Received: 11 November 2019 | Accepted: 13 May 2020
DOI: 10.1111/1365-2435.13603

RESEARCH ARTICLE

Functional Ecology BRITISH ECOLOGICAL SOCIETY

Ecology eclipses phylogeny as a major driver of nematode parasite community structure in a graminivorous primate

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Funding information
Leakey Foundation; National Institutes of Health, Grant/Award Number: 5R00AG051764-04; National Geographic Society, Grant/Award Number: 8100-06 and 8989-11; National Science Foundation, Grant/Award Number: BCS- 1723237, BCS-0715179, BCS-1723228, IOS-1255974 and IOS-1854359; National Science Foundation Directorate of Social and Behavioral Sciences Fellowship Program; Arizona State University Center for Evolution and Medicine

Handling Editor: Seth Barribeau

Abstract

1. Understanding how ecology and phylogeny shape parasite communities can inform parasite control and wildlife conservation initiatives while contributing to the study of host species evolution.
2. We tested the relative strengths of phylogeny and ecology in driving parasite community structure in a host whose ecology diverges significantly from that of its closest phylogenetic relatives.
3. We characterized the gastrointestinal (GI) parasite community of wild geladas *Theropithecus gelada*, primates that are closely related to baboons but specialized to graminovory in the Ethiopian Highlands.
4. Geladas exhibited very constrained GI parasite communities: only two genera (*Oesophagostomum* and *Trichostrongylus*) were identified across 305 samples. This is far below the diversity reported for baboons (*Papio* spp.) and at the low end of the range of domestic grazers (e.g. *Bos taurus*, *Ovis aries*) inhabiting the same region and ecological niche.
5. Using deep amplicon sequencing, we identified 15 amplicon sequence variants (ASVs) within the two genera, seven of which matched to *Oesophagostomum* sp., seven to *Trichostrongylus* sp., and one to *T. vitrinus*.
6. Population was an important predictor of ASV richness. Geladas in the most ecologically disturbed area of the national park exhibited approximately four times higher ASV richness than geladas at a less disturbed location within the park.
7. In this system, ecology was a stronger predictor of parasite community structure than was phylogeny, with geladas sharing more elements of their parasite communities with other grazers in the same area than with closely related sister taxa.

KEYWORDS
cercopithecines, gastrointestinal parasites, habitat sharing, nemabiome, parasite community structure, parasite ecology, parasite evolution, primate parasite ecology

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1 | INTRODUCTION

Wild animals are almost invariably infected with at least one parasite throughout their lives (Jenkins, Simon, Bachand, & Stephen, 2015). The immunological and reproductive costs of these infections have driven physiological and behavioural adaptations in hosts, ultimately centring parasites as major drivers of host evolution (Anderson & May, 1979; Ezenwa et al., 2016; Thomas, Verneau, Thierry, & Francois, 1996). The structure of parasite communities within a host species or population can thus be understood both as an evolutionary product and as a dynamic network to which changes can affect host health and survival. Understanding the major drivers of parasite community structure can accordingly offer insights into host evolution and into contemporary changes in susceptibility and disease (Patz, Graczyk, Geller, & Vittor, 2000).

The parasite community of a host population is shaped by a number of factors related to host ecology and phylogeny (Arneberg, 2002; Lindenfors et al., 2007). Habitat sharing may drive the structure of host–parasite communities by increasing the likelihood that host species in a given geographic area encounter infective parasitic stages shed by other host species in that area. This is particularly salient in the case of generalist parasites, which are able to infect multiple host species upon exposure (Ezenwa, 2003; VanderWaal, Omondi, & Obanda, 2014; Woolhouse, Taylor, & Haydon, 2001; Zaffaroni et al., 2000). Ecological changes that result in newly sympatric host communities allow parasites to accumulate adaptations to new hosts, a phenomenon that underlies the emergence of novel infectious diseases in wildlife, domestic animals, and humans (Daszak, Cunningham, & Hyatt, 2001; Mayer, 2000). Animals living in habitats in which multiple generalist parasites are endemic are thus expected to share parasite species and other elements of parasite community structure such as species richness and prevalence (Ezenwa, 2003; Poulin, 1995; VanderWaal et al., 2014).

Animals that share a recent common ancestor are also expected to share elements of parasite community structure, relative to more distantly related animals, even if they live in ecologically divergent habitats (Beer, Ingram, & Randhawa, 2018; Poulin, 1995). Certain parasites evolve with their hosts, accumulating adaptations that facilitate continued infection. During speciation, populations that evolve to be distinct species are expected to retain the parasite communities of their most recent ancestor, which then embark on their own evolutionary trajectories (Poulin, 1995). Thus, phylogeny is expected to be a powerful driver of parasite community structure across hosts, driven by co-evolutionary patterns of hosts and their parasites (Poulin, 1995). Supporting this expectation, the inclusion of phylogeny in analyses of parasite species richness has revealed a consistently strong effect of shared ancestry across terrestrial mammals (Morand & Poulin, 1998) and within certain mammalian clades (Nunn, Altizer, Jones, & Sechrest, 2003).

The similarity of parasite community structures between two species is thus a consequence of their phylogenetic proximity and shared ecology, but little is known about the relative strength of these factors within particular systems. To investigate the interplay between phylogeny and ecology in shaping the dynamics of parasite–host systems, we turned to a host species whose ecology differs substantially from its sister taxa and for which predictions about parasite community based on ecology or phylogeny diverge sharply. Geladas *Theropithecus gelada* are primates that diverged from other members of the Papionini tribe (baboons *Papio* spp. and mangabeys *Lophocebus* sp. and *Rungwecebus* sp.) approximately 4–7 mya (Zinner et al., 2018) and were long considered to be a species of baboon. However, where baboon species are highly omnivorous and consume fruits, leaves, and meat (Swedell, 2011), geladas specialize on graminoid leaves and supplement with underground plant parts (Fashing, Nguyen, Venkataraman, & Kerby, 2014; Jarvey, Low, Pappano, Bergman, & Beehner, 2018). While baboon species are widely dispersed across the African continent, geladas are found only in the Ethiopian highlands. On these high-altitude plateaus, geladas share their niche with domestic grazers (i.e. sheep *Ovis aries*, cows *Bos taurus*, donkeys *Equis asinus*, and horses *Equis equis* and less frequently with wild grazers (klipspringer *Oreotragus oreotragus*, bushbuck *Tragelaphus sylvaticus*) and closely related omnivorous primates (i.e. baboons). Geladas thus provide a useful model system in which to examine the relative power of phylogeny and ecology in shaping parasite communities.

We first characterized the gastrointestinal nematode parasite community of gelada populations in the Simien Mountains National Park (SMNP), Ethiopia. We focused on nematode parasites (Phylum *Nematoda*) because many species are generalists, meaning that they possess the capacity to infect a multitude of hosts across phylogenetic divisions (Walker & Morgan, 2014; Zaffaroni et al., 2000). In addition, many nematode parasite species are transmitted through the faecal-oral route, increasing the likelihood of exposure for animals that inhabit the same area (Anderson, 1988). Thus, any observed divergences from expectations based on phylogeny or niche sharing will reflect the biological forces shaping parasite communities apart from species specificity of certain parasites or differences in exposure related to transmission route.

We evaluated the role of habitat sharing in shaping parasite communities by comparing the gelada parasite community structure to that reported for domestic grazers in the same region of Ethiopia where our population of geladas is located (Amhara). We then performed a parallel analysis to evaluate the role of phylogeny in shaping parasite communities, comparing the gelada parasite community to that reported for baboons across Africa (*Papio* spp.). To assess on a finer scale the relative roles of microhabitat and demography on the gelada parasite community, we characterized the parasite genetic population structure using a recently developed deep amplicon sequencing approach.

2 | MATERIALS AND METHODS

2.1 | Study sites and populations

All samples were collected from wild geladas inhabiting the Simien Mountains National Park (SMNP), Ethiopia (13.1833′N, 38.0667′E). The SMNP is located in the North Gondar Zone of the Amhara region, covers 13,600 ha, and is characterized by Afromontane and Afro-Alpine habitats. The park faces intense anthropogenic pressure from villages within its boundaries and at its peripheries as well as from high tourist presence with low infrastructural development. The SMNP is home to the largest remaining population of geladas, which number approximately 10,000 across the park (J.C. Beehner & T.J. Bergman, pers. comm.).

Geladas are graminivorous and terrestrial primates endemic to the Ethiopian Highlands. The most basic unit of their multi-tiered social system is a reproductive unit, which comprises one dominant ('leader') male, ~2–12 related adult females, and dependent offspring. The reproductive unit can also include one or more subordinate ('follower') males, which are typically previous leaders (Snyder-Mackler, Beehner, & Bergman, 2012). Male offspring disperse upon reaching maturity, joining all-male ('bachelor') groups and eventually becoming leader males of non-natal groups by overthrowing current leaders. Single reproductive units associate to form 'bands' that tend to forage, travel, and sleep together.

The samples analysed for this study come from three areas within the SMNP (Figure 1): Sankaber, Chenek and Limalimo. Sankaber (~3,250 m a.s.l.) is home to the Simien Mountains Gelada Research Project (SMGRP) field site, a small park ranger village, and a tourist campsite. Limalimo (~3,000 m a.s.l.) sits at the park's western boundary and has the closest proximity to large villages. Chenek (~3,600 m a.s.l.) has a park ranger village and campsite, and serves both as the ultimate destination for many of the park's tourists and as a transportation hub for commercial traffic crossing the park. The linear distance between Sankaber and Limalimo is approximately 16 km; however, since geladas travel along the plateaus of the park, the actual travelling distance between the two sites is approximately 40 km. Similarly, the linear distance between Chenek and both Sankaber and Limalimo is 17 km, but geladas would need to travel 21 km along the plateau to get from Chenek to Sankaber, and 60 km to get from Chenek to Limalimo. Geladas have notably small day ranges, and the home ranges of units at Chenek and Limalimo fall well outside those of units at Sankaber (Snyder-Mackler et al., 2012). Thus, the geladas sampled at each of these sites are likely to belong to separate populations.

In Sankaber, we collected samples from known individuals under long-term study by the SMGRP. In Chenek and Limalimo, we habituated individuals over 2–3 days prior to sample collection, and recorded descriptions that included age category (i.e. adult, juvenile, infant), sex, and distinguishing features (e.g. obvious injuries, facial scars, hair patterns). We cross-checked descriptions upon sample collection to minimize the possibility that individuals were erroneously sampled more than once (Miller, Schneider-Crease, Nunn, & Muehlenbein, 2018).

2.2 | Gastrointestinal nematode identification

We characterized the gelada gastrointestinal nematode community using both traditional microscopy and a recently developed deep amplicon sequencing approach (Avramenko et al., 2015, 2017; Pafčo et al., 2018). We used this combination of techniques to ensure the most rigorous assessment possible: microscopy is limited to differentiating between egg types but can guide the selection of primers for deep amplicon sequencing; and deep amplicon sequencing provides higher resolution on the level of genetic diversity and genera present.

2.2.1 | Microscopy

We non-invasively collected 49 fresh faecal samples from 43 individually identifiable geladas under long-term study by the SMGRP. A bolus weighing ~1 g was taken from the centre of each faecal

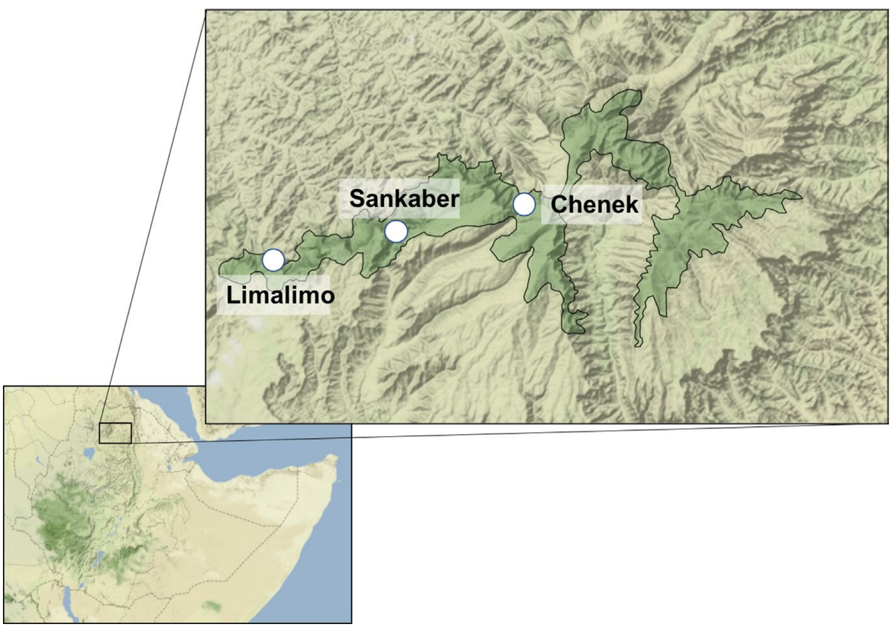


FIGURE 1 Simien Mountains National Park, Ethiopia. National park boundaries indicated with dark green, sampling sites indicated with white circles

sample and placed in 5 ml tube with ~4 ml of 10% buffered formalin. We performed modified Wisconsin sugar flotations at 1 egg per gram sensitivity at Duke University. Briefly, samples were placed into 15 ml tubes with 10 ml of water. Tubes were spun at 1,500 rpm for 10 min in a swing-bucket centrifuge, after which the supernatant was discarded and Sheather's sugar flotation solution added. Pellets were broken up by mixing with wooden applicators, and additional flotation solution was added in order to form a positive meniscus. Cover slips were placed on the tubes, which were centrifuged at 1,500 rpm for 10 min. Cover slips were then placed on labelled slides and read using a compound microscope.

2.2.2 | Amplicon sequencing: DNA extraction and library preparation

We collected 396 faecal samples from 160 individually identifiable geladas in the SMNP (9 individuals in Limalimo, 92 in Sankaber and 59 in Chenek). Of these faecal samples, 74 were cultured in the field and processed with the Baermann technique (Appendix S1), with the resulting larvae stored in RNAlater. An additional 322 faecal samples were stored in RNAlater without culturing (Appendix S2). In the laboratory, larval DNA was extracted using the MoBio DNeasy Blood and Tissue Kit and faecal DNA was extracted with the Powerlyser PowerSoil kit as per the manufacturer protocols, and DNA concentration was measured using a Qubit Fluorometer (Thermofisher Scientific). Samples were processed for next generation sequencing following Avramenko et al. (2015) with primers for the internal transcribed spacer 2 (ITS-2), a region commonly used for Class V nematode identification, designed by Avramenko et al. (2015) and Pafčo et al. (2018). Briefly, 2ng of each DNA sample was added to 10 µl NEBNext buffer and 1 µl of each ITS-2 nematode primer (NC1_ITS-2: ACG TCT GGT TCA GGG TTG TT; NC2_ITS-2: ATG CTT AAG TTC AGC GGG TA). We performed the first PCR under the following conditions: 95°C for 3 min (1x), 98°C for 20 s, 62°C for 15 s and 72°C for 15 s (25x), and then 72°C for 2 min (1x). After a 1x bead-based purification (AMPure XP Magnetic Beads; Beckman Coulter, Inc.), we performed a second PCR to add unique dual molecular indexes using the following conditions: 98°C for 45 s (1x), 98°C for 20 s, 63°C for 20 s and 72°C for 2 min (7x). After another 1x bead clean up, barcoded samples were pooled in equimolar quantities and sequenced on an Illumina Novaseq SP flowcell using paired-end 250 bp reads. To increase diversity in the flowcell, we sequenced this pool along with a different, non-ITS-2 library pool and 10% PhiX. The full protocol can be accessed at https://smack-lab.com/protocols/.

2.2.3 | Amplicon sequencing: Sequence clustering

We used QIIME2 to align the sequencing reads, generate amplicon sequence variants (ASVs), and match ASVs with taxonomic data available through the NCBI Nucleotide database (https://doi.org/10.5281/zenodo.3827516). Our initial filtering steps included removing matches with an e-value of $<10^{-100}$ and a percent identity under 85% (Figure S3), based on a bimodal e-value and percent identity distributions across the dataset. This filtering rendered a dataset with percent identity matches between 92.04% and 100%, and all potential matches for each sequence were within the same genus. The best taxonomic match for each ASV was then determined using the reported percent identity matches. If no match had a species percent identity above 98%, the sequence was assigned at the genus level (all genera matches had percent identity matches of above 92.04% in this dataset). To reduce noise caused by minor differences in nucleotide bases and low frequency ASVs, we filtered our dataset to include only those ASVs that comprised at least 1% of the total abundance of at least 10% of samples in any of our sampling sites. We imposed this threshold on each site independently to ensure that we were able to capture any site-based differences in ASV abundance that would otherwise be eliminated by analysing all of the samples together (given the difference in sample sizes collected at each site). This filtering step eliminated 1,128 ASVs from our dataset, rendering a final taxonomic set of 15 ASVs (Table S4). We then removed all samples that failed to amplify (n = 30) or were not sequenced deeply enough (<1,000 reads; n = 61), rendering a final dataset of 305 samples. All downstream analyses were done in *R* using the PHYLOSEQ package (McMurdie & Holmes, 2013) and the DESEQ2 package (Love, Huber, & Anders, 2014).

2.3 | Gelada-nematode parasite community: Composition, richness, and prevalence

Because we were able to resolve certain ASVs only to the genus level, we report parasite richness at the genus level ('genus richness'). We calculated genus richness by summing the number of genera represented in our final dataset of 305 gelada faecal samples. We also calculated the ASV richness by summing the number of ASVs represented across the dataset. We then calculated the prevalence of each taxon and ASV as the number of samples in which it appeared divided by the total number of samples.

2.4 | Cross-species comparisons: Composition and richness

We first obtained average genus richness estimates for (a) domestic grazing species in Ethiopia that share their habitat with geladas (*O. aries*, *B. taurus*, *E. asinus*, *E. equis*) and (b) baboon species (*Papio* spp.) across Africa. Data on grazer parasites were gathered through a Google Scholar search using the terms 'gastrointestinal parasites', 'helminths', 'nematodes', and 'Amhara, Ethiopia' paired with Latin and common names for each target species, and baboon data were gathered from the Global Mammal Parasite Database (https://gmpd.nunn-lab.org/). Because some papers included in these analyses report parasite species identification based on morphology alone, we calculated richness on the genus level for both ruminant and *Papio* studies. Where studies reported strongyles to their genus or species without morphological examination of cultured larvae, we reduced all strongyle species to a 'strongyle' category. Since our morphological and molecular analyses only targeted nematodes (and our molecular approach only targeted Class V nematodes), we further restricted these datasets to taxa in the nematode phylum. We then qualitatively compared the composition and richness of the gelada parasite community to the composition and richness reported for grazers in Amhara, Ethiopia and *Papio* spp. across Africa.

2.5 | Within-species drivers of ASV richness and abundance: Habitat and demography

To identify the drivers of gelada parasite community structure on a finer scale, we assessed the importance of sampling site and demographic predictors (i.e. age, sex) on two metrics of parasite community: richness (the number of unique ASVs in each faecal sample) and relative abundance (the number of reads of each ASV normalized by the total number of reads in the sample). Because robust ages (estimated based on validated morphological cues or known by date of birth) are only known for Sankaber geladas, models analysing demographic predictors include only samples collected at Sankaber (n = 237). This dataset includes 146 females and 91 males, of which 215 were adults, five were infants, and 17 were juveniles. The overall dataset used for site-based analyses contained all 305 samples, with nine samples from Limalimo, 237 samples from Sankaber, and 59 from Chenek. Of these, 175 were females, 112 were males, and 18 were individuals of unknown sex. This dataset included 252 adults, seven infants, 33 juveniles, and 13 individuals of unknown age category. We worked to reduce the likelihood of collecting repeated samples from individuals at Limalimo and Chenek by cross-checking individual descriptions upon sample collection.

First, we evaluated the impact of sampling site, age, and sex on ASV richness. We modelled the log-transformed ASV richness of each sample as a function of sampling site and included individual ID as a random effect ('lmer' function in the LME4 package; Bates, Mächler, Bolker, & Walker, 2015, with default parameters). We then modelled the log-transformed ASV richness of each sample as a function of individual age (in years), sex, and the interaction between the two, with individual ID as a random effect. We performed the same analyses on other measures of sample alpha diversity (i.e. Shannon, Simpson, Inverse Simpson and Chao1 indices of diversity). We also ensured that our approach was equally likely to pick up similar ASV compositions in both cultured and uncultured samples by performing an analysis that modelled log-transformed ASV richness as a function of type of sample (0/1; uncultured/cultured) while controlling for individual ID as a random effect.

Second, we evaluated the impact of sampling site, age, and sex on relative ASV abundance. We modelled the number of reads of each ASV, normalized with a scaling factor (DESEQ2 package), as a function of site and with individual ID as a random effect. We fitted a GLMM with a negative binomial distribution and a term for zero inflation using the GLMMTMB package (Brooks et al., 2017). To address the variation in sample sizes between sites, we performed a secondary analysis that excluded samples from Limalimo (n = 9). For this analysis, we subsampled 60 samples from Sankaber without replacement 10,000 times to match the Chenek sample size and examined the distribution of coefficients to assess the role of sampling site in predicting abundance of ASVs.

We then modelled normalized ASV abundance as a function of individual age (in years) and sex across the Sankaber-only dataset, with individual ID as a random effect. We again used the GLMMTMB package, fitting a GLMM with a negative binomial distribution. We adjusted all p-values with a Bonferroni correction to account for multiple comparisons.

3 | RESULTS

3.1 | Microscopy

Strongyle-type eggs were virtually the sole egg type observed with microscopy across 48 faecal samples from individuals across age–sex groups. At least one strongyle-type egg was observed in 94.2% (17/18) of samples from females and in 96.8% (29/30) of samples from males. Similarly, at least one strongyle-type egg was observed in 90.9% (11/12) of samples from juveniles and in 97.3% (36/37) of samples from adults. A single instance of a possible non-strongylid egg type (ascarid-type morphology) was recorded during the validation stages, with a prevalence of 2.7% of the formalin-fixed sample set (1/37) and 0.3% of all analysed samples (1/347).

3.2 | Amplicon sequencing

With the deep amplicon approach, we confidently identified 15 ASVs mapping to two genera and one species (*Oesophagostomum* sp., *Trichostrongylus* sp., and *Trichostrongylus vitrinus*; Table S4; Figure S5). All sequences are available on NCBI (BioProject Accession: PRJNA609008). The application of our approach to cultured samples (vs. uncultured samples) did not have an impact on the richness of ASV communities (t = −1.618, p = 0.1).

3.3 | Gelada-nematode parasite community: Composition, richness, and prevalence

Our examination of the gelada gastrointestinal nematode community revealed a high degree of homogeneity. Across the entire SMNP gelada population, only two genera were ever observed (*Trichostrongylus* and *Oesophagostomum*), and both genera were found in almost all samples: *Oesophagostomum* appeared in 97.7% of samples (298/305 samples), while *Trichostrongylus* appeared in 98.7% of samples (301/305 samples).

At the individual sequence level, the ASV community was more complex: 15 ASVs observed across the sample set. Seven of these ASVs matched to *Oesophagostomum* sp., seven to *Trichostrongylus* sp., and one to *T. vitrinus*. The low relative identity match (~94%) of the ASV that mapped to *T. vitrinus* may suggest that this ASV represents a strain belonging to a species closely related to *T. vitrinus*. ASV prevalence ranged from 0% to 100% within each of the three sites (Table S6).

3.4 | Cross-species comparisons: Composition and richness

Our dataset of grazers in the Amhara region of Ethiopia contained 10 independent studies on domestic sheep, cows, donkeys, horses, and mules. Nematode genus richness ranged from 2.0 to 7.0, with an average of 4.8 (Table S7). Our dataset of *Papio* species (*P. anubis*, *P. cynocephalus*, *P. papio*, *P. ursinus*) included 30 independent studies. Nematode parasite genus richness ranged from 4.0 to 8.2, with an average of 6.3 (Table S8). Comprised of only two genera, gelada-nematode parasite genus richness fell within the distribution of grazer genus richness and below the range of the distributions for *Papio* species (Figure 2).

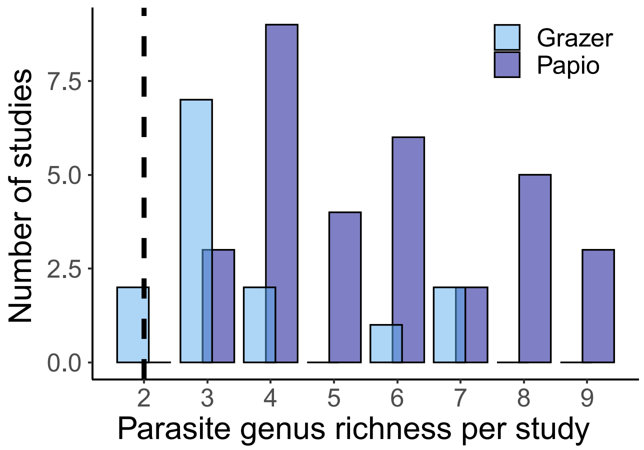


FIGURE 2 Nematode parasite richness by genus for five grazing species in Amhara, Ethiopia (light blue) and four *Papio* species (purple). The parasite genus richness of geladas is indicated by the dashed line

3.5 | Within-species drivers of ASV richness and abundance: Habitat and demography

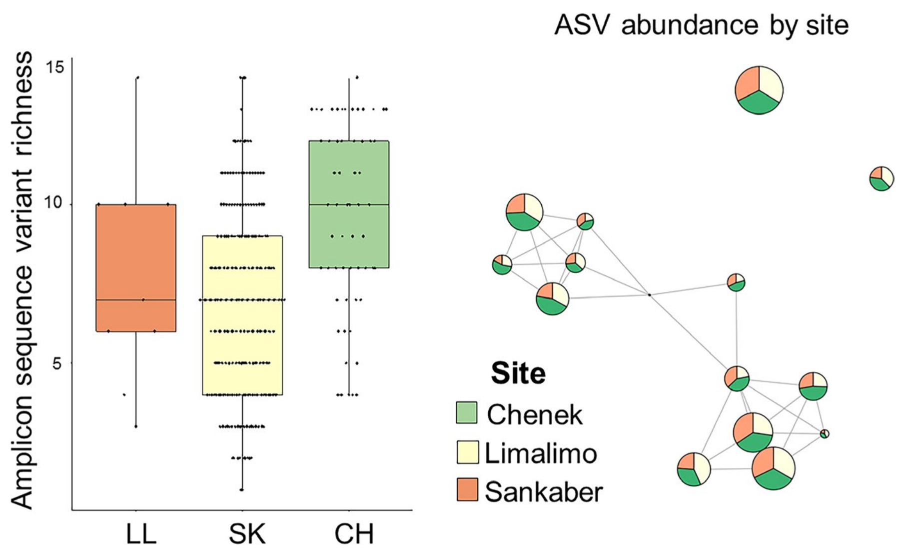
Sampling site was a strong predictor of ASV richness (Figure 3). Chenek samples had approximately 39% more ASVs than those from Sankaber (estimate: 0.39; $p < 0.001$). No strong difference was observed between Limalimo and either Sankaber or Chenek, possibly due to the small sample size at Limalimo. Sampling site had a similar effect on other metrics of alpha diversity (Figure S9). No demographic predictors were strongly associated with ASV richness (Table S10).

The effect of sampling site on normalized relative abundance varied by ASV. Subsampling demonstrated that only three ASVs showed a trend towards differential relative abundance by site. Two *Trichostrongylus* ASVs and one *Oesophagostomum* ASV trended towards higher relative abundances in Sankaber across 10,000 random subsample iterations (Table S11). No demographic predictors were associated with changes in normalized abundance for any ASV (Table S12).

4 | DISCUSSION

Wild geladas in the SMNP exhibited a highly constrained parasite community, below the range of diversity reported for *Papio* spp. and even at the low end of the range reported for other grazers. Geladas were infected by only two nematode genera, *Trichostrongylus* and *Oesophagostomum*, both of which are generalist parasites that infect a broad array of herbivores as well as humans. Infections were ubiquitous, occurring in geladas at all three sampling sites and of all ages and sexes, but ASV diversity was highest at the Chenek site (Figure 3a). Within these two genera, we identified 15 ASVs: seven from *Oesophagostomum* and eight from *Trichostrongylus*.

FIGURE 3 (a) The highest richness of parasite amplicon sequence variants (ASVs) was observed at Chenek (in green). (b) Relative ASV abundance differed at each site, here represented with a network based on the Jaccard index of dissimilarity (using the IGRAPH package; Csardi & Nepusz, 2006). Nodes represent the 15 unique ASVs, colours reflect the relative proportion of ASVs at each site and node size reflects the relative abundance of each ASV.



4.1 | Cross-species comparisons of nematode parasite communities: Composition and richness

The nematode parasite community of geladas fell within the distribution of nematode genus richness observed in grazing species across the Amhara region of Ethiopia, where our study gelada population is located, and fell below the distribution observed in *Papio* species across Africa. Strongyles, including *Oesophagostomum* and *Trichostrongylus*, were found across geladas, grazers, and *Papio* sp., but geladas lacked any of the other parasites found in either group. In addition to strongyles, grazers were commonly observed to harbour infections with *Ascaris* sp., *Dictyocaulus* sp., *Parascaris* sp., and *Trichuris* sp. In *Papio* species, the most common non-strongyle parasites were *Ascaris* sp., *Enterobius* sp., *Physaloptera* sp., *Ternidens* sp., *Streptopharagus* sp., and *Trichuris* sp. Geladas were infected with only the most general of gastrointestinal nematode parasites.

The absence of eggs characteristic of *Ascaris* sp. or *Trichuris* sp. in gelada samples analysed with microscopy (with one possible exception) is notable, particularly because these parasites are found in both grazers and *Papio* species. One possible avenue of explanation for the unusually low nematode parasite diversity in geladas is their evolutionary history. The extinct sister taxa to geladas ranged across much of Africa, Europe, and India, and all possessed morphological adaptations that suggest similar levels of terrestriality and graminivory (Krentz, 1993). Thus, geladas have likely been terrestrial grazers since their origin, a characteristic that likely was accompanied by high levels of exposure to soil-transmitted helminth parasites. If the costs associated with infection were high, resulting in damages to reproductive success or survival, parasitism may have exacted sufficient pressure for selection of immunological mechanisms to mitigate those costs in this particular system.

4.2 | Within-species drivers of ASV richness and abundance: Habitat and demography

While both *Oesophagostomum* sp. and *Trichostrongylus* sp. were observed across sampling sites, ages, and sexes, certain ASVs appeared in varying frequencies and abundances at different sites (Figure 3b). The overall homogeneity of the parasite communities of geladas across all three sites indicates a lack of parasite population structure at the genus level. This could be attributable to gelada dispersal patterns, in which males may travel extensively to reach new groups upon maturity. Because both genera found in geladas were also found in other ruminants in Ethiopia, it is also possible that multiple host species transport these parasites across the park and contribute to the homogeneity of the gelada parasite community.

Our ASV-level analysis revealed fine-grained parasite population structure. The higher ASV richness in Chenek individuals compared to Sankaber could be tied to higher overall host species diversity; Chenek is the only of our sampled sites where geladas overlap with Walia ibex *Capra walia* and Ethiopian wolves *Canis simensis*. In Limalimo and Sankaber, geladas overlap only with klipspringer *Oreotragus oreotragus*, bushbuck *Tragelaphus sylvaticus*, and domestic ruminants, all of which are also found in Chenek. Another possible explanation hinges on the exposure of Chenek geladas to human trash and faecal waste. The Chenek campsite, unlike the park's other campsites, contains multiple open trash pits and is plagued by the extensive and open deposition of human excrement. The campsite is also a transportation hub, and local travellers regularly feed the geladas. As a result, geladas at the Chenek campsite display atypical behaviour that includes raiding trash pits and spending time concentrated around human waste. The higher ASV richness of Chenek could thus be tied to exposure to strains of *Oesophagostomum* sp. and *Trichostrongylus* sp. in human waste.

While richness and abundance typically vary according to age- and sex-based differences in immune function and pathogen exposure, geladas were uniformly infected across demographic categories and neither ASV richness nor abundance was affected by age or sex. The lack of demographic structure in ASV richness and abundance may be related to gelada ecology. As terrestrial grazers, geladas are likely to encounter infective stages of these parasites at the beginning of their lives. Even while dependent offspring are still nursing and not yet feeding on grass, they frequently dismount from their mothers and experiment with placing objects (e.g. soil, faeces, grasses) in their mouths. This may provide sufficient exposure to eggs and larvae for infections to establish, with continued exposure throughout their lives.

The near-ubiquitous infection of geladas with species of both parasite genera, coupled with the lack of detectable demographic structure in ASV abundance, suggests that the gelada immune system may tolerate infections as opposed to working to resist or eliminate them. Resistance and tolerance are the two principal approaches available to hosts upon the establishment of a parasite infection (Best, White, & Boots, 2008; Kutzer & Armitage, 2016; Medzhitov, Schneider, & Soares, 2012; Råberg, Graham, & Read, 2008; Read, Graham, & Råberg, 2008). Resistance includes the reduction or elimination of infections, which are accompanied by collateral costs to the host, while tolerance includes the mitigation of damage caused by infection while avoiding the costs of active defence (Medzhitov et al., 2012; Råberg, 2014; Read et al., 2008).

The evolution of resistance in a host–parasite system is expected when the costs of infection are higher than the costs of mounting an immune response to limit the infection, whereas the evolution of tolerance is expected when the costs of infection are not higher than the costs of the immune response (Råberg et al., 2008). In practice, resistance should reduce parasite prevalence while tolerance is expected to increase it or have no effect (Råberg et al., 2008). The high prevalence of both *Oesophagostomum* sp. and *Trichostrongylus* infections fits the pattern expected in a system in which tolerance—as opposed to resistance—has evolved. To test the hypothesis that the gelada–nematode parasite system is characterized by tolerance-focused strategies, further work must be done to quantify infection intensity (a measure of parasitism that we did not gather here) and to assess the relationship between intensity and host fitness (Jackson et al., 2014; Råberg et al., 2008) or other correlates of health such as body condition (Hayward et al., 2014; Jackson et al., 2014; Råberg, 2014) across this population.

In this system, habitat sharing appears to be a stronger force in shaping the gastrointestinal nematode parasite community of geladas than phylogeny. The gelada gastrointestinal nematode parasite community diverged sharply in richness and composition from those of their sister taxa in the *Papio* genus, but fell within the distribution grazer parasite genus richness in the same area and was similarly composed. However, it should be noted that we collapsed any egg-based identification of strongyle-type egg to genus- or species-level to a 'strongyle' category for our analyses. In many of the grazer studies we included, this category likely represents more than one genus. Thus, it is possible—and perhaps even likely—that geladas fall even below the distribution of grazer nematode parasite genus richness. Our results emphasize the importance of a dynamic confluence of factors—including abiotic factors such as altitude and climate as well as biotic factors such as niche sharing and host diversity—in shaping parasite communities.

ACKNOWLEDGEMENTS

We thank the Ethiopian Wildlife Conservation Authority, the Amhara National Regional Parks State Development and Protection Authority, and all park officials who have aided our research. We thank all past and present field crew of the Simien Mountains Gelada Research Project for their dedication to over 12 years of data collection, Kenneth L. Chiou for intellectual support, Charlie L. Nunn for mentorship, and the Kaplan lab at the University of Georgia College of Veterinary Medicine and Henry S. Bishop at the Centers for Disease Control and Prevention for guidance on parasitological techniques. Long-term gelada research was supported by the National Science Foundation (IOS-1255974, BCS-0715179, BCS-1723237, BCS-1723228, IOS-1854359), the National Institutes of Health (5R00AG051764-04), the Leakey Foundation, and the National Geographic Society (Gr. #8989-11, Gr. #8100-06) and the University of Michigan I.S.-C. acknowledges funding from the National Science Foundation Directorate of Social and Behavioral Sciences Fellowship Program and the Arizona State University Center for Evolution and Medicine. Finally, we thank the reviewers and editors of this journal for their indispensable comments. The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

I.S.-C. conceptualized the study and designed the methodology with N.S.-M.; I.S.-C. and M.A.G. collected the data, and A.L., J.C.B., N.S.-M. and T.J.B. facilitated data collection; I.S.-C. and L.K. analysed data with contributions from N.S.-M.; I.S.-C. wrote the manuscript with N.S.-M., and A.L., J.C.B., and T.J.B. provided important intellectual input.

DATA AVAILABILITY STATEMENT

Data and code are available on GitHub and archived on Zenodo: https://doi.org/10.5281/zenodo.3827516 (Schneider-Crease, 2020).

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SUPPORTING INFORMATION

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How to cite this article: Schneider-Crease I, Beehner JC, Bergman TJ, et al. Ecology eclipses phylogeny as a major driver of nematode parasite community structure in a graminivorous primate. *Funct Ecol*. 2020;34:1898–1906. https://doi.org/10.1111/1365-2435.13603