

# *Nosema ceranae* in South American Native Stingless Bees and Social Wasp

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**Abstract** Besides the incipient research effort, the role of parasites as drivers of the reduction affecting pollinator populations is mostly unknown. Given the worldwide extension of the beekeeping practice and the diversity of pathogens affecting *Apis mellifera* populations, honey bee colonies are a certain source of parasite dispersion to other species. Here, we communicate the detection of the microsporidium *Nosema ceranae*, a relatively new parasite of honey bees, in stingless bees (Meliponini) and the social wasp *Polybia scutellaris* (Vespidae) samples from Argentina and Brazil by means of duplex PCR. Beyond the geographic location of the nests, *N. ceranae* was detected in seven from the eight Meliponini species analyzed, while *Nosema apis*, another common parasite of *A. mellifera*, was absent in all samples tested. Further research is necessary to determine if the presence of the parasite is also associated with established infection in host tissues.

The obtained information enriches the current knowledge about pathologies that can infect or, at least, be vectored by native wild pollinators from South America.

**Keywords** Stingless bee · *Polybia scutellaris* · Microsporidia · *Nosema apis*

Recent studies have suggested that *Apis mellifera* L. hives represent a source of pathogens for other bee species such as solitary bees [1], bumble bees [2, 3], and stingless bees [4]. However, there is still insufficient information about the distribution and impact of honey bee diseases on native Hymenoptera populations.

Given a host-jump event from its historical host and a surprisingly late discovered territorial expansion, *Nosema*

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*ceranae* has demonstrated to be an epidemiologically relevant parasite affecting *A. mellifera* colonies [5–7]. Furthermore, there is a large discussion in course related to the role of this parasite in honey bee colony collapse disorder as reviewed by Holt and Grozinger [8].

*Nosema ceranae* spores are highly resistant [9] and spread as a consequence of the oral–fecal cycle, contaminating pollen, honey, hive materials, and the surrounding areas of the hive because of the wide working range of foragers [10–13] including also the dispersion throughout certain insectivorous birds [14]. Spreading pathways of spores and common use of resources enlarge the possibilities to reach other nectarivorous pollinators. Among them, stingless bees (Meliponini) and the social wasp *Polybia scutellaris* (White) (Vespidae) have been observed to perform robbing behavior to honey bee empty combs with traces of

honey, commonly available in apiaries after harvest season, being also common the honey bee robbing from stingless bees destroyed nests (F. Muller and Dr. Porrini D., pers. obs.).

Meliponini comprises a tribe with nearly 600 species with distribution on tropical and subtropical regions of the world and on temperate regions of Southern hemisphere [15, 16]. In Argentinian territory, 37 species are known to be present (Alvarez L. et al. data not published) with major representation in Misiones Province and in the Yungas region [17], while in Brazil, a huge diversity is present, since more than 300 species were described [18]. The social wasp *P. scutellaris* presents a wide distribution in Argentina [19]. They are animals of prey, although references indicate that wasps and honey bees commonly share floral resources [20] since they also collect large quantities of nectar [21].

**Table 1** Detail of bees and wasp species analyzed, geographical origin, sampling date, and PCR results. Argentinian samples (stingless bees) were obtained from Chaco Province, Sáenz Peña city (26° 47' 27" S, 60° 26' 29" W); Misiones Province, Puerto Rico city (26° 49' 30.5" S, 55° 01' 44.2" W), and Capióví city (26° 56' 40.8" S, 55° 02' 63.1" W); *Polybia scutellaris* wasp samples were obtained from Buenos Aires Province, Mar del Plata city (37° 56' 41" S, 57° 46' 51" W). Brazilian samples were obtained from Goiás State, Goiânia city (16° 35' 16.1" S; 49° 17' 32.7" W). From each hive or nest, 30–40 individuals were collected in sterile flasks and stored in 90% ethanol for transportation until analyses. Wasp individuals were collected and freeze (–18 °C)

until analyses. Ten or 20 bees from each sample were pooled and macerated to obtain a filtered clean pellet to perform DNA extraction (High Pure PCR template preparation kit, Roche). The 16S rRNA locus was used to perform *N. apis*–*N. ceranae* duplex PCR according to Martín-Hernández et al. [41]. Negative controls (from DNA extraction) were included in all PCR experiments. Amplification products were visualized by electrophoresis in 2% agarose gel. To perform the PCR gel, *N. ceranae*-positive control, *N. ceranae* + *N. apis* co-infection sample, and a negative control sample were included. For further information see [Supplementary Material](#)

| Species “common native name”                                       | Location/nest emplacement or nesting substrate  | Sampling year | <i>N. ceranae</i> gel result (no. of pooled individuals) |
|--|---|---------------|--|
| <i>Tetragonisca fiebrigi</i> (Schwarz) “Jateí”                     | Argentina, Misiones, Capióví/hive in Kaki tree ( <i>Diospyros kaki</i> )                  | 2014          | Positive (20)  |
| <i>Tetragonisca fiebrigi</i> (Schwarz) “Jateí”                     | Argentina, Misiones, Puerto Rico/hive in trunk  | 2014          | Positive (20)  |
| <i>Tetragonisca fiebrigi</i> (Schwarz) “Jateí”                     | Argentina, Misiones, Capióví/hive in Kaki tree ( <i>Diospyros kaki</i> )                  | 2014          | Positive (20)  |
| <i>Tetragonisca fiebrigi</i> “Rubiecito”                           | Argentina, Chaco, S. Peña/hive in urban tree (Ceibo tree— <i>Erythrina crista-galli</i> ) | 2015          | Positive (20)  |
| <i>Tetragonisca fiebrigi</i> “Rubiecito”                           | Argentina, Chaco, S. Peña/rural, trunk of Quebracho tree— <i>Schinopsis lorentzii</i>     | 2015          | Positive (20)  |
| <i>Scaptotrigona jujuyensis</i> “Yana”                             | Argentina, Chaco, S. Peña/wooden (pine) rational hive box                                 | 2015          | Positive (10)  |
| <i>Scaptotrigona jujuyensis</i> “Yana”                             | Argentina, Chaco, S. Peña/rural zone, trunk   | 2015          | Positive (10)  |
| <i>Tetragonisca fiebrigi</i> “Rubiecito”                           | Argentina, Chaco, S. Peña/wooden (pine) rational hive box                                 | 2015          | Positive (20)  |
| <i>Tetragonisca angustula</i> (Latreille) “Jataí”                  | Brasil, Goiás, Goiânia/wooden ( <i>Cedrela</i> spp.) rational hive box                    | 2015          | Negative (20)  |
| <i>Melipona fasciculata</i> Smith “Uruçu-cinza”                    | Brasil, Goiás, Goiânia/wooden ( <i>Cedrela</i> spp.) rational hive box                    | 2015          | Positive (10)  |
| <i>Melipona quadrifasciata anthidioides</i> Lepeletier “Mandaçaia” | Brasil, Goiás, Goiânia/wooden ( <i>Cedrela</i> spp.) rational hive box                    | 2015          | Positive (10)  |
| <i>Melipona marginata</i> Lepeletier “Manduri”                     | Brasil, Goiás, Goiânia/wooden ( <i>Cedrela</i> spp.) rational hive box                    | 2015          | Positive (10)  |
| <i>Melipona rufiventris</i> Lepeletier “Uruçu-amarela”             | Brasil, Goiás, Goiânia/wooden ( <i>Cedrela</i> spp.) rational hive box                    | 2015          | Positive (10)  |
| <i>Melipona mandacaia</i> Smith “Mandaçaia-da-bahia”               | Brasil, Goiás, Goiânia/wooden ( <i>Cedrela</i> spp.) rational hive box                    | 2015          | Positive (10)  |
| <i>Polybia scutellaris</i> (White) “Camuati”                       | Argentina, Buenos Aires, Mar del Plata, Natural Reserve “Laguna de los Padres”            | 2010          | Positive (20)  |

Given the close phylogenetic relation of Meliponini to Apini [22], the overlap in the spatial distribution range and feeding resources, the forager and robbing behavior of stingless bees and wasps, and the previous report of honey bee diseases in Meliponini species [4], the objective of this study was to assess *N. ceranae* presence on those native pollinators sampled from different South American sites. Fourteen stingless bee samples and one *Polybia* wasp were analyzed by means of duplex PCR. Details about species identification, analysis technique, sampling areas, and number of individuals are described in Table 1. PCR products whose length matched with those expected for *N. ceranae* (218 bp) were purified and then sequenced. The alignments of the sequences obtained are shown in [Supplementary Material](#). In the present work, the duplex PCR analyses performed using primers for 16S ribosomal RNA (rRNA) locus on pooled samples of hole bodies revealed the solely presence of *N. ceranae* in seven from the eight screened Meliponini species and in the sample of *Polybia* wasps. These results constitute the first detection of a Nosematidae parasite on samples of those native Hymenoptera.

An increasing number of studies reveal the occurrence of Microsporidian species, or specifically *N. ceranae* in wild pollinators, such as bumblebees [2, 3, 23, 24], wasps, and solitary bees [25]. Nevertheless, our results contrast with recent data from a survey performed in two Brazilian states, where a large number of Meliponini samples, analyzed for Nosematidae presence using primers for 18S rRNA locus, resulted in absence of detection [4]. In the cited work, most of the species sampled were different from the included in our work, being *Tetragonisca fiebrigi* (Schwarz), the only common species included in both works being positive to *N. ceranae* in our survey. Contradictory results could be clarified by larger studies, expanding the prospected area, the number of species and individuals sampled in different seasons. Furthermore, differences in molecular detection protocols should be attended.

Given the relatively long presence of *N. ceranae* in South America [26–28] and the widespread capability of the disease, there are many possible ways of interaction driving the contact between honey bees and native species resulting in cross contamination, since flowers are known to be an important site for interspecies transmission of bee pathogens [29, 30]. Also, robbing food resources is a common way of disease transmission between bee colonies [31, 32], being suggested as the cause of host-jump of *N. ceranae* between *A. mellifera* and *Apis cerana* colonies [33]. In the same way, some stingless bee species have evolved to a diverse robbing and usurping lifestyles, particularly stealing resources from other stingless bees and occasionally from honey bee nests [34–37]. Given that nests of stingless bees sampled from Misiones Province (Argentina) and *Polibya* wasp were settled near productive or feral honey bee hives, we propose the interspecies robbing

behavior as a certain possibility of contact with *N. ceranae* spores. Nevertheless, sampled colonies from Brazil and Chaco Province (Argentina) were settled far away of the presence of managed honey bee hives and the sampling sites were surrounded mainly by native vegetation, and therefore, it is probable that another way of contact with spores could occur.

In reference to the other *Nosema* parasite, the absence of *Nosema apis*-positive results in our samples is consistent with reports indicating an absence of this species in South America [7, 26, Porrini M. et al., unpublished] or some scarce co-infection on isolated sites [27].

Pathological effects of *N. ceranae* in honey bees and bumble bees have been described [38–40]. Plischuk and Lange [2, 3] have already reported the presence of this microsporidium in bumble bees, infecting four native South American species, describing also spores inside epithelial midgut cells. Here, we report the first detection of *N. ceranae* in stingless bees and wasp samples of pooled individuals, but procedures do not allow us to determine if positive samples comprise a vector role of the insects, established infection in the host tissues, or both possibilities. Therefore, the invasion of host organs and possible effects on survival should be properly studied to test the hypothesis of the existence of a genus barrier which prevents the development of an intracellular infection or instead to describe a new host-jump of *N. ceranae* from honey bees to wild native stingless bees and wasps.

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#### Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

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