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Original Article

First record of *Ornithonyssus bursa* (Berlese, 1888) (Mesostigmata: Macronyssidae) parasitizing invasive monk parakeets in Santiago, Chile

Primeiro registro de *Ornithonyssus bursa* (Berlese, 1888) (Mesostigmata: Macronyssidae) parasitando caturritas invasoras em Santiago, Chile.

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Abstract

Myiopsitta monachus is an invasive psittacine with wide distribution due to the pet trade. Its large communal nests and synanthropic nature contribute to its successful colonization of cities, from where it seems to be expanding in range and numbers. This is relevant with regard to pathogens that invasive species may harbor, especially when host populations thrive. We aimed to identify an abundant mite found in invasive monk parakeet chicks that had been collected in Santiago during 2017 and 2018. Through morphological and molecular identification of the 18S ribosomal RNA gene, we confirmed the presence of *Ornithonyssus bursa*. This was the first report of this mite in Chile. This mite is common in native and invasive monk parakeet populations and may affect other birds, including domestic fowl. Further, this mite bites people and can be a potential vector of pathogens such as bacteria or viruses. We conclude that this parasite was likely introduced with the parakeet and discuss possible ecological, health and economic consequences of this new potential pest.

Keywords: Invasive species, monk parakeet, tropical fowl mite, Chile.

Resumo

Myiopsitta monachus é um psitacídeo invasor amplamente distribuído devido ao tráfico de animais selvagens. Os grandes ninhos comunitários construídos e sua condição de espécies sinantrópicas contribuem para a colonização bem-sucedida das cidades, onde parece estar expandindo sua distribuição e número de indivíduos. Isso é relevante, quando se trata de patógenos que os invasores podem abrigar, especialmente quando as populações hospedeiras prosperam. O objetivo deste trabalho foi identificar um ácaro abundante, encontrado em filhotes de periquitos-monge introduzidos em Santiago, Chile, coletados durante 2017 e 2018. Por meio da identificação morfológica e molecular do gene do RNA ribossômico 18S, foi confirmada a presença de *Ornithonyssus bursa*, sendo o primeiro registro para o Chile. Esse ácaro é comum em populações nativas e introduzidas de periquitos-monge e pode afetar outras aves, incluindo aves domésticas. Além disso, esse ácaro

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†*In memoriam*

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pode picar pessoas e pode ser um potencial vetor de patógenos, como bactérias ou vírus. Conclui-se que esse parasita provavelmente foi introduzido com o periquito e foram discutidas as possíveis consequências ecológicas de saúde e econômicas dessa nova praga em potencial.

Palavras-chave: Espécies invasoras, periquito-monge, ácaro de aves tropicais, Chile.

Introduction

Invasive species are considered to be the second largest cause of biodiversity loss globally (Vitousek et al., 1997) and the first in biogeographic islands (Vitousek, 1988). Central Chile is recognized as a biogeographic subregion, and Santiago province is one of its two biogeographic units (Morrone, 2006). These unique habitats are considered to be a valuable global ecoregion (Olson & Dinerstein, 1998). Central Chile is now identified as one of the 25 biodiversity hotspots of highest conservation priority, due its high levels of endemism and high anthropic pressure (Myers et al., 2000). Nonetheless, threats to Chilean biodiversity are still underestimated (Brooks et al., 2002).

Biological invasions relate to the emergence of diseases (Dunn & Hatcher 2015) that have the potential to contribute to wildlife extinctions, particularly when they interact with additional driving factors (Daszak et al., 2000, Harvell et al., 2002; Smith et al., 2009). In this regard, invasive species have been identified as sources of spread of zoonoses. Thus, these species have the potential to affect the health of animals and people (Keesing et al., 2010; Dunn & Hatcher 2015; Estrada-Peña et al., 2014). Consequently, invasive parasites are also considered to constitute biological invasions, with a crucial role in species loss (Taraschewski, 2006; Landaeta-Aqueveque et al., 2018).

Monk parakeets are widely distributed as an invasive species as a consequence of the pet trade (Edelaar et al., 2015). Currently, alien populations are found in 19 countries (ISSG, 2015). These parakeets are considered to be ecosystem engineers (Briceño et al., 2019) because of their unique ability among psittacids for building large communal nests weighing up to several hundred kilograms (Spreyer & Bucher, 1998). These nests are entwined with twigs and branches by a parakeet couple or several family members, to construct chambers that may be aggregated over several seasons, thus enlarging these nests (Spreyer & Bucher, 1998). Monk parakeets use their nests for breeding and roosting year-round (Navarro et al., 1995) and are considered to be highly philopatric (Martin & Bucher, 1993). This bird species is deemed to be synanthropic (Briceño et al., 2019) and thus is mainly found in urban environments. However, they may also have an important impact in rural areas, as observed in their native distribution in Argentina, where they are considered to produce US$ 1 billion of annual costs associated with crop losses (Iriarte et al., 2005).

In Chile, the invasive population of monk parakeets grew from pets that were released in eastern Santiago during the early 1980s (Iriarte et al., 2005; Briceño et al., 2017). Official records estimate that at least 15,000 parakeets were imported into Chile, until the species was declared harmful and importation banned. Since then, its major impact has been perceived on fruit and ornamental trees (Iriarte et al., 2005).

Although little is known about monk parakeets' pathogen load and their role in pathogen transmission (Briceño et al., 2017), they have been found to harbor ectoparasites in their area of native distribution, and some are specific to these birds. Hence, in Argentina the cimicid bug *Psitticimex uritui* (Hemiptera: Cimicidae) has been classified as one of the most abundant parasites of monk parakeet nests, even though it only parasitizes this species (Spreyer & Bucher, 1998; Aramburú et al., 2003). The blood-sucking mite *Ornithonyssus bursa* (Acarina: Macronyssidae) has also been described in parakeets' nests in Argentina (Aramburú et al., 2002). Two chewing lice, *Heteromenopon macrurum* (Phthiraptera: Menopodidae) and *Paragoniocotes fulvofasciatum* (Phthiraptera: Philopteridae), are permanent parasites of monk parakeets in their area of native distribution (Aramburú et al., 2003). The latter has recently also been found in introduced monk parakeet populations in Chile and Italy (Mori et al., 2015; Briceño et al., 2017). Finally, the soft tick *Argas monachus* (Ixodoidea: Argasidae) is also specific to monk parakeets and all stages of this parasite have been found dwelling in nests of these birds in Argentina and Paraguay (Keirans et al., 1973; Mastropaolo et al., 2011). Furthermore, in their invasive distribution, monk parakeets are also capable of acquiring local parasites. Consequently, it was found in Rome, Italy, that monk parakeets harbored not only the invasive parasites *P. fulvofasciatum* and *O. bursa*, but also the flies *Crataerina pallida* and *Ornithophila metallica* (Diptera: Hippoboscidae) and the louse *Columbicola columbae* (Phthiraptera: Philopteridae) (Ancillotto et al., 2018).

The aim of the present study was to report on the presence of abundant mites found on monk parakeet chicks in Santiago, characterize them morphologically and genetically and discuss the potential ecological and health implications of this blood-sucking mite for local fauna.

Material and Methods

Study site

This study was conducted in the Metropolitan Region of Santiago (33º27′ S; 70º38′ W), Chile. This region is located in the Mediterranean bioclimatic zone of Central Chile, which is characterized by dry summers and wet winters, with marked interannual variability due to the El Niño-Southern Oscillation phenomenon (Amigo & Ramírez, 1998). The mean annual temperature is 13.2 ºC and the mean annual precipitation is 531 mm (Schulz et al., 2010). Temperature and humidity depend upon topography, resulting in a vegetation mosaic of *Acacia caven* shrubland on lower hillslopes, and evergreen sclerophyllous forest, mainly on watersheds and south-facing slopes (Badano et al., 2005; Schulz et al., 2010).

Sample collection

As part of a research project aiming to asses monk parakeets' ecological and sanitary impact, monk parakeet nestlings (<40 days old, as suggested by Eberhard (1998) were manually collected from nests throughout the city of Santiago, during two of their reproductive seasons (austral springs of 2017 and 2018). During the collection process, abundant mites on some of the nestlings were noted. These arthropods were collected and preserved in 70% ethanol. Some of them were subsequently clarified in Nesbitt solution and mounted in Berlese medium (Krantz & Walter, 2009) for viewing and photographing using bright-field microscopy (Bf; Leica DM1000; Figures 1 and 2) and scanning electron microscopy (SEM; Hitachi SU3500; Figure 1A).

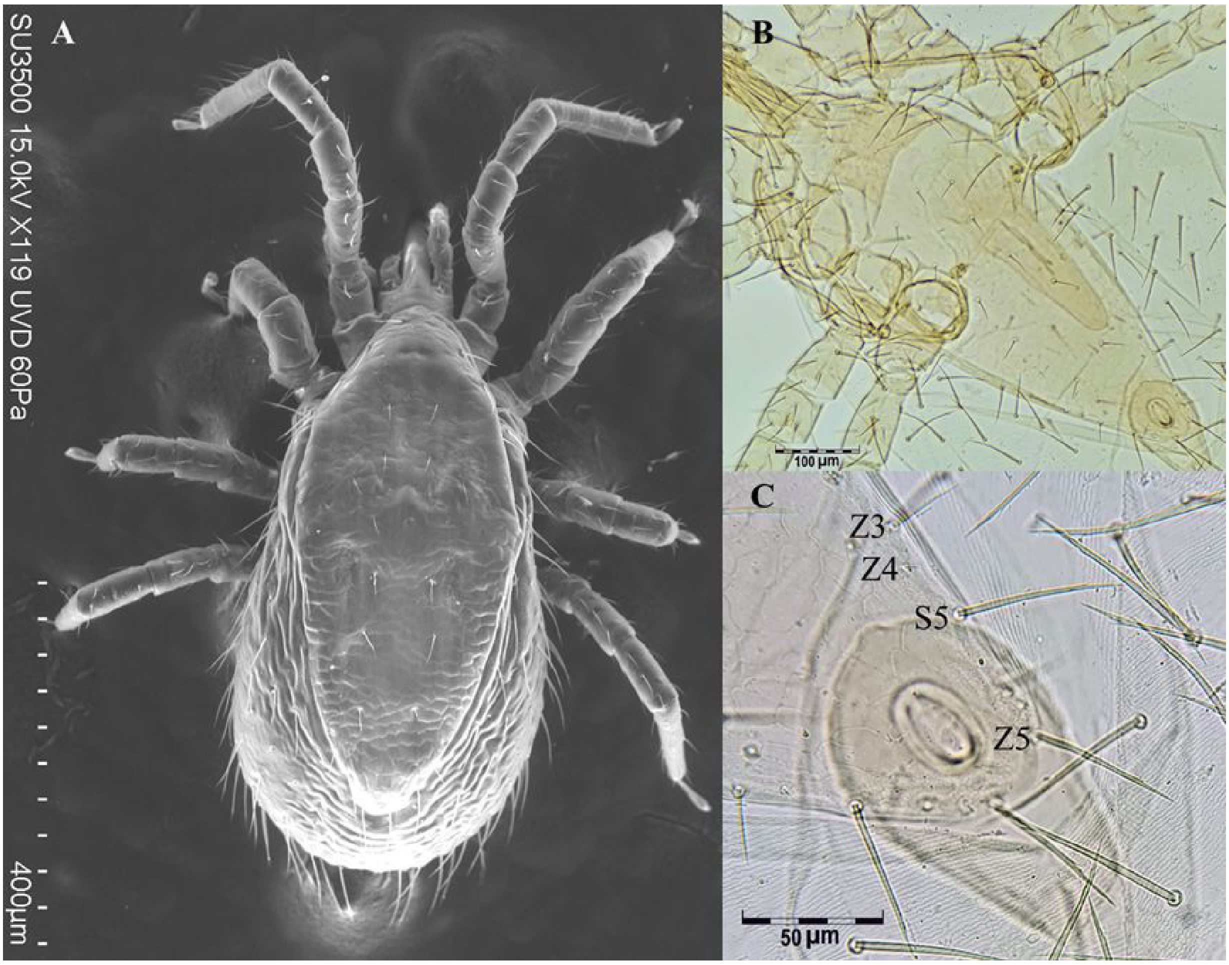

Figure 1. Female of *Ornithonyssus bursa.* (A) Dorsal general view (SEM); (B) Dorsal general view (Bf); (C) Posterior margin of the dorsal plate with Z3, Z4, Z5 and S5 setae.

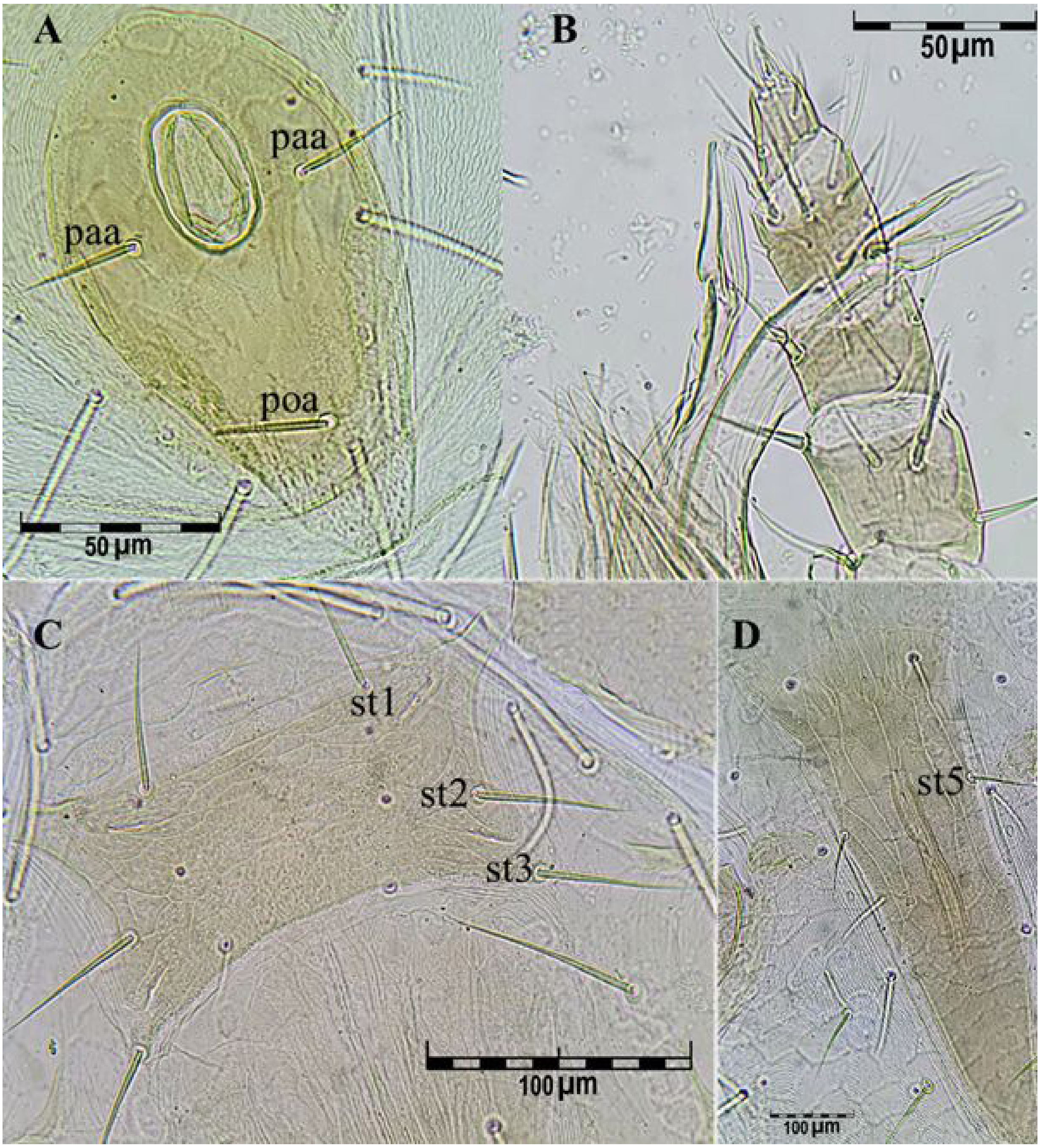

Figure 2. Female of *Ornithonyssus bursa*. (A) Anal plate with paranal setae (paa) and postanal seta (poa); (B) General view of the chelicera; (C) Sternal plate with three sternal setae (st1-st3); (D) Epigynal plate with epigynal seta (st5).

Molecular analyses

To contribute to identification of mite species, five mites were individually pressed between two frozen glass slides and squashed for DNA extraction (Briceño et al., 2020). Biological material was retrieved by pipetting 180 µL of T1 buffer provided in the NucleoSpin® tissue DNA kit (Macherey-Nagel Ltd., Düren, Germany) on the rim of the slides, and collecting the material through capillary action. Extraction of DNA from each mite was completed using the NucleoSpin® tissue DNA kit, following the manufacturer's instructions.

In order to perform PCR, a fragment of the 18S rRNA gene was amplified using the following primers: 5'-CTA TTG GAG GGC AAG TCT GG-3' (forward) and 5´-TGG CAT AGT TTA TGG TTA G-3´ (reverse). These are modified versions of the primers Mite18S-1F and Mite18S-1R published by Otto & Wilson (2001). PCR was performed using Taq DNA polymerase with the ThermoPol® buffer kit (New England BioLabs®), following the manufacturer's instructions.

Reactions were set up in a volume of 25 µl and contained the following: 1X of ThermoPol® reaction buffer, 200 µM of each dNTP, 0.2 µM of each primer, 2 µL of genomic DNA extracted as described above and 0.625 units of Taq polymerase. Negative controls, in which nuclease-free water was added in place of the DNA template, were used to confirm that no contamination of reagents had occurred. The PCR sequence consisted of a modified version of what was described by Otto & Wilson (2001): 94 °C for 1 min; 35 cycles of 94 °C for 20 sec, 50 °C for 30 sec and 72 °C for 1 min and 30 secs; and a final elongation cycle of 72 °C for 5 min. The PCR products were analyzed by separately distributing 5 µL of each amplicon on 1.5% agarose gel stained using GelRed®, and visualizing the results under ultraviolet light. Successful PCR reactions (four in total) were then purified using the DNA Clean & Concentrator™-5 kit (Zymo Research), following the manufacturer's instructions. Purified PCR products were then sequenced using both forward and reverse primers (Macrogen Inc., Seoul, South Korea). DNA sequences were edited manually by reviewing chromatograms and were aligned using MEGA 7 (Kumar et al., 2016).

Results

Most of the manually collected monk parakeet chicks had abundant mites on them, as did the nest materials. During transportation to the laboratory for subsequent analyses, many of these mites were found creeping on research team members, who received bites that left marks lasting for days. The mites also remained on the team members' clothing for days. Under a microscope (Figure 1A, B), these were identified as mites of the order Mesostigmata, potentially of the species *Ornithonyssus bursa*.

The following characteristics were described. Dorsal plate complete with short setae and with gradual distal narrowing, posterior margin of dorsal shield with four pairs of posterior setae, Z4 present and short (Figure 1A-C); sternal plate with three sternal setae inside the plate (Figure 2C); chelicerae not enlarged distally (Figure 2B); epigynal plate with tapered distal end (Figure 2D); anal plate longer than wide (Figure 2A) (Strandtmann & Wharton, 1958; Denmark & Cromroy, 2003; Radovsky, 2010; Lareschi et al., 2017; Bassini-Silva et al., 2019).

The preparations were deposited in the Collection of the Animal Science Department (Colección del Departamento de Ciencia Animal, CDCA; Universidad de Concepción, Chillán) under the numbers CDCA-352-353. DNA amplification by means of PCR provided four identical sequences (GenBank accession number MT176123; 457 bp), each obtained from individual mites, with 100% homology to *O. bursa* at the 18S ribosomal RNA gene (Reference FJ911854; 1597 bp).

Discussion

The present manuscript provides the first report of occurrence of the tropical fowl mite (*Ornithonyssus bursa* Berlese, 1888) in Chile. This mite belongs to the order Mesostigmata and parasitizes passerines and domestic birds (Sreenivasa Murthy & Panda, 2016). It is found in warmer areas of the world including North, Central and South America, in Argentina, Brazil and Colombia (Aramburú et al., 2002; Mascarenhas et al., 2009; Sreenivasa Murthy & Panda, 2016; Bassini-Silva et al., 2019; Mesquita-Sousa et al., 2020). It was recently described as an invasive parasite in Italy (Castelli et al., 2015) and Spain (Mori et al., 2019). It has also been found in domestic fowl in Indonesia, India (Sreenivasa Murthy & Panda, 2016; Selfiannisa et al., 2018) and various locations in Africa and Australia (Denmark & Cromroy, 2003).

The tropical fowl mite produces feather asymmetry (Proctor & Owens, 2000), weight loss and reduced numbers of eggs and sperm counts in birds (Sreenivasa Murthy & Panda, 2016). This parasite may impact the poultry industry (Santillán et al., 2015; Sreenivasa Murthy & Panda, 2016; Selfiannisa et al., 2018), considering that it has frequently been found parasitizing backyard chickens in Brazil (Guimarães et al., 2001). Moreover, it has the potential to feed on mammals such as mice and rabbits (Sikes & Chamberlain, 1954).

This parasite also bites humans (Fox, 1957; Oliveira et al., 2012; Castelli et al., 2015; Mentz et al., 2015; Bassini-Silva et al., 2019). It can move into houses from nests when these are close to human settlements (Oliveira et al., 2012; Castelli et al., 2015). Because it is hematophagous, it has also the potential to transmit pathogens such as bacteria and viruses (Santillán et al., 2015; Lareschi et al., 2017).

Ornithonyssus bursa has been described in monk parakeets in their area of native distribution (Aramburú et al., 2002). It also affects several bird species with diverse host infestation rates (Santillán et al., 2015). This mite has been detected parasitizing American kestrels (*Falco sparverius*) (Liébana et al., 2011) and has been found at high prevalence rates (90%) in native blue-crown parakeet chicks (*Aratinga acuticaudata*) in Argentina (Aramburú et al., 2003). Also in Argentina, this mite has been identified as an ectoparasite of the invasive European starling (*Sturnus vulgaris*), and further, as vector of *Wolbachia* sp. bacteria (Lareschi et al., 2017).

The tropical fowl mite is considered to be a common nest parasite of passerines (Proctor & Owens, 2000), affecting sparrows (*Passer domesticus*) and northern mockingbirds (*Mimus polyglottos*) in Florida, USA (Phillis & Cromroy, 1972), rock pigeons (*Columba livia*) in Colombia (Pérez-García et al., 2015) and Spain (Mori et al., 2019) and swallows (*Hirundo rustica*) in Denmark; in the latter it reduced clutch size (Moller, 1991). In New Zealand, it has been found to diminish the body size of chicks of North Island robins (*Petroica longipes*) and force them to fledge at an early age, thus increasing their vulnerability to predators (Berggren, 2005).

Ornithonyssus bursa has been recently found at a high infectious rate (97%) in chicks of invasive monk parakeets in Barcelona, Spain (Mori et al., 2019), and it was suggested that this was an invasive parasite. In addition, it seems that *O. bursa* is already established in Europe: human dermatitis cases have already been diagnosed in Sicily (Castelli et al., 2015), where free-ranging invasive monk parakeet populations have been established (ISSG, 2015), thus representing a potential source of the introduced mite. In Rome, introduced urban populations of monk parakeets have also been found to be parasitized by *O. bursa* (Ancillotto et al., 2018). One alternative hypothesis could be that this mite reached these places carried by migratory birds such as the European starling or the barn swallow (*Hirundo rustica*), which are known to be hosts of tropical fowl mites (Moller, 2010; Lareschi et al., 2017).

The dynamics of macronyssid mites are strongly influenced by the environment (Arce et al., 2018) and *O. bursa* has been shown to have decreasing virulence with increasing temperatures (Moller, 2010). This may be relevant in hot environments such as urban Santiago, where some meteorological stations report average maximum temperatures higher than 30 ºC in January and February (McPhee et al., 2014). Here, the tropical fowl mite might find relief in controlled environments. Nest chambers constructed by monk parakeets have been shown to be somewhat controlled environments in relation to external conditions. They are able to buffer maximum ambient temperatures such that the temperature in the nest may be as much as seven degrees lower than the ambient temperature during the breeding season (Viana et al., 2016).

Monk parakeets are known to share space and interact with other birds at the same trees or feeding sites (Briceño et al., 2019; Mori et al., 2019). Because *O. bursa* is known to be abundant on monk parakeet chicks and in their nests (Aramburú et al., 2003), there is therefore a risk for other birds that use parakeets' nests, given that these ectoparasites can overwinter in nesting holes (Mori et al.. 2019). In Santiago, both native and introduced bird species occupy monk parakeets' nest for breeding (Briceño et al., 2019). Introduced rock pigeons and house sparrows, which are both synanthropic species, are known to become infested with *O. bursa* mites (Phillis & Cromroy, 1972; Pérez-García et al., 2015; Santillán et al., 2015; Mori et al., 2019). It is therefore likely that populations of these two introduced species are already affected by this parasite in Santiago. *O. bursa* was also found in 16% of a sample of free-ranging native eared doves (*Zenaida auriculata*) in Brazil (Goulart et al., 2011), although it was not found in an extensive study on the same species in Chile, some 400 km south of Santiago (González et al., 2004). Eared doves are known to breed in monk parakeets' nests in Santiago (Briceño et al., 2019), so it is also likely that populations in sympatry with monk parakeets may have the mite already.

The American kestrel and Harris's hawk (*Parabuteo unicinctus*) are predators of monk parakeets (Celis-Diez, 2016; Briceño et al., 2019), even though other birds of prey such as the Austral pigmy owl (*Glaucidium nana*) and aplomado falcon (*Falco femoralis*) have been found breeding in monk parakeets' nests (Briceño et al., 2019): These raptors may be at risk through predation on parakeet chicks in an mite-infested environment before nest occupation. In fact, populations of the ferruginous pigmy owl and aplomado falcon in Argentina have been described as hosts of *O. bursa* (Santillán et al., 2015). Other species that have been found to breed in monk parakeets' nests in Santiago are the American kestrel and the house wren (*Troglodytes aedon*) (Briceño et al., 2019), which are both species on which *O. bursa* has been found in Argentina (Santillán et al., 2015). The chimango caracara (*Milvago chimango*), an abundant bird species that is suspected of thriving in urban and periurban areas and is known to share space and interact with monk parakeets (Briceño et al., 2019), has been found to be parasitized by *O. bursa* in Argentina, in both chicks and adults (Santillán et al., 2015).

Monk parakeets are highly gregarious, and *O. bursa* has been found to be more abundant in denser populations of monk parakeets (Ancillotto et al., 2018; Mori et al., 2019). Also, introduced populations of monk parakeets seem to have higher reproductive rates, resulting in denser populations than in their areas of native distribution (Senar et al., 2019). Rock pigeons have been shown to have higher abundance of ectoparasites in areas of higher monk parakeet densities (Mori et al., 2019), and this could apply to other species as well. Moreover, in Barcelona, the *O. bursa* infection rate in rock pigeons was 10%. Because this is a wide ranging bird, it has the potential to spread *O. bursa* over large areas, even outside urban settlements (Mori et al., 2019).

Although field studies have determined that the tropical fowl mite has some degree of affinity for certain host species, the bird species with the highest mite prevalences are not the ones presenting the highest mite intensity. Additionally, it appears that nestlings of bird species with higher mite prevalences are the ones with higher encounter rates (Arce et al., 2018). This could also be relevant in relation to gregarious species such as the monk parakeet, which in its invasive distribution seems to have higher densities of nestlings, as well as longer reproductive periods (Senar et al., 2019).

Even though in this study we did not quantify the intensity of mite infestation, it appears that the abundance was greater (we retrieved many mites from the chicks) than the one mite that was found among ninety-two adult monk parakeets collected in Santiago during 2006 and 2007 (Briceño et al., 2017). This finding is consistent with the biology of the parasite, which mainly affects non-flying chicks (Aramburú et al., 2003). Nonetheless, a couple of mite individuals on adult birds is all that is required for mite abundance to increase rapidly (Arce et al., 2018).

O. bursa has been found at high prevalence in native and invasive populations of monk parakeet nestlings in Argentina (48%) (Aramburú et al., 2003) and Barcelona (97%) (Mori et al., 2019), respectively. Moreover, these parakeets' nests are occupied by several other species (Briceño et al., 2019). It therefore seems more likely that *O. bursa* is a co-introduced parasite (with *M. monachus*), with the potential to become a co-invasive parasite that infects native birds (Lymbery et al., 2014).

This was the first report of *O. bursa* in Chile, found in monk parakeets' nestlings. The monk parakeet is considered to be one of the most invasive bird species, in part due to its condition of being an ecosystem engineer (Briceño et al., 2019). Invasive monk parakeet populations produce economic losses (Iriarte et al., 2005; Senar et al., 2019), although little is known about their impact on animal or public health (Briceño et al., 2017). This species continues to expand its distribution in Santiago, with consequent potential spread of the tropical fowl mite. This hematophagous mite has the potential to affect the conservation of native and/or endangered bird species through increased predation, due to forced early fledging of nestlings (Berggren, 2005). It also has the potential to affect the health status of resident birds, not only through blood sucking but also as a potential vector of pathogens, with possible risk for other animals, including humans (Fox, 1957; Oliveira et al., 2012; Castelli et al., 2015; Mentz et al., 2015; Bassini-Silva et al., 2019). Finally, our finding is also of concern for the poultry industry, which may become affected by this introduced parasite. These impacts may already be happening and further studies to understand them in detail are warranted.

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