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Research note

The tropical fowl mite *Ornithonyssus bursa* (Acari: Mesostigmata: Macronyssidae) parasitizing the European starling *Sturnus vulgaris* (Aves: Passeriformes: Sturnidae), an invasive bird in central Argentina. An approach to the bacterial fauna of this mite

El ácaro de aves tropicales Ornithonyssus bursa *(Acari: Mesostigmata: Macronyssidae) parasitando al estornino pinto* Sturnus vulgaris *(Aves: Passeriformes: Sturnidae), un ave invasiva en el centro de Argentina. Una aproximación a la fauna bacteriana de este ácaro*

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Abstract

The tropical fowl mite (*Ornithonyssus bursa*) is a common parasite of domestic and wild birds in tropical and subtropical regions. This mite can cause irritation, severe dermatitis and anemia. The European starling (*Sturnus vulgaris*) is an invasive bird in Argentina. Both *O. bursa* and *S. vulgaris* have high reproductive rates with implications for public health. The goals of our study were to report the occurrence of this mite parasitizing the starlings, and test for the presence of bacteria that could be potentially associated with the mites. The study was conducted in Estación de Cría de Animales Silvestres (Buenos Aires, Argentina). Mites were collected from starlings and stored in 96% ethanol, and prepare for their identification under an optic microscope. Molecular studies were conducted in order to examine the presence of bacteria. All mites were identified as *O. bursa.* We detected for the first time *Wolbachia* sp., associated with the genus *Ornithonyssus*. In contrast, mites were negative for species of *Rickettsia, Bartonella* and *Borrelia*. In addition to the damage that the starlings produce as an invasive species in central Argentina, herein we report the association of these birds with *O. bursa*, favoring the dispersal of the mites and their colonization to other birds.

Keywords: Macronyssidae; Mites; Passeriformes; *Wolbachia*; Bacteria; Argentina

Resumen

Ornithonyssus bursa es un parásito común en aves domésticas y silvestres de regiones tropicales y subtropicales que puede causar irritación, dermatitis severa y anemia. El estornino pinto *(Sturnus vulgaris)* es un ave invasora en Argentina. Tanto *O. bursa* como *S. vulgaris* tienen altas tasas de reproducción con implicaciones en salud pública. Los objetivos del estudio fueron reportar la presencia de este ácaro parasitando a los estorninos y comprobar la potencial asociación de bacterias a los ácaros. El estudio se realizó en la Estación de Cría de Animales Silvestres

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(Buenos Aires, Argentina). Los ácaros se obtuvieron de estorninos, se almacenaron en etanol 96% y se prepararon para su identificación al microscopio óptico. Se realizaron estudios moleculares con el fin de examinar la presencia de bacterias. Todos los ácaros se identificaron como *O. bursa*. Se detectó por primera vez la presencia de *Wolbachia* sp. asociada con el género *Ornithonyssus*. En contraste, los resultados fueron negativos para las especies de *Rickettsia*, *Bartonella* y *Borrelia*. Además del daño que producen los estorninos como una especie invasora en el centro de Argentina, se presenta la asociación de estas aves con *O. bursa*, lo que favorece la dispersión de los ácaros y su colonización hacia otras aves.

Palabras clave: Macronyssidae; Ácaros; Passeriformes; *Wolbachia*; Bacterias; Argentina

The family Macronyssidae (Parasitiformes, Mesostigmata) includes haematophagous mites parasitic of reptiles, birds, bats and rodents. Some species live in their nests and burrows. From an epidemiological point of view, macronyssids are important as parasites themselves, as well as vectors of pathogens that affect domestic animals and humans (Chaisiri, McGarry, Morand, & Makepeace, 2015; Guimarães, Tucci, & Barros-Battesti, 2001; Strandtmann & Wharton, 1958). The tropical fowl mite, *Ornithonyssus bursa* (Berlese, 1888) is a common mite parasite of a variety of domestic and wild birds in tropical and subtropical regions. Records of *O. bursa* from Canada could have been from migratory birds returning from overwintering in a warm region, or a misidentification of these mites (Strandtmann & Wharton, 1958). In the Neotropical Region, *O. bursa* is the mite of most frequent occurrence in farms, causing significant economic losses (Guimarães et al., 2001). The tropical fowl mite is an important parasite causing irritation, severe dermatitis and anemia (Bohrer-Mentz, Liberato da Silva, & Silva, 2015; Guimarães et al., 2001; Mauri & Mosquera, 1985; Semenas & Rocha, 1998). *O. bursa* is considered a common mite in wild birds (Strandtmann & Wharton, 1958). However, there is scarce information about its importance as vector of pathogens which may affect domestic animals and humans, such as protozoans, bacteria and viruses (Chaisiri et al., 2015; Guimarães et al., 2001; Santillán et al., 2015). The role of this mite as reservoir and vector of encephalitis viruses has been reported (Santillán et al., 2015; Valiente-Moro, Chauve, & Zenner, 2005). Bacteria of the species *Rickettsia, Bartonella, Wolbachia* and *Borrelia*, among others, have been detected associated with other *Ornithonyssus* species, but not with *O. bursa* (Chaisiri et al., 2015). Thus, we suspect that some of these bacteria may also be associated with *O. bursa*.

The tropical fowl mite inhabit mostly in nests and nesting birds, and has become a pest to people in areas of high bird populations or where birds are allowed to roost on roofs, around the eaves of homes, schools, hospitals and office buildings. After the birds abandon their nests, the hungry mites frequently move into buildings through windows, doors, and vents and bite the occupants (Mauri & Mosquera, 1985; Semenas & Rocha, 1998; Strandtmann & Wharton, 1958). The use of the nests of the hosts and the roosts, as well as its high reproductive rate, will benefit *O. bursa* with the possibility of colonization of new hosts of the same or different taxa (Radovsky, 1985).

In Argentina, *O. bursa* has been recorded infecting wild birds (Arámburu, Calvo, Alzugaray, & Cicchino, 2003; Arrabal, Manzoli, Antoniazzi, Lareschi, & Beldomenico, 2012; Santillán et al., 2015) and also humans (Bohrer-Mentz et al., 2015; Mauri & Mosquera, 1985; Semenas & Rocha, 1998). Studies carried out in central areas in Santa Fe Province, support that the tropical fowl mite is more frequently associated with nests of the Rufous hornero (*Furnarius rufus* (Gmelin, 1788), Furnariidae) than with nests of any other examined bird. High prevalence of *O. bursa* was also observed in other birds parasitic of the Rufous hornero, as well as in those which use abandoned hornero nests (Arrabal et al., 2012).

The European starling (*Sturnus vulgaris* (L., 1758)) (Passeriformes, Sturnidae) is a native species to Europe, Western Asia and northern Africa (Feare, 1984). This bird is an invasive species in Argentina (Ibañez, 2015), where the first specimens were recorded in Buenos Aires Province in 1987 (Pérez, 1988). A few years later, the number of European starlings increased and they were frequently observed in flocks in central and northern area of Argentina (Peris, Soave, Camperi, Darrieu, & Arámburu, 2005). The starling is usually undergoing an exponential expansion in Argentina (Ibañez, 2015). The European starling has the world's largest distribution of all species of the genus (Craig & Feare, 2009), and is included in the list of the 100 most damaging invasive species in the world (Lowe, Browne, Boudjelas, & De Poorter, 2000). In addition, in some of the countries where it was introduced it has shown an explosive population growth (Feare, 1984).

The tropical fowl mite was first recorded on humans in 1950, in New Zealand (Murray, 1950). The source of infection was a deserted starling nest in the ceiling of the house. Thus, it is supposed that the mites may parasite starlings and inhabit their nests. Since then, the tropical fowl mite was also known as "starling mite", and was recorded in New Zealand in a variety of birds (Powlesland, 1977).

Since the invasive species *S. vulgaris* and the mites have high reproductive rate with implication in public health, the aim of our study is to examine the occurrence of this mite parasitizing European starlings in central Argentina. In addition, we investigate the presence of bacteria, including potentially pathogenic taxa, associated with the mites.

Fieldwork was conducted in Estación de Cría de Animales Silvestres (ECAS 34°50′N, 58°06′W), situated at the Parque Provincial Reserva Forestal Pereyra Iraola, Berazategui, Buenos Aires Province, Argentina. ECAS is located on 230 hectares of pastures, small lagoons and patches of forest composed mainly by exotic species as Glossy privet (*Ligustrum lucidum* Ait),

Honey locust (*Gleditsia triacanthos* L.), Cypress (*Cupressus* sp.), European hackberry (*Celtis australis* L.), and in a lower proportion by native species as Tala (*Celtis enrenbergiana* Gillet). Mean temperature of the warmest and coldest months are 23 °C and 9 °C, respectively, and the mean annual rainfall in the area is ∼90 mm (Ministerio de Asuntos Agrarios, 2007).

Mites were collected during a survey on the reproductive biology of the starlings. 40 wooden nest-boxes were placed at a height of 2.5/3 m in European hackberries and Talas during the breeding season of the starlings. 40 samples of mites were collected from nestlings of European starlings born in nest-boxes from August to December 2013. Each nestling was placed into a plastic bag with a piece of cotton soaked in ethyl acetate, leaving the head outside the bag. Another piece of cotton soaked in ethyl acetate was passed through the neck and the head. After 6 min feathers were brushed, so mites fell down into the plastic bag. Mites were isolated and preserved in 96% ethanol in individual tubes. In the laboratory, mites were cleared in lactophenol, mounted in Hoyer's medium, studied by light microscopy and photographed. Identifications were carried out following Baker (1999), Guimarães et al. (2001) and Micherdzinski (1980).

Mites were pooled in 4 groups of 10–13 mites each one. DNA extraction of the pools was performed using the High Pure PCR Template Preparation Kit (Roche Applied Science, Mannheim, Germany). DNA obtained was analyzed by means of 2 PCRs. The genus *Rickettsia* amplification was performed with oligonucleotides for a portion of the intergenic space 23S-5S rRNA (Jado et al., 2006). *Rickettsia parkeri* was used as positive control. Family Anaplasmataceae oligonucleotides were used for a fragment of 16S rRNA (Parola et al., 2000). *Anaplasma bovis* was used as positive control. Nuclease-free water was used as a negative control. For the genus *Bartonella* primers were used for a portion of the 16S rRNA (García-Esteban et al., 2008). *Bartonella henselae* was used as positive control. For the genus *Borrelia* primers were used for a portion of the 16S rRNA (Gil, Barral, Escudero, García-Pérez, & Anda, 2005). *Borrelia burgdorferi* was used as positive control.

The amplified products were purified with Zymoclean™ Gel DNA Recovery Kit (Zymo Research, Irvine, USA) and sequenced in a 3500 Genetic Analyzer sequencer (Applied Biosystems, Foster City, USA) in Neurovirosis Department (National Institute of Infectious Diseases, ANLIS, Dr. Carlos G. Malbrán, Argentina). The obtained sequences were compared with sequences available in GenBank, using BLAST (www.ncbi.nlm.nih.gov/blast) software.

Representative sequence obtained in this study has been deposited in the GenBank database under the following accession number: KU953378 (16S rRNA fragment of *Wolbachia* sp.).

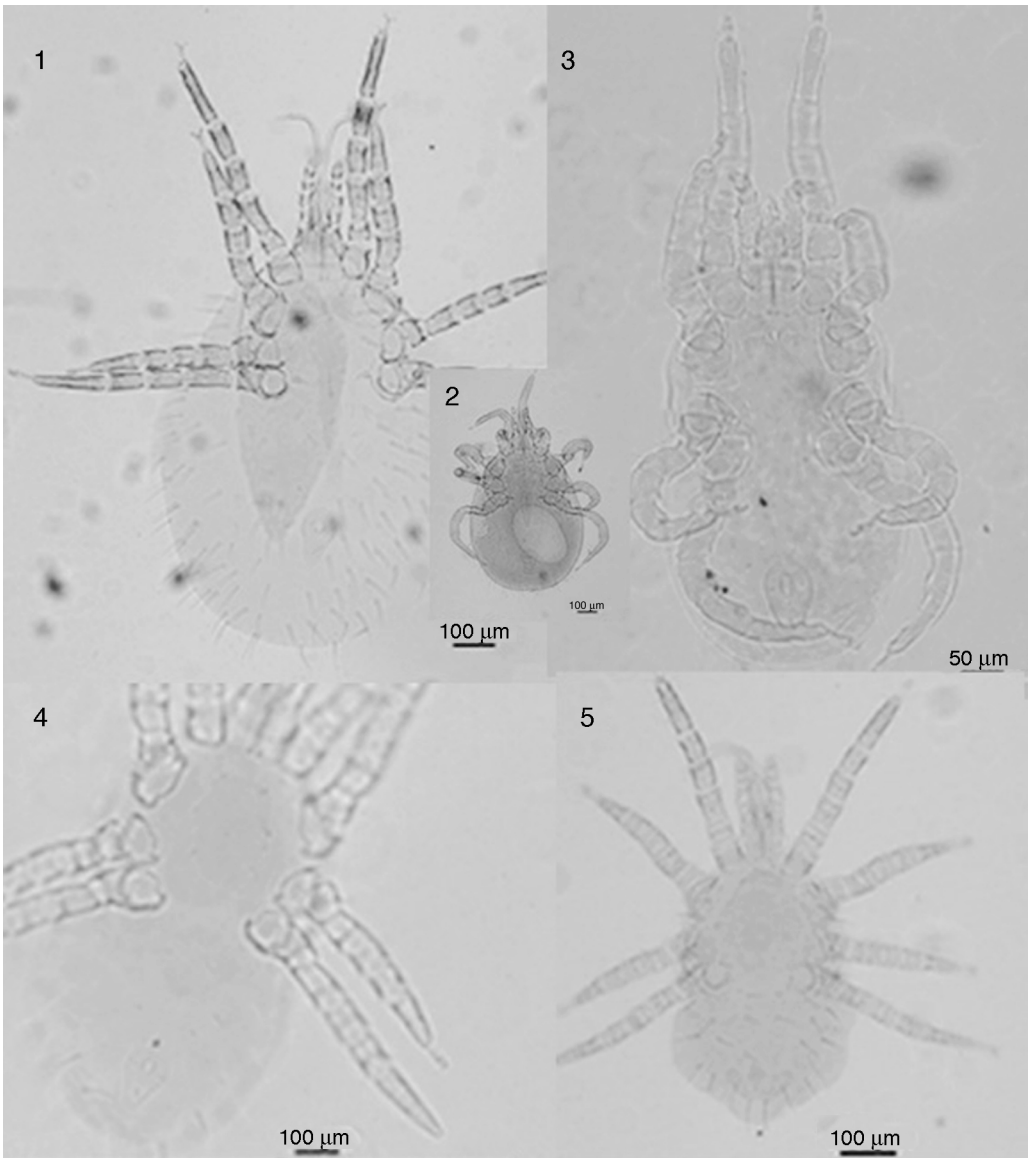
Adults (males and females) and protonymphs collected from the European starlings were identified as *O. bursa* (Figs. 1–5). Their morphology fit descriptions, drawings and keys provided in the literature. Females (Fig. 1) with entire dorsal shield with the posterior end gradually tapering and with short setae; chelicera not enlarged distally; sternal shield with 3 pairs of setae; epigynal shield with the posterior end acutely tapering; anal shield longer than wide; anal opening located near the proximal border of the anal shield; 2 posterior pairs of setae on the dorsal shield are longer than the preceding ones. Some females were observed with an egg inside (Fig. 2). Males as in Figure 3, with entire dorsal shield and a holoventral shield. Protonymphs with 2 dorsal shields (Fig. 4) and 1 short ventral shield (Fig. 5).

Three of the 4 pools examined were positive to PCR for the family Anaplasmataceae. The products detected were sequenced, and sequences resulted in a 100% identity among themselves, and 99.7% with different endosymbionts of arthropods of the genus *Wolbachia*, 98.7% with *Wolbachia pipientis* (HQ121414) from *Ornithonyssus sylviarum*, and 97.4–95.4% with other *Wolbachia* sp. detected in other species of mites (KP114101, DQ288985, KF135426, EU499319, EU499317 and KF511580, among others). In contrast, mites were negative for species of *Rickettsia, Bartonella* and *Borrelia*.

Only protonymphs, male and female adults of the tropical fowl mite were collected from the European starlings. The absence of larvae and deutonymphs of *O. bursa* in the samples is in accordance with the literature for macronyssids. The larvae do not feed and may molt to protonymphs in a day or so. Protonymphs are haemotophagous, and usually leave the host prior to molting, so that the deutonymph is found in the nest or roost and usually don't feed. The deutonymphs usually have weak sclerotization, poorly developed plates and setation and nonfunctional mouthparts, while adult macronyssids, which emerge after a relatively brief deutonymphal stage, are rapid feeders. The protonymphal biology allows macronyssids to maintain a higher reproductive rate. In addition, the use of the nests of the hosts and the roosts, will benefits the tropical fowl mite with the possibility of colonization new hosts of the same and/or different taxa (Radovsky, 1985).

The European starlings nest on cavities on trees formed by decay or built by birds that excavate their own cavities, such as woodpeckers (*Colaptes* spp., Picidae) (Wesołowski, 1989). They also use for nesting artificial buildings like roofs of houses, lamp posts and nest-boxes (Ibañez, 2015; Ingold, 1998). In Argentina, European starlings were observed nesting in nests of Rufous horneros (*F. rufus*) (Rizzo, 2010), woodpeckers (*Colaptes* spp.) (Rebolo-Ifrán & Fiorini, 2010; Schmidtutz & Agulián, 1988) and of the Firewood-gatherer *Annumbius annumbi* (Vieillot, 1817) (Di Sallo & Segura, 2014). In Parque Provincial Reserva Forestal Pereyra Iraola, where this study was carried out, there are several species of birds that nest in cavities with which European starlings may be competing (Ibañez, 2015). Probably parasitic nesting habits of the European starlings will benefit the tropical fowl mite with the possibility of colonization these birds as new hosts in Argentina. An analogous situation was revealed at Espinal Region of Santa Fe Province, Argentina, where *O. bursa* is highly associated with the nests of *F. rufus*, and through the nests the mite colonizes other birds (Arrabal et al., 2012).

In the present study we report for the first time the presence of *Wolbachia* sp. associated with *O. bursa.* At the GenBank there is an unpublished sequence of *W. pipientis* from *O. sylviarum* (HQ121414) which is not published, and not included in Chaisiri et al. (2015). With the exception of this record, our results appear



Figures 1–5. 1, *Ornithonyssus bursa*. Female. 2, *Ornithonyssus bursa.* Female with an egg inside. 3, *Ornithonyssus bursa*. Male. 4, *Ornithonyssus bursa*. Protonymph, dorsal view. 5, *Ornithonyssus bursa*. Protonymph, ventral view.

to represent the first mention of *Wolbachia* associated with the genus *Ornithonyssus.*

Species of *Wolbachia* are the most prevalent endosymbionts of terrestrial arthropods, incluing Mesostigmata, which show host preference and are known to manipulate mite reproduction by inducing cytoplasmic incompatibility, parthenogenesis, sex-ratio distortion (e.g. male-killing and feminization), and an increase in female fecundity (Chaisiri et al., 2015). In further studies it will be interesting to analyze the effect of these bacteria on *O. bursa*.

In contrast, all samples of the tropical fowl mite examined were negative for *Rickettsia, Bartonella* and *Borrelia*. The low number of samples examined does not allow further conclusions at the moment. Further studies will confirm the absence of bacteria of these genera. Previously, Chaisiri et al. (2015) reported *Coxiella burneti*, *Bartonella* sp., *Rickettsia* spp., *B. burgdorferi* and *Francisella tularensis* associated with *Ornithonyssus bacoti*, and *Chlamydia psittaci* with *O. sylviarum.*

In addition to the damage that the European starlings produce as an invasive species in central Argentina, competing and displacing other birds (Ibañez, 2015), herein we reported the association of these birds with the tropical fowl mite, favoring the dispersal of the mites and potentially their colonization of other bird species. Since *O. bursa* impact on public health, the results obtained are important from an epidemiological point of view.

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