

Internal parasites of the two subspecies of the West Indian manatee *Trichechus manatus*

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ABSTRACT: The West Indian manatee *Trichechus manatus* is divided into 2 subspecies: the Antillean (*T. m. manatus*) and Florida (*T. m. latirostris*) manatees. This study reports sample prevalence of manatee parasites from populations of these 2 subspecies in different geographical locations. Although necropsy is a valuable diagnostic tool for parasite infections, the need for antemortem diagnostic techniques is important. Fecal samples collected during necropsies of Antillean manatees (n = 3) in Puerto Rico and Florida manatees (n = 10) in Crystal River, Florida, as well as from live-captured Florida manatees (n = 11) were evaluated using centrifugal flotation with sucrose and ethyl acetate sedimentation to compare parasites from each of the populations. Although both fecal examination methods provided similar results, the centrifugal flotation method required less time for diagnosis. The most common parasite eggs found in both populations included the trematodes *Pulmonicola cochleotrema* and *Nudacotyle undicola*, oocysts of the coccidian *Eimeria* spp., and eggs of the ascarid *Heterocheilus tunicatus*. Eggs of the trematode *Chiorchis groschafti* were found in both populations of manatees; however, eggs of a related species, *Chiorchis fabaceus*, were abundant in the Florida samples, but not found in Puerto Rico populations. Trematode eggs of *Moniligerum blairi* were found in both populations, but were more common in the Florida manatee (42%) than the Antillean manatee (33%). To our knowledge, this is the first report of both *Eimeria manatus* and *Eimeria nodulosa* oocysts in Antillean manatees from Puerto Rico.

KEY WORDS: Parasites · *Trichechus* · Trematode · *Heterocheilus* · *Eimeria* · *Chiorchis*

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INTRODUCTION

The West Indian manatee *Trichechus manatus* has 2 recognized subspecies: the Florida manatee *T. m. latirostris* and the Antillean manatee *T. m. manatus*; however, arguments for 3 subspecies have been proposed based on mitochondrial DNA studies of the different West Indian manatee populations in Brazil (Barros et al. 2017). Gene flow is restrictive between the 2 studied populations (Hunter et al. 2012), and

suggests little or no intermingling; however, most populations seem to share the same parasites. Feeding behavior and diet differ between the 2 subspecies in that the Florida manatees in Crystal River feed primarily on freshwater vegetation that is floating or submerged, while Antillean manatees mainly feed on sea grasses from the ocean floor (Alves-Stanley et al. 2010). Parasites of terrestrial mammals are typically transmitted via fecal contamination of food or water sources or predation. Since manatees

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are herbivores, ingestion of parasite infective stages contained in fecal material (or freed from fecal material and loose in the water column), parasite stages encysted on aquatic vegetation, or the snail intermediate host (IH; trematodes) are suspected to be the likely modes of transmission for most manatee endoparasites (Stunkard 1929, Beck & Forrester 1988). Additionally, the bipolar filaments found on multiple manatee trematode eggs could facilitate egg attachment to vegetation to await ingestion by grazers.

In general, host species, age, and immune status can affect the virulence of parasites. Furthermore, factors such as parasite species and behavior, infection intensity, and metabolism can also complicate pathogenicity. Most cases of parasitism in marine mammals are considered non-pathogenic, but in some cases clinical signs can be observed, and some parasites can be fatal to their host (O'Shea et al. 1991, Miller et al. 2001a,b, Dubey et al. 2003). For example, one study reported that the death of a Florida manatee was due to verminous pneumonia caused by a substantial concentration of *Pulmonicola cochleotrema* (Buergelt et al. 1984). Although the West Indian manatee was reclassified in 2017 as threatened through the Endangered Species Act in the USA (US Fish & Wildlife Service 2017), protection for these animals is still in effect, and the IUCN Red List still has both subspecies listed as Endangered. Given their protected status, it is especially important to understand all aspects of mortality due to parasitism in these animals (IUCN 2008a,b, US Fish & Wildlife Service 2017).

Chiorchis fabaceus was the first intestinal trematode reported in manatees in 1838 (Price 1932); the closely related *Chiorchis groschafti* was not recognized until much later (Coy-Otero 1989). Other trematodes reported in manatees include *Pulmonicola cochleotrema*, which infects the lungs/nasal passages, and the intestinal trematodes *Moniligerum blairi* and *Nudacotyle undicola* (Beck & Forrester 1988, Dailey et al. 1988). The ascarid *Heterocheilus tunicatus* was first described in the Antillean manatee by Khalil & Vogelsang (1932). Although most parasite diagnoses in manatees take place during necropsies, antemortem diagnosis has made it easier to detect coccidian species and the helminths listed above by centrifugal flotation of fresh feces. Bando et al. (2014) conducted the first comprehensive study on the diagnostic stages of parasites of the Florida manatee, and their study is 1 of only 4 studies that have examined feces for parasite diagnosis (Lainson et al. 1983, Upton et al. 1989, Borges et al. 2011, Bando et al. 2014). Upton et al. (1989) discovered 2 new coccidian spe-

cies, *Eimeria manatus* and *E. nodulosa*, by fecal examination in the Florida manatee.

Due to the difficulties involved in studying aquatic organisms, most of these parasite life cycles are unknown, and most of what is known has been derived from knowledge of terrestrial parasite life cycles. This makes epidemiology and the geographical reach of the parasite difficult to determine. In this study, we compared the parasites present in fecal samples from 2 subspecies of manatees and report parasite prevalence in the samples tested.

MATERIALS AND METHODS

Collections

Opportunistic fecal samples were collected from necropsied Antillean manatees (n = 3) at the Puerto Rico Manatee Conservation Center (PRMCC) and necropsied adult (n = 1), juvenile (n = 6), and calf (n = 3) Florida manatees at the Marine Mammal Pathobiology Laboratory (MMPL). Adult parasite specimens from 2 of the necropsied Florida manatees were also collected. Designation of age classes for Antillean manatees are as described by Mignucci-Giannoni et al. (2000). Fecal samples were shipped on ice and refrigerated at 4°C until examination. Storage times before processing ranged from 1 d to 3 mo. Adult nematodes were stored in either 70% ethanol or alcohol glycerin until identification. Adult trematodes were stored in either 70% ethanol or alcohol formalin acetic acid (AFA) until staining using the procedure described by Pritchard & Kruse (1982) for staining of trematodes.

During live-capture health assessments completed in the winter in Crystal River, Florida, by the US Geological Survey (USGS), manatees of all age classes were captured, and defecated feces (n = 11) were collected during beach examination (Bonde et al. 2012, Merson et al. 2014).

Testing

Parasite screening procedures

Centrifugal flotation with Sheather's sucrose (sp. gravity = 1.275) was used for parasite screening of fecal samples (Bowman 2014). Fecal examination by sedimentation using 10% formalin and ethyl acetate was used for the recovery of the heavier trematode and acanthocephalan eggs (Bowman 2014).

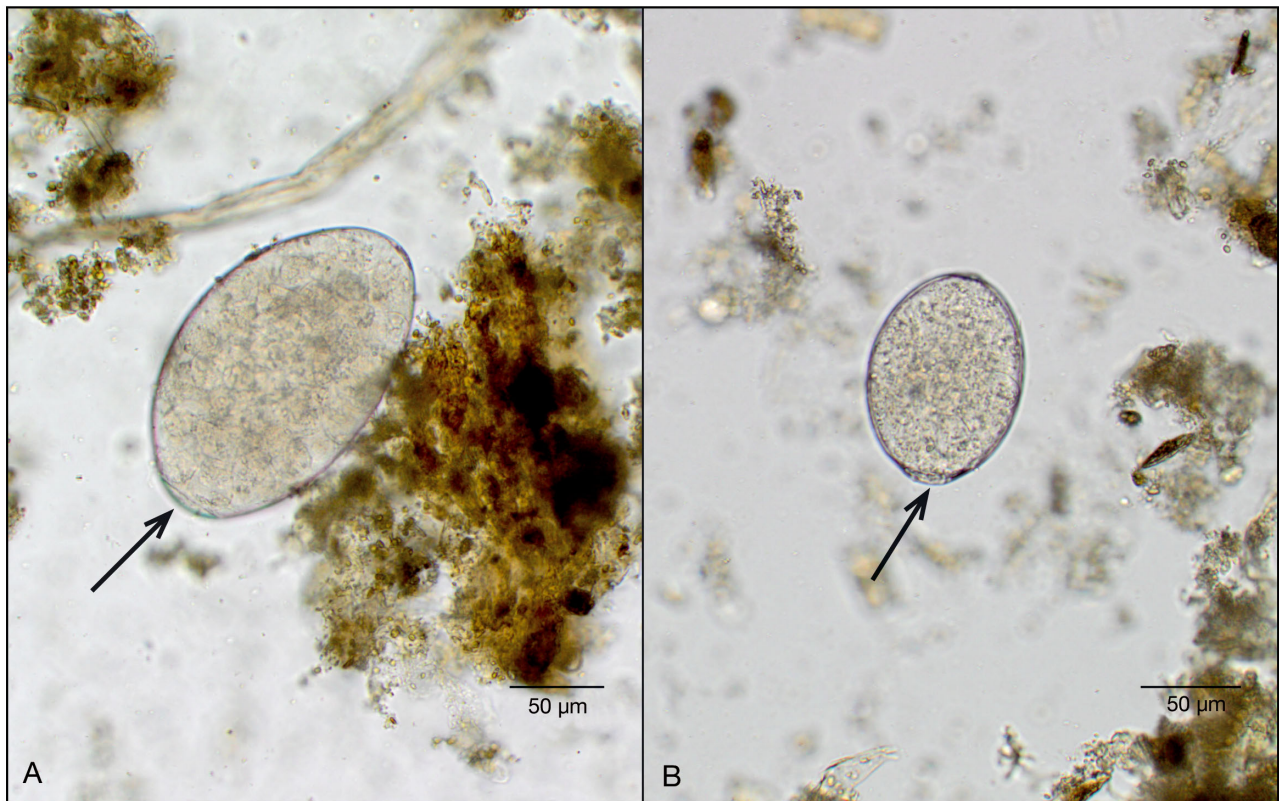


Fig. 1. Trematode parasite eggs from the Florida manatee *Trichechus manatus latirostris*. (A) *Chiorchis fabaceus* egg (168 × 114 µm) from live adult male; (B) *C. groschafti* egg (110 × 78 µm) from live adult male. Arrows: large opercula

Parasite identification

Parasites detected in fecal examinations were identified using Bando et al. (2014) for Florida manatees. *Chiorchis fabaceus* and *C. groschafti* were distinguishable based on egg size (Fig. 1). *Pulmonicola*

cochleotrema eggs were distinguished from eggs of *Moniligerum blairi* and *Nudacotyle undicola* by the appreciable difference in the base widths of the bipolar filaments (Fig. 2A); *N. undicola* (Fig. 2B) and *M. blairi* (Fig. 2C) were distinguishable by capsule lengths. A miracidium could also be seen in some *M.*

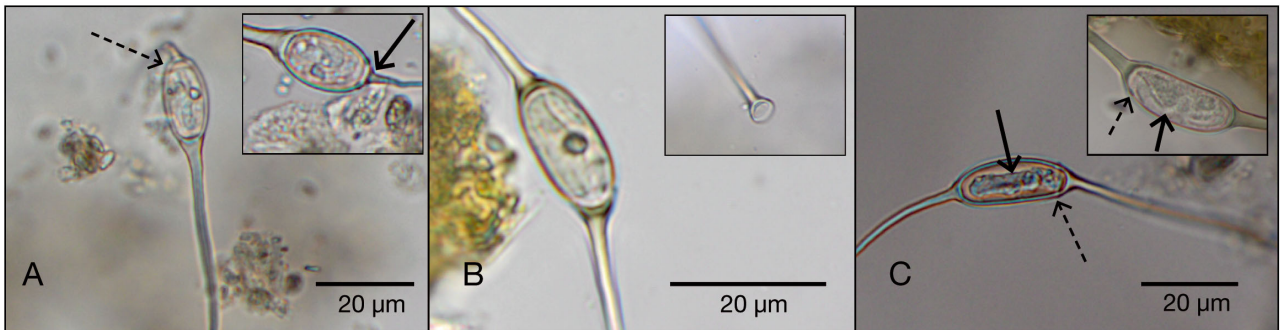


Fig. 2. Trematode eggs with bipolar filaments from the Florida manatee *Trichechus manatus latirostris*. (A) *Pulmonicola cochleotrema* eggs; operculum (dashed arrow) on end with smaller filament base from a live juvenile/subadult male. Inset: uneven base of bipolar filaments visible on higher magnification from a dead adult male; smaller base width ~1 µm (solid arrow), larger base width ~4 µm, capsule length ~18 µm. (B) *Nudacotyle undicola* eggs from a live adult male. Base widths of bipolar filaments averaging 3.5 µm, capsule length average = 18.5 µm, miracidium beginning to form. Inset: single filament broken off capsule often noted from a live male calf. (C) *Moniligerum blairi* eggs from dead female calf. Base widths of bipolar filaments are equal, averaging 3.5 µm, capsule length average = 22 µm. Inset: miracidia (solid arrows) and opercula (dashed arrow) visible on higher magnification

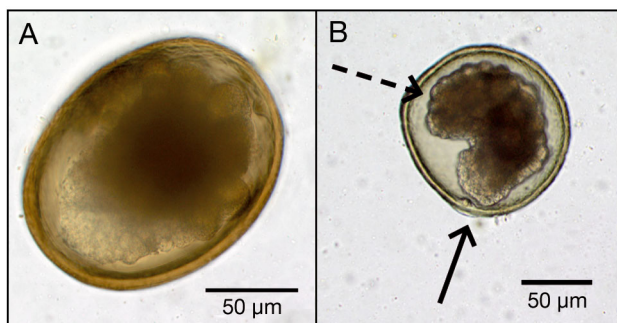


Fig. 3. (A) *Heterocheilus tunicatus* eggs ($163 \times 140 \mu\text{m}$) from a live adult male Florida manatee *Trichechus manatus latirostris*. (B) Atypical characteristics of *Heterocheilus tunicatus* eggs from live adult female Florida manatee. Solid arrow: clear button; dashed arrow: larval development within egg

blairi eggs (Fig. 2C). Eggs of *Heterocheilus tunicatus* were identified by their dark brown color and large size with a thick shell and internal morula (Fig. 3). *Eimeria* spp. (Fig. 4) were distinguishable by size, and by the presence or absence of characteristic nodules on the wall surface of *E. nodulosa* (Fig. 4A).

Identification of adult parasites was made using morphological keys and original descriptions assisted by host species and location (Yorke & Maplestone 1962, Jones et al. 2005). An Olympus BH2 microscope and a DP25 camera were used for parasite imaging.

Statistical analyses

Analysis of variance (ANOVA) was used to examine the significance of age class (calf, juvenile/subadult, adult) and the number of parasites detected

in the samples from Florida manatees using SAS v.9.4. Fisher's exact test was used to determine if the infection of parasite species was influenced by the age class or sex of the manatees. Logistic regression was used for multiple pairwise comparisons. Data met the assumptions of Shapiro-Wilks test for normality and Levene's test for homogeneity of variance. An alpha value of 0.05 was used for all statistical analyses.

RESULTS

All manatee fecal samples (100%) had at least 2 parasite species detected, and all samples except one contained the coccidian *Eimeria manatus* (Table 1). Eggs of *Pulmonicola cochleotrema*, *Nudacotyle undicola*, *Chiorchis groschafti*, and oocysts of *E. manatus* were found in all (100%; $n = 3$) Antillean manatee samples (Table 1). This is the first report of oocysts consistent in size and morphology with *E. manatus* and *E. nodulosa* (Fig. 4) in Antillean manatees from Puerto Rico. Several coccidian oocysts that were similar, but inconsistent with size and morphology of reported *Eimeria* spp. or *Cryptosporidium* spp. in manatees, were also observed in the Florida and Antillean manatee populations. Sizes of these oocysts ranged from $6 \mu\text{m}$ to as large as $20 \mu\text{m}$. Adult nematodes recovered from the 2 Florida manatees at necropsy were morphologically identical to *Heterocheilus tunicatus* (Yorke & Maplestone 1962). Trematodes recovered from the nasal cavity of these animals were identified as *P. cochleotrema* (Carvalho et al. 2009). The colon trematodes were more consistent

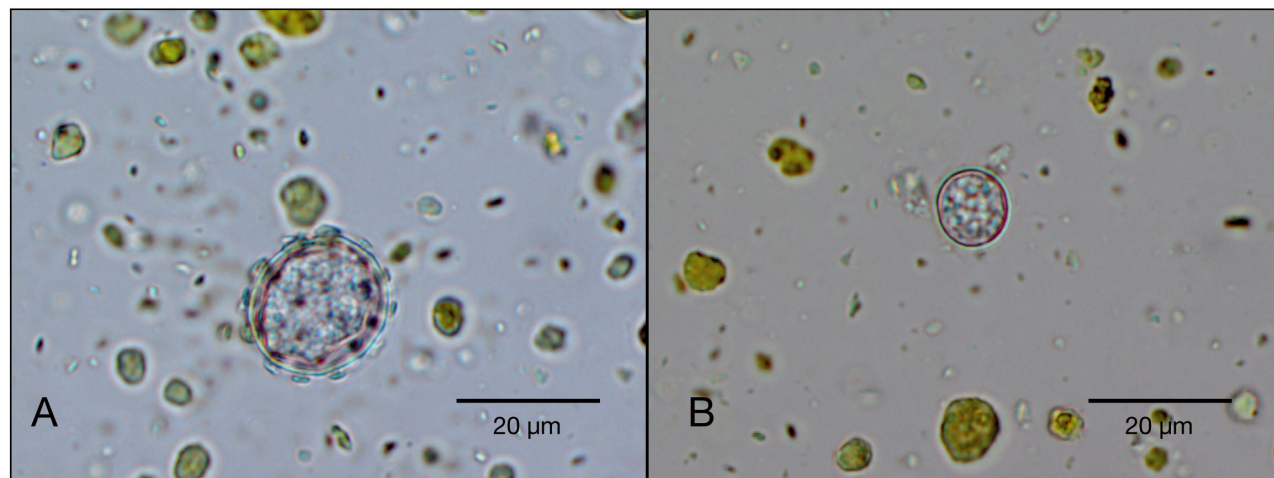


Fig. 4. Protozoa from the Florida manatee *Trichechus manatus latirostris*. (A) *Eimeria nodulosa* oocyst with mushroom-like nodules covering wall from a live adult male (diameter $\sim 15 \mu\text{m}$); (B) *Eimeria manatus* oocyst with smooth wall from a live adult male (diameter range 8 to $10 \mu\text{m}$)

Table 1. Parasite prevalence in the Florida manatee *Trichechus manatus latirostris* and the Puerto Rico populations of the Antillean manatees *Trichechus manatus manatus* in 2016 compared to data from Bando et al. (2014)

Parasite	Prevalence (%)					Total (n = 24)	Florida (Bando et al. 2014)
	Calf (n = 4)	Florida manatee Juvenile (n = 10)	Adult (n = 7)	Total (n = 21)	Antillean manatee (n = 3)		
Trematoda							
<i>Pulmonicola cochleotrema</i>	50	80	43	62	100	67	14
<i>Moniligerum blairi</i>	75	50	14	43	33.3	42	19
<i>Nudacotyle undicola</i>	50	80	43	62	100	67	29
<i>Chiorchis fabaceus</i>	25	70	71	62	0	54	43
<i>Chiorchis groschafti</i>	25	10	71	33	100	42	33
Nematoda							
<i>Heterocheilus tunicatus</i>	0	40	100	52	67	54	38
Protozoa							
<i>Eimeria manatus</i>	75	100	100	95	100	96	83
<i>Eimeria nodulosa</i>	25	60	57	38	33.3	37.5	50

with descriptions of *C. groschafti* (Coy-Otero 1989, E. Greiner pers. comm.) than *C. fabaceus*.

Variation in bipolar filament morphology in the trematode eggs and appreciable larval development within the ascarid eggs were observed in samples from both dead and live animals and with a process time of less than 1 d to as long as 3 mo (Figs. 3B & 5). A number of unidentified cysts were also detected

and are suspected to be amoebae (Fig. 6). Cysts collapsed in sugar solution, but intact cysts had 3 to 5 clearly visible nuclei (Fig. 6C,D).

In the current study, there was no significant difference in numbers of parasite species (co-infections) among manatee age classes (ANOVA; $F_{2,18} = 2.65$, $p = 0.098$); however, there was an age association with infection of 2 parasite species (Fisher's exact

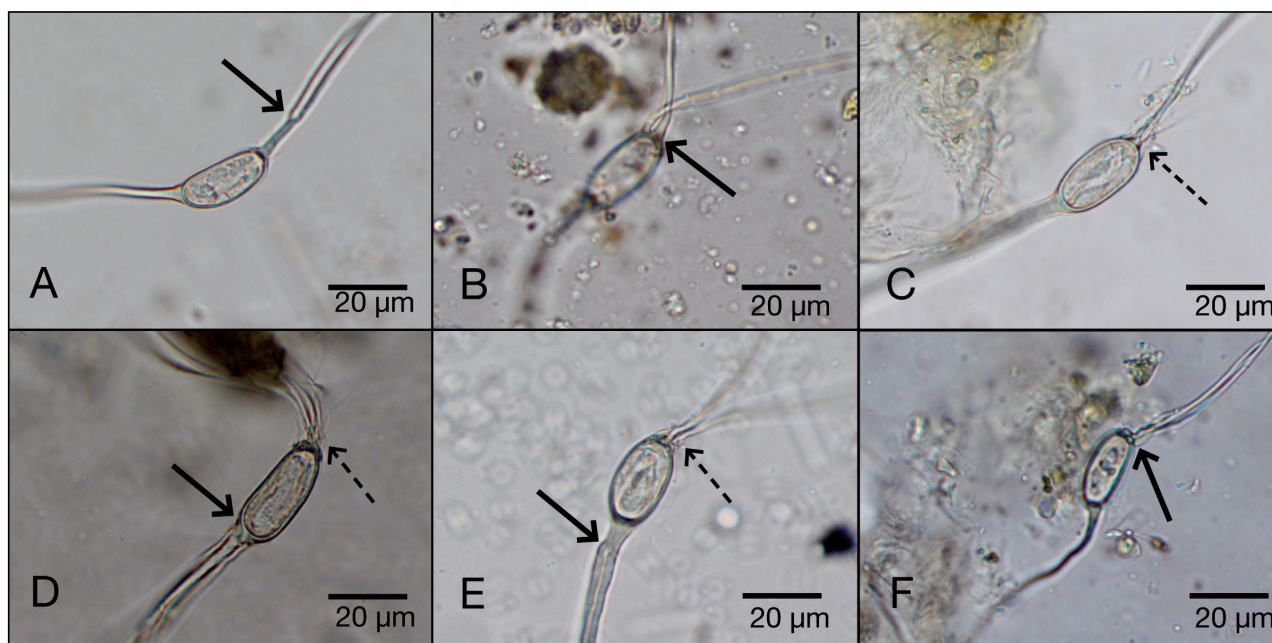


Fig. 5. Atypical characteristics of trematode eggs from the Florida manatee *Trichechus manatus latirostris*. (A) Trematode egg with split filament (solid arrow), capsule = 22.47 µm. (B) Trematode egg with extra polar filament on one end (solid arrow) from dead female calf, capsule = 18 µm. (C) Trematode egg with several small hair-like projections (dashed arrow), capsule = 17 µm. (D) Trematode egg with 3 filaments on one end (solid arrow) of capsule and small hair-like projections on the other end (dashed arrow), capsule = 19 µm. (E) Trematode egg with split filament (solid arrow) on one end of capsule and an extra filament and small hair-like projections on the other end (dashed arrow), capsule = 22.99 µm. (F) Trematode egg with multiple filaments on one end (solid arrow), capsule = 20.9 µm. (A) and (C–E) from same dead female calf

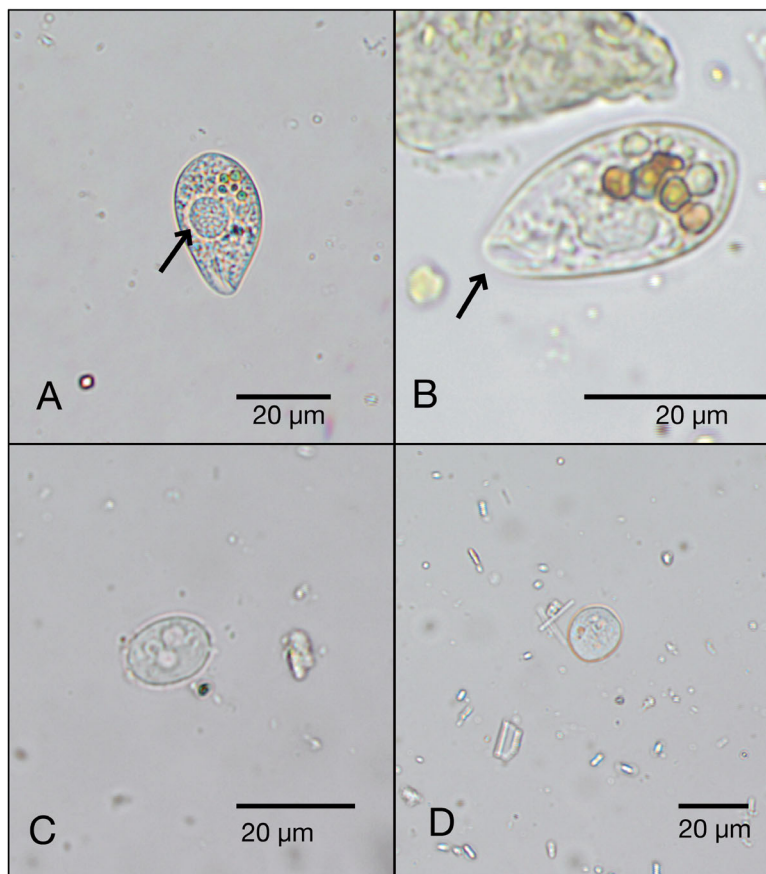


Fig. 6. Unidentified cysts from the feces of the Florida manatee *Trichechus manatus latirostris*. (A) Ameoba-like cyst (length = 31.5 µm) with large nucleus (arrow). (B) Ameoba-like cyst from (A) with fold (arrow) easily visible on higher magnification. (C) & (D) Small cysts on higher magnification with 3 nuclei clearly visible in each. All pictures from live adult males

test). According to Fisher's exact test with logistic regression, adult manatees had significantly higher prevalence of *C. groschafti* (71.4%) than juvenile/subadults (10%; $p = 0.021$) and adult manatees also had significantly higher prevalence of *H. tunicatus* (100%) than calves (0%; $p = 0.024$). Age class, sex, or status (dead/alive) were not significant in determining infection status.

DISCUSSION

In Florida manatees, our study found higher egg and oocyst prevalence of *Chiorchis fabaceus*, *Pulmonicola cochleotrema*, *Moniligerum blairi*, *Nudacotyle undicola*, *Heterocheilus tunicatus*, and *Eimeria manatus* than previously reported by Bando et al. (2014); however, our study found the same prevalence of *Chiorchis groschafti* (Table 1). Our small sample size and the lack of previous publications on parasite

prevalence in Antillean manatees from Puerto Rico make it impossible to derive firm conclusions for the parasite results from these individuals.

Colón-Llavina et al. (2009) corrected previous reports of *C. fabaceus* in the Antillean manatee by Mignucci-Giannoni et al. (1999a,b) to *C. groschafti* after a reclassification by Mora-Pinto (2000). Mora-Pinto (2000) described 2 distinct *Chiorchis* spp. from each manatee subspecies; however, it appears that although *C. fabaceus* has never been described in the Antillean manatee (post correction), both manatee subspecies share *C. groschafti* (Bando et al. 2014). Our results are consistent with the corrected reports. The restriction of *C. fabaceus* to Florida manatees suggests a lack of the proper snail or mollusk intermediate host in the Antillean manatee's habitat to perpetuate the trematode's life cycle; however, the subspecies share the closely related trematode *C. groschafti*, which may use the same intermediate host species of snail or mollusk as *C. fabaceus*.

Although no *Cryptosporidium* spp. oocysts were detected using these methods, this could be an underestimate of this parasite in our sample population given the difficulty of detection. The high sample prevalence of both *Eimeria* spp. is significant given what we know

about the transmission of its terrestrial counterparts. *Eimeria* spp. in cattle, for instance, is best transmitted when animals are overcrowded and housing conditions are poor. Understanding the transmission of the *Eimeria* spp. in these aquatic herbivores requires knowledge of aggregation behavior. The high prevalence of the *Eimeria* spp. suggests that oocyst contamination is high in aggregation areas, such as warm water sites, and maximizes transmission during these brief periods of accumulation.

Similarly, there are several possible explanations for how these 2 presumably divided manatee populations could share parasite species. Rostral deflection and dental adaptations have provided unique insight into manatee evolution and distribution (Domning 1982, Domning & Hayek 1984). Significant differences in adaptations to temperatures and feeding behaviors suggest that temperature, water depth, and the strength of water currents effectively segregate the 2 West Indian manatee subspecies (Dom-

ning & Hayek 1984), so perhaps the connection is evolutionary. Although these divisions in genetic variation suggest complete segregation, it is likely that the 2 subspecies cross-migrated or filled niches previously utilized by other manatee subspecies prior to recent isolation events. A parasite's ability to utilize these host habitats and ecosystems to await the next host could facilitate parasite transmission to presumably disconnected host populations.

Many organisms found in the fecal flotations from the Florida manatees were inconsistent with any previously reported descriptions (Figs. 3B & 5–7). A single egg consistent with the descriptions of *C. fabaceus* and *C. groschafti* except for size was found in one of the samples. The typical trematode eggs reported in manatees (*P. cochleotrema*, *N. undicola*, *M. blairi*) have 2 bipolar filaments, one on each end of the capsule; however, many trematode eggs were observed with more than one filament per capsule end (Fig. 5B,D,F), splits in one filament (Fig. 5A,E), or with many smaller hair-like projections attached at one end surrounding the filament (Fig. 5C,D,E). It is unknown if these are atypical mutations of known trematode eggs, stages of known species more developed than typically seen from fresh feces, or new species of trematode eggs not yet described. Since adult trematodes were not collected from the specific animals passing these unique eggs, new parasite species cannot be confirmed without more sampling and further testing. An atypical ascarid egg characteristic (Fig. 3B) is described as having an opercular region with a visible cap or button similar to the egg-shell structure reported in several other ascarid species (Sprent 1973, Ubelaker & Allison 1975, Wharton 1979, Kazacos & Turek 1983).



Fig. 7. Unidentified arthropod egg (160 × 128 μm) from the feces of the Florida manatee *Trichechus manatus latirostris*. Arrow shows appendages within egg shell

A few unidentified eggs with visible jointed legs on the internal organism were likely arthropod eggs (Fig. 7); however, it is unknown if this is a parasitic arthropod of the manatee or an incidental finding from the environment.

Little is known of parasites in manatees and their effect on the animal's overall health. Our results are consistent with other parasite reports in the 2 separate populations; however, this is the first report of *E. manatus* and *E. nodulosa* in the Antillean manatees from Puerto Rico. Research on aquatic parasite life cycles is vital to understanding the transmission of these organisms between individuals and subspecies populations. Further investigations could examine parasite transmission and pathogenicity in these animals. Given the large number of eggs, oocysts, cysts, and larvae that were not identified and the very few studies of manatee parasites from fecal examination, further research in this field is warranted.

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