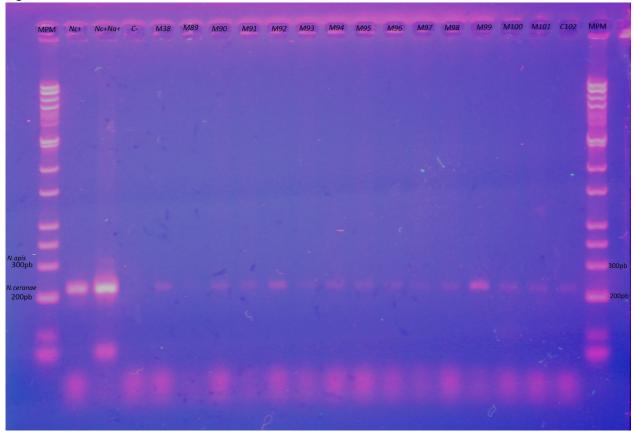
1 Supplementary material

2 Samples processing

- 3 Ten to twenty bees from each sample were randomly selected to conduct spores collection. Samples of
- 4 the whole body was allowed to evaporate the ethanol used to store samples under room temperature and
- 5 then were manually macerated in 1ml of sterile water using glass rods in a 15ml sterile glass tubes during 5
- 6 minutes. Then, another 1ml was added to homogenize. The suspension was filtered and the remaining
- 7 solution centrifuged at 2000 rpm for 6 minutes. The supernatant was discarded, the pellet was re-
- 8 suspended in 1 ml of sterile water and the solution was vortexed with glass beads for 1 min at maximum
- 9 speed. All samples were stored at 4°C until DNA extraction.
- 10 DNA Extraction, Duplex PCR design and PCR conditions. The extraction was carried out with a High Pure
- 11 PCR template preparation kit (Cat Nro. 11796828001, Roche), using 400µl of sample material under the
- 12 "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
- 16 System 2700 using 50μl reaction cocktail containing: 25 μl of Fast Start PCR Master Mixture (Cat Nro.
- 17 04710444001; Roche Diagnostic), 0.8 μM of each primer, 0.4 mM of each deoxynucleoside triphosphate, 3
- 18 mM Cl2Mg, 0.1% Triton X-100, and 3 µl of N. apis or N. ceranae DNA template.
- 19 The thermocycler program consisted of 94°C for 2 min, followed by 10 cycles of 15 s at 94°C, 30 s at
- 20 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
- 21 elongation for each successive cycle, and a final extension step at 72°C for 7 min. Negative controls (from
- 22 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
- 24 size ladder (100pb) and GelRed pre-staining.
- 25 To perform the PCR gel, N. ceranae positive control, N. ceranae + N. apis co-infection sample and a
- 26 negative control sample were included (Figure 1 and Table 1).
- 27 Samples sequencing. The pathogen identity of at least one sample from every meliponini and wasp genera
- 28 was determined by capillary electrophoresis sequencing. Results are showed in Figure 2.

30 Figure 1.



33 Table 1. Gel description.

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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 (<i>Diospyros kaki</i>) / 2014	MEL100	YES
Tetragonisca fiebrigi (Schwarz) "Jateí"	Argentina, Misiones, Puerto Rico / hive in trunk / 2014	MEI101	
Tetragonisca fiebrigi (Schwarz) "Jateí"	Argentina, Misiones, Capioví / hive in Kaki tree (<i>Diospyros kaki</i>) / 2014	M38	YES
Tetragonisca fiebrigi "Rubiecito"	Argentina, Chaco, S. Peña / hive in urban tree (Ceibo tree- <i>Erythrina crista-galli</i>) / 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 (<i>Cedrela</i> spp) rational hive box / 2015	MEL89	
Melipona fasciculata Smith "Uruçu- cinza"	Brasil, Goiás, Goiânia / wooden (<i>Cedrela</i> spp) rational hive box / 2015	MEL90	YES
Melipona quadrifasciata anthidioides Lepeletier "Mandaçaia"	Brasil, Goiás, Goiânia/ wooden (<i>Cedrela</i> spp) rational hive box / 2015	MEL91	YES
<i>Melipona marginata</i> Lepeletier "Manduri"	Brasil, Goiás, Goiânia / wooden (<i>Cedrela</i> spp) rational hive box / 2015	MEL92	
<i>Melipona rufiventris</i> Lepeletier "Uruçu-amarela"	Brasil, Goiás, Goiânia / wooden (<i>Cedrela</i> spp) rational hive box / 2015	MEL93	
<i>Melipona mandacaia</i> Smith "Mandaçaia-da-bahia"	Brasil, Goiás, Goiânia / wooden (<i>Cedrela</i> 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

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