

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
13 locus was selected to perform *N. apis*-*N. ceranae* duplex PCR according to Martín-Hernández et al. (2007)
14 using 321APIS primers for *N. apis* and 218MITOC primers for *N. ceranae*. Data available in the GenBank
15 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.

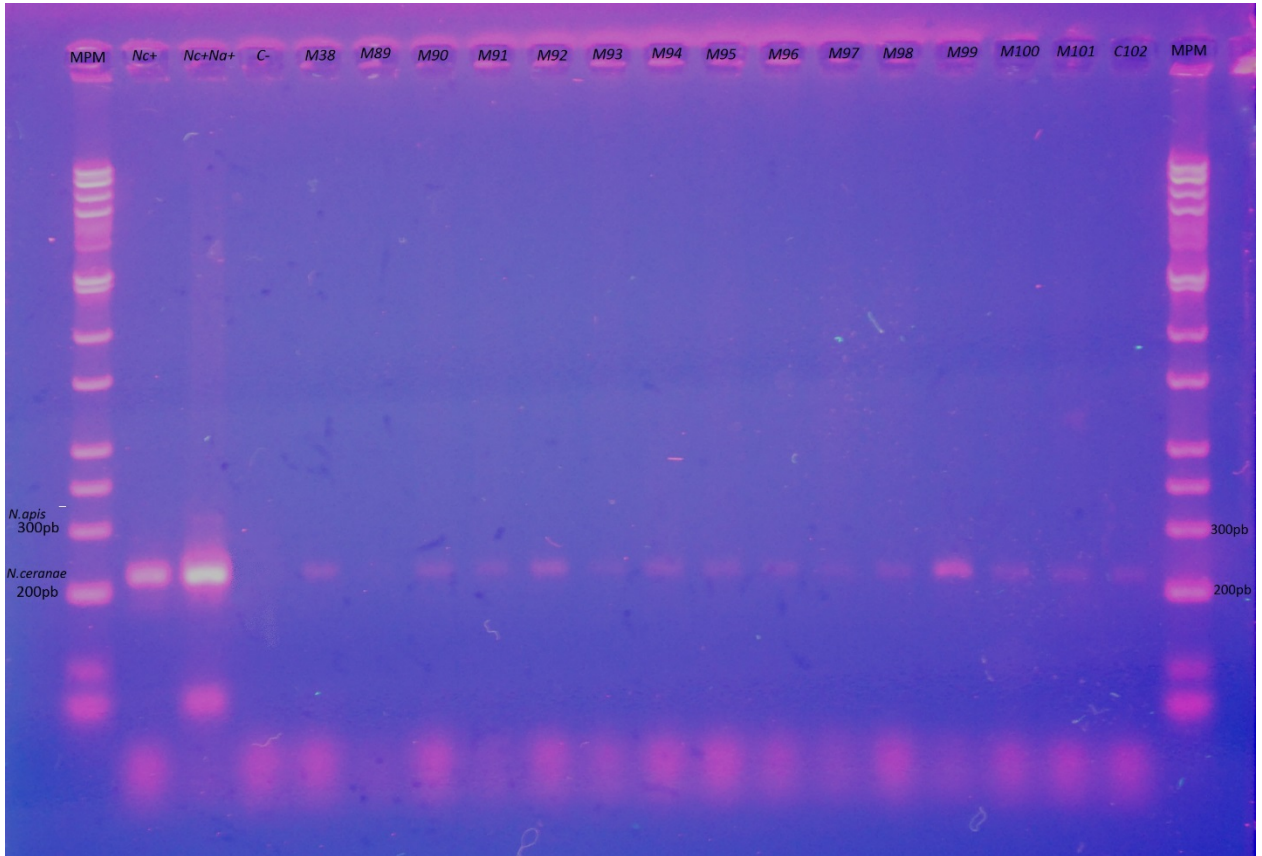
19 The thermocycler program consisted of 94°C for 2 min, followed by 10 cycles of 15 s at 94°C, 30 s at
20 61.8°C, and 45 s at 72°C, 20 cycles of 15 s at 94°C, 30 s at 61.8°C, and 50 s at 72°C plus an additional 5 s of
21 elongation for each successive cycle, and a final extension step at 72°C for 7 min. Negative controls (from
22 DNA extraction) were included in all PCR experiments. Amplification products were visualized by
23 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 (Figure1 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.

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30 Figure 1.



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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	
<i>N. ceranae</i> (positive control)	-	Nc+	
<i>N. ceranae</i> + <i>N. apis</i> (co-infection positive control)	-	Nc+ Na+	
Negative control	-	C-	
<i>Tetragonisca fiebrigi</i> (Schwarz) "Jateí"	Argentina, Misiones, Capióví / hive in Kaki tree (<i>Diospyros kaki</i>) / 2014	MEL100	YES
<i>Tetragonisca fiebrigi</i> (Schwarz) "Jateí"	Argentina, Misiones, Puerto Rico / hive in trunk / 2014	MEI101	
<i>Tetragonisca fiebrigi</i> (Schwarz) "Jateí"	Argentina, Misiones, Capióví / hive in Kaki tree (<i>Diospyros kaki</i>) / 2014	M38	YES
<i>Tetragonisca fiebrigi</i> "Rubiecito"	Argentina, Chaco, S. Peña / hive in urban tree (Ceibo tree- <i>Erythrina crista-galli</i>) / 2015	MEL95	YES
<i>Tetragonisca fiebrigi</i> "Rubiecito"	Argentina, Chaco, S. Peña / rural, Trunk of Quebracho tree- <i>Schinopsis lorentzii</i> / 2015	MEL96	
<i>Scaptotrigona jujuyensis</i> "Yana"	Argentina, Chaco, S. Peña / wooden (pine) rational hive box / 2015	MEL97	
<i>Scaptotrigona jujuyensis</i> "Yana"	Argentina, Chaco, S. Peña / rural zone, Trunk / 2015	MEL98	YES
<i>Tetragonisca fiebrigi</i> "Rubiecito"	Argentina, Chaco, S. Peña / wooden (pine) rational hive box / 2015	MEL99	YES
<i>Tetragonisca angustula</i> (Latreille) "Jataí"	Brasil, Goiás, Goiânia / wooden (<i>Cedrela</i> spp) rational hive box / 2015	MEL89	
<i>Melipona fasciculata</i> Smith "Uruçu-cinza"	Brasil, Goiás, Goiânia / wooden (<i>Cedrela</i> spp) rational hive box / 2015	MEL90	YES
<i>Melipona quadrifasciata anthidioides</i> 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	
<i>Polybia scutellaris</i> (White) "Camuatí"	Argentina, Buenos Aires, Mar del Plata, Natural Reserve "Laguna de los Padres" / 2010	CAM102	YES

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

	10	20	30	40
<i>N.ceranae</i> 16S ribRNA gene	CAT AAT AGAA	ATTT GAGTTT	TTTGGCTCTG	GGGATAGTAT
MI6 Ncisol <i>A.mellifera</i>	NNNNNNNNNN	ANTT GAGTTT	TTTGGCTCTG	GGGATAGTAT
NA58 Ncisol <i>A.mellifera</i>	NNNNNNNNNA	ATTT GAGTTT	TTTGGCTCTG	GGGATAGTAT
MEL38 <i>Tetragonisca fiebrigi</i>	NNNNNNNNNN	NNNT GAGTTT	TTTGGCTCTG	GGGATAGTAT
MEL90 <i>Melipona fasciculata</i>	NNNNNNNNNN	NNNT GAGTTT	TTTGGCTCTG	GGGATAGTAT
MEL91 <i>Melipona quadrifasciata</i>	NNNNNNNNNN	NNNT GAGTTT	TTTGGCTCTG	GGGATAGTAT
MEL95 <i>Tetragonisca</i> sp.	NNNNNNNNNN	NNNT GAGTTT	TTTGGCTCTG	GGGATAGTAT
MEL98 <i>Scaptotrigona</i> sp.	NNNNNNNNNN	ANNNNGTTT	TTTGGCTCTG	GGGATAGTAT
MEL99 <i>Tetragonisca</i> sp.	NNNNNNNNNN	NNNT GAGTTT	TTTGGCTCTG	GGGATAGTAT
MEL100 <i>Tetragonisca fiebrigi</i>	NNNNNNNNNN	NNNT GAGTTT	TTTGGCTCTG	GGGATAGTAT
CAM102 <i>Polybia scutellaris</i>	NNNNNNNNNN	NNTNNNGTTT	TTTGGCTCTG	GGGATAGTAT
	50	60	70	80
<i>N.ceranae</i> 16S ribRNA gene	GATCGCAAGA	TTGAAAATTA	AAGAAATTGA	CGGAAGAATA
MI6 Ncisol <i>A.mellifera</i>	GATCGCAAGA	TTGAAAATTA	AAGAAATTGA	CGGAAGAATA
NA58 Ncisol <i>A.mellifera</i>	GATCGCAAGA	TTGAAAATTA	AAGAAATTGA	CGGAAGAATA
MEL38 <i>Tetragonisca fiebrigi</i>	GATCGCAAGA	TTGAAAATTA	AAGAAATTGA	CGGAAGAATA
MEL90 <i>Melipona fasciculata</i>	GATCGCAAGA	TTGAAAATTA	AAGAAATTGA	CGGAAGAATA
MEL91 <i>Melipona quadrifasciata</i>	GATCGCAAGA	TTGAAAATTA	AAGAAATTGA	CGGAAGAATA
MEL95 <i>Tetragonisca</i> sp.	GATCGCAAGA	TTGAAAATTA	AAGAAATTGA	CGGAAGAATA
MEL98 <i>Scaptotrigona</i> sp.	GATCGCAAGA	TTGAAAATTA	AAGAAATTGA	CGGAAGAATA
MEL99 <i>Tetragonisca</i> sp.	GATCGCAAGA	TTGAAAATTA	AAGAAATTGA	CGGAAGAATA
MEL100 <i>Tetragonisca fiebrigi</i>	GATCGCAAGA	TTGAAAATTA	AAGAAATTGA	CGGAAGAATA
CAM102 <i>Polybia scutellaris</i>	GATCGCAAGA	TTGAAAATTA	AAGAAATTGA	CGGAAGAATA
	90	100	110	120
<i>N.ceranae</i> 16S ribRNA gene	CCACAAGGAG	TGGATTGTGC	GGCTTAATTT	GACTCAACGC
MI6 Ncisol <i>A.mellifera</i>	CCACAAGGAG	TGGATTGTGC	GGCTTAATTT	GACTCAACGC
NA58 Ncisol <i>A.mellifera</i>	CCACAAGGAG	TGGATTGTGC	GGCTTAATTT	GACTCAACGC
MEL38 <i>Tetragonisca fiebrigi</i>	CCACAAGGAG	TGGATTGTGC	GGCTTAATTT	GACTCAACGC
MEL90 <i>Melipona fasciculata</i>	CCACAAGGAG	TGGATTGTGC	GGCTTAATTT	GACTCAACGC
MEL91 <i>Melipona quadrifasciata</i>	CCACAAGGAG	TGGATTGTGC	GGCTTAATTT	GACTCAACGC
MEL95 <i>Tetragonisca</i> sp.	CCACAAGGAG	TGGATTGTGC	GGCTTAATTT	GACTCAACGC
MEL98 <i>Scaptotrigona</i> sp.	CCACAAGGAG	TGGATTGTGC	GGCTTAATTT	GACTCAACGC
MEL99 <i>Tetragonisca</i> sp.	CCACAAGGAG	TGGATTGTGC	GGCTTAATTT	GACTCAACGC
MEL100 <i>Tetragonisca fiebrigi</i>	CCACAAGGAG	TGGATTGTGC	GGCTTAATTT	GACTCAACGC
CAM102 <i>Polybia scutellaris</i>	CCACAAGGAG	TGGATTGTGC	GGCTTAATTT	GACTCAACGC
	130	140	150	160
<i>N.ceranae</i> 16S ribRNA gene	GAGGTAACCT	ACCAATATTT	TATTATTTTG	AGAGAACGGT
MI6 Ncisol <i>A.mellifera</i>	GAGGTAACCT	ACCAATATTT	TATTATTTTG	AGAGAACGGT
NA58 Ncisol <i>A.mellifera</i>	GAGGTAACCT	ACCAATATTT	TATTATTTTG	AGAGAACGGT
MEL38 <i>Tetragonisca fiebrigi</i>	GAGGTAACCT	ACCAATATTT	TATTATTTTG	AGAGAACGGT
MEL90 <i>Melipona fasciculata</i>	GAGGTAACCT	ACCAATATTT	TATTATTTTG	AGAGAACGGT
MEL91 <i>Melipona quadrifasciata</i>	GAGGTAACCT	ACCAATATTT	TATTATTTTG	AGAGAACGGT
MEL95 <i>Tetragonisca</i> sp.	GAGGTAACCT	ACCAATATTT	TATTATTTTG	AGAGAACGGT
MEL98 <i>Scaptotrigona</i> sp.	GAGGTAACCT	ACCAATATTT	TATTATTTTG	AGAGAACGGT
MEL99 <i>Tetragonisca</i> sp.	GAGGTAACCT	ACCAATATTT	TATTATTTTG	AGAGAACGGT
MEL100 <i>Tetragonisca fiebrigi</i>	GAGGTAACCT	ACCAATATTT	TATTATTTTG	AGAGAACGGT
CAM102 <i>Polybia scutellaris</i>	GAGGTAACCT	ACCAATATTT	TATTATTTTG	AGAGAACGGT
	170	180		
<i>N.ceranae</i> 16S ribRNA gene	TTTTTGTTTG	AGAATGATAA	TAGA	
MI6 Ncisol <i>A.mellifera</i>	TTTTTGTTTG	AGAATGACCG	GGN	
NA58 Ncisol <i>A.mellifera</i>	TTTTTGTTTG	AGAATGACCG	GGN	
MEL38 <i>Tetragonisca fiebrigi</i>	TTTTTGTTTG	AGAATGACCG	GGA	
MEL90 <i>Melipona fasciculata</i>	TTTTTGTTTG	AGAATGACCG	GGA	
MEL91 <i>Melipona quadrifasciata</i>	TTTTTGTTTG	AGAATGACCG	GGA	
MEL95 <i>Tetragonisca</i> sp.	TTTTTGTTTG	AGAATGACCG	GGA	
MEL98 <i>Scaptotrigona</i> sp.	TTTTTGTTTG	AGAATGACCG	GGA	
MEL99 <i>Tetragonisca</i> sp.	TTTTTGTTTG	AGAATGACCG	GGN	
MEL100 <i>Tetragonisca fiebrigi</i>	TTTTTGTTTG	AGAATGACCG	GGN	
CAM102 <i>Polybia scutellaris</i>	TTTTTGTTTG	AGAATGACCG	NN	