Prevalence of Gastrointestinal Parasites in Civets of Fragmented Rainforest Patches in Anamalai Hills, Western Ghats, India

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Prevalence of Gastrointestinal Parasites in Civets of Fragmented Rainforest Patches in Anamalai Hills, Western Ghats, India

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ABSTRACT: Parasitism, driven by anthropogenic habitat modifications, is being increasingly recognized as a major threat to wildlife. Unfortunately, even baseline parasite data for most wildlife species are lacking in India, including the civets, which are particularly vulnerable due to their proximity to human habitations. Civet fecal samples were collected from 10 forest fragments that vary in size and disturbance level in Anamalai Hills, Western Ghats, India. These samples were screened for the presence of gastrointestinal parasites using fecal floatation and fecal sedimentation techniques. From a total of 180 civet fecal samples, 15 gastrointestinal parasite taxa were recovered, and these species are also known to infect domesticated animals. Additionally, small, disturbed forest fragments recorded higher mean gastrointestinal parasite taxa and greater prevalence when compared to large, undisturbed forest fragments, indicating a potential relationship between anthropogenic activities and gastrointestinal parasitism of civets in the Anamalai Hills.

Three species of civets are known to occur in the Western Ghats, a highly bio-diverse region in peninsular India. Among them, the small Indian civet *Viverricula indica* and Asian palm civet *Paradoxurus hermaphroditus* have the widest distribution across south and southeast Asia. Both these species are found across different habitats including evergreen and deciduous forests (Kumara and Singh, 2007). These 2 species are also known to live in close proximity to human habitations, agricultural lands, parks, and plantations. The brown palm civet *Paradoxurus jerdoni,* an endemic species of Western Ghats, is known to occur largely in the tropical rainforest habitats and sometimes in the adjacent coffee plantations (Rajamani et al., 2002). Very little information is available on the ecology of these elusive small carnivores (Kumara and Singh, 2007; Patou et al., 2008). They are solitary and nocturnal mammals that prey on insects, earthworms, molluscs, and small vertebrates like rodents (Balakrishnan and Sreedevi, 2007; Patou et al., 2010). However, palm civet species are largely frugivorous and feed on berries, figs, and palms. They are incapable of digesting the ingested seeds, and viable seeds are expelled in their feces, thereby facilitating dispersal in the immediate environment, which has a cascading effect on all the trophic levels in their ecosystem (Mudappa et al., 2010; Jothish, 2011). While hunting is a threat to some of these species, the existence of all 3 civet species is seriously threatened by anthropogenic activities.

Civet species like *P. hermaphroditus* are captured from their wild habitats and reared in captivity. They are known to feed on coffee cherries and have an inherent ability to select and feed on the ripe fruit. The undigested inner coffee beans that are egested by this animal have a unique aroma and flavor that is appreciated by the people worldwide (Ongo et al., 2012). Similarly, the perineal gland secretion, "civet" of *V. indica* is used as a fixative in the perfume industry and is also used widely within India in Ayurveda traditional medicine practices. The increasing popularity and demand of civet coffee and the demand for "civet" in the international perfume industries have endangered their population in the wild (Balakrishnan and Sreedevi, 2007).

In addition to the aforementioned factors, habitat loss and habitat fragmentation brought about by mining, large-scale plantations, rampant urbanization, and hydroelectric projects also affect the survival and perpetuation of the civet species (Menon and Bawa, 1997; Rajamani et al., 2002). Parasitic diseases, on the other hand, while known to be of significance to wildlife conservation, have not been given their due importance (Deem et al., 2001). Consequently, little is known regarding the parasites of civets in India (Coumarane and Mohan, 2008). Rather, the rampant modification of their habitats in the Western Ghats and a resultant potential shift in civet populations may actually facilitate the emergence and re-emergence of parasitic diseases or the spread of less prevalent ones among civets (Patz et al., 2000). The proximity of some of the civet species to humans and domestic animals may also cause cross-species transmission of parasites to which civets are susceptible and thus increase the risk to their health manifold (Daszak et al., 2000). This study generates baseline information regarding the parasites of these animals, to help in their conservation, and also to facilitate the management of whole small carnivore community in the Western Ghats.

The field survey was carried out in the Anamalai Tiger Reserve (987 km^2; 10°12′–10°35′N, 76°49′–77°24′E) and the adjoining Valparai plateau of the Anamalai Hills, southern Western Ghats, India (Fig. 1). The Valparai plateau, once covered by continuous tropical rainforest vegetation, presently contains several rainforest patches as a result of vegetation clearing between 1890s and 1930s to develop the area for tea, coffee, and cardamom plantations. The patches are between 2 and 2,000 ha in size and are interspersed by tea, coffee, and cardamom estates, settlements of estate workers, the Valparai Township, the Pollachi-Chalakkudy road, and numerous trails cutting across the estates and the rainforest fragments (Umapathy and Kumar, 2000).

Fresh fecal samples were collected during December 2014 to March 2015 from the rainforest fragments of Anamalai Hills. Randomly placed transects, which varied from 400 m to 3,000 m, across 10 forest fragments were traversed to collect fecal samples and preserved in 10% formalin. The feces were identified as that of civets by the characteristic size/diameter, shape, texture, nearby tracks, and characteristic odor. As it was not possible to visually differentiate between feces of the 3 civet species, all the samples were recorded as that from civets as a group. The location of each sample was recorded using a handheld GPS unit (Garmin Montana 650, Garmin, Kansas City, Kansas). Data on presence of human settlement in and around the forest fragments were recorded for the analysis of data on number of gastrointestinal parasite taxa and percentage prevalence in civets (Umapathy and Kumar, 2000). The preserved samples were then immediately sent to Laboratory for the Conservation of Endangered Species at the Centre for Cellular and Molecular Biology, Hyderabad, India, for parasite recovery.

The fecal samples were screened for the presence of helminth eggs, larvae, and protozoan cysts by both fecal floatation and fecal sedimentation techniques (Gillespie, 2006). Half of each fecal sample was stored in the original vial for any future examination. The fecal floatation technique was performed as described earlier with a minor modification (Dryden et al., 2005). About 2 g of fecal sample was weighed and transferred to a fresh 15 ml centrifuge tube. Subsequently, 10 ml ultra-purified "Milli-Q" water was added to the tube, the content was mixed with the help of a

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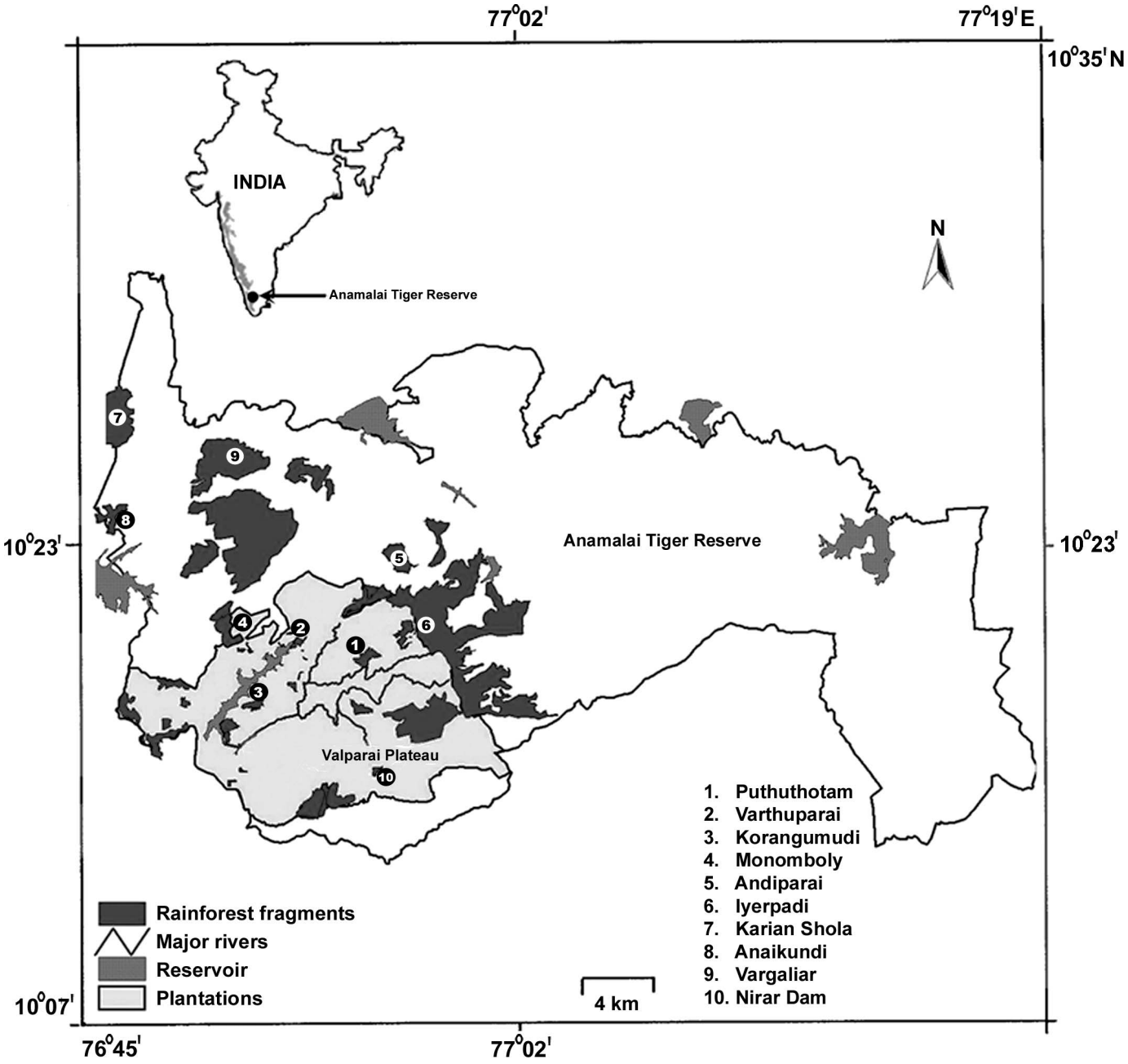

FIGURE 1. Rainforest fragments in Anamalai Tiger Reserve, Western Ghats, India.

clean glass rod, and the tubes were vortexed vigorously for 10 min to homogenize the content. The homogenized contents were then filtered through a piece of cheese cloth into a fresh 15 ml centrifuge tube, which was then spun in a laboratory centrifuge at 650 *g* for 10 min (Eppendorf centrifuge 5810 R, Eppendorf AG, Hamburg, Germany) and the supernatant was discarded. Sucrose solution (3.73 M) was added to the sediment and mixed well with the help of a clean glass rod. These tubes with well-mixed contents were spun again in a laboratory centrifuge at 3,220 *g* for 10 min (Eppendorf centrifuge 5810 R, Eppendorf AG) to precipitate debris and facilitate the floatation of parasitic forms in the sample. Next, the sucrose solution was added to the tubes to form a positive meniscus, cover slips were gently placed over each of these tubes and waited for 10 min. The cover slips were then lifted carefully and placed on a clean grease-free glass slide for microscopic examination using ×20 and ×40 objectives of a light microscope.

A similar protocol was followed for the screening of fecal samples by the sedimentation technique, in which, diluted soapy water solution (Foreyt, 2001) was added to the sediment following first centrifugation step. The sediment was mixed evenly with a clean glass rod following which the tubes were centrifuged at 3,220 *g* for 10 min (Eppendorf centrifuge 5810 R, Eppendorf AG) to precipitate the fecal content. The supernatant was discarded, and a small amount of the sediment was taken on a grease-free slide for microscopic examination (Foreyt, 2001; Gillespie, 2006). Parasitic forms, including eggs, cysts, and larvae were identified based on their morphological characteristics (Sloss et al., 1994; Inpankaew et al., 2014). We used iodine to identify the protozoan cysts. With the exception of coccidian parasites, isolated gastrointestinal

TABLE I. Number and percentage of prevalence of gastrointestinal parasite taxa in civets from forest fragments of Anamalai Tiger Reserve, India.

Fragments	Area (ha)	Human presence	Total no. of fecal samples	No. of gastrointestinal parasite taxa	Prevalence (%)
Vargaliar	2,000	N	11	1	09.09
Karian Shola	1,520	N	16	1	12.50
Anaikundi	225	N	13	1	46.15
Monomboly	200	Y	23	5	43.47
Andiparai	185	Y	17	5	47.05
Iyerpadi	100	Y	20	5	35.00
Puthuthotam	65	Y	20	8	60.00
Korangumudi	35	Y	21	5	52.38
Varathuparai	24	Y	22	6	40.90
Nirar dam	8	Y	17	7	70.58

parasites were identified at the genus level. Prevalence was defined as the percentage of samples with any gastrointestinal parasite taxon and species richness as the number of unique gastrointestinal parasite taxa recovered from a sample (Watve and Sukumar, 1995; Hussain et al., 2013; Chakraborty et al., 2015).

To examine the difference in parasite prevalence and richness with reference to area of the forest fragment, we classified fragments into large ($\geq$200 ha) and small ($<$200 ha). All the larger fragments had higher tree density, tree basal area, and canopy cover compared to small fragments (Umapathy and Kumar, 2000; Hussain et al., 2013). Most of the small fragments are owned by private estates and have human settlements within or bordering the forest fragment (Umapathy and Kumar, 2000). The Mann–Whitney *U*-statistic was used to test for the difference between 2 sets of data, and Spearman rank correlation coefficient (r_s) was used to examine association between 2 variables. The value of $P < 0.05$ was set as

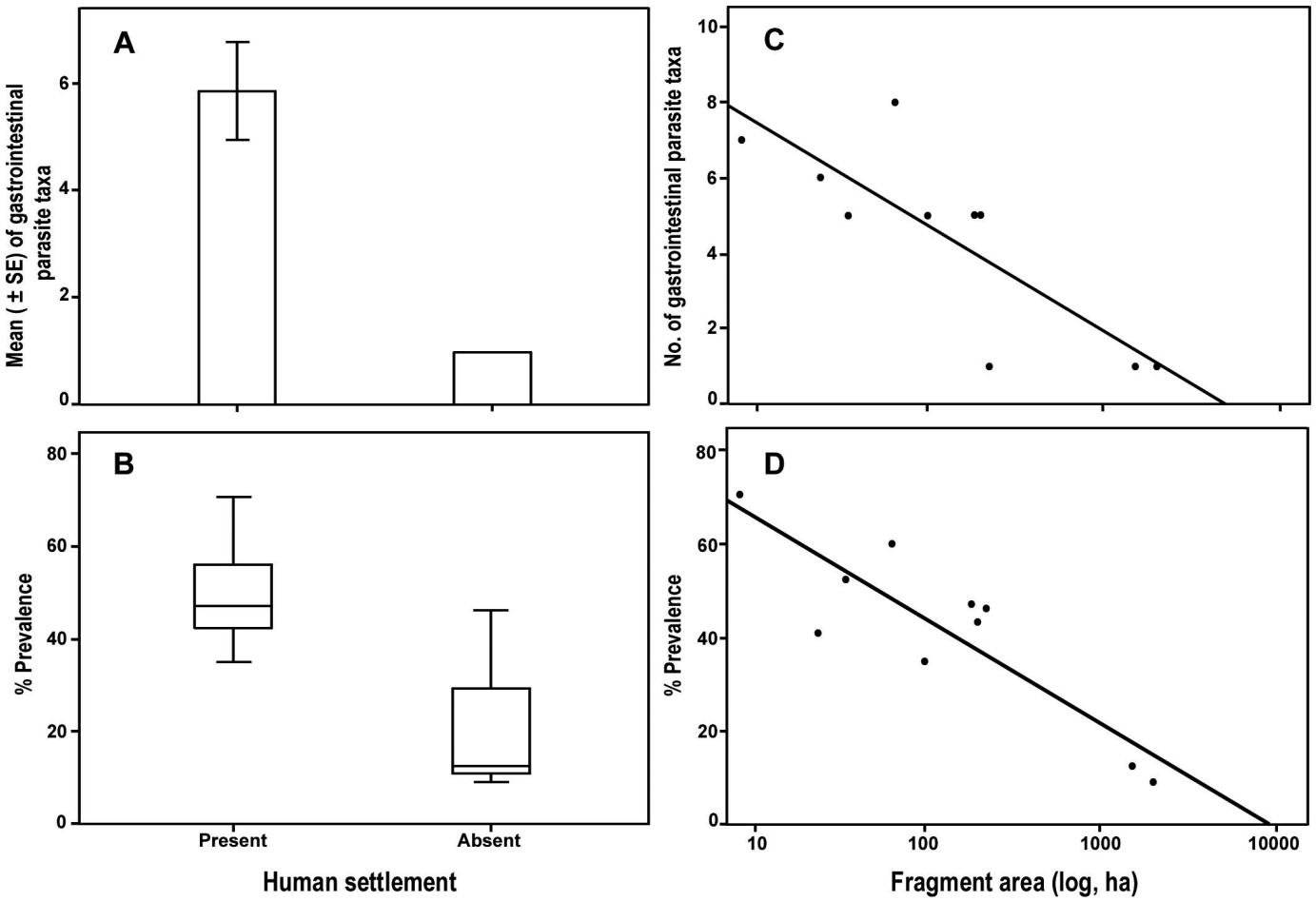

FIGURE 2. The relationship between gastrointestinal parasite and attributes of forest fragments: (**A**) the average (mean ± SE) number of gastrointestinal parasite taxa and forest fragments with and without human settlement, (**B**) box plot showing % of prevalence of gastrointestinal parasite taxa and forest fragments with and without human settlement, (**C**) the number of gastrointestinal parasite taxa and forest fragment area, and (**D**) percentage prevalence of gastrointestinal parasite taxa and forest fragment area.

TABLE II. Percentage prevalence of gastrointestinal parasite taxa in civets from forest fragments in Anamalai Tiger Reserve, India.

Phylum	Gastrointestinal parasite taxa	Vargaliar	Karian Shola	Monomboly	Andiparai	Iyerpadi	Puthuthottam	Korangumudi	Nirar dam	Varathuparai	No. of fragments recorded
Nematoda	*Trichuris* sp.	0	0	0	0	0	10.00	0	0	0	1
	Ascaris sp.	0	0	0	05.88	0	05.00	0	0	13.63	3
	Toxocara sp.	0	0	0	05.88	0	10.00	0	05.88	0	3
	Strongyloides sp.	0	0	22.23	0	15.00	0	15.78	0	04.54	4
	Capillaria sp.	0	0	05.56	11.76	0	0	0	05.88	04.54	4
	Physaloptera sp.	09.09	0	11.12	0	0	0	15.78	35.29	09.09	5
Cestoda	*Dipylidium* sp.	0	0	0	0	0	0	0	05.88	0	1
	Hymenolepis sp.	0	0	0	0	0	05.00	05.26	0	0	2
Trematoda	*Nanophyetus* sp.	0	0	0	0	0	0	05.26	0	0	1
	Schistosoma sp.	0	0	0	0	10.00	0	0	0	0	1
Protozoa	*Cyclospora* sp.	0	12.50	05.56	0	0	0	0	0	0	2
	Eimeria sp.	0	0	0	29.41	0	05.00	0	05.88	09.09	4
	Coccidia	0	0	16.67	05.88	10.00	05.00	31.57	23.52	18.18	7
	Balantidium sp.	0	0	0	0	05.00	35.00	0	05.88	0	3
	Giardia sp.	0	0	0	0	05.00	05.00	0	0	0	2

significant for all our tests. SPSS, version 17.0 (SPSS, Inc., Chicago, Illinois) was used for the statistical analyses.

We collected 180 civet fecal samples from 10 forest fragments, 43.34% of which had at least 1 gastrointestinal parasite taxon and 21.50% of the infected samples had multiple gastrointestinal parasites. We recorded 15 gastrointestinal parasite taxa: 6 nematodes (*Ascaris* sp., *Trichuris* sp., *Capillaria* sp., *Strongyloides* sp., *Physaloptera* sp., *Toxocara* sp.), 2 cestodes (*Hymenolepis* sp. and *Dipylidium* sp.), 2 trematodes (*Schistosoma* sp. and *Nanophyetus* sp.), and 5 protozoa (*Balantidium* sp., *Giardia* sp., *Cyclospora* sp., *Eimeria* sp., and Coccidia). Twelve of these parasites were observed in forest fragments with human settlements nearby, and the other 3 parasites were observed in both forest fragments with and without human settlement.

The number of parasite taxa recorded in forest fragments ranged from 1 to 8; the lowest was recorded in 3 large, undisturbed forest fragments—Varagaliar, Anaikundi, and Karina Shola—and the highest was recorded in Puthuthottam, a small, disturbed forest fragment (Hussain et al., 2013). Overall, prevalence of parasites ranged widely from 09.09% to 70.58%; the lowest was recorded in Varagaliar, the largest undisturbed forest fragment and highest in Nirar dam, a small highly disturbed fragment (Table I). The percentage prevalence of parasitic infection increased with a decrease in forest fragment size ($r_s = -0.690$, $P = 0.029$), and it was higher in forest fragments with human settlement nearby (Fig. 2), but the difference was not statistically significant (M–W U-test $U = 4.000$, $P = 0.080$).

Coccidia was the most prevalent parasite found in 7 forest fragments followed by *Physaloptera* sp. which was recorded in 5 forest fragments. *Trichuris* sp., *Nanophyetus* sp., *Schistosoma* sp., and *Dipylidium* sp. were found in 1 forest fragment each (Table II). Furthermore, coccidia was also the most common parasite taxa, recorded in 38.46% of all the infected fecal samples collected followed by *Physaloptera* sp. (17.94%) and *Strongyloides* sp. (14.10%).

This is the first study on the gastrointestinal parasites in civets of the fragmented rainforest in the Western Ghats. We recorded 15 gastrointestinal parasite taxa from 10 forest fragments and found more parasite taxa and higher prevalence in small forest fragments in close proximity to human settlements. Almost all the parasites recorded in this study are known to infect domestic dogs and cats (Zain et al., 2013; Ngui et al., 2014). Some of these parasites have also been reported in small carnivores (Patton et al., 1986; Patton and Rabinowitz, 1994; Garjito et al., 2008; Colón and Patton, 2012). Colón and Patton (2012), for instance, recorded 13 endoparasite taxa in civets of Sabah forests, Borneo and found more parasite richness and higher prevalence of parasite infection in the logged forests as compared to the unlogged forests. In another study, the Chinese lesser civets in northeastern Taiwan were infected with 11 parasite species, and the most prevalent parasites were *Capillaria* sp. followed by *Toxocara* sp., *Ancylostoma* sp., and *Strongyloides* sp (Su et al., 2013). In this study, coccidia was the most dominant parasite taxa recorded in 7 of the 10 forest fragments followed by *Strongyloides* sp., which was found in 5 forest fragments. Interestingly, *Ancylostoma* sp., a common parasite reported in civets was not recorded in this study (Courmarane and Mohan, 2008; Colón and Patton, 2012); instead 4 lesser known parasite taxa, *Physaloptera* sp., *Nanophyetus* sp., *Dipylidium* sp., and *Cyclospora* sp., were reported.

A previous study on lion-tailed macaque groups in the forest fragments of Anamalai Hills found a higher parasite species richness and prevalence of parasitic infection in groups near human settlements than the groups far from human settlements (Hussain et al., 2013). High prevalence and parasite species richness in these macaque groups near human settlement were attributed to the contamination of water and soil by intermediate hosts (Hussain et al., 2013). In the present study, the presence of humans, cattle, and other domestic animals were observed in 7 of the 10 forest fragments; furthermore few of these forest fragments had either public road bordering or bisecting them thereby potentially facilitating the exposure of infective parasites from domestic animals to wildlife (Traub et al., 2002). Such anthrpogenic interactions are

particularly intense in the Puthuthottam and Nirar dam forest fragments where highest parasite richness and prevalence were also recorded. Furthermore, in these forest fragments, stream water is known to often be shared by humans, cattle, domestic dogs, domestic cats, and wild animals, which may arguably lead to transmission and sharing of gastrointestinal parasites between multiple species (Hussain et al., 2013).

It must be emphasized that the primary goal of this study was to report baseline information on parasites of wildlife, a grossly understudied aspect of parasitology in India. Additionally, although we could not differentiatiate feces from different species of civets (the parasites were described from different species of civets pooled together), we were able to explore the pattern of parasitism in an important peridomestic wildlife in India within a severely human-modified landscape. Future studies might consider collecting larger datasets to differentiate between the effects of humans and nature, and to identify both the host and the gastrointestinal parasites at the species level with reference to seasonal factors.

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