

Parasitic survey on introduced monk parakeets (*Myiopsitta monachus*) in Santiago, Chile

Levantamento parasitário da caturrita (*Myiopsitta monachus*) introduzida em Santiago, Chile

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Abstract

Central Chile has been identified as a unique ecosystem with high conservation priority because of its high levels of endemism and intensive anthropic pressure. Over a period of almost four decades, the monk parakeet has been successful in establishing and dispersing in urban Santiago, although little is known about its potential impact. Furthermore, nothing is known about its epidemiological risks towards animals or even humans. For this reason, we conducted the first parasitic survey of monk parakeets in Chile through capture, necropsy and thorough external and internal inspection of 92 adult individuals. Among these, 45.7% presented lice that were identified as *Paragoniocolletes fulvofasciatus*, 1.1% had mesostigmatid acari and 8.9% had free-ranging acari. Among 89 parakeets, 19.1% had structures identified as *Cryptosporidium* sp. This study provides the first description of *Cryptosporidium* sp. in monk parakeets. Along with the presence of a mesostigmatid acarid in one parakeet, this serves as a public health warning, given that both of these parasites have zoonotic potential.

Keywords: Psittacidae, *Paragoniocolletes fulvofasciatus*, mesostigmatid acarid, oribatid acari, *Cryptosporidium*.

Resumo

A porção central do Chile é reconhecidamente uma área com ecossistemas únicos de alta prioridade para conservação. Isso se deve aos altos níveis de endemismo na região e pressões antrópicas intensas. Durante quase quatro décadas, a caturrita tem obtido sucesso em seu estabelecimento e dispersão na área urbana de Santiago, apesar da falta de conhecimento com relação ao seu potencial impacto. Além disso, não há informações sobre riscos epidemiológicos para animais e tampouco para humanos. Motivado por essa questão, foi realizado o primeiro levantamento parasitário de caturritas no Chile a partir da captura, necropsia e inspeção interna e externa de 92 indivíduos adultos. Deste total, 45,7% apresentaram piolhos da espécie *Paragoniocolletes fulvofasciatus*, 1,1% apresentaram ácaros da ordem Mesostigmata, e 8,9% apresentaram ácaros de vida livre. Dentre 89 caturritas, 19,1% apresentaram estruturas identificadas como *Cryptosporidium* sp. Este estudo apresenta a primeira descrição de *Cryptosporidium* sp. em caturritas. Ademais, a presença de ácaros da ordem Mesostigmata em uma das aves serve como um alerta para saúde pública, considerando que estes dois parasitas apresentam potencial zoonótico.

Palavras-chave: Psittacidae, *Paragoniocolletes fulvofasciatus*, ácaro mesostigmatídeo, ácaro orobatídeo, *Cryptosporidium*.

Introduction

The monk parakeet (*Myiopsitta monachus*) is a medium-sized sexually monomorphic parrot originally distributed in Paraguay, Uruguay, Bolivia, southern Brazil and northern and central Argentina (EBERHARD, 1998). As non-migrants, they remain in their nests both for nesting and for roosting year-round (NAVARRO et al.,

1995). It is the only parrot, among over 350 species, that is able to build its own communal nests, thus making them independent of the need for tree or cliff cavities (MARTIN & BUCHER, 1993).

In Argentina, the monk parakeet's original range is increasing southwards towards southern Patagonia (BUCHER & ARAMBURÚ, 2014) and, within its native range, it is currently considered to be a pest (ISSG, 2011). Consequently, it is estimated that in Argentina this invader causes 2-15% crop losses, with an annual cost of over US\$ 1 billion (IRIARTE et al., 2005).

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Currently, invasive populations of monk parakeets can be found worldwide as an unintentional by-product of large-scale pet trade (EDELAAR et al., 2015). In both native and invasive sites, the monk parakeet is considered to be a problem for agriculture and for electricity transmission lines (BUCHER & ARAMBURÚ, 2014). In England, this species has been classified as one of the six priority invaders for rapid reaction (VAN HAM et al., 2013). In United States, parakeets build nests mostly on manmade structures being considered a nuisance by utility companies (BURGER & GOCHFELD, 2009; AVERY et al., 2012; REED et al., 2014). In Florida, the species has thrived and has become urban and suburban without any observed limits to their population growth (AVERY et al., 2012). In Spain, most information on monk parakeets has been produced in Barcelona, where they are mainly concentrated in urban areas. This population is estimated to double every nine years and was found to be highly dependent on bird feed (DOMÈNECH et al., 2003; RODRÍGUEZ-PASTOR et al., 2012).

In Chile, there is little information about the invasion of monk parakeets. However, these birds are considered to be the newest and most troublesome invasive species. Monk parakeets are blamed for a major negative impact on fruit and ornamental trees. They were first released by private citizens in eastern Santiago in 1972 (IRIARTE et al., 2005). The Chilean official Agriculture and Livestock Bureau (Servicio Agrícola y Ganadero, SAG) has reported that the first naturalized colony was established during the early 1980s, in a radio antenna in La Reina commune, eastern Santiago. From there, the birds would have slowly colonized the landscape through dispersal to nearby areas (TALA et al., 2005). During the 1990s, sightings of parrot flocks became more frequent in the higher parts of La Reina and Lo Barnechea communes in Santiago. In total, it has been estimated that 15,000 individuals were imported from the time of their first introduction until the time when the species was declared harmful and its importation was banned through the Hunting Law (No. 19.473) in 1997 (IRIARTE et al., 2005; TALA et al., 2005). Through either intentional releases or escaping parrots, by 1998 parrots became common mainly in the eastern communes of the city of Santiago (specifically in Las Condes, La Reina and Ñuñoa). Since then, the population has been increasing with parakeets spreading through central Chile further towards the south (La Pintana commune), the west (Maipú commune) and the north (Lampa commune) of Santiago (TALA et al., 2005), with breeding colonies reaching as far as the west coast in Valparaíso region (IRIARTE et al., 2005).

Biological invasions are closely linked to the emergence of diseases and have the potential to affect the health of people and domestic animals (KEESING et al., 2010; DUNN & HATCHER, 2015), given that they are a source of spreading of zoonoses (ESTRADA-PENÁ et al., 2014) including parasites (THOMPSON, 2013). Ecological interactions of monk parakeets with other introduced birds, such as sparrows or pigeons, could pose a risk of transmission of zoonotic pathogens, especially to immunologically susceptible individuals such as young children, elderly people or sick individuals (HAAG-WACKERNAGEL & MOCH, 2004; COSTA et al., 2010).

So far, no information on potential microorganisms that monk parakeets may be harboring is available in Chile. Central

Chile is one of the 25 unique biodiversity hotspots in the world, because of its high levels of endemism and intensive level of anthropic pressure (MYERS et al., 2000). Despite identification of this highly endemic and endangered biota, the threats to Chile's biodiversity may still have been underestimated (BROOKS et al., 2002). In addition, biological invasions and the emergence of diseases have the potential to contribute to wildlife extinctions, particularly when these invasions interact with other driving factors (DASZAK et al., 2000; HARVELL et al., 2002; SMITH et al., 2009). Furthermore, parasites have the potential to affect wildlife populations and have been identified as causative agents of population declines (THOMPSON, 2013).

So far in Chile, the monk parakeet invasion has been completely overlooked and, moreover, it appears that it is expanding to new urban areas, and even rural areas (TALA et al., 2005).

The objective of this study was to survey internal and external parasites in urban monk parakeets that were caught in central Chile.

Materials and Methods

Sampling of individuals was conducted at La Dehesa Golf Club (33°20' S; 70°30' W), a private area located in the municipality of Lo Barnechea in the Andes foothills, on the eastern side of Santiago, Chile. Monk parakeets were hunted between August 2006 and April 2007, and were handled in accordance with the recommendations from the official body (Servicio Agrícola y Ganadero; SAG), under University's Ethics Committee authorization (No. 4042006). Dead parakeets were processed immediately, while injured parakeets were euthanized and were placed in closed containers filled with cotton wool that was saturated with chloroform (BLACKSHAW et al., 1988), after obtaining blood for smears. All inanimate birds were then inoculated intraperitoneally using 10% formalin, individually stored in sealed plastic bags, and transported inside a cooler to the Parasitological Laboratory of the School of Animal and Veterinary Sciences, University of Chile.

In the laboratory, exhaustive inspection was conducted in order to observe and isolate ectoparasites. Additionally, feathers were combed thoroughly and loose feathers and scales that were left in the plastic bags were inspected. All ectoparasites found were placed in vials with 70% ethanol and labeled according to their origin.

The ectoparasites were soaked in 20% KOH (day one, 24 h) to clean them and remove debris, and were then left in distilled water for 24 h (day 2). During day 3, the parasites were immersed in ascending ethanol concentrations (40%, 70% and 96%) for five to ten minutes in each of these, and were finally deposited in poppy seed oil to be cleared for 24 h. On the fourth day, the ectoparasites were mounted in Canada balsam to be observed under a bifocal magnifier (PALMA, 1978). Identification of ectoparasites was conducted following Price et al. (2003), whereas species was determined following Guimarães (1947) and Palma (1973) for *Paragoniocoltes fulvofasciatum*, in particular.

Among all the birds collected, complete necropsies were performed on most of them. Sex was determined through visual examination of the gonads, and organs were examined exhaustively for endoparasites. Five complementary methods for parasite detection were applied:

- a) Direct examination of four sections of the digestive tract: Esophagus and crop; stomachs; small intestine; and large intestine. All sections were tied up separately and each organ was opened with scissors and individually washed to place all its content on a white tray for observation. After the particles had been separated according to fragment size, drops of Lugol's iodine were applied and left to act for one minute (the excess was then washed off), to aid visualization of internal parasites.
- b) Flotation method on the contents of each of the four tied-up sections was applied and observation performed under a microscope with 10X and 40X objective lenses (SOULSBY, 1987).
- c) Sedimentation method on the same remaining subset of samples was applied and sediment was observed under a bifocal magnifier (SOULSBY, 1987).
- d) Ziehl-Neelsen technique (adapted from FAYER & XIAO, 2008) was applied to smears from feces and from digestive content collected with stick swabs from each of the digestive segments that were observed on the trays. The smears, once dry, were covered with basic fuchsin, heated enable to vapor emission and stained for 20 minutes. Subsequently the slides were washed with tap water. Acid alcohol was then added for 30 seconds and rinsed with tap water. The slides were then covered with methylene blue for two to five minutes and rinsed in tap water. Finally, the smears were air-dried and observed under an optical microscope using a 100X objective lens.
- e) Hemoparasites were also surveyed using blood smears produced in the field. In the laboratory, these smears were stained using Giemsa.

The results from these analyses were recorded and the frequency of parasites found was established, along with the intensity of infection and abundance, based upon definitions given by Bush et al. (1997). Additionally, the frequencies were compared using a

chi-squared analysis, to explore sex-related differences and also any possible association between ectoparasites and endoparasites found in the parakeets (THRUSFIELD, 2005).

Results

Ninety-two monk parakeets (35 males, 50 females and 7 unsexed) were collected between August 2006 and April 2007. Among these, 88 birds were bled in the field to obtain blood smears, and 89 underwent necropsies and thorough internal examination of organs in the laboratory.

All 92 individuals caught were examined externally for parasites and 51 (55.4%; CI 95%; 45.2-65.6) were found to be parasitized by arthropods (Table 1). Forty-nine (53.3%; CI 95%; 43.1-63.5) of the birds presenting parasitic arthropods had the louse *Paragoniocytes fulvofasciatum* (Insecta: Phthiraptera: Philopteridae; Table 2; Figures 1A, 1B, 1C). One (1.1%; CI 95%; -1.0-3.2) of these birds also had a parasitic mite (Arachnida: Acarina: Mesostigmata; Figure 1D). Eight birds (8.7%; CI 95%; 2.9-14.5) presented free-ranging mites (Arachnida: Acarina: Oribatida; Figure 1E).

From the 51 monk parakeets that had arthropods, 119 lice, one mesostigmatid mite and 16 oribatid mites were obtained. Among the 49 parasitized birds, the infestation rate ranged from one to nine lice per parakeet, with a mean intensity of 2.43 parasites/parakeet and a mean abundance of 1.29.

Necropsies and complete examination of internal organs were performed on 89 monk parakeets, which were the individuals in which the digestive organs analyzed were undamaged. Thus, 84 esophagi and crops, 89 stomachs, 88 small intestines and 87 large intestines were included in the endoparasite analyses. No endoparasites were found through direct observation, flotation or sediment examination.

Among the 89 parakeets from which smears were produced using digestive and fecal material, 17 (19.1%) had acid-alcohol resistant structures of 5 µm in diameter that were compatible with *Cryptosporidium* spp. oocysts (Figure 1F). On the other

Table 1. Frequency of ectoparasites and endoparasites in free-ranging monk parakeets (*Myiopsitta monachus*) caught in Santiago, Chile.

	Parasite	No. positive (% positive)	Abundance	No. sampled
Ectoparasites	<i>P. fulvofasciatum</i>	42 (45.7)	119	92
	Mesostigmatid acari	1 (1.1)	1	92
	Free-range acari	8 (8.7)	16	92
Total	Ectoparasites	51 (55.4)	136	92
Endoparasites	<i>Cryptosporidium</i>	17 (19.1)	-	89

Table 2. Body measurements (in µm) of *Paragoniocytes fulvofasciatum* found in *M. monachus* caught in Santiago, Chile.

		Males (n=54)	Females (n=62)	Nymphs (n=3)
Head	Length	347.07 ± 17.32	401.53 ± 14.01	340 ± 55.67
	Width	302.96 ± 14.87	328.06 ± 15.55	283.33 ± 47.25
Thorax	Length	292.41 ± 19.12	315.96 ± 14.98	250 ± 65.57
	Width	320 ± 20.64	347.74 ± 21.38	280 ± 62.45
Abdomen	Length	805 ± 85.4	1030.48 ± 127.63	570 ± 121.24
	Width	368.51 ± 26.16	404.19 ± 33.21	326.66 ± 66.58
Total length		1471.48 ± 98.55	1747.98 ± 31.38	1160 ± 242.48

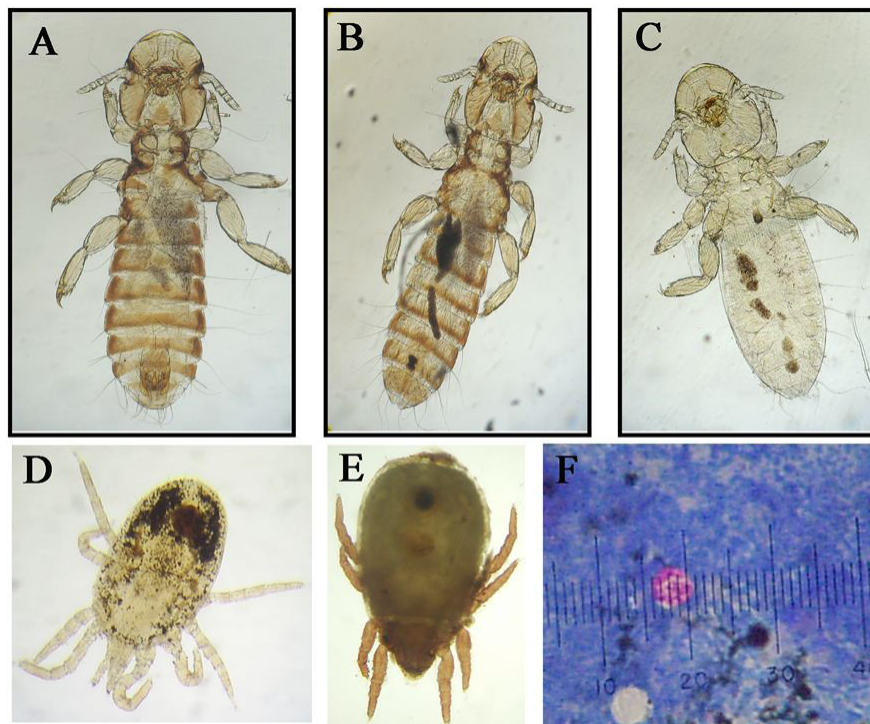


Figure 1. Ectoparasites and endoparasites found in adult monk parakeets (*Myiopsitta monachus*) caught in Santiago, Chile. The three images (A) to (C) show specimens of *Paragoniocytes fulvofasciatus* (Insecta: Phthiraptera: Philopteridae): (A) male; (B) female; and (C) nymphal stage. (D) Parasitic acarid belonging to the suborder Mesostigmata. (E) Free-ranging acarid belonging to the suborder Oribatida. (F) Oocyst of *Cryptosporidium* spp. detected from fecal material using Ziehl-Neelsen staining. All images were obtained using 10X, except (F), which is at 100X.

hand, no hemoparasites were observed from 88 blood smears stained using Giemsa.

There was no statistically significant association between parasite presence (response variable) and the sex of the parakeets (explanatory variable), as explored using the chi-square test.

Discussion

In this report, we provide the first description of *Paragoniocytes fulvofasciatus* in Chile. Although, almost half of the parakeets sampled had this louse, its abundance and intensity per individual was low. This parasite was first described by Picaglia in 1885 in Italy and was found parasitizing a monk parakeet brought to Italy from South America (PALMA, 1973). Guimarães (1947) provided morphometric measurements for a male, and the description of *P. fulvofasciatus* was completed when Palma (1973), included the description of females from analyses on 91 adults (39 males and 52 females) and 125 larvae that were obtained from 16 monk parakeets from the Paraná delta in Argentina, although he did not included morphometric values (see Table 2). This parasite would have been introduced with the monk parakeet and, although lice are species specific, they may be a source of transmission of pathogens amongst birds. For instance, helminths have been isolated from bird lice of the suborder Ischnocera, the same suborder to which *Paragoniocytes* sp. belongs (CLAYTON et al., 2008).

Regarding the single mesostigmatid acarid that was found in one parakeet, it was not possible to determine its species

based upon internal structures, since its abdomen was full of content that concealed the structures necessary for identification. This mesostigmatid subfamily, when found in birds or rodents, is often unspecific and zoonotic (BOWMAN, 2014). This may apply to this individual, although further studies including nesting material would provide more individuals for species identification. Regarding the oribatid acarid found as a free-ranging subfamily, it is possible that these may have been obtained from the environment, during the short time for which these parakeets remained on the ground prior to collection. It is also possible, that these acarid may have been carried from other areas in nesting material brought by the parakeets.

The present study provides the first report of *Cryptosporidium* spp. in monk parakeets. This protozoan was the only endoparasite found in this study, despite complete analysis of the digestive system and internal organs. *Cryptosporidium* spp. is a widely distributed zoonotic microorganism that has been used as an environmental sentinel for biotic pollution in wild birds in Chile (FREDES et al., 2007, 2008). Water is a major source of *Cryptosporidium* spp. contamination and wild birds can contaminate water with oocysts of this protozoan through their droppings. In fact, environmental samples are more likely to be positive for *Cryptosporidium* spp. when birds are present (JELLISON et al., 2004). Recent evidence has suggested that *Cryptosporidium* spp. would be more prevalent in intensive dairy production than in more extensive production, probably because of density of hosts (DÍAZ-LEE et al., 2011). In this case, future studies on infection rates due to *Cryptosporidium* spp. in this species could provide tools

to pinpoint urban contamination and risk factors associated with this biotic pollutant. Further, it would be important to identify the species of *Cryptosporidium* found in monk parakeets, as this information will contribute to understanding the epidemiology of the infection. Three species of avian *Cryptosporidium* spp. have been described: *C. meleagridis*, *C. baileyi* and *C. galli*. From these, *C. baileyi* is perhaps the most prevalent in birds while *C. galli* is the most prevalent in passerines. *Cryptosporidium meleagridis* has been detected in many avian hosts, is a zoonotic species and represents the third most prevalent species of *Cryptosporidium* in humans (CACCIÒ & WIDMER, 2014), relevant in case urban citizens are the source of infection. Although *C. baileyi* has been suggested to be zoonotic based upon morphology, size and affinity to organs in chickens inoculated from a human case (DITRICH et al., 1991), this was not confirmed (DITRICH et al., 1993). Thus, future studies in monk parakeets should aim to genetically characterize *Cryptosporidium* species given that *C. meleagridis*, and perhaps *C. baileyi*, are two species found in birds which have also been found in humans, representing a zoonotic risk (CACCIÒ & WIDMER, 2014).

Given that this parakeet is a gregarious species living in dense colonies, it was unexpected to find so few parasites in the parakeets collected, after thorough internal and external examination (CÔTÉ & POULIN, 1995; EZENWA, 2004; WHITEMAN & PARKER, 2004; RIFKIN et al., 2012). For instance, no *Argas monachus* was found. This tick has been associated exclusively with *Myiopsitta monachus* and all stages of this parasite have been found dwelling in the nests of this parrot (KEIRANS et al., 1973; MASTROPAOLO et al., 2011). One explanation is that this tick is found mainly in nests, rather than attached to adults, which were the sample targeted on the present study. Alternatively, it may be possible that this parasite was not imported along with the parakeet invasion.

The lack of parasitic diversity found in this study could be related to the fact that all birds were obtained from one location. Alternatively, the low parasitic diversity and load obtained in this study may be explained by the “parasitic release hypothesis”, in which an invasive species would be less parasitized in a new area, compared with its native populations. Further, this new invasive species would be less parasitized than other similar native species in the area (TORCHIN et al., 2003). One reason for this is that parasites often have complex life cycles that include more than one host, and in the absence of any of these hosts in the new environment, the parasite cycle would be limited. This mechanism may lead to an advantage over native biota (MITCHELL & POWELL, 2003; TORCHIN et al., 2003), and may have contributed to the demographic explosion observed in populations of this introduced species in Chile. Moreover, parakeets have been observed using certain plants brought daily to nests when breeding. This plant material may act as natural insecticides and bactericides, thus contributing towards reducing biotic hazards and increasing nestling survival and hence their invasive success in new environments (VIANA et al., 2016).

Lastly, considering that monk parakeets are an invasive species that is well adapted to urban environments (i.e. with close proximity to humans and domestic animals), complete surveillance of pathogens in this bird and subsequent risk analyses are warranted

for the sake of public health (HULME, 2014). This surveillance should include not only individuals but also their nests.

The present study provides the first description of the presence of *Cryptosporidium* sp. oocysts in monk parakeets and also the first description of *Paragoniocoltes fulvofasciatum* in Chile.

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