Large scale patterns of abundance and distribution of parasites in Mexican bumblebees

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ABSTRACT

Bumblebees are highly valued for their pollination services in natural ecosystems as well as for agricultural crops. These precious pollinators are known to be declining worldwide, and one major factor contributing to this decline are infections by parasites.

Knowledge about parasites in wild bumblebee populations is thus of paramount importance for conservation purposes. We here report the geographical distribution of *Crithidia* and *Nosema,* two common parasites of bumblebees, in a yet poorly investigated country: Mexico.

Based on sequence divergence of the Cytochrome b and Glycosomal glyceraldehyde phosphate deshydrogenase (gGPDAH) genes, we discovered the presence of a new *Crithidia* species, which is mainly distributed in the southern half of the country. It is placed by Bayesian inference as a sister species to *C. bombi*. We suggest the name *Crithidia mexicana* for this newly discovered organism.

A population of *C. expoeki* was encountered concentrated on the flanks of the dormant volcanic mountain, Iztaccihuatl, and microsatellite data showed evidence of a bottleneck in this population.

This study is the first to provide a large-scale insight into the health status of endemic bumblebees in Mexico, based on a large sample size (n = 3,285 bees examined) over a variety of host species and habitats.

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1. Introduction

Bumblebees (*Bombus* spp.) are ecologically and economically important as they provide pollination services in the wild as well as for agriculture, such as for greenhouse crops (Garibaldi et al., 2013; Vethuis and Van Doorn, 2006). Hence, this makes the abundance, diversity and health status of bumblebee populations an obvious matter of concern in many parts of the world. The concerns are fuelled by the fact that many bumblebee populations are known to be declining in abundance in various parts of their native range (Williams and Osborne, 2009). Several factors have been blamed for this decline: competition with exotic, invasive species (Schmid-Hempel et al., 2014), habitat fragmentation, loss of nesting sites and the increasing use of pesticides (Goulson et al., 2008). A particular concern is the effect of parasitic infections and diseases (Meeus et al., 2011), either alone or in combination

with other ecological stress factors. In fact, the globalization of the commercial use of bumblebees for pollination can potentially lead to pathogen spill-overs from imported to wild bumblebees, as has been reported in several cases (Colla et al., 2006; Murray et al., 2013; Schmid-Hempel et al., 2014; Winter, 2006). Clearly, to assess the risks posed by pathogens, it is of crucial importance to survey the health status of bumblebee species in their native range, especially also where species alien to the region had been introduced for pollination at some points. In this project, we investigate the abundance and distribution of two common bumblebee parasites, *Crithidia* (Trypanosomatidae) and *Nosema* (Microsporidia) in a yet poorly investigated but economically and zoogeographically important region: Mexico.

Crithidia is an intestinal protozoan parasite with a monoxenous life cycle, i.e. only one host is used and no vector is present. Parasite cells are ingested by the host, colonize the hindgut, grow in numbers by clonal and sexual reproduction, and are subsequently shed in faeces to infect a next host within the colony or between colonies through the shared use of flowers (Schmid-Hempel, 2001). This parasite is known to substantially reduce the fitness

of infected queens, and is therefore an important pathogen for bumblebees (Brown et al., 2003). The microsporidian *Nosema* is an obligatory intracellular parasite. Its life cycle starts when spores are ingested by the host and penetrate the epithelial cells of the gut to infect various tissues of the host body. The parasite then multiplies inside host cells and forms spores that will eventually be shed through faeces (Fries et al., 1996). Transmission also occurs by contact between workers within colonies, and between colonies likely through the shared use of flowers (Schmid-Hempel and Loosli, 1998). *Nosema bombi*, the most common species found in bumblebees, is suspected to be the main cause of the population decline observed in the late 1990s for *Bombus occidentalis* in North America (Cameron et al., 2011). Effects of parasite infection include reduction in individual reproduction rate. Infected colonies of *B. terrestris* are smaller and produce fewer reproductive offspring (Otti and Schmid-Hempel, 2007, 2008). Besides, Rutrecht and Brown (2009) showed that the parasite *N. bombi* reduces the lifespan of infected individuals and the colony growth. *N. ceranae* is a common parasite of honeybee and may be involved in the colony collapse disorder of honeybees by inducing an increased mortality rate of infected bees (Higes et al., 2008). It has been detected in European commercial *B. terrestris* (Graystock et al., 2013) and in South-American native bumblebees (Plischuk et al., 2009). Multiple species of *Crithidia* and *Nosema* are known to infect bumblebees (Imhoof and Schmid-Hempel, 1999; Rutrecht and Brown, 2008; Schmid-Hempel and Tognazzo, 2010).

Mexico is a leading country both in open-field and protected plantings. As a result, about 50,000 colonies of *B. impatiens* per year are produced locally by two commercial companies and imported in large numbers from North America for crop pollination in greenhouses (Torres-Ruiz and Jones, 2012). A recent study of the exotic *B. impatiens* used for pollination in greenhouse has shown a high prevalence, with 45% of the samples being infected by one or more pathogens (Sachman-Ruiz et al., 2015). However, the spillover to wild bumblebees in Mexico has not been studied. Both internal parasites, *Crithidia* and *Nosema*, have already been diagnosed in native Mexican bumblebee populations, but it is not yet known if the strains are endemic or were imported with their host, then spilling over and expanding into new host populations.

Mexico is also an important biogeographic region at the cross roads of the Neotropical and Nearctic zoogeographical regions. More specifically, the *Bombus*-clade evolved in Western China around 40 My ago. It expanded its range during the existence of land bridges (Beringia) towards the North American continent. During its expansion, the Isthmus of Panama was crossed into South America; in the course of events, *Bombus* has reached the southern tip of the American continent with one species, *B. dahlbomii* (Hines, 2008). Tropical lowland habitats are generally unsuitable for bumblebees (with few exceptions). Hence, the Isthmus of Panama must have represented a barrier for the host and, therefore, for its obligate parasite *Crithidia*, too. Whilst tracing the evolutionary and phylogeographic history of *C. bombi*, Schmid-Hempel et al. (2014) noted that the strain found in South America (Chile and Argentina) was not endemic but likely was introduced with *B. terrestris* from Europe (Schmid-Hempel et al., 2014; Schmid-Hempel, unpublished data). Because of the phylogeographic history of *Bombus*, scrutinizing the origin of parasitic strains found in Mexico would help to more firmly answer the question whether *Crithidia* reached South America naturally (with the historic spread of its host), or not, i.e. was imported from somewhere else. This would clarify whether there was and is a pathogen spill-over threat for native species in Central and South America; such insights would furthermore help to understand the global phylogeographic pattern of the parasite itself.

2. Materials and methods

2.1. Geographic distribution

The sampling for the project was carried out by several Mexican institutions, including ECOSUR (El Colegio de la Frontera Sur), UNAM (Universidad Nacional Autónoma de México), UADY (Universidad Autónoma de Yucatán), and UDLAP (Universidad de las Américas Puebla). Specimens of bumblebees were sampled at 85 different sites across Mexico from July to December 2012, and from July to October 2013. Sampling sites were separated by at least 30 km and were located at altitudes over 1500 m, which is a prime habitat for bumblebees. All sites were georeferenced (coordinates given in Table S1). A minimum of 5 individuals per site was used for population and statistical analyses. At each location, specimens of all species were collected at random, during one hour, so as to represent the local community of *Bombus* species present in the area in the best possible, yet practical manner. Bees were captured when foraging on flowers by netting; species and sex were determined in the field and confirmed later in the laboratory. The insects were subsequently sacrificed using sodium cyanide and preserved in 95% ethanol. Because the results of the 2012 campaign revealed an interesting localized pattern of parasite distribution, a more intensive sampling effort was made in a mountainous volcanic region separating the Valley of Mexico and Puebla, with three altitudinal transects from 1000 m to 4020 m. a.s.l. Throughout the whole country, a total of 3285 individuals belonging to 15 different species were collected. These species were: *B. ephippiatus, B. weisi, B. huntii, B. pensylvanicus, B. diligens, B. wilmattae, B. trinominatus, B. brachycephalus, B. vosnesenskii, B. macgregori, B. fervidus, B. steindachneri, B. medius* and *B. pullatus*.

Genomic DNA extraction was carried out at ETH Zurich from the whole guts using the DNeasy 96 Blood & Tissue Kit® (Qiagen GmbH, Hilden, Germany) following instructions by the manufacturer. We screened all samples for *Crithidia* spp. presence by amplifying a mitochondrial gene, Cyt b, and the partial sequence of a nuclear gene, gGAPDH, as described in Schmid-Hempel and Tognazzo (2010). Besides, *Nosema* spp. presence was determined by amplification of the small rRNA subunit using primers from Baker et al. (1995) labelled 18f and 1537r. Subsequently, we visualized PCR products on PCR CheckIT® gels (Elchrom Scientific, Baar, Switzerland) together with the necessary controls. PCR-products from all positive samples were then purified (ExoSAP method by Hanke and Wink (1994) and Werle et al. (1994)) and sequenced using BigDye® chemistry on a 3130/3730 ABI Sequencer (Applied Biosystems). Sequences were checked manually and blasted for parasite identification using the software MacVector 12.5.1 (Mac Vector Inc., Cary, NC).

2.2. Molecular analyses

Several samples of *Crithidia* sequences obtained for the Cyt b and the gGAPDH gene carried mutations at several sites compared to the published GenBank™ sequence for the already defined species *C. expoeki* and *C. bombi*. Thus, sequences obtained from the Cyt b gene and gGAPDH were aligned using CLUSTALX of MacVector 10 (MacVector Inc.) for the inference of phylogenetic relationship between the different types of *Crithidia*. In addition, MrBayes for inference of the phylogenetic relationships was used for the samples of *Crithidia* (Huelsenbeck and Ronquist, 2001). This program estimates the posterior probability distribution of trees using a Markov Chain Monte Carlo (MCMC) procedure. We ran the model for 131,100 generations until an average standard deviation of split frequencies below 0.01 was achieved. We used a mixed model, flat priors, and sampling of trees every 100 generations; a burn-in