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Short communication

The tracheal mite *Locustacarus buchneri* in South American native bumble bees (Hymenoptera: Apidae)

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ABSTRACT

As in other regions of the world, bumble bees (*Bombus* spp.) are important pollinators in the neotropics. Despite its relevance, knowledge on their health is still limited in the region. While external acari are known to occur in these insects, presence of the internal, tracheal mite *Locustacarus buchneri* is here reported for first time. After the examination of 2,508 individuals of eight *Bombus* species from Argentina, two workers of *Bombus bellicosus* and one of *Bombus atratus* were found parasitized by *L. buchneri* in localities within San Luis and Buenos Aires provinces, respectively. The rare occurrence recorded agrees with findings from elsewhere in the world.

Bumble bees of genus *Bombus* reach more than 250 species worldwide and nearly 40 in the neotropics. The importance of these insects as pollinators is well known throughout the world, and it is particularly relevant in some crops as tomato and red clover [1]. *Bombus* species have an annual life cycle in which mated queens hibernate commonly in the soil and emerge during warmer weather. Knowledge about bumble bee health in South America was virtually inexistent until recent years when the emergent microsporidium *Nosema ceranae* was discovered parasitizing three *Bombus* species in Argentina [2]. Also after invasion of the exotics *Bombus terrestris* and *Bombus ruderatus* in that country, surveys were intensified and different pathogens and parasites were detected [3–6]. In addition, a variety of external mites is associated with *Bombus* species. These mites are generally mild and they often live in association with the bee's nest using the insect mainly as a dispersal agent. Seven species of external mites, *Kuzinia laevis*, *Kuzinia americana*, *Parasitellus fucorum*, *Pneumolaelaps longanalis*, *Pneumolaelaps longipilus*, *Scutacarus acarorum*, and *Tyrophagus putrescentiae*, are known to be present in Argentina [6]. Internal mites are less common and more harmful than external ones [7,8]. The purpose of this communication is to present the first report of a tracheal mite associated with South American native bumble bees.

Samplings were conducted at 65 localities throughout Argentina. Bumble bees were captured with entomological nets while foraging between early spring and early autumn. Soon after collection, they were frozen (−32 °C). Once in laboratory, insects were identified, dissected, and examined under the stereoscopic microscope (×10–×40) to check for pathogens and parasites [1,9]. When mites were observed, they were extracted with a pipette or fine-point tweezers. Upon isolation, mites were deposited in Petri dishes with one-quarter-strength Ringer's solution [10]. Initial observations were performed in a stereoscopic microscope. After that isolated individuals (including eggs) were cleared in lactophenol and mounted in slides to be observed under the compound microscope (×400, ×1000) [6]. Measurements were taken with an ocular micrometer.

A total of 2,608 adult bumble bees of eight *Bombus* species were examined. We scrutinized individuals of *Bombus atratus* (n = 1,713), *B. terrestris* (704), *Bombus bellicosus* (105), *Bombus morio* (45), *Bombus opifex* (25), *B. ruderatus* (8), *Bombus tucumanus* (6), and *Bombus dahlbomii* (2) (see Appendix for details). Only two (*Bombus brasiliensis* and *Bombus baeri*) out of the ten species of *Bombus* known to inhabit Argentina [1] could not be collected (for supplementary information, see Tables A1 and A2).

Two workers of *B. bellicosus* and one of *B. atratus* were found parasitized by internal mites. Parasitized *B. bellicosus* were from El Amago (32°42′33″S; 66°09′34″W), between the localities of La Carolina and San Francisco, San Luis province in the center of the country during the first half of March 2012. The parasitized *B. atratus* was from Colonia Urquiza (34°56′24″S; 58°04′54″W), northeastern Buenos Aires province in late January 2012 (Fig. AI). None of the three bumble bees appeared to show obvious external signs or symptoms of parasitism. Two and six physogastric females were found in each of the two *B. bellicosus*, respectively, and eight in *B. atratus*. Physogastric females

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were always localized in the bee's metasomal tracheal system. Larviform females (adult but non-gravid stages), males, and numerous eggs were also observed in the tracheae as well as free in the metasomal hemocoel (Fig. 1). Tracheae harboring mites were partially melanized. Of the eight gravid females isolated from *B. atratus*, four were surrounded by eggs and appeared yellowish and larger. The other four were not associated with eggs and were somewhat paler. Larviform females were not found in *B. atratus*.

The family Podapolipidae is a highly specialized group of mites that includes both external and internal species. They parasitize arthropods, particularly insects such as cockroaches, grasshoppers, beetles, and bees. Within the family, genus *Locustacarus* comprises three species that are characterized by the presence of three pairs of functional legs in both sexes, with tarsal claws and a large, pedicellate pulvillus. The gravid female is enlarged, almost non-motile, and the life cycle is oviparous-type, with adults hatching directly from eggs. *Locustacarus trachealis* and *Locustacarus masoni* are associated to the tracheal system of grasshoppers, parasitizing species of Acrididae in the subfamilies Oedipodinae and Cyrtacanthacridinae (*L. trachealis*), and Catantopinae (*L. masoni*) [11,12]. *Locustacarus buchneri* has been found associated with nearly 30 *Bombus* species, all native to the Northern Hemisphere [7,9,11], invading tracheae and air sacs of larvae, pupae, and adult bees, where the mite develops and reproduces. Parasitization may alter host physiology, causing lethargic behavior, reduced life span, decreased foraging, and weakened colony [8,12]. This species hibernates in over-wintering queens and seems to be specific of genus *Bombus* [8,11,13] although a record is known in honey bees [14]. Detections in the Southern Hemisphere were only recorded in New Zealand in *B. ruderatus* and *Bombus subterraneus* [15], which were introduced from European stocks at the beginning of the Twentieth Century [16]. Morphological characters of stages (egg; male; larviform and gravid female) strongly suggest that the species found in Argentina is *L. buchneri* [11,17]. *L. buchneri* is distinguished from the remaining species of the genus by the following characters: larviform female with anterolateral gnathosoma setae equal in length to gnathosoma; the first two pairs of propodosomal setae short, setae ventricales externae, do not overlap setae scapulares externae; metapodosoma shield shaped with conspicuous setae; setae humerales externae as long as tarsi III; plate 1 divided mesially by two sulci; opisthosoma with plate 1 quadrate, with setae sacrales about half the length of plate; coxal plate III pointed anteriorly; coxae I with setae barely reaching the posterior margin of its plate; and tibiae I with five setae. Male: gnathosoma slightly wider than long, with ventral setae less than half the length of gnathosoma; propodosoma with three pairs of short setae plus setae scapulares externae; plate formed by fusion of coxae III with a pair of setae with about half the length of plate; leg I slightly more than half the width of idiosoma; legs II and III more than half the length of body; tarsus I without terminal spines. Gravid female: more than three times long as larviform; number of setae and legs drastically reduced; idiosoma hemispherical with a lateral genital opening (Fig. 1).

The morphology of the mite here reported fits properly with the description of *L. buchneri* [11,17]. However, size differences between South and North American isolates do exist (Table 1). Previous authors [11,17] observed a marked predominance of larger males in their samples while we detected all males of the smaller size kind in the three parasitized bumble bees. Similarly, larviform and gravid females, and eggs showed smaller sizes in all our observations. These differences could be attributable to crowding of mites, deficient nutrition, or aging of progenitors as suggested by Husband and Sinha [11] and Husband and Dastych [18], or even variation due to differences in handling techniques. In *B. bellicosus*,

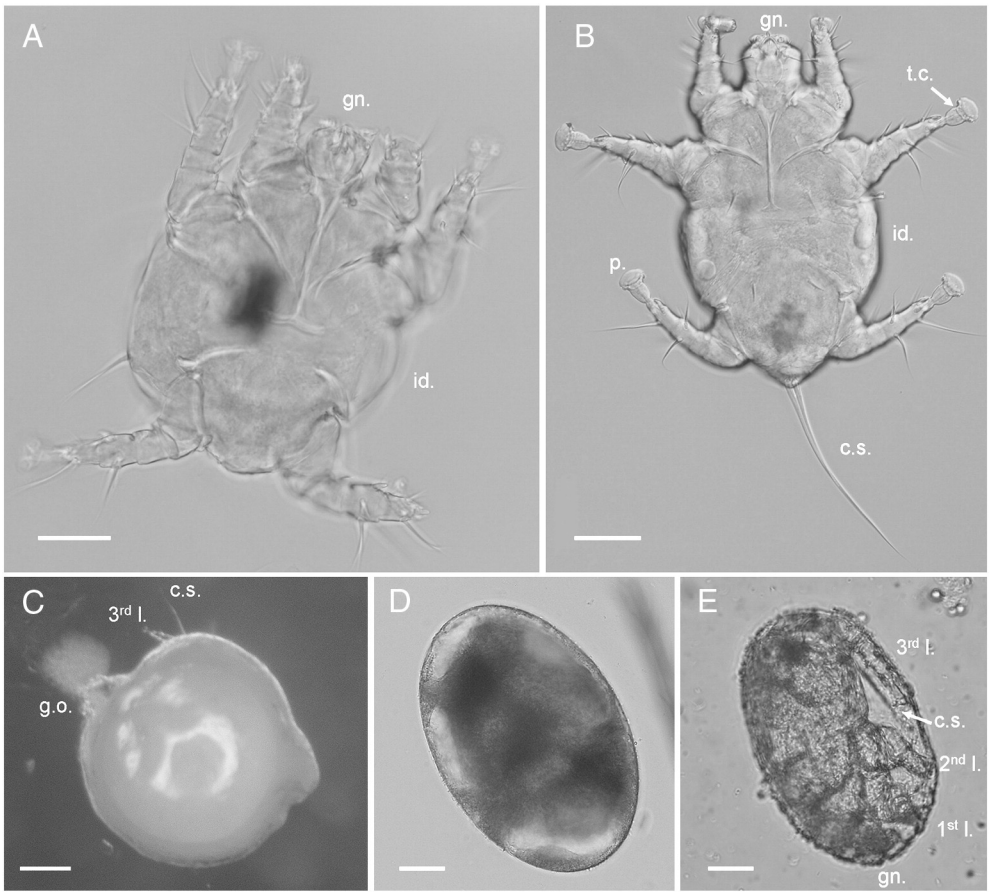

Fig. 1. *Locustacarus buchneri* from *Bombus bellicosus* and *Bombus atratus* of Argentina. (A) Male, ventral view. (B) Larviform female, ventral view. (C) Gravid female, dorsal view. (D) Egg, early embryo stage. E, egg, advanced embryo stage; gn, gnathosoma; id, idiosoma; c.s., caudal setae; p., pulvillus; t.s., tarsal claw; g.o., genital opening; 1st l., first leg; 2nd l., second leg; 3rd l., third leg. [A, B, D, E: bars = 25 μm; C: bar = 100 μm].

Table 1
Measurements (in μm) of *Locustacarus buchneri* based on specimens from Argentina and compared with those published by Husband and Sinha (1970) [11].

Character		Husband and Sinha (1970)	This study
Egg		*(n = 10)*	*(n = 10)*
	Length	185.0	145.2
	Width	120.4	98.8
Larviform female		*(n = 10)*	*(n = 6)*
	Idiosoma length	160.0	115.0
	Idiosoma width	89.3	75.7
	Gnathosoma length	27.0	26.3
	Gnathosoma width	31.3	29.3
	Chelicerae length	12.0	10.0
	Leg I length	51.9	36.0
	Leg II length	70.0	65.0
	Leg III length	75.0	72.0
Male		*(n = 10)*	
	Idiosoma length	140.3	–
	Idiosoma width	102.9	–
	Gnathosoma length	23.8	–
	Gnathosoma width	25.1	–
Small male		*(n = 1)*	*(n = 10)*
	Idiosoma length	84.2	83.2
	Idiosoma width	70.5	72.2
	Gnathosoma length	20.0	19.7
	Gnathosoma width	21.3	20.3
	Aedeagus length	42.5	44.0
	Aedeagus width	13.3	10.7
	Leg I length	–	40.0
	Leg II length	–	58.0
	Leg III length	–	80.5
Gravid female		*(n = 5)*	*(n = 2)*
	Idiosoma length	520.0	446.0
	Idiosoma width	442.0	430.0
	Gnathosoma length	57.7	46
	Gnathosoma width	43.3	38

the sex ratio showed a predominance of male over female mites, and in *B. atratus*, even though the insect was harboring eight gravid females, no larviform was observed. Husband and Sinha [11] suggested the possibility that these mothers would be unfertilized, producing only males. Anyway, because setae arrangement and other morphological characters are coincident with previous descriptions [11,17], we still believe that the found mite might be a morphotypic variation of *L. buchneri*. Based on MtDNA studies, the existence of intraspecific genetic variability in this mite has already been revealed [9,19].

The apparent rare occurrence of *L. buchneri* in Argentine bumble bees seems to be in agreement with records from other regions and could be also related to host preferences [8,15]. According to Otterstatter and Whidden [8], *L. buchneri* seems to show higher prevalence in populations of certain species, most of them included in the subgenus *Bombus* sensu stricto [*B.* (*Bombus*) Latreille]. The only species in the neotropics belonging to this subgenus appears to be *B. terrestris*, which has been identified as a carrier of pathogens (*Crithidia bombi* and *Apicystis bombi*) [3,20], but to our knowledge there are no regional surveys reporting the presence of *L. buchneri* in this species. Genetic and epizootiological studies will be needed and are envisaged to clarify the associations between *L. buchneri* and bumble bees in South America.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.parint.2013.07.006.

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