Supplementary material

Samples processing

Ten to twenty bees from each sample were randomly selected to conduct spores collection. Samples of the whole body was allowed to evaporate the ethanol used to store samples under room temperature and then were manually macerated in 1ml of sterile water using glass rods in a 15ml sterile glass tubes during 5 minutes. Then, another 1ml was added to homogenize. The suspension was filtered and the remaining solution centrifuged at 2000 rpm for 6 minutes. The supernatant was discarded, the pellet was re-suspended in 1 ml of sterile water and the solution was vortexed with glass beads for 1 min at maximum speed. All samples were stored at 4°C until DNA extraction.

DNA Extraction, Duplex PCR design and PCR conditions. The extraction was carried out with a High Pure PCR template preparation kit (Cat Nro. 11796828001, Roche), using 400µl of sample material under the "Isolation of nucleic Acids from Mammalian Tissue" protocol detailed by manufacturer. The 16S rRNA locus was selected to perform *N. apis-N. ceranae* duplex PCR according to Martín-Hernández et al. (2007) using 321APIS primers for *N. apis* and 218MITOC primers for *N. ceranae.* Data available in the GenBank database (http://www.ncbi.nlm.nih.gov/GenBank/). Amplifications were carried out with Gene Amp PCR System 2700 using 50µl reaction cocktail containing: 25 µl of Fast Start PCR Master Mixture (Cat Nro. 04710444001; Roche Diagnostic), 0.8 µM of each primer, 0.4 mM of each deoxynucleoside triphosphate, 3 mM Cl2Mg, 0.1% Triton X-100, and 3 µl of *N. apis* or *N. ceranae* DNA template.

The thermocycler program consisted of 94°C for 2 min, followed by 10 cycles of 15 s at 94°C, 30 s at 61.8°C, and 45 s at 72°C, 20 cycles of 15 s at 94°C, 30 s at 61.8°C, and 50 s at 72°C plus an additional 5 s of elongation for each successive cycle, and a final extension step at 72°C for 7 min. Negative controls (from DNA extraction) were included in all PCR experiments. Amplification products were visualized by electrophoresis in 2% agarose gel with TBE (Tris 10.8g, boric acid 5.5g, EDTA 0.5 M pH 8 4ml/100ml), using size ladder (100pb) and GelRed pre-staining.

To perform the PCR gel, *N. ceranae* positive control, *N. ceranae* + *N. apis* co-infection sample and a negative control sample were included (Figure1 and Table 1).

Samples sequencing. The pathogen identity of at least one sample from every meliponini and wasp genera was determined by capillary electrophoresis sequencing. Results are showed in Figure 2.

Figure 1.

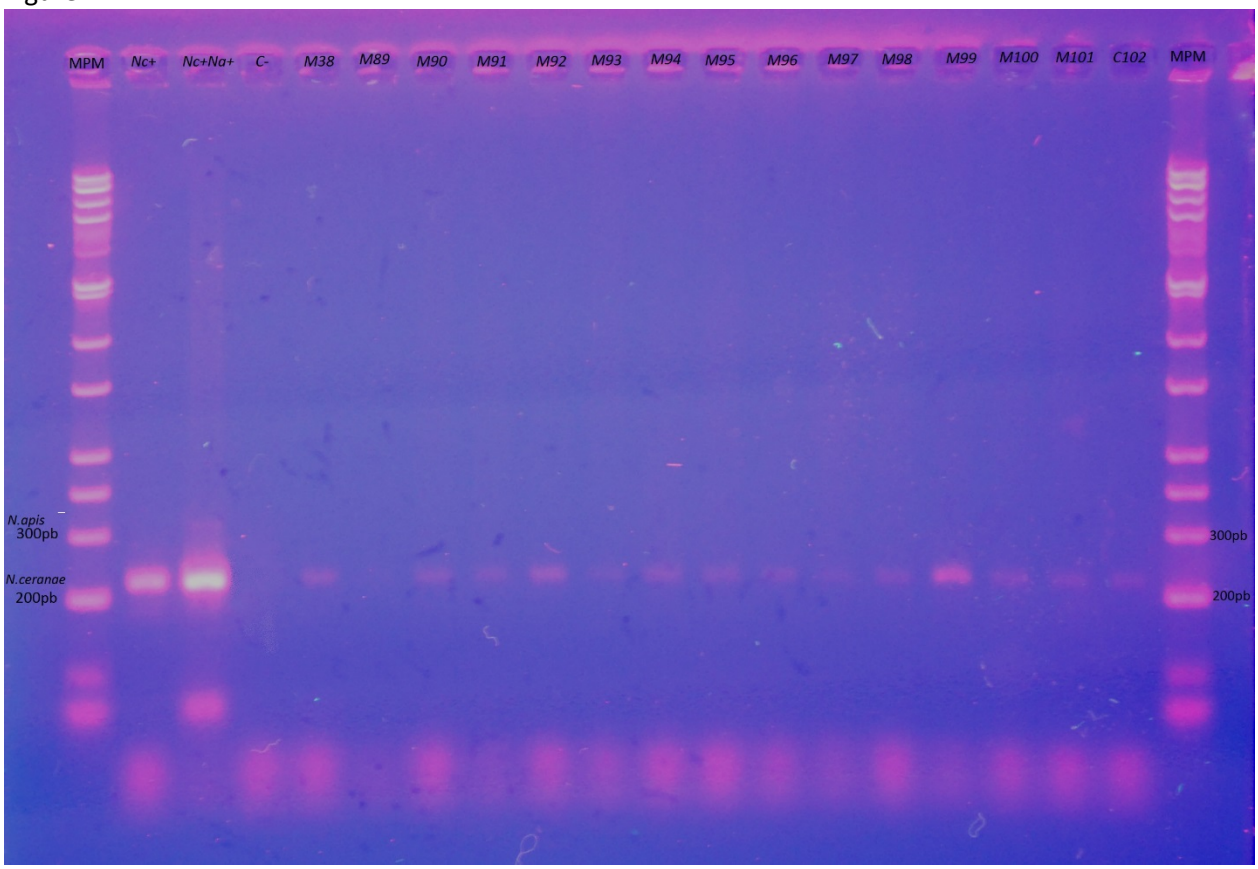

Table 1. Gel description.

Inoculum description	Location/Nest emplacement or nesting substrate/Sampling year	Figure references	*Sample sequenced*
Molecular weight marker	-	MPM	
N. ceranae (positive control)	-	Nc+	
N. ceranae + N. apis (co-infection positive control)	-	Nc+ Na+	
Negative control	-	C-	
Tetragonisca fiebrigi (Schwarz) "Jateí"	Argentina, Misiones, Capioví / hive in Kaki tree (*Diospyros kaki*) / 2014	MEL100	YES
Tetragonisca fiebrigi (Schwarz) "Jateí"	Argentina, Misiones, Puerto Rico / hive in trunk / 2014	MEl101	
Tetragonisca fiebrigi (Schwarz) "Jateí"	Argentina, Misiones, Capioví / hive in Kaki tree (*Diospyros kaki*) / 2014	M38	YES
Tetragonisca fiebrigi "Rubiecito"	Argentina, Chaco, S. Peña / hive in urban tree (Ceibo tree- *Erythrina crista-galli*) / 2015	MEL95	YES
Tetragonisca fiebrigi "Rubiecito"	Argentina, Chaco, S. Peña / rural, Trunk of Quebracho tree- *Schinopsis lorentzii* / 2015	MEL96	
Scaptotrigona jujuyensis "Yana"	Argentina, Chaco, S. Peña / wooden (pine) rational hive box / 2015	MEL97	
Scaptotrigona jujuyensis "Yana"	Argentina, Chaco, S. Peña / rural zone, Trunk / 2015	MEL98	YES
Tetragonisca fiebrigi "Rubiecito"	Argentina, Chaco, S. Peña / wooden (pine) rational hive box /2015	MEL99	YES
Tetragonisca angustula (Latreille) "Jataí"	Brasil, Goiás, Goiânia / wooden (*Cedrela* spp) rational hive box / 2015	MEL89	
Melipona fasciculata Smith "Uruçu-cinza"	Brasil, Goiás, Goiânia / wooden (*Cedrela* spp) rational hive box / 2015	MEL90	YES
Melipona quadrifasciata anthidioides Lepeletier "Mandaçaia"	Brasil, Goiás, Goiânia/ wooden (*Cedrela* spp) rational hive box / 2015	MEL91	YES
Melipona marginata Lepeletier "Manduri"	Brasil, Goiás, Goiânia / wooden (*Cedrela* spp) rational hive box / 2015	MEL92	
Melipona rufiventris Lepeletier "Uruçu-amarela"	Brasil, Goiás, Goiânia / wooden (*Cedrela* spp) rational hive box / 2015	MEL93	
Melipona mandacaia Smith "Mandaçaia-da-bahia"	Brasil, Goiás, Goiânia / wooden (*Cedrela* spp) rational hive box / 2015	MEL94	
Polybia scutellaris (White) "Camuatí"	Argentina, Buenos Aires, Mar del Plata, Natural Reserve "Laguna de los Padres" / 2010	CAM102	YES

Figure 2. Sequences alignment of *N. ceranae* fragments obtained from Meliponini and wasp samples compared with *N. ceranae* consensus sequence described in Spain (GenBank, acc. Num. DQ286728).