


Parasites of the Shiny Cowbird, *Molothrus bonariensis*, and the Austral Blackbird, *Curaeus curaeus*, (Passeriformes: Icteridae) in Chile

Parasitas do chupim *Molothrus bonariensis* e do pássaro-preto-austral *Curaeus curaeus* (Passeriformes: Icteridae) no Chile

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Abstract

Comparative studies of parasites in sympatric bird species have been generally scarce. Parasitic infection/transmission can be spread in a number of ways that suggests possible direct and indirect, horizontal transmission between avian hosts. In order to determine whether two sympatric icterids from Central and Southern Chile share their parasite fauna (ecto- and endoparasites), we examined parasites of 27 Shiny Cowbirds, *Molothrus bonariensis*, and 28 Austral Blackbirds, *Curaeus curaeus*, including individuals captured in the wild and carcasses. We found that Shiny Cowbirds were infected with the chewing lice *Brueelia bonariensis*, *Philoapterus* sp. 1, the feather mites *Amerodectes molothrus*, *Proctophyllodes* spp. (species 1 and 2), and the helminths *Mediorhynchus papillosus*, *Plagiorhynchus* sp., *Dispharynx nasuta* and *Tetrameres paucispina*, while Austral Blackbirds had the chewing lice *Myrsidea* sp., *Philoapterus* sp. 2, the feather mites *Proctophyllodes* sp. 3, *Amerodectes* sp., and three helminths: *Anochotaenia* sp., *Capillaria* sp. and *M. papillosus*. The flea *Dasytysyllus* (*Neornipsyllus*) *cteniopus* was found only on the Austral Blackbird. The only parasite species shared by both icterids was the acanthocephalan *M. papillosus*, possibly due to their feeding on the same intermediate insect hosts. With the exception of *B. bonariensis* and *Philoapterus* sp. 1 found on the Shiny Cowbird, all species reported in this study represent new parasite-host associations and new records of parasite diversity in Chile.

Keywords: Parasite diversity, sympatry, Icteridae, Phthiraptera, Acari, roundworm.

Resumo

Estudos comparativos de parasitas em espécies de aves simpátricas são escassos. A infecção/transmissão de parasitas pode acontecer de diversas maneiras, incluindo possível transmissão direta, indireta ou horizontal entre as aves hospedeiras. Com o objetivo de determinar se dois icterídeos simpátricos do centro e sul do Chile compartilham a sua fauna parasitária (ecto- e endoparasitas), foram examinados os parasitas de 27 chupins *Molothrus bonariensis* e 28 pássaros-pretos-austral *Curaeus curaeus*, incluindo indivíduos capturados com rede de neblina e em carcaças. Nos chupins analisados, foram encontrados os piolhos de penas *Brueelia bonariensis*, *Philoapterus* sp. 1, os ácaros *Amerodectes molothrus*, *Proctophyllodes* spp. (espécie 1 e 2), e os helmintos *Mediorhynchus papillosus*, *Plagiorhynchus* sp., *Dispharynx nasuta* e *Tetrameres paucispina*. Em contraste, os pássaros-pretos-austral estavam infectados com os piolhos *Myrsidea* sp., *Philoapterus* sp. 2, os ácaros *Proctophyllodes* sp. 3,

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Amerodectes sp., e os helmintos *Anonchotaenia* sp., *Capillaria* sp. e *M. papillosus*. Adicionalmente, um espécime de pássaro-preto-austral estava parasitado pela pulga *Dasypsyllus (Neornipsyllus) cteniopus*. A única espécie de parasita que foi encontrada nas duas espécies de aves foi o acantocéfalo *M. papillosus*, possivelmente devido ao fato de que ambas as aves se alimentam dos insetos que são os hospedeiros intermediários deste parasita. Exceto os registros de *B. bonariensis* e *Philoaterus* sp. 1 encontrados no chupim, todas as espécies reportadas neste estudo correspondem à novas associações de parasita/hospedeiro e novos registros para a diversidade parasitológica do Chile.

Palavras-chave: Diversidade de parasitas, simpatria, Icteridae, Phthiraptera, Acari, nematódeos.

Introduction

Parasitism is ubiquitous in nature and although recent advances have revealed important details of some host-parasite associations (e.g. Doña et al., 2019), we are still far from understanding in depth key points of host-parasite dynamics. The relatively vast avian diversity and even richer diversity of gastrointestinal and external parasites make the study of parasite transmission dynamics an obscure subject. For example, transmission of ectoparasites generally happens through direct contact between individuals, either between adult birds (horizontal transmission) or between parents and their offspring (vertical transmission) (Eichler, 1963; Marshall, 1981; Darolova et al., 2001). In the case of gastrointestinal parasites, because they often have complex life cycles and generally need a fecal-oral route to infect, transmission also occurs from ingestion of eggs or other infective stages of the parasite, often within an intermediate host (Atkinson et al., 2008). The Shiny Cowbird, *Molothrus bonariensis* (Gmelin, 1789), and the Austral Blackbird, *Curaeus curaeus* (Molina, 1782), are two locally common icterids that have a similar size, behavior, and diet (Fraga, 2011). Although the Shiny Cowbird and Austral Blackbird are largely distributed in South America and Patagonia, respectively, they are sympatric only in Chile, where they share a large part of their distribution range and are often observed in mixed flocks foraging together (Fraga, 2011; Couve et al., 2016). This close proximity between these two species might suggest interspecific gastrointestinal and ectoparasite exchange. However, to date, the few studies of parasites of Shiny Cowbirds and Austral Blackbirds were centered

Table 1. Checklist of parasites recorded in Shiny Cowbirds and Austral Blackbirds.

Parasites	Host	Origin	Reference
Phthiraptera			
<i>Brueelia bonariensis</i>	Shiny Cowbird	Chile	Cicchino & Castro (1996); This study
<i>Brueelia trinidadensis</i>	Shiny Cowbird	Trinidad and Tobago	Cicchino & Castro (1996)
<i>Brueelia marcoi</i>	Austral Blackbird	Chile; Argentina	Cicchino & Castro (1996)
<i>Philoaterus</i> sp. 1	Shiny Cowbird	Chile	This study
<i>Philoaterus</i> sp. 2	Austral Blackbird	Chile	This study
<i>Myrsidea</i> sp.	Austral Blackbird	Chile	This study
<i>Myrsidea bonariensis</i>	Shiny Cowbird*	Possibly in Argentina	Clay (1968)
<i>Machaerilaemus laticorpus</i>	Austral Blackbird	Chile	Price et al. (2002)
Acari (feather mites)			
<i>Amerodectes molothrus</i>	Shiny Cowbird	Brazil; Chile	Mironov et al. (2008); This study
<i>Amerodectes</i> sp.	Austral Blackbird	Chile	This study
<i>Proctophyllodes</i> sp. 1	Shiny Cowbird	Chile	This study
<i>Proctophyllodes</i> sp. 2	Shiny Cowbird	Chile	This study
<i>Proctophyllodes</i> sp. 3	Austral Blackbird	Chile	This study
<i>Syringophilopsis bonariensis</i> (ticks)	Shiny Cowbird	Brazil	Skoracki et al. (2016)
<i>Ixodes auritulus</i>	Austral Blackbird	Chile	González-Acuña et al. (2004)
Helminths			
<i>Dispharynx nasuta</i>	Shiny Cowbird	Chile	This study
<i>Tetrameres paucispina</i>	Shiny Cowbird	Chile	This study
<i>Mediorhynchus papillosus</i>	Shiny Cowbird; Austral Blackbird	Chile	This study
<i>Plagiorhynchus</i> sp.	Shiny Cowbird	Chile	This study
<i>Capillaria</i> sp.	Austral Blackbird	Chile	This study
<i>Anonchotaenia</i> sp.	Austral Blackbird	Chile	This study
<i>Anonchotaenia longiovata</i>	Austral Blackbird	Not given	Rausch & Morgan (1947)

*Possible erroneous host. See discussion in Clay (1968).

on taxonomical descriptions based on morphological traits of new species instead of the host-parasite dynamics (see Table 1 and references therein).

In the present study, we examined the external and gastrointestinal parasites of sympatric Shiny Cowbirds and Austral Blackbirds in Chile, with the aim to determine whether sharing environment and diet could influence interspecific parasite transmission between these hosts.

Materials and Methods

We examined a total of 27 Shiny Cowbirds and 28 Austral Blackbirds. The first group of birds analyzed corresponded to seven Shiny Cowbirds and 20 Austral Blackbirds captured using mist nets in four localities of Central and Southern Chile (2013-2014): Termas del Flaco (1720 meters above sea level (ma.s.l.); 34°57'S, 70°26'W), Sierras de Bellavista (900-1100 ma.s.l.; 34°48'S, 70°45'W), Parque Inglés (1100-1300 ma.s.l.; 35°28'S, 70°59'W) and Chiloé National Park (sea level; 42°37'S, 74°3'W) (Figure 1). The birds were examined for ectoparasites for no longer than five minutes to avoid an excessive stress. The ectoparasites were removed from feathers with anatomic tweezers and kept in microcentrifuge tubes with 70% ethanol for subsequent identification. The second group examined corresponded to 20 Shiny Cowbirds and eight Austral Blackbirds carcasses (2007-2014), dead after vehicle-collision, poaching or later in rescue centers. The birds were received by the Faculty of Veterinary Science, University of Concepción, Campus Chillán, and each individual was independently stored in a plastic bag and frozen until necropsy, which followed the protocol proposed by Kinsella & Forrester (1972), enabling an efficient

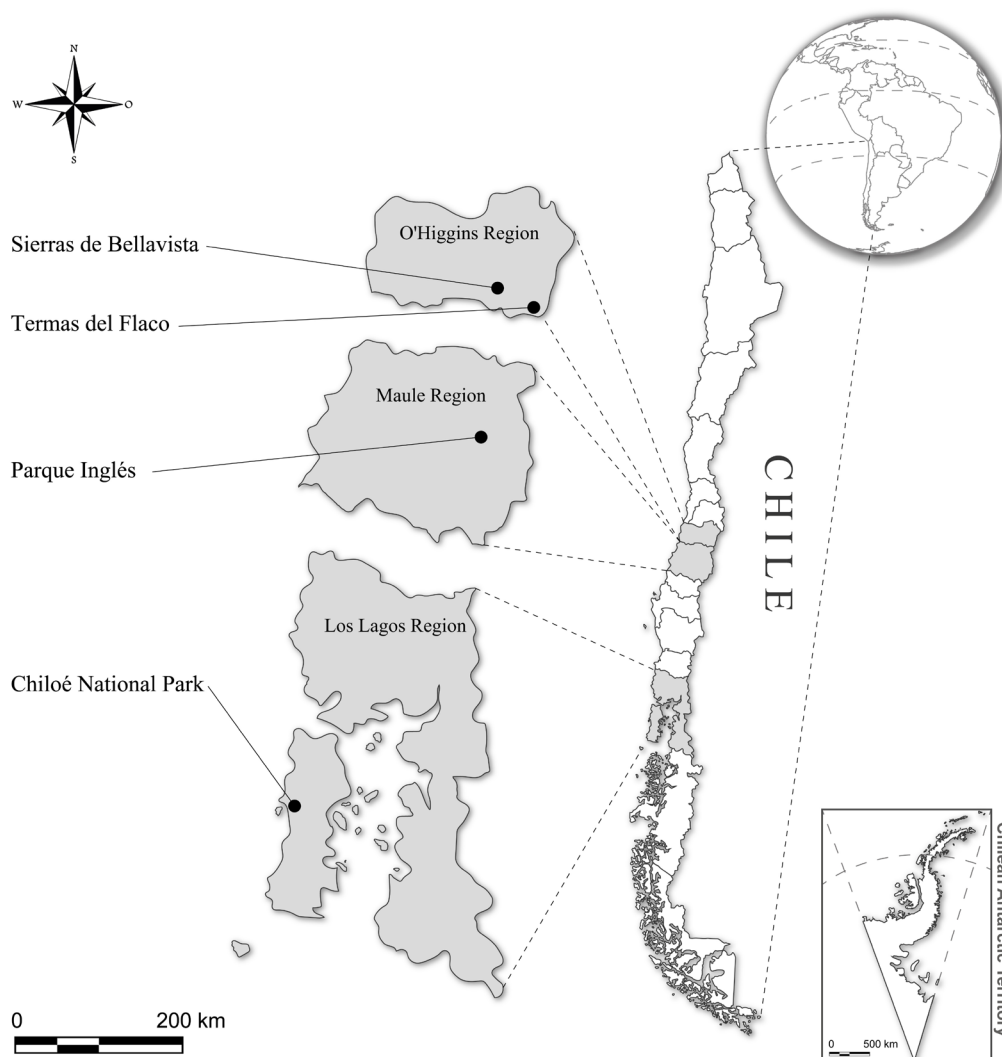


Figure 1. Sampling localities of Shiny Cowbirds and Austral Blackbirds in Central and Southern Chile.

collection of both ectoparasites and gastrointestinal parasites. Lice were mounted in Canada balsam following the protocol described by Palma (1978) and Price et al. (2003). Mites were cleared in Nesbitt's solution (40 g of chloral hydrate, 25 mL of distilled water, and 2.5 mL of hydrochloric acid) for 72 h and were later mounted in Berlese's solution (Walter & Krantz, 2009). Fleas were mounted in Canada balsam following the procedures described by Furman & Catts (1982). All parasites were examined using the standard light microscopy methods, recommended for each parasite type (magnifications 40X-100X). For the identification of Phthiraptera, including sex determination and state of development, were used keys proposed by Nitzsch (1818), Cicchino & Castro (1996) and Price et al. (2003); for feather mites, keys proposed by Gaud & Atyeo (1996), Mironov et al. (2008), Valim & Hernandez (2010), Mironov & Chandler (2017); and for fleas, keys from Hastriter & Schlatter (2006). Endoparasites were processed according to Hendrix & Robinson (2012) and identified with keys proposed by Yamaguti (1961, 1963), Khalil et al. (1994), Van Cleave (1916), Sandground (1928), and Zhang et al. (2004). All collected specimens were stored in the collection of the Laboratory of Zoology, Faculty of Veterinary Science, University of Concepción, under the codes CDCA 140 to 146 for mites, UdeCPHsa 150 to 153 for lice, CDCA 186 to 193 for helminths, and CDCA 50 for the flea.

Results and Discussion

Phthiraptera

On 33.3% of Shiny Cowbirds (9/27), we found two species of chewing lice (Table 2a and Figure 2): *Brueelia bonariensis* Cicchino & Castro, 1996 and *Phlopterus* sp. 1. Whereas on Austral Blackbirds, the lice prevalence was lower (Table 2b), with 14.3% of birds (4/28) parasitized with two species (Figure 2): *Myrsidea* sp. and *Phlopterus* sp. 2.

In Shiny Cowbirds, the *Brueelia* lice found (Figure 2A, 2B) corresponded to the classic descriptions of this genus in New World Icterids (for details, see Cicchino & Castro, 1996). In particular, the macule on the pulvinal edge of the frontoclypeal suture placed these parasites in the *amazonae* subgroup. Within this group, males of *B. bonariensis* and *B. americana* Cicchino & Castro, 1996 could be very similar, however, it was possible to clearly discriminate from the latter by its head's shape, the II-IV brownish tergites, and lack of the postspiracular setae in the IV tergite

Table 2. Descriptive statistics of parasites of (a) Shiny Cowbirds and (b) Austral Blackbirds in Chile.

Parasites	Prevalence (%)	Mean intensity	Range	Males	Females	Immature
a) Shiny Cowbird						
Phthiraptera						
<i>Brueelia bonariensis</i>	25.9	3.9	0-11	8	11	8
<i>Phlopterus</i> sp. 1*	11.1	1.3	0-2	0	1	3
Acari						
<i>Amerodectes molothrus</i> *	3.8	1	0-1	1	0	0
<i>Proctophyllodes</i> sp. 1*	22.2	4.33	0-12	7	18	1
<i>Proctophyllodes</i> sp. 2*	18.5	1.6	0-2	1	7	0
Helminths						
<i>Dispharynx nasuta</i> *	5	4	0-4	1	3	0
<i>Tetrameres paucispina</i> *	5	2	0-2	2	0	0
<i>Mediorhynchus papillosus</i> *	5	1	0-1	0	1	0
<i>Plagiorhynchus</i> sp.*	5	2	0-2	0	2	0
b) Austral Blackbird						
Phthiraptera						
<i>Myrsidea</i> sp.*	3.6	3	0-3	1	0	2
<i>Phlopterus</i> sp. 2*	10.7	1.7	0-2	1	1	2
Acari						
<i>Amerodectes</i> sp.*	53.6	11.7	0-44	60	59	56
<i>Proctophyllodes</i> sp. 3*	7.1	2.5	0-4	1	4	0
Helminths						
<i>Capillaria</i> sp.*	25	3	0-4	2	4	0
<i>Ananchotaenia</i> sp.*	12.5	1	0-1	0	1	0
<i>Mediorhynchus papillosus</i> *	12.5	2	0-2	0	2	0

*New parasite-host association.

(Cicchino & Castro, 1996). From each bird host, we collected lice that were classified within the *Philopterus*-complex (Figure 2C-E) due to their e.g., preantennal head very narrow, marginal carina with indentation on median side, and terminal segment of female abdomen with paired pseudostyli (more details in Mey, 2004). The lice from this complex are specialized to live on the head of their hosts and are usually found on perching birds (Mey, 2004). Although these lice found shared mutual similarities, they represented two distinctive morphotypes, clearly differing morphologically from one another (see Price et al., 2003), and did not correspond to any known species. In the Austral Blackbird, a louse showed morphological characteristics that suggested a good fit to males of the genus *Myrsidea* (Figure 2F), according to Waterston (1915). Unfortunately, this louse was distinctive enough not to fit any of the nearly 350 described species for *Myrsidea* (Valim et al., 2011; Valim & Weckstein, 2013). Nevertheless, this goes in line with the ideas by the latter authors, suggesting that to date only a small fraction of species in the genus *Myrsidea* have been described.

Acari

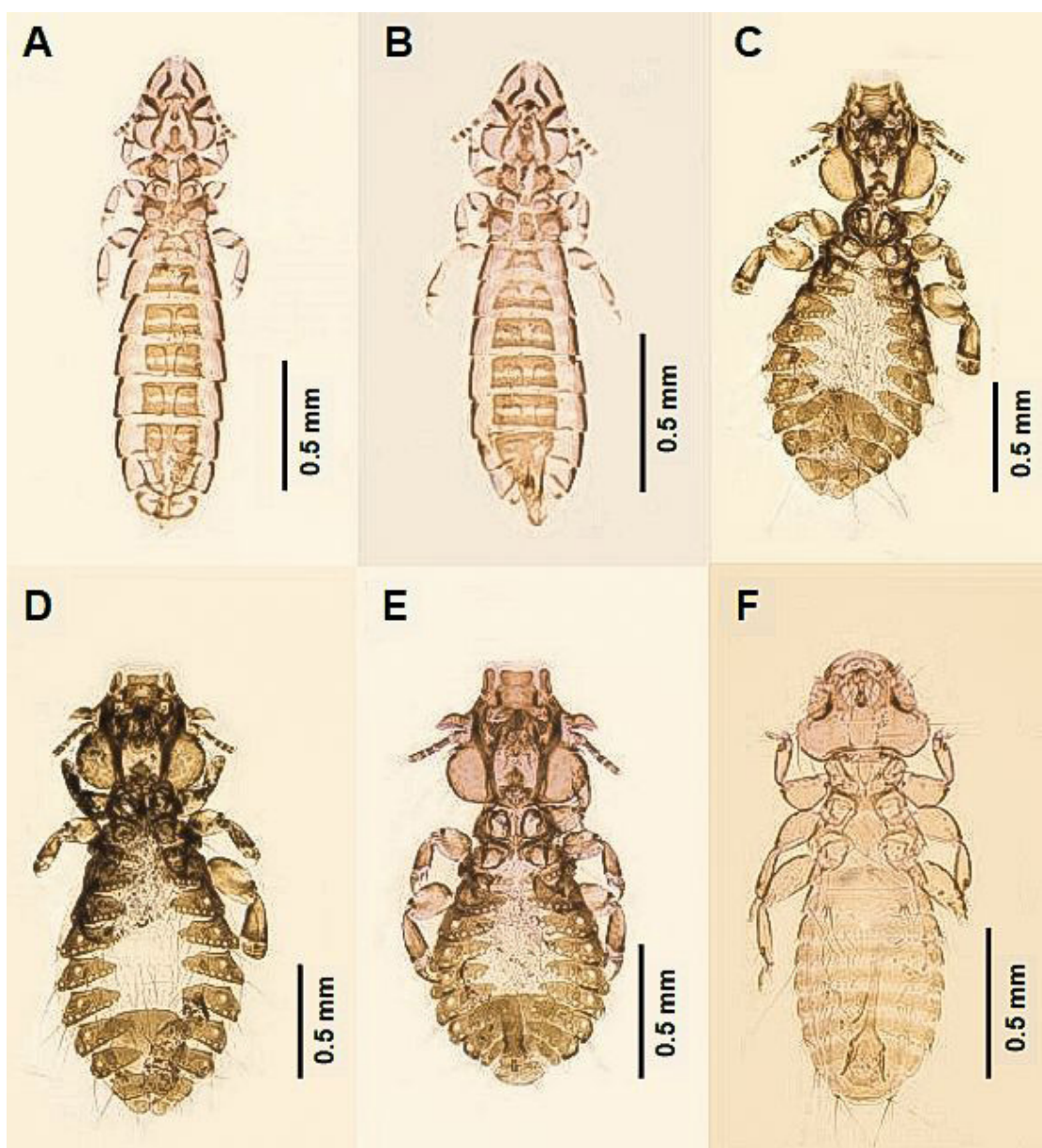


Figure 2. Chewing lice found in Shiny Cowbirds and Austral Blackbirds: *Brueelia bonariensis*, female (A) and male (B); *Philopterus* sp. 1, female (C); *Philopterus* sp. 2, female (D) and male (E); and *Myrsidea* sp., male (F).

Shiny Cowbirds, 37% of individuals (10/27), were infested with three feather mite species of the family Proctophyllodidae (Table 2a and Figure 3): *Amerodectes molothrus* (Mironov, 2008) (Pterodectinae), and two morphotypes of *Proctophyllodes* (hereafter *Proctophyllodes* sp. 1, and *Proctophyllodes* sp. 2) (Proctophyllodinae). On Austral Blackbirds, 53.6% of birds (15/28) had feather mites (Table 2b and Figure 3), belonging to the species *Amerodectes* sp. and *Proctophyllodes* sp. 3. *Amerodectes* mites found on both bird species (Figure 3G-I) met the morphological criteria of the genus (see Valim & Hernandez, 2010). The only female found on the Shiny Cowbird generally corresponds to the description of *A. molothrus*, described from this bird species in Brazil (Mironov et al., 2008), in having similar shape of the dorsal shields, lobar region, and the pattern of ornamentation on the hysteronotal shield. However, in contrast to the original description, our specimen lacks the rudiments of humeral shields and has noticeably shorter terminal appendages that could probably be a manifestation a geographic variability of this mite species widely distributed on *Molothrus* species. Females of the genus *Amerodectes* found on the Austral Blackbird have different shape of the lobal region (with much shorter opisthosomal lobes than

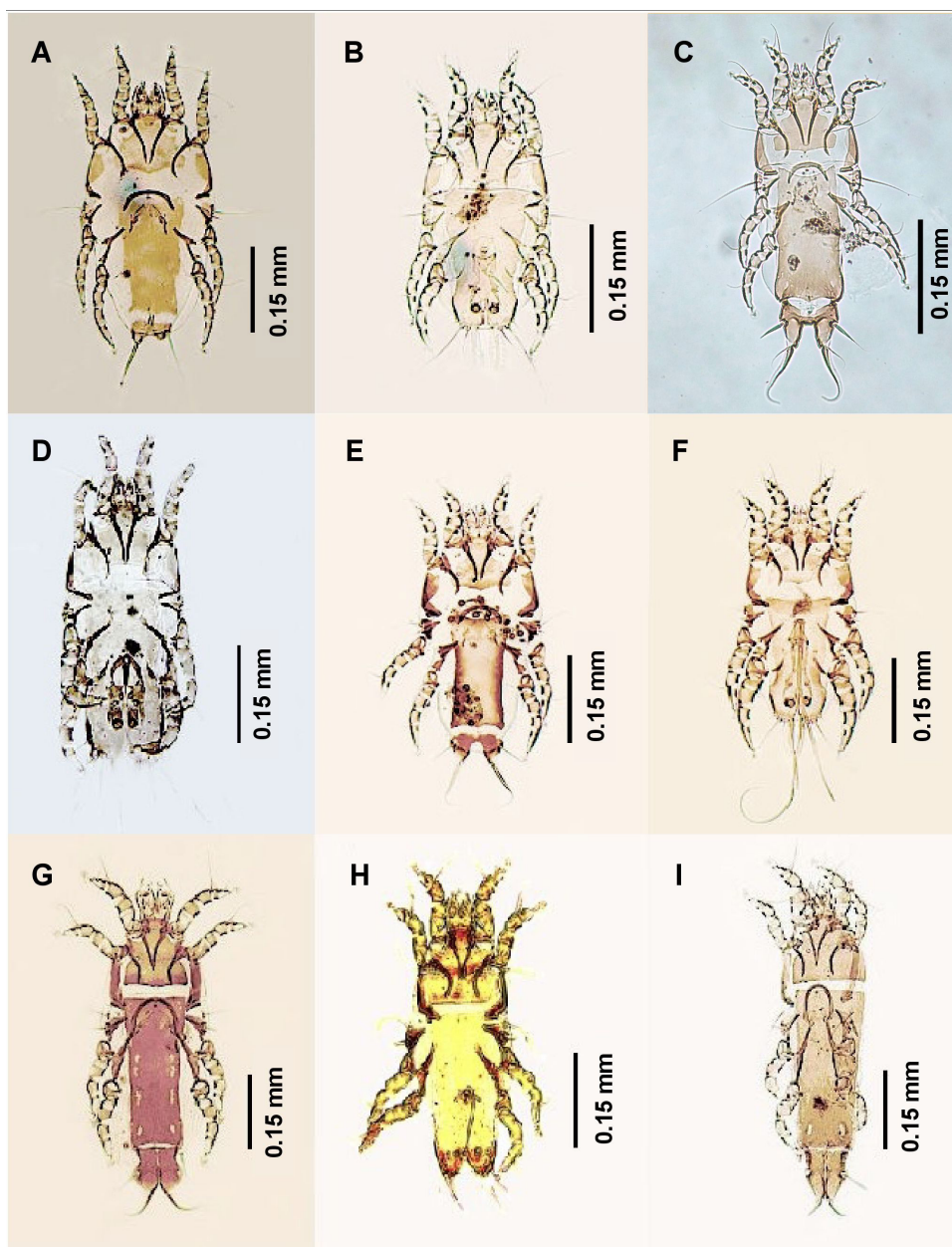


Figure 3. Feather mite species found in Shiny Cowbirds and Austral Blackbirds: *Proctophyllodes* sp. 1, female (A) and male (B); *Proctophyllodes* sp. 2, female (C) and male (D); *Proctophyllodes* sp. 3, female (E) and male (F); *Amerodectes* sp., female (G) and male (H); and *Amerodectes molothrus*, female (I).

in specimen from the Shiny Cowbird), and the hysteronotal shield entirely covered with ornamentation of small lacunae. Males of this mite belong to the species group having long filiform setae *h3*, and by the shape of dorsal shields and the length of the genital apparatus they are most similar to *Amerodectes xanthocephali* Mironov and Overstreet, 2015 described from the Yellow-headed Blackbird *Xanthocephalus xanthocephalus* (Bonaparte, 1826) in Oregon, USA (Mironov & Overstreet, 2015). Males of *Proctophyllodes* sp. 1 (Figure 3B) have the genital apparatus not extending to the level of setae *ps3* and the opisthogastric shield split into two longitudinal pieces that formally allows to refer this mite to the *musicus* species group by Atyeo & Braasch (1966). However, extremely long, narrow and widely separated pieces of the opisthogastric shield and narrow terminal lamellae in males give evidence that this mite could belongs to the *thraupis* species group (phylogenetic clade) (Klimov et al., 2017). *Proctophyllodes* sp. 2, also found on the Shiny Cowbird (Figure 3C, 3D), has an H-shaped opisthogastric shield and wedge-shaped genital sheath with a strongly sclerotized basal ring in males that univocally refers it to the *pinnatus* group (Atyeo & Braasch, 1966). Males of *Proctophyllodes* sp. 3, found in the Austral Blackbird (Figure 3F), have extremely long whip-like genital sheath extending far beyond the posterior end of the body of males. Therefore, following the revision by Atyeo & Braasch (1966), this mite could be formally referred to the *glandarinus* species group. However, taking in attention the structure of the opisthogastric shield represented by a pair of extremely long and thin sclerites bearing setae *g* and *ps3*, and narrow ribbon-like terminal lamellae, this mite definitely belongs to the *thraupis* species group as interpreted by Klimov et al. (2017). Within this group, *Proctophyllodes* sp. 3 is most similar to *Proctophyllodes longiphylus* Atyeo and Braasch, 1966 associated with the Baltimore Oriole *Icterus galbula* (Linnaeus, 1758) and Hispaniolan Oriole *Icterus dominicensis* (Linnaeus, 1766) in North America.

Siphonaptera

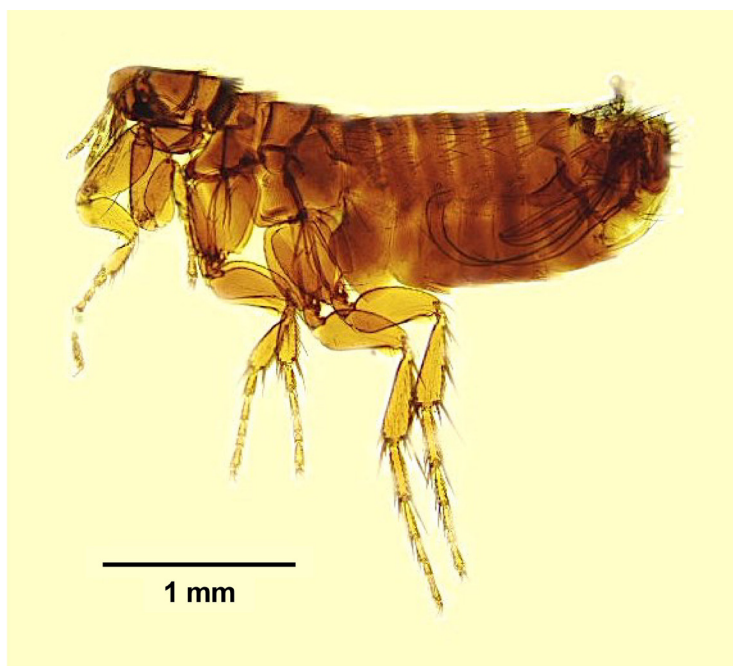


Figure 4. *Dasypsyllus (Neornipsyllus) cteniopus*, male, found on one Austral Blackbird.

On one Austral Blackbird carcass, we found a male specimen of flea (Figure 4) that, according to Hastriter & Schlatter (2006), was classified as *Dasypsyllus (Neornipsyllus) cteniopus* Jordan & Rothschild, 1920. This represents a rare finding on host, because fleas often abandon their host once dead. These fleas are commonly found in bird nests (Lewis & Stone, 2001). *Dasypsyllus* species often parasitize passerine birds, however, is not uncommon to find them on hosts of other avian orders, and even on mammals, although the latter hosts have been reported as rare and sporadic (Hastriter & Schlatter, 2006). In Chile, this parasite has been found in nests of the Magellanic Tapaculo *Scytalopus magellanicus* (Gmelin, 1789), Chucao Tapaculo *Scelorchilus rubecula* (Kittlitz, 1830), Thorn-tailed Rayadito *Aphrastura spinicauda* (Gmelin, 1789), Rufous-Collared Sparrow *Zonotrichia capensis* (Müller, 1776), and

House Wren *Troglodytes aedon* (Vieillot, 1809) (Turienzo & Di Iorio, 2013; Beaucournu et al., 2014). We argue that the present record is possibly an accidental finding, because Austral Blackbirds have been reported preying on nests of the Thorn-Tailed Rayadito (Vergara, 2007). Thus, we cannot confirm the present finding as a natural parasite-host association.

Helminths

In 10% of Shiny Cowbirds necropsied (2/20), we found two species of nematodes and two of acanthocephalans: *Dispharynx nasuta* Rudolphi, 1819, and *Tetrameres paucispina* Sandground, 1928, and *Mediorhynchus papillosus* Van Cleave, 1916, and *Plagiorhynchus* sp. (Table 2a). Whereas 50% of Austral Blackbirds (4/8) were parasitized, presenting the helminths *Capillaria* sp. (Nematoda), *Anonchotaenia* sp. (Cestoda), and *M. papillosus* (Acanthocephala) (Table 2b).

The two nematodes found in Shiny Cowbirds agreed with descriptions by Macko et al. (1977) and Zhang et al. (2004) for *D. nasuta*, and of Sandground (1928) for male *T. paucispina*. In the case of acanthocephalans, we identified only gravid females of *M. papillosus*, and two females of one fusiform species, presenting a lacunar system with reticular anastomosis, long cylindrical proboscis, with numerous small hooks at the base, and a terminal genital pore. These features agreed with characteristics of the genus *Plagiorhynchus* (Yamaguti, 1963). In the Austral Blackbird, the *Capillaria* specimens found were males (terminal anus, membranous caudal alae, long and thin spicule with spiny sheath) and females (vulva without a flap or protrusions), but their morphology did not match any previously known species described for related host (Yamaguti, 1961). Only one specimen of cestode was found, a female of *Anonchotaenia* sp. This parasite did not have a rostellum or hooks, had very short proglottids (except for the posterior one), and longitudinal muscles arranged in two distinct layers (Yamaguti, 1959). *Mediorhynchus papillosus* was also present in the Austral Blackbird but at higher prevalence and intensity than in the Shiny Cowbird. As typical for acanthocephalans, *M. papillosus* requires an invertebrate as intermediate host to complete its cycle (Atkinson et al., 2008). Therefore, it is possible to conclude that both birds share at least one invertebrate species in their diets. We did not find *Anonchotaenia longiovata* (Fuhrmann, 1901) in the birds sampled, the only helminth previously reported in the Austral Blackbird (Rausch & Morgan, 1947).

Shared parasite fauna

Host shifts are widespread across parasite taxa (Sieber & Gudelj, 2014). Accordingly, we found that most genera of ectoparasites on Shiny Cowbirds and Austral Blackbirds were shared by these birds. However, the chewing louse genera *Brueelia* and *Myrsidea* were specific to Shiny Cowbirds and Austral Blackbirds, respectively. Although ectoparasites are generally highly species-specific, lice tend to be very mobile, with many accidental reports, e.g. in birds of prey (see discussion on *Heteromenopon macrurum* in Valdebenito et al., 2015). Therefore, it is interesting that these phylogenetically closely related icterid birds (subfamily Agelaiinae) (Remsen et al., 2016) did not share a higher number of ectoparasites. Bush et al. (2019) recently showed that feather lice require a relatively low number of generations to develop adaptations to a new host, suggesting a high plasticity from lice in case of host shifts. However, these are only speculations and we need further studies to prove these ideas on these icterids, since factors that determine success or failure of these events are complex and strongly dependent on, for example, ecological processes (climate, geographical, vectors) and host immune mechanisms (Moens et al., 2016; Sieber & Gudelj, 2014). Gastrointestinal parasites are the most likely to be transmitted between hosts because, contrary to ectoparasites, their life cycle allows indirect transmission. Interestingly, only *M. papillosus* was found in both bird species. Perhaps, only this parasite had the ideal conditions for the transmission to occur, which may include sharing similar diets (to ingest the intermediate host) and physiological conditions to allow its development and further infestation (Holmes, 1987; Hurd, 1990).

The present study contributed to the biodiversity of parasites of Chile, establishing new parasite-host associations. We also showed that, although these birds live in sympatry and share similar behaviors and diets, only one species of gastrointestinal parasite was shared. Parasite-host interactions of icterids of South America are widely understudied. Future studies involving representative samples across the distribution range of both icterids could provide more thorough description on the composition and ecology of their parasite fauna.

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