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Short Communication

Bombus brasiliensis Lepeletier (Hymenoptera, Apidae) infected with *Nosema ceranae* (Microsporidia)

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ABSTRACT

Heavy infections caused by a microsporidium were detected in midgut epithelium cells of two adult workers of the bumble bee *Bombus brasiliensis* Lepeletier collected near Puerto Iguazú, Misiones province, Argentina. Microsporidium rRNA (16S small subunit) was amplified by 218MITOC primers and produced amplicons indicating presence of *Nosema ceranae* Fries et al., a virulent pathogen of more than 20 bee species, possibly involved in *Apis mellifera* L. Colony Collapse Disorder. Campaigns in search of *B. brasiliensis* between 2008 and 2015 have revealed a possible narrower range in the southeastern area of its known distribution. Effects of *N. ceranae* infections could be modulating their populations and should not be overlooked. In addition, the wide host range of this microsporidium makes it a potential threat to several endemic bees such as stingless (Meliponini) and orchid bees (Euglossini).

When compared to other regions of the world, southern South America seems to depict low bumble bee diversity (Williams, 1998). Only ten out of the *ca.* 250 species of *Bombus* described worldwide have been reported to inhabit Argentina. Two of them, *Bombus ruderatus* (Fabricius, 1775) and *B. terrestris* (L., 1758), are invasive species of relatively recent entry into the southwest of the country from Chile, while the remaining eight are native (Abrahamovich et al., 2007; Schmid-Hempel et al., 2014).

According to the last available surveys on their geographic distribution in Argentina (Moure and Sakagami, 1962; Abrahamovich and Díaz, 2001; Abrahamovich et al., 2004, 2007), *B. pauloensis* Friese, 1913 (=*B. atratus* Franklin, 1913)[1], *B. bellicosus* Smith, 1879, *B. morio* (Swederus, 1787), and *B. opifex* Smith, 1879 are known to exhibit wide ranges. *Bombus tucumanus* Vachal, 1904, *B. baeri* Vachal, 1904, and *B. dahlbomii* Guérin, 1835 appear to show more limited ranges, while *B. brasiliensis* Lepeletier, 1836 may possibly occur only in Misiones province at the northeastern tip of the country. Although *B. brasiliensis*, an assiduous visitor of bromeliad flowers (Bromeliaceae) like *Aechmea* spp. (Kaehler et al., 2005; Schmid et al., 2011) appears to be widespread in Brazil (Abrahamovich et al., 2004; Santos Júnior et al., 2015), surveys carried out by our group since January 2008 suggest that its distribution in Argentina may be nowadays considerably reduced. This communication reports the detection of the microsporidium *Nosema ceranae* infecting *B. brasiliensis* and argues on a possible effect on the distribution of this bee species on the southeastern part of its range.

After campaigns in search of *B. brasiliensis* since 2008 done by authors and other team members surveying 32 localities in the provinces of Formosa (Bañado La Estrella, Colonia Perin, El Colorado, Gran Guardia, Ibarreta, Ingeniero Juárez, Laguna Yema, Las Lomitas, Palo Santo, Pirané, Posta Cambio Zalazar, Pozo del Tigre), Chaco (38 km North of Resistencia, Colonia Elisa, Juan José Castelli, Resistencia, Presidencia Roque Sáenz Peña), Corrientes (Colonia Carlos Pellegrini, Corrientes, Estero Santa Lucía, Laguna Iberá, Santo Tomé), and Misiones (Aristóbulo del Valle, Cuña Pirú, El Alcázar, 30 km East of María Magdalena, Montecarlo, Leandro N. Alem, Posadas, Urugua-í, Wanda) (Fig. 1A, Table 1) with no positive results, only six adult workers were collected in February 2015 in the surroundings of Puerto Iguazú, Misiones, northeastern Argentina (Fig. 1A). They were captured while foraging using entomological nets, conserved frozen (−32° C), and identified based on information provided by Moure and Sakagami (1962), Abrahamovich et al. (2005), and Santos Júnior et al. (2015).

Examination of each individual was performed following dissection techniques under stereoscopic microscopy (×10, ×40) (Lacey and Solter, 2012). Briefly, small portions of different tissues and organs were extracted in order to prepare fresh smears

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[1] Although the name *B. atratus* is widely adopted, the valid name seems to be *B. pauloensis*. See Moure and Melo (2012) for detailed information.

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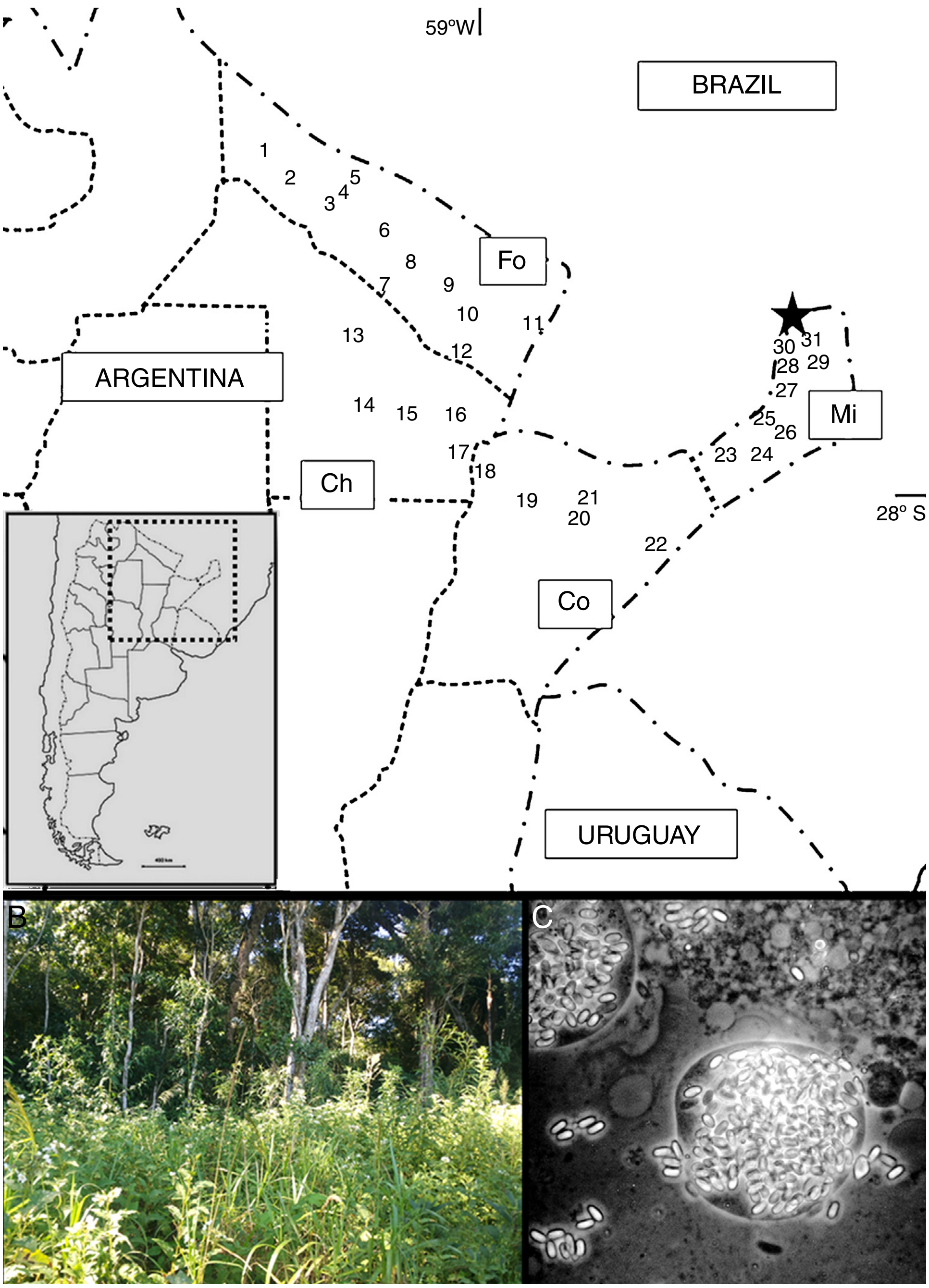

Fig. 1. (A) The 32 surveyed localities in northeastern Argentina. Formosa province [Fo]: (1) Ingeniero Juárez; (2) Laguna Yema; (3) Las Lomitas; (4) Bañado La Estrella; (5) Posta Cambio Zalazar; (6) Pozo del Tigre; (7) Colonia Perin; (8) Ibarreta; (9) Palo Santo; (10) Pirané; (11) Gran Guardia; (12) El Colorado. Chaco province [Ch]: (13) J. J. Castelli; (14) Presidencia Roque Saenz Peña; (15) Colonia Elisa; (16) 38 km North of Resistencia; (17) Resistencia. Corrientes province [Co]: (18) Corrientes; (19) Estero Santa Lucía; (20) Colonia Carlos Pellegrini; (21) Laguna Iberá; (22) Santo Tomé. Misiones province [Mi]: (23) Posadas; (24) Leandro N. Alem; (25) Cuña Pirú; (26) Aristóbulo del Valle; (27) El alcázar; (28) Montecarlo; (29) 30 km East of María Magdalena; (30) Wanda; (31) Urugua-í. (⋆) indicates Puerto Iguazú, the only locality where six *Bombus brasiliensis* workers were found. (B) Habitat where *B. brasiliensis* was found. (C) Two distended midgut cells of *B. brasiliensis* with spores of *Nosema ceranae* inside.

with one-quarter-strength Ringer's solution (Poinar and Thomas, 1984) for detection of microsporidia and protists (Lange and Lord, 2012; Solter et al., 2012). Observations were done using phase-contrast microscopy (×400, ×1000). Each infected individual was then homogenized in 2 mL of double distilled water and infection intensity (spore load) was quantified using an Improved Neubauer hemocytometer (Undeen and Vávra, 1997). Spore suspensions were obtained by repeated filtration and centrifugation (15 min; 7500 × *g*) (Lange and Henry, 1996). Double distilled water was replaced by absolute ethanol in spore suspensions and stored at −32 °C until genetic analysis were performed. Microsporidium rRNA (16S small subunit) was amplified by real time PCR according to Medici et al. (2012) with specific primers for *Nosema apis* Zander, 1907 (321APIS) and *N. ceranae* (218MITOC). Amplicons were separated on ethidium bromide-stained 1% agarose gel. Genetic material was purified with an ExoSap-IT kit (Amersham, Biosciences) and sequenced in an automatic MegaBACE Sequence Analyzer (Amersham, Biosciences). Sequences were aligned using SMS software (Stothard, 2000) and submitted to Genbank.

Microsporidian infections were detected in two individuals of *B. brasiliensis* collected while foraging on *Solanum* sp. (Solanaceae)

Table 1
Date and location of the 32 surveyed localities in northeastern Argentina looking for *Bombus brasiliensis*. Bold indicates the only campaign with findings (see text for details).

Province	Locality	GPS location	Date
Chaco	38 km North of Resistencia	27°05′10″ S; 58°57′24″ W	Feb 2015
	Colonia Elisa	26°51′57″ S; 59°31′51″ W	Jan 2008
	J. J. Castelli	25°54′02″ S; 60°32′06″ W	Feb 2012 Feb 2015
	Presidencia Roque Saenz Peña	26°47′04″ S; 60°28′17″ W	Jan 2008 Feb 2012 Feb 2015
	Resistencia	27°23′05″ S; 58°59′22″ W	Feb 2015
Corrientes	Colonia Carlos Pellegrini	28°32′14″ S; 57°11′04″ W	Jan 2008 Oct 2009
	Corrientes	27°27′38″ S; 58°47′35″ W	Jan 2008 Feb 2012 Feb 2015
	Estero Santa Lucía	28°01′15″ S; 58°01′35″ W	Jan 2008
	Laguna Iberá	28°30′01″ S; 57°04′50″ W	Oct 2009
	Santo Tomé	28°32′41″ S; 56°01′45″ W	Feb 2015
Formosa	Bañado La Estrella	24°30′32″ S; 60°25′36″ W	Feb 2015
	Colonia Perin	25°36′08″ S; 60°04′15″ W	Feb 2015
	El Colorado	26°19′57″ S; 59°21′37″ W	Feb 2015
	Gran Guardia	25°50′19″ S; 58°48′24″ W	Feb 2012
	Ibarreta	25°11′49″ S; 58°48′24″ W	Feb 2012 Feb 2015
	Ingeniero Juárez	23°55′230″ S; 61°47′58″ W	Feb 2015
	Laguna Yema	24°11′10″ S; 61°18′48″ W	Feb 2015
	Las Lomitas	24°43′58″ S; 60°33′22″ W	Feb 2012 Feb 2015
	Palo Santo	25°33′57″ S; 59°16′52″ W	Feb 2012 Feb 2015
	Pirané	25°40′24″ S; 59°05′50″ W	Feb 2012 Feb 2015
	Posta Cambio Zalazar	24°12′47″ S; 60°11′46″ W	Feb 2015
	Pozo del Tigre	24°53′55″ S; 60°18′25″ W	Feb 2012 Feb 2015
Misiones	30 km East of María Magdalena	26°11′26″ S; 54°17′17″ W	Feb 2015
	Aristóbulo del Valle	27°07′02″ S; 54°52′45″ W	Jan 2008
	Cuña Pirú	27°05′17″ S; 54°57′09″ W	Jan 2008
	El alcázar	26°43′43″ S; 54°47′55″ W	Jan 2008
	Leandro N. Alem	27°35′17″ S; 55°15′00″ W	Jan 2008
	Montecarlo	26°33′42″ S; 54°43′29″ W	Sept 2009 Feb 2015
	Posadas	27°24′04″ S; 55°55′23″ W	Jan 2008 Feb 2015
	Puerto Iguazú	**25°38′52″ S; 54°32′40″ W**	Jan 2008 **Feb 2015**
	Urugua-í	25°52′24″ S; 54°33′31″ W	Jan 2008 Feb 2015
	Wanda	25°57′05″ S; 54°34′32″ W	Jan 2008 Feb 2015

in a farmland 5 km southeast of Puerto Iguazú (25°38′52″ S; 54°32′40″ W) (Fig. 1B). Diseased individuals did not show obvious external signs of infection. The microsporidium was found infecting cells of the midgut epithelium, many of which were distended due to the heavy presence of spores (Fig. 1C). Infection intensity for each bumble bee was 5.5×10^7 and 4.4×10^7 spores/insect. Samples of spores from both infections were amplified by 218MITOC primers and produced amplicons indicating presence of *N. ceranae*. One of the sequences (220 bp) was deposited on GeneBank under accession number KX024757.

Nosema ceranae was originally described from the Asian honey bee *Apis cerana* Fabricius, 1793 (Fries et al., 1996). Ten years after its description, it was also detected in the European honey bee *A. mellifera* L., 1758 in Spain (Higes et al., 2006). Teixeira et al. (2013) evidenced that *N. ceranae* has been present in Brazil for at least 36 years infecting Africanized honey bees. Plischuk et al. (2009) reported for the first time the presence of this microsporidium in bumble bees infecting three native South American species of genus *Bombus* [*B. pauloensis* (named as *B. atratus*), *B. bellicosus*, *B. morio*].

Transmission of *N. ceranae* is mainly horizontal (Higes et al., 2008). This microsporidium completes its lifecycle inside midgut epithelial cells. Spores leave the host with the feces and may remain viable in the environment until they enter a new individual *per os*. During the last decade, numerous studies worldwide have demonstrated high virulence of *N. ceranae* against *A. mellifera* (Paxton et al., 2007; Higes et al., 2007), suggesting a role in the honey bees Colony Collapse Disorder (CCD) (Higes et al., 2008). Effects of *N. ceranae* in honey bees are relatively well known and include lesions in the midgut epithelium causing metabolic stress and suppressing immune response (Antúnez et al., 2009; Mayack and Naug, 2009). Higes et al. (2007) have reported 100% bee mortality 8 days post inoculation under experimental conditions. On the contrary, pathological effects of *N. ceranae* in bumble bees are not completely clear. Experimental infections on *B. terrestris* have shown that this pathogen would be highly virulent, colonizing the midgut epithelium and reducing host survival by 48% within one week after exposure (Graystock et al., 2013).

Because these are the first detections of *N. ceranae* in *B. brasiliensis*, effects on the host are yet unknown. However based on the observation that infections in both individuals were advanced and heavy, a significant virulence possibly similar to that described in other hosts would not be unexpected.

An important subject is the possible spread of *N. ceranae* to other species. Since the vectoring of pathogens by the sharing use of flowers seems to be a relatively common process, the transferring of infective spores between bees and other pollinators appears highly likely (Graystock et al., 2013, 2015), and *B. brasiliensis* [possibly along with *A. mellifera* (Teixeira et al., 2013)] could act as source of *N. ceranae* to other sympatric species. Recent studies have demonstrated that *N. ceranae* is capable to infect not only *Bombus* and *Apis* species but also solitary bees belonging to genus *Andrena* (Andrenidae), *Osmia*, and *Heriades* (Megachilidae) in Belgium (Ravoet et al., 2014). Numerous endemic bees inhabit the Neotropical region, as several stingless bees (Meliponini) (Freitas et al., 2009) and orchid bees (Euglossini) (Nemésio, 2009) that could be potential hosts. If these bees are susceptible, the effects of *N. ceranae* might be enhanced as seen in other, several new host–parasite associations (Goulson and Hughes, 2015).

Although data about the presence of *B. brasiliensis* is fragmented, the extension of its geographic distribution appears to be from the southern portions of the Araguaia-Tocantins basin in Mato Grosso, Brazil to northern Argentina and Uruguay in the South. The western limit would be along the Paraná River basin (Mato Grosso do Sul and southern Goiás), whereas to the East it would reach the Atlantic coast (Santos Júnior et al., 2015).

However, recent studies have shown that the eastern range would actually be narrower than previously thought since that area is inhabited by *B. bahiensis* Santos Júnior et al., 2015, a recently described sister species (Santos Júnior et al., 2015). The southwestern range into Argentina also seems to have historically diminished from Chaco, Formosa and Misiones provinces (Holmberg, 1903; Moure and Sakagami, 1962; Abrahamovich and Díaz, 2001; Abrahamovich et al., 2004) to only the latter (Abrahamovich et al., 2007). Absence of detections of *B. brasiliensis* after several campaigns since 2008 at more than 30 localities (Fig. 1A, Table 1) within the original range may suggests a more restricted presence nowadays, apparently limited to a small area at the northeastern tip of the country (Fig. 1A). *Bombus brasiliensis* was also present in Uruguay during the last century (Moure and Sakagami, 1962) but it has not been detected in that country since at least 2005, suggesting that the southernmost distribution could also have suffered a retraction (Santos et al., 2013). In Uruguay, *N. ceranae* is highly prevalent in *B. pauloensis* and *B. bellicosus* (Arbulo et al., 2015).

Although evidencing a cause–effect link between occurrence of *N. ceranae* and the apparent range retraction of *B. brasiliensis* constitute an elusive goal, we feel it is important to report on the infections we have found. *Nosema ceranae* may be at least one of the drivers modulating the populations of this host.

Conflicts of interest

The authors declare no conflicts of interest.

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