RESEARCH ARTICLE

Altitude and human disturbance are associated with helminth diversity in an endangered primate, *Procolobus gordonorum*

Claudia Barelli[ORCID][1,2]*, **Viviana Gonzalez-Astudillo**[3,4], **Roger Mundry**[5], **Francesco Rovero**[2,6], **Heidi C. Hauffe**[1], **Thomas R. Gillespie**[ORCID][3,7]*

1 Department of Biodiversity and Molecular Ecology, Research and Innovation Centre, Fondazione E. Mach, San Michele all'Adige, Trento, Italy, **2** MUSE–Science Museum, Tropical Biodiversity Section, Trento, Italy, **3** Department of Environmental Sciences and Program in Population Biology, Ecology and Evolutionary Biology, Emory University, Atlanta, GA, United States of America, **4** Pathology Resident, California Animal Health & Food Safety Laboratory, University of California, Davis, CA, United States of America, **5** Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany, **6** Department of Biology, University of Florence, Florence, Italy, **7** Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA, United States of America

* barelli.cla@gmail.com (CB); thomas.gillespie@emory.edu (TRG)

Abstract

Gastrointestinal parasites colonizing the mammalian gut influence the host immune system and health. Parasite infections, mainly helminths, have been studied intensively in both humans and non-human animals, but relatively rarely within a conservation framework. The Udzungwa red colobus monkey (*Procolobus gordonorum*) is an endangered endemic primate species living in the Udzungwa Mountains of Tanzania, a global biodiversity hotspot. Since this endemic primate species is highly sensitive to human disturbance, here we investigate whether habitat type (driven by natural and human-induced factors) is associated with helminth diversity. Using standard flotation and sedimentation techniques, we analyzed 251 fecal samples belonging to 25 social groups from four different forest blocks within the Udzungwa Mountains. Five parasitic helminth taxa were recovered from Udzungwa red colobus, including *Trichuris* sp., *Strongyloides fulleborni*, *S. stercoralis*, a strongylid nematode and *Colobenterobius* sp. We used Generalized Linear Mixed Models to explore the contribution of habitat type, altitude and fecal glucocorticoid levels (as biomarkers of stress) in predicting gut parasite variation. Although some parasites (e.g., *Trichuris* sp.) infected more than 50% of individuals, compared to others (e.g., *Colobenterobius* sp.) that infected less than 3%, both parasite richness and prevalence did not differ significantly across forests, even when controlling for seasonality. Stress hormone levels also did not predict variation in parasite richness, while altitude could explain it resulting in lower richness at lower altitudes. Because human activities causing disturbance are concentrated mainly at lower altitudes, we suggest that protection of primate forest habitat preserves natural diversity at both macro- and microscales, and that the importance of the latter should not be underestimated.

Introduction

Gastrointestinal parasitic worms (mainly helminths) represent the most prevalent infectious agents affecting nearly one-third of the human population, as well as most livestock and wildlife, especially in the tropics and in developing countries [1, 2, 3]. Although helminths cause considerable human morbidity and mortality worldwide [4] with a notable economic impact, their absence in the gut biota of humans consuming a 'western' diet has been widely associated with an increased prevalence of auto- and hyper- immune diseases [5, 6]. This suggests that helminths are an essential player in host gut homeostasis and health. While it is widely accepted that human activities causing habitat disturbance or loss (especially encroaching agriculture and logging) lead to loss in species diversity at a macro scale [7], we know far less about the impact of these activities on biodiversity at a micro-scale, such as that of the gastrointestinal tract.

Animals living in fragmented and disturbed habitats are more likely to experience habitat saturation leading to higher population density, reduced food availability and elevated stress hormone levels (leading to lowered immune system function) [8, 9]; all factors which facilitate the transmission of helminths [10, 11, 12]. Some studies indicate that the proportion of infected hosts (parasite prevalence) and/or the number of different parasite taxa infecting each host (parasite richness) increase with increasing habitat disturbance [11, 13–16]. However, opposite associations [17, 18; 19] or no relationships have also been found [20].

Tropical non-human primates live in pristine forests that are constantly reduced by human activities, making primates one of the most threatened taxa on the planet [21]. Exploring whether human-induced habitat changes may affect also biodiversity at micro scale, such as that of gastrointestinal parasites, is urgent and relevant for the plausible implications on animal health and conservation. We addressed this question in the Udzungwa red colobus (*Procolobus gordonorum*), an endangered primate endemic to the forests of the Udzungwa Mountains (hereafter Udzungwas) in south-central Tanzania. The area is of critical importance for biological diversity and endemism [22, 23, 24]. However, it is also increasingly threatened by intensive agriculture [25], subsistence logging and harvesting [21, 24] making primate populations particularly vulnerable to hunting [26, 27]. Forests within the Udzungwas are currently fragmented into numerous blocks, widely different in habitat structure and exposure to anthropogenic disturbance (from national park to forest reserve or completely unprotected). Because the climate of the area shows seasonal variations and the study sites vary widely in altitude gradient (within and among study sites), natural environmental factors need to be also taken into account when predicting parasite infections [28–32]. Hence, we here explore the variation in parasite prevalence and richness in the endemic and endangered Udzungwa red colobus living in natural and modified habitats.

Materials and methods

Study site

The Udzungwas represent the southernmost mountain block in the Eastern Arc Mountains and occupy an area of approximately 19,000 km^2 (7˚40' S to 8˚40' S and 35˚10' E to 36˚50' E; Fig 1). Average annual rainfall varies from 1,500 to 2,000 mm per year, distributed into two main seasons: November-December and March-May.

Social groups of the endangered and endemic Udzungwa red colobus monkeys were studied in four forest blocks: Magombera (MA), Uzungwa Scarp Nature Reserve (US), Matundu (MT) and Mwanihana (MW). Long-term studies on such forest blocks have already assessed and revealed differences in habitat structure, vegetation parameters and human disturbance,
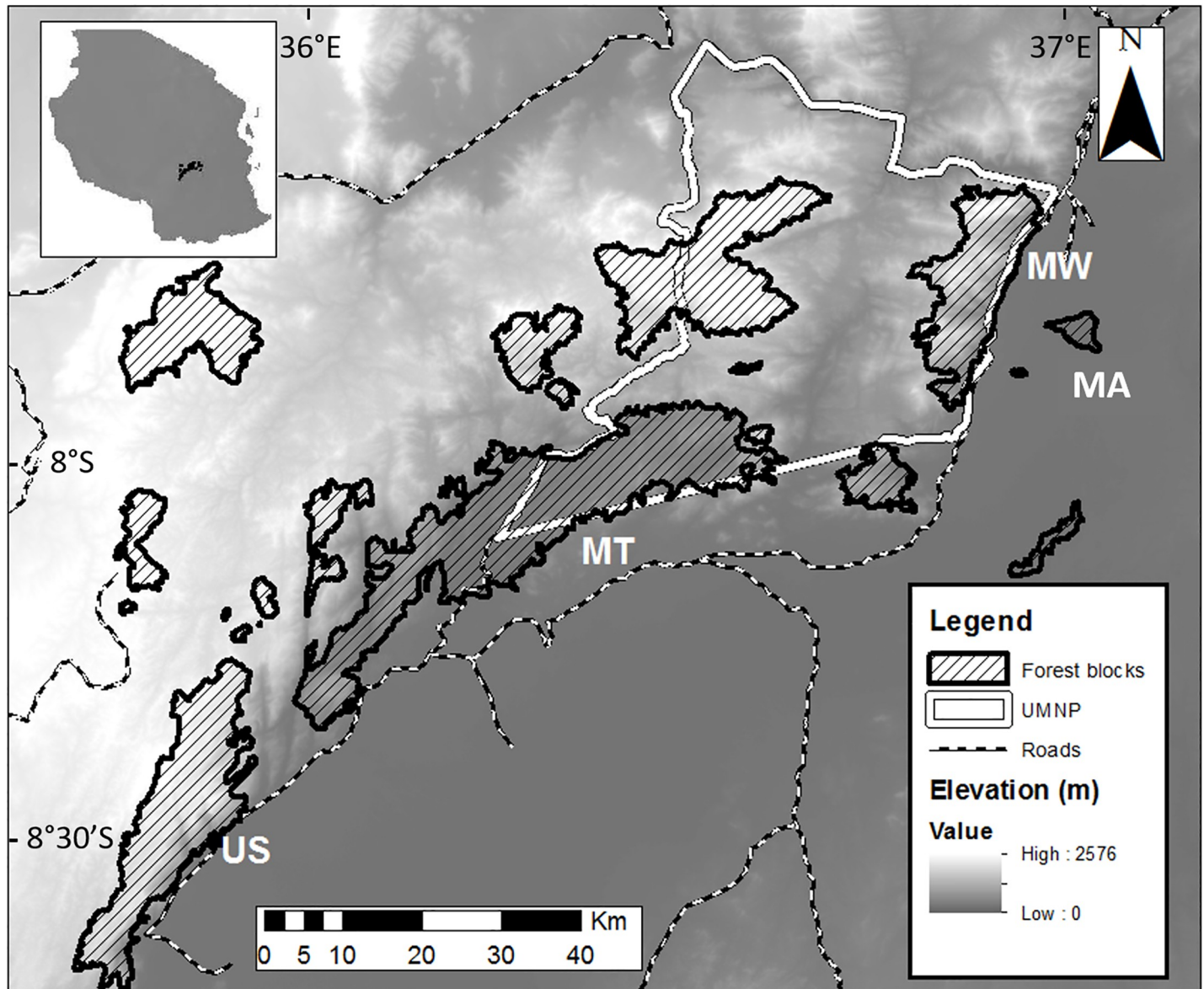
Fig 1. Map of the study sites. Map of the Udzungwa Mountains of Tanzania showing the four forest blocks sampled (MA: Magombera, MT: Matundu, MW: Mwanihana, US: Uzungwa Scarp Nature Reserve). The borders of the Udzungwa Mountain National Park (UMNP) are highlighted in white. (Original map from [33]).

https://doi.org/10.1371/journal.pone.0225142.g001

highlighting plausible associations between both primate population density and their genetic diversity to habitat type and human-driven changes [Table 1; 34, 35,36]. Moreover, all forest blocks have been separated from each other for over 60 years by at least 6 km (longer than the maximum dispersal range of the study species), with urbanized or agricultural areas concentrated at lower altitude or at forest edges [35].

Sample collection and parasitological analyses. In order to avoid disturbing this shy and elusive species, from July 2011 to October 2012, four trained field assistants followed social groups from a distance of approximately 10–15 meters as they moved through the forest canopy, and collected fecal samples for parasitological analysis opportunistically. Since fecal samples could not be assigned to a specific individual or sex, to avoid any potential re-sampling, samples were collected during a single defecation event from each group, and sampled groups

Table 1. Characteristics of the four study forests in the Udzungwa Mountains of Tanzania (MA, US, MT, MW) ordered by the degree of human impact and level of protection, from the most disturbed and least protected (MA) to the least disturbed and most protected (MW).

Forest	Area (km^2)	Altitude (m a. s.l.)	Density (groups/km^2) (SE)	Habitat structure, protection level and human activities
Magombera (MA)	11.9	269–302	4.88 (0.97)	Ground-water lowland evergreen forest; one of the few remaining patches of once continuous lowland forest; surrounded by villages and intensive agriculture. No formal protection; the forest is shrinking in size and frequently encroached for firewood collection, pole cutting and hunting
Uzungwa Scarp (US)	314.5	290–2,144	1.2 (0.34)	Lowland, semi-deciduous, sub-montane and montane evergreen forest, including upper montane, bamboo-dominated forest. Nature Reserve since 2016; no resource extraction allowed, but pole and timber cutting, as well as hunting are illegally practiced; several villages along the border
Matundu (MT)	526.3	279–1,046	2.4 (0.41)	Lowland to sub-montane evergreen to deciduous forest, with large portions logged in the past, now secondary, regenerating vegetation with low tree diversity. National Park since 1992, with a smaller portion (unsampled in this study) falling in Kilombero Nature Reserve.
Mwanihana (MW)	150.6	351–2,263	1.83 (0.33)	Continuous forest escarpment, similar to US. National Park since 1992; several villages along the eastern edge.

Data on forest area and altitude from [36, 37].

https://doi.org/10.1371/journal.pone.0225142.t001

were at least 2 km apart (N = 25 groups and 251 fecal samples; S1 Table). Sampling procedures are described in more detail in [35, 38, 39]. All samples were examined macroscopically for consistency, and presence of blood, mucus, tapeworm proglottids and adult and larval nematodes. An aliquot of 2 g of fresh feces was placed in a polypropylene tube containing 10 ml of 10% neutral buffered formalin and stored it at ambient temperature (20–25˚C) until shipment to Emory University, USA for parasitological analysis.

The eggs and larvae of metazoan parasites were recovered via sodium nitrate flotation and fecal sedimentation utilizing standard techniques as previously described [40]. Slides were examined under a compound microscope at 400X magnification, and parasite identification was performed on the basis of the size, shape, and contents of the egg and larvae. If needed, a drop of Lugol's iodine solution was added to the sample to assist in parasite identification. Representative examples were measured to the nearest 0.1 μm with an ocular micrometer with Leica computer software. Parasite quantification was carried out by examining one slide per sample as previously described [41], by removing the cover slip from the flotation tube and placing it on a slide. We scanned slide using the × 10 objective lens of a compound microscope and identified and counted all parasite eggs and larvae. We then used the × 40 objective lens for measurement and confirmation of identifications. We measured the length and width of individual eggs and larvae using a calibrated ocular micrometer and photographed representatives.

Assessment of stress hormone levels. To assess the link between physiological stress and parasite infections, fecal glucocorticoid (FGC) levels were determined for a subset of 202 samples collected from 20 groups between 8h00 and 10h00 directly following defecation (S1 Table). Fresh feces were homogenized with gloved hands, and any obvious undigested matter was removed. For each sample, approximately 0.5 g of stool was placed in a 15ml polypropylene tube pre-filled with 4 ml of 80% ethanol, and the tube was shaken manually for 30 seconds to produce a fecal suspension [38, 42, 43]. We used Parafilm (Pechiney Plastic Packaging Company, USA) to avoid evaporation and leakage until return to the research station where we kept all samples in the dark at room temperature for 15 days before extraction. All samples underwent exactly the same duration of storage in alcohol, eliminating any potential storage-time dependent variation in FGC levels [44]. Prior to extraction, tubes containing the fecal suspension were weighed to determine fecal wet weight to the nearest 0.001g, and fecal samples were extracted following established procedures [43, 45]. In summary, the tube containing the fecal suspension was shaken firmly for 2 minutes, centrifuged at high speed for 2 min using a

manually operated centrifuge (Hettich GmbH & Co. KG Tuttlingen, Germany). Two ml of the supernatant (containing the dissolved steroids) was pipetted into a 2 ml polypropylene tube (PPT; SafeSeal Micro Tube; Ref. No. 72.695.200 from Sarstedt AG & Co. Nuernbrecht, Germany), and sealed them with Parafilm and stored them in the dark at ambient temperature (20–25˚C). The latter procedure has been shown to be effective for storing fecal extracts long-term without affecting FGC levels [46, 47].

Ethics statement. The authors confirm they did not interact with the red colobus social groups in any way. Fecal sample collection was conducted using non-invasive methods and adhered to the 'Code of Best Practices for Field Primatology' of the International Primatological Society (IPS) and to the 'Principles for the Ethical Treatment of Primates' of the American Society of Primatologists (ASP). The study was conducted with permission to CB and FR from the Tanzania Commission for Science and Technology (COSTECH Permits No. 2011-85-NA-2011-33; 2011-84-NA-2011-33; 2011-351-NA-2011-68; 2011-346-NA-2011-183), Tanzania Wildlife Research Institute (TAWIRI) and Tanzania National Parks (TANAPA). All applicable institutional and/or national guidelines for the care and use of animals were followed.

Data analyses

Comparing parasite prevalence among forests (model 1). To compare parasite prevalence (number of hosts infected with one or more individuals of a particular parasite taxon in each fecal sample [48, 49] (for definition) among forests, we used a Generalized Linear Mixed Model (GLMM) [50], one for each parasite taxon, with binomial error structure and logit link function [51, 52]. As response variables we used parasite prevalence per fecal sample for the three most common taxa (*Trichuris* sp., *Strongyloides fulleborni*, strongylid nematode) separately, because the other two (*S. stercoralis* and *Colobenterobius* sp.) were found in only two and four samples respectively. We included forest block as a fixed effect, and social group as a random intercepts effect. To further account for potential seasonal variation in parasite load, we also included the sine and cosine of Julian date (after turning it into radians by first dividing by 365.25 and then multiplying by $2 \times \pi$) [53]. The data for these models included 211 fecal samples from 21 groups.

Comparing parasite richness among forests (model 2). Similarly, we compared parasite richness (number of different parasite types per sample) among forests by using a GLMM with binomial error structure and logit link function and with the same fixed and random effects used in model 1. However, we included one additional random effect for the sample ID to account for the non-independence of parasite presence and absence from the same sample. This model essentially modeled the proportion of all five parasite taxa found per sample, including samples in which no parasites were found. Overdispersion was also not an issue (dispersion parameter = 0.838) and data comprised 251 fecal samples from 25 groups.

Comparing parasite prevalence and richness among groups within forests. To compare parasite prevalence among groups of the same forest, we considered the variance estimated for the contribution of group in the above models (for reasons of comparability with the estimates obtained for the fixed effects, we report the respective standard deviations). We do not provide a formal significance test because the degrees of freedom associated with an estimated random effect are currently not known [54].

Parasite richness as a function of altitude and FGC level (model 3). This model estimated the proportion of possible parasites a sample was infected with as a function of altitude or FGC levels (i.e., it was a GLMM fitted with binomial error structure and logit link function). As fixed effects predictors, we included forest, FGC levels per group (averaged after square root transformation), altitude (square root transformed after subtracting the minimum

altitude) per group, and season as described for model 1. Furthermore, we included random intercepts effects for group and sample ID. As an overall test of the effect of altitude and stress hormone level, we compared this full model with a null model lacking these factors but being otherwise identical [55]. The predictors date and altitude were moderately collinear with one another and with population [56, 57] with a maximum Generalized Variance Inflation Factor (GVIF; taken to the power of 1/twice the degrees of freedom of the respective effect, then squared) of 5.51 [58]; however, a visual inspection of Julian date plotted against altitude indicated that there was sufficient variation in both across their ranges. Furthermore, both altitude and sample date varied considerably within populations. Hence, model results are unlikely to suffer from collinearity. Overdispersion was no issue (dispersion parameter = 0.843). The data for this model comprised 202 samples taken from 20 groups.

Parasite prevalence as a function of altitude and stress hormones (model 4). These models were identical to model 1 with the exception that we added altitude and stress hormone levels as described in model 3. The data used for all these models comprised 162 samples taken from 16 social groups. Collinearity among predictors was not evident (maximum GVIF taken to the power of 1/twice the degrees of freedom of the respective effect and then squared = 6.186; see model 3), nor was overdispersion (range of dispersion parameters: 0.761 to 0.938). We conducted a full-null model comparison as described for model 3.

General considerations. We conducted the analyses in R (version 3.5.3) [59]. For GLMMs we used the function 'glmer' in the package lme4 (version 1.1–21) [60] or glmmTMB (version 0.2.3 from the package glmmTMB (0.2.3) [61] in case of models with a negative binomial error distribution. We determined Generalized Variance Inflation Factors using the function 'vif' of the package car [62]. We estimated model stability by excluding groups one at a time and comparing model estimates obtained for the subsets with those obtained for the full data set. We used a parametric bootstrap to obtain confidence intervals of model estimates (function bootMer of the package lme4 or simulate.glmmTMB of the package glmmTMB). Throughout we determined the significance of individual effects or groups of terms by using likelihood ratio tests [63] comparing a respective full model with a reduced model lacking the effect(s) to be tested [64]. The reason that we fitted models 3 and 4 in addition to models 1 and 2 rather than including altitude and stress hormone levels into model 1 and 2 was that we did not have FGC levels for all samples but wanted to use a sample as large as possible for the group comparisons.

Results. Five different gastrointestinal parasites were recovered from fecal samples of the Udzungwa red colobus monkeys: *Trichuris* sp., *Strongyloides fulleborni*, *S. stercoralis*, *Colobenterobius* sp. and one strongylid nematode (egg morphology did not permit species identification; Table 2; Fig 2). We identified at least one nematode species in 72% (181/251) of fecal samples.

Table 2. Overall prevalence (expressed in %) of gastrointestinal parasites in the endemic and endangered Udzungwa red colobus monkeys collected in four forests within the Udzungwa Mountains of Tanzania (MA: Magombera, US: Uzungwa Scarp Nature Reserve, MT: Matundu, MW: Mwanihana).

Parasite type	Forests*			
	MA (*n* = 66)	US (*n* = 44)	MT (*n* = 60)	MW (*n* = 81)
Trichuris sp.	39.4	63.6	43.3	60.5
strongylid nematode	7.6	29.5	3.3	37.0
Strongyloides fulleborni	15.2	13.6	28.3	18.5
Strongyloides stercoralis	0.0	4.5	0	0
Colobenterobius sp.	1.5	2.3	0	2.5

* Forests are ordered by protection level and degree of human impact, from least protected and most disturbed (MA forest) to most protected and least disturbed regarding human impact (MW forest).

Number of samples (*n*) analyzed per forest are expressed in brackets.
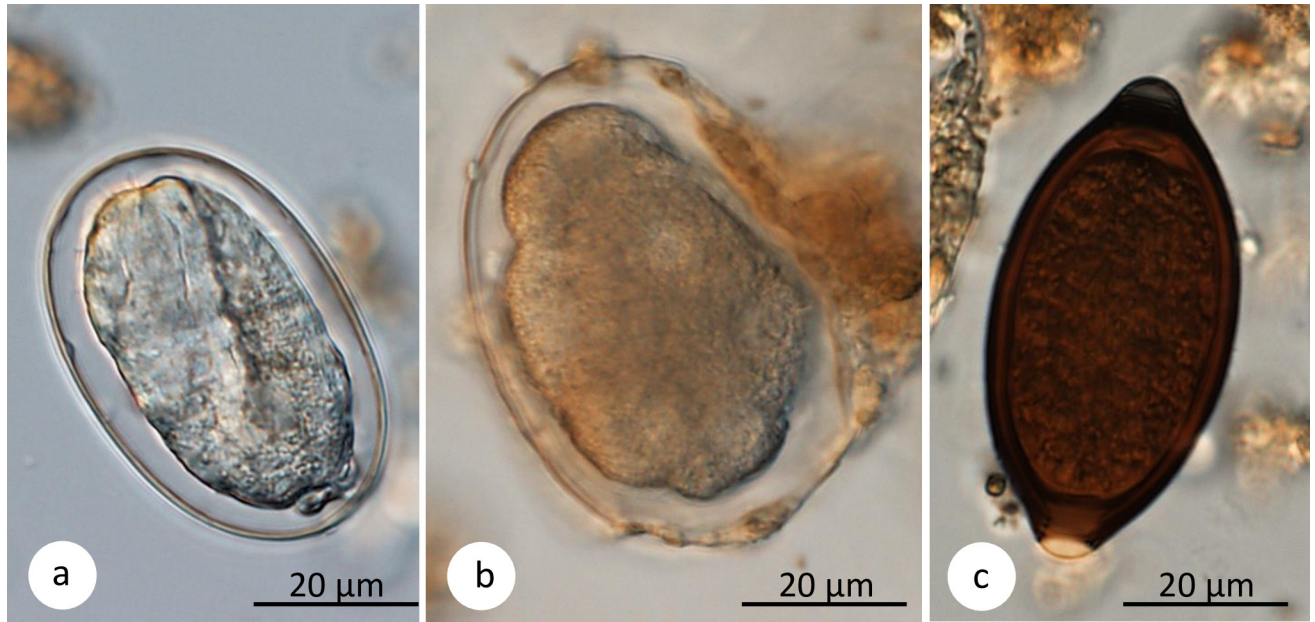
Fig 2. Parasite eggs. Sample pictures of gastrointestinal parasites found by sedimentation and flotation methods in Udzungwa red colobus monkeys: (a) *Strongyloides fulleborni*, (b) strongylid nematode, (c) *Trichuris* sp. Scale bars: 20 μm.

The prevalence of these parasites differed markedly with *Trichuris* sp. infecting more than 60% of individuals in two forests (MW and US) and *Colobenterobius* sp. infecting 2.5% of individuals (in MW; Table 2).

Parasite prevalence among forest blocks (model 1). Parasite prevalence did not differ significantly among forests (likelihood ratio tests, *Trichuris* sp.: $\chi^2 = 4.155$, df = 3, P = 0.245; *S. fulleborni*: $\chi^2 = 4.371$, df = 3, P = 0.224; strongylid nematode: $\chi^2 = 3.779$, df = 3, P = 0.286). Furthermore, none of the three models revealed a significant seasonal variation in parasite prevalence (*Trichuris* sp.: $\chi^2 = 2.422$, df = 2, P = 0.298; *S. fulleborni*: $\chi^2 = 5.616$, df = 3, P = 0.060; strongylid nematode: $\chi^2 = 0.847$, df = 2, P = 0.655; Table 3).

Parasite richness among forest blocks (model 2). We found no significant differences in parasite richness among individuals of different forests ($\chi^2 = 5.949$, df = 3, P = 0.114). Moreover, the effect of season also appeared non-significant ($\chi^2 = 0.158$, df = 2, P = 0.924; Table 4, Fig 3).

Comparisons of groups within forests. Furthermore, across groups living in the same forest, we found clear differences in parasite prevalence only in part (estimated standard deviations for the contribution of the random effect of group, *Trichuris* sp.: <0.001.; *S. fulleborni*: 0.361; strongylid nematode: 1.486). In fact, most of these estimates were roughly of the same order of magnitude as the estimated fixed effects (Table 3), showing that variation among groups was about as large as variation due to differences among forests or seasonal variation. However, with regard to parasite richness, variation among groups was very low (0.0001).

Parasite richness as a function of altitude and stress hormones (model 3). Overall, parasite richness was influenced by the factors investigated (full null comparison: $\chi^2 = 7.727$, df = 2, P = 0.021). More specifically, parasite richness increased with altitude (Table 5; Fig 4). Stress hormone levels was not significantly associated with parasite richness, nor were season or forest (Table 5).

Table 3. Results of models predicting parasite prevalence (response) across forests (MA: Magombera; US: Uzungwa Scarp; MT: Matundu, MW: Mwanihana).

Term[1]	Estimate	SE	lower Cl	upper Cl	min	max
Trichuris sp.						
Intercept	0.038	0.432	-0.817	0.976	-0.282	0.435
MT	-0.665	0.792	-2.455	0.844	-1.317	-0.213
MW	0.429	0.446	-0.462	1.334	0.233	0.984
US	0.474	0.585	-0.741	1.649	0.255	1.089
sin(date.rad)	0.074	0.456	-0.792	1.017	-0.119	0.537
cos(date.rad)	-0.493	0.323	-1.226	0.115	-0.788	-0.149
strongylid nematode						
intercept	-3.184	1.206	-7.699	-0.761	-4.083	-2.389
MT	-1.035	2.405	-19.536	3.773	-16.977	1.460
MW	1.660	1.458	-2.041	5.157	0.326	4.730
US	0.446	1.877	-4.446	5.275	-0.897	4.146
sin(date)	-1.157	1.439	-5.191	1.301	-1.779	1.751
cos(date)	-0.530	0.929	-2.901	1.212	-1.291	1.418
Strogyloides fulleborni						
Intercept	-2.667	0.693	-4.593	-1.457	-3.729	-2.318
MT	2.245	1.225	-0.677	5.251	1.652	4.129
MW	1.163	0.600	-0.143	2.430	0.882	1.767
US	1.367	0.872	-0.558	3.332	1.089	2.092
sin(date)	0.330	0.659	-1.227	1.713	-0.222	0.690
cos(date)	1.156	0.507	0.177	2.710	0.882	2.099

[1] population was dummy coded with MA being the reference category; sin(date) and cos(date) model potential seasonal variation in parasite prevalence.

Estimates, together with standard error (SE), lower and upper confidence interval (Cl) and minimum (min) and maximum (max) of estimates obtained when excluding groups one at a time are reported.

https://doi.org/10.1371/journal.pone.0225142.t003

Parasite prevalence as a function of altitude and stress hormones (model 4)

The results for the models estimating the effects of altitude and stress hormone levels on parasite prevalence depended on the taxon considered. While for two taxa the full null model comparison was not significant (*Trichuris* sp.: $\chi^2 = 0.733$, df = 2, P = 0.693; *S. fulleborni*: $\chi^2 = 0.085$, df = 2, P = 0.958), for the strongylid nematode it was clearly significant ($\chi^2 = 14.448$, df = 2, P = 0.001; Table 6).

Table 4. Results of the model predicting parasite richness (response) across forests (MA: Magombera; US: Uzungwa Scarp; MT: Matundu, MW: Mwanihana).

	Estimate	SE	lower Cl	upper Cl	min	max
Intercept[1]	-1.972	0.261	-2.556	-1.467	-2.330	-1.779
US	0.622	0.367	-0.177	1.380	0.482	1.535
MT	0.293	0.363	-0.396	1.103	-0.376	0.517
MW	0.727	0.285	0.130	1.295	0.495	1.485
sin(date)	-0.118	0.295	-0.754	0.430	-0.355	0.647
cos(date)	-0.011	0.182	-0.403	0.351	-0.151	0.452

[1] population was dummy coded with MA being the reference category; sin(date) and cos(date) model potential seasonal variation in parasite richness

Estimate, together with standard error (SE), lower and upper confidence limit (Cl) and minimum (min) and maximum (max) of estimates obtained when excluding groups one at a time are reported.

https://doi.org/10.1371/journal.pone.0225142.t004
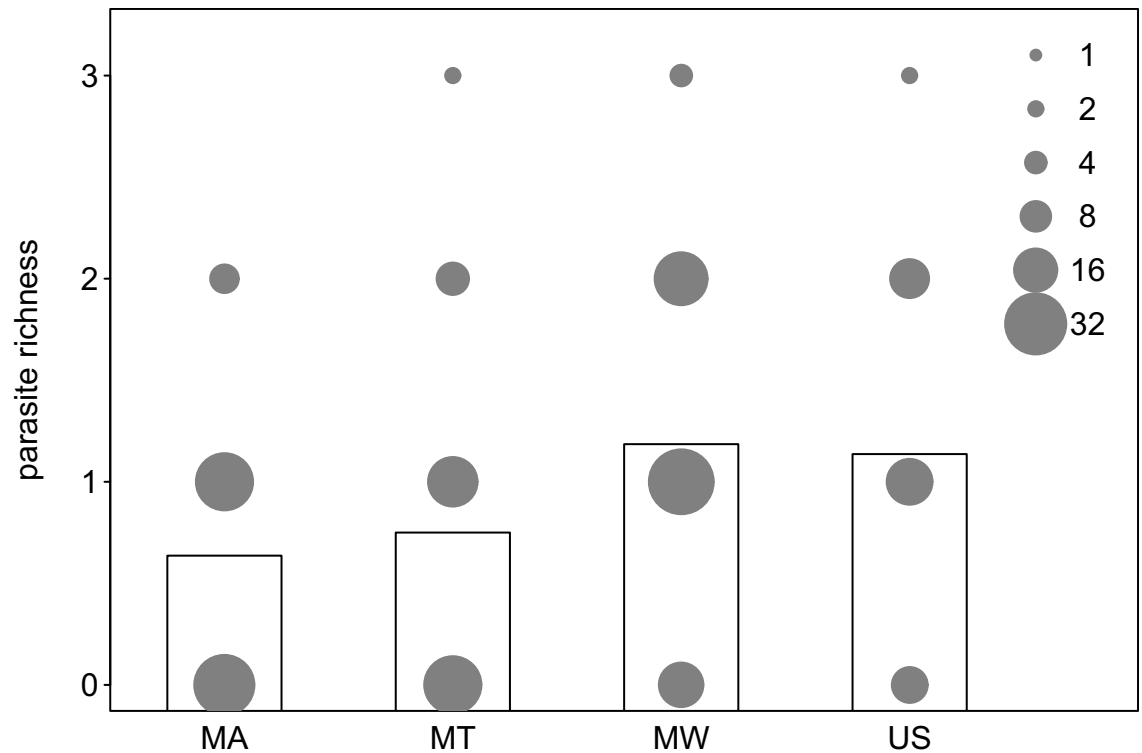
Fig 3. Parasite richness. Parasite richness for the four forests (MA: Magombera, US: Uzungwa Scarp Nature Reserve; MT: Matundu, MW: Mwanihana). The area of the dots corresponds to the number of samples with the respective parasite richness in each forest, and the bars indicate the average parasite richness per forest.

In this model we found that the prevalence of the strongylid nematode increased with increasing altitude (Fig 5). Furthermore, the control predictor population revealed significance, while season did not have a significant effect in two of the three models but tended to have an impact on *Trichuris* sp. prevalence (Table 6).

Table 5. Results of the model predicting parasite richness (response) across forests (MA: Magombera; US: Uzungwa Scarp; MT: Matundu, MW: Mwanihana), including altitude and stress hormone (faecal glucocorticoid level, FGC) as additional predictors.

|	Estimate	SE	lower Cl	upper Cl	χ^2	df	P	min	max
Intercept	-2.064	0.537	-3.118	-1.051				-2.727	-1.726
FGC	0.010	0.022	-0.035	0.052	0.208	1	0.648	-0.004	0.031
altitude[1]	0.041	0.016	0.009	0.074	6.746	1	0.009	0.032	0.057
US	-0.316	0.444	-1.114	0.468				-0.719	0.469
MT[2]	-0.597	0.440	-1.508	0.222	5.256	3	0.154	-1.001	-0.201
MW	0.011	0.360	-0.648	0.687				-0.162	0.678
sin(date)[3]	0.233	0.313	-0.344	0.848	0.620	2	0.734	-0.116	0.772
cos(date)	-0.042	0.230	-0.510	0.412				-0.179	0.366

[1] square root transformed after subtracting its minimum.

[2] population was dummy coded with MA being the reference category; the indicated test refers to the overall effect population.

[3] sin(date) and cos(date) model potential seasonal variation in parasite richness; the indicated test refers to the overall effect.

Estimate, standard error (SE), lower and upper confidence limit (Cl), results of significance tests, and minimum (min) and maximum (max) of estimates obtained when excluding groups one at a time are reported.
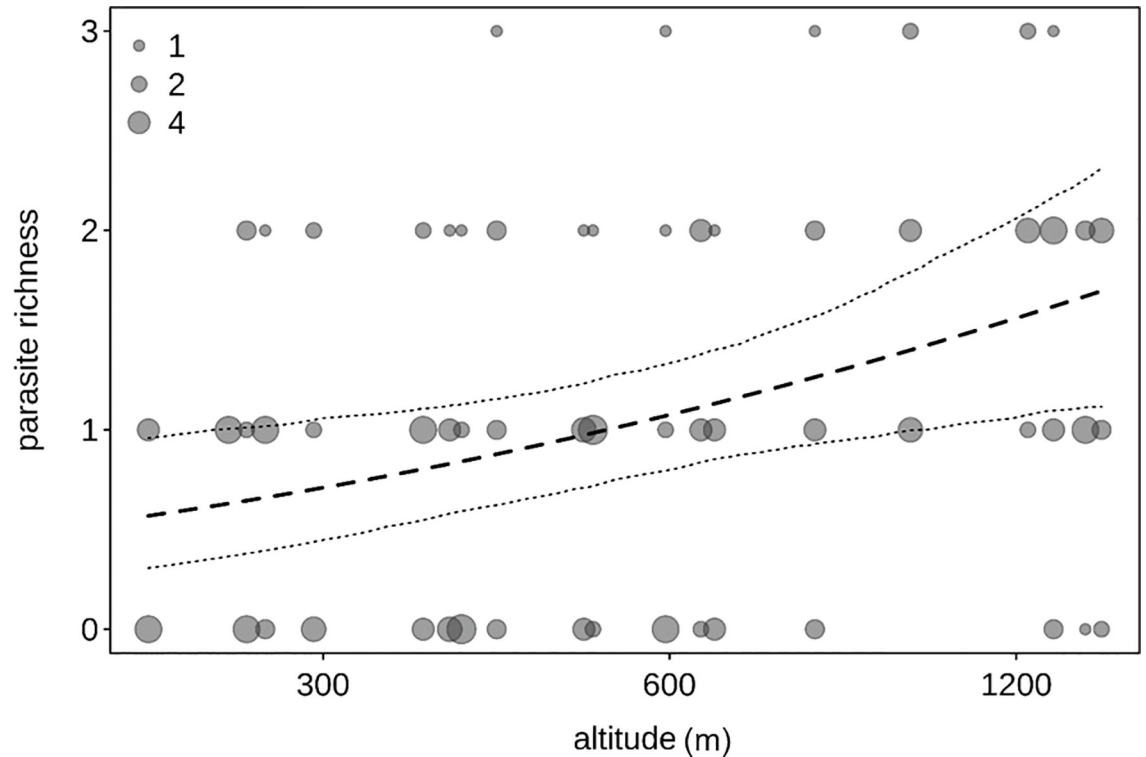
Fig 4. Parasite richness and altitude. Parasite richness as a function of altitude across the four forest blocks (MA: Magombera, US: Uzungwa Scarp Nature Reserve; MT: Matundu, MW: Mwanihana) within the Udzungwa Mountains in Tanzania. The area of the dots corresponds to the number of samples (from 1 to 7) per bin of altitude. The dashed line and dotted lines represent the fitted model and its confidence limits with all other predictors centered.

Discussion

Under the hypothesis that human activities contributing to habitat loss and degradation would also influence biodiversity at micro scales [9, 11, 16, 19, 65], we investigated gastrointestinal parasites of the endangered Udzungwa red colobus monkeys living in protected and human-impacted habitats. Despite the complexity of parasite infection risks and the potential limitation of the methods applied [66], from a comparison among forest types neither of the parasite indices examined (i.e., parasite prevalence and richness) significantly varied between fragmented, unprotected forests and intact, protected ones, as noted previously for other primate species [9, 11, 13, 15, 16, 67, 68]. However, considering that human activities are concentrated at forest edges (in both protected and unprotected forests) at lower altitudes [36, 69], the finding of a reduction in parasite richness at lower altitudes could still be explained as an indirect influence of human activities. Other studies have explained such reduction in parasite richness as a consequence of a higher diet quality in open, fragmented areas as in roe deer (*Capreolus capreolus*) populations [70], or in the coastal lowland Chacma baboons (*Papio cynocephalus ursinus*) compared to montane ones [71]. Indeed the reduction of nematodes in Australian skinks *Lampropholis guichenoti* was inferred to habitat disruption [72], as well as for some helminth species in the sigmodontinae rodent species (*Akodon cursor, A. montensis* and *Oligoryzomys*) from Brazil [73] and in a black howler monkey (*Alouatta pigra*) population [19]. Although the ecological importance of parasites and their critical roles in food webs and ecosystem processes have been largely discussed [74], still few data are available on the impact of habitat disturbance and parasite infections.

Table 6. Results of the models predicting parasite prevalence (response) across forests (MA: Magombera; US: Uzungwa Scarp; MT: Matundu, MW: Mwanihana), including altitude and stress hormone (faecal glucocorticoid levels, FGC) as additional predictors.

	Estimate	SE	lower Cl	upper Cl	χ^2	df	P	min	max
Trichuris sp.									
Intercept	2.030	1.544	-1.024	5.906				0.915	2.936
FGC[1]	-0.050	0.060	-0.186	0.073	0.703	1	0.402	-0.093	-0.020
altitude[1]	0.020	0.037	-0.054	0.099	0.303	1	0.582	0.000	0.053
MT[2]	-2.548	1.282	-6.224	-0.370	6.596		0.086	-3.439	-1.659
MW[2]	-0.102	0.764	-1.796	1.522		3		-0.625	1.418
US[2]	-0.725	0.958	-3.100	1.216				-1.212	0.900
sin(date)[3]	0.636	0.664	-0.627	2.310	5.460	2	0.065	0.256	1.648
cos(date)	-1.193	0.581	-2.862	-0.229				-1.538	-0.588
Strongyloides fulleborni									
Intercept	-2.160	1.719	-6.814	1.255				-4.861	0.449
FGC[1]	0.020	0.073	-0.132	0.167	0.073	1	0.787	-0.051	0.086
altitude[1]	-0.002	0.050	-0.113	0.120	0.002	1	0.968	-0.107	0.036
MT[2]	0.753	1.317	-2.285	4.863	0.655		0.884	-0.452	3.806
MW[2]	0.747	0.953	-1.504	2.769		3		-0.443	2.441
US[2]	0.806	1.110	-1.648	3.831				-0.483	2.476
sin(date)[3]	0.776	0.777	-0.937	2.835	1.484	2	0.476	-0.526	1.467
cos(date)	0.492	0.648	-0.697	2.886				-0.647	1.677
strongylid nematode									
Intercept	1.216	1.271	-8.079	7.862				-2.953	2.476
FGC[1, 4]	-0.298	0.322	-1.220	0.370	0.775	1	0.379	-0.750	0.194
altitude[1, 5]	3.323	0.826	2.137	10.361	13.438	1	*<0.001*	2.286	4.585
MT[2]	-3.337	1.894	-21.471	-0.089	7.866		0.049	-19.803	2.699
MW[2]	-3.025	1.597	-15.560	6.026		3		-4.905	0.254
US[2]	-4.698	1.875	-21.309	1.507				-8.053	-0.618
sin(date)[3]	0.706	1.320	-4.004	5.217	0.812	2	0.666	-1.942	3.189
cos(date)	0.837	0.883	-1.482	3.376				-0.198	3.480

[1] square root transformed after subtracting its minimum.

[2] population was dummy coded with MA being the reference category; the indicated test refers to the overall effect population.

[3] sin(date) and cos(date) model potential seasonal variation in parasite prevalence; the indicated test refers to the overall effect.

[4] z-transformed to a mean of zero and a standard deviation of one, mean and sd of the original variable were 21.102 and 3.487, respectively.

[5] z-transformed to a mean of zero and a standard deviation of one, mean and sd of the original variable were 17.813 and 11.515, respectively.

Estimate, standard error (SE), lower and upper confidence limit (Cl), results of significance tests, and minimum (min) and maximum (max) of estimates obtained when excluding groups one at a time are reported.

https://doi.org/10.1371/journal.pone.0225142.t006

The five gastrointestinal parasites (all nematodes) identified have all been found in other red colobus species, such as Ugandan red colobus (*Piliocolobus tephrosceles*), eastern black-and-white colobus (*Colobus guereza*) and Angolan black-and-white colobus (*C. angolensis*) [9, 75]. However, in agreement with Altizer and colleagues [76] who propose that threatened species have lower richness of parasites than non-threatened species, Udzungwa red colobus host fewer parasite types than colobines of least concern (*Cercopithecus ascanius*: 10 parasite types [13], *Colobus vellerosus*: 11 [77]). Despite their predominant arboreality (with occasional descent to the ground), more than 70% of the Udzungwa red colobus individuals were infected with at least one nematode species. Thus, their arboreal behaviour habit does not prevent them from becoming infected with nematode eggs or larvae from the feces of other infected group members or other animals. Indeed, most individuals (52%) were infected with *Trichuris* sp.
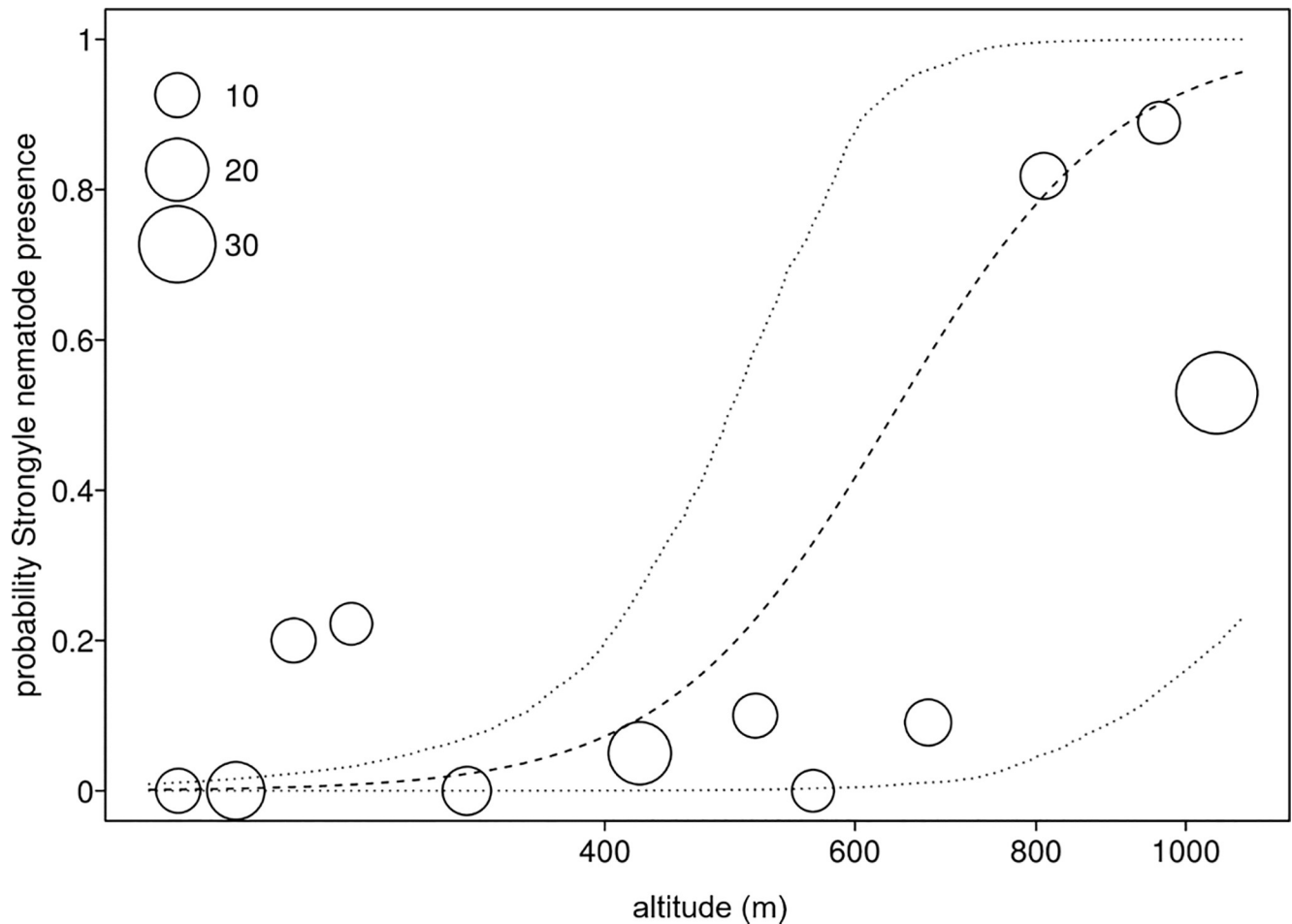
Fig 5. Strongylid nematode prevalence and altitude. Prevalence of the strongylid nematode as a function of altitude across the four forest blocks (MA: Magombera, US: Uzungwa Scarp Nature Reserve; MT: Matundu, MW: Mwanihana) within the Udzungwa Mountains in Tanzania. The area of the dots corresponds to the number of samples (from 9 to 34) per bin of altitude. Dashed and dotted lines represent the fitted model and its confidence limits with all other predictors centered.

that does not need an intermediate host to develop, but are transmitted through the ingestion of eggs and passed via hosts' feces onto soil, water and food items. Also *S. fulleborni* and the unidentified strongylid nematode were well represented in all four forests, as documented for other monkeys [16, 78] and apes [79]. However, because the identification of strongylid species based on the eggs' morphology is difficult, discussing the potential impact of this unidentified species on host health is purely speculative. Although flotation and sedimentation methods are not the optimal methods to infer the prevalence of pinworms, *Colobenterobius* sp. were found in three out of four forests (MA, MW and US). Moreover, *S. stercoralis*, a common human parasite, rarely found in wild primates, infected individuals inhabiting one forest (US) where humans are often present because illegal hunting of primates is still practiced.

When considering the potential association between parasite infections and measures of stress levels [10, 11, 12], Udzungwa red colobus did not show any association between FGC levels and parasite infections. A lack of association could be due to the sampling method since sex and reproductive condition of individuals were unknown [80]. However, our findings showing no association between FGC and parasite infection may still suggest that the interaction between immune- and endocrine functions of the host are activated independently as also

suggested in other primate (lemurs [19]; gibbons [30]; howler monkeys [32]; colobines [78]) and non-primate species (mouse [81]; raccoons [82]; African ungulates [83]). Moreover, it is possible that the lack of association is an artefact of our sampling design, since more frequent longitudinal sampling would be required to firmly establish both parasite infection and stress levels.

Indeed, models of parasitism in animals indicate that parasitic infections may be affected by climatic conditions [84] as well as geophysical characteristics of the environment as shown in non-human primates (e.g., howler monkeys [28], colobines [78], vervet monkeys [31] and lemurs [20]). Our finding of a positive association between altitude and parasite richness can be explained by the variation of the forest physical characteristics across an altitudinal gradient. In fact, it is likely that parameters such as humidity, water availability and canopy cover do change between habitats at different altitudes, making the contrasting habitats more suitable for parasites and thus facilitating host infections [82]. However, the unexpected result of a reduction in parasite richness in animals inhabiting forests at lower instead of higher altitude, made us formulate an alternative explanation. In the Udzungwas, human presence and activities are intensified at lower altitude in both protected and unprotected forests [35, 85]; such anthropogenic pressure may play an active role in shaping habitat features over time and consequently reducing parasite presence and potential transmission.

We cannot exclude that clearance of bigger trees during logging activities and consequent creation of forest edges change the forest structure, and thus parasite life cycles. For example, higher exposure to sun radiation and increased wind, due to reduced canopy cover in disturbed forests, may promote drier environments [86]. Based on these altered conditions, low humidity that reduces survival of free-living stages of parasites would be expected and consequently lower parasite infections in affected habitats. Moreover, the study areas at lower altitudes are surrounded by cultivated fields usually treated with pesticides, fertilizers and antihelminthics resulting in a lower contamination of helminths, which is particularly true for the small lowland forest fragment (MA), surrounded by sugar cane plantations [87]. Thus, when all of this evidence is considered together, it appears that a combination of factors means that the results could still reflect habitat disturbance.

Finally, our findings of reduced helminth richness at lower altitude where human disturbance is higher agree with lower gut bacterial diversity found in the same animal populations inhabiting these forests [39]. In light of plausible interactions between helminths and bacteria in natural environments [88, 89], it cannot be excluded that such losses in micro-biodiversity could hamper the long-term viability of threatened populations. Thus, we recommend and foresee more detailed investigations to understand whether such interactions do exist.

Supporting information

S1 Table. Number of samples analyzed per group across forests (MA: Magombera; US: Uzungwa Scarp; MT: Matundu, MW: Mwanihana) on models 1 and 2 (N1) and models 3 and 4 (N2).
(DOCX)

Acknowledgments

Author Contributions

Conceptualization: Claudia Barelli.

Data curation: Thomas R. Gillespie.

Formal analysis: Viviana Gonzalez-Astudillo, Roger Mundry, Thomas R. Gillespie.

Funding acquisition: Claudia Barelli, Francesco Rovero.

Methodology: Thomas R. Gillespie.

Supervision: Claudia Barelli.

Writing – original draft: Claudia Barelli.

Writing – review & editing: Claudia Barelli, Roger Mundry, Francesco Rovero, Heidi C. Hauffe, Thomas R. Gillespie.

References

1. Maizels RM, Balic A, Gomez-Escobar N, Nair M, Taylor MD, Allen JE. Helminth parasites–masters of regulation. Immunol Rev. 2004; 201: 89–116. https://doi.org/10.1111/j.0105-2896.2004.00191.x PMID: 15361235

2. Elias D, Britton S, Kassu A, Akuffo H. Chronic helminth infections may negatively influence immunity against tuberculosis and other diseases of public health importance. Expert Rev Anti Infect Ther. 2007; 5: 475–484. https://doi.org/10.1586/14787210.5.3.475 PMID: 17547511

3. WHO. Soil-transmitted helminth infections. Fact sheet No 366. http://www.who.int/mediacentre/factsheets/fs366/en/ (accessed 2019); 2019.

4. Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J. Helminth infections: the great neglected tropical diseases. J Clin Invest. 2008; 118: 1311–1321. https://doi.org/10.1172/JCI34261 PMID: 18382743

5. Bilbo SD, Wray GA, Perkins SE, Parker W. Reconstitution of the human biome as the most reasonable solution for epidemics of allergic and autoimmune diseases. Med Hypotheses. 2011; 77: 494–504. https://doi.org/10.1016/j.mehy.2011.06.019 PMID: 21741180

6. Loke P, Lim YAL. Helminths and the microbiota: parts of the hygiene hypothesis. Parasite Immunol. 2015; 37: 314–323. https://doi.org/10.1111/pim.12193 PMID: 25869420

7. Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venail P, et al. Biodiversity loss and its impact on humanity. Nature. 2012; 486: 59–67. https://doi.org/10.1038/nature11148 PMID: 22678280

8. Rogovin K, Randall JA, Kolosova I, Moshkin M. Social correlates of stress in adult males of the great gerbil, *Rhombomys opimus*, in years of high and low population densities. Horm Behav. 2003; 43: 132–139. https://doi.org/10.1016/s0018-506x(02)00028-4 PMID: 12614643

9. Chapman CA, Saj TL, Snaith TV. Temporal dynamics of nutrition, parasitism, and stress in colobus monkeys: implications for population regulation and conservation. Am J Phys Anthropol. 2007; 134: 240–250. https://doi.org/10.1002/ajpa.20664 PMID: 17596853

10. Trejo-Macías G, Estrada A, Cabrera MÁM. Survey of helminth parasites in populations of *Alouatta palliata mexicana* and *A. pigra* in continuous and in fragmented habitat in southern Mexico. Int J Primatol. 2007; 28: 931–945.

11. Mbora DNM, McPeek MA. Host density and human activities mediate increased parasite prevalence and richness in primates threatened by habitat loss and fragmentation. J Anim Ecol. 2009; 78: 210–218. https://doi.org/10.1111/j.1365-2656.2008.01481.x PMID: 19120603

12. Marcogliese DJ, Pietrock M. Combined effects of parasites and contaminants on animal health: parasites do matter. Trends Parasitol. 2011; 27: 123–130. https://doi.org/10.1016/j.pt.2010.11.002 PMID: 21144800

13. Gillespie TR, Chapman CA, Greiner EC. Effects of logging on gastrointestinal parasite infections and infection risk in African primate populations. J Appl Ecol. 2005; 42: 699–707.

14. Gillespie TR, Chapman CA. Prediction of parasite infection dynamics in primate metapopulations based on attributes of forest fragmentation. Conserv Biol. 2006; 20: 441–448. https://doi.org/10.1111/j.1523-1739.2006.00290.x PMID: 16903105

15. Gillespie TR, Chapman CA. Forest fragmentation, the decline of an endangered primate, and changes in host-parasite interactions relative to an unfragmented forest. Am J Primatol. 2008; 70: 222–230. https://doi.org/10.1002/ajp.20475 PMID: 17879941

16. Hussain S, Ram MS, Kumar A, Shivaji S, Umapathy G. Human presence increases parasitic load in endangered lion-tailed macaques (*Macaca silenus*) in its fragmented rainforest habitats in southern India. PLoS ONE. 2013; 8: e63685. https://doi.org/10.1371/journal.pone.0063685 PMID: 23717465

17. Salzer JS, Carroll D, Williams-Newkirk A, Lang S, Kerbis-Peterhans J, Rwego I, et al. Effects of anthropogenic and demographic factors on patterns of parasitism in African small mammal communities. Parasitology. 2015; 142: 512–522. https://doi.org/10.1017/S0031182014001450 PMID: 25262668

18. Jrijer J, Bordes F, Morand S, Neifar L. Gastro-intestinal helminth parasites of the common North African gerbil *Meriones shawi* (Duvernoy) in Tunisia: Parasites diversity and habitat anthropization effect. Trop Biomed. 2016; 33: 702–710.

19. Martínez-Mota R, Pozo-Montuy G, Bonilla Sánchez YM, Gillespie TR. Effects of anthropogenic stress on the presence of parasites in a threatened population of black howler monkeys (*Alouatta pigra*). Therya. 2018; 9: 161–169.

20. Rakotoniaina JH, Kappeler PM, Ravoniarimbinina P, Pechouskova E, Hämäläinen AM, Grass J, et al. Does habitat disturbance affect stress, body condition and parasitism in two sympatric lemurs? Conserv Physiol. 2016; 4: cow034. https://doi.org/10.1093/conphys/cow034 PMID: 27656285

21. Estrada A, Garber PA, Rylands AB, Roos C, Fernandez-Duque E, Di Fiore A, K. et al. Impending extinction crisis of the world´s primates: why primates matter. Sci Adv. 2017; 3: e1600946. https://doi.org/10.1126/sciadv.1600946 PMID: 28116351

22. Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GA, Kent J. Biodiversity hotspots for conservation priorities. Nature. 2000; 403: 853–858. https://doi.org/10.1038/35002501 PMID: 10706275

23. Mittermeier RA, Robles Gil P, Hoffmann M, Pilgrim J, Brooks T, Mittermeier CG, et al. Hotspots Revisited: Earth's Biologically Richest and Most Endangered Ecoregions. Mexico City, Mexico: CEMEX; 2004.

24. Rovero F, Menegon M, Fjeldså J, Collett L, Doggart N, Leonard C, et al. Targeted vertebrate surveys enhance the faunal importance and improve explanatory models within the Eastern Arc Mountains of Kenya and Tanzania. Divers Distrib. 2014; 20: 1438–1449.

25. Laurance WF, Useche DC, Rendeiro J, Kalka M, Bradshaw CJ, Sloan SP, et al. Averting biodiversity collapse in tropical forest protected areas. Nature. 2012; 489: 290–294. https://doi.org/10.1038/nature11318 PMID: 22832582

26. Rovero F, Mtui AS, Kitegile AS, Nielsen MR. Hunting or habitat degradation? Decline of primate populations in Udzungwa Mountains, Tanzania: An analysis of threats. Biol Conserv. 2012; 146: 89–96.

27. Rovero F, Mtui A, Kitegile A, Jacob P, Araldi A, Tenan S. Primates decline rapidly in unprotected forests: Evidence from a monitoring program with data constraints. PLoS ONE. 2015; 10: e0118330. https://doi.org/10.1371/journal.pone.0118330 PMID: 25714404

28. Cristóbal-Azkarate J, Hervier B, Vegas-Carrillo S, Osorio-Sarabia D, Rodríguez-Luna E, Vea JJ. Parasitic infections of three Mexican howler monkey groups (*Alouatta palliata mexicana*) living in forest fragments in Mexico. Primates. 2010; 51: 231–239. https://doi.org/10.1007/s10329-010-0193-7 PMID: 20224913

29. Trejo-Macías G, Estrada A. Risk factors connected to gastrointestinal parasites in mantled *Alouatta palliata mexicana* and black howler monkeys *Alouatta pigra* living in continuous and in fragmented rainforests in Mexico. Curr Zool. 2012; 58: 375–383.

30. Gillespie TR, Barelli C, Heistermann M. Effects of social status and stress on patterns of gastrointestinal parasitism in wild white-handed gibbons (*Hylobates lar*). Am J Phys Anthropol. 2013; 150: 602–608. https://doi.org/10.1002/ajpa.22232 PMID: 23440877

31. Gaetano TJ, Danzy J, Mtshali MS, Theron N, Schmitt CA, Grobler JP, et al. Mapping correlates of parasitism in wild South African vervet monkeys (*Chlorocebus aethiops*). S Afr J Wildl Res. 2014; 44: 56–70.

32. Martínez-Mota R, Garber PA, Palme R, Gillespie TR. The relative effects of reproductive condition, stress, and seasonality on patterns of parasitism in wild female black howler monkeys (*Alouatta pigra*). Am J Primatol. 2017; 79: e22669.

33. Marshall AR, Jørgensbye HIO, Rovero F, Platts PJ, White PCL, Lovett JC. The species-area relationship and confounding variables in a threatened monkey community. Am J Primatol. 2010; 72: 325–36. https://doi.org/10.1002/ajp.20787 PMID: 20039329

34. Barelli C, Mundry R, Araldi A, Hodges K, Rocchini D, Rovero F. Modeling primate abundance in complex landscapes: A case study from the Udzungwa Mountains of Tanzania. Int J Primatol. 2015; 36: 209–226.

35. Ruiz-Lopez MJ, Barelli C, Rovero F, Hodges K, Roos C, Peterman WE, Ting N. A landscape genetics approach demonstrates the effects of human disturbance on a primate indicator species in an East African biodiversity hotspot. Heredity. 2016; 116: 167–176. https://doi.org/10.1038/hdy.2015.82 PMID: 26374237

36. Cavada N, Tenan S, Barelli C, Rovero F. Effects of anthropogenic disturbance on primate density at the landscape scale. Conserv Biol. 2018; https://doi.org/10.1111/cobi.13269 PMID: 30561170

37. Araldi A, Barelli C, Hodges K, Rovero F. Density estimation of the endangered Udzungwa red colobus (*Procolobus gordonorum*) and other arboreal primates in the Udzungwa Mountains using systematic distance sampling. Int J Primatol. 2014; 35: 941–956.

38. Barelli C, Rovero F, Hodges K, Araldi A, Heistermann M. Physiological stress levels in the endemic and endangered Udzungwa red colobus vary with elevation. Afr Zool. 2015; 50: 23–30.

39. Barelli C, Albanese D, Donati C, Pindo M, Dallago C, Rovero F, et al. Habitat fragmentation is associated to gut microbiota diversity of an endangered primate: implications for conservation. Sci Rep. 2015; 5: 14862. https://doi.org/10.1038/srep14862 PMID: 26445280

40. Gillespie TR. Noninvasive assessment of gastrointestinal parasite infections in free-ranging primates. Int J Primatol. 2006; 27: 1129–1143.

41. Gillespie TR, Lonsdorf EV, Canfield EP, Meyer DJ, Nadler Y, Raphael J, et al. Demographic and ecological effects on patterns of parasitism in eastern chimpanzees (*Pan troglodytes schweinfurthii*) in Gombe National Park, Tanzania. Am J Phys Anthropol. 2010; 143: 534–544. https://doi.org/10.1002/ajpa.21348 PMID: 20623606

42. Ziegler TE, Wittwer DJ. Fecal steroid research in the field and laboratory: Improved methods for storage, transport, processing, and analysis. Am J Primatol. 2005; 67: 159–174. https://doi.org/10.1002/ajp.20175 PMID: 16163716

43. Shutt K, Setchell JM, Heistermann M. Non-invasive monitoring of physiological stress in the Western lowland gorilla (*Gorilla gorilla gorilla*): validation of a fecal glucocorticoid assay and methods for practical application in the field. Gen Comp Endocrinol. 2012; 179: 167–177. https://doi.org/10.1016/j.ygcen.2012.08.008 PMID: 22926327

44. Khan MZ, Altmann J, Isani SS, Yu J. A matter of time: evaluating the storage of fecal samples for steroid analysis. Gen Comp Endocrinol. 2002; 128: 57–64. https://doi.org/10.1016/s0016-6480(02)00063-1 PMID: 12270788

45. Rimbach R, Heymann EW, Link A, Heistermann M. Validation of an enzyme immunoassay for assessing adrenocortical activity and evaluation of factors that affect levels of fecal glucocorticoid metabolites in two New World primates. Gen Comp Endocrinol. 2013; 191: 13–23. https://doi.org/10.1016/j.ygcen.2013.05.010 PMID: 23707497

46. Wheeler BC, Tiddi B, Kalbitzer U, Visalberghi E, Heistermann M. Methodological considerations in the analysis of fecal glucocorticoid metabolites in tufted capuchins (*Cebus apella*). Int J Primatol. 2013; 34: 879–898. https://doi.org/10.1007/s10764-013-9703-y PMID: 24098064

47. Kalbitzer M, Heistermann M. Long-term storage effects in steroid metabolite extracts from baboon (*Papio* sp.) faeces–a comparison of three commonly applied storage methods. Meth Ecol Evol. 2013; 4: 593–600.

48. Bush AO, Lafferty KD, Lotz JM, Shostak AW. Parasitology meets ecology on its own terms: Margolis et al. Revisited. J Parasitol. 1997; 83: 575–583. PMID: 9267395

49. Greiner EC, McIntosh A. Collection methods and diagnostic procedures for primate parasitology. In: Huffman MA, Chapman CA, editors. Primate Parasite Ecology: the Dynamics and Study of Host-parasite Relationships. Cambridge: Cambridge University Press; 2009. pp. 3–27.

50. Baayen RH. Analyzing Linguistic Data. Cambridge: Cambridge University Press; 2008.

51. McCullagh P & Nelder JA. Generalized linear models. Chapman and Hall. London; 1989.

52. Hilbe JM. Negative Binomial Regression. Cambridge University Press, Cambridge, New Jersey; 2011.

53. Stolwijk AM, Straatman H, Zielhuis GA. Studying seasonality by using sine and cosine functions in regression analysis. J Epidemiol Community Health. 1999; 53: 235–238. https://doi.org/10.1136/jech.53.4.235 PMID: 10396550

54. Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White J-SS. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol Evol. 2009; 24: 127–135. https://doi.org/10.1016/j.tree.2008.10.008 PMID: 19185386

55. Forstmeier W, Schielzeth H. Cryptic multiple hypotheses testing in linear models: overestimated effect sizes and the winner's curse. Behav Ecol Sociobiol. 2011; 65: 47–55. https://doi.org/10.1007/s00265-010-1038-5 PMID: 21297852

56. Quinn GP, Keough MJ. Experimental Designs and Data Analysis for Biologists. Cambridge: Cambridge University Press; 2002.

57. Field A. Discovering Statistics using SPSS. London: Sage Publications; 2005.

58. Fox J, Monette G. Generalized Collinearity Diagnostics. J Am Stat Assoc. 1992; 87: 178–183.

59. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria; 2019.

60. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme 4; 2015.

61. Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Maechler M, Bolker BM. glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling; 2017.

62. Fox J, Weisberg S. An R Companion to Applied Regression. 2nd ed. Thousand Oaks CA: Sage; 2011.

63. Dobson AJ. An Introduction to Generalized Linear Models. Chapman & Hall/CRC, Boca Raton; 2002.

64. Barr DJ, Levy R, Scheepers C, Tily HJ. Random effects structure for confirmatory hypothesis testing: Keep it maximal. J Memory Lang. 2013; 68: 255–278.

65. Clough D, Heistermann M, Kappeler PM. Host intrinsic determinants and potential consequences of parasite infection in free-ranging red-fronted lemurs (*Eulemur fulvus rufus*). Am J Phys Anthropol. 2010; 142: 441–452. https://doi.org/10.1002/ajpa.21243 PMID: 20091843

66. Young H, Griffin RH, Wood CL, Nunn CL. Does habitat disturbance increase infectious disease risk for primates?. Ecol Lett. 2013; 16: 656–663. https://doi.org/10.1111/ele.12094 PMID: 23448139

67. Vitazkova SK, Wade SE. Parasites of free-ranging black howler monkeys (*Alouatta pigra*) from Belize and Mexico. Am J Primatol. 2006; 68:1089–1097. https://doi.org/10.1002/ajp.20309 PMID: 17044010

68. Kowalewski MM, Gillespie TR. Ecological and anthropogenic influences on patterns of parasitism in free-ranging primates: a meta-analysis of the genus *Alouatta*. In: Garber PA, Estrada A, Bicca-Marques JC, Heymann EW, Strier KB, editors. South American Primates. New York, NY: Springer; 2009. pp. 433–461.

69. DeFries R, Rovero F, Wright P, Ahumada J, Andelman S, Brandon K, Dempewolf J, Hansen A, Hewson J, Liu J. From plot to landscape scale: linking tropical biodiversity measurements across spatial scales. Front Ecol Environ. 2010; 8: 153–160.

70. Navarro-Gonzalez N, Verheyden H, Hoste H, Cargnelutti B, Lourtet B, Merlet J et al Diet quality and immunocompetence influence parasite load of roe deer in a fragmented landscape. Eur J Wildl Res. 2011; 57: 639–645.

71. Appleton CC, Henzi SP. Environmental correlates of gastrointestinal parasitism in montane and lowland baboons in Natal, South Africa. Int J Primatol. 1993; 4: 623–635.

72. Resasco J, Bitters ME, Cunningham SA, Jones HI, McKenzie VJ, Davies KF. Experimental habitat fragmentation disrupts nematode infections in Australian skinks. Ecology. 2019; 100: e02547. https://doi.org/10.1002/ecy.2547 PMID: 30488947

73. Cardoso TS, Simões RO, Luque JLF, Maldonado A, Gentile R. The influence of habitat fragmentation on helminth communities in rodent populations from a Brazilian Mountain Atlantic Forest. J Helminthol. 2016; 90: 460–468. https://doi.org/10.1017/S0022149X15000589 PMID: 26206199

74. Hudson PJ, Dobson AP, Lafferty KD. Is a healthy ecosystem one that is rich in parasites?. Trends Ecol Evol. 2006; 21: 381–385. https://doi.org/10.1016/j.tree.2006.04.007 PMID: 16713014

75. Gillespie TR, Greiner EC, Chapman CA. Gastrointestinal parasites of the colobus monkeys of 608 Uganda. J Parasitol. 2005; 91: 569–573. https://doi.org/10.1645/GE-434R PMID: 16108549

76. Altizer S, Nunn CL, Lindenfors P. Do threatened hosts have fewer parasites? A comparative study in primates. J Anim Ecol. 2007; 76: 304–314. https://doi.org/10.1111/j.1365-2656.2007.01214.x PMID: 17302838

77. Teichroeb JA, Kutz SJ, Parkar U, Thompson RA, Sicotte P. Ecology of the gastrointestinal parasites of *Colobus vellerosus* at Boabeng-Fiema, Ghana: Possible anthropozoonotic transmission. Am J Phys Anthropol. 2009; 140: 498–507. https://doi.org/10.1002/ajpa.21098 PMID: 19434756

78. Hodder SA, Chapman CA. Do nematode infections of red colobus (*Procolobus rufomitratus*) and black-and-white colobus (*Colobus guereza*) on humanized forest edges differ from those on nonhumanized forest edges? Int J Primatol. 2012; 33: 845–859.

79. Modrý D, Pafćo B, Petrželková KJ, Hasegawa H. Parasites of Apes: An Atlas of Coproscopic Diagnostics. Frankfurt: Chimaira; 2018.

80. Raminelli JLF, de Sousa MBC, Cunha MS, Barbosa MFV. Morning and afternoon patterns of fecal cortisol excretion among reproductive and non reproductive male and female common marmosets, *Callithrix jacchus*. Biolo Rhythm Res. 2001; 32: 159–167.

81. Pedersen AB, Greives TJ. The interaction of parasites and resources cause crashes in a wild mouse population. J Anim Ecol. 2008; 77: 370–377. https://doi.org/10.1111/j.1365-2656.2007.01321.x PMID: 18028357

82. Monello R, Millspaugh J, Woods R, Gompper M. The influence of parasites on faecal glucocorticoid metabolite levels in raccoons: an experimental assessment in a natural setting. J Zool. 2010; 282: 100–108.

83. Cizauskas CA, Turner WC, Pitts N, Getz WM. Seasonal patterns of hormones, macroparasites, and microparasites in wild African ungulates: the interplay among stress, reproduction, and disease. PLoS ONE. 2015; 10: e0120800. https://doi.org/10.1371/journal.pone.0120800 PMID: 25875647

84. Mas-Coma S, Valero MA, Bargues MD. Effects of climate change on animal and zoonotic helminthiases. Rev Sci Tech. 2008; 27: 443–457. PMID: 18819671

85. Cavada N, Havmøller RW, Scharff N, Rovero F. A landscape-scale assessment of tropical mammals reveals the effects of habitat and anthropogenic disturbance on community occupancy. PLoS ONE. 2019; 14: e0215682. https://doi.org/10.1371/journal.pone.0215682 PMID: 31002707

86. Matlack GR. Microenvironment variation within and among forest edge sites in the eastern United States. Biol Conserv. 1993; 66: 185–194.

87. Marshall AR. Ecological report on Magombera forest. 2008.

88. Kreisinger J, Bastien G, Hauffe HC, Marchesi J, Perkins SE. Interactions between multiple helminths and the gut microbiota in wild rodents. Philos Trans Royal Soc B. 2015; 370: 20140295.

89. Rapin A, Harris NL. Helminth–Bacterial interactions: cause and consequence. Trends Immunol. 2018; 9: 724–733.