

BLOOD AND INTESTINAL PARASITES IN WILD PSITTACIFORMES: A CASE STUDY OF BURROWING PARROTS (*CYANOLISEUS PATAGONUS*)

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Resumen. – Hemoparásitos y parásitos intestinales en Psittaciformes silvestres: el caso de los Loros Barranqueros (*Cyanoliseus patagonus*). – Cuantificar parásitos es esencial para comprender las implicancias ecológicas y evolutivas de los parásitos sobre sus hospedadores. Es también crucial para muchos esfuerzos de conservación en grupos de aves en peligro, como es el caso de los Psittaciformes. La finalidad del presente estudio ha sido investigar la presencia de hemoparásitos y parásitos intestinales en Loros Barranqueros (*Cyanoliseus patagonus*) (Psittaciformes) nidificando en una colonia en la costa Atlántica de la Patagonia, Argentina. No se detectaron hemoparásitos en extendidos de sangre ni mediante el uso de una técnica de detección basada en una PCR a pesar de que los Loros Barranqueros de esta colonia poseen varios ectoparásitos hematófagos que podrían transmitirlos. Asimismo las muestras fecales de Loros Barranqueros no contuvieron ni huevos ni oocistos de parásitos intestinales. En una revisión bibliográfica sobre hemoparásitos y parásitos intestinales de Psittaciformes que aquí presentamos, ninguno de los diez considerados encontró hemoparásitos y 20 de los 28 trabajos considerados no encontraron tampoco parásitos intestinales. La aparente ausencia de hemoparásitos observada en Loros Barranqueros y otros Psittaciformes Neotropicales esta en línea con la teoría que propone que los hemosporidios aviares pudieron haber evolucionado en los trópicos del Viejo Mundo, donde se encuentran ampliamente distribuidos, y que probablemente penetraron recientemente a Centro y Sudamérica a través de la región Neártica del Holártico. La aparente ausencia de hemoparásitos observada podría ser también explicada por una inmunidad innata en Psittaciformes, tal como fuera recientemente sugerido para otras aves longevas.

Abstract. – Quantifying parasites is essential for understanding the ecological and evolutionary implications of parasites on their hosts. It is also crucial for many conservation attempts carried out in endangered groups of birds, like the Psittaciformes. The aim of the present study was to test for the presence of blood and intestinal parasites of Burrowing Parrots (*Cyanoliseus patagonus*) (Psittaciformes) breeding in a large colony at the Atlantic coast of Patagonia, Argentina. Although Burrowing Parrots in this colony have

several blood-sucking ectoparasites, no blood parasite was detected in blood smears or with the use of a PCR detection method. Likewise, faecal samples of Burrowing Parrots contained no eggs or oocysts of intestinal parasites. We also review the literature on blood and intestinal parasites in wild Psittaciformes, showing that blood parasites were absent in all cases, and 20 out of 28 studies were negative for intestinal parasites. The observed apparent absence of blood parasites in Burrowing Parrots and other Neotropical Psittaciformes is in line with the theory that avian hemosporidians could have been evolved in the tropics of the Old World, where they are widely distributed and prevalent, and they probably penetrated to Central and South America through the Nearctic region of the Holarctic recently. The observed apparent absence of blood parasites could also be explained by innate immunity in Psittaciformes, as has recently been suggested for other long-lived birds. *Accepted 12 June 2006.*

Key words: Argentina, avian haematzoa, blood parasites, hemoparasites, intestinal parasites, Parrots, Patagonian Conure, Psittaciformes.

INTRODUCTION

Most free-living individual organisms are the habitat for different species with which they hold different kinds of relationships from parasitism to mutualism (Jovani 2003). Parasites can affect host morphology, behavior or fitness even when the parasites are sub-lethal (e.g., Loye & Carroll 1995), exerting important ecological and evolutionary pressures on their hosts. Quantifying parasites is essential for understanding the ecological and evolutionary implications of parasites on their hosts and, ultimately, on ecosystems (see Sol *et al.* 2003). Effects of parasites on host fitness can range from castration to decreased reproductive success (e.g., Lehmann 1993, Møller 1997). Hosts, in turn, have evolved anti-parasite behavioral, physiological or immunological defenses to counteract the negative effects of parasites (e.g., de Lope *et al.* 1998, Scheuerlein & Ricklefs 2004, Kleynhans & Tieleman 2005). Some factors that influence parasite load in nature include genetic background, investment in immune defense, season, migration, host age, size, sex, and hormonal state (see also e.g., Klukowski & Nelson 2001, Tieleman *et al.* 2005).

Blood parasites were considered to be organisms of low pathogenicity in wild populations but it has been demonstrated that haematzoa can have important affects on life

history traits of avian hosts (e.g., Hōrak *et al.* 2001, Sanz *et al.* 2001a, 2001b; Sol *et al.* 2003). Birds are usually infected by a number of blood parasites but infection varies greatly among different bird orders (e.g., Bennett 1993). Variation in the prevalence of haematzoa among species of birds has been used to test hypotheses about the effects of sexual selection (e.g., Merilä *et al.* 1999, Weatherhead & Bennett 1991), as haematzoan prevalence primarily reflects immunocompetence. However, the factors related to interspecific variation in parasite prevalence or diversity are still poorly understood (Scheuerlein & Ricklefs 2004). Intestinal parasites affect many avian species and the variation in the prevalence of these infections has also been shown to play a role in sexual selection (e.g., Zuk *et al.* 1998, Brawnner III *et al.* 2000).

Psittaciformes have become one of the most endangered orders of birds. The Parrot Action Plan 2000–2004 (Snyder *et al.* 2000) considered that 29% of parrots worldwide are at some risk of extinction. This situation is even worse in the Neotropics where 34% of the species are considered at risk of global extinction (see Snyder *et al.* 2000). The principal sources of threat are loss, fragmentation or degradation of habitat, introduction of exotic species and diseases, persecution and hunting (see Snyder *et al.* 2000), and collection of birds for the pet trade (see Wright *et al.*

2001). The increased pet trade of parrots, cockatoos, lorries, conures, and macaws has, on the one hand, endangered many species in the wild by reducing their numbers and, on the other hand, made them quite common in zoological parks and in homes as pets. Nevertheless, our knowledge of parasitic infestation in wild Psittaciformes remains scarce, with most information available on blood and intestine parasites in the order Psittaciformes coming from captive birds. This scarcity of information poses a risk for conservation attempts in Psittaciformes, an order where diseases have been a major factor in the decline of some species (Snyder *et al.* 2000).

Burrowing Parrots (*Cyanoliseus patagonus*) are colonial Neotropical Psittaciformes. Casamagnaghi (1947) carried out a first search of blood parasites on wild individual of this species but found none. However, he surveyed only a few individuals. Intestinal parasites of wild Burrowing Parrots are, to our knowledge, unknown.

The aim of the present study was to examine the prevalence of blood and intestinal parasites of Burrowing Parrots in their largest breeding colony. We also review the literature in order to summarize the available knowledge about blood and intestine parasites in the order Psittaciformes in the wild.

METHODS

Study species and site. Burrowing parrots are highly gregarious colonial Psittaciformes. In Argentina, the species occurs from the Andean slopes in the northwest of the country to the Patagonian steppes in the south (Bucher & Rinaldi 1986). Generally, Burrowing Parrots inhabit bushy steppes, marginal xerophytes forests, grassland and farmland but they require sandstone, limestone or earth cliffs where they excavate their nest burrows. The species is migratory, occupying the colonies some months before egg laying and leav-

ing the breeding site gradually as the young fledge (Bucher & Rinaldi 1986, Bucher & Rodríguez 1986). Adult Burrowing Parrots excavate their own nest burrows by tunneling into the faces of sandstone, limestone or earth cliffs. The nesting pairs use burrows that they have dug in previous seasons, but they enlarge the burrows every year. Each burrow is occupied by a single pair (Masello & Quillfeldt 2002). Burrowing Parrots do not use nesting material but, rather, deposit their eggs on the sandy bottom of the nest chamber (Masello *et al.* 2001). Burrowing Parrots lay one clutch of two to five eggs per year (Masello & Quillfeldt 2002). Burrowing Parrots have a socially and genetically monogamous breeding system with intensive biparental care (see Lubjuhn *et al.* 2002, Masello *et al.* 2002, 2004; Masello & Quillfeldt 2002, 2003, 2004a). The nestlings remain in the nest for about 63 days (see also Masello & Quillfeldt 2002, Masello & Quillfeldt 2004b). Burrowing Parrots feed on seeds, berries and fruits of wild shrubs, on seeds of introduced thistles and on buds and other soft vegetable matter (for details see Bucher *et al.* 1987, Masello *et al.* 2006).

The study was carried out from October 1998 to February 1999, November 1999 to January 2000, November 2001 to January 2002, and November 2003 to January 2004 at the largest colony of Burrowing Parrots. The colony (41°04'S, 62°50'W) is located in a cliff facing the Atlantic Ocean, 3 km west of the mouth of the Río Negro River, 30 km southeast from Viedma, on the Atlantic coast of the province of Río Negro, Patagonia, Argentina. The colony covers 9 km of cliffs with an average of 37,000 active nests, and is therefore considered the largest known colony in the world for the entire order Psittaciformes (Masello *et al.* 2006). The habitat in the surroundings of the colony is primarily Patagonian steppe.

The four studied breeding seasons were characterized by very contrasting weather

conditions and, as a consequence, by contrasting breeding success (for details on breeding success see Masello & Quillfeldt 2002, 2004b). In particular, the breeding season 1998–1999 was subject to the highly unfavorable environmental conditions related to La Niña phase of ENSO that strongly reduced breeding success (see Masello & Quillfeldt 2004b).

Study methods. According to accessibility, 79 nests were selected and marked in the densest sector of the colony as part of an ongoing study of the breeding behavior of the species (see e.g., Masello *et al.* 2006 and references therein). Nests were inspected every five days by climbing the cliff face. Burrowing Parrots tend to desert in response of disturbance during the incubation period and during the first week after hatching (Masello *et al.* 2002). In order to reduce observer influence, nests were not disturbed until about five days after the estimated hatching date of the last nestling of a clutch. Adult Burrowing Parrots were captured in their nests while attending nestlings. Nestlings were sampled between the ages of 38–60 days. All birds were released in their original burrows.

A total of 133 Burrowing Parrots were sampled in search of blood parasites. During the breeding season, we obtained blood samples from 15 breeding adults (8 females and 7 males) and 23 nestlings in 1998–1999, 13 breeding adults (10 females and 3 males), and 25 nestlings in 1999–2000, and 15 breeding adults (6 females and 9 males) and 42 nestlings in 2001–2002. Blood samples of adult and nestling Burrowing Parrots were collected via puncture of the cutaneous ulnar vein immediately after capture i.e., between 11:00 and 19:00 h. Every individual was sampled once. Blood was smeared immediately after sampling, air dried, and fixed with absolute methanol later in the lab. All smears were stained with Giemsa. Slides were examined

for the presence and abundance of extra cellular and intracellular stages of blood parasites with a light microscope (Zeiss Axioplan) at 400 × and 1000 × magnification for 10 min, scanning across the slide by one of us (RGC), who had the extensive previous experience in hematological techniques required to results on blood parasites be reliable (Cooper & Anwar 2001). This was equivalent to the observation of 70–80 microscopic fields in each smear. The average of 20 smears indicated that $10,430 \pm 251$ erythrocytes were inspected for each blood smear. Only good quality smears were used, and those with staining problems (five in total) were discarded. When no parasites were detected after this time, the smear was considered negative.

A total of 145 Burrowing Parrots were sampled in search of intestinal parasites. During the breeding season 1998–1999, we sampled 44 breeding adults, and 101 nestling Burrowing Parrots. Faecal samples were collected during nest controls i.e., between 11:00 and 19:00 h. Again, adults were sampled when in the nest and nestlings were sampled between the age of 38 and 60 days. After collection, faecal samples were immediately preserved in 2% potassium dichromate ($K_2Cr_2O_7$) and stored at 4°C. Following Misof (2004), these samples were examined later on for the presence of eggs of endoparasites. In the lab, we took the supernatant with Pasteur pipettes and washed coarse particles (berry seeds, grass, etc.) with water in order not to loose eggs still attached to those particles. Then we mixed the supernatant and the washing water and centrifuged at 1500 U/min for 10 minutes. From the pellet obtained, we took between 0.2 and 0.4 g to which we added 6 ml of flotation medium, consisting of a saturated solution of NaCl and $ZnCl_2$. Four compartments of McMasters counting chambers, each with a volume of 0.15 ml, were filled. We searched eggs in this solution with

the use of a light microscope at 100 × magnification.

Additional blood samples from 9 nestling Burrowing Parrots from the breeding season 2003–2004 were tested for the presence of *Plasmodium* spp. and *Haemoproteus* spp. using a PCR detection method. Blood samples for molecular assays were collected via puncture of the cutaneous ulnar vein immediately after capture i.e., between 11:00 and 19:00 h., and stored in lysis buffer (10 mM Tris-HCL pH 8.0, 100 mM EDTA, 2% SDS). DNA was subsequently extracted from the samples using a DNeasy kit protocol (Qiagen, Valencia, California). We used the primers 621 and 983 that amplify a fragment of the parasites' cyt *b* gene and protocols with appropriate controls as described in Richard *et al.* 2002.

RESULTS

We detected no blood parasites in smears of peripheral blood of 43 breeding adults, and 90 nestling of wild Burrowing Parrots from three breeding seasons. Additional testing of 9 samples of nestling Burrowing Parrots with PCR detected no *Plasmodium* spp. or *Haemoproteus* spp.

Neither parasite eggs nor oocysts were detected in faecal samples of 44 breeding adults, and 101 nestling Burrowing Parrots from one breeding season.

DISCUSSION

In the present study, we did not detect any blood or intestinal parasites in Burrowing Parrots. This was surprising, as we expected to find such parasites given 1) the large size of the colony, together with a number of possible insect vectors for blood parasites (see below), 2) the conspicuous ornamentation of Burrowing Parrots, correlated with female body condition, male size and nestling pre-fledging condition (see Masello & Quillfeldt

2003, 2004a, Masello *et al.* 2004), suggesting secondary sexual signals of potential individual differences in the prevalence of parasitic infection, according to the Hamilton-Zuk hypothesis. Hamilton and Zuk (1982) suggested that parasites might be an important criterion to use in mate choice, proposing that elaborate ornaments and colorful plumage have evolved to signal differences in genetic quality in terms of resistance to parasites and diseases. The Hamilton-Zuk hypothesis predicts that species most subject to infection should have more developed and/or conspicuous secondary sexual characters (e.g., brighter coloration, more elaborate plumage, etc.; Brawner III *et al.* 2000). Individuals need to invest in immunity, and only individuals in good condition can invest time and energy or spare antioxidants such as carotenoids or psittacofulvins to develop ornaments like bright plumage to their full extent, and this would enable the birds to select partners of high genetic quality (sexual selection). Parrots use psittacofulvins (Krukenberg 1882, Stradi *et al.* 2001), which also have antioxidant activity (Morelli *et al.* 2003), therefore the same hypothesis may apply if psittacofulvins prove to be costly to produce.

Nevertheless, apparent absence or scarcity of blood parasites has been reported frequently (see Martínez-Abraín *et al.* 2004, Valkiunas *et al.* 2003, 2004). Absence or scarcity of blood parasites has been reported to correlate with different habitats such as the Arctic tundra (e.g., Bennett *et al.* 1992), arid environments (e.g., Little & Earlé 1995), island environments (e.g., Little & Earlé 1994) or marine environments (e.g., Piersma 1997, Figuerola 1999, Jovani *et al.* 2001). It has been also reported to correlate with different groups of birds such as South African sandgrouse and weavers (Little & Earlé 1995), swifts (Tella *et al.* 1995), waders (Figuerola *et al.* 1996), raptors (e.g., Tella *et al.* 1996), nightjars (Forero *et al.* 1997), seabirds (e.g., Merino

TABLE 1. Reports of blood and intestinal parasites in wild Psittaciformes. NF: not found. Prevalence noted in brackets where known. Travassos (1930) mentioned four species of Nematoda and Cestoda from the intestine of a number of Psittaciformes, which we do not include in the present table. Travassos (1930) did not mention for those records if the parasites were recovered from wild or captive psittaciform species, and citation was not clear enough to allow us to track back the records. We had no access to Pereira (1933) where, according to Serra-Freire & Bianchin (1978), *Ascaridia sergiomeirai* was found in some Brazilian Psittaciformes.

Psittaciformes species	Blood parasites		Birds sampled		Intestinal parasites		Birds sampled		References
	Adults	Nestlings	Adults	Nestlings	Adults	Nestlings	Adults	Nestlings	
Rainbow Lorikeet (<i>Trichoglossus haematodus</i>)	–	–	–	–	<i>Eimeria haematodi</i> (43.3%)	30	–	–	Varghese (1977)
Musk Lorikeet (<i>Glossopsitta concinna</i>)	–	–	–	–	<i>Eimeria dunsingi</i>	5	–	–	Gartrell <i>et al.</i> (2000)
Swift Parrot (<i>Lathamus discolor</i>)	–	–	–	–	NF	34	–	–	Gartrell <i>et al.</i> (2000)
Hyacinth Macaw (<i>Anodorhynchus hyacinthinus</i>)	–	–	–	–	NF	4	–	–	Travassos (1930)
Yellow-collared Macaw (<i>Propyrrhura auricollis</i>)	–	–	–	–	NF	2	–	–	Travassos (1930)
White-eyed Conure (<i>Aratinga leucophthalmus</i>)	–	–	–	–	<i>Ascaridia hermaphrodita</i> , <i>A. sergiomeirai</i>	?	?	–	Magalhães Pinto <i>et al.</i> (1993)
Peach-fronted Conure (<i>Aratinga aurea</i>)	–	–	–	–	NF	2	–	–	Travassos (1930)
Dusky-headed Parakeet (<i>Aratinga weddelli</i>)	NF	–	38	–	NF	38	–	–	Gilardi <i>et al.</i> (1995)
Orange-fronted Conure (<i>Aratinga canicularis</i>)	NF	–	13	5	NF	13	5	–	Joyner <i>et al.</i> (1992)
	–	–	–	–	<i>Eimeria aratingi</i> (14%)	21	–	–	Upton & Wright (1994)
Thick-billed Parrot (<i>Rhynchopsitta pachyrhyncha</i>)	–	–	–	–	NF	–	35	–	Stone <i>et al.</i> (2005)
Burrowing Parrot (<i>Cyanoliseus patagonus</i>)	NF	–	2	–	–	–	–	–	Cassamagnaghi (1947)
	NF	–	43	90	NF	44	101	–	This study
Monk Parakeet (<i>Myiopsitta monachus</i>)	–	–	–	–	NF	9	–	–	Travassos (1930)
Green-cheeked Conure (<i>Pyrrhura molinae</i>)	–	–	–	–	NF	1	–	–	Travassos (1930)
White-eared Conure (<i>Pyrrhura leucotis</i>)	–	–	–	–	<i>Ascaridia hermaphrodita</i>	?	?	–	Magalhães Pinto <i>et al.</i> (1993)
Green-rumped Parrotlet (<i>Forpus passerinus</i>)	–	–	–	–	NF	15	–	–	Travassos (1930)
Plain Parakeet (<i>Brotogeris tirica</i>)	–	–	–	–	NF	2	–	–	Travassos (1930)
Yellow-chevroned Parakeet (<i>Brotogeris chiriri</i>)	–	–	–	–	NF	2	–	–	Travassos (1930)
Orange-chinned Parakeet (<i>Brotogeris jugularis</i>)	NF	–	4	4	NF	4	4	–	Joyner <i>et al.</i> (1992)
	–	–	–	–	NF	11	–	–	Upton & Wright (1994)
Tui Parakeet (<i>Brotogeris sanctithomae</i>)	NF	–	13	–	NF	13	–	–	Gilardi <i>et al.</i> (1995)
Scaly-headed Parrot (<i>Pionus maximiliani</i>)	–	–	–	–	<i>Ascaridia hermaphrodita</i> (64%)	11	–	–	Travassos (1930)

TABLE 1. Continued.

Psittaciformes species	Blood parasites	Birds sampled		Intestinal parasites	Birds sampled		References
		Adults	Nestlings		Adults	Nestlings	
	–	–	–	<i>Ascaridia hermaphrodita</i> , <i>A. sergiomeirai</i>	?	?	Magalhães Pinto <i>et al.</i> (1993)
<i>White-fronted Amazon (Amazona albifrons)</i>	NF	–	–	NF	–	3	Joyner <i>et al.</i> (1992)
	–	–	–	NF	26	6	Upton & Wright (1994)
Red-crowned Amazon (<i>Amazona viridigenalis</i>)	–	–	–	NF	–	10	Stone <i>et al.</i> (2005)
Red-lored Amazon (<i>Amazona autumnalis</i>)	–	–	–	NF	–	3	Stone <i>et al.</i> (2005)
Yellow-headed Amazon (<i>Amazona oratrix</i>)	–	–	–	NF	–	7	Stone <i>et al.</i> (2005)
Yellow-naped Amazon (<i>Amazona auropalliata</i>)	NF	–	9	NF	–	9	Joyner <i>et al.</i> (1992)
	–	–	–	NF	4	7	Upton & Wright (1994)
Orange-winged Amazon (<i>Amazona amazonica</i>)	–	–	–	<i>Ascaridia hermaphrodita</i>	1	–	Serra-Freire & Bianchin (1978)

et al. 1997, Merino & Minguez 1998, Engström *et al.* 2000), and storks (Jovani *et al.* 2002). Scarcity of hematozoa appears to be common in the Neotropics, where the prevalence of blood parasites until now reported (approximately 10%) is significantly less than that in any other zoogeographical region (Valkiunas *et al.* 2003, 2004). In a recent review regarding the apparent lack of blood parasites in avian species, Martínez-Abraín *et al.* (2004) discussed the main hypotheses to explain this absence: 1) the absence or scarcity of proper vectors, 2) a highly specific association between host and parasites with host switching being infrequent (host-parasite assemblage), 3) host immunological capabilities preventing infection by parasites, and 4) competitive exclusion of blood parasite vectors mediated by ectoparasites. In order to explain the scarcity of blood parasites in the Neotropics, Valkiunas *et al.* (2004) proposed that avian hemosporidians could have been evolved in the tropics of the Old World, where they are widely distributed and prevalent, and they probably penetrated to Central and South America through the Nearctic region of the Holarctic recently. Therefore, they may be less adapted and more virulent for indigenous species of birds (Valkiunas *et al.* 2003, 2004). Our results and those from a literature review shown in Table 1 are in line with this explanation.

Close to the region with the Burrowing Parrots, an apparent absence of blood parasites in 13 seabird species breeding in colonies on the Atlantic coast of the province of Chubut, Patagonia, Argentina, has been reported (Jovani *et al.* 2001, Tella *et al.* 2001). The scarcity of vectors (only 12% of birds infested with ectoparasites in the case of Magellanic Penguins (*Spheniscus magellanicus*), Tella *et al.* 2001) due to the marine environment and the dry conditions around the colonies was postulated as the most plausible hypothesis for explaining the apparent

absence of blood parasites. The Burrowing Parrots studied here breed in a cliff also facing the Atlantic Ocean on the Atlantic coast of Patagonia, in a very large colony (9 km long and an estimated average of 37,000 active nests, see Masello *et al.* 2006), but have several blood-sucking ectoparasites, such that easy transmission of parasites was expected. In the case of the Burrowing Parrots of northeast coastal Patagonia, the absence of vectors is an improbable explanation for the apparent absence of blood and intestinal parasites observed. Nestlings are infested with sand fleas (Siphonaptera, Tungidae, Blank *et al.* in press), ectoparasitic chewing lice *Paragoniocotes meridionalis* (Ischnocera, Philoptera) and *Heteromenopon macrurum* (Amblicera, Menoponidae, see Mey *et al.* 2002), very high numbers of the bug *Psitticimex uritui* (Hemiptera, Cimicidae, Haematosiphoninae, see also Masello & Quillfeldt 2004a), mosquitoes and other biting dipterans are very common in particular during the breeding season.

Migratory birds, which are exposed to a more diverse parasite fauna, invest more in immune defense than resident birds (e.g., Møller & Erritzoe 1998, Kleynhans & Tieleman 2005) but it has also been found that in species undergoing longer distance migrations, a higher proportion of individuals were infected by haematozoa (Figuerola & Green 2000). Southern populations of Burrowing Parrots are migratory (Bucher & Rinaldi 1986, Bucher & Rodríguez 1986) and, as such, might invest more in immune defense. The observed apparent absence of blood and intestinal parasites in the Burrowing Parrots of northeast coastal Patagonia could be explained by their necessity to cope with the different parasite faunas they may find during the annual cycle. As short distance migrants, the here reported apparent absence of blood parasites in Burrowing Parrots is also in line with the findings of Figuerola & Green (2000) who found that the number of

parasite species or genera reported per host was positively related to migration distance. But to test this hypothesis it would be necessary to compare levels of parasitism between resident and non-resident populations of Burrowing Parrots.

Higher transmission rates of blood parasites have been found among colonial birds (Tella 2002). The larger blood parasitization pressure in colonial birds could have selected for an improved immune system in this group of birds, as denoted by the larger immune system organs in colonial avian species (see Tella 2002). This might be the case of the Burrowing Parrots of northeastern Patagonia.

Host diet may influence parasite species richness, with top predator harboring rich parasite communities because parasite species may accumulate through predator-prey relationships (see e.g., Simková *et al.* 2001). Burrowing Parrots are herbivorous and no items of animal origin have been reported in their diet (see Bucher *et al.* 1987, Masello *et al.* 2006), thus reducing the probabilities of infestation with intestinal parasites.

The host specificity and the competitive exclusion hypotheses proposed by Martínez-Abraín *et al.* (2004) remain also possible explanations for the apparent absence of blood and intestinal parasites observed in Burrowing Parrots of northeast coastal Patagonia, but require further testing.

Aspects of methodology. It has been suggested that blood smears may not detect parasites like Plasmodium or Trypanosoma (see Dawson & Bortolotti 2001, Jovani *et al.* 2001, 2002). Nevertheless, the technique has been used successfully to detect of the genera Trypanosoma (e.g., Merino & Potti 1995, Dufva 1996, Sanz *et al.* 2001a, b) and Haemoproteus (see e.g., Merilä *et al.* 1999, Dawson & Bortolotti 2001, Hōrak *et al.* 2001), among others. But the technique is still considered the best method to record the diversity of species of

these parasites in each individual avian host (see e.g., Valkiunas 2005a). The prepatent period for blood parasites varies between 5–14 days (Fallis & Bennett 1961), and therefore blood parasite counts in nestlings are often low (e.g., Bennett *et al.* 1974, Weatherhead & Bennett 1991). However, haematozoa were evident in 20% of 13-day old nestlings of the Pied Flycatcher *Ficedula hypoleuca* (Merino & Potti 1995). Our samples from Burrowing Parrot nestlings were obtained between 38–60 days of age, largely after the minimal prepatent period reported for several blood parasites (e.g., Fallis & Bennett 1961, Merino & Potti 1995, Valkiunas *et al.* 2004). In addition, no Plasmodium spp. or Haemoproteus spp. were detected in nestling Burrowing Parrots searched with the use of a PCR detection method, thus confirming our results based on blood smears.

Faecal analysis for parasite eggs, like the one here reported is an indirect method to estimate the presence of some intestinal parasites. The coprological analyses for a novel species should assume that parasite eggs are equally distributed in all portions of faeces. In a recent study (Cringoli *et al.* 2004), it has been found that the flotation medium used in our analyses floated the least number of eggs in comparison with other 14 flotation solutions. Nevertheless, all the 14 flotation solutions tested, including the NaCl-ZnCl₂ solution used in our analyses, were capable of floating eggs where present (Cringoli *et al.* 2004).

In the present study, adult Burrowing Parrots samples were obtained during the breeding season, a period when maximum intensity of parasites has been predicted for several bird species (see Jovani *et al.* 2001, Scheuerlein & Ricklefs 2004). It remains possible that, having taken our samples in one of the densest sectors of the colony, only individuals of the highest quality were sampled (i.e., with access to the best sector of the colony) which could account for the apparent absence of

blood and intestine parasites (see e.g., Bosch *et al.* 1997). However, the study sector is also used by first-time breeders (J. F. Masello & P. Quillfeldt, unpubl.), and thus, our sample most likely included a range of individual qualities (see also Masello & Quillfeldt 2003, Masello *et al.* 2004). The possibility that heavily infected individuals skip breeding during the acute stage of infection and are thus not readily available for researchers to sample cannot be discarded (see also Valkiunas *et al.* 2003, 2004), but our large sample set and the molecular methods should detect even low levels of prevalence.

Thus, we can conservatively affirm that the prevalence of blood and intestinal parasites in the Burrowing Parrots of northeast coastal Patagonia is, at least, very low.

Psittaciformes. The minority of psittaciform species revised by Bennett (1993) were infested with *Haemoproteus* (43 of 143 species, representing 30% of the revised species or 12% of psittaciform species), while in contrast, most species of Passeriformes were infested with *Haemoproteus* (2047 of 2409 examined species or 85%). Seven species of haemosporidians have been originally described from parrots but at least in some cases parasites were found either in imported birds kept in captivity or in birds previously housed in zoological gardens and might have being infested through contact with poultry or pets (see e.g., *Haemoproteus* (*Parahaemoproteus*) *psittaci* Bennett and Peirce, 1992, *Haemoproteus* (*Parahaemoproteus*) *handai* Maqsood, 1943, in Valkiunas 2005b). However, a literature review of blood and intestinal parasites in wild Psittaciformes shows that blood parasites were absent in all cases (Table 1). In the same literature review, faecal exams were negative in 20 out of 28 studies (Table 1).

Other sources, uncited in Table 1, sometimes found parasites, but examined individu-

als had been brought into wildlife hospital or relocation facilities, and thus, might have acquired parasites through direct or indirect contact with poultry or pets (intestinal parasites: e.g., *Amazona aestiva* in Travassos 1930, Kreis 1955, Lederer 2000, Rooney *et al.* 2001, McDonnell 2003). Nevertheless, in confiscated birds housed in relocation facilities, Rooney *et al.* 2001 also recorded absence of blood parasites in 64 *Amazona a. autumnalis*, four *A. p. senilis*, 16 *A. f. ferinosa guatemala*, 10 *A. a. albifronsus*, and one *A. xantholora*. These authors found also no fecal parasites in four *A. p. senilis*, 12 *A. f. guatemala*, eight *A. a. albifronsus*, and one *A. xantholora*, belonging to the same group of confiscated birds. Coccidia were present in 6.0% (3/50) of the *Amazona a. autumnalis* survey by Rooney *et al.* (2001).

Psittaciformes are long-lived birds. It has been suggested that development, maintenance and use of the immune system incur costs in terms of both time and nutrients (Klasing & Leshchinsky 1999). Therefore, inter-taxa variation in immune function might be related to longevity in such a way that higher levels or a better quality of immune response evolved in longer-lived species having increased investment in self-maintenance over reproduction (Tieleman *et al.* 2005). Thus the observed apparent absence of blood parasites could be explained by innate immunity species-specific in Psittaciformes.

Further research. Further research in Burrowing Parrots of other colonies should consider possible geographical variation in parasite prevalence (see Gregory 1990, Merilä *et al.* 1995) and to compare migratory with resident populations. It also might investigate other types of parasites including bacteria, virus and numerous other taxa that could play a role in the evolution of the ornamental coloration of Burrowing Parrots (e.g., Thompson *et al.*

1997). Burrowing Parrot blood samples should be searched with use of PCR-based identification methods for the presence of parasites such as *Cryptosporidium* spp. as they were found in pet parrots (Abe & Iseki 2004). Coprological analyses should be repeated using some of the most effective flotation solutions recently tested by Cringoli *et al.* (2004).

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