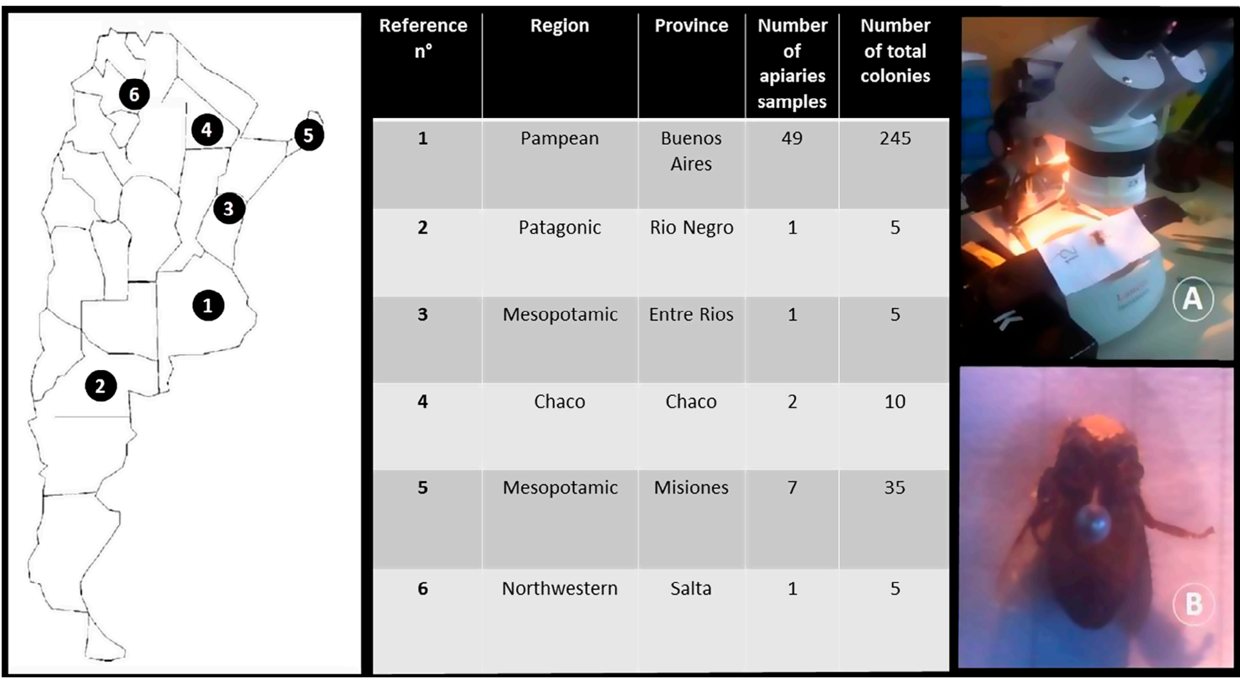
Detection methods

The morphological technique. Screening individual bees

For each honey bee colony of Rio Negro, Misiones, Entre Rios, Chaco and Salta provinces, 30 bees were chosen randomly to detect *A. woodi* by visual inspection (n = 1800 bees). In the case of colonies from Bs As (n = 245), five bees per colony were analyzed (n = 1225 bees). This classical method consists of pulling off the head and collar of the bee and examining the trachea. One proceeds according to the technique developed by Milne (1948) to locate the internal mites in individual bees. The bee is placed under a dissecting microscope, held prone with forceps (across abdomen) and the head and the first pair of legs is scraped off using a scalpel or razor blade (Figure 1(A)). Then, the ring of prothoracic sclerite (collar) is also removed using a fine forceps (Figure 1(B)). The exposed tracheae of both sides are removed after carefully detaching them from the thoracic wall. The removed tracheae are placed on a glass slide and examined under a microscope for mites.

Molecular detection

For this part of the study, five bees per colony were chosen from samplings belonging to Río Negro, Misiones, Salta, Entre Ríos and Chaco provinces (n = 300).



Reference n°	Region	Province	Number of apiaries samples	Number of total colonies
1	Pampean	Buenos Aires	49	245
2	Patagonic	Rio Negro	1	5
3	Mesopotamic	Entre Rios	1	5
4	Chaco	Chaco	2	10
5	Mesopotamic	Misiones	7	35
6	Northwestern	Salta	1	5

Figure 1. Collection sites in Argentina where *A. mellifera* were collected. Table summary show the number of apiaries and colonies per province sampled. (A) Individual bee placed under binocular lens slightly perpendicular from the back punctured through mesothorax on a wooden support covered with EVA rubber. (B) Dissection of the head and first pair of legs with a bistoury, showing the ring around the neck opening.

Due to the high number of samples taken for the province of Bs As, only ten random colonies were selected from the total and five bees from each one were analyzed by the PCR technique ($n = 50$). In this way, only ten localities/apiaries were analyzed by this technique from the 49 available.

DNA extraction

Total genomic DNA was isolated from the honey bees preserved in 70% ethanol using the commercial kit ADN HP PCR template preparation (Roche Diagnostics).

Internal control

To verify the success of DNA extraction from samples of individual bees and lack of inhibition of the PCR reactions, amplifications of beta actin gene of *A. mellifera* were carried out (Yang & Cox-Foster, 2005). The thermal cycling conditions were as follows: 1 cycle of initial denaturation at 95 °C for 2 min, and 45 cycles of denaturation at 94 °C for 10 s, annealing at 60 °C for 15 s, and extension at 72 °C for 15 s. After amplification, a melting curve analysis to confirm specific amplification of the PCR product was performed. Bee samples with Ct values (threshold cycle) beta actin < 35 were considered appropriate for further analysis.

A. woodi *real-time PCR assay*

PCR amplification of the mitochondrial CO1 gene from *A. woodi* was performed. The selected primers were: forward primer (AcarFor) 5′-CGGGCCCGAGCTTATT TTACTGCTG-3′; reverse primer (AcarRev) 5′-GCG CCTGTCAATCCACCTACAGAAA-3′. The expected size of the amplicon was 162 bp (Garrido-Bailón et al., 2012). The thermal cycling conditions were as follows: 1 cycle of initial denaturation at 95 °C for 2 min, and 45 cycles of denaturation at 95 °C for 10 s, annealing at 57 °C for 15 s, and extension at 72 °C for 20 s. The specific temperature dissociation of *A.woodi* PCR product was 76°C. All real-time PCR reactions were carried out in a thermocycler Rotor Gene (Qiagen, Hilden, Germany) in a final volume of 20 μl using EvaGreen as fluorescent intercalating dye (KAPA Fast, Biosystems, Woburn, E.E.U.U.).

Results

A total of 3025 bees were analyzed for *A. woodi* presence by morphological analysis while 350 bees were analyzed by PCR technique (Table 1). In none of the samples studied the presence of the mite was detected by neither of the two methods (Table 1). In the sample from Chile used as positive control, adult females of *A. woodi* were detected (Figure 2), subsequently confirmed by molecular analysis (Figure 3).

Discussion

Little is known about the actual prevalence of *A. woodi* in honey bee colonies from Argentina. A recent study carried out in Spain on honey bee samples collected in

Table 1. Table summary with information on sample sites in Argentina and Chile (localities and georeference) and result of presence of *A. woodi* according to the morphological and molecular analysis.

			Methods for detecting *A. woodi*	
Country/Province	Locality	Georeference	Morphological technique	Molecular technique
Chile (control)			Positive	Positive
Argentina Buenos Aires (1)	Salv. María	35° 19′ 03′′ S 59° 09′ 46′′ W	Negative	NA
	Saladillo	35° 38′ 20′′ S 59° 46′ 46′′ W	Negative	Negative
	Maza	36° 48′ 1′′ S 63° 20′ 16′′ W	Negative	NA
	25 de Mayo	35° 26′ 26′′ S 60° 09′ 45′′ W	Negative	NA
	Gral. Alvear	36° 01′ 19′′ S 60° 00′ 52′′ W	Negative	NA
	Bolivar	36° 13′ 52′′ S 61° 07′ 14′′ W	Negative	Negative
	Urdampilleta	36° 25′ 50′′ S 61° 25′ 48′′ W	Negative	NA
	Carhué	37° 10′ 46′′ S 62° 45′ 37′′ W	Negative	NA
	Bordenave	37° 48′ 13′′ S 63° 02′ 27′′ W	Negative	NA
	Darragueira	37° 41′ 22′′ S 63° 09′ 39′′ W	Negative	NA
	Villa Iris	38° 10′ 16′′ S 63° 14′ 9′′ W	Negative	NA
	17 de Agosto	37° 54′ 38′′ S 62° 56′ 10′′ W	Negative	NA
	Felipe Solá	38° 0′ 27′′ S 62° 48′ 58′′ W	Negative	Negative
	Tres Arroyos	38° 22′ 39′′ S 60° 16′ 31′′ W	Negative	NA
	Pedro Luro	39° 27′ 01′′ S 62° 40′ 34′′ W	Negative	NA
	La Rica	34° 52′ 56′′ S 60° 01′ 10′′ W	Negative	NA
	9 de Julio	35° 27′ 28′′ S 60° 53′ 14′′ W	Negative	Negative
	Lobos	35° 02′ 57′′ S 58° 45′ 34′′ W	Negative	NA
	Mercedes	34° 39′ 4′′ S 59° 25′ 50′′ W	Negative	NA
	Lujan	34° 33′ 48′′ S 59° 07′ 15′′ W	Negative	NA
	Villa	34° 14′ 37′′ S 59° 28′ 26′′ W	Negative	NA
	Ing. Otamendi	34° 13′ 45′′ S 58° 54′ 17′′ W	Negative	NA
	Zárate	34° 05′ 45′′ S 59° 01′ 27′′ W	Negative	Negative
	Baradero	33° 48′ 25′′ S 59° 30′ 08′′ W	Negative	NA
	San Pedro	33° 40′ 33′′ S 59° 39′ 46′′ W	Negative	NA
	Manuel Ocampo	33° 46′ 00′′ S 60° 39′ 00′′ W	Negative	NA
	Rojas	34° 11′ 52′′ S 60° 44′ 02′′ W	Negative	NA
	San Nicolás	33° 19′ 47′′ S 60° 13′ 52′′ W	Negative	Negative
	Villa Constitución	33° 13′ 52′′ S 60° 20′ 40′′ W	Negative	NA
	Los Hornos	34° 57′ 14′′ S 57° 58′ 29′′ W	Negative	NA
	Monte Veloz	35° 28′ 10′′ S 57° 17′ 18′′ W	Negative	NA
	Las Armas	37° 04′ 55′′ S 57° 49′ 39′′ W	Negative	NA
	Gral. Pirán	37° 16′ 34′′ S 57° 46′ 26′′ W	Negative	NA
	Coronel Vidal	37° 26′ 47′′ S 57° 43′ 45′′ W	Negative	NA
	Balcarce	37° 50′ 47′′ S 58° 15′ 20′′ W	Negative	Negative
	Lobería	38° 01′ 1′′ S 58° 38′ 45′′ W	Negative	NA
	San Manuel	37° 47′ 12′′ S 58° 50′ 52′′ W	Negative	NA
	Necochea	38° 33′ 16′′ S 58° 44′ 23′′ W	Negative	Negative
	Quequén	38° 33′ 20′′ S 58° 42′ 60′′ W	Negative	NA
	La Dulce	38° 16′ 60′′ S 59° 11′ 60′′ W	Negative	NA
	J.N.Fernandez	38° 0′ 28′′ S 59° 15′ 50′′ W	Negative	NA
	San Cayetano	38° 20′ 43′′ S 59° 36′ 50′′ W	Negative	NA
	Miramar	38° 16′ 13′′ S 57° 50′ 22′′ W	Negative	NA
	Villa Gesell	37° 15′ 36′′ S 56° 58′ 17′′ W	Negative	NA
	Gral. Madariaga	37° 00′ 07′′ S 57° 08′ 10′′ W	Negative	Negative
	Gral. Lavalle	36° 24′ 2′′ S 56° 58′ 01′′ W	Negative	NA
	Villa Cacique	37° 40′ 16′′ S 59° 23′ 54′′ W	Negative	NA
	Rauch	36° 46′ 30′′ S 59° 05′ 23′′ W	Negative	Negative
	Gral. Belgrano	35° 46′ 02′′ S 58° 29′ 46′′ W	Negative	NA
Rio negro (2)	Choele-choele	39° 17 ′17′′ S 65° 39′ 55′′ W	Negative	Negative
Entre Rios (3)	Los Charrúas	31° 10′ 0′′ S 58° 10′ 60′′ W	Negative	Negative
Chaco (4)	Presidencia Roque Sáenz Peña	26° 47′ 26′′ S 60° 26′ 25′′ W	Negative	Negative
	Las Breñas	27° 05′ 11′′ S 61° 05′ 09′′ W	Negative	Negative
Misiones (5)	El dorado	26° 24′ 14′′ S 54° 37′ 36 W	Negative	Negative
	Capioví	26° 56′ 49′′ S 55° 04′ 70′′ W	Negative	Negative
	San Ignacio	27° 15′ 10′′ S 55° 32′ 97′′ W	Negative	Negative
	Florentino Ameguino	27° 35' 27′′ S 55° 08′ 76′′ W	Negative	Negative

(*Continued*)

Table 1. (*Continued*).

Country/Province	Locality	Georeference	Methods for detecting *A. woodi*	
			Morphological technique	Molecular technique
	San Javier	27° 52′ 73′′ S 55° 08′ 47′′ W	Negative	Negative
	Apóstoles	27° 54′ 86′′ S 55° 45′ 59′′ W	Negative	Negative
	25 de Mayo	27° 22′ 39′′ S 54° 44′ 36′′ W	Negative	Negative
Salta (6)	Salta	24° 46′ 59′′ S 65° 24′ 37 ′′ W	Negative	Negative

Note: NA = not analyzed by PCR technique.

Figure 2. Dissection of bees infected by the tracheal mite, samples from Chile (positive control). (A) Trachea which flows into the blowhole of prothorax of the body of a bee infested by *A. woodi*. It can be seen the yellowing color characteristic of the presence of the mite in the respiratory conduct (400×). (B) A female individual *A. woodi* (red arrow) and an embryonated egg (green arrow) (400×).

2006 and 2007 by Garrido-Bailon et al. (2012) revealed a prevalence of *A. woodi* higher than expected, even in areas with unfavorable climatic conditions for the multiplication of this parasite. These authors stated that the presence of *A. woodi* in bee colonies should not be underestimated as a cofactor in colony loss. Indeed, honey bee colonies parasitized with *Varroa destructor* and *A. woodi* suffer considerably greater mortality during winter (Downey & Winston, 2001).

Until the development of this study there was only the work of Eguaras et al. (1998) in Argentina about the presence of *A. woodi* infesting bee colonies in the country. Eguaras and collaborators analyzed the prevalence of the mite over a year, reporting the highest prevalence of the parasite occurred during the winter months. A mean of 2000 bees per month were analyzed by the morphological technique and the registered ranges of prevalence of the parasite were from 0.4 to 4%. These authors and also Sammataro et al., (2013) reported that the higher prevalence of mites in winter or early spring is due to reduced bee brood production. During this time, a high proportion of older over wintering bees are present in the colonies, and the mites have had a long time to reproduce. Infestation of mites decreases in summer due to the dilution of mite populations as they enter the large population of emerging host bees.

In our study, we have analyzed a total of 3375 bees belonging to 61 apiaries, sampled across Argentine territory. In none of the samples analyzed the presence of *A. woodi* mites was found, even by using two different methods of detection (visual and PCR method). Not detecting positive samples with *A. woodi* presence from the total samples analyzed in this study, is very interesting. Seventeen years ago, this mite species had normally been found parasitizing bee colonies in Argentina (Eguaras et al., 1998). In the present study, the same geographic area that Eguaras and his co-workers covered in their research was included. Even more, comparing both studies, the number of bees analyzed was similar between them (approximately 1800 bees sampled in June respectively in each study). The sampling performed by Eguaras during June 1998 detected an *A. woodi* prevalence of 2.5%.

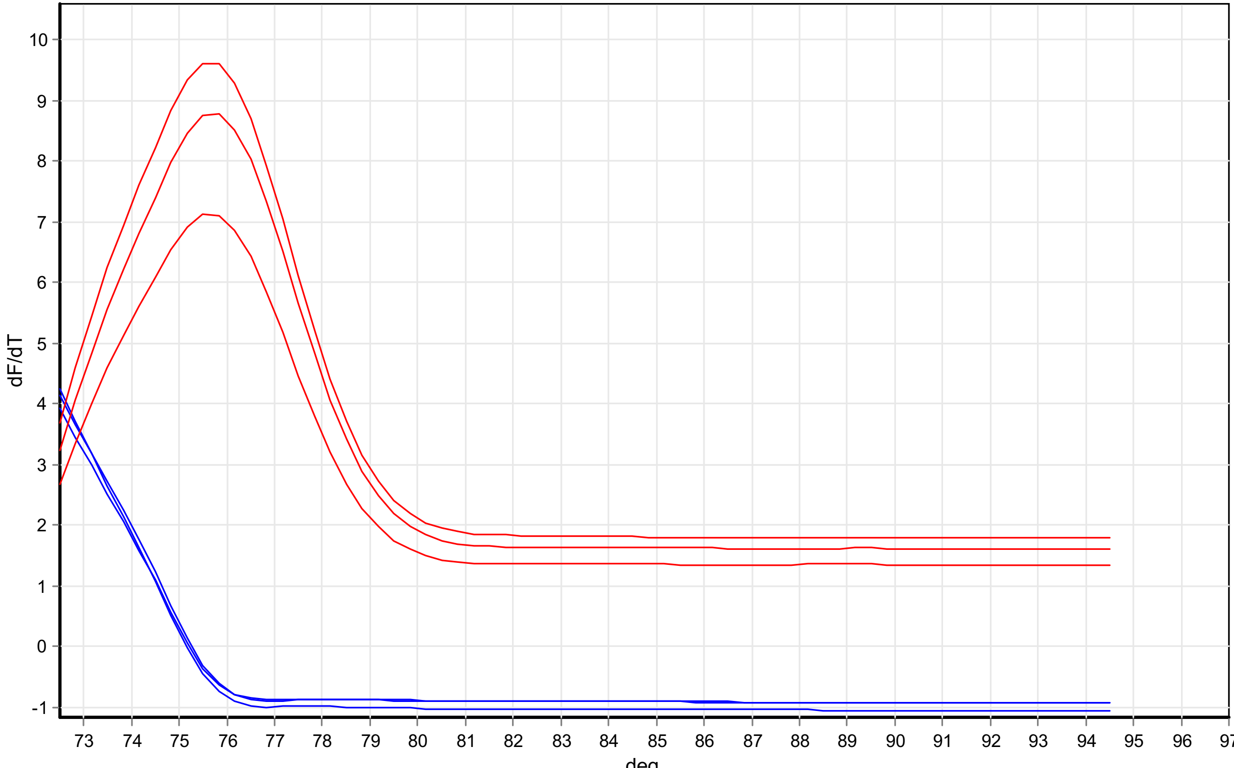


Figure 3. Melting analysis of the specific amplification of *A. woodi* PCR product. In the vertical axis is represented the rate of change in relative fluorescence units (RFU) with time (*T*) (−*d* (RFU)/*dT*) vs. temperature (°C) in the horizontal axis. This rate is marked as a peak at the specific dissociation T° (76 °C). Positive samples from Chile show the corresponding melting peak (red), samples from Argentina are negative (blue).

Although it was not possible to survey all the country, we have covered a wide geographical range of Argentina. Moreover, we have even incorporated in our sampling wild bee populations from Chaco province, which had never received acaricide treatments. Even though our study used a smaller number of bees per beehive for the detection of *A. woodi* compared to that of Eguaras et al. (1998), it should be noted that global sampling included a total of bees similar to those analyzed by them. This *n* was sufficient to detect the presence of the parasite seventeen years ago. In this sense, the main difference between the two studies was that our research maximized the sampling effort in terms of territory coverage. We have even added PCR techniques to reinforce the results found by visual detection, improving in this way the robustness of the data presented in the present study. Here, we are providing results that could suggest changes in the population dynamics of *A. woodi* in Argentina during the winter, a very different situation from that reported years ago by Eguaras and co-workers. Likewise, considering these observations, in the future monthly sampling and more sampling sites should be considered to definitively establish a potential regression in the presence of the mite in Argentina.

One possible reason for the absence of *Acarapis* in the samples analyzed in this study could be the heavy use of miticides in the country to control Varroosis. In this country, the use of pyrethroids, fluvalinate and flumethrin, amitraz and coumaphos is deeply rooted (Maggi et al., 2008, 2016). In fact, the excessive use of these synthetic drugs has led to the emergence of resistant populations of *Varroa* and to the contamination of bee products (Maggi et al., 2009, 2011; Medici et al., 2014). Similar phenomena have been reported to explain the absence of other parasites of bees as *Braula coecca* (Diptera: Braulidae), that were usually found inside of bee hives (Eguaras & Ruffinengo, 2006). In the case of *A. woodi*, it has been reported in other countries, that the continuous use of acaricides against *Varroa* have indirectly impacted in *Acarapis* populations (Garrido-Bailón et al., 2012; Kojima et al., 2011).

Considering that in recent years there has been reported an increased mortality in bee colonies in this country (Maggi et al., 2013), we might suggest that currently *A. woodi* may not be a major factor impacting colony losses reported in Argentina.

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Disclosure statement

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