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SHORT COMMUNICATION

Detection of honey bee viruses in Argentinian stingless bees (Hymenoptera: Apidae)

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Abstract The Meliponini is a eusocial group of bees tropically distributed. In Argentina, 37 species have been recorded, mostly in Misiones province. They use a great variety of sites to build their nests including tree hollows, cavities formed in rocks, human constructions or underground spaces. Numerous natural enemies are associated with stingless bees, including viruses. Until now, some viruses present in honey bees and related to the colony collapse disorder (CCD), have been found in several wild bees around the world. Here, we have studied the presence of honey bee viruses in stingless bees from different locations of Misiones province, Argentina. On this research, 73 samples of ten workers belonging to 12 species of stingless bees and feral honey bees, have been analyzed. Our results confirm the presence of three viruses: ABPV, IAPV and DWV Type A in four species of stingless bees and feral honey bees. More studies are required to establish if ABPV, IAPV and DWV are natural pathogens of stingless bees that have been spilled over to honey bees, or were transmitted by *Apis mellifera* to stingless bees.

Keywords Meliponini · Apiformes · Health · Virus · Misiones province

Introduction

Meliponini is mainly a tropical group of bees which contains over 500 species, with the highest richness in the Neotropical region (~400 species, 33 genera) (Camargo and Pedro 2007; Melo 2016). Particularly, Argentina represents a marginal area in the distribution of meliponines (Roig-Alsina et al. 2013) and up to now 37 species were recorded being Misiones the province with more diversity of Meliponini (Alvarez L, unpublished). Stingless bees, as they are commonly named, are eusocial bees with perennial colonies that have different female castes. Nesting behaviour involves nest construction in a wide variety of sites, not only mainly inside tree hollows or branches but also in cavities formed underground, rocks, human constructions and exposed nests (Michener 2007, 2013; Roubik 2006). Meliponines are one of the most abundant and common bees in the Neotropical region. They play a very important role as pollinators in tropical forests (Roubik 1989). Their social behaviour, the great difference between the number of individuals of the colony, the morphological diversity and the great range of species of plants visited by this group of bees make them good candidates as a future alternative in commercial pollination (Slaa et al. 2006). Culturally important due to honey production, stingless bees have been exploited and bred by different cultures representing an important food and medicinal resource (Arenas 2003; Zamudio et al. 2010). In Argentina, particularly in Misiones province, meliponiculture is a common practice in rural communities and the indigenous population (Zamudio and Alvarez 2016).

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As other native bees, meliponines have numerous natural enemies including predators and parasites, (Brown 1997; 2006; Noguera-Neto 1997; Brown and Kung 2006) however, associations with pathogens are scarcely known for this group of bees (Nunes-Silva et al. 2016; Porrini et al. 2017). Actually, there is little information about bee viruses that infect stingless bees. Ueira-Vieira et al. (2015) found the Acute Bee Paralysis Virus (ABPV) in *Melipona scutellaris* Latreille in Brazil and Guzman-Novoa et al. (2015) reported the presence of Deformed Wing Virus (DWV) and Black Queen Cell Virus (BQCV) in *Scaptotrigona mexicana* (Guérin) in Mexico. In contrast, 24 viruses have already been reported on honey bees (*Apis mellifera* L.), and almost all of them are positive-strand RNA viruses commonly named *Picorna-like virus*. Many of these honey bee pathogenic viruses only cause covert infections, which show no clinical signs and have no detectable impact on infected bees (McMenamin and Genersch 2015). However, the presence of some viruses was related to the colony collapse disorder (CCD), a serious disease in which adult worker bees abruptly disappear and die, leading to the collapse of the entire colony (Brutscher et al. 2016). According to the negative impact that CCD reached worldwide in the last years, the epidemiologic study of these viruses became a matter of vital importance. In this sense, Fürst et al. (2014) have alerted about the emerging infectious disease (EIDs) associated with "spill-over" from domestic animals to wildlife populations living in proximity. In this way, pathogens of honey bees could spill-over to wild bees such as bumblebees and meliponines causing a decline in their number.

To increase our knowledge on pathogens that can affect populations of native bees in Argentina, and to get information about honey bee viruses in wild bees, the aim of the present study was to detect the presence of honey bee viruses in stingless bees from different locations of Misiones province, Argentina.

Materials and methods

Bees were sampled from 11 locations in Misiones province between December 2015 and April 2017 (Fig. 1; Table 1). No commercial honey bee hives were found surrounding the sampling areas. Stingless bees were collected from: natural nests found in trunk cavities, brick walls, or aerial external nest, managed beehives under meliponiculture conditions and foragers on flowers/fecal feline and perspiration (Fig. 2; Table 1). In addition, foraging feral *A. mellifera* adults were collected on flowers. Fifteen to twenty bees from each species were collected using plastic bags. All specimens were stored on ice, freeze-killed at − 20 °C and finally transferred to − 80 °C to ensure optimal viral RNA preservation until its use. Some collected individuals were pinned for taxonomic

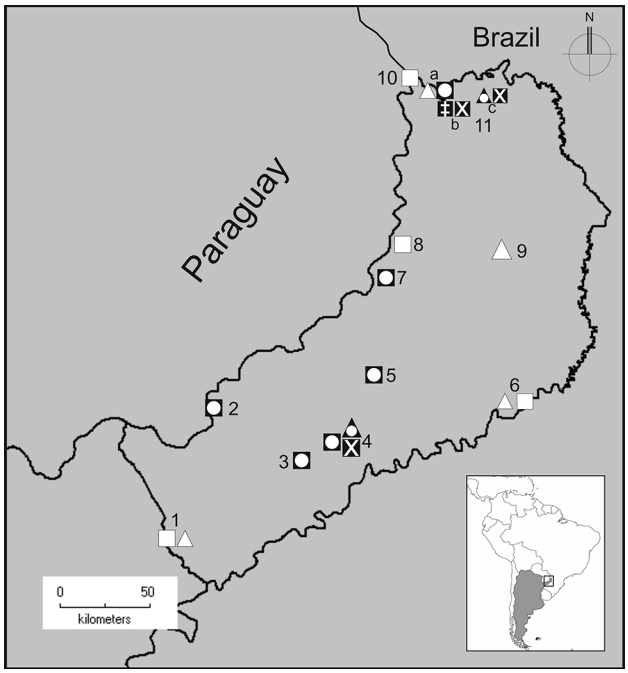

Fig. 1 Locations in Argentina where stingless bees where collected. Square: stingless bees and triangle: *Apis mellifera*. Black: positive viruses. White: negative viruses. Double cross: ABPV. Diagonal cross: IAPV. Circle: DWV positive. Location references: (1) Estación Apóstoles (− 27.912°, − 55.804°); (2) San Ignacio (− 27.243°,− 55.565°); (3) Oberá (− 27.484°, − 55.115°); (4) Campo Ramón (− 27.451°, − 55.024°); (5) Picada Libertad (− 27.104°, − 54.802°); (6) Rta Prov. 2 (− 27.227°, − 54.017°); (7) Montecarlo (− 26.573°, − 54.74°); (8) Eldorado (− 26.405°, − 54.664°); (9) Rta Prov. 20 (− 26.462°, − 54.138°); (10) Puerto Iguazú (− 25.595°, − 54.59°); 11. Parque Nacional Iguazú (site a: − 25.678°, − 54.449°; site b: − 25.675°, − 54.23° and site c: − 25.722°, − 54.404°)

identification and deposited in the collection of the Museo de La Plata, Argentina (MLP). Samples were analyzed using a multiplex PCR (mPCR) according to Sguazza et al. (2013). To test the six viruses already detected in Argentinian honey bees, some modifications were done. Briefly, each sample (ten workers) was crushed separately in stomacher bags with 1 ml of phosphate-buffered saline and clarified by centrifugation at 1500 g for 15 min. Total RNA was extracted using Viral Nucleic Acid Extraction Kit II (Real Biotech) according to manufacturer's protocol, and the RNA was eluted with 50-µl nuclease-free water. Two negative mPCR controls were used: (1) sample prepared by excluding the cDNA from the reaction and (2) negative samples from stingless bees previously analyzed in the laboratory. Positive controls were taken from honey bee-positive samples detected in previous studies in Argentina. The mPCR was carried out using primers previously described in Sguazza et al. (2013) with a thermal protocol which consist of a denaturalization cycle of 5 min at 95 °C, followed by 40 amplification cycles [30 s at 95 °C, 30 s at 53 °C, extension of 60 s at 72 °C] and a

Table 1 Summary of localities and species of bees collected in Misiones province, with information on bees sampled, nesting substrate and detection of virus by mPCR

Locality	Species	N° of Individuals	Habitat	Substrate	Viruses
1. Estación Apóstoles	*Apis mellifera*	10	Foragers	On flowers	–
	Trigona spinipes	20	Foragers	On flowers	–
2. San Ignacio	*Tetragonisca fiebrigi*	10	Natural nest	Tree trunk	DWV
3. Oberá	*Tetragona clavipes*	10	Meliponary	Hive	–
	Tetragonisca fiebrigi	40	Meliponary	4 hives	DWV (1/4)
4. Campo Ramón	*Apis mellifera*	10	Foragers	On flowers	DWV
	Tetragonisca fiebrigi	40	Meliponary	4 hives	IAPV(1/4) DWV(2/4)
	Trigona spinipes	10	Foragers	On flowers	–
5. Picada Libertad	*Tetragonisca fiebrigi*	40	Natural nests	2 nests in brick walls and 2 in tree trunks	DWV (2/4)
6. Rta Prov. 2	*Apis mellifera*	10	Foragers	On flowers	–
	Lestrimelitta chacoana	10	Natural nest	Tree trunk	–
	Plebeia emerinoides	10	Foragers	Perspiration	–
	Tetragonisca fiebrigi	30	Natural nests	3 in tree trunks	–
7. Montecarlo	*Plebeia droryana*	20	Meliponary	2 hives	–
	Plebeia emerinoides	10	Meliponary	Hive	–
	Tetragona clavipes	10	Meliponary	Hive	–
	Tetragonisca fiebrigi	40	Meliponary	4 hives	DWV (4/4)
8. El Dorado	*Lestrimelitta chacoana*	10	Natural nest	Brick wall	–
	Melipona q. quadrifasciata	20	Meliponary	2 hives	–
	Plebeia droryana	10	Meliponary	Hive	–
	Plebeia emerinoides	10	Meliponary	Hive	–
	Scaptotrigona aff. postica	10	Natural nest	Tree trunk	–
	Tetragona clavipes	10	Natural nest	Tree trunk	–
	Tetragonisca fiebrigi	40	Natural nests	3 in brick walls and 1 in tree trunk	–
	Tetragonisca fiebrigi	10	Meliponary	Hive	–
9. Rta Prov. 20	*Apis mellifera*	10	Foragers	On flowers	–
10. Puerto Iguazú	*Plebeia emerinoides*	30	Natural nests	3 nests in stone walls	–
	Scaptotrigona depilis	10	Natural	Tree trunk	–
	Tetragona clavipes	10	Natural nest	Tree trunk	–
	Tetragonisca fiebrigi	10	Natural nest	Tree trunk	–
11. Parque Nacional Iguazú					
Site a	*Apis mellifera*	10	Foragers	On flowers	–
	Tetragonisca fiebrigi	70	Natural nests	3 nests in brick walls and 4 in tree trunks	DWV (3/7)
	Trigona spinipes	10	Natural nest	External nest on branches of tree	–
Site b	*Apis mellifera*	10	Foragers	On flowers	DWV
	Plebeia droryana	20	Foragers	Perspiration	IAPV (1/2)
	Plebeia emerinoides	10	Natural nest	Dead tree trunk	ABPV
	Tetragona clavipes	10	Foragers	On flowers	–
Site c	*Cephalotrigona capitata*	10	Foragers	On feline fecal matter	–
	Melipona torrida	10	Foragers	On feline fecal matter	–
	Plebeia emerinoides	10	Foragers	Perspiration	IAPV
	Scaptotrigona depilis	10	Forager	On feline fecal matter	–
	Schwarziana quadripunctata	10	Forager	On feline fecal matter	–
	Tetragonisca fiebrigi	10	Natural	Tree trunk	–
	Trigona spinipes	10	Forager	On feline fecal matter	IAPV

ABPV acute bee paralysis virus, *DWV* deformed wing virus, *IAPV* Israeli acute paralysis virus

−: Negative sample

Fig. 2 Examples of substrates where stingless bees were collected. **a** Hive of *Tetragonisca fiebrigi* in a meliponary; **b** natural nest of *T. fiebrigi* inside tree trunk; **c** entrance of natural nest of *Lestrimelitta chacoana* on tree trunk; **d** aerial external nest of *Trigona spinipes*; **e** foragers stingless bees and honey bees on feline fecal matter; **f** nest entrance of *Scaptotrigona aff. postica* in a tree trunk

final extension of 5 min at 72 °C. The amplified product was analyzed using 2% agarose gel electrophoresis and ethidium bromide staining. The positive PCR products were purified using a gel extraction kit (Wizard® SV Gel&PCR Clean Up, Promega Madison, Wisconsin, USA) and sequenced (Biotechnology Resource Center, University of Cornell, Ithaca, USA). The sequences were analyzed using Basic Local Alignment Search Tool (BLAST) software.

Results

In total, 73 samples of 10 workers of bees were analyzed. Six samples belong to *Apis mellifera* L. and the others 67 samples belong to 12 species of stingless bees: *Cephalotrigona capitata* (Smith) ($n=1$), *Lestrimelitta chacoana* Roig-Alsina ($n=2$), *Melipona quadrifasciata quadrifasciata* Lepeletier ($n=2$), *M. torrida* Friese ($n=1$), *Plebeia droryana* (Friese) ($n=5$), *P. emerinoides* (Silvestri) ($n=8$), *Scaptotrigona depilis* (Moure) ($n=2$), *S. aff. postica* ($n=1$), *Schwarziana quadripunctata* (Lepeletier) ($n=1$), *Tetragona clavipes* (Fabricius) ($n=5$), *Tetragonisca fiebrigi* (Schwarz) ($n=34$), *Trigona spinipes* Fabricius ($n=5$) were analyzed (Table 1).

We registered the presence of three viruses, ABPV, Israeli Acute Paralysis Virus (IAPV) and DWV, in specimens of stingless bees and feral honey bees (Table 1). Four species of meliponines were detected positive, for honey bee virus for the first time: ABPV for *P. emerinoides*, IAPV for *P. emerinoides, P. droyana, T. fiebrigi* and *T. spinipes* and DWV for *T. fiebrigi*, whilst *A. mellifera* only was positive for DWV, in foragers, natural nest and managed beehives. BLAST results confirmed the identity of the mPCR-amplified sequences. Amplified products of DWV were identified at each other and showed 100% of homology with the JQ413340 sequence and belong to type A, ABPV showed 99% of homology with the AF486072 sequence and IAPV KY243933 showed 99% of homology with the AF486072 sequence (Fig. 3).

Discussion

Several viruses previously found in *A. mellifera* have been detected in flower-visiting insects (mainly wild bees and wasps) in North America (Dolezal et al. 2016) and around the world (McMahon et al. 2015; Miao et al. 2016; Singh et al. 2010; Tehel et al. 2016). Particularly, in South America, up to now six viruses have been recorded for honey bees (ABPV, BQCV, CBPV, DWV, IAPV and SBV) being ABPV, BQCV, DWV and SBV widely spread in commercial hives (Maggi et al. 2016). On the other hand, the presence of honey bee viruses in wild bees were reported by Reynaldi et al. (2013) that recorded a viral co-infection in individuals of *Bombus pauloensis* Friese (as *B. atratus*) by BQCV, SBV and DWV. Recently, Gamboa et al. (2015) added the ABPV and Like Sinai Virus to bumblebees from Colombia; Lucia et al. (2014) reported the presence of DWV in the large carpenter bee *Xylocopa augusti* Lepeletier and Ueira-Vieira et al. (2015) recorded ABPV in the stingless bee *M. scutellaris* in Brazil. Nowadays, three types of DWV (A, B and C) were reported worldwide, however, the primers used in this study to amplify DWV recognized only the types A and B. Type C was not considered because, up to now, few records exist worldwide and particularly in South America there are no records. Regarding ABPV primers, Sguazza et al. (2013) explains that the primers used to avoid the potential cross-amplification related to the ABPV-KBV-IAPV virus complex due to the fact that they were designed based on a pattern of sequence conservation and variation within and between these groups of viruses.

In honey bees, some viruses follow a vertical, horizontal, or both, transmission pathways. Horizontal transmission pathways include food-borne and fecal-oral routes inside the colony (Chen 2011). However, vector-borne transmission via *Varroa* destructor is considered the main vector of ABPV, IAPV and DWV in *A. mellifera* (McMenamin and Genersch 2015). Due to the absence of *Varroa* mites in stingless bees, there might be alternative routes of infection of these viruses, probably through the robbery of honey from infected *Apis* colonies or by collection and ingestion of pollen and nectar from flowers previously visited by infected bees (Singh et al. 2010; Tehel et al. 2016). Lanzi et al. (2006) mentioned that the presence of viral RNA in an individual does not necessarily indicate an active infection. We detected DWV in foraging bees that clearly did not show the deformed wing symptoms; so, we considered that these bees are asymptomatic carriers. Even though these asymptomatic bees do not show

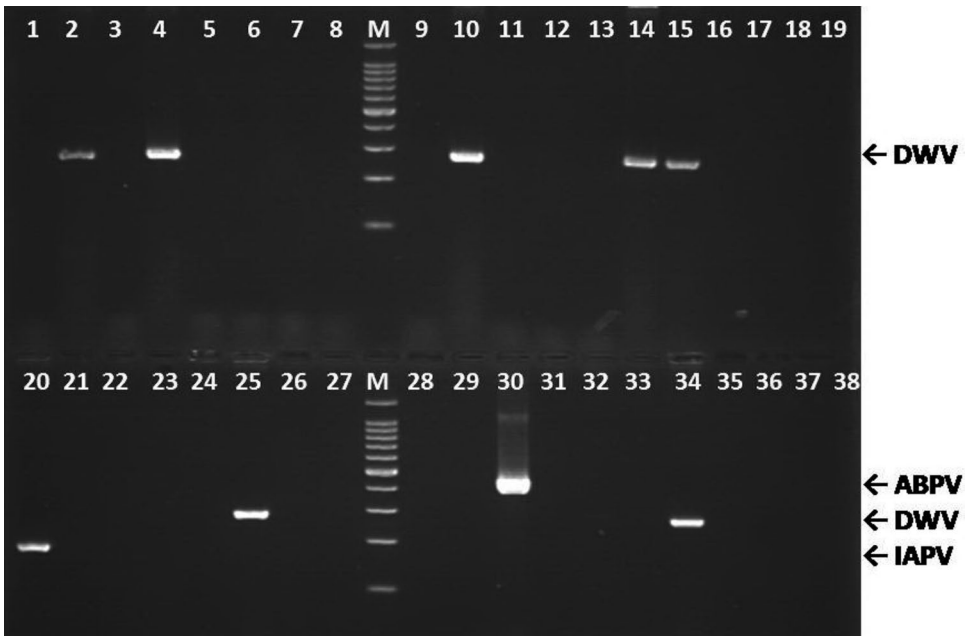

Fig. 3 2.5% agarose gel electrophoresis of mPCR product of 38 out of 67 stingless bees and 6 honey bee samples, stained with ethidium bromide. Lane M: molecular weight marker (100–1500), Lanes 2, 4, 10, 14, 15, 25 and 34 DWV-positive samples; Lane 30: ABPV-positive sample. Lane 20: IAPV-positive sample

substantial mortality in a short period of time, the viral presence could have an effect in lifespan, reproduction and overwintering (Dolezal et al. 2016). The absence of commercial hives of *A. mellifera* in the sampled locations and the wide distribution of positive DWV samples (210 km) could indicate that this virus might be more common in native bees than expected. Taylor et al. (2007) suggested that feral honeybee colonies could represent a risk to the managed population, harbouring disease agents and re-infecting managed stocks. Recently, Thompson et al. (2014) reported that feral honey bees contained a significant higher level of DWV than managed honey bee colonies. However, as the density of feral honey bee colonies was not calculated in Misiones province yet, it is not possible to estimate the importance of these populations of bees in the dispersion and reservoir of honey bee viruses to other wild bees.

Regarding ABPV, Tehel et al. (2016) proposed that this virus spill-over from wild bees to honey bees, according to their prevalence and pathogeny. However, we found only a positive sample to ABPV out of 73 samples. Considering that the prevalence of this virus in honey bees is near 20% in Argentina (Castilla et al. 2015), we supposed that the natural history of this virus in South America could be different from the one suggested for Tehel et al. (2016). Up to now, IAPV has only been detected in honey bee workers from Buenos Aires province (Reynaldi et al. 2011), but our results revealed that this virus is present in stingless bee of Misiones. Thus, this wide range of distribution inside Argentina, the lack of detection in the bordering countries and the very low complains of CCD could be related to a particular natural history of these viruses in our region.

In conclusion, our results confirm the presence of honey bee viruses in wild bees never studied yet. Further work will be needed to establish if ABPV, IAPV and DWV type A are natural pathogens of stingless bees and have spilled over to honey bees, or are transmitted by *A. mellifera* to stingless bees.

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References

Arenas P (2003) Etnografía y Alimentación entre los Toba-Ñachilamole#ek y Wichí-Lhuku'tas del Chaco Central (Argentina). Latín Grafica S.R.L. Argentina, p 562

Brown BV (1997) Parasitic phorid flies: a previously unrecognized cost to aggregation behavior of male stingless bees. Biotropica 29:370–372

Brown B (2006) Revision of the untreated taxa of *Melaloncha s. s.* bee-killing flies (Diptera: Phoridae) Zootaxa 1280:1–68

Brown B, Kung G (2006) Revision of the *Melaloncha ungulata*-group of bee-killing flies (Diptera: Phoridae). Contrib Sci (Los Angeles) 507:1–31

Brutscher LM, McMenamin AJ, Flenniken ML (2016) The buzz about honey bee viruses. PLoS Pathog 12:e1005757

Camargo JMF, Pedro SRM (2007) Meliponini lepeletier, 1836. In: Moure JS, Urban D, Melo GAR (eds) Catalogue of bees (Hymenoptera, Apoidea) in the Neotropical Region. Sociedade Brasileira de Entomologia, Curitiba, pp 272–578

Castilla R, Reynaldi FJ, Sguazza GH, Pecoraro M, Galosi C (2015) Determinación de virus que afectan a las abejas durante el período 2009–2014. XXXV Reunión Científica Anual Sociedad Argentina de Virología, Ciudad de Buenos Aires, Argentina

Chen Y (2011) Viruses and viral diseases of the honey bee, *Apis mellifera*. In: Liu T, Kang L (eds) Recent Advances in entomological research. Higher Education Press, Beijing and Springer-Verlag, Berlin, pp 105–120

Dolezal AG, Hendrix SD, Scavo NA, Carrillo-Tripp J, Harris MA, Wheelock MJ, O'Neal ME, Toth AL (2016) Honey bee viruses in wild bees: viral prevalence, loads, and experimental inoculation. Plos One 11(11):e0166190. doi:10.1371/journal.pone.0166190

Fürst MA, McMahon DP, Osborne JL, Paxton RJ, Brown MJF (2014) Disease associations between honeybees and bumblebees as a threat to wild pollinators. Nature 506:364–368

Gamboa V, Ravoet J, Brunain M, Smagghe G, Meeus I, Figueroa J, Riaño D, de Graaf DC (2015) Bee pathogens found in *Bombus atratus* from Colombia: a case study. J Invertebr Pathol 129:36–39

Guzman-Novoa E, MbHamiduzzaman M, Angiano-Baez R, Correa-Benítez A, Castañeda-Cervantes E, Arnold NI (2015) First detection of honey bee viruses in stingless bees in North America. J Apic Res 54:93–95

Lanzi G, de Miranda JR, Boniotti MB, Cameron CE, Lavazza A, Capucci L, Camazine SM, Rossi1 C (2006) Molecular and biological characterization of deformed wing virus of honeybees (*Apis mellifera* L.). J Virol 80:4998–5006

Lucia M, Reynaldi FJ, Sguazza GH, Abrahamovich AH (2014) First detection of deformed wing virus in *Xylocopa augusti* larvae (Hymenoptera: Apidae) in Argentina. J Apic Res 53:466–468

Maggi MD, Antúnez K, Invernizzi C, Aldea P, Vargas M, Negri P, Brasesco C, De Jong D, Message D, Teixeira EW, Principal J, Barrios C, Ruffinengo S Rodríguez Da Silva R, Eguaras MJ (2016) Honeybee health in South America. Apidologie 47:835–854

McMahon DP, Fürst MA, Caspar J, Theodorou P, Brown MJF, Paxton RJ (2015) A sting in the spit: widespread cross-infection of multiple RNA viruses across wild and managed bees. J Anim Ecol 84:615–624

McMenamin AJ, Genersch E (2015) Honey bee colony losses and associated virus. Curr Opin Insect Sci 8:121–129

Miao W, Junlong B, Lei W, Danyin Z, Xiaotian M, Wengui L, Wenzheng Z, Gefen Y, Jianping L, Shaoyu H (2016) Prevalence of four common bee RNA viruses in eastern bee populations. J Veterinar Sci Technol 7:1. doi:10.4172/2157-7579.1000284

Michener CD (2007) The Bees of the world. 2nd edn. Johns Hopkins University Press, Baltimore, p 953

Michener CD (2013) The meliponini. In: Vit P, Pedro SRM, Roubik DW (eds) Pot-honey: a legacy of stingless bees. Springer, Berlin pp 3–17

Melo GAR (2016) *Plectoplebeia*, a new Neotropical genus of stingless bees (Hymenoptera: Apidae). Zoologia 33:e20150153

Nogueira-Neto P (1997) Vida e criação de abelhas indígenas sem ferrão. Editora Nogueirapis, São Paulo, p 446

Nunes-Silva P, Piot N, Meeus I, Blochtein B, Smagghe G (2016) Absence of Leishmaniinae and Nosematidae in stingless bees. Sci Rep 6:32547

Porrini MP, Porrini LP, Garrido P, De Melo e Silva Neto C, Porrini D, Muller F, Nuñez LA, Alvarez LJ, Fernández Iriarte P, Eguaras MJ (2017) *Nosema ceranae* on Argentinian and Brazilian native stingless bees and social wasp. Microb Ecol. doi:10.1007/s00248-017-0975-1

Reynaldi FJ, Sguazza GH, Tizzano MA, Fuentealba N, Galosi CM, Pecoraro MR (2011) First report of Israeli acute paralysis virus in asymptomatic hives of Argentina. Rev Argent Microbiol 43:84–86

Reynaldi FJ, Sguazza GH, Albicoro FJ, Pecoraro MR, Galosi CM (2013) First molecular detection of co-infection of honey bee viruses in asymptomatic *Bombus atratus* in South America. Braz J Biol 73:797–800

Roig-Alsina A, Vossler FG, Gennari GP (2013) Stingless bees in Argentina. In: Vit P, Pedro SRM, Roubik DW (eds) Pot-honey: a legacy of stingless bees. Springer, Berlin, pp 125–134

Roubik DW (1989) Ecology and natural history of tropical bees. Cambridge University Press, New York, p 514

Roubik DW (2006) Stingless bee nesting biology. Apidologie 37:124–143

Sguazza GH, Reynaldi FJ, Galosi CM, Pecoraro MR (2013) Simultaneous detection of bee viruses by multiplex PCR. J Virol Methods 194:102–106

Singh R, Levitt AL, Rajotte EG, Holmes EC, Ostiguy N, Vanengelsdorp D, Lipkin WI, Depamphilis CW, Toth AL, Cox-Foster DL (2010) RNA viruses in hymenopteran pollinators: evidence of inter-Taxa virus transmission via pollen and potential impact on non-*Apis* hymenopteran species. PLoS One 5:e14357

Slaa EJ, Sánchez Chaves LA, Malagobi-Braga KS, Hofstede FE (2006) Stingless bees in applied pollination: practice and perspectives. Apidologie 37:293 – 315

Tehel A, Brown MJ, Paxton RJ (2016) Impact of managed honey bee viruses on wild bees. Curr Opin Virol 19:16–22

Thompson CE, Biesmeijer JC, Allnutt TR, Pietravalle S, Budge GE (2014) Parasite pressures on feral honey bees (*Apis mellifera* sp.). Plos One 9:e105164

Taylor MA, Goodwin RM, McBrydie HM, Cox HM (2007) Destroying managed and feral honey bee (*Apis mellifera*) colonies to eradicate honey bee pests. N Z J Crop Hortic Sci 35:313–323

Ueira-Vieira C, Oliveira-Almeida L, Correadealmeida F, Marques I, Brandemburgo MAM, Onetti AM (2015) Scientific note on the first molecular detection of the acute bee paralysis virus in Brazilian stingless bees. Apidologie 46:628–630. doi:10.1007/s13592-015-0353-2

Zamudio F, Kujawska M, Hilgert NI (2010) Honey as medicinal and food resource. Comparison between Polish and multiethnic settlements of the Atlantic forest, Misiones, Argentina. Open Complement Med J 2:58–73

Zamudio F, Alvarez LJ (2016) Abejas sin aguijón de Misiones: Guía etnotaxonómica. Editorial de la UNC, Córdoba, Argentina, p 217