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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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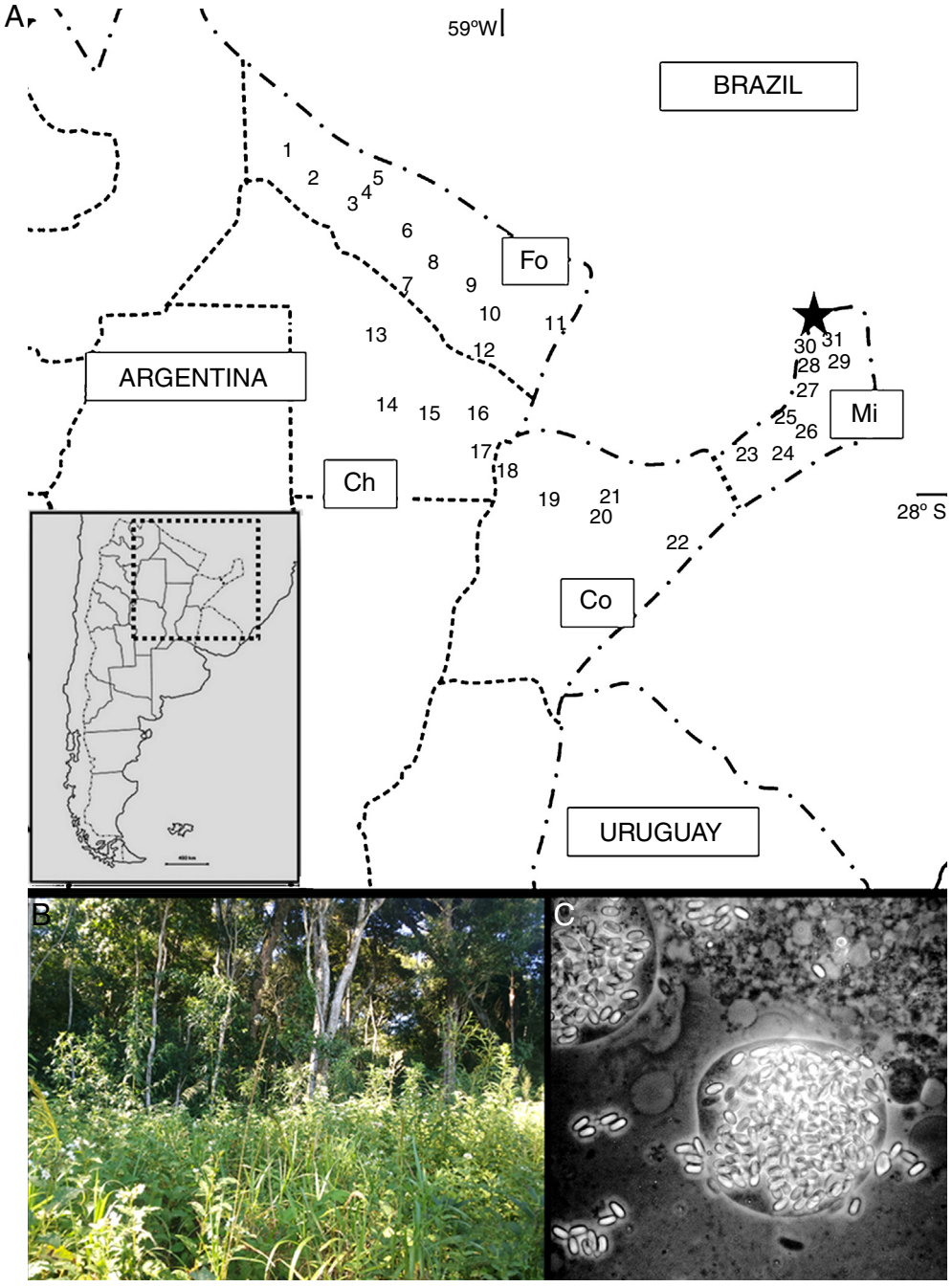

Fig. 1. (A) The 32 surveyed localities in northeastern Argentina. Formosa province [Fo]: (1) Ingeniero Juárez; (2) Laguna Yema; (3) Las Lomitas; (4) Bañado La Estrella; (5) Posta Cambio Zalazar; (6) Pozo del Tigre; (7) Colonia Perin; (8) Ibarreta; (9) Palo Santo; (10) Pirané; (11) Gran Guardia; (12) 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)