GASTROINTESTINAL PARASITES IN THE GALÁPAGOS PENGUIN *SPHENISCUS MENDICULUS* AND THE FLIGHTLESS CORMORANT *PHALACROCORAX HARRISI* IN THE GALÁPAGOS ISLANDS

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The Galápagos Penguin *Spheniscus mendiculus* and the Flightless Cormorant *Phalacrocorax harrisi* are endemic to the Galápagos Islands, Ecuador (Harris 1973). Both species have particular characteristics that make them unique. The former is the most northerly of penguin species (Boersma *et al*. 2013); the latter is the only extant flightless cormorant and has a sequential polyandrous mating system (Valle 1994). The International Union for Conservation of Nature has listed them as endangered and vulnerable, respectively, due to their limited geographical distribution and small fluctuating populations (IUCN 2012). More than 90% of Galápagos Penguins and the entire population of Flightless Cormorants occur on Isabela and Fernandina islands (Boersma 1976, Snow 1965, Harris 1973, Valle 1994). The remaining penguins are found on Floreana, Bartolomé and Santiago islands (Harris 1973, Boersma 1976, Castro & Phillips 1996, Bingham 2001, Wiedenfeld & Jiménez-Uzcátegui 2008). According to the latest survey, the populations of these species are estimated to be 1 870 Galápagos Penguins and 2 080 Flightless Cormorants (Jiménez-Uzcátegui 2013).

As part of a continuing effort, annual ecological monitoring has been developed (Harris 1974, Boersma, 1976, Vargas 2006, Jiménez-Uzcátegui 2013) as well as evaluations of the health status of these species. Included in the latter are the species' life cycles, possible sources of infection, and emerging parasites (Greiner & Ritchie 1994, Zucca & Delogu 2010). Pathogens such as microfilaria *Toxoplasma gondii* have been identified in both species (Merkel *et al*. 2007, Deem *et al*. 2010), and *Plasmodium* sp. and *Haemoproteus* sp. have been found in the Galápagos Penguin (Levin *et al*. 2009). However, to our knowledge, there are no studies of gastrointestinal parasites in either of these avian species. In other species of penguins such as Humboldt *Spheniscus humboldti* and Magellanic *Spheniscus magellanicus* Penguins, the gastrointestinal parasites *Contracaecum* spp., *Tetrabothrius* spp. and *Cardiocephaloides* spp. have been detected (Pazos *et al*. 2003, González-Acuña *et al.* 2008a, Medeiros & Amato 2010, Díaz *et al*. 2010). Likewise, in the Neotropic Cormorant *Phalacrocorax brasilianus,* parasites such as *Andracantha phalacrocorasis, Contracaecum* spp., *Paradilepis* spp., *Hysteromorpha* spp. and *Ascocotyle* spp. have been found (González-Acuña *et al*. 2008b, Monteiro *et al.* 2011, Violante-González *et al*. 2011). In the studies cited, samples were taken during necropsies of dead individuals, but our objective was to determinate and identify the gastrointestinal parasites in live Galápagos Penguins and the Flightless Cormorants by fecal examination.

The fieldwork was carried out during December 2011, and March and June 2012. Fecal samples of 167 Galápagos Penguins were taken by cloacal swabs (Pur-wraps Sterile Swabs, Puritan Medical Products Company LLC, Guilford, Maine, USA) and spontaneous evacuation from subpopulations at Caleta Iguana (0°97′668″S, 91°44′682″W), Puerto Pajas (0°75′480″S, 91°37′505″W), and Marielas islets (0°59′570″S, 91°08′750″W), located along the shore of Isabela Island. The feces of 142 Flightless Cormorants were collected directly from the cloacae of nesting birds at Playa Escondida (0°26′269″S, 91°46′822″W), Carlos Valle (0°26′076″S, 91°45′950″W), Punta Albemarle 1 (0°15′400″N, 91°36′652″W) and Punta Albemarle 2 (0°16′223″N, 91°35′947″W), on the coasts of Fernandina and Isabela islands (Fig. 1).

All birds were tagged (if not previously tagged), measured and weighed. Sexes and age groups were distinguished on the basis

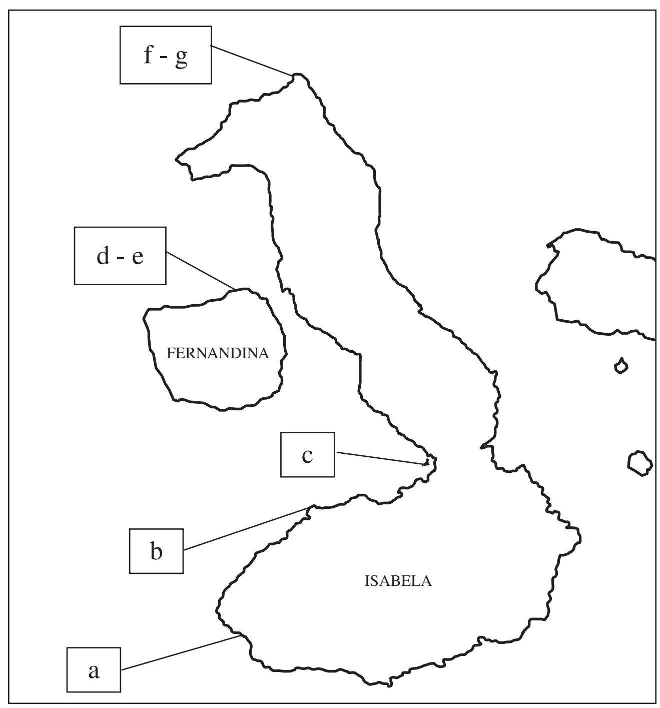

Fig. 1. Map of the Isabela and Fernandina islands showing colonies of the Galápagos Penguin (a. Caleta Iguana. b. Puerto Pajas. c. Marielas.) and the Flightless Cormorant (d.-e. Playa Escondida-Carlos Valle. f,-g. Punta Albemarle 1 and 2).

of plumage and body size (Boersma 1976, Snow 1966, Valle 1994, Boersma *et al*. 2013): Galápagos Penguin: 101 males and 63 females, 5 chicks and 162 adults; Flightless Cormorant: 54 males and 79 females, 16 chicks and 126 adults.

All samples were fixed in 10% formaldehyde, stored in plastic cryovials at 4 °C and analyzed at the Charles Darwin Research Station Laboratory and the Parasitology Laboratory, International Zoonosis Center of the Central University of Ecuador. Samples were processed using the qualitative flotation technique (Greiner 1989, Greiner & Ritchie 1994, Permin & Hansen 1998, Morales 2010). The positive samples of helminth parasitic infection (eggs) were quantified using an eyepiece micrometer, graphically recorded and identified by taxonomic keys. The differences relative to age groups, sex and sampling months were analyzed statistically by the chi-square test. Body weight distribution and its relation to parasitized versus non-parasitized individuals was evaluated by Kolmogorov-Smirnov *z*- and Student *t*-tests, respectively (Thrusfield 1990).

Of 167 Galápagos Penguin samples, only 26 (15.57%) were positive for any form of gastrointestinal parasitic infection; of positive samples, 15/63 (23.8%) were in females and 11/101 (10.9%) were in males. Two helminths and two protozoans were identified: a nematode *Contracaecum* sp. (50 µm length, 45 µm width), a Cestode proglottid (100 µm length, 70 µm width), a cyst of *Entamoeba* spp., and oocysts of Sporozoa (Apicomplexa) of different sizes that could not be identified because of the lack of sporulation (Fig. 2). There were no combinations of two different parasites per positive plate; there were two (7.7%) plates entirely of helminths and 24 (92.3%) plates of protozoans. The frequency of positives did not differ among sampling months (χ^2 = 5.78, P = 0.05). Parasitic infection was most prevalent among females (χ^2 = 4.85, P = 0.02). All positives cases involved adults. Host body weight varied from 1 000 g to 3 800 g (mean ± standard deviation = 1 973 ± 502 g, n = 167) with a normal distribution (P = 0.06); the prevalence of parasites was not related to the weight of individuals (t = -1.65, P = 0.1). Parasitism presence varied by location (χ^2 = 10.82, P < 0.004): Marielas 26.47% of samples (18/68), Caleta Iguana 10.63% (5/47) and Puerto Pajas 5.77% (3/52).

Of the 142 Flightless Cormorant samples, 48 (33.8%) were positive for gastrointestinal parasites, 20/54 (37.0%) in males and 27/79 (34.18%) in females. Identified were two helminths: a nematode *Contracaecum* sp. (56.4 ± 3.6 µm length, 53.4 ± 3.4 µm width), and a trematode Heterophyidae (33.4 ± 3.3 µm length, 19.6 ± 0.9 µm width). Three different protozoans were found: cysts of Entamoebidae *Entamoeba* sp., oocyst of Eimeriidae *Eimeria* sp., and oocyst of Sporozoa (Apicomplexa) (Fig. 3). Only two (4.2%) of the positive samples showed interaction between helminths and protozoans per plate. The remainder had exclusively helminths, 39.6% (19/48), or protozoans, 56.2% (27/48). The prevalence of

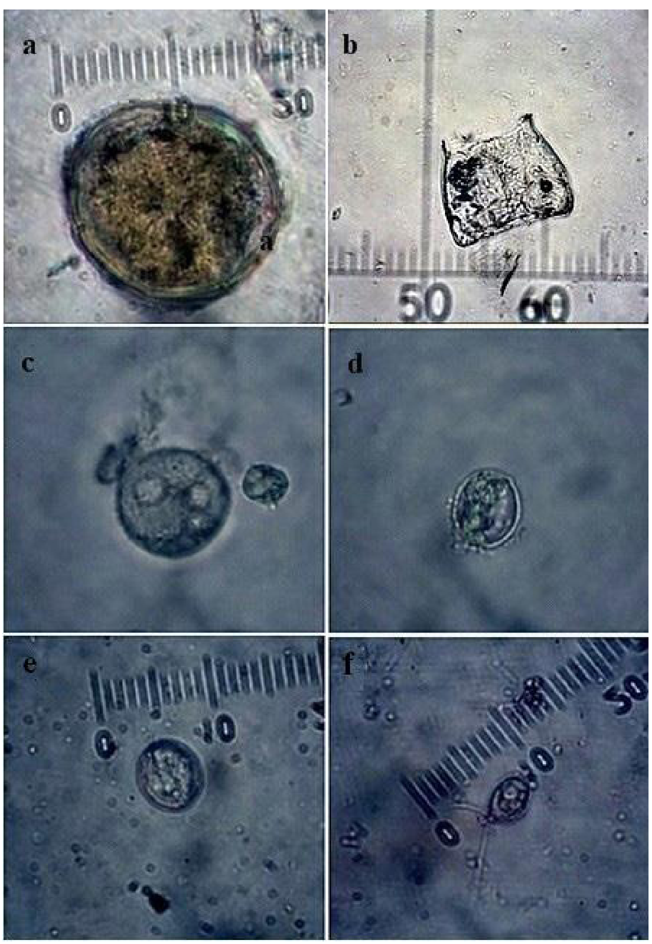

Fig. 2. Parasites found in the Galápagos Penguin: a, eggs of *Contracaecum* sp. (40×); b, Cestode proglottid (10×); c, cyst of *Entamoeba* spp. (40×); d.-e.-f., oocysts of Sporozoa (Apicomplexa) (40×).

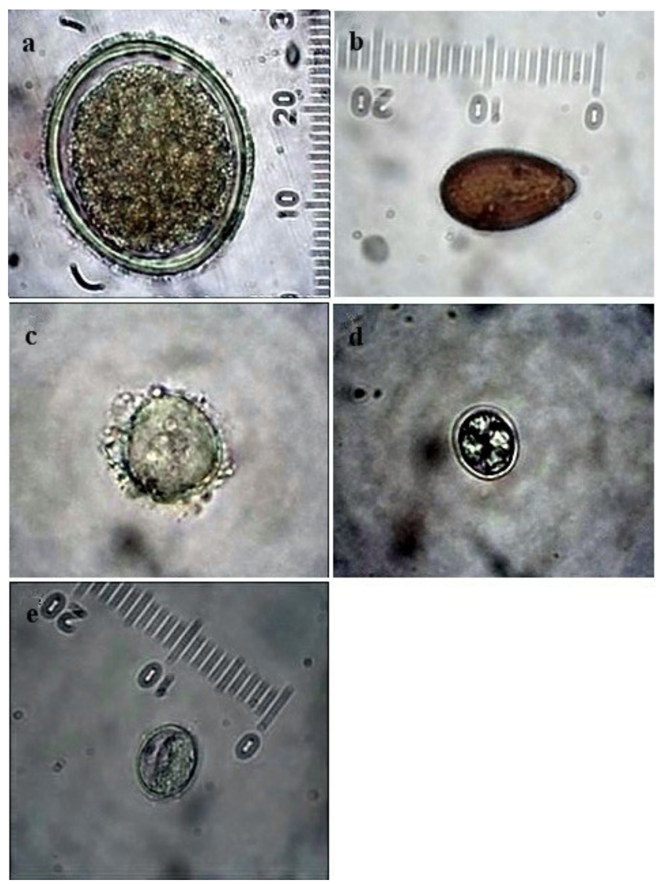

Fig. 3. Parasites found in the Flightless Cormorant: a, eggs of *Contracaecum* sp. (40×); b, egg of Heterophyidae (40×); c, cyst of *Entamoeba* spp. (40×); d, oocyst of *Eimeria* sp. (40×); e, oocyst of Sporozoa (Apicomplexa) (40×).

parasites varied significantly by month (χ^2 = 13.40, P < 0.001), with the highest frequency of parasites found in samples collected in June (39.6%) followed by those collected in March (37.5%) and in December (22.9%). Neither the host sex (χ^2 = 0.12, P = 0.73) nor sexual maturity (χ^2 = 0. 62, P = 0. 43) influenced the presence of parasitism. Host weight varied from 2 250 g to 4 600 g (mean = 3 216 ± 598 g, n = 142) with a normal distribution (P = 1.80), but was not affected by the presence of parasites (t = 0.25, P = 0.8). Finally, the presence of parasitism was not affected by colony location (χ^2 = 1.39, P = 0.71).

All helminths found in this study are new geographical records for the Galápagos Penguin and the Flightless Cormorant. Their taxonomic groups, however, were similar to those reported for Humboldt and Magellanic Penguins and for the Neotropic Cormorant (Clarke & Kerry 1993, Díaz *et al.* 2010, Violante-González *et al.* 2011). The genus *Contracaecum* spp. was common in both species, showing that this parasite could be widely distributed among the Galápagos Islands. The Heterophyidae digeneans from the Flightless Cormorant are common in fish-eating birds. *Ascocotyle longa* (Heterophyidae) has been reported in the Neotropic Cormorant in coastal lagoons of Mexico (Violante-González *et al*. 2011), so it could be the same genus in the Flightless Cormorant. The coccidians such as *Eimeria* spp. and Sporozoa oocyst are common in wild birds (Yabsley 2008). *Eimeria* sp. oocysts have been identified previously in the Galápagos Penguin (Vargas, 2006); however, in the present study these were found only in the Flightless Cormorant, perhaps because formaldehyde negatively affects sporulation. For this purpose, the use of potassium dichromate would have been a better choice to allow species identification. The amoebae *Entamoeba* spp., found in both the Galápagos Penguin and Flightless Cormorant, are mostly commensal protozoans located in the intestine of vertebrates (Ponce-Gordo & Martínez-Díaz 2010). Although the flotation method used is not as accurate as necropsy for the detection of parasite structures (adults, larvae, eggs, proglotide), it was the only appropriate method, given the extremely low population sizes of these birds. Molecular tests will permit a new level of differentiation of parasites species in future studies.

Parasite prevalence might have been affected by climate variation. Galápagos Penguin and Flightless Cormorant populations have declined during strong climate variations, such as El Niño (Valle & Coulter 1987, Vargas *et al.* 2007). According to the meteorological base at Charles Darwin Foundation (CDF), the rainfall at Puerto Ayora in 2012 was 514.3 mm, higher than the median annual precipitation (CDF 2012). Most of the year was considered normal, with the exception of a weak La Niña in December 2011/January 2012 and a weak-to-moderate El Niño in May/July (Walter & Timlin 1993, 1998; ESRL 2013). In the Galápagos Penguin, parasite prevalence did not vary by month, but for the Flightless Cormorant the presence of parasites was highest in June. This peak coincides with the strongest El Niño signal in 2012, and is presumably related to the decline of body condition and effects on the immune system, favouring gastrointestinal parasite infection (Boersma 1998, Vargas *et al.* 2007). All parasites found in this study may affect the health of seabirds under conditions of starvation or stress (Fagerholm & Overstreet 2008, Huffman 2008, McLaughlin 2008, Wobeser 2008, Yabsley 2008).

The sex-related differences found in the parasitism frequency in Galápagos Penguins could be related to distinct sexual survival strategies or individual feeding habits of the birds (Wobeser 2008). The Marielas colony showed more parasitism, which could be associated with the Bolivar Canal, where marine productivity is high and intermediate hosts (crustaceans) are likely abundant (Houvenaguel 1984). More research is needed to confirm this.

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