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Nosema ceranae has been present in Brazil for more than three decades infecting Africanized honey bees

Erica Weinstein Teixeira [a,*], Lubiane Guimarães dos Santos [b], Aroni Sattler [c], Dejair Message [d], Maria Luisa Teles Marques Florencio Alves [a], Marta Fonseca Martins [e], Marina Lopes Grassi-Sella [f], Tiago Mauricio Francoy [f]

[a] Laboratório de Sanidade Apícola (LASA), Pólo Regional do Vale do Paraíba, Agência Paulista de Tecnologia dos Agronegócios (APTA), Secretaria de Agricultura e Abastecimento do Estado de São Paulo, Caixa Postal 07, CEP 12.400-970 Pindamonhangaba, SP, Brazil
[b] Programa de Pós-graduação em Entomologia, Universidade Federal de Viçosa (UFV), Avenida Peter Henry Rolfs s/n, CEP 36.571-000 Viçosa, MG, Brazil
[c] Depto. de Fitossanidade, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bento Gonçalves, 7712, CEP 91540-000 Porto Alegre, RS, Brazil
[d] Depto. de Ciências Animais, Universidade Federal Rural do Semiárido (UFERSA), BR110 KM 47, CEP 59.625-900 Mossoró, RN, Brazil
[e] Laboratório de Genética Molecular, EMBRAPA Gado de Leite, Rua Eugênio do Nascimento, 610, CEP 36.038-330 Juiz de Fora, MG, Brazil
[f] Escola de Artes, Ciências e Humanidades, Universidade de São Paulo (USP), Rua Arlindo Bétio, 1000, CEP 03.828-000 São Paulo, SP, Brazil

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ABSTRACT

Until the mid-1990s, the only microsporidium known to infect bees of the genus *Apis* was *Nosema apis*. A second species, *Nosema ceranae*, was first identified in 1996 from Asian honey bees; it is postulated that this parasite was transmitted from the Asian honey bee, *Apis cerana*, to the European honey bee, *Apis mellifera*. Currently, *N. ceranae* is found on all continents and has often been associated with honey bee colony collapse and other reports of high bee losses. Samples of Africanized drones collected in 1979, preserved in alcohol, were analyzed by light microscopy to count spores and were subjected to DNA extraction, after which duplex PCR was conducted. All molecular analyses (triplicate) indicated that the drones were infected with both *N. ceranae* and *N. apis*. PCR products were sequenced and matched to sequences reported in the GenBank (Acc. Nos. JQ639316.1 and JQ639301.1). The venation pattern of the wings of these males was compared to those of the current population living in the same area and with the pattern of drones collected in 1968 from Ribeirão Preto, SP, Brazil, from a location close to where African swarms first escaped in 1956. The morphometric results indicated that the population collected in 1979 was significantly different from the current living population, confirming its antiquity. Considering that the use of molecular tools for identifying *Nosema* species is relatively recent, it is possible that previous reports of infections (which used only light microscopy, without ultrastructural analysis) wrongly identified *N. ceranae* as *N. apis*. Although we can conclude that *N. ceranae* has been affecting Africanized honeybees in Brazil for at least 34 years, the impact of this pathogen remains unclear.

1. Introduction

Until the mid-1990s it was believed that *Nosema apis* was the only microsporidian species associated with bees of the genus *Apis* (Fries et al., 1996). However, there are reports of commercial colonies infected with a different type of micrsporidium dating from the 1970s, when it was suggested to be a species other than *N. apis* due to the capacity to infect larvae at 3 or more days of age (Buys, 1972, 1977). Subsequently, Clark (1980) demonstrated morphological details of spores, by means of electron microscopy and proposed the existence of a second microsporidian species that afflicts honey bees. He postulated that the low number of spores found in the hundreds of bees that he analyzed was an indication that honey bees were not a usual host, although he did not identify the species at that time.

Morphological and molecular characterization of a second *Nosema* species that afflicts bees of the *Apis* genus, *Nosema ceranae*, was first reported in 1996, from Asian honey bees (Fries et al., 1996). This species was detected naturally infecting European honey bees in central and northern Spain in 2004 and 2005, respectively (Higes et al., 2006). Experimental contamination of colonies resulted in up to 100% infection of the bees (Higes et al., 2007).

It is postulated that *N. ceranae* was transmitted from *Apis cerana* to *Apis mellifera* (Higes et al., 2006; Klee et al., 2007).

* Corresponding author. Fax: +55 12 36421812.
E-mail addresses: erica@apta.sp.gov.br (E.W. Teixeira), lubi.guimaraes@gmail.com (L.G. dos Santos), aronisattler@yahoo.com.br (A. Sattler), dejair.message@gmail.com (D. Message), marta.martins@embrapa.br (M.F. Martins), marinalg@usp.br (M.L. Grassi-Sella), tfrancoy@usp.br (T.M. Francoy).

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The process of infection with *N. ceranae* begins when spores are ingested by the bees during the cleaning process (when they remove sick or dead hive members), while foraging or when they consume contaminated food (Higes et al., 2010). This microorganism enters the host's digestive tract, germinates into the epithelial cells where it reproduces and mature spores are then released along with the feces, potentially contaminating the entire colony (Somerville and Hornitzky, 2007). Infection by *Nosema* spp. can damage the bees' digestive system, reducing bee longevity and colony populations, diminishing both honey production and pollination activity (Chen et al., 2008; Higes et al., 2008; Whitaker et al., 2010).

Currently, *N. ceranae* is known to infect bees on five continents (Klee et al., 2007; Martín-Hernández et al., 2007; Giersch et al., 2009) and has frequently been associated with the phenomenon of bee disappearance (Higes et al., 2006, 2007; Cox-Foster et al., 2007; Martín-Hernández et al., 2007; Chen et al., 2009; Paxton, 2010). In South America, studies have confirmed the presence of *N. ceranae* in Brazil (Klee et al., 2007), Uruguay (Invernizzi et al., 2009), Argentina (Medici et al., 2012) and Chile (Martínez et al., 2012).

Here, we report the presence of the species *N. apis* and *N. ceranae* in Africanized *A. mellifera* drones that were collected in 1979 in southern Brazil. We also characterized the drones using geometric morphometric analyses; the venation pattern of the wings of these drones was examined, allowing us to confirm that these drone specimens differ from the population that currently occur in that region.

2. Material and methods

The drone specimens collected in 1979, conserved in ethanol solution and stored in the collection of Rio Grande do Sul Federal University, Porto Alegre, Brazil, were sent to the Honey Bee Health Laboratory (LASA) of the São Paulo State Agribusiness Technology Agency (APTA) located in Pindamonhangaba, São Paulo, Brazil. The sample, coded as "col. CDR", was from a collection maintained by Professor Dauro Correia Redaelli (now deceased) of the Phytosanitary Department, who used bees from the university's apiary in his research.

Ten drones were pooled and macerated, using 1 mL of sterile distilled water per drone. The macerate was filtered and after constant agitation of the filtrate, a micropipette was used to remove a small aliquot of the suspension, which was deposited in a Neubauer chamber. The spore suspension was analyzed under light microscopy at 400× (Cantwell, 1970), to count the spores. The remainder of the spore suspension was centrifuged at 2518*g* for 40 min at room temperature. The supernatant was discarded and the pellet was resuspended in 1 mL of sterile water. This suspension was centrifuged at 10,000*g* for 5 min, after which the supernatant was discarded and the pellet was submitted for DNA extraction employing a Qiagen DNeasy® Plant Mini Kit, according to the manufacturer's recommendations. After extraction, the sample was submitted to duplex-PCR (Martín-Hernández et al., 2007). The primer sequences utilized to amplify the 218 bp fragment corresponding to the 16S ribosomal gene of *N. ceranae* were 218MITOC-FOR 5′-CGGCGACGATGTGATATGAAAATATTAA-3′ and, 218MITOC-REV 5′-CCCGGTCATTCTCAAACAAAAAACCG-3′. The primer sequences used to amplify the 321 bp fragment corresponding to the 16S ribosomal gene of *N. apis* were 321APIS-FOR 5′-GGGGGCATGTCTTTGACGTACTATGTA-3′ 321APIS-REV 5′-GGGGGGCGTTTAAAATGTGAAACAACTATG-3′. Reactions were performed with a final volume of 20 μL in a Veriti® thermal cycler (Applied Biosystems, Foster City, CA, USA). The routine consisted of an initial denaturing step at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 58 °C for 30 s and 72 °C for 1 min, and a final extension step at 72 °C for 5 min. The PCR products were submitted for electrophoresis in 2% agarose gel (m/v), stained with SYBR SAFE® in 1X TBE buffer and visualized in a Safe Imager™ 2.0 Blue-Light Transilluminator (Invitrogen, Carlsbad, CA, USA). All analyses were carried out in triplicate, including positive and negative controls. These procedures were repeated in the Molecular Genetics Laboratory of the Embrapa Dairy Cattle Research Unit (Embrapa Gado de Leite), located in Juiz de Fora, Minas Gerais, Brazil. The PCR products were purified using a QIAquick Gel Extraction Kit, according to the manufacturer's recommendations, and then were submitted to sequencing reaction using a BigDye® Terminator v3.1 Cycle Sequencing Kit and a Veriti® thermal cycler (both from Applied Biosystems, Foster City, CA, USA). The reaction products were purified by precipitation in ethanol and injected into an Applied Biosystems 3130xl automatic sequencer. The sequences were analyzed with the LaserGene package (DNASTAR, Madison, Wisconsin, USA). BLAST searches were performed in the GenBank database (http://www.ncbi.nlm.nih.gov) to identify the samples.

The venation patterns of the wings of the drones were evaluated using morphometric analyses in order to demonstrate that these drone specimens do not belong to the population that currently lives in that region. We compared these wing patterns with those of the current population living in the same region and with those of specimens collected in 1968, in Ribeirão Preto, São Paulo State (Francoy et al., 2009, 2012). These older specimens came from a site near where African swarms escaped in 1956, initiating the Africanization process in Brazil. For this analysis, 20 wings from the group of drones sampled in 1979 were compared with 25 wings from drones from four current colonies in Porto Alegre collected in 2012, and 16 wings from the specimens collected in Ribeirão Preto in 1968. The comparisons were carried out according to the method described by Francoy et al. (2008). Briefly, 19 homologous landmarks were plotted at the wing vein intersections of each individual, using the software tpsDig2 version 2.16 (Rohlf, 2010a). The images were Procrustes aligned and the Cartesian coordinates X and Y from each landmark were used as inputs in a discriminant analysis. The squared Mahalanobis distances were used to determine the morphological proximity of these groups. The alignments and feature extractions were performed using tpsRelw version 1.49 (Rohlf, 2010b), and the statistical analyses were performed using Statistica version 7.0.

3. Results

The spore counts by light microscopy indicated the average of 350,000 spores/bee, without distinction between species. Molecular analyses identified both of the microsporidian species that are known to infect honey bees, *N. ceranae* and *N. apis*. The PCR products were sequenced and matched to sequences contained in the GenBank (accession numbers JQ639316.1 and JQ639301.1). Fig. 1 shows the gel with the respective amplified fragments.

The venation patterns of the wings of the drones from which the *Nosema* samples were collected differed from those of the population that currently inhabits the same region. Based on the-morphometric wing analysis, the three groups of drones were significantly different (Wilks' Lambda: 0.00456; $P < 0.00001$). Six Cartesian coordinates significantly contributed to the separation of the groups (Y7, 1X, 10X, 6X, 7X and 3X). The drones collected in 1979, which provided the *Nosema* spores, belonged to an Africanized honey bee population. Based on wing venation they differed significantly from the current population (Mahalanobis square distance (D2) = 10.46). Greater differences were found between these two population that was and the oldest population, collected from the region where Africanization began in Brazil

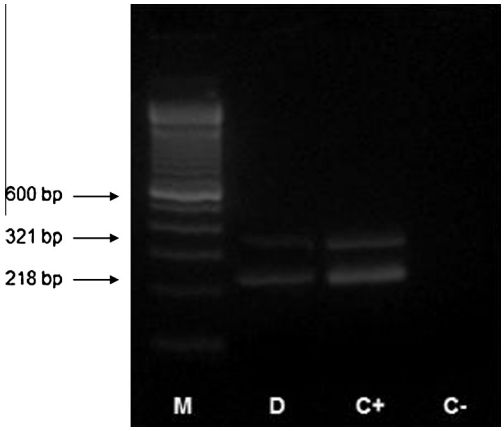

Fig. 1. Detection of *Nosema ceranae* and *Nosema apis* by PCR amplification of nucleic acids from pooled Africanized *A. mellifera* drones sampled in 1979. 2% Agarose gel stained with SYBR SAFE®. M: 100 bp marker, D: sample (drones), C+: positive control – *Nosema ceranae* (218 bp) and *Nosema apis* (321 bp), C−: negative control.

(Pop 1968 × Pop 1979 D2 = 45.47; Pop 1968 × Pop 2012 D2 = 57.74). All individuals were classified into their respective groups with probability greater than 0.98. The only exception was one individual from 1979, which gave $P = 0.60$.

4. Discussion

Our morphometric results were similar to those reported by Francoy et al. (2009), who compared Africanized worker bees collected in the 1960s with the ancestral subspecies and the current population. Although the bees collected in 1979 clearly were Africanized honey bees, they exhibited a profile closer to European bees than the current population, indicating increasing Africanization of the local populations with passage of time. Our results clearly show that the current population differs from the 1979 population.

The first report of *N. ceranae* in Brazil was reported by Klee et al. (2007); the authors confirmed the presence of this species of microsporidium on four continents. Our results indicate that both *N. apis* and *N. ceranae* have infected *A. mellifera* in Brazil for at least 34 years, the oldest finding of *N. ceranae* globally.

Previous reports suggested that *N. ceranae* passed from *A. cerana* to *A. mellifera* in Asia, probably in the 1990s, thereafter dispersing throughout the world (Fries et al., 1996; Higes et al., 2006; Klee et al., 2007; Paxton et al., 2007; Fries, 2010). However, it is not possible to determine precisely when or where *N. ceranae* passed from *A. cerana* to European honey bees Fries (2010), and this microsporidium has been found in various geographically isolated European bee populations. These facts, along with the chronic nature of *N. ceranae* infection have led to the suggestion that this species is not a new pathogen in a new host (Fries et al., 2006).

The present widespread distribution of *N. ceranae* indicates an overlap with *N. apis* in *A. mellifera*. *N. apis* is becoming less frequent, demonstrating successful adaptation of *N. ceranae* to various environments, including hot and dry climates (Martín-Hernández et al., 2009). This apparent competitive advantage related to wider temperature resistance remains an enigma, since the *N. ceranae* spores are less resistant than those of *N. apis*, as reported by Fries (2010). However, even though *N. ceranae* presents tissue tropism identical to that of *N. apis*, overall spore production and timing of release from host midgut cells is sufficiently different to provide an advantage for *N. ceranae* (Huang and Solter, 2013). These authors reported that *N. ceranae*-infected bees produced higher percentage of mature infective spores than *N. apis*-infected bees.

Other studies conducted by our group demonstrate ample prevalence of *N. ceranae* throughout Brazil. Among 637 samples analyzed between 2009 and 2012, from 47 municipalities in 10 Brazilian states (São Paulo, Santa Catarina, Paraná, Rio de Janeiro, Maranhão, Ceará, Mato Grosso do Sul, Bahia, Minas Gerais and Goiás), 79.9% of the samples were infected with *Nosema*, of which 98.82% were *N. ceranae* and only 0.39% were *N. apis*. Only 0.79% of the samples were infected by both species (S1). *N. apis* was found in two municipalities in two southern states, Santa Catarina (Caçador municipality) and Paraná (Tamarana municipality) (Teixeira et al., 2013) (S1).

Previous studies in Brazil, based only on light microscopic analyses (Flechtmann, 1964; Anjos and Silva, 1973; Gama et al., 1994; Teixeira et al., 1997), reported a high prevalence of *N. apis* in Brazilian apiaries, but without clinical signs or a need to implement chemical therapy. The greater disease tolerance found in Africanized compared to European *A. mellifera* (Guzmán-Novoa et al., 1999; Rosenkranz, 1999; Moretto and Mello, 1999, 2001; Aumeier, 2001; Vandame et al., 2002; Mondragón et al., 2005) could have been a factor in this lack of *Nosema*-infection symptomatology. Currently, even with high prevalence of *N. ceranae* in Brazilian apiaries, the official policy is not to use chemical products or drugs, due to uncertainties about whether this pathogen actually impacts colony health. Although fumagillin has been administered to control *N. ceranae* infection in some countries, Huang et al. (2013) have shown that this antibiotic can negatively affect bee physiology and may actually increase the prevalence of *N. ceranae*. There is also concern about the risk of bee product contamination, especially honey.

In Brazil, as also reported by Fries (2010) for Europe, *N. ceranae* infection appears to produce different effects in different geographic regions. In previous evaluations of samples from the state of São Paulo, we generally observed higher infection intensity in the autumn and winter; however, in some regions the greatest pathogen load was found in summer. In the region of Altinópolis, SP, for example, the prevalence of *N. ceranae* in 2007 was 100% in 1106 hives in 20 apiaries, with peaks of infection intensity distributed throughout the year (D. Message, unpublished data). In the central-western region of the same state, the highest infection intensity was found during the autumn fall season (Santos et al., 2011). Both localities have a humid subtropical climate (Köeppen climate classification), with temperatures varying from 15 to 29 °C during the year. Evident clinical signs, such dysentery or population collapse, which could be directly associated with *Nosema* infection or weather conditions, were not found. Although Chen et al. (2012) reported a significant negative correlation of *N. ceranae* pathogen load with temperature and suggested that average temperatures could predict the infection dynamics of *N. ceranae*, we have observed a total lack of pattern in infection intensity during the year in Brazil. Although this pathogen is amply present, the biological impacts remain unknown (Chen et al., 2009).

Studies in other South American countries (Martínez et al., 2012; Medici et al., 2012) present results similar to those reported here, namely detection of *N. apis*, but at very low prevalence, and co-infection with *N. ceranae*. According to Giersch et al. (2009), transport of bee products and contaminated beekeeping equipment, along with the practice of migratory apiculture, have facilitated the dispersion of this microsporidium. According to Fries (1997), *N. ceranae* develops faster in *A. mellifera* than does *N. apis* in *A. cerana*, concluding, in agreement with Higes et al. (2007), that *N. ceranae* is highly pathogenic and poses a significant threat to apiculture. However, Forsgren and Fries (2010) did not find significant differences when comparing the virulence of these two species in European bees.

Because of the difficulty of differentiating the species by light microscopy techniques (Fries et al., 1996), previous reports of infections by *N. apis* could have misdiagnosed the species.

Our findings confirm the efficacy of molecular diagnostic techniques applied to sample specimens stored for long periods (Chen et al., 2008) and point to new interpretations concerning dispersion of such pathogens around the world, or even about their origin.

The original host of *N. ceranae* is unknown, but it is presumed to be *A. cerana* because it was first isolated from that species. It could also be another bee species (Paxton, 2010). This pathogen might also circulate in the pollinator community and be transferred back to commercial honey bees (Li et al., 2012). The international movement of queens, colonies and honey bee products could have contributed to or intensified the spread of this pathogen, and particular conditions could have had an important role in the replacement of *N. apis* as the dominant microsporidian infection in many regions of the world. It is also possible that before the availability of molecular techniques, previous reports of infections in *A. cerana* and possibly in *A. mellifera* were actually caused by *N. ceranae* (Fries, 2010).

The microsporidium *N. ceranae* has been infecting Africanized *A. mellifera* bees in Brazil for at least the past 34 years. Because the use of molecular techniques for identifying species of the genus *Nosema* is relatively recent, further studies on presence, prevalence, and dispersion of these pathogens are warranted. More information is needed on the factors involved in the transmission and survival of this parasite to help clarify the impact of this pathogen in Africanized honey bee colonies. Morphometric analyses of the wing venation pattern were very effective in evaluating temporal changes in the Africanized honey bee groups.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jip.2013.09.002.

References

Anjos, L.D., Silva, R.M.B., 1973. Estudo sobre a ocorrência de nosemose em abelhas *Apis mellifera* L. no município de Pindamonhangaba. Bol. Ind. Anim. 30, 191–194.

Aumeier, P., 2001. Bioassay for grooming effectiveness towards *Varroa destructor* mites in Africanized and Carniolan honey bees. Apidologie 32, 81–90.

Buys, B., 1972. Nosema in brood. S. Afr. Bee J. 44, 2–4.

Buys, B., 1977. A nosema disease affecting honeybee brood. In: Symposium of Bee Biology and Pathology. Apimondia Publishing House, Romania, pp. 73–76.

Cantwell, G.R., 1970. Standard methods for counting nosema spores. Am. Bee J. 110, 222–223.

Chen, Y., Evans, J.D., Smith, I.B., Pettis, J.S., 2008. *Nosema ceranae* is a long-present and wide-spread microsporidean infection of the European honey bee (*Apis mellifera*) in the United States. J. Invertebr. Pathol. 97, 186–188.

Chen, Y.P., Evans, J.D., Zhou, L., Boncristiani, H., Kimura, K., Xiao, T., Litkowski, A.M., Pettis, J.S., 2009. Asymmetrical coexistence of *Nosema ceranae* and *Nosema apis* in honey bees. J. Invertebr. Pathol. 101, 204–209.

Chen, Y.W., Chung, W.P., Wang, C.H., Solter, L.F., Huang, W.F., 2012. *Nosema ceranae* infection intensity highly correlates with temperature. J. Invertebr. Pathol. 111, 264–267.

Clark, T.B., 1980. A second microsporidian in the honeybee. J. Invertebr. Pathol. 35, 290–294.

Cox-Foster, D.L., Conlan, S., Holmes, E.C., Palacios, G., Evans, J.D., Moran, N.A., Quan, P.-L., Briese, T., Hornig, M., Geiser, D.M., Martinson, V., vanEngelsdorp, D., Kalkstein, A.L., Drysdale, A., Hui, J., Zhai, J., Cui, L., Hutchison, S.K., Simons, J.F., Egholm, M., Pettis, J.S., Lipkin, W.I., 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. Science 318, 283–287.

Flechtmann, C.H.W., 1964. Ocorrência das principais doenças das abelhas. R. Agric. Piracicaba 39, 5–8.

Forsgren, E., Fries, I., 2010. Comparative virulence of *Nosema ceranae* and *Nosema apis* in individual European honey bees. Vet. Parasitol. 170, 212–217.

Francoy, T.M., Wittmann, D., Drauschke, M., Müller, S., Steinhage, V., Bezerra-Laure, M.A.F., De Jong, D., Gonçalves, L.S., 2008. Identification of Africanized honey bees through wing morphometrics: two fast and efficient procedures. Apidologie 39, 488–494.

Francoy, T.M., Wittmann, D., Steinhage, V., Drauschke, M., Müller, S., Cunha, D.R., Nascimento, A.M., Figueiredo, V.L.C., Simões, Z.L.P., De Jong, D., Arias, M.C., Gonçalves, L.S., 2009. Morphometric and genetic changes in a population of *Apis mellifera* after 34 years of Africanization. Genet. Mol. Res. 8, 709–717.

Francoy, T.M., Gonçalves, L.S., De Jong, D., 2012. Rapid morphological changes in populations of hybrids between Africanized and European honey bees. Genet. Mol. Res. 11, 3349–3356.

Fries, I., 1997. Protozoa. In: Morse, R.A., Flottum, K. (Eds.), Honey Bee Pests, Predators and Diseases, third ed. A.I. Root Company, Medina, Ohio, USA, pp. 59–76.

Fries, I., 2010. *Nosema ceranae* in European honey bees (*Apis mellifera*). J. Invertebr. Pathol. 103, 573–579.

Fries, I., Feng, F., Silva, A., Slemenda, S.B., Pieniazek, N.J., 1996. *Nosema ceranae* n. sp. (Microspora, Nosematidae), morphological and molecular characterization of a microsporidian parasite of the Asian honey bee *Apis cerana* (Hymenoptera, Apidae). Eur. J. Protistol. 32, 356–365.

Fries, I., Martín, R., Meana, A., García-Palencia, P., Higes, M., 2006. Natural infections of *Nosema ceranae* in European honey bees. J. Apic. Res. 45, 230–233.

Gama, P.H.G., Silva, R.M.B., Silva, E.C.A., Moreti, A.C.C.C., Alves, M.L.T.M.F., 1994. Preliminary studies of the *Nosema apis* sporulation curve in Pindamonhangaba, SP, Brasil. In: Proceedings of the Fifth International Conference on Apiculture in Tropical Climates. IBRA, Cardiff, pp. 126–128.

Giersch, T., Berg, T., Galea, F., Hornitzky, M., 2009. *Nosema ceranae* infects honey bees (*Apis mellifera*) and contaminates honey in Australia. Apidologie 40, 117–123.

Guzmán-Novoa, E., Vandame, R., Arechavaleta, E.M., 1999. Susceptibility of European and Africanized honey bees (*Apis mellifera* L.) to *Varroa jacobsoni* Oud. in Mexico. Apidologie 30, 173–182.

Higes, M., Martín-Hernández, R., Meana, A., 2006. *Nosema ceranae*, a new microsporidian parasite in honeybees in Europe. J. Invertebr. Pathol. 92, 93–95.

Higes, M., García-Palencia, P., Martín-Hernández, R., Meana, A., 2007. Experimental infection of *Apis mellifera* with *Nosema ceranae* (*Microsporidia*). J. Invertebr. Pathol. 94, 211–217.

Higes, M., García-Palencia, P., Martín-Hernández, R., Botías, C., Garrido-Bailón, E., González-Porto, A.V., Barrios, L., del Nozal, M.J., Berna, J.L., Jiménez, J.J., Palencia, P.G., Meana, A., 2008. How natural infection by Nosema ceranae causes honeybee colony collapse. Environ. Microbiol. 10, 2659–2669.

Higes, M., Matin-Hernandez, R., Meana, A., 2010. Nosema ceranae in Europe: an emergent type C nosemosis. Apidologie 41, 375–392.

Huang, W.F., Solter, L.F., 2013. Comparative development and tissue tropism of *Nosema apis* and *Nosema ceranae*. J. Invertebr. Pathol. 113, 35–41.

Huang, W.F., Solter, L.F., Yau, P.M., Imai, B.S., 2013. *Nosema ceranae* escapes fumagillin control in honeybee. Plos Pathog. 3, e1003185. <http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1003185>, (acecssed 04.06.13).

Invernizzi, C., Abud, C., Tomasco, I.H., Harriet, J., Ramallo, G., Campa, J., Katz, H., Gardiol, G., Mendonça, Y., 2009. Presence of *Nosema ceranae* in honeybees (*Apis mellifera*) in Uruguay. J. Invertebr. Pathol. 101, 150–153.

Klee, J., Besana, A.M., Genersch, E., Gisder, S., Nanetti, A., Tam, D.Q., Chinh, T.X., Puerta, F., Ruz, J.M., Kryger, P., Message, D., Hatjina, F., Korpela, S., Fries, I., Paxton, R.J., 2007. Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the western honey bee, *Apis mellifera*. J. Invertebr. Pathol. 96, 1–10.

Li, J., Chen, W., Wua, J., Peng, W., An, J., Schmid-Hempel, P., Schmid-Hempel, R., 2012. Diversity of *Nosema* associated with bumblebees (*Bombus* spp.) from China. Int. J. Parasitol. 42, 49–61.

Martínez, J., Leal, G., Conget, P., 2012. *Nosema ceranae* an emergent pathogen of *Apis mellifera* in Chile. Parasitol. Res. 111, 601–607.

Martín-Hernández, R., Meana, A., Prieto, L., Salvador, A.M., Garrido-Bailon, E., Higes, M., 2007. Outcome of colonization of *Apis mellifera* by *Nosema ceranae*. Appl. Environ. Microbiol. 73, 6331–6338.

Martín-Hernández, R., Meana, A., García-Palencia, P., Marín, P., Botías, C., Garrido-Bailón, E., Barrios, L., Higes, M., 2009. Temperature effect on biotic potential of honey bee microsporidia. Appl. Environ. Microbiol. 75, 2554–2557.

Medici, S.K., Sarlo, E.G., Porrini, M.P., Braunstein, M., Eguaras, M.J., 2012. Genetic variation and widespread dispersal of *Nosema ceranae* in *Apis mellifera* apiaries from Argentina. Parasitol. Res. 110, 859–864.

Mondragón, L., Vandame, R., Spivak, M., 2005. A multifactorial study of the resistance of honeybees *Apis mellifera* to the mite *Varroa destructor* over one year in Mexico. Apidologie 36, 345–358.

Moretto, G., Mello Jr., L.J., 1999. *Varroa jacobsoni* infestation of adult Africanized and Italian honey bees (*Apis mellifera*) in mixed colonies in Brazil. Genet. Mol. Biol. 22, 321–323.

Moretto, G., Mello Jr., L.J., 2001. Infestation and distribution of the mite *Varroa jacobsoni* in Africanized honey bee (*Apis mellifera*) colonies. Interciencia 26, 394–396.

Paxton, R.J., 2010. Does infection by *Nosema ceranae* cause "Colony Collapse Disorder" in honey bees (*Apis mellifera*). J. Apic. Res. 49, 80–84.

Paxton, R., Klee, J.S., Korpela, S., Fries, I., 2007. *Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis*. Apidologie 38, 558–565.

Rohlf, F.J., 2010a. tpsDig, Version 2.16. Department of Ecology and Evolution, State University of New York, Stony Brook.

Rohlf, F.J., 2010b. tpsRelw, Version 1.49. Department of Ecology and Evolution, State University of New York, Stony Brook.

Rosenkranz, P., 1999. Honey bee (*Apis mellifera* L.) tolerance to *Varroa jacobsoni* Oud. in South America. Apidologie 30, 159–172.

Santos, L.G., Alves, M.L.T.M.F., Message, D., Teixeira, E.W., 2011. Apicultura migratória: aspectos sanitários. In: 4° Congresso Interinstitucional de Iniciação Científica, Campinas. <http://www.cnpm.embrapa.br/5ciic/anais/Artigos/RE11301.pdf>, (accessed 27.03.12).

Somerville, D., Hornitzky, M., 2007. Nosema Disease. Primefact 699. <http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0003/177519/nosema-disease.pdf>, (accessed 27.03.12).

Teixeira, E.W., Silva, E.C.A., Alves, M.L.T.M.F., Moreti, A.C.C.C., Silva, R.M.B., Gama, P.H.G., 1997. Epidemiologia da nosemose em abelhas africanizadas (*Apis mellifera* L.) na região de Pindamonhangaba, SP, Brasil. Bol. Ind. Anim. 54, 99–102.

Teixeira, E.W., Message, D., Alves, M.L.T.M.F., Santos, L.G., 2013. Sanidade apícola no Brasil: cenário segundo análises realizadas no LASA. In: Proc. I Workshop Sobre Insetos Sociais (Seção Brasileira) – IUSSI), Ribeirão Preto. <http://www.iussi-workshop-brazil.yolasite.com/>, (accessed 22.03.13).

Vandame, R., Morand, S., Colin, M.E., Belzunces, L.P., 2002. Parasitism in the social bee *Apis mellifera*: quantifying costs and benefits of behavioral resistance to *Varroa destructor* mites. Apidologie 33, 433–445.

Whitaker, J., Szalanski, A.L., Kence, M., 2010. Molecular detection of *Nosema ceranae* and *Nosema apis* from Turkish honey bees. Apidologie 41, 364–374.