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# Mammalian gastrointestinal parasites in rainforest remnants of Anamalai Hills, Western Ghats, India

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Habitat fragmentation is postulated to be a major factor influencing infectious disease dynamics in wildlife populations and may also be responsible, at least in part, for the recent spurt in the emergence, or re-emergence, of infectious diseases in humans. The mechanism behind these relationships are poorly understood due to the lack of insights into the interacting local factors and insufficient baseline data in ecological parasitology of wildlife. Here, we studied the gastrointestinal parasites of nonhuman mammalian hosts living in 10 rainforest patches of the Anamalai Tiger Reserve, India. We examined 349 faecal samples of 17 mammalian species and successfully identified 24 gastrointestinal parasite taxa including 1 protozoan, 2 trematode, 3 cestode and 18 nematode taxa. Twenty of these parasites are known parasites of humans. We also found that as much as 73% of all infected samples were infected by multiple parasites. In addition, the smallest and most fragmented forest patches recorded the highest parasite richness; the pattern across fragments, however, seemed to be less straightforward, suggesting potential interplay of local factors.

[Chakraborty D, Hussain S, Reddy DM, Raut S, Tiwari S, Kumar V and Umaphathy G 2015 Mammalian gastrointestinal parasites in rainforest remnants of Anamalai Hills, Western Ghats, India. *J. Biosci.* **40** 399–406] DOI 10.1007/s12038-015-9517-5

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## 1. Introduction

Deforestation and habitat fragmentation are increasingly transforming large and continuous habitats into smaller patches that are interspersed with human settlements, agricultural lands, plantations, mines and roads, thus bringing humans closer to wildlife (Fahrig 2003). Such human-modified landscapes may often harbour a higher density of wildlife in fragmented and smaller habitats (Connor *et al.* 2000; Brotons *et al.* 2003), which can, in turn, considerably enhance the chance of potentially new hosts, including forest-dwellers, encountering infective stages of parasites

(Morand and Poulin 1998; Poulin 1998; Arneberg 2002; Nunn *et al.* 2003). This alteration in parasitism in the natural hosts, arguably as a result of diminished biodiversity, may also drive some of the parasites to spill over to livestock and people eventually, not to mention new wild hosts (Friggens and Beier 2010; Keesing *et al.* 2010; Ostfeld and Keesing 2012, 2013). Studies have showed a high prevalence and richness of directly transmitted parasites among wild animals in human-modified landscapes (Packer *et al.* 1999; Mbora and McPeck 2009; Hussain *et al.* 2013; Laurance *et al.* 2013), indicating the role of anthropogenic habitat modification in driving infectious disease risk to wildlife.

**Keywords.** Gastrointestinal parasite; mammals; prevalence; rainforest; Western Ghats

Supplementary materials pertaining to this article are available on the Journal of Biosciences Website at <http://www.ias.ac.in/jbiosci/jun2015/supp/Chakraborty.pdf>

It must be noted that the association between biodiversity loss and zoonotic disease risk is a complex one, often varying between host–parasite groups and geographic areas (Salkeld *et al.* 2013; Lafferty and Wood 2013; Wood and Lafferty 2013; Young *et al.* 2013). Additionally, studies that claimed a direct connection between loss of biodiversity and zoonoses risk have been found to be largely based on smaller sample sizes (Salkeld *et al.* 2013) and were restricted mostly to few geographic regions of the world.

One region, experiencing similar biodiversity loss, in the face of a rapid increase in human population, is South Asia. Unfortunately, little parasitological research, specifically related to wildlife, has been conducted in this highly biodiverse region (Watve and Sukumar 1995; Hussain *et al.* 2013). Consequently, the broad objective of the present study was to develop baseline data of gastrointestinal parasites of nonhuman host communities from a highly fragmented tropical rainforest habitat of the Western Ghats, India. We therefore explored the diversity and prevalence of gastrointestinal parasite communities of wild mammalian host species across rainforest fragments that varied in size and degree of anthropogenic disturbance.

## 2. Materials and methods

### 2.1 Study site

The present study took place within the tropical rainforest remnants of the Anamalai Tiger Reserve (987 km<sup>2</sup>; 10°12'–10°35'N and 76°49'–77°24'E) and the adjoining Valparai plateau in the Anamalai hills, southern Western Ghats, India (figure 1). The plateau was once covered by continuous tropical rainforest vegetation, most of which was cleared between the 1890s and 1930s for tea, coffee and cardamom plantations (Congreve 1938). As a result, the plateau now contains several rainforest patches with sizes varying between 2 and 2000 ha 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 remnant rainforest fragments (Umapathy and Kumar 2003). In spite of such severe habitat fragmentation, the rainforest patches, quite astonishingly, have been successful in retaining much of their original wildlife, along with some of the wide-ranging species such as the Asian elephant (*Elephas maximus*), tiger (*Panthera tigris*) and leopard (*Panthera pardus*).

### 2.2 Sampling strategy

We conducted sampling of faeces between July and September 2011 from rainforest fragments (including privately owned ones) of Anamalai Tiger Reserve, Western Ghats, India. Samples were collected from un-habituated

populations of mammalian wildlife, according to standard protocols (Gillespie 2006; Sá *et al.* 2013). We collected fresh faecal samples, non-invasively and opportunistically, during the day, on trails (that varied from 400 m to 3000 m in length) across the 10 forest fragments. To avoid sampling the same individual, only one sample was collected from each spot. Further, to avoid contamination from soil, samples were collected, whenever possible, from the inside of the bolus or pellet, depending on the species. We immediately fixed each sample in a 50 mL Falcon tube containing 10% formalin and stored it at room temperature until samples were transported to the Laboratory for the Conservation of Endangered Species (LaCONES), Hyderabad, India, for parasitological screening.

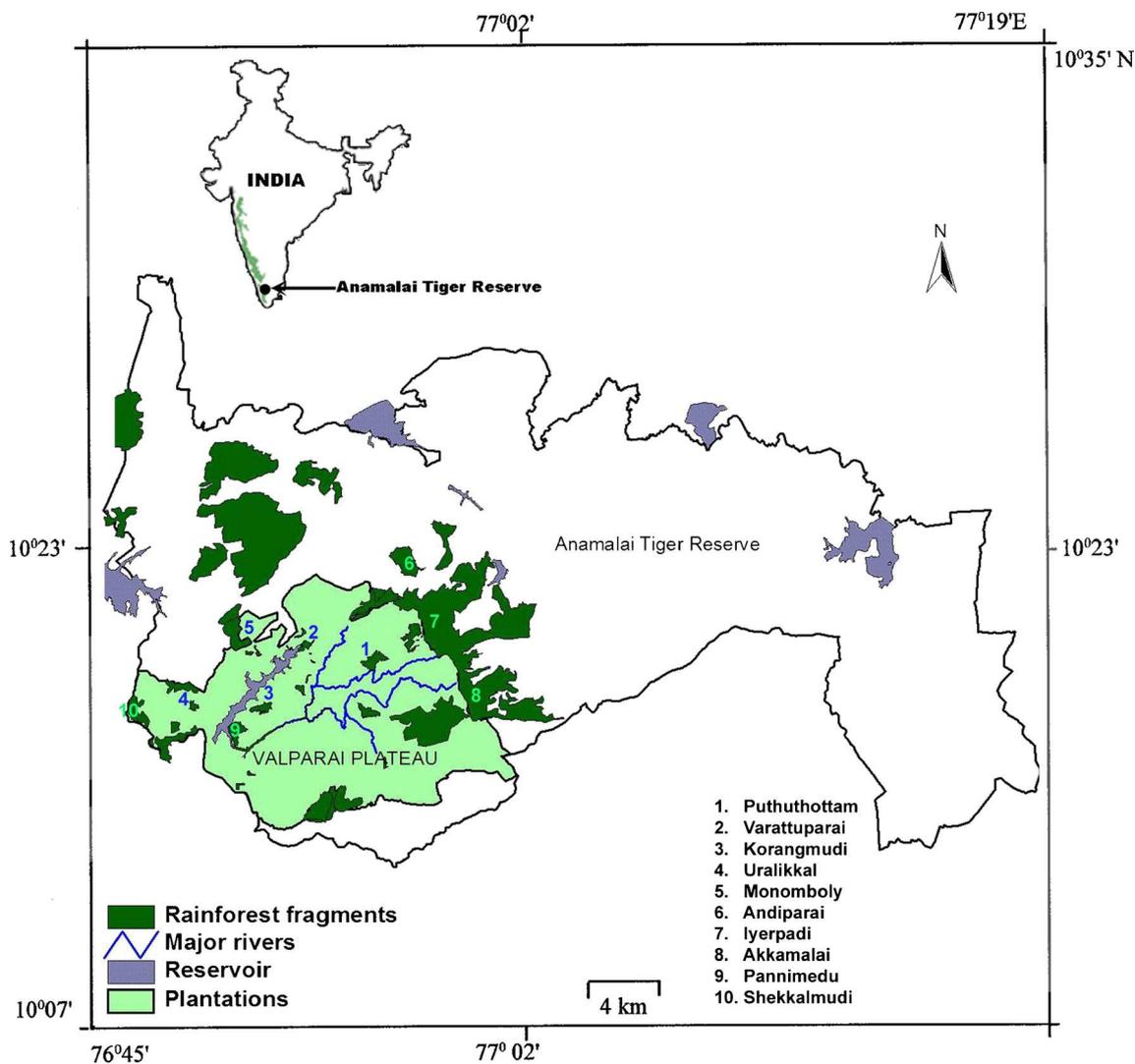
### 2.3 Parasite recovery and identification

About half of each sample was stored in the original vial for possible future examination. We used 2 g of fecal samples for parasite recovery by sucrose floatation and sedimentation techniques to examine for helminth eggs, larvae and protozoan cysts present in the sample (Gillespie 2006). We identified parasites based on their morphological traits such as size, wall structure, internal content and shape of various infective stages including eggs, cysts and larvae (Sloss *et al.* 1994; Nunn and Altizer 2005). We used iodine to identify protozoan cysts.

### 2.4 Data analyses

We defined parasite prevalence as the percentage of samples with a given parasite taxon and sample richness as the number of unique parasite taxa recovered from a sample (Sá *et al.* 2013). We compared the prevalence of each parasite taxon between forest fragments to test if they (parasite prevalence) are independent of each other. We applied chi-square tests of independence to test this hypothesis. The richness data did not follow the criterion of normality (data not shown), and hence, differences in richness among fragments were investigated using the nonparametric Kruskal-Wallis test, with the least significant difference (LSD) *post hoc* test used for pairwise comparisons. We used the Mann-Whitney U-test to detect differences in number of parasite taxa between fragments with and without human settlement.

We used data from all the hosts initially, while we removed data for wide-ranging host species (elephant, Indian gaur, leopard, wild dog) to investigate if their dispersal abilities between different forest fragments influence the estimated sample richness. We set the value of  $P < 0.05$  as significant for all our tests. Finally, we used Pearson correlation to explore the relationships between size of forest fragments and prevalence of parasites infecting mammalian hosts. We used SPSS version 17 (SPSS, Inc., Chicago, IL) for the statistical analyses. It is noteworthy, however, that complex parameters such as parasite prevalence and sample richness are likely



**Figure 1.** Locations of the 10 study sites across Anamalai Tiger Reserve, Western Ghats, Tamil Nadu, India.

influenced by multiple variables simultaneously and hence warrant, ideally, more advanced analytical methods such as generalized linear models. But, we also realize that our smaller sample size is inadequate for such powerful analysis. We, consequently, depended on simple yet standard methods of analyses, as our goal was to arrive at a basic and preliminary understanding of the system under study and establish a starting point for a larger and more intense future project.

### 3. Results

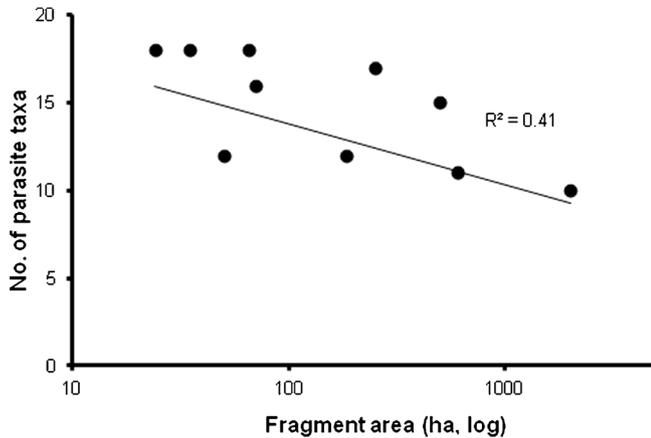
#### 3.1 Parasite richness

We identified 24 parasite taxa from 349 faecal samples, collected from 17 mammalian host species (supplementary tables 1 and 2). At least 20 of these parasite taxa are known

to be pathogenic to humans. Three forest fragments, namely, Varattuparai, Puthuthottam and Korangumudi, had the highest parasite richness of 18 taxa. On the other hand, both Akkamalai and Shekkalmudi recorded the lowest of 10. The number of parasite species observed from each fragment did not increase with sample size ( $r=0.58$ ,  $P=0.073$ ). Further, we found a negative correlation between size of the forest fragments and parasite richness ( $r=-0.64$ ,  $P=0.052$ ; figure 2). Forest fragments near human settlement had significantly higher parasite taxa ( $17.4\pm 0.89$ ) than those without settlement ( $12\pm 1.2$ ; Mann-Whitney U-test  $P=0.008$ ; figure 3).

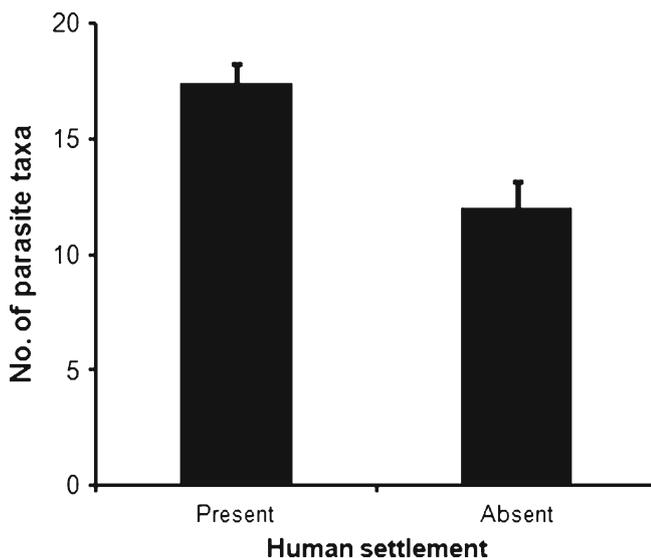
#### 3.2 Parasite prevalence

Almost 78% of the samples had, at least, one parasite taxon present. About 57% of the samples had more than one



**Figure 2.** Relationship between parasite richness and forest fragment area in Anamalai Tiger Reserve, Western Ghats, India.

parasite taxon. The presence of multiple-parasite infections was found to be as high as 73% when only infected samples were considered. We found that the roundworm *Ascaris* sp. was present in all of the hosts except the leopard (however, sample size,  $n=2$ ). *Ascaris* sp., along with *Gongylonema* sp., *Trichuris* sp. and *Toxocara* sp., was present across all of the 10 forest fragments we studied. Parasite taxa that were most common across forest fragments also appeared to be the prevalent ones, although, clearly, a considerable skew in prevalence exists across taxa (supplementary table 3). Unsurprisingly, therefore, we could not detect any



**Figure 3.** Number of parasitic taxa recorded in wild mammals from rainforest fragments with and without nearby human settlement in Anamalai Tiger Reserve, Western Ghats, India.

significant difference in parasite prevalence, on the basis of the chi-square test of independence, among forest fragments. Parasite richness across sampling sites, on the other hand, was significantly different (Kruskal-Wallis ANOVA,  $H=17.332$ ,  $P=0.044$ ). The least significant difference (LSD) test, consequently, revealed five pairs of fragments that differed from each other significantly. Monomboly, for instance, had higher sample richness ( $P=0.013$ ) than the geographically distant Iyerpadi fragment but closer Pannimedu ( $P=0.013$ ) and Shekkalmudi ( $P=0.047$ ) forest fragments (figure 1). Another smaller forest fragment, namely, Uralikkal, also exhibited a significantly higher sample richness than the geographically distant Iyerpadi ( $P=0.024$ ) but closer Pannimedu ( $P=0.025$ ; figure 1).

### 3.3 Number of parasite taxa per host

All of the host species studied harboured more than one parasite taxon, except the stripe-necked mongoose *Herpestes vitticollis* ( $n=2$ ). On average, we recorded approximately 9 parasite taxa per host species (mean=9.44,  $SD=5.45$ ), out of the total of 24 recovered taxa. The median number of parasites also was 9 ( $Q1=6.25$ ,  $Q3=14.25$ ). The host species that exhibited the presence of more than 14 different parasite taxa included barking deer (*Muntiacus muntjak*,  $n=44$ ), gaur (*Bos gaurus*,  $n=46$ ), sambar (*Cervus unicolor*,  $n=35$ ) and Indian porcupine (*Hystrix indica*,  $n=44$ ). The most common parasite taxa that were found to be present in more than 9 host species (median=5.5,  $Q1=2.75$ ,  $Q3=9.25$ ) were *Ascaris* sp., *Gongylonema* sp., *Trichuris* sp., *Baylisascaris* sp., *Fasciola* sp. and *Toxocara* sp.

## 4. Discussion

Our study reveals considerable gastrointestinal parasite diversity, chiefly helminths, in the nonhuman mammalian community in the fragmented rainforest landscape of the Anamalai Tiger Reserve, India. The parasite diversity was significantly higher in forest fragments with human settlement than those away, and the most common parasite taxa were also the most prevalent among the hosts and forest fragments. Furthermore, we also recorded 20 parasite taxa that are reported as pathogenic in humans and livestock.

We found all the major soil-transmitted helminth (STH) groups pathogenic to humans – common round worms (*Ascaris* sp.), whipworms (*Trichuris* sp.), hookworms (*Ancylostoma* sp. and *Necator* sp.), and pinworms or threadworms (*Strongyloides* sp.) – to be prevalent in the nonhuman mammalian community of the fragmented rainforest of Anamalai Tiger Reserve. We recorded the presence of common roundworms, a common human parasite worldwide, in all the 16 mammalian hosts except

leopards (n=2) and from all the 10 forest fragments (mean prevalence, 53.43%) with the highest prevalence of 69.23% in the Monomboly fragment. This 500 ha forest fragment (Umapathy and Kumar 2000) has moderate human presence in its fringes and a motorable road cuts across it (pers. obs.).

The other common parasites in all the forest fragments include *Gongylonema* sp., *Trichuris* sp. and *Toxocara* sp., which have mean prevalence of 35.89%, 27.64% and 9.57%, respectively. *Gongylonema* sp. is a spirurid nematode of vertebrate hosts with an indirect mode of transmission, usually through insect vectors (Mowlavi *et al.* 2009). We recorded the presence of these species in all the mammalian hosts studied except the wild boar (n=8), stripe-necked mongoose (n=2), wild dog (n=2) and leopard (n=2). Trichuriasis is one of the most important neglected tropical diseases (Hotez *et al.* 2009) and can transmit both indirectly (vector borne) or via soil (Bethony *et al.* 2006). Incidentally, we also recorded the highest prevalence (43.9%) of this parasite in the Monomboly fragment. *Toxocara* sp. belong to another group of zoonotic nematodes causing debilitating toxocariasis or larva migrans in humans (Smith *et al.* 2009), one of the major causes of infective blindness in humans (Schantz 1994).

We further recorded other parasites of considerable zoonotic risks from multiple forest fragments in our study sites. The hookworm *Ancylostoma* sp. was recorded from all the forest fragments except the Akkamalai forest fragment, which was poorly sampled (n=7). The other hookworm parasite, *Necator* sp., was only recorded from Varattuparai forest fragment, which is of smaller size (24 ha, Umapathy and Kumar, 2000), highly fragmented, and lies at the heart of the human-modified Valparai landscape of the Anamalai hills (pers. obs.). We additionally found *Nematodirus* sp., *Baylisascaris* sp., *Fasciola* sp., *Trichostrongylus* sp., *Moniezia* sp., *Enterobius* sp., *Strongyloides* sp. and *Bunostomum* sp., all of which are either parasitic to humans or their livestock (Ash and Orihel 1990; Bethony *et al.* 2006; Hotez *et al.* 2008, 2009). The presence of *Strongyloides* sp. and *Schistosoma* sp., one of the 13 core neglected tropical parasites in wild mammals, is important for public health management (Hotez *et al.* 2009). While *Strongyloides* sp. were present in all the forest fragments, *Schistosoma* sp. were present only in the Varattuparai forest fragment. Among cestodes, we recorded the presence of *Moniezia* sp., *Hymenolepis* sp., and *Diphyllobothrium* sp., which are known parasites to humans, across our study sites. Among these parasitic worms, *Hymenolepis* sp. and *Diphyllobothrium* sp. appeared to be relatively rare in the Anamalai Tiger Reserve, recorded only in three of the forest fragments each – *Hymenolepis* sp. in Iyerpadi, Uralikkal and Varattuparai forest fragments, and *Diphyllobothrium* sp. in Monomboly, Puthuthottam and Korangumudi forest fragments.

We recorded only one protozoan, *Giardia* sp., in the faecal samples. This may be the outcome of the destruction of other protozoan cysts due to storage of faecal samples in formalin and, in all likelihood, not a true representative of protozoan diversity in the nonhuman mammalian hosts of the Anamalai hills. *Giardia* sp. are common symbionts/parasites of multiple vertebrate taxa and often engage in cross-species transmissions and zoonosis (Hunter and Thompson 2005). They were present in the forest fragments of Monomboly, Uralikkal, Varattuparai, Puthuthottam, Korangamudi and Shekkalmudi, all of which are exposed to between moderate and high levels of human presence. Humans, living around these forest fragments, frequently enter these patches with their livestock and exploit forest resources including water holes (Hussain *et al.* 2013; pers. obs.). Additionally, many mammalian hosts, particularly species with large home ranges, often stray into or raid human habitations for food or water and consequently share water resources with humans and their livestock (Kumar *et al.* 2010; Hussain *et al.* 2013). These water resources, which potentially are the shedding grounds of *Giardia* sp. cysts, eventually may result in spread of human *Giardia* sp. infection in wildlife hosts. Of the seven species of *Giardia* sp., *G. intestinalis* is known to infect humans, cattle, dogs and cats, and cause gastrointestinal distress. However, species like *G. muris* is host specific and transmission to humans is rather rare (Johnston *et al.* 2010; Lane *et al.* 2011). Studies have shown that *Giardia* sp. infections increase with increasing contact with humans and livestock across multiple wild primate species (Johnston *et al.* 2010; Kowalewski *et al.* 2011; Lane *et al.* 2011). Interestingly, four out of five host species – barking deer, sambar, Indian porcupine and Asian elephant – that were found to be infected with *Giardia* sp. are known to regularly use roads, plantations and agricultural fields in the study area (pers. obs.). Furthermore, the lion-tailed macaque (n=7), which has been found recently to have increasing contacts with humans, particularly in the Valparai Plateau, exhibited the presence of *Giardia* sp. Interestingly, the Nilgiri langur, a wild primate, potentially with the least human contact among the three primate species studied, did not exhibit any *Giardia* sp. infections, in spite of its fairly large sampling (n=34), suggesting, perhaps, another instance of increase in *Giardia* sp. infection with increasing human contact with wild primates. Surprisingly, however, bonnet macaques, the wild primate species from Anamalai hills that comes in regular contact with humans, did not show any presence of these infections. This outcome may be attributed to bias from the limited sampling (n=7) of the species.

The distribution of parasite taxa across individual samples is also highly dispersed as our results show that most samples contained few, between one and three parasite species, while only few others harboured more than five. It, thus,

appears that the observed aggregation level of multiple parasites within each sample is restricted to a relatively narrow range. Such a pattern is recurrent in ecological parasitology, for, if the aggregation level of multiple parasite species in one individual is high, then that may lead to increased inter-specific competition between parasite species and high host mortality (Anderson and May 1978; Poulin 2007; Tompkins et al. 2011).

Parasite richness was found to be highest in Varattuparai, Puthuthottam and Korangamudi forest fragments, which are small in size, isolated and located in the middle of the human-dominated landscape. This result is in agreement with previous studies from Anamalai hills and elsewhere, which found high parasite richness in species that live in fragmented habitats and closer to human habitations (Gillespie et al. 2005; Hussain et al. 2013; Sá et al. 2013). Thus, it is possible that smaller forest patches, which are highly fragmented, may, actually, manifest considerably higher parasite richness than their larger and continuous counterparts. However, we also found that other fragments, such as Monomboly and Uralikkal, also harbour considerable diversity of parasites in spite of them being relatively larger and continuous. The number of host species may also be significantly related to number of parasites detected from a habitat (Telfer and Bown 2012). We, however, did not find that the number of mammalian species had a considerable influence on the detection of number of parasites in the present study, given that the number of mammal species sampled per fragment was more or less the same (mean=8.77, SD=1.09), except, of course, the poorly sampled Akkamalai fragment (number of mammalian species sampled=2). Additionally, Shekkalmudi (9 species of mammals), with the lowest richness for parasites, in spite of being equal in number of mammalian species to Puthuthottam and Korangamudi, that themselves recorded highest parasite richness, attests to this fact. Additionally, we found significant difference in sample richness between only few forest fragments. Among these fragments, Monomboly again showed significantly higher sample diversity from other closer and one distant (Iyerpadi) fragments. Such contradictions, therefore, hint at the effect of local factors and their complex interplay with more large-scale factors such as fragment size, in influencing parasite diversity in the tropical rainforest fragments of the Anamalai Tiger Reserve.

A previous study (Hussain et al. 2013) from the Anamalai Tiger Reserve found the prevalence and richness of gastrointestinal parasites of the endangered lion-tailed macaques to be correlated with habitat fragmentation and high anthropogenic activities. While our present study suggests a potential relation between habitat fragmentation and parasite diversity, it further emphasizes the presence of complex mechanisms driving parasite infection among different mammalian hosts. It is also

clear from our parasitological exploration that many mammalian wild species can play an important role as reservoirs for many critically important, yet neglected, tropical diseases.

Finally, it must also be noted that many parasites recorded in the present study may actually originate from humans and their livestock living within the landscape and coming in regular contact with the wildlife, directly and indirectly. Such transmissions of parasites from humans to wildlife, many of which are already highly threatened, can cause a crash of wildlife populations and may drive species locally extinct (Hussain et al. 2013; Daszak et al. 2000). It is thus clear that the presence of potentially shared parasites in the wildlife community of Anamalai Tiger Reserve poses a serious threat to the conservation of already stressed wildlife populations. It is thus imperative that a detailed and analytical investigation into both the potential ecological drivers of parasite infection and their relation to emerging infectious diseases is essential at this point. Such understanding will be important for the conservation of already endangered wildlife as well as for staving off zoonotic disease risk, particularly to marginal societies that live close to wilderness.

#### Acknowledgements

We thank the Chief Wildlife Warden, Tamil Nadu Forest Department, India, for permitting sample collection from the Anamalai Tiger Reserve, India. We also thank the private owners of Valparai plateau forest remnants for their permission, help and cooperation during this study. This study was funded by the Department of Biotechnology, India, and the Central Zoo Authority of India. We also thank Mr Prakash for his help in sample collection and guidance in the field.

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*MS received 16 August 2014; accepted 03 March 2015*

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