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GASTROINTESTINAL PARASITES OF ECUADORIAN MANTLED HOWLER MONKEYS (*ALOUATTA PALLIATA AEQUATORIALIS*) BASED ON FECAL ANALYSIS

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ABSTRACT: An analysis of gastrointestinal parasites of Ecuadorian mantled howler monkeys, *Alouatta palliata aequatorialis*, was conducted based on examination of fecal smears, flotations, and sedimentations. At least 1 type of parasite was detected in 97% of the 96 fecal samples screened across 19 howler monkey groups using these techniques. Samples averaged 3.6 parasite species per individual (±1.4 SD). Parasites included species representing genera of 2 apicomplexans: *Cyclospora* sp. (18% of individual samples) and *Isospora* sp. (3%); 6 other protozoa: *Balantidium* sp. (9%), *Blastocystis* sp. (60%), *Chilomastix* sp. (4%), *Dientamoeba* sp. (3%), *Entamoeba* species (56%), *Iodamoeba* sp. (5%); 4 nematodes: *Enterobius* sp. (3%), *Capillaria* sp. (78%), *Strongyloides* spp. (88%) which included 2 morphotypes, *Trypanoxyuris* sp. (12%); and the platyhelminth *Controrchis* sp. (15%). A statistically significant positive correlation was found between group size and each of 3 different estimators of parasite species richness adjusted for sampling effort (ICE: $r^2 = 0.24$, $P = 0.05$; Chao2: $r^2 = 0.25$, $P = 0.05$, and Jackknife: $r^2 = 0.31$, $P = 0.03$). Two significant associations between co-infecting parasites were identified. Based on the prevalence data, individuals infected with *Balantidium* sp. were more likely to also be infected with *Isospora* sp. ($\chi^2 = 6.02$, $P = 0.01$), while individuals harboring *Chilomastix* sp. were less likely to have *Capillaria* sp. present ($\chi^2 = 4.03$, $P = 0.04$).

In the last decade, an increasing number of studies have focused on parasitism in wild primate populations, emanating mainly from an interest in zoonotic pathogen transmission (Muriuki et al., 1998; Pedersen et al., 2005; Howells et al., 2011), conserving host species (Wallis and Lee, 1999; Gillespie et al., 2005; Smith et al., 2008), and ecosystem health (Marcogliese, 2005). Many of the parasitic diseases in primates are zoonotic (Pedersen, 2005; Wolfe et al., 2007), and concern is growing that anthropogenic disturbances are changing the dynamics of these ecological systems (Morse, 1995; Patz et al., 2000; Daszak et al., 2001; Chapman et al., 2005; Puttker et al., 2008). A first step in understanding this relationship is examining the parasite community of a primate that is intricately associated with humans, such as howler monkeys (*Alouatta* spp.), and is often detrimentally affected by their actions.

The genus *Alouatta* consists of 6 species and 22 subspecies spanning most of Central and South America (Crockett, 1998). Five subspecies of *Alouatta palliata* are recognized, *Alouatta palliata palliata* found in Honduras, Nicaragua, Costa Rica, Panama, and El Salvador; *Alouatta palliata mexicana* found in southern Mexico and Guatemala; *Alouatta palliata aequatorialis* found in Panama, W. Colombia, W. Ecuador and N.W. Peru; and *Alouatta palliata trabeata* and *Alouatta palliata coibensis* found solely in Panama (Crockett, 1998; IUCN, 2012). *Alouatta* spp. are among the largest New World primates, living in groups averaging 14 individuals and ranging from 4–40 individuals per group (Rowe, 1996; IUCN, 2012). Howlers are arboreal, spending the vast majority of their time in the forest canopy, seasonal and non-seasonal forests, and in mangroves and swamps, providing key ecological services such as seed dispersal (Anzures-Dadda et al., 2011). They are largely folivorous, though it has also been shown that upwards of 40% of their diet is fruit during favorable seasons (Estrada et al., 1999; Pinto et al., 2003; Vitazkova, 2009). An indiscriminate diet is likely one of the main reasons that they are so adaptable to changing ecological landscapes, whether natural or anthropogenic.

The gastrointestinal parasite communities of several howler monkey species (*Alouatta* spp.) have been studied throughout Central and South America including *A. palliata* (Stuart et al., 1998; Trejo-Macias et al., 2007), *Alouatta pigra* (Eckert et al., 2006; Stoner and Gonzalez Di Pierro, 2006; Vitazkova and Wade, 2006; Trejo-Macias et al., 2007; Cristobal-Azkarate et al., 2010), *Alouatta caraya* (Stiles et al., 1929; Cruz et al., 2000), and *Alouatta seniculus* (Gilbert, 1994; Phillips et al., 2004). These howler monkey studies have found an array of gastrointestinal parasites including protozoans, nematodes, and platyhelminths (Pope, 1966; Stuart et al., 1990; Amato et al., 2002; Godoy et al., 2004; Phillips et al., 2004; Eckert et al., 2006; Vitazkova and Wade, 2007; Cristobal-Azkarate et al., 2010; Valdespino et al., 2010). A complete literature review, including non-gastrointestinal parasites, can be found in Stuart et al. (1998) and Vitazkova (2009).

Gastrointestinal parasite communities of mantled howler monkeys (*A. p. aequatorialis*) from Ecuador have yet to be described; therefore, the main objective of this study was to determine what parasites are found in this threatened howler monkey subspecies. These data will also be used in our long-term research project designed to study the effects of habitat disturbance (i.e., human proximity and basal area) on parasite communities in the mantled howler monkey and to assess zoonotic transmission of gastrointestinal parasites. We anticipate that a broad array of protozoans, nematodes, and platyhelminths will be recovered based on findings from other related howler monkey studies throughout Central and South America.

We also examined the relationship between group size and gastrointestinal parasite species richness because several studies have documented increased parasite species richness in larger groups (Freeland, 1979; Nunn et al., 2003). Individual parasitism is expected to increase with larger social groups due to higher densities and increased contact rate (Cote and Poulin, 1995; Arneberg et al., 1998; Nunn et al., 2003). Unfortunately, the effect may or may not be real, as some of these studies fail to account for sampling effort—larger groups are expected to have more parasites simply because more individuals are sampled. Modeling by Wilson et al. (2003) suggests that per capita risk of infection

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from directly transmitted parasites is actually decreased in larger groups, and Mbora et al. (2009) found smaller groups had higher parasite prevalence and species richness, though the results were not significant. Presumably, maintaining groups could be advantageous against the spread of parasitism if clustering of individuals reduced the likelihood of an infected individual entering a new group (Black, 1966; Freeland, 1976, 1979; Wilson et al., 2003). Conversely, larger group limitations include increased intra-group parasite transmission along with higher localized host density, increased stress levels, and larger travel requirements in search of food (Wrangham, 1980; Cote and Poulin, 1995; Sterck et al., 1997; Nunn and Dokey, 2006; Borries et al., 2008; Majolo et al., 2008). If gastrointestinal parasites infecting howler monkeys are transmitted directly or via fecal–oral route, then larger groups are expected to have more parasite species per individual.

Lastly, gastrointestinal parasite species have previously been shown to interact with each other both experimentally and in the wild (Poulin, 2001; Nacher, 2011). Two main relationships have been previously reported: competitive interactions where 1 parasite species outcompetes another, causing an inhibitory effect (Poulin, 2001); and where infection of a host by 1 parasite species increases host susceptibility to secondary infection (Murphy et al., 2013). We will investigate this phenomenon using fecal analysis to evaluate whether certain parasites co-occur or some occur in the absence of others.

MATERIALS AND METHODS

The Bilsa Biological Station, a private reserve of 3,300 hectares in northwestern Ecuador (35 km west of Quininde, 0°21′N, 79°44′W), is located in a pre-montane tropical forest along the Pacific coast at an altitude of 350–576 m (Fig. 1A, B). This area is part of the Tumbes-Choco-Magdalena bioregion and is currently under threat due to increased logging pressure, but it still maintains a mixture of disturbed and undisturbed forest (Ortega-Andrade et al., 2010). At Bilsa, 20% of the reserve is comprised of secondary forest, while only 4% of primary rainforest remains throughout the rest of northwest Ecuador (Ortega-Andrade et al., 2010). Annual precipitation ranges from 1,500–2,000 cm, making it one of the wettest areas in the world.

Field teams consisting of 2 to 5 people collected 96 fecal samples from 19 primate groups, plus 4 samples from solitary individuals, starting June until August 2010. Primate groups were sampled along 2 (5 km) transects at the Bilsa Biological Reserve (Fig. 1C). Howler monkey groups were found based on vocalization or visualizations while walking transects or by scent with the use of a dog. All primate groups found along each transect were sampled, and every effort was made to ensure that the same group wasn't re-sampled. In order to avoid re-sampling, we systematically worked our way down transects, noting the location of the last sampled group at night, then moving further down the transect to the next group the following morning. Knowing the location of the last sampled group ensured that individuals from that group would not be re-sampled with nearby groups the following day. It is possible that groups were missed, but our main purpose was to avoid repeat sampling. Although these transects were initially designed for use as part of a larger study to evaluate impacts of human proximity to primate groups, they also served to provide an objective protocol for detecting howler groups to include in the study. All individuals detected in a foraging group were sampled when feasible, though some individuals did not defecate or we could not locate a fecal sample. Three additional samples were collected away from our transects near the village of Dogola (Fig. 1C). Group size ranged from solitary to 10 individuals. Four individuals were solitary, 5 groups had 2–3 individuals, 7 groups had 4–5 individuals, 6 groups had 6–7 individuals, and 1 group had 10 individuals.

Fecal samples were immediately collected following defecation, and environmental contamination was minimized by only collecting the upper portion of the feces that hadn't contacted the ground and leaf litter. Location of each sample was recorded using GPS and howler monkey group demographics were noted. Contamination was minimized by wearing disposable gloves and collecting each sample with a new set of wooden tongue depressors to manipulate into a new set of the 3 (50 ml) tubes for every sample. Zinc polyvinyl alcohol (Zn-PVA) was used to preserve the feces for parasite recovery, RNAlater® (Qiagen Inc., Valencia, California) was used to preserve parasite DNA for *Blastocystis* spp. PCR analysis, and a third preservation solution of 50% ethanol was used for a separate study. All individuals within a group were sampled as logistically feasible. A description of each sample was recorded and included consistency of feces and presence of blood, mucus, and macroscopic parasites.

Fecal samples were examined for parasites at the Fish and Wildlife Disease Laboratory at State University of New York College of Environmental Science and Forestry (SUNY-ESF), Syracuse, New York for helminth eggs and larvae, and protozoan cysts were examined using trichrome stain on fecal smears, centrifugal flotations, and sedimentations (single slide each), as described by Garcia (1999) and Hendrix and Robinson (2006) with the following modifications. An $NaNO_3$ solution (SG 1.2) was used for optimal retrieval of parasite eggs in flotations (Hendrix and Robinson, 2006). Cover slips were placed on tubes for 10 min following centrifugation as opposed to before centrifugation. Flotations are optimal for retrieving nematode eggs and protozoan cysts, and sedimentations are optimal for obtaining trematodes which are too heavy to be retrieved from flotations (Hendrix and Robinson, 2006). We also used smears, which are useful for obtaining protozoan parasites (Garcia et al., 1993). One gram of Zn-PVA preserved sample was used in the fecal flotation and the remaining pellet was used in the sedimentation. Results from fecal smears, flotations, and sedimentation were combined to confirm presence or absence and are subsequently reported as a single value for all calculations. Slides were scanned at ×20 objective lens using a Nikon 80i compound microscope (Nikon Instruments, Melville, New York) with Nomarski and phase objectives. Images were captured at ×40 objective lens with a 3MP IDEA digital camera and analyzed with photomicrography software (Diagnostic Instruments, Inc., Spot RT Software 4.6, Sterling Heights, Michigan). Identification was based on size, shape, color, and interior structure. An index of individual parasite abundance was made by counting the number of parasite eggs or cysts on a single cover slide at ×20 magnification (22 × 22 mm) (Gillespie, 2006; Hendrix and Robinson, 2006).

A PCR-based method of detection was used to confirm the presence of *Blastocystis* spp. because it is a cryptic but common gastrointestinal parasite found in primates (Stensvold et al., 2009). In addition, generated molecular data are being used in a related study on *Blastocystis* spp. phylogenetics, so the data were included for completeness. For *Blastocystis* spp. PCR detection, DNA was extracted from approximately 200 mg of feces using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. *Blastocystis* spp. were confirmed using protocols and PCR primers BH1F/BHRDr and BLF/BLR and protocols described in Whipps et al. (2010) and Menounos et al. (2008), respectively.

Every attempt was made to ensure that duplicate samples were not collected from the same individual by attempting to identify specific monkeys prior to defecation and then collecting samples immediately. However, because it is possible that more than 1 sample may have been collected for some individuals, we consider our calculations to be an index of prevalence where an equal sampling bias exists across all individuals and groups (Chapman et al., 2011). Parasite species richness and prevalence were calculated across all samples and howler groups. In order to assess prevalence, we considered an individual positive for a parasite if it was found for any 1 fecal extraction method. Prevalence values are given with 95% confidence intervals which were calculated with the modified Wald method.

The relationship of parasite species richness to howler monkey group size was evaluated as follows. Larger howler groups may be expected to harbor more parasites simply because there are more samples collected (Gregory, 1990; Walther et al., 1995; Poulin, 1998; Walther and Martin, 2001; Colwell et al., 2004; Yurkov et al., 2011). Several methods have been proposed to control for the uneven sampling effort attributable to number of individual monkeys sampled per group. We controlled for larger sample sizes in bigger groups by using 3 non-parametric species richness estimators, available in the software program EstimateS (Colwell, 2009), that adjust for different sampling effort including Chao 2 (Chao, 1987),

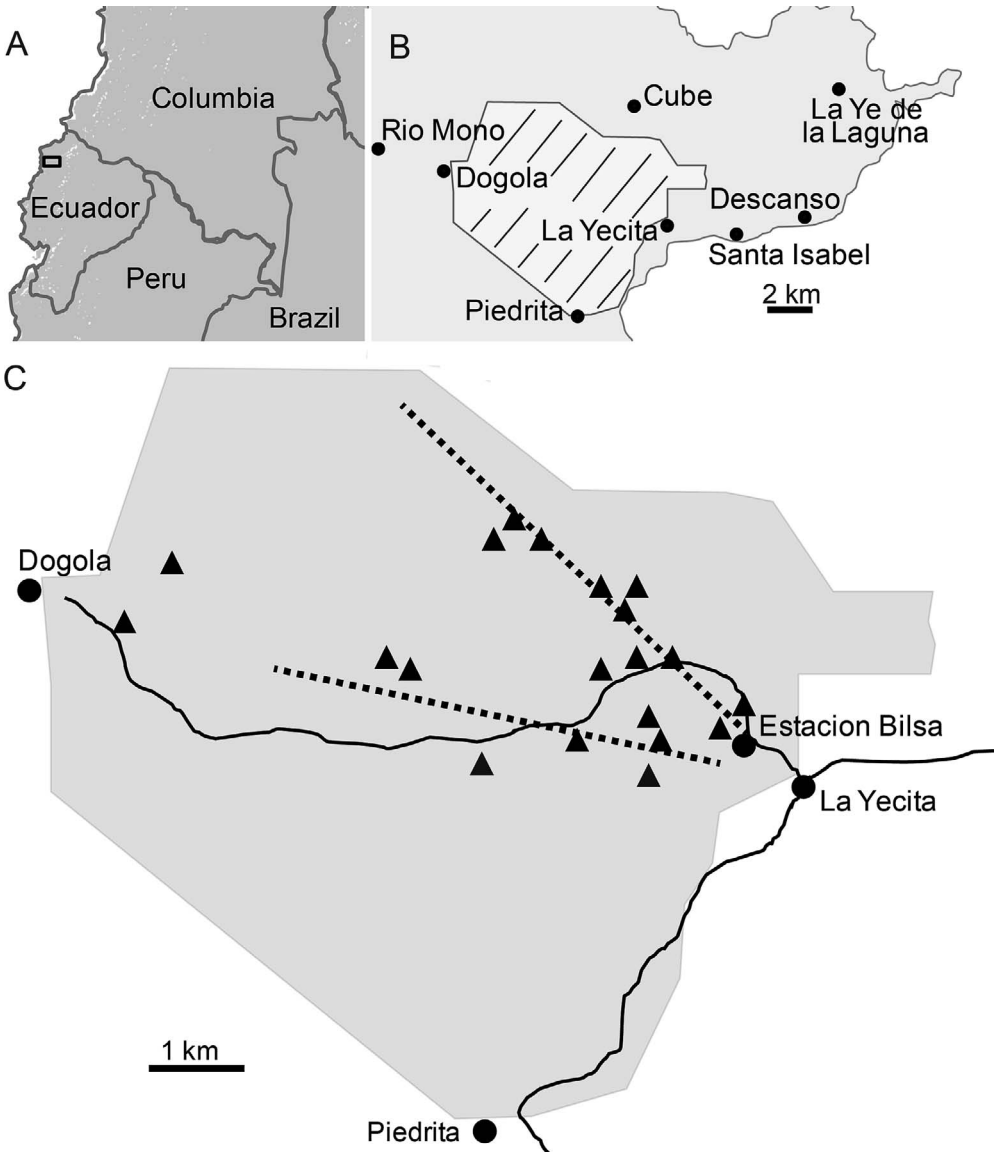

FIGURE 1. (**A**) The Mache Chindul Reserve in northwestern Ecuador is part of the Tumbes-Choco-Magdalena bioregion and surrounds the (**B**) Bilsa Ecological Station highlighted with diagonal lines. The field station is within a 1 km of La Yecita on the Eastern edge of the reserve. (**C**) Two transects are indicated, running through both secondary and primary forest, along which mantled howler monkey groups were sampled (triangles). Communities surrounding the reserve include Dogola, Cube, La Yecita, and Piedrita with additional homes found along much of the southeastern border.

Jackknife (Chao and Lee, 1992), and Incidence-based Coverage Estimator (ICE) (Gotelli and Colwell, 2001, 2011; Walther and Martin, 2001). We investigated 3 adjusted species richness estimators to ensure that the observed results were robust to choice of adjustment. Groups with 2 or fewer individuals were not included in analyses because estimates of adjusted species richness would be unreliable based on so few individuals. Estimated species richness adjusted for sampling effort was then modeled as a function of group size to assess the relationship between species richness and group size. Estimated species richness adjusted for sampling effort is a continuous variable, so ordinary least squares regression was used to quantify the association between estimated species richness and group size.

Attribute agreement analysis coupled with Cohen's kappa was used to evaluate the ability of the 3 methods employed to detect parasites. No gold-standard for presence of each parasite species is available, so each method was compared to the final assessment, which was a summation of fecal smears, flotations, and sedimentation results. In the case of *Blastocystis* sp., we used PCR-based results as a gold standard. Co-infection was assessed using the chi-square (χ^2) test of independence (with the Yates adjustment) for pairs of parasite species. Unless otherwise specified, all statistical analyses were conducted using STATISTICA 10 for Windows (StatSoft, Inc., Tulsa, Oklahoma).

RESULTS

Almost all samples (97%) contained at least 1 type of gastrointestinal parasite. Parasite species richness per fecal sample ranged from 0 to 7 species. Of the 96 fecal samples, 23 had 5–7 parasite species, 68 had 2–4 parasite species, 3 had a single parasite, and 2 had no parasites detected. Samples averaged 3.6 parasite species per individual (±1.4 SD). Thirteen parasites encompassing 13 genera were identified, representing 2 apicomplexans, 6 other protozoa, 4 nematodes, and 1 platyhelminth (Fig. 2; Table I). At least 1 apicomplexan was found in 20% of all samples, other protozoans were present in 88% of samples, and nematodes were detected in 95% of the samples.

Average length and range of parasites are listed in Table I. Three parasite groups require additional explanation. First, *Strongyloides* spp. egg length ranged from 14.4 μm to 71.3 μm and, when we plotted a frequency distribution of the lengths, this produced a bimodal curve with peaks at 36 μm and at 52.8 μm. Over half (52%) of identified eggs fell below 48 μm in length, a

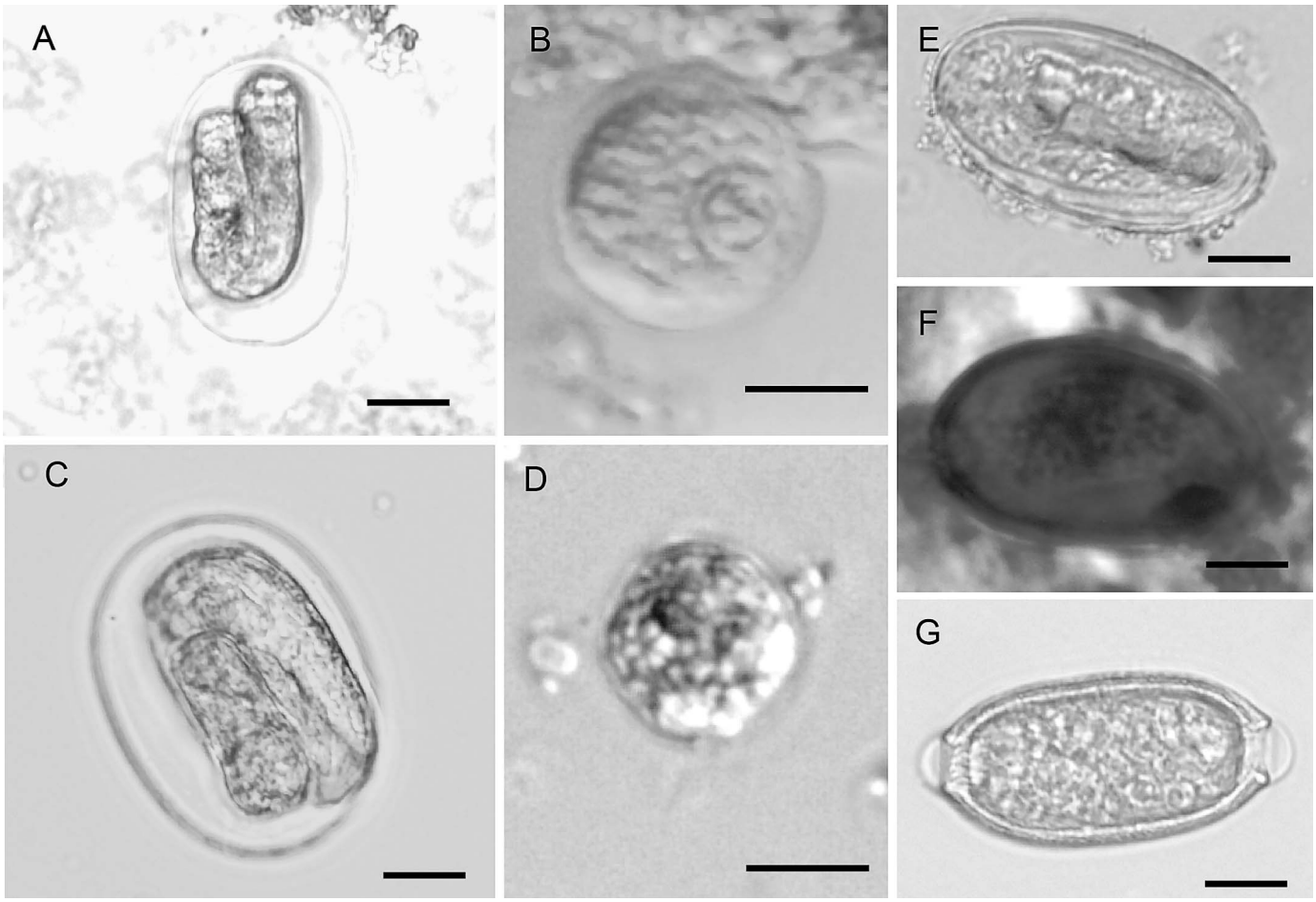

FIGURE 2. Examples of parasites and parasite eggs recovered from howler monkeys. (**A**) *Strongyloides* sp. 1, (**B**) *Entamoeba* sp. 1, (**C**) *Strongyloides* sp. 2, (**D**) *Entamoeba* sp. 2, (**E**) *Trypanoxyuris* sp., (**F**) *Enterobius* sp., (**G**) and *Capillaria* sp. Bar = 10 μm.

reported minimum for *Strongyloides* species in howlers. The 2 apparent morphotypes observed were considered a single entity for the purposes of analysis here. Secondly, 58% of samples contained *Entamoeba* spp. Several morphotypes existed with cysts that averaged 12.8 μm in length and ranged from 9.2–16.3 μm. The number of nuclei within each cyst could not reliably be determined; thus, all findings were categorized as *Entamoeba* spp. Lastly, PCR-based confirmation was conducted for *Blastocystis* spp. Seven percent (7%) of fecal smears were positive, along with 1% of flotations and 4% of sedimentations, while 60% were positive using PCR detection.

Recovery efficiency was dependent on fecal extraction method (Table I). For example, *Strongyloides* spp. were found in 88% of samples based on all methods combined, but only 58% of smears, 3% of flotations, and 73% of fecal sedimentations were positive. Similarly, a *Capillaria* sp. was present in 78% of samples yet only 64% of smears, 56% of flotations, and 38% of sedimentations were positive. Thirteen parasite species were recovered from fecal smears compared to 9 species from flotations and 10 from sedimentations. The highest parasite prevalence for 6 species was found using smears, while only 1 species had the highest prevalence from flotations and 3 species from sedimentations. There is clearly a benefit to using multiple extraction methods as each is suited to recovering different parasite species (Table II).

Considering the 19 howler groups with at least 2 individuals, parasite species richness varied from 3 to 11 parasite species and averaged 3.5 species per group (±0.6 SD; Fig. 3). Sixteen groups (84%) harbored 5 or more distinct parasite species and 3 groups (16%) had 2–4 species of parasite. Eleven groups (58%) exhibited at least 1 apicomplexan. Nematode and protozoan species were found in all other groups. Parasite species richness increased with group size, regardless of the method used to adjust for sampling effort (Fig. 4): Jackknife ($F_{1,14} = 6.2$, $r^2 = 0.31$, $P = 0.03$); Chao2 ($F_{1,14} = 4.7$, $r^2 = 0.25$, $P = 0.05$); and ICE ($F_{1,14} = 4.5$, $r^2 = 0.24$, $P = 0.05$).

Co-infection status was evaluated for all parasite pair combinations. Two significant associations were found. Of those individuals infected with *Balantidium* sp., 22% were also infected with *Isospora* sp., which is significantly greater than expected (3%) under the hypothesis of no association between the 2 species ($\chi^2 = 6.02$, $P = 0.01$). A negative association was found between *Chilomastix* sp. and *Capillaria* sp. Only 25% of the individuals infected with *Chilomastix* sp. were also infected with *Capillaria* sp., far fewer than the expected *Capillaria* sp. prevalence of 78%

TABLE I. Percent parasite prevalence (P) with 95% confidence intervals (CI), mean intensity (MI) estimated as number of eggs per gram of feces using three fecal extraction techniques, and mean and range of length of eggs or cysts.

		Smear			Flotation			Sedimentation			Total sampled (n = 96)			
Parasite group	Parasite species	P %	CI	MI	P %	CI	MI	P %	CI	MI	P %	CI	Mean length (μm)	Range (μm)
Apicomplexa	*Cyclospora* sp.	8.3	4–16	7.0	3.1	1–9	134.0	9.4	5–17	13.4	17.7	11–27	14.7	10.2–23.4
	Isospora sp.	1.0	0–6	1.0	1.0	0–6	1.0	1.0	0–6	1.0	3.1	1–9	35.2	11.2–68.6
Other Protozoa	*Balantidium* sp.	8.3	4–16	2.4	1.0	0–6	1.0	0.0	0–5	0.0	9.4	5–17	44.5	30.1–107.3
	Blastocystis sp.	7.3	3–15	1.1	1.0	0–6	1.0	4.2	1–11	8.5	60.4*	60–78	18.6	14.0–33.4
	Chilomastix sp.	3.1	1–9	3.0	0.0	0–5	0.0	1.0	0–6	1.0	4.2	1–11	23.6	16.0–27.3
	Dientamoeba sp.	3.1	1–9	1.0	0.0	0–5	0.0	0.0	0–5	0.0	3.1	1–9	11.8	5.4–15.9
	Entamoeba sp.	55.0	45–65	6.8	2.0	0–5	1.0	7.3	2–13	1.3	56.3	46–66	12.8	9.2–16.3
	Iodamoeba sp.	5.2	2–12	1.0	0.0	0–5	0.0	0.0	0–5	0.0	5.2	2–12	19.2	11.2–30.1
Nematoda	*Enterobius* sp.	1.0	0–6	1.0	0.0	0–5	0.0	2.1	0–8	1.0	3.1	1–9	41.5	36.7–46.3
	Capillaria sp.	63.5	53–72	7.3	56.3	46–66	17.2	37.5	28–48	3.2	78.1	69–85	48.8	24.5–54.0
	Strongyloides sp.	58.3	48–68	3.8	3.1	1–9	1.0	72.9	63–81	4.4	87.5	79–93	44.6	14.4–71.3
	Trypanoxyuris sp.	6.3	3–13	1.8	1.0	0–6	7.0	6.3	3–13	6.0	11.5	6–20	42.5	41.7–43.3
Platyhelminth	*Controrchis* sp.	3.1	1–9	1.0	9.4	5–17	3.0	2.1	0–8	1.0	14.6	9–23	35.5	25.9–54.1

* *Blastocystis* confirmation using PCR-based detection.

under the null hypothesis of no association between the 2 species ($\chi^2 = 4.03$, $P = 0.04$).

DISCUSSION

Parasite species from 13 genera were found in 19 groups of Ecuadorian mantled howler monkeys. These 13 genera represent a higher number than observed in previous howler monkey studies. The higher parasite species richness observed for these howler groups relative to previous studies could be due to several factors including differences in sampling effort and technique, extraction methodology, environmental conditions (Stuart et al., 1990, 1998; Stoner, 1996; Eckert et al., 2006; Cristobal-Azkarate et al., 2010), anthropogenic disturbance (Daszak et al., 2001; Chapman et al., 2005; Puttker et al., 2008), host densities, and parasite attributes such as intermittent shedding of parasites by hosts in various seasons, differences in host immunity (Stear et al., 1995), initial infection dose (Christensen et al., 1995), and parasite fecundity (Dineen et al., 1965). Our study may simply have recovered more parasites because we used 3 different methods to retrieve parasites from feces (plus PCR-based detection for *Blastocystis* spp.) in order to maximize the likelihood of recovering various types of parasites, whereas the other studies limited retrieval and detection to 1 or 2 extraction methods. We are not suggesting that this is the only explanation for increased species richness. However, looking only at our fecal flotation results, 9 species were recovered as opposed to 13 species recovered when all 3 extraction methods were combined. Sedimentation methods resulted in the recovery of 10 parasite species. Fecal smears did result in the detection of all 13 species and also had the highest number of positive matches (when compared to a combination of all methods) for 7 parasite species (Table II). Yet, only 2 other howler monkey studies have

TABLE II. Percent agreement and kappa statistics for parasite detection using 3 extraction methods (fecal flotation, sedimentation, and smear). Aggregated data from all methods were used as the gold-standard. N = 96 fecal samples assessed by all 3 methods. Kappa values >0.81 are considered near perfect agreement, 0.41–0.60 corresponds to moderate agreement, and <0.20 is considered poor.

	Flotation		Sedimentation		Smear	
Genera	Agreement %	Kappa	Agreement %	Kappa	Agreement %	Kappa
Cyclospora	85	0.26	92	0.65	91	0.59
Isospora	98	0.49	98	0.49	98	0.49
Balantidium	92	0.18	91	0.00	99	0.94
Blastocystis†	40	−0.02	39	−0.05	38	−0.08
Chilomastix	96	0.00	97	0.39	99	0.85
Dientamoeba	97	0.00	97	0.00	100	1.00
Entamoeba	44	0.03	49	0.11	97*	0.94
Iodamoeba	90	0.15	95	0.68	95	0.68
Enterobius	97	0.00	99	0.79	98	0.49
Capillaria	77	0.51	58	0.27	85*	0.65
Strongyloides	18	0.02	88*	0.63	71*	0.39
Trypanoxyuris	95	0.00	95	0.00	100	1.00
Controrchis	95	0.75	88	0.22	89	0.32

* Significantly higher prevalence than other unmarked methods based on 95% confidence interval.
† For *Blastocystis* sp., the gold-standard was PCR data.

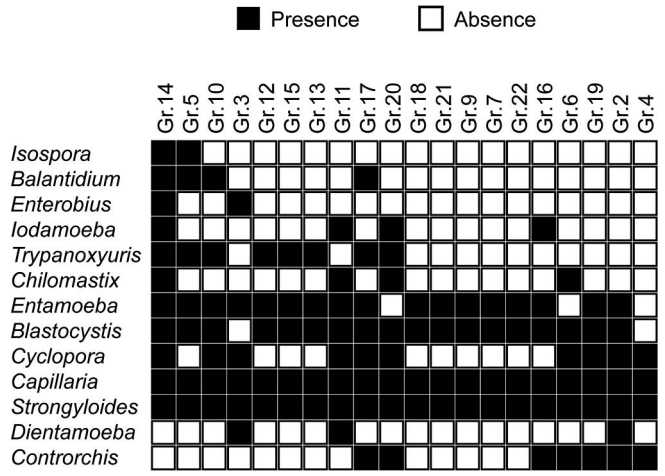

FIGURE 3. Similarity of parasite communities in howler monkey groups based on parasite species presence–absence. A group was considered positive if a parasite was found present in at least 1 individual using any of the 3 fecal extraction methods (i.e., smear, flotation, or sedimentation).

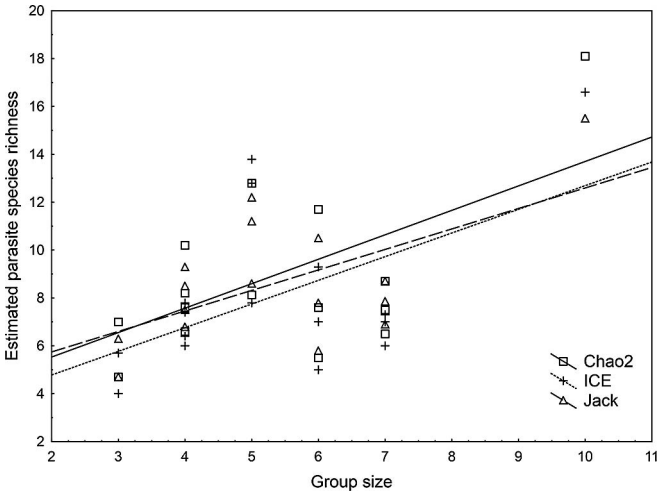

FIGURE 4. Association of Y = estimated parasite species richness (adjusted for sampling effort) with x = howler monkey group size. Larger groups have more gastrointestinal parasite species even after accounting for sampling effort using 3 non-parametric richness estimators. The ordinary least squares prediction equations are Chao 2 estimated species richness (solid line, square) ($Y = 3.48 + 1.02x$); Jackknife (dashed line, triangle) ($Y = 4.03 + 0.86x$); and ICE estimated species richness (dotted line, plus sign) ($Y = 2.80 + 0.99x$).

utilized this method (Eckert et al., 2006; Stoner and Gonzalez Di Pierro, 2006).

Group size

Group size was associated with parasite species richness; larger groups harbored a greater number of parasite species (Fig. 4). This finding is consistent with the hypothesis proposed by Freeland (1979) and summarized by Altizer et al. (2003) in which the number of intestinal protozoan species is a function of group size. In larger groups, individuals are hypothetically more likely to be infected either from increased environmental contamination, from an increased exposure to immigrants (Freeland, 1976), or from expanded traveling needed to secure food resources (Chapman et al., 2008). Conversely, other studies have found no association of parasitism with group size (Chapman et al., 2008) or a negative association (Snaith et al., 2008). The positive relationship found between group size and parasite richness is likely a product of several contributing factors closely associated with the number of individuals within a group (Cote and Poulin, 1995; Altizer et al., 2003) including group density, individual stress levels, food availability, and subsequent altered ranging behaviors that could bring individuals into contact with fecal-contaminated areas. This could mean that fluctuations in certain environmental factors, such as food sources, would result in a change in stress levels, group density, and number of individuals (Chapman et al., 2006).

Parasite interactions

There was 1 case where a host was more likely to harbor a parasite species in the presence of another—individuals positive for *Isospora* sp. were much more likely to be found in individuals with *Balantidium* sp. There was also only 1 case where 2 parasites showed a negative association. Individuals infected with *Chilomastix* sp. were much less likely to be infected with *Capillaria* sp. There is little evidence in the literature to suggest that presence of a *Chilomastix* sp. actually inhibits or influences the presence of *Capillaria* sp. In fact, *Chilomastix mesnili* is found in primates of all sorts and is often associated with other parasitic infections (Cox, 2001; Ekanayake et al., 2006; Chapman et al., 2011). In either case, it is not possible to definitively identify what type of interaction is occurring simply based on patterns of co-infection; however, there is certainly evidence elsewhere to suggest that competitive interactions between parasites occurs (Petney and Andrews, 1998; Pedersen and Fenton, 2007; Graham, 2008) as well as parasite-induced immunosuppression—where the presence of 1 parasite species benefits another (Cox, 2001). Poulin (2001) summarizes possible causes of gastrointestinal parasite relationships, including the possibility that certain hosts might be more susceptible to parasitism than are other individuals.

Parasite species identification

We may have encountered 2 distinct *Strongyloides* morphotypes in our study, as the eggs we measured ranged in length from 14 μm to 71.3 μm, and the distribution of egg length was bimodal with modes at 36 μm and at 52.8 μm. Because of significant overlap, we could not confidently separate large and small eggs for the purposes of our analysis here. Multiple *Strongyloides* species have been found in the same red colobus monkey population, though it was not reported whether both were in a single individual (Gillespie and Chapman, 2008). The howler parasite literature typically only lists genus because these are difficult to identify to species level from eggs, and there are more than 50 described *Strongyloides* species throughout the world (Speare, 1989). It is possible that some of the recovered eggs we found that were longer than 48 μm are attributable to *Strongyloides stercoralis*, and this species does have a distribution throughout humans in the Neotropics (Olsen et al., 2009). However, 52% of identified *Strongyloides* eggs were less than 48 μm long, which puts them outside the previous size estimates for any species reported from howlers. Besides egg length, there were

no other discernible morphological differences between the morphotypes (Fig. 2A, B).

All but 3 parasite genera (*Cyclospora* sp., *Capillaria* sp., and *Balantidium* sp.) have been previously reported in other howler monkey species (Gilbert, 1994; Stoner 1996; Eckert et al., 2006; Stoner and Gonzalez Di Pierro, 2006; Vitazkova and Wade, 2006; Trejo-Macias et al., 2007; Cristobal-Azkarate et al., 2010; Kowalewski et al., 2010). In most cases we were not able to identify to a species level based simply on morphology, yet we can speculate based on other factors. *Entamoeba coli* could have been responsible for some howler infections, as cyst morphology was consistent with other reports (Table I). It has also been reported in humans living in the same province as our field study (Gatti et al., 2002). Nonetheless, we cannot rule out the presence of other *Entamoeba* spp.

Trypanoxyuris sp. eggs averaged 42.5 μm in length, similar to previous findings which ranged from 31.2–49.3 μm in *A. palliata* in Mexico (Table I; Cristobal-Azkarate et al., 2010). *Trypanoxyuris minutus* has been reported in nearly every other Central and South American country, which leads us to believe that this is *T. minutus* (Thatcher and Porter, 1968; Gilbert, 1994; Stuart et al., 1998; Cristobal-Azkarate et al., 2010). This parasite is relatively important because it has previously been reported as the cause of death of a howler in Brazil (Amato et al., 2002).

We observed eggs of a *Capillaria* species in 78% of our samples with eggs averaging 48.8 μm long. Over 250 *Capillaria* spp. have been described in vertebrates, though only eggs from *Capillaria hepatica* and *Capillaria brochieri* have been reported from primates (Brack et al., 1994; Graczyk et al., 1999). *Capillaria hepatica* eggs were reported to be from 50 μm (Brack et al., 1994) to 54.3 ± 0.5 μm (Graczyk et al., 1999). *Capillaria brochieri* eggs in chimpanzees were documented from 45–55 μm, which would encompass our observations (Justine, 1987). However, *Capillaria* species have only been found in *A. caray* but not in *A. palliata* (Godoy et al., 2004).

Balantidium coli is the only known ciliated protozoan to infect humans and has been found in Ecuadorian human populations (Chiriboga Urquizo et al., 1985). However, no cases of *B. coli* have been reported in howler monkeys, though *Balantidium* species have been reported in *A. caraya* (Stiles et al., 1929). Trophozoites averaged 44.5 μm (30.1–107.3 μm), which is within the normal variable size limit of 30–300 μm in length (Fig. 1). *Cyclospora* species have been previously reported from Ethiopian and Kenyan primates (Eberhard et al., 1999, 2001). However, *Cyclospora* in South American non-human primates has not been reported. Human infections of *Cyclospora cayetanensis* have been reported in South American countries including Peru (Ortega et al., 1994) and Venezuela (Chacin-Bonilla et al., 2001), which suggests that this parasite might be found in Ecuador. *Chilomastix* sp. was also identified to the genus level. Only 1 other howler study (*A. caraya*) reported this parasite, a study in Brazil (Stiles et al., 1929).

Kowalzik et al. (2010) previously hypothesized that black howler monkeys (*A. pigra*) who eat the leaves, fruit, and stems of the cecropia tree, *Cecropia peltata*, are likely to become infected with *Controrchis* sp. through the ingestion of infected ants. Our study found only 15% of samples positive for *Controrchis* sp. compared to 80–89% prevalence in black howler monkeys (Vitazkova and Wade, 2006; Kowalzik et al., 2010). Aside from the difference in host species, there is the possibility that forest structure differences between the studies might play a role. The cecropia tree is a pioneer species and thus we would expect *Controrchis* sp. to be more prevalent in disturbed forest. Only 20% of the Bilsa Biological Reserve is considered secondary forest (Ortega-Andrade et al., 2010), while Kowalzik et al. (2010) describe their site as a "hurricane-damaged" forest and Vitazkova and Wade (2006) sampled monkeys in secondary growth forest, cattle pastures, and plantations. If indeed these *Controrchis* species were limited to degraded environments, then this might very well explain the reduced prevalence in our study. Vitazkova and Wade (2006) identified the parasite from black howlers as *Controrchis biliophilus*. Based on descriptions and size estimates of eggs (41 μm– 50 μm) from other studies (Stuart et al. 1990), the eggs we observed are consistent with *Co. biliophilus*.

Pathogenicity

Although we took a conservative approach to identify species only to genus, some of the species in this study are from genera where most of the members are considered asymptomatic. Others may be associated with host impacts ranging from mild signs of infection to death (Abbott and Majeed, 1984; Foreyt, 2001; Chapman et al., 2005; Trejo-Macias et al., 2007). In the case of *Blastocystis* sp., *Iodamoeba* sp., and *En. coli* there is little evidence to suggest pathogenicity (Toft, 1982; Chapman et al., 2005; Coyle et al., 2011). Alternatively, *Strongyloides* spp. (Abbott and Majeed, 1984; Chapman et al., 2005), *Capillaria* spp. (Abbott and Majeed, 1984), *Balantidium* sp. (Kuntz, 1982; Toft, 1982), *Enterobius* sp. (Toft, 1982; Chapman et al., 2005), and *Chilomastix* sp. (Chapman et al., 2005) have been shown to be pathogenic in other primates. *Entamoeba* spp. (Kuntz, 1982) could be either asymptomatic (*En. coli*) or cause amoebiasis (*Entamoeba histolytica*), but is limited in this case by our inability to identify these parasites to a species level (Chapman et al., 2005). Similarly, *Isospora* sp. may or may not lead to coccidiosis in non-human primates (Lindsay et al., 1997; Duszynski et al., 1999). Information on the effect of these parasite species in *A. palliata* is limited, especially as it relates to multiple infections; however, based on evidence found in other organisms there is the possibility that host health and fitness could be affected (Murray et al., 1998; Chapman et al., 2007; Cristobal-Azkarate, 2010). Multiple infections in combination or single infections coupled with reduced host immune response could influence pathogenicity, leading to illness or in some circumstances death (Amato et al., 2002).

In conclusion, our study found several parasites which may have relevance to primate and human health: *Balantidium* sp., *Isospora* sp., *Enterobius* sp., and *Strongyloides* sp. We also found that group size was positively correlated with gastrointestinal parasitism—a finding which builds on previous primate studies by accounting for sampling effort. Describing gastrointestinal parasites infecting non-human primate species, and understanding factors that impact parasite communities, is important to both human health and primate conservation. Future natural and man-made changes to the environment have been predicted to increase spill-over events from wildlife populations to people and vice versa (Daszak et al., 2000). The data presented here provide the foundation for future studies on howler monkey parasites in Ecuador.

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