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Short Communication

Bee pathogens found in *Bombus atratus* from Colombia: A case study

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ABSTRACT

Bombus atratus bumblebees from Colombia that were caught in the wild and from breeding programs were screened for a broad set of bee pathogens. We discovered for the first time Lake Sinai Virus and confirmed the infection by other common viruses. The prevalence of *Apicystis bombi*, *Crithidia bombi* and *Nosema ceranae* was remarkably high. According to other studies the former two could have been co-introduced in South America with exotic bumble bees as *Bombus terrestris* or *Bombus ruderatus*. Given the fact that none of these species occur in Colombia, our data puts a new light on the spread of these pathogens over the South American continent.

1. Introduction

The neotropical bumblebee *Bombus atratus* is one of the 12 *Bombus* species from Colombia. Besides, it is one of those species that allowed to develop technologies for breeding in captivity in order to be applied for the pollination of different cultivated crops and fruits (Liévano et al., 1991).

In the context of the global decline of pollinators, there is an increasing interest in determining the pathogen diversity of bumblebee species. Imported bee species are considered a putative threat for native plant–pollinator networks through the spillover of pathogens (Meeus et al., 2011; Arbetman et al., 2013). Especially in North and South America there is considerable data to suggest that pathogens are an important driver of bumble bee decline (Cameron et al., 2011; Arbetman et al., 2013) and that at least some of them could have been introduced with exotic, Paleartic bumble bee species (*Bombus terrestris*; *Bombus ruderatus*) commercially reared and shipped worldwide for pollination services (Plischuk et al., 2011; Arbetman et al., 2013). Given the fact that none of these allochthonous species occur in Colombia makes this country particularly interesting for further research.

Here we present a comprehensive pathogen screen of a limited set of samples of *B. atratus* from Colombia that were caught in the wild and from breeding programs. We discovered for the first time Lake Sinai Virus (LSV) in this bumblebee species and found a strain of *Apicystis bombi* with most resemblance to the European clade.

2. Materials and methods

2.1. Sampling

In 2013, five foraging *B. atratus* specimens were collected with entomological nets in the municipality of Chía (Colombia). We also sampled seven nests reared and maintained in the facilities of the campus of the Military University Nueva Granada located in the municipality of Cajicá (Colombia). Two bumblebees were collected from each colony. All bumblebees were killed by freezing and preserved in RNAlater (Ambion) at 4 °C for transport to Belgium.

2.2. Nucleic acid extraction

The bumblebees were individually homogenized in PBS. RNA was isolated using the RNeasy Lipid Tissue kit (Qiagen) and DNA

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was extracted using the DNeasy Blood and Tissue kit (Qiagen), all in accordance to the manufacturer's instructions.

2.3. PCR analysis

Around 500 ng RNA was retro-transcribed using random hexamer primers with the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). Using previously described PCR protocols, samples were screened for the presence of bacteria, microsporidia, protists and viruses (detailed description is given as Supplementary data).

Amplicons of the ABPV complex, *A. bombi* ITS region and *Spiroplasma* spp. were cloned using the TOPO TA Cloning Kit for sequencing (Invitrogen, USA) and isolated with the GeneJet Plasmid Miniprep kit (Thermo Scientific). DNA sequences obtained by direct sequencing of amplicons or by sequencing plasmids with M13 primers were BLAST-searched for confirmation.

2.4. Sequence analysis

Trypanosomatid amplicons were aligned with 18S sequences from *Crithidia bombi* and *Crithidia expoeki* (unpublished information) in order to resolve their identity.

Sequences of *A. bombi* ITS regions were aligned in Geneious R7 using clustalW and subsequently trimmed. The best fitting nucleotide substitution model with maximum likelihood was selected using the Bayesian Information Criterion as implemented in MEGA6. Phylogenetic trees were inferred via maximum likelihood (ML) with PhyML 3.0 (Guindon et al., 2010) using the HKY85 model with approximate non-parametric likelihood ratio test branch support based on a Shimodaira-Hasegawa-like (aLRT SH-like) procedure (Anisimova and Gascuel, 2006).

3. Results and discussion

An overview of the detected pathogens is given in Table 1. All tested bumblebees were found infected by at least four different species of pathogen, but up to eight species could be found in a single specimen. Deformed Wing Virus (DWV) and *Nosema ceranae* were omnipresent (both in captive-bred and wild samples), and *C. bombi* was discovered in all captive-bred samples. Overall, *A. bombi* was detected in 12 out of 19 samples (63%) and LSV in 13 out of 19 samples (68%). To the best of our knowledge this is the first report of the occurrence of LSV in *B. atratus*. After its first discovery in the USA (Runckel et al., 2011), the field of action of LSV was subsequently extended to honey bees and solitary bees of Europe (Ravoet et al., 2013, 2014; Granberg et al., 2013), and now to bumblebees of the northwest of South America.

The common honey bee viruses like Acute Bee Paralysis Virus (ABPV), Black Queen Cell Virus (BQCV), DWV and Sacbrood Virus (SBV) were already detected in South American honey bees (Antúnez et al., 2006; Teixeira et al., 2008; Mendoza et al., 2014). They are now considered multi-host pathogens, infecting many arthropod species even beyond the pollinator community (Li et al., 2011; Peng et al., 2011; Zhang et al., 2012; Levitt et al., 2013). All except ABPV were found in *B. atratus* from Brazil (Reynaldi et al., 2013). The scope of the microsporidium *N. ceranae* is so far only restricted to Hymenopteran insect species. Originating from Asian honey bees (*Apis cerana*) (Fries et al., 1996), it was subsequently found in European honey bees (*Apis mellifera*) (Higes et al., 2006; Huang et al., 2007), bumblebees (Graystock et al., 2013; Fürst et al., 2014) including *B. atratus* (Plischuk et al., 2009) and solitary bees (Ravoet et al., 2014). Its occurrence in South American honey bees is well-documented (Klee et al., 2007; Invernizzi et al., 2009; Martinez et al., 2012;

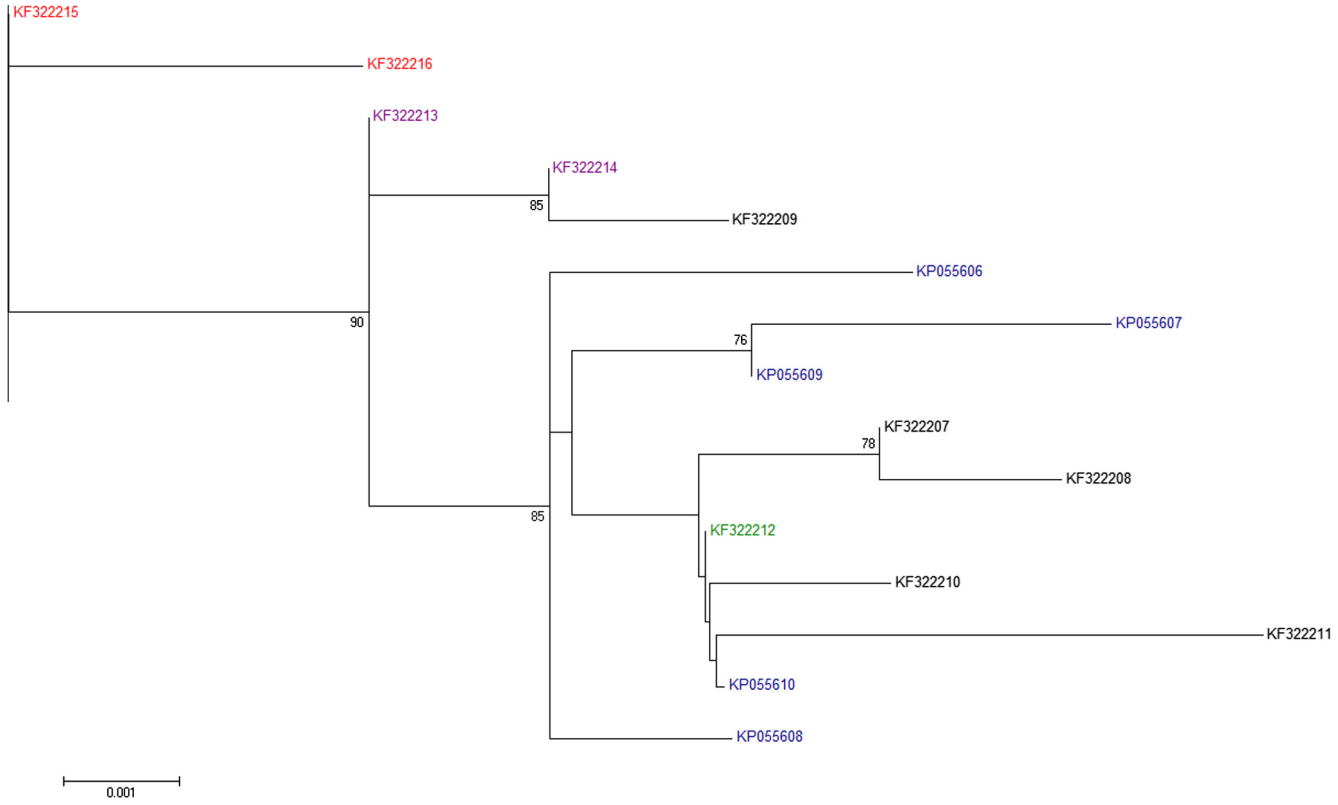

Fig. 1. Phylogenetic analysis of *Apicystis bombi* internal transcribed spacers (ITS). This region includes the partial 18S rRNA, complete ITS 1, 5.8S rRNA gene, ITS 2, and the partial 28S rRNA gene. Samples originating from Argentina are indicated in purple, from Colombia in blue, from Europe in black, from Mexico in red and the universal haplotype in green. Branch support is designated by aLRT statistics. Only values higher than 70% are shown.