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Gastrointestinal helminths of Emperor Penguin (*Aptenodytes forsteri*) from Antarctica

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Abstract
Although research on parasitic diseases in Antarctic birds is scarce and the information largely fragmented, an increasing number of studies are currently in progress in this area. The host/parasite relationship is very important for understanding the impact of parasitism on the seabirds' feeding ecology, especially in those isolated regions where the host is extremely sensitive to new diseases. Penguins are a big favorite for these studies because of their importance as indicators of change in the Antarctic ecosystem. This study aims at adding information on gastrointestinal parasites of the Emperor Penguin *Aptenodytes forsteri* from Snow Hill colony. Based on a sample of 43 individuals found freshly dead (3 adults and 40 chicks) from predation or starvation, the gastrointestinal helminth community in Emperor penguins was composed of two species of cestodes (*Parorchites zederi* and *Tetrabothrius* sp.) and three species of nematodes (*Stegophorus macronectes, Tetrameres* sp. and *Contracaecum osculatum*). This is the first record of *S. macronectes* in the Emperor Penguin. The low parasite richness observed might be related to the stenophagic and pelagic diet of this host.

Keywords Emperor penguin · *Parorchites zederi* · *Tetrabothrius* sp. · *Stegophorus macronectes* · *Contracaecum osculatum* · *Tetrameres* sp. · Antarctica

Introduction

Penguins are widely distributed around the Antarctica and they are important consumers within the marine trophic web (Brooke 2004). The Emperor Penguin *Aptenodytes forsteri* (Gray, 1844) is the species with southernmost geographical distribution. Emperor populations breed during the Antarctic winter. There are approximately 238.000 breeding pairs, distributed in 46 colonies (Fretwell et al. 2012). These colonies are located in a few favorable Antarctic coastal areas and sea ice between 64°S and 78°W (Kooyman et al. 1992; Kooyman and Kooyman 1995; Wienecke et al. 2007; Libertelli and Coria 2014). Their diet is composed of squid (e.g., *Psychroteuthis glacialis* and *Alluroteuthis antarcticus*), fish (e.g., *Pleuragramma antarcticum*), and crustaceans (e.g., *Euphausia superba*), proportions vary depending on the colony's location (Cherel and Kooyman 1998). Emperor penguins adapt to extreme environmental conditions, which makes them very sensitive to climatic variations.

Climate change is much more pronounced in polar regions than elsewhere. Changes caused by El Niño event influence marine and terrestrial parasites (Daszak et al. 2000) because gastrointestinal infections depend mainly on foraging habits (Hoberg 1996). Particularly, the Antarctic Peninsula and its islands are areas with rapid regional climate changes (Klimpel et al. 2017). Host diet variations owing to climate change or anthropogenic impact, such as fisheries, lead to changes in the occurrence of parasites in Antarctic penguins (Vidal et al. 2012). Parasites can be used as indicators of environmental changes because any given variation in parasite diversity may be a sign of 'ecosystem distress syndrome' (Rapport 2007).

Parasitological researches in Antarctica are relatively recent. Inevitably, reports and descriptions of parasites appear after the host's biology. It is, therefore, no surprise that there are so few published reports on parasites of Antarctic organisms since the middle of the twentieth century (Mackenzie 2017). Although penguins represent about 80%

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of the vertebrate biomass and are the most studied birds in Antarctica (Barbraud and Weimerskirch 2006), there is little information about their parasitic diseases (Diaz et al. 2017). Recent studies have expanded our knowledge of parasites of some Antarctic penguin species, providing taxonomic information as well as data on prevalence and intensities (Fonteneau et al. 2011; Vidal et al. 2012; Gonzalez-Acuña et al. 2013; Diaz et al. 2013, 2016, 2017). However, most information corresponds to Pygoscelidae species.

The aim of the present study is to describe the helminth community of *A. forsteri* from Snow Hill colony, based on chick and adult penguin samples. These results might serve as baseline data for future studies on ecosystem health.

Materials and methods

This study was carried out in the colony number 44 of emperor penguins in Snow Hill Island, during the winter season of the years 2014 and 2016. It is known as the northernmost emperor penguin colony of the Antarctica and it is located at 64° 30´S, 57° 26´W in the south coast of the island (Coria and Montalti 2000; Todd et al. 2004) (Fig. 1).

A total of 43 recently dead specimens (3 adults and 40 chicks less than 20 days old) were collected (see Table 1). The samples were placed in plastic bags and frozen at – 20 ℃ until evisceration was carried out. In the laboratory, digestive track was removed and fixed in 10% formalin or preserved in 96% ethanol for parasitological examination. The digestive track was separated in esophagus, stomach and intestine. Helminths were removed from each portion using a stereomicroscope, fixed in 5% formalin (12 h), and thereafter preserved in 70% ethanol. Cestodes were stained in Semichon's carmine, dehydrated in an ethanol-graded series, clarified in methyl salicylate and mounted in balsam of Canada. Nematodes were clarified in 25% glycerine ethanol or lactophenol. All specimens were studied using an optic microscope and identified following taxonomic keys and specific related bibliography (Johnston and Mawson 1945; Delymure 1955; Petter 1959; Yamaguti 1959, 1961; Chabaud 1974; Cielecka et al. 1992; Khalil et al. 1994). The parasite samples were deposited in the Helminthological Collection of Museo de La Plata (MLP He 7451, 7452, 7453, 7454, 7455), Argentina. Skeletons of birds were deposited in the Ornithology section of Museo de La Plata (MLP-O 1526-1535) Argentina, and in the Ornithological

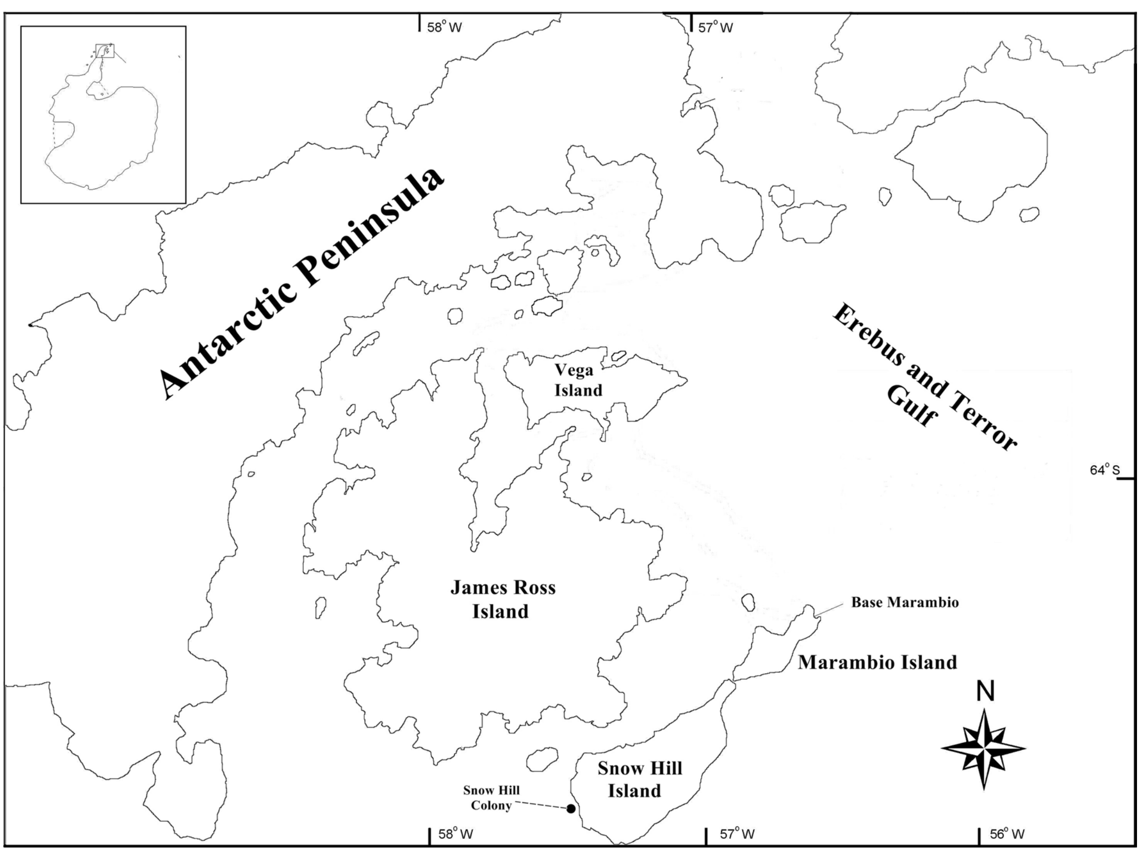

Fig. 1 Sample site of the Emperor Penguins (*Aptenodytes forsteri*) in Snow Hill Island during the breeding season 2014 and 2016

Table 1 Parasite composition and ecological parameters of the Emperor Penguin *Aptenodytes forsteri* by year and age group

			2014			2016		
	Adults	Chicks	Total	Adults	Chicks	Total	Adults	Chicks
Host analyzed [n°(P; MI)]	3 (100; 28.6)	40 (65; 10.7)	29 (62; 15.7)	1 (100; 64)	28 (58.6; 12.8)	14 (78; 7.4)	2 (100; 11.5)	12 (75; 6.3)
Stegophorus macronectes	(0; 0)	(10; 5.75)	(10.3; 6.3)	(0; 0)	(10.7; 6.3)	(7.1; 4)	(0; 0)	(8.3; 4)
Tetrameres sp.	(66.6; 8.5)	(50; 12.1)	(48.3; 13.4)	(100; 1)	(46.4; 14.3)	(57.1; 8.4)	(50; 16)	(58.3; 7.3)
Contracaecum osculatum	(0; 0)	(15; 2.5)	(10.3; 3)	(0; 0)	(10.7; 3)	(14.3; 1)	(0; 0)	(16,6; 1)
Parorchites zederi	(100; 3.6)	(0; 0)	(3.4; 57)	(100; 57)	(0; 0)	(14.3; 1)	(100; 2)	(0; 0)
Tetrabothrius sp. (P; MI)	(33.3; 6)	(2.5; 1)	(6.9; 3.5)	(100; 6)	(3.5; 1)	(0; 0)	(0; 0)	(0; 0)

Collection of Museo Argentino de Ciencias Naturales Bernardino Rivadavia (MNBR 8450-8463), Argentina.

The terms prevalence (*P*), mean intensity (MI), richness (*S*), infracommunity and component community were interpreted according to Bush et al. (1997) and calculated using the Quantitative Parasitology 3.0 Budapest software (Rózsa et al. 2000). The MI and mean S are followed by the range.

Results

A total of 363 helminths were recovered from all analyzed hosts. Four adult parasite species were identified: three nematode species: *Stegophorus macronectes* (Johnston and Mawson, 1942) (Acuariidae) and *Contracaecum osculatum* (Rudolphi, 1802) (Asnisakidae) in the lumen of de stomach, and *Tetrameres* sp. (Tetrameriidae) in the glandular stomach; and one cestode species: *Parorchites zederi* (Baird, 1853) (Dilepididae) in the intestine. Additionally, immature cestodes identified as *Tetrabothrius* sp. (Tetrabothridae) were recovered from the intestine of two penguins.

Out of the total number of penguins examined, 67.4% were parasitized by at least one of the species identified (MI = 12.5, 1–97). *Parorchites zederi* (*P* = 9.67%; MI = 21, 1–57) were diagnosed based on the scolex, proglottids and the presence of a pseudoscolex. This helminth species was only present in adult birds. *Stegophorus macronectes* (*P* = 9.3%; MI = 5.75, 1–14) was diagnosed by the morphology of ornamental cephalic structures, cephalic collar and deirids, position of the vulva in females, papillae distribution and spicules in males (see Vidal et al. 2016). This parasite species was only present in chicks. *Tetrameres* sp. (*P* = 51.7%; MI = 11.7, 1–83) were identified by the features of the buccal capsule and spination of the body surface. *Contracaecum osculatum* (*P* = 11.6%; MI = 3.6, 1–7) were identified based on the distribution and number of caudal papillae and length of the spicules in male. This parasite was only found in chicks. *Tetrabothrius* sp. specimens were found weakly adhered to the surface of the intestinal mucosa as well as free in the lumen (*P* = 4.65%; MI = 3.5, 1–6). In this case, specific identification was not possible since specimens were immature.

Most infracommunities (75.86%) consisted of one parasite species only, *S* = 1 (1–3): 77.3% were parasitized by *Tetrameres* sp., 13.6% by *C. osculatum*, 3.4% by *S. macronectes* and 3.4% by *Tetrabothrius* sp. Only 17.2% of infracommunities were parasitized by two different species: 40% by *Tetrameres* sp. and *S. macronectes*, 40% by *Tetrameres* sp. and *P. zederi,* and 20% by *Tetrameres* sp. and *C. osculatum*. Only 6.9% of infracommunities were infected by three different species: 50% of them by *Tetrameres* sp., *C. osculatum* and *S. macronectes*, and 50% of them by *Tetrameres* sp., *P. zederi* and *Tetrabothrius* sp.

The richness of the component community was greater for chicks than for adults (*S* = 4 vs. *S* = 3). The sample size did not allow us to perform statistical analysis between ages or between years. However, could be observed that *P. zederi* was the more prevalent species in adults, whereas *Tetrameres* sp. was the more prevalent species in chicks, both in 2014 and 2016. The less prevalent species both in adult than chicks was *Tetrabothrius* sp. species. This species was present in samples from 2014 but absent in samples from 2016. The nematodes *S. macronectes* and *C. osculatum* were good represented in chicks in both years. The composition and ecological parameters by year and age group are shown in Table 1.

Despite low number of adult samples, it is interesting to note that one parasite species was only present in adult birds (i.e., *P. zederi*), whereas two species were only present in chicks (*S. macronectes* and *C. osculatum*).

Discussion

In Emperor Penguins, the helminth richness is in accords with the relatively low number of parasite species (from 3 to 6) found in other Antarctic and subantarctic penguins (Fonteneau et al. 2011; Vidal et al. 2012; Diaz et al. 2013, 2016). Two of the parasite species (*S. macronectes* and *P. zederi*) registered in this work are widely distributed among Antarctic penguins (Diaz et al. 2017).

Spirurid nematodes occur in the esophagus and stomach of seabirds and are one of the most abundant components in the helminth communities of penguins. Among them, *S macronectes* has been reported in the three pygoscelid species, also in rockhopper penguin *Eudyptes chrysocome* (Forster) and in macaroni penguin *Eudyptes crysolophus* (Brandt) (Diaz et al. 2017). While the prevalence of this parasite species in pygoscelid penguins has always been high (e.g., *P* = 78% in chicks of *P. antarctica*; *P* = 30% in chicks of *P. adelia*, and *P* = 48.6% in both chicks and adults of *P. papua*) (Vidal et al. 2012; Diaz et al. 2013, 2016), it has been markedly low in *A. forsteri* (*P* = 10% in chicks). The genus *Tetrameres* parasitized the proventricular glands of Antarctic penguins (Schmidt 1965). *Tetrameres wetzeli* (Schmidt 1965) is the only species on the genus described parasitizing penguins and has been recovered from rockhopper, King and Gentoo Penguins (Schmidt 1965; Fonteneau et al. 2011; Diaz et al. 2013). Unidentified species of *Tetrameres* have also been found in Adélie Penguins (Diaz et al. 2016). Although most of the *Tetrameres* specimens collected in this work were immature, their presence has been accompanied by a high prevalence in comparison with the other parasitic species. Mature specimens were only present in one adult bird. Life cycles of spirurida nematodes include crustaceans like *E. superba* as intermediate hosts, in whose hemocoel the infective larva develops (Anderson 2000; Hoberg 2005; Vidal et al. 2016; Diaz et al. 2017).

The nematodes of the genus *Contracaecum* (ascaridoid) are commonly found in the stomach of piscivorous birds (Garbin et al. 2007, 2008; Diaz et al. 2010). *Contracaecum heardi* (Johnston and Mawson, 1942) is the best documented species among subantarctic penguins parasitizing King, Macaroni and Gentoo penguins (Mawson 1953; Fonteneau et al. 2011). *Contracaecum osculatum* is a very common parasite in Antarctic pinnipeds, as the Weddell seal *Leptonychotes weddellii* and the sea lion *Mirounga leonina*, distributed in the Ross and Weddell seas. These marine mammals act as definitive hosts for *C. osculatum*, whereas crustaceans, fish and squid serve as intermediate/paratenic hosts (Mattiucci et al. 2017). The presence of this parasite supports the hypothesis of a food niche superposition between *A. forsteri* and *L. weddellii*.

Parorchites zederi is the only member of the order Cyclophyllidea in pelagic systems (Hoberg 2005) and it is a very common parasite in pygoscelid species (Diaz et al. 2017). These cestodes penetrate the intestinal mucosa reaching the serosa, where they generate large nodules associated with lesions (Tzvetkov et al. 1999; Martin 2015; Martin et al. 2016). In pygoscelid penguins, each lesion is occupied by multiple cestodes (up to 16), whereas in present study a maximum of three specimens per lesion were found. Euphausiid species have been recorded as suitable intermediate hosts of this parasite species (Hoberg 2005).

Members of the Tetrabothriidea are also important components of the helminth communities of Antarctic penguins (Baer 1954). *Tetrabothrius pauliani* (Joyeux and Baer, 1954) was registered parasitizing all pygoscelid species and also the King Penguin (Prudhoe 1969; Cielecka et al. 1992), *Tetrabothrius joubini* (Railliet and Henry, 1912) was only reported in the Chinstrap Penguin (Cielecka et al. 1992; Georgiev et al. 1996) and *Tetrabothrius wrighti* (Leiper and Atkinson, 1914) was registered for adélie, King and Emperor penguins (Leiper and Atkinson 1914; Johnston 1937; Prudhoe 1969; Fonteneau et al. 2011). The low prevalence and development of the specimens found in this study did not allow their identification at species level. The complete life cycle of *Tetrabothrius* species is still unclear and further investigations are needed. It is known that plerocercoid larvae use a variety of fish/squid prey (Williams 1995; Hoberg 2005; Kleinertz et al. 2014).

Taking into account that the finding of a parasite in a definitive host indicates that other hosts required for the transmission are present in that community, having a trophic relationship between them (Marcogliese and Cone 1997), the presence of *Tetrameres* sp., *S. macronectes* and *P. zederi* in the Emperor Penguin suggest the ingestion of Antarctic krill, although in smaller quantity compared to other penguin species such as pygoscelids (Diaz et al. 2017). It is in accordance with recent investigation on diet of Emperor Penguin in the present colony, in which crustaceans were represented although in low proportion (Libertelli et al. unpub. data).

Despite *S. macronectes* and *P. zederi* use, the same intermediate host draws attention that the former was present only in chicks, whereas the later was found only in adult birds. It could be related with the site if infection of each parasite species. Host must travel a long extension of sea from the feeding place to the colony. Taking into account that *P. zederi* parasitized the intestine, it is unlikely that after several days of being recruited by the parents—into their intermediate host—cestode larvae still persist in the stomach of the parents and could be transferred to the chicks. In contrast, site of infections of nematodes is the stomach, so they have more possibilities to be transferred from parents to the chicks during feeding. On the other hand, the nematodes have a strong cuticle that confers them greater resistance to the digestion and decomposition process. Finally, the early age of chicks did not allow parasites to reach maturity. Consequently, the presence of immature or juvenile parasites was high.

Climate change in the Antarctic Peninsula and surrounding areas produces intense environmental changes that affect the trophic web from bottom to top and cause a significant reduction in the primary production of the food web (Montes-Hugo et al. 2009; Klimpel et al. 2017). With the consequent reduction in krill abundance (Atkinson et al. 2004; Flores et al. 2012), top predators such as penguins are changing their population trends (Carlini et al. 2009; Trivelpiece et al. 2011; Barbosa et al. 2012). Such changes would certainly affect not only the overall nutritional and health status of these seabirds, but also the rate of ingestion of parasite cysts/larvae and their exposure to new parasites and new diseases. Helminth effects on Antarctic penguins have been reported at the level of tissue damage, specifically. Martin et al. (2016) described lesions accompanied by hemorrhage, edema, degeneration and necrosis of the pygoscelis penguin intestine. Effects on the immune system of Antarctic penguins have also been demonstrated in terms of an increased foot-web swelling response to phytohemagglutinin and a decreased concentration of eosinophils and monocytes in the blood of individuals treated with anti-helminthic drugs (Bertellotti et al. 2016). Parasites seem to play a key role in the population dynamics of their hosts by affecting factors such as survival, reproductive success or behavior (Morand and Deter 2009). This can produce a decline in host populations or affect hosts in different subtle ways through resources consumption and altering metabolic rate, territorial behavior, phenology, intra- and interspecific interactions, mating and foraging success, among others (Møller 1997).

It is, therefore, necessary to develop a long-term, geographically representative and monitoring program to investigate the occurrence of parasites and pathogens as well as their ecology, life cycle, epidemiology and health impacts. In this way, the different species of penguins and their parasites could be taken as bioindicators of the health of the Antarctic trophic ecosystem.

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Compliance with ethical standards

Conflict of interest The authors declare that no conflicts of interest exist. Procedures used in this study comply with the current laws for working in Antarctica. Permission to work in the study area and for penguin handling was granted by the Environmental Management and Tourism Program of the Dirección Nacional del Antártico (DNA), Argentina.

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