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Intestinal Parasites in Galapagos Sea Lions (*Zalophus wollebaeki*) Sivertsen, 1953 on San Cristóbal Island, Galapagos, Ecuador

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ABSTRACT: Knowledge regarding endoparasites of Galapagos sea lions, *Zalophus wollebaeki*, is limited to 1 report. Herein, we examined feces extracted from the lower gastrointestinal tract of 15 Galapagos sea lions plus 14 fecal mounds voided by Galapagos sea lions at 4 locations on San Cristobal Island, Galapagos in May and June of 2016. With the use of standard fecal flotation and sedimentation techniques, lungworm larvae suggestive of *Parafilaroides* and *Otostrongylus* sp., eggs of pseudophyllidea cestodes and anisakid nematodes, and coccidian oocysts were collected from study samples. This is the first report of potential lungworm larvae, anisakids, pseudophyllidean cestodes, and coccidian parasites in Galapagos sea lions and demonstrates the importance of fecal survey techniques in describing patterns of parasitism in endangered or protected host populations.

The Galapagos archipelago harbors a variety of endemic plants and animals. Galapagos sea lions, *Zalophus wollebaeki* Sivertsen, 1953 inhabit the islands, and information regarding these endangered (IUCN, 2017) animals, including parasite studies, is limited. Marine mammals are infected by a variety of parasites (Dailey, 2001), and a majority of the parasitic infections include respiratory and gastrointestinal helminths. These parasites are capable of causing severe disease (Jacobus et al., 2016). In pinnipeds, pneumonia can develop from lungworm infections, and its severity is often dependent upon parasite species, intensity, and the overall health of the host (Gulland and Beckman, 1997; Jacobus et al., 2016). There have also been associations between anisakid nematodes and gastric lesions in pinnipeds (Young and Lowe, 1969). The common endoparasites of Galapagos sea lions have not been documented. The aim of this study was to assemble a preliminary register of endoparasites in Galapagos sea lions on San Cristobal Island, Galapagos.

During May and June 2016, fecal samples were digitally collected from the rectums of 15 Galapagos sea lions at 4 locations on San Cristobal Island: Playa de los Marinos: 0°54′04″S 89°36′43″W (n = 7); Playa Punta Carola: 0°53′25″S 89°36′44″W (n = 1); Playa Mann: 0°53′48″S 89°36′30″W (n = 4); and Punta Pitt: 0°43′01″S 89°14′47″W (n = 3). In addition, 14 environmental fecal samples were opportunistically collected from 3 of the 4 locations: Playa de los Marinos (n = 2); Playa Punta Carola (n = 4); and Punta Pitt (n = 8). Fecal samples were evaluated by centrifugal flotation using Sheather's sugar (sp. 1.25), and simple sedimentation techniques (Zajac and Conboy, 2012). Approximately 1 g of feces was used for each diagnostic procedure. When minimal feces were available, only a sedimentation was performed to recover as many parasite stages (eggs, larvae, oocysts) as possible. Of Galapagos sea lion fecal samples collected directly from recta, 5 provided sufficient feces for both fecal flotation with centrifuge and fecal sedimentation, and 10 had enough feces for fecal sedimentation only (Table I). Diagnostic stages of parasites were identified based on morphological characteristics with the use of published keys and descriptions (Delyamure, 1968; Bergeron et al., 1997; Gosselin and Measures, 1997) with a compound microscope at a total magnification of ×400.

Parasite stages were collected from Galapagos sea lions at all 4 locations (Table I). Nematode larvae measuring 240–401.8 μm long and 12–16.8 μm wide were collected from the feces of 3 sea lions at Playa de los Marinos, 1 sea lion at Punta Carola, 1 sea lion at Playa Mann, and 1 sea lion at Punta Pitt (Figs. 1, 2). One larva had a bent, pointed posterior; the others had a pointed posterior with no obvious bend or curve. Ascaridoid eggs were collected from 2 sea lions at Playa de los Marinos, 1 sea lion at Punta Carola, 2 sea lions at Playa Mann, and 3 sea lions at Punta Pitt (Fig. 3). These eggs had a thick shell, were mostly spherical, and measured 48–67.2 μm × 40.8–67.2 μm. Elliptical nematode eggs with a thin shell and containing a multicelled morula were collected in the feces of 1 Galapagos sea lion from Playa Mann (Fig. 4). These eggs measured 40.8 × 50.4 μm. Operculate cestode eggs were collected from 1 sea lion sample at Punta Pitt. These eggs were 52.8 × 43.2 μm. A single sea lion sample from Playa de los Marinos contained coccidian oocysts (Fig. 5). Oocysts were spherical, nonsporulated, and measured 16.8–19.2 μm. Of the 14 environmental fecal samples examined, pseudophyllidean eggs were collected from 2 samples collected from Punta Pitt and 1 from Playa de los Marinos and measured 57.6 × 40.8 μm (Fig. 6), and nematode larvae collected in 1 sample at Playa de los Marinos that measured 240–360 μm in length.

To date, limited reports of parasites infecting and infesting Galapagos sea lions are available. Dailey et al. (2005) collected the trematode *Philophthalmus zalophi* from the eye of Galapagos sea lions, as well as 2 ectoparasites, *Antarctophthirus microchir* (Anoplura: Echinophthiriidae) and *Orthohalarachne diminuata* (Acarina: Halarachnidae). In this study, larvae morphologically consistent with those of *Parafilaroides* sp. or *Otostrongylus* sp. lungworms were found at each location. First-stage larvae of *Parafilaroides gymnurus* has been reported to have a pointed tail bent slightly dorsally, 220–304 μm long and 11–19 μm wide (Gosselin and Measures, 1997). Size of first and second stage

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TABLE I. Parasite taxa collected from feces collected rectally from *Zalophus wollebaeki* at 4 locations on San Cristobal Island, Galapagos.

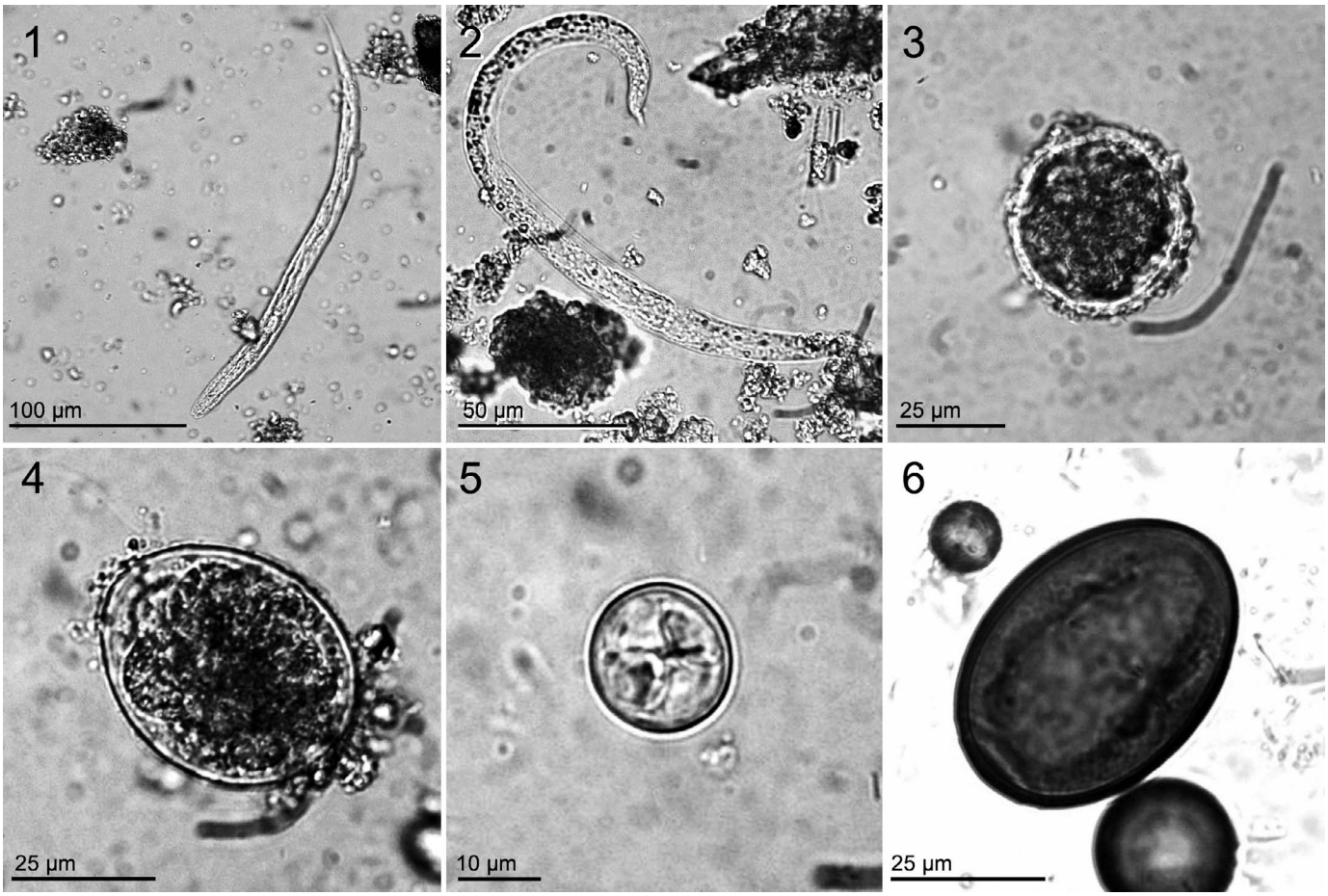
Location	ID	Sex (M/F)	Age class	Parasite stage	Diagnostic test*	Characteristics of parasite stages collected
Playa de los Marinos	1	M	Juvenile	–	Sed/CFlt	–
	2	F	Juvenile	–	Sed	–
	3	F	Juvenile	Nematode larva	Sed/CFlt	Pointed posterior, 264 μm long and 14.4 μm wide
	4	F	Juvenile	Nematode larva	Sed/CFlt	Pointed posterior, 264 μm long and 16 μm wide
				Nematode egg		Thick shell, spherical, 57.6 μm Anisakidae sp.
				Oocyst		Smooth shell, 16.8–19.2 μm Coccidia sp.
	5	F	Juvenile	–	Sed	–
	6	M	Juvenile	–	Sed	–
	7	M	Juvenile	Nematode larva	Sed	Bent, pointed posterior, 264 μm long and 12 μm wide
				Nematode egg		Thick shell, spherical, 48 μm Anisakidae sp.
Punta Carola	1	F	Juvenile	Nematode larva	Sed/CFlt	Pointed posterior, 264 μm long and 16.8 μm wide
				Nematode egg		Thick shell, 48 μm long and 40.8–45.6 μm wide Anisakidae sp.
Playa Mann	1	M	Juvenile	–	Sed/CFlt	–
	2	M	Juvenile	Nematode larva	Sed	Pointed posterior, 401.8 μm long
	3	M	Juvenile	Nematode egg	Sed	Thick shell, spherical, 48 μm Anisakidae sp.
				Nematode egg		Thin shell, cellular morula, 50.4 μm long and 40.8 μm wide, unknown
	4	M	Juvenile	Nematode egg	Sed	Thick shell, spherical, 48 μm Anisakidae sp.
Punta Pitt	1	M	Juvenile	Cestode egg	Sed	Operculated, 52.8 μm long and 43.2 μm wide, Pseudophyllidea sp.
				Nematode larva		Bent, pointed posterior, 240 μm
				Nematode egg		Thick shell, spherical, 67.2 μm, Anisakidae sp.
	2	F	Adult	Nematode egg	Sed	Thick shell, spherical, 57 μm Anisakidae sp.
	3	F	Adult	Nematode egg	Sed	Thick shell, 57.6 μm long and 36.8 μm wide Anisakidae sp.

* Sed = fecal sedimentation; CFlt = fecal flotation with centrifuge.

larvae of *Otostrongylus circumlitus* have been reported to range from 211 to 469 μm long and from 15 to 27 μm wide (Delyamure, 1968; Bergeron et al., 1997). Infections with these nematodes have been associated with pneumonia in other sea lion species and nematodes consistent with these species were detected histologically in South American Sea Lions (*Otaria flavescens*) in Peru (Gonzales-Viera et al., 2011). Although there are no documented reports of these parasitic nematodes in Galapagos sea lions, *Parafilaroides* sp. have been documented in Steller sea lions (*Eumetopias jubata*) and California sea lions (*Zalophus californianus*) (Dougherty, 1947). *Otostrongylus* sp. larvae have also been reported in the feces of *O. flavescens* (Hermosilla et al., 2016). In a previous study, the prevalence of *Parafilaroides decorus* reached 94% in 993 yearling California sea lions stranded and diagnosed with undernutrition (Greig et al., 2005). *Parafilaroides gymnurus* and *O. circumlitus* are common lungworms of many seal species, where they may cause significant respiratory disease and promote secondary bacterial infections (Stroud and Dailey, 1978; Bergeron et al., 1997; Gosselin and Measures, 1997; Gosselin et al., 1998).

Ascaridoid eggs consistent with those of Anisakidae species were collected from fecal samples from all 4 locations. In a study identifying intestinal helminths of *O. flavescens* from Argentina, the most common anisakids included *Contracaecum ogmorhini*, *Pseudoterranova cattani*, and *Anisakis* sp. (Hernández-Orts et al., 2013). *Contracaecum ogmorhini* sensu lato was reported in *Z. californianus* in the Pacific waters of Mexico and California (Fagerholm and Gibson, 1987); however, through genetic analysis, *C. ogmorhini* sensu lato appears to be a complex comprised of *C. ogmorhini* sensu stricto and *Contracaecum margolisi* (Mattiucci et al., 2003) with *C. margolisi* found in *Z. californianus*. *Pseudoterranova* sp. have been found in *Z. californianus* from the north Pacific (Nadler et al., 2005) and *Anisakis* sp. have been found in the gastrointestinal tract of Steller sea lions, *E. jubata*, from the Oregon coast (Stroud and Dailey, 1978). The single sample containing thin-shelled eggs with central morula collected from a Galapagos sea lion from Playa Mann are suggestive of strongyloid species or similar (Delyamure, 1968; Dailey, 2001).

Occasional cestode eggs, consistent with those of pseudophyllidea species, and coccidian oocysts were collected from Galapagos sea lions or environmental samples. Cestode eggs (Hermosilla et al., 2016) or adults (Hernández-Orts et al., 2013) of *Diphyllobothrium* sp. have been reported from *O. flavescens*. *Adenocephalus* spp. are also common in pinnipeds (Hernández-Orts et al., 2015) and species of both genera have zoonotic potential, as do many anisakid species (Sakanari and McKerrow, 1989). The coccidian oocysts were collected from a single sample from Playa de los Marinos and were 16.8 to 19.2 μm in diameter. Girard et al. (2016) identified coccidian oocysts of unknown species, genetically similar to *Neospora caninum*, in the feces of *Z. californianus*; however, the sizes were markedly smaller than ours, ranging from 8 to 10 μm in diameter for nonsporulated oocysts. Coccidian parasites are known in some pinniped species; however, some authors suggest these coccidians may be spurious parasites passing through the intestine as a result of feeding on infected fish (Dailey, 2001).



FIGURES 1–6. Parasites collected from the feces of the Galapagos sea lion, *Zalophus wollebaeki*. (**1**) Nematode larva from Punta Carola. Note pointed posterior with no obvious bend or curve, 264 × 16.8 µm. (**2**) Nematode larva from Playa de los Marinos. Note pointed posterior with a slight bend, 264 × 12 µm. (**3**) Nematode egg from Punta Carola. Note thick shell and generally spherical shape, 40.8 × 48 µm. (**4**) Nematode egg collected from Playa Mann. Note thin shell and central cellular morula, 40.8 × 50.4 µm. (**5**) Coccidian oocyst collected from Playa de los Marinos. This oocyst was nonsporulated, spherical, and measured 19.2 µm. (**6**) Cestode egg collected from environmental fecal sample from Playa de los Marinos. This is a dark brown, operculate egg, 40.8 × 57.6 µm.

This is the first survey of the endoparasitic fauna of Galapagos sea lions in their free-ranging environment, and thus the first report of potential lungworm larvae, anisakids, pseudophyllidean cestodes, and coccidian infections in these endangered hosts. For many protected species, it has become progressively more difficult to conduct investigations based on parasites acquired post-mortem from hosts. This work demonstrates the value of fecal survey as a source of important information describing patterns of parasitism in protected hosts. For endangered and protected host populations, higher-order identification of host–parasite relationships using eggs and larvae from fecal samples may be the best taxonomic resolution possible without opportune access to a chance carcass of random morbidity. It would be preferable to identify parasites to genus or species; this is usually not possible as the diagnostic stages recovered from fecal samples have not been consistently matched to their corresponding adults in the literature. Thus the report presented here constitutes a preliminary survey of the endoparasitic fauna of Galapagos sea lions and lays the groundwork for future opportunistic studies that may collect adult parasites and verify the actual species present in Galapagos sea lions.

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