

SHORT REPORT

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# Assessment of intestinal parasites in the coexisting *Bombus terrestris* (Apidae) and *Xylocopa augusti* (Apidae) in central Chile

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## Abstract

*Bombus terrestris* is a European bumblebee extensively commercialized worldwide for crop pollination. In Chile, this species was introduced in 1997 and after confinement escape, it has spread and established in several localities of central-southern Chile and in the Argentine Patagonia. The South American carpenter bee *Xylocopa augusti*, in turn, has been recently reported in central Chile, and as *B. terrestris*, this species has become increasingly common, often found in sympatry with *B. terrestris* in some localities. While intestinal parasites such as the flagellate trypanosome *Crithidia bombi*, the microsporidium *Nosema bombi*, and the neogregarine protozoan *Apicystis bombi*, show high levels of specialization on the *Bombus* genus, parasites often increase their host range, especially after invading novel habitats, hence creating new infection disease scenarios. In this work, we used molecular techniques to detect the presence of the intestinal pathogens of *B. terrestris* in coexisting *X. augusti* from different localities in the Metropolitan Region of Chile. Our results revealed the presence of the three pathogens in *B. terrestris* only, with population prevalence broadly similar to that reported in other studies. The carpenter bee *X. augusti* did not show evidence of any of the three parasites examined, indicating that this invader species is not recipient of any of the parasite species present in *B. terrestris*.

**Keywords:** *Apicystis bombi*, *Crithidia bombi*, *Nosema bombi*, Pathogen spillover

## Introduction

Pathogen spillover, broadly defined as the transfer of one or more pathogens from one reservoir host to a recipient host is one of the major factors influencing disease spread in natural populations. Spillover often occurs when infected host species invade novel habitats and transmit exotic parasites to native hosts, which often experience substantial reduction in reproduction and survival [1, 2]. Pathogen spillover occurs commonly in bee colonies transported from one region to another for commercial purposes because commercially-reared colonies often harbor parasites harmful to wild bees, resulting in disease epidemics, and native bee declines in wild

populations [3–5]. In this regard, *Bombus terrestris* Linnaeus (Apidae) (Fig. 1a), one of the most extensively used bumblebee species for crop pollination worldwide has been suggested to transmit their parasites to recipient native bees in southern South America [6, 7]. One of the parasites commonly harbored by *B. terrestris* is *Crithidia bombi* Lipa & Triggiani, a flagellated trypanosome species that infects exclusively species of the *Bombus* genus. This protozoan is horizontally transmitted by ingestion of infectious cells present in the feces deposited on flowers by infected workers of other colonies or bumblebee species, by contaminated honey stores and nest material, and through vertical transmission from one generation to another within the same colony [8, 9]. Like *C. bombi*, the microsporidium *Nosema bombi* Fantham & Porter, infects exclusively species of the *Bombus* genus. The spores (infective stages) locate mostly in the

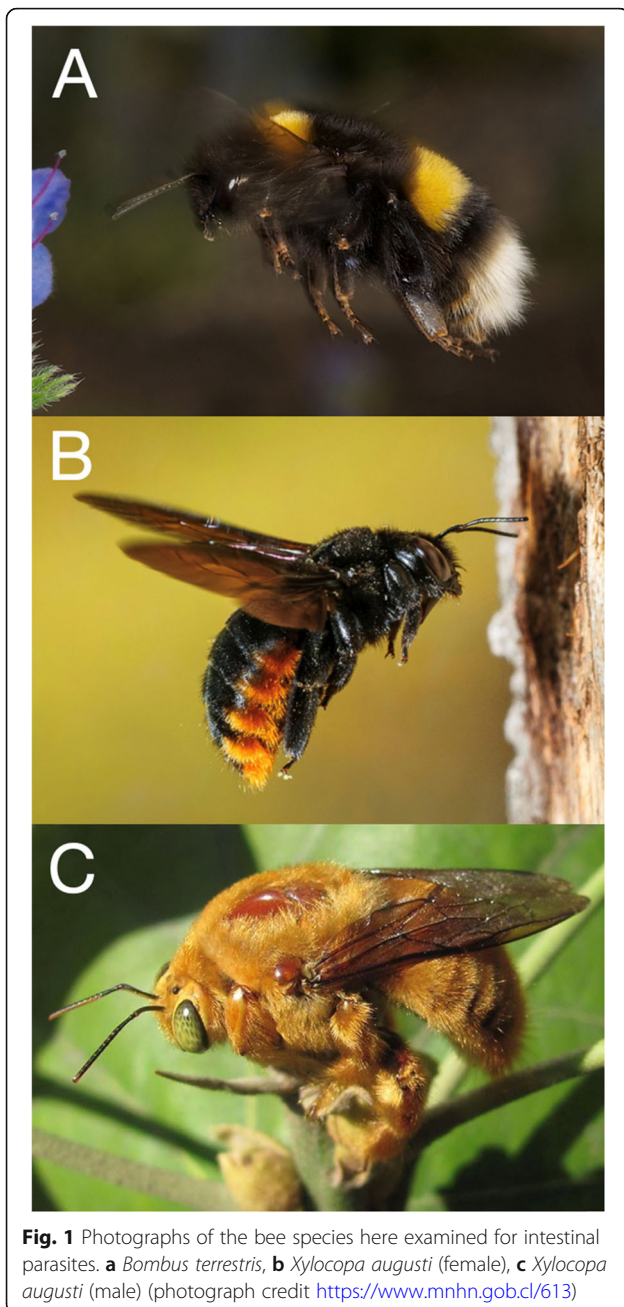
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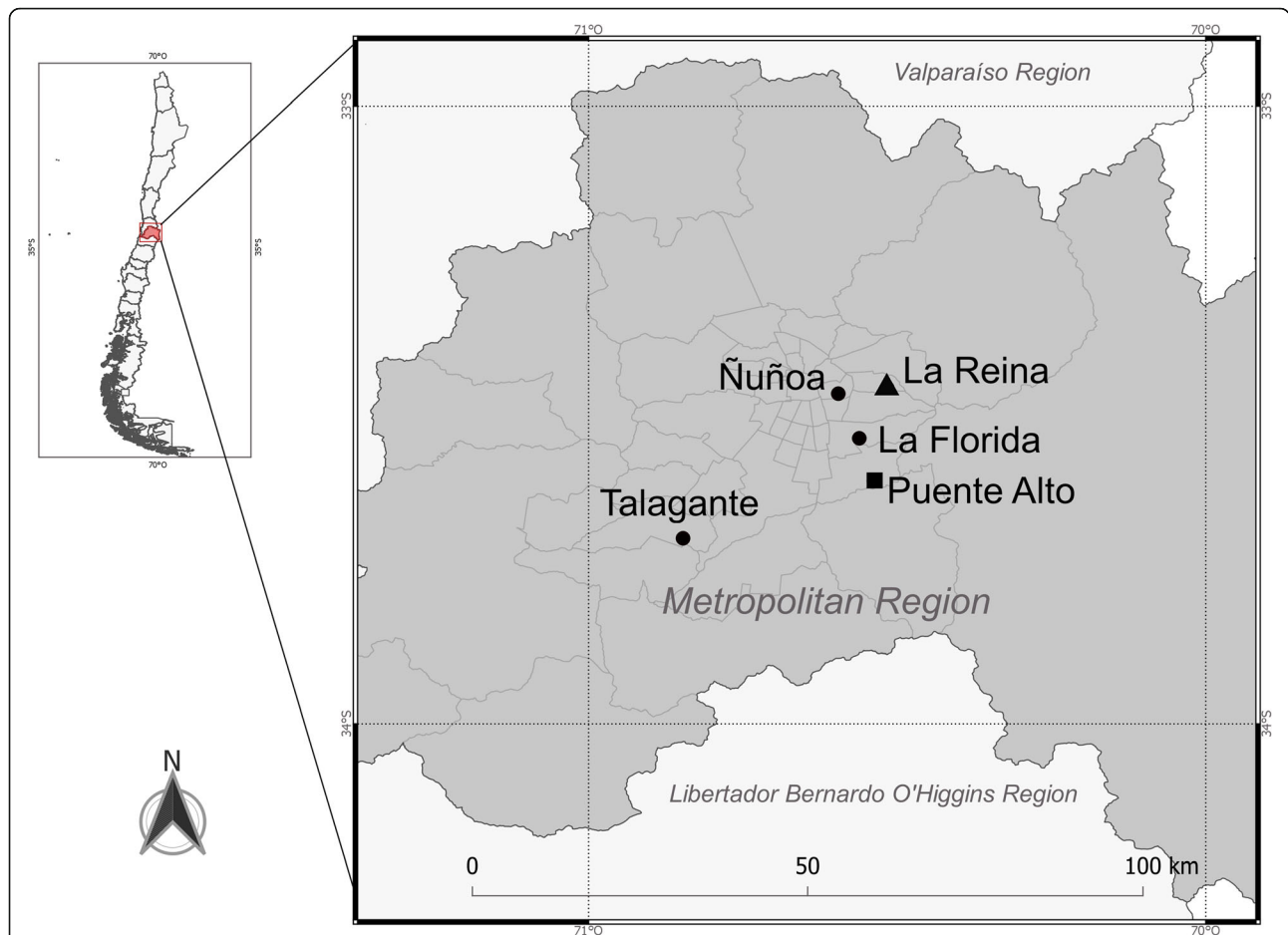
digestive tract of the bumble bees, and after proliferation within the host, are released into the environment [10, 11]. As in *C. bombi*, this parasite transmits vertically across generations in bumble bees [12]. Finally, *Apicystis bombi* Liu, Macfarlane & Pengelly, is a neogregarine protozoan that in addition to *Bombus* species, infects *Apis mellifera*, and *Osmia bicornis* Linnaeus (Megachilidae). It locates in the host adipose tissue, reducing the colony establishment success [12]. Its transmission is horizontal through shared flower resources, but vertical transmission has also been suggested [13].

In Chile, *B. terrestris* was imported for tomato pollination in 1997/1998. After greenhouse confinement escape, it has spread and become established in several localities of southern Chile and Argentina (see reviews in [6, 14]). In 2009, the presence of *C. bombi* and *A. bombi* was reported for the first time in South America [15]. Since then, further reports have confirmed that invasion of *B. terrestris* to novel habitats in southern Chile and Argentina carried exotic intestinal parasites [6, 16], hence increasing the chance of pathogen spillover to other bee species. For example, after *B. terrestris* invasion, *C. bombi* was detected in the native bumblebee *Bombus dahlbomii* Guérin-Ménéville in Chile [6], and *A. bombi* was recorded in the honeybee *Apis mellifera* in Argentina [17, 18], which strongly suggests parasite spillover from *B. terrestris* to other bee species in southern south America [2].

A recently reported invasive bee species in Chile is the South American carpenter bee *Xylocopa augusti* Lepeletier (Fig. 1b and c). Originally distributed in Argentina, Brazil, Uruguay, and Paraguay, this species was recorded for the first time in central Chile in 2013, probably transported through undetected nests in packaging structures from Argentina [19]. After its arrival, new ecological scenarios of coexistence between otherwise allopatric *X. augusti* and *B. terrestris* are frequently found in central Chile, providing new opportunities for parasite transmission. It is known that parasite transmission is an expected result when founding populations in novel habitats are genetically depauperate [20, 21]. In principle, judging by the pathogens found in *B. terrestris* and other *Xylocopa* species, *X. augusti* has the potential to become infected by some viruses such as DWV (Deformed wing virus), BQCV (Black queen cell virus), and SBPV (Slow bee paralysis virus), all of them detected in *B. terrestris* also [22, 23]. However, as most studies inquiring on the potential transmission of parasites from *B. terrestris* to other bee species in southern South America have focused almost exclusively on intestinal parasites, and molecular diagnosis techniques for viral testing require a methodology different to that used in this study [24, 25], we restricted the analysis to the molecular detection of the protozoans *C. bombi* and *A. bombi*, and the microsporidium *N. bombi* in *B. terrestris* and *X. augusti*.

## Methods

Overall, 59 specimens of the two species combined were collected from the total 26 campaigns performed to different localities in the Metropolitan Region, Santiago, during October–December 2018 (Fig. 2, Table 1). Individuals were collected in three sites where both species were observed foraging in the same plants and in sites where only one of the species was found, one site per



**Fig. 2** Map showing the study sites in the Metropolitan Region where specimens were collected. In total, 13 campaigns were carried out. Parentheses indicate the number of visits to the same site: Ñuñoa (3), Puente Alto (2), La Florida (3), Talagante (3), and La Reina (2). Circles: sites where *B. terrestris* and *X. augusti* were collected; triangle: sites where only *B. terrestris* was collected; square: sites where only *X. augusti* was collected

species. Even though campaigns were conceived to check the presence or absence of the focal species, *B. terrestris* and *X. augusti*, no native bee species was observed. The only additional bee present in some sites was the

honeybee *A. mellifera*. Specimens of the focal bees were captured using entomological nets, euthanized in killing jars, and stored in 95% alcohol at  $-20\text{ }^{\circ}\text{C}$  until processing. At the laboratory, separate dissecting tools were

**Table 1** Number of individuals screened and infected with each parasite per site and bee species. The number of PCR products sequenced is indicated in parenthesis

Locality	Coordinates	Sample size ( <i>B. terrestris</i> / <i>X. augusti</i> )	<i>Apicystis bombi</i>	<i>Crithidia bombi</i>	<i>Nosema bombi</i>
Ñuñoa	33° 27' 56" S 70° 35' 44" W	10/11	3/0	1/0	0/0
La Reina	33° 27' 22" S 70° 30' 60" W	5/0	4/0	2/0	0/0
La Florida	33° 32' 16" S 70° 33' 42" W	3/9	0/0	1/0	1/0
Talagante	33° 41' 60" S 70° 50' 50" W	8/7	0/0	2/0	1/0
Puente Alto	33° 36' 24" S 70° 32' 16" W	0/6	0/0	0/0	0/0
Total		26/33	7/0 (4)	6/0 (5)	2/0 (2)

used to avoid cross-contamination between samples, and utensils and working place were submitted to 30 min UV-light before sample processing. Each insect was cleaned with bi-distilled water and then subjected to abdominal extrusion to obtain the intestine and intestinal content. To evaluate the status of infection of the collected samples, and the parasite species involved, whole DNA was extracted from the insect guts dissected, using the EZNA Tissue DNA Kit (Omega Bio-tek) following the manufacturer's instructions. The presence of the specific parasites was evaluated by PCR amplification of the small ribosome subunit of each parasite. Primers used in this work are listed in Table 2. They were designed from the following sequences: *Apicystis bombi* (accession no. FN546182), *Crithidia bombi* (accession no. GU321194) and *Nosema bombi* (accession no. AY741110). Specific primers for *B. terrestris* and *X. augusti* to be used as positive PCR controls, were designed from the partial mitochondrial genome from *B. terrestris* (accession no. KT164618) and from the partial COI gene from *Xylocopa violacea* Linnaeus (accession no. EU122101), respectively. The COI gene from *X. violacea* was used for primer design as no *X. augusti* DNA sequences were found at the NCBI database when this study was performed. PCR reactions were performed using a Thermal Cycler 2720 (Applied Biosystems) in a final volume of 25 µl containing 1x PCR buffer (20 mM Tris-HCl, 10 mM KCl), 2 mM MgCl<sub>2</sub>, 200 µM of each dNTP, 1 µM of each primer, 2 U *Taq* DNA polymerase, and 100–130 ng of template DNA. The PCR parameters were: initial denaturation at 94 °C for 3 min, 35 cycles of denaturation at 95 °C for 30 s, annealing at 53 °C for 30 s and extension at 72 °C for 3 min. Then, a final extension step at 72 °C for 10 min was applied and samples were kept at 4 °C until evaluated by 2.0% agarose gel electrophoresis in TAE buffer stained with SafeView™ Plus (Applied Biological Materials). To confirm the obtained results, some amplicons were sequenced at the service provided by the Sequencing Unit, Pontificia Universidad Católica de Chile (Santiago, Chile) using ABI

PRISM 3500 xl (Applied Biosystems). The obtained sequences were analyzed and compared with the GenBank database using the BLAST algorithm (<http://www.ncbi.nlm.nih.gov/BLAST>).

## Results and discussion

Among the total 26 bumblebees screened, seven tested positive for the neogregarine *A. bombi* (26.9%), six for the trypanosome *C. bombi* (23.07%), and two for the microsporidium *N. bombi* (7.69%) (Table 1). Among the seven specimens infected with *A. bombi*, two of them were also infected with *C. bombi* and both specimens infected with *N. bombi*, were also infected with *C. bombi*. To confirm these results, four *A. bombi* (accession numbers MT856196, MT856197, MT856198 and MT856199), five *C. bombi* (accession no. MT856200, MT856201, MT856202, MT856203 and MT856204) and the two *N. bombi* (accession no. MT856205 and MT856206) PCR products were sequenced. In all cases, the BLAST search revealed over 99% of identity with the corresponding DNA sequences from *A. bombi*, *C. bombi* and *N. bombi* deposited at the NCBI-database, being 100% of identity in most cases (Table 3), confirming that the primers used in this work were successful to identify the parasites under study.

Unlike *B. terrestris*, no parasite was detected in the 33 specimens of *X. augusti* here examined, suggesting that regardless of the status of coexistence with *B. terrestris*, parasites are not present in *X. augusti* (Table 1). To confirm this result, primers were designed in order to differentiate between *B. terrestris* and *X. augusti* DNA (primer pairs BT-F/BT-R and XA-F/XA-R, respectively) (Table 2). PCR products of the expected size were obtained from the 26 *B. terrestris* samples and none from the 33 *X. augusti* samples when using primer pair BT-F/BT-R. Accordingly, PCR products of the expected size were only obtained from the 33 *X. augusti* samples and none from the 26 *B. terrestris* samples when using primer pair XA-F/XA-R. A PCR product from each case was randomly selected and sequenced. The BLAST analysis of the PCR product from

**Table 2** List of primers used in PCR. See [Methods](#) section for details

Name	Sequence (5' → 3')	Target	Expected amplicon size (BP)	Reference
AbSSU-F	GCGGTAATCCAGCTCCAATA	18 rDNA gene, <i>A. bombi</i>	323	This work
NeoR	GACAGCTTCCAATCTCTAGTCG			[23]
CbSSU-F	TTAGGGTTCGATTCCGGAGAG	18 rDNA gene, <i>C. bombi</i>	477	This work
CbSSU-R	CATGCGTGGATCAGGAAAGGA			This work
NbSSU-F	TGAGGTGATTAATTGGAGGGC	18 rDNA gene, <i>N. bombi</i>	404	This work
NbSSU-R	CTCCTTGTGGTATTCTCCGT			This work
BT-F	AGGATTAGACGTTGATACACGAGC	Mitochondrial genome, <i>B. terrestris</i>	447	This work
BT-R	GGATAATCTGAGTATCGTCGAG			This work
XA-F	TCCAATAGGAGGAGGAGATC	COI gene, <i>X. augusti</i>	657	This work
XA-R	CGTCGAGGTATTCCTATCAATCC			This work

**Table 3** BLASTn search results of parasite PCR products. The best alignment between the query sequence and the found sequence is shown in each case

Sample (GenBank accession N°)	BLAST Result – Coverage; %Identity	BLAST Result - GenBank accession N°	BLAST Result - Description
MT856196	478/478; 100%	FN546182	<i>Apicystis bombi</i> SSU rRNA gene, partial sequence
MT856197	468/469; 99,79%	FN546182	<i>Apicystis bombi</i> SSU rRNA gene, partial sequence
MT856198	461/461; 100%	FN546182	<i>Apicystis bombi</i> SSU rRNA gene, partial sequence
MT856199	468/468; 100%	FN546182	<i>Apicystis bombi</i> SSU rRNA gene, partial sequence
MT856200	303/306; 99%	MH010977	<i>Crithidia bombi</i> SSU rRNA gene, partial sequence
MT856201	377/377; 100%	GU321194	<i>Crithidia bombi</i> SSU rRNA gene, partial sequence
MT856202	376/377; 99,73%	GU321194	<i>Crithidia bombi</i> SSU rRNA gene, partial sequence
MT856203	388/388; 100%	GU321194	<i>Crithidia bombi</i> SSU rRNA gene, partial sequence
MT856204	366/366; 100%	MH010977	<i>Crithidia bombi</i> SSU rRNA gene, partial sequence
MT856205	321/321; 100%	KF188740	<i>Nosema bombi</i> SSU rRNA gene, partial sequence
MT856206	385/385; 100%	KF188740	<i>Nosema bombi</i> SSU rRNA gene, partial sequence

a *B. terrestris* sample (accession no. MT862773) revealed 100% of identity with the partial mitochondrial genome from *B. terrestris* and the analysis of the PCR product from a *X. augusti* sample (accession no. MT862774) revealed 90.7% of identity with the partial COI gene sequence from *X. violacea*. These results indicate that the quality of DNA samples from *X. augusti* specimens was adequate to perform PCR analysis, which supports the absence of the three parasites in these samples.

The prevalence of *A. bombi* in *B. terrestris* specimens collected in this work was 26.9%, which is less than the prevalence obtained in a comparable study carried out in Argentina where 14 out of 30 individuals were infected (47%) [16]. Regarding the detection of *C. bombi*, all the sites where *B. terrestris* was present had individuals infected with the protozoan, reaching an overall prevalence of 23.1%, which is in the range of values previously reported for populations sampled near Santiago ([6], 0–41.7%). This result is consistent with the observation that *C. bombi* is very widespread and abundant wherever *B. terrestris* is found [6]. Regarding *N. bombi*, the results obtained in this work indicate a low prevalence of this microsporidium (7.7%), probably because this parasite is more infectious in larvae than in adult bumblebees [26]. Interestingly, Schmid-Hempel et al. [6] did not record the presence of this parasite in *B. terrestris* populations near Santiago. Likewise, the prevalence of *N. bombi* in the 12 Chilean populations sampled in 2010 ranged from 0 to

3.7%, with an average prevalence of 1.7% [6], indicating that even if small, our recordings are the highest prevalence recorded for *B. terrestris* in Chile.

Results from this study indicate that the exotic bee, *X. augusti*, is not infected by any of the three parasites infecting *B. terrestris*, suggesting that transmission of pathogens typical of the *Bombus* genus does not proceed from *B. terrestris* to sympatric *X. augusti*. This result is not necessarily unexpected as the pathogens here examined are often considered specific to the *Bombus* genus. However, as intestinal parasites represent only a fraction of the potential pathogens transmitted (i.e., viruses should also be considered), we cannot rule out completely the idea of parasite transmission from one species to another, especially considering that susceptibility to new parasites is high in novel hosts [27], and new parasites often experience drastic changes in life history traits that permit them to infect novel hosts [28]. While the co-invasion of *B. terrestris* and *X. augusti* to Chile has created a new zone of geographical sympatry for the two otherwise allopatric species, the consequence of their coexistence for disease spread is unknown. Although our data suggests absence of intestinal parasite transmission between hosts, works that consider a broader spectrum of intestinal and viral parasites are needed. This information is crucial to understand the extent to which new contexts of host sympatry in species outside their original habitats influence and amplify parasite transmission in novel environments.

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### Authors' contributions

KF collected the samples, carried out the experiments and interpreted the results. DS contributed with the molecular analysis. JA and RM contributed to the study design and analysis of the results. RM conceived the study and participated in the experimental design and coordination. KF, JA and RM drafted the manuscript. All authors read and approved the final manuscript.

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### Availability of data and materials

All data generated or analyzed during this study are included in this published article. Sequence data that support the findings of this study have been deposited in GenBank with the accession codes MT856196, MT856197, MT856198, MT856199, MT856200, MT856201, MT856202, MT856203, MT856204, MT856205, MT856206, MT862773, MT862774.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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