Parasite community structure in sympatric Bornean primates

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ABSTRACT

Parasites are important components of ecosystems, influencing trophic networks, competitive interactions and biodiversity patterns. Nonetheless, we are not nearly close to disentangling their complex roles in natural systems. Southeast Asia falls within global areas targeted as most likely to source parasites with zoonotic potential, where high rates of land conversion and fragmentation have altered the circulation of wildlife species and their parasites, potentially resulting in altered host-parasite systems. Although the overall biodiversity in the region predicts equally high, or even higher, parasite diversity, we know surprisingly little about wild primate parasites, even though this constitutes the first step towards a more comprehensive understanding of parasite transmission processes. Here, we characterise the gastrointestinal helminth parasite assemblages of a community of Bornean primates living along the Kinabatangan floodplain in Sabah (Malaysian Borneo), including two species endemic to the island. Through parasitological analyses, and by using several measures of parasite infection as proxies for parasite diversity and distribution, we show that (i) most parasite taxonomic groups are not limited to a single host, suggesting a greater flexibility for habitat disturbance, (ii) parasite infracommunities of nocturnal primates differ from their diurnal counterparts, reflecting both phylogenetic and ecological constraints, and (iii) soil-transmitted helminths such as whipworm, threadworm and nodule worm are widespread across the primate community. This study also provides new parasite records for southern pig-tailed macaques (*Macaca nemestrina*), silvered langurs (*Trachypithecus cristatus*) and Western tarsiers (*Cephalopachus bancanus*) in the wild, while adding to the limited records for the other primate species in the community. Given the information gap regarding primate-parasite associations in the region, the information presented here should prove relevant for future studies of parasite biodiversity and infectious disease ecology in Asia and elsewhere.

1. Introduction

The world's most biodiverse places are under extreme and continuous pressure from anthropogenic habitat conversion. This is no exception in Borneo, an island of remarkable diversity and importance to the evolutionary history of Southeast Asia's biodiversity in general (Myers et al., 2000). Borneo is also a primate biodiversity hotspot, harbouring up to 13 species of non-human primates, many of them endemic to the island and most of them threatened with extinction (Meijaard and Nijman, 2003). There is still a lack of essential information on a large proportion of the species inhabiting this area, but especially for primate parasites (Hopkins and Nunn, 2007; Cooper and Nunn, 2013). This is a point of concern, as Southeast Asia has also been identified as a hotspot

for emerging and re-emerging diseases (Jones et al., 2008; Acuin et al., 2011), where high rates of land conversion and forest fragmentation have increased the circulation of wildlife species and their parasites, potentially resulting in altered host-parasite systems (Patz et al., 2000; Keesing et al., 2010; Cable et al., 2017). It is under this scenario that collection of reference data on patterns of parasitism in natural populations is critical, particularly on community level interactions where multiple parasites infect and are transmitted by multiple host species (Johnson et al., 2015).

In fact, most parasites of zoonotic potential depend on multiple host species (Holt et al., 2003; Rigaud et al., 2010), where their association with a given host can determine their spread or demise in a transmission network (Pedersen and Fenton, 2007). By targeting communities, we can begin quantifying the relative contribution of each host species to the persistence of parasites in a natural system and explore the role of parasite communities in mediating host interactions (Viana et al., 2014; Seabloom et al., 2015). For example, hosts contributing disproportionately to parasite persistence in the environment can become targets in the development of disease monitoring and control strategies (Fenton and Pedersen, 2005; Streicker et al., 2013), while asymmetrical patterns of parasite transmission between host species can ultimately alter both host and parasite community structure (Hatcher et al., 2006).

However, to understand complex ecological interactions in nature it is necessary to know what is out there, and for primate parasites there is still much to be discovered (Hopkins and Nunn, 2007; Cooper and Nunn, 2013). The attention that primate parasites have received over the last two decades stems from a desire to understand the effects of habitat degradation on both human and non-human primates, and it has been addressed mainly from the perspectives of conservation and health (Wolfe et al., 1998; Chapman et al., 2005; Gillespie and Chapman, 2008). As endangered primates increasingly lose their habitat to deforestation and land conversion, they become vulnerable to a series of threats including hunting, trade and infectious disease (Estrada et al., 2017). At the same time, anthropogenic change can alter host-parasite assemblages, and become a threat to non-human primates and humans by extension. This scenario leads research efforts towards targeting the next disease outbreak, focusing on those pathogens more likely to cross species boundaries, and on the most vulnerable primate species (Wolfe et al., 2007). As a result, we are left with considerable knowledge gaps about what happens in natural communities, and the isolation of certain wildlife and parasite species from their ecological communities.

The overall biodiversity in Southeast Asia predicts high parasite diversity and yet we know surprisingly little about parasites infecting wild primates. This baseline information seems deceptively simple to obtain, nonetheless studies keep pointing to large information gaps (Hopkins and Nunn, 2007; Cooper and Nunn, 2013). The recent expansion of the Global Mammal Parasite Database (GMPD, Stephens et al., 2017), a database containing over 24,000 host-parasite associations collected from the scientific literature from wild mammals (primates, ungulates and carnivores) and their parasites (both micro- and macroparasites), suggests we will continue to find new host-parasite associations, and at the same time indicates where we are still lagging behind. Among the 504 primate species identified to date (Estrada et al., 2017), parasite surveys including arthropods, helminths, protists, bacteria, viruses and fungi have covered 78.9% of African primates (continental Africa and Madagascar) but only 36.8% of Neotropical primates and 30.2% of Asian primates (GMPD, Stephens et al., 2017).

Species inventories and diversity monitoring rarely include parasites; in addition to the logistic difficulties of studying primate species in the wild, parasite sampling comes with special ethical considerations. Invasive studies aimed at collecting adult worms from the host's gastrointestinal tract, such as those performed on rodents, are ethically impossible in primates. Necropsies are also rare, apart from those conducted at long-term research sites where individual primates are continuously followed and dead individuals can occasionally be recovered. Therefore, most parasite studies in the wild rely on non-invasive, opportunistic collection of faecal samples. Gastrointestinal helminths are a convenient group of parasites to study under this framework. These are usually generalist parasites with a widespread and abundant distribution (Gaston et al., 2000), which tend to produce chronic, sublethal and often subclinical infections. Most gastrointestinal helminths develop outside the host, accumulating and persisting in the environment for relatively long periods of time, and making close spatiotemporal contact between sympatric hosts unnecessary for cross-species transmission.

By characterising the diversity and distribution of parasites in a community of free-living primates in Borneo, our study contributes to the systematic collection of parasite data, and provides valuable information to help in establishing biodiversity baselines, identifying rare taxa, and monitoring changes in parasite biodiversity (Gehman et al., 2019; Carlson et al., 2020).

2. Materials and methods

2.1. Study area and sample collection

Sampling was conducted in the Lower Kinabatangan Wildlife Sanctuary (LKWS, Fig. 1), located in the eastern part of the Malaysian state of Sabah (5°18′N-5°42′N and 117°54′E-118°33′E). The Kinabatangan floodplain consists of several patches of protected forest interspersed with a mosaic of agricultural land and natural forest. The broad habitat spectrum of the LKWS makes it a biodiversity hotspot, where up to 10 different primate species are known to live in sympatry. Primates found in the area include four species of colobines (silvered langur (*Trachypithecus cristatus*), maroon langur (*Presbytis rubicunda*), Hose's langur (*Presbytis hosei*), and proboscis monkey (*Nasalis larvatus*)), two species of macaques (long-tailed macaque (*Macaca fascicularis*) and southern pig-tailed macaque (*Macaca nemestrina*)), two species of apes (Bornean orangutan (*Pongo pygmaeus*) and Bornean gibbon (*Hylobates muelleri*)), one nocturnal haplorrhine (Western tarsier (*Cephalopachus bancanus*)) and one nocturnal strepsirrhine (Philippine slow loris (*Nycticebus menagensis*)). Three members of the primate community, however, were excluded from this study (maroon langur, Hose's langur and Bornean gibbon) due to their rarity in the area and thus difficulty in sample collection.

Between September 2014 and February 2017, we conducted three seasons (September 2014, October 2015, and December 2016 to February 2017) of non-invasive faecal sampling of colobines and macaques, mostly occupying riparian zones along the Kinabatangan River, and orangutans and nocturnal primates, inhabiting areas within the forest. We collected a total of 315 faecal samples from primates, including long-tailed macaques (*N* = 140), pig-tailed macaques (*N* = 14), proboscis monkeys (*N* = 91), silvered langurs (*N* = 35), Bornean orangutans (*N* = 11), Philippine slow lorises (*N* = 16), and Western tarsiers (*N* = 8). Samples from colobines and macaques were collected beneath sleeping trees in the early morning (between 06:00 and 08:00) and, because direct observation of defecation was not possible, stored in ASL buffer (Qiagen, Japan) to genetically confirm the identity of the host species. Faecal sampling from orangutans and nocturnal primates was limited to one patch of forest (Lot 6), where orangutans were tracked on foot and faecal samples were collected immediately after defecation. Slow lorises and tarsiers were continuously captured as part of a radio-tracking study of nocturnal primates in
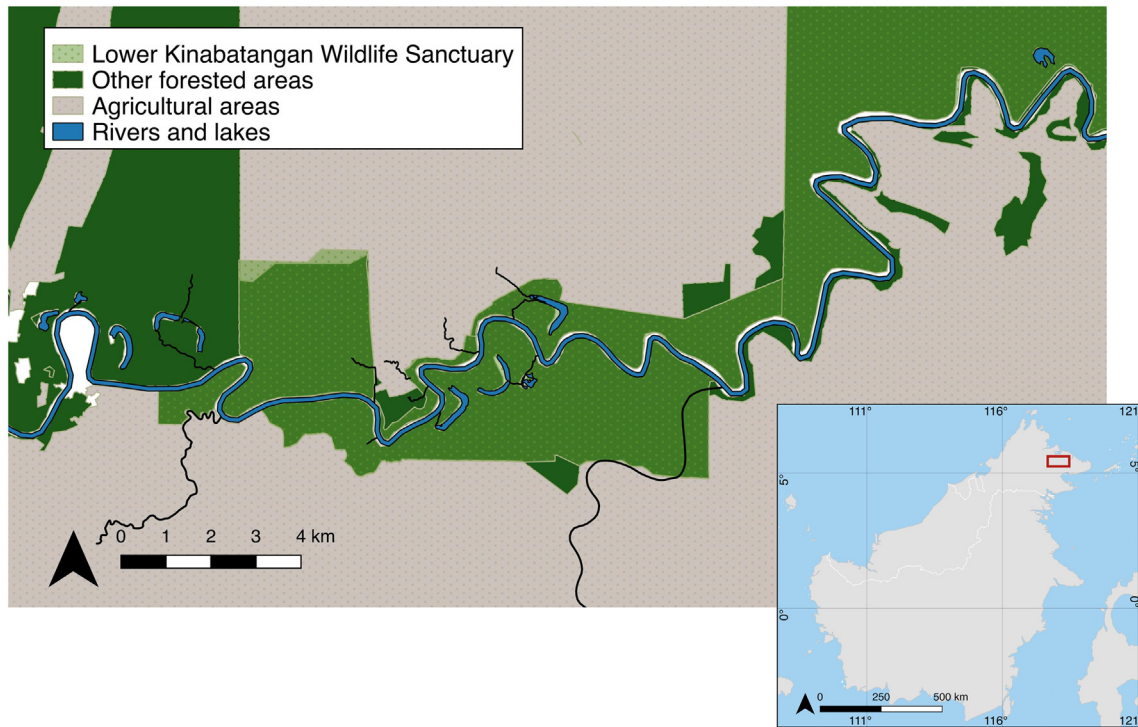
Fig. 1. Study area: the Lower Kinabatangan Wildlife Sanctuary (Sabah, Malaysia).

the area, during which time feces were opportunistically collected. The majority of samples (N = 197) were stored in sodium acetate–acetic acid–formaldehyde (SAF) for parasitological analyses, while other fixatives were used for the rest (27 samples were stored in formalin and 91 in EcoFix®, Meridian Bioscience, USA). Note that fixative type was shown to be unrelated to the recovery of parasite ova in our laboratory as long as the concentration protocols used were consistent (MacIntosh et al., unpublished data).

2.2. Host DNA identification

Host species identification was conducted for all faecal samples. Total genomic DNA was extracted from each sample using a QIAamp DNA stool mini kit (Qiagen, Japan), following the manufacturer's recommendations. A small fragment of the *cytochrome b* (*cytb*) gene was amplified for all samples, using the primer pair L14724/H15915 (Irwin et al., 1991), and following the protocol described in Frias et al. (2018). After PCR amplification, contaminants were removed from the amplicons using the Agencourt AMPure system (Agencourt Bioscience Corp., Beverly, MA, USA), and aliquots were sequenced in an ABI 3730xl DNA Analyzer (Applied Biosystems, CA, USA). To identify primate species, resulting *cytb* sequences were compared with template sequences in GenBank.

2.3. Parasitological analysis

We used a modified formalin-ethyl acetate sedimentation protocol to concentrate parasite eggs (Young et al., 1979; Manser et al., 2016), and examined the samples with a sequential sedimentation-flotation procedure. Five drops of Triton X-100 were added to each sample, which were mixed thoroughly before being strained through a 330 µm Saran™ mesh (Asahi Kasei, Japan) into a 15 mL centrifugation tube. The filtrate was centrifuged at 1,900g for 3 min. After the supernatant was discarded, the faecal pellet was weighed before being re-suspended in 8 mL of saline,

4 mL of ethyl acetate, and five more drops of Triton X-100. Each sample was mixed thoroughly, centrifuged, and the supernatant was discarded again. The final pellet was re-suspended in SAF, and a 1 mL aliquot was placed into a vial containing saline and put on a magnetic stirrer to keep well homogenised throughout the analysis. In order to estimate the number of eggs per gram of feces (EPG) in each sample, an aliquot was removed from the homogeneous suspension, placed in a McMaster counting chamber and examined at $100\times$ magnification. The average of five replicate counts of all nematode eggs observed under the chamber's grid was used to calculate the EPG, given the known weight of faecal sediment in the 0.15 mL volume of suspension in the McMaster chamber. After quantification, each sample was centrifuged, the supernatant was discarded, and the concentrated pellet was re-suspended in Sheather's solution with a specific gravity of 1.27. Two slides were examined to minimise the risk of missing less abundant helminth eggs. Parasite identification was conducted using standard keys (Modrý et al., 2018).

2.4. Measures of parasite distribution and diversity

To compare parasite distribution and diversity among primates in the community, we evaluated five measures of parasite infection:

(1) Parasite species richness was expressed as the number of parasite taxonomic groups identified through morphological assessment in a given sample, i.e. "observed richness". We also calculated species accumulation curves to model the relationship between the number of samples collected and the number of parasite species retrieved.

(2) Parasite prevalence, or the proportion of the host population infected with a given parasite, was measured as the percentage of positive samples for a given parasite taxonomic group in a given host population. Here we only present sample prevalence, as individuals were not identified. However,

given the breadth rather than depth of our sampling regime, it is highly unlikely that the same individual was sampled more than once in this study.

(3) Parasite abundance, or the number of individuals of each parasite taxonomic group detected in a given sample, was estimated using the EPG as a surrogate measure. While several studies have shown that EPG can provide a reliable estimate of adult parasite infection in a range of host species (Seivwright et al., 2004; Denwood et al., 2012), others have shown incongruences between EPG and true parasite intensities (Cripps et al., 2015), and thus results must be interpreted with caution.

(4) Parasite diversity was calculated using the Shannon index (H'), a measure that incorporates both species richness and their relative abundance, expressed here as parasite taxonomic groups and parasite prevalence, respectively.

(5) Parasite aggregation, resulting in a small percentage of the host population infected with most of the parasites, reflects both differential exposure to infective stages in the environment and susceptibility to infection (Crofton, 1971). In order to assess this measure, we compared two of the most used indices in the parasitological literature: (i) the ratio of the variance to the mean (s^2/x) number of parasites (here, EPG) per host, which indicates aggregation among hosts when greater than one, and (ii) the parameter k of the negative binomial distribution, which tends towards zero as aggregation increases (Crofton, 1971).

Data were analysed in R version 3.6.3 (https://www.R-project.org/). Diversity measures and accumulation curves were calculated using the package "vegan" version 2.5-6 (https://CRAN.R-project.org/package=vegan) and "BiodiversityR" version 2.12.1 (Kindt and Coe, 2005).

2.5. Ethics statement

Authorization to conduct research in Sabah, collect samples and export them to Japan was provided by the Sabah Biodiversity Centre (SaBC) and the Sabah Wildlife Department. Our field protocols adhered to the guidelines set by these agencies, as well to those set by the Field Research Committee at the Kyoto University Primate Research Institute, Japan.

3. Results

We analysed the parasite taxonomic group composition as estimated from samples collected from seven primate species, but because sampling effort was heterogeneous across taxa (Fig. 2),
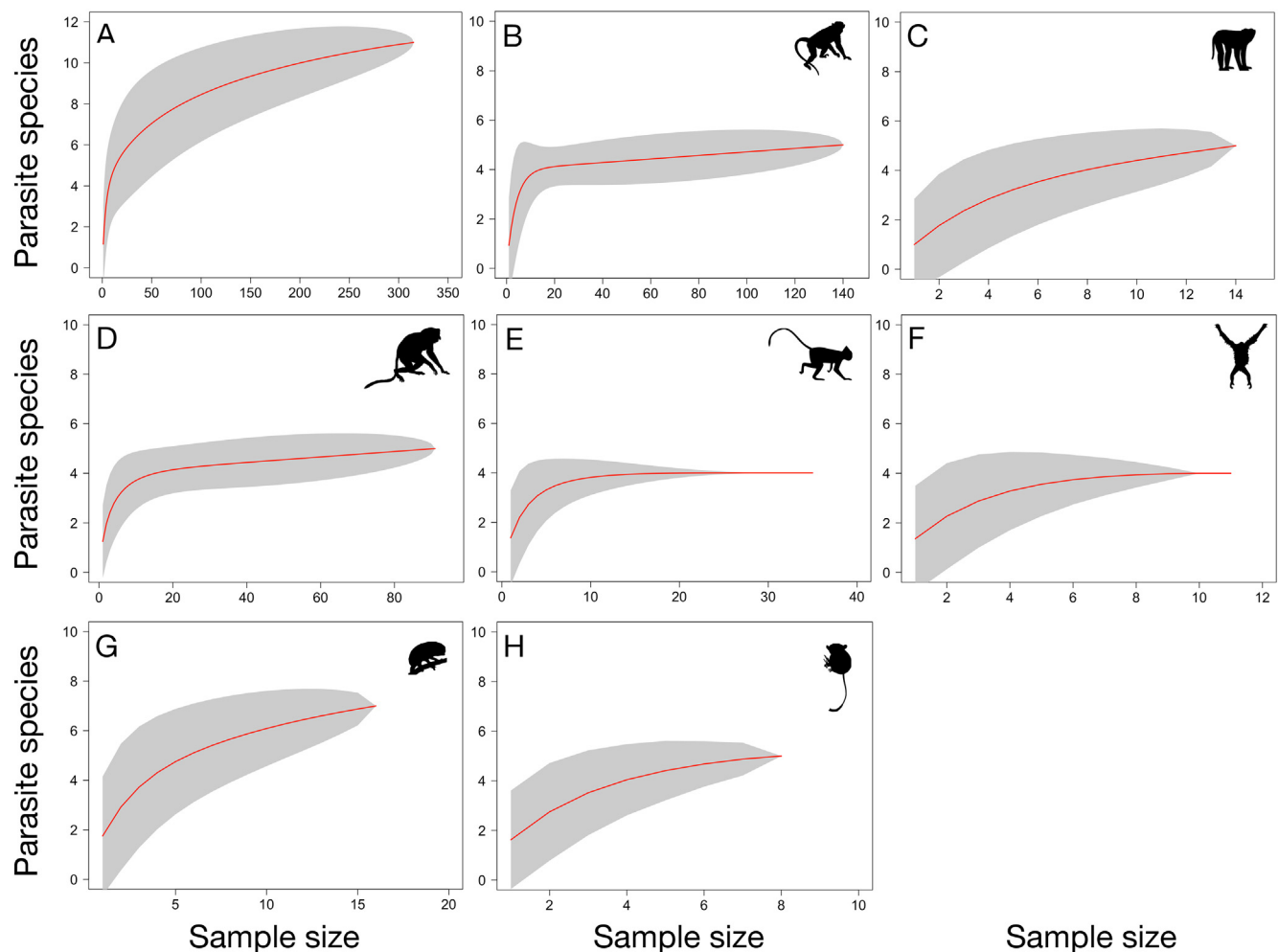

Fig. 2. Parasite species accumulation curve for the primate community studied and the parasites identified in feces through microscopy. The red line shows the mean curve and the shaded region the S.D. Accumulation curves correspond to (A) all primate species, (B) long-tailed macaques, (C) pig-tailed macaques, (D) proboscis monkeys, (E) silvered langurs, (F) Bornean orangutans, (G) Philippine slow lorises, and (H) Western tarsiers.

we regard the following results as the lower limit of parasite diversity across host species. We identified a total of nine nematode and at least two different trematode taxonomic groups in the dataset (Table 1, Supplementary Fig. S1). The prevalence, mean intensity and intensity range for each parasite group and host species are given in Supplementary Table S1. The highest prevalence observed among target taxa was for soil-transmitted helminths, a group of environmentally transmitted parasites, where *Trichuris* sp. 1 was the most prevalent parasite (48.5%), followed by parasites of the order Strongylida (25%), and *Strongyloides* spp. (21.5%).

Within the primate community, parasite species richness was highest in slow lorises (*N* = 7 taxa), and lowest in orangutans and silver langurs (*N* = 4). Several parasite groups were only identified in a few species of hosts; *Anatrichosoma* spp. in macaques, *Trichuris* sp. 2 in slow lorises, *Capillaria* spp. and trematodes in nocturnal primates, and oxyurids in nocturnal primates and one proboscis monkey. Members of the order Strongylida and the genus *Strongyloides*, widespread in primates, were present across all hosts in the primate community. Spirurid nematodes, which are transmitted through the ingestion of infected arthropod intermediate hosts, were only absent in tarsiers. In addition, while the most common morphotype of *Trichuris* spp. (Supplementary Fig. S1A) was not detected in nocturnal primates, we did observe a different morphotype (*Trichuris* sp. 2) producing significantly larger eggs in slow lorises (Supplementary Fig. S1B-C; 91.91 ± 6.03 µm × 40.90 ± 2.99 µm versus 56.39 ± 4.32 µm × 25.76 ± 1.96 µm) that was not present in any other members of the primate community. Note that the presence of these parasites in faecal samples does not necessarily confirm infection in primates, and that rare parasites (e.g. trematodes and spirurids), although included here, may not in fact be parasites of the primate community. We have nonetheless included all parasite material observed in feces in our results for full disclosure.

To emphasise the discrepancy in parasite richness values obtained from microscopy in this study and those obtained from genetic analyses (Frias et al., 2018, 2019a, 2019b), Table 1 presents the results obtained through microscopy alone. Parasite taxonomic groups showing distinct morphological differences were presented as different morphotypes (e.g. *Trichuris* sp. 1 and *Trichuris* sp. 2), and those that did not, such as oxyurid eggs, were clumped together into one group, even though the specialist nature of this parasite in primates likely ensures the presence of distinct species per host. Genetic studies conducted in parallel to assess parasite

species (Frias et al., 2019a) and potential cryptic diversity (Frias et al., 2018, 2019b) have further confirmed that morphological evaluation of eggs shed in feces underestimates richness values. Parasites identified here as "strongyles" have already been characterised as *Oesophagostomum aculeatum* and *Ternidens deminutus,* while "oxyurids" include *Lemuricola* (*Protenterobius*) *nycticebi* and *Enterobius* (*Colobenterobius*) *serratus*, and "*Strongyloides* spp." include *Strongyloides fuelleborni* and an as yet unidentified *Strongyloides* sp. (Fig. 3).

Finally, the observed frequency distribution of the most abundant parasites across the primate community was highly aggregated, as indicated by both measures of aggregation (Fig. 4, Supplementary Table S1). The parameter k had a negative correlation with the variance to mean ratio ($r = -0.518$, $P < 0.05$). As measured by k, aggregation decreased as the number of samples increased ($r = -0.436$, $P < 0.05$). Prevalence was positively correlated with k ($r = 0.556$, $P < 0.01$), but there was no correlation between variance to mean ratio and host sample or prevalence of infection. Aggregation as measured by k was not correlated to mean intensity, but if measured by the variance to mean ratio, correlation increased significantly with mean intensity ($r = 0.870$, $P < 0.001$).

4. Discussion

The first step towards understanding complex ecological interactions in nature is to know what is out there. This includes knowing what infectious agents occur naturally in populations and to what extent (i.e. what is their host range and how does it vary geographically), as well as their expected distribution in hosts and how it varies among hosts in a community. Communities of closely related species, or hosts having overlapping ranges, are often assumed to share parasite species. However, the presence of the same parasite morphotype in multiple sympatric hosts does not necessarily translate to cross-species transmission. The host-parasite associations we present in this study (Fig. 3A-B) should be interpreted with caution, as they are far from an accurate representation of true parasite species richness. In fact, if "hidden" parasite species richness is considered (Fig. 3C), the number of primate-parasite associations increases, which may significantly alter the structure of transmission networks, as well as our understanding of interaction patterns among species (Lafferty et al., 2006).

Table 1
Parasites found through microscopy in primates living in the Lower Kinabatangan Wildlife Sanctuary, Sabah, Malaysia. Values presented here include parasite prevalence (%) per host species (and number of positive samples observed), parasite species richness[a] and parasite diversity.

Parasite taxonomic group	Long-tailed macaque (*N* = 140)	Pig-tailed macaque (*N* = 14)	Proboscis monkey (*N* = 91)	Silvered langur (*N* = 35)	Bornean orangutan (*N* = 11)	Philippine slow loris (*N* = 16)	Western tarsier (*N* = 8)	Total (*N* = 315)
Nematoda								
Anatrichosoma spp. (Trichurida)	0.7 (1)	7.1 (1)	0	0	0	0	0	0.6
Capillaria spp. (Trichurida)	0	0	0	0	0	6.2 (1)	50 (4)	1.6
Trichuris sp. 1 (Trichurida)	31.4 (44)	50 (7)	80.2 (73)	74.2 (26)	27.2 (3)	0	0	48.5
Trichuris sp. 2 (Trichurida)	0	0	0	0	0	37.5 (6)	0	1.9
Strongyloides spp. (Rhabditida)	22.1 (31)	7.1 (1)	20.8 (19)	28.5 (10)	18.1 (2)	18.7 (3)	25 (2)	21.5
Oxyurida	0	0	1 (1)	0	0	56.2 (9)	50 (4)	4.5
Spirurida sp. 1	16.4 (23)	7.1 (1)	2.1 (2)	2.8 (1)	57.1 (4)	0	0	10.6
Spirurida sp. 2	0	0	0	0	0	12.5 (2)	0	0.6
Strongylida	22.8 (32)	28.5 (4)	29.6 (19)	28.5 (10)	54.5 (6)	37.5 (6)	25 (2)	25
Trematoda								
Trematoda sp. 1	0	0	0	0	0	6.2 (1)	0	0.3
Trematoda sp. 2	0	0	0	0	0	0	12.5 (1)	0.3
Parasite species richness[a]	5	5	5	4	4	7	5	
Shannon (*H'*)	1.39	1.27	0.99	1.06	1.30	1.69	1.49	

[a] Values correspond to observed richness.

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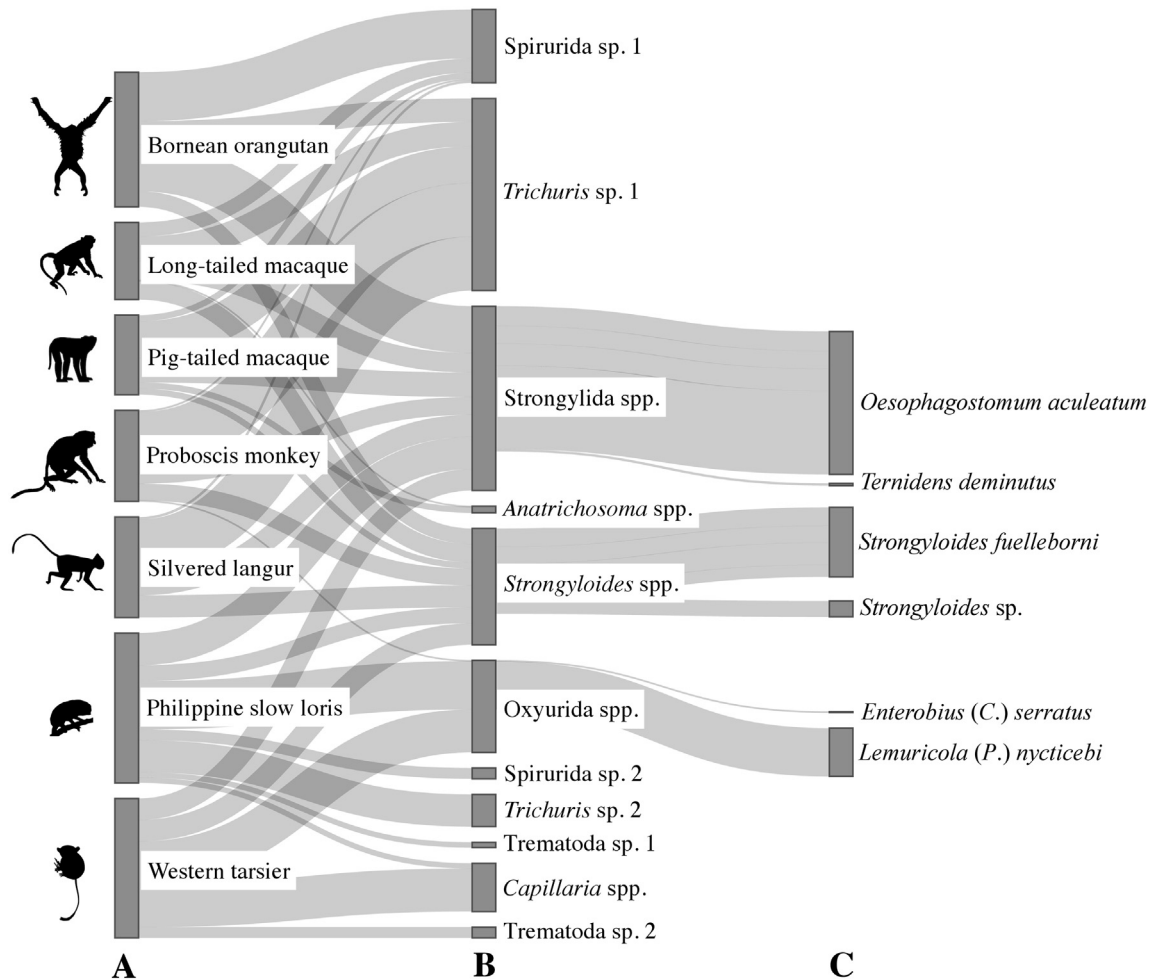International Journal for Parasitology xxx (xxxx) xxx

Fig. 3. Primate-parasite associations, showing (A) primate hosts, (B) observed parasite species richness per host species (detected through microscopy), and (C) hidden parasite species richness per parasite taxonomic group (detected through molecular analyses). Widths of bars correspond to parasite species richness for each primate host (A; range = 4–7) and parasite prevalence (B; range for observed prevalence = 0.6–48.5%). Genetic studies conducted in parallel have revealed higher parasite species richness (C) than that observed by microscopy alone (Frias et al., 2018, 2019a, 2019b).

The presence of cryptic species, i.e. species that are morphologically identical but genetically distinct, also challenges our estimates of biodiversity (Scheffers et al., 2012) and our ability to determine host ranges and interaction networks (Poulin and Keeney, 2008). Cryptic genetic lineages have been described for primate soil-transmitted helminths (*Trichuris* spp. (Ghai et al., 2014a), *Oesophagostomum* spp. (Ghai et al., 2014b; Ota et al., 2015; Frias et al., 2019b), and *Strongyloides* spp. (Frias et al., 2018)), showing the hidden complexities of parasite population structure. The demonstration that *Oesophagostomum bifurcum* from humans and several non-human primates have distinct transmission patterns, and thus non-human primates are not reservoir hosts for human infection in Ghana as was once proposed, had practical public health implications for the control of the disease in the country (Gasser et al., 2006). Such examples underscore both the need to exercise caution when interpreting the results of studies such as this, and the need to increase efforts to uncover the true biological diversity of parasitic organisms in host communities, especially when dealing with parasites of wildlife, domestic animal and public health concern (Wolfe et al., 2007).

As much of the present range of primates is being greatly influenced by anthropogenic activities, increasing the risk for zoonotic parasite transmission in both directions (Gottdenker et al., 2014; Han et al., 2016), efforts to preserve endangered primates and monitor patterns of parasite transmission at the wildlife-human interface could benefit greatly from surveillance of natural populations (Woolhouse et al., 2001; Karesh et al., 2012). This means that the full potential for bidirectional exchange of parasites and pathogens involving Asian primates in particular has yet to be sufficiently explored, despite the human population density in Asia far exceeding that of other regions (http://www.worldpopdata.org) and the proportion of threatened primates in Asia being greater than that of either mainland Africa or the Americas (Estrada et al., 2017).

The recent record of malaria caused by *Plasmodium cynomolgi* in Borneo (Law, 2018), which also corresponds to the second zoonotic malaria identified in the region, *Plasmodium knowlesi* (Singh et al., 2004), reminds us of the need for more extensive surveys and inventories; biodiversity cannot be fully comprehended without a systematic foundation. This becomes essential to recognise the potential emergence of parasites, and interactions between parasite assemblages circulating at the interface of agricultural and wild ecosystems (Brooks and Hoberg, 2000). The compilation of non-invasive wildlife data does not always allow for systematic collection schemes, and unfortunately this study does not account for sampling bias across host species or seasonal variation, whereas several parasite species do show marked seasonal fluctuations (Huffman et al., 1997). Parasites are also likely to be overlooked if the sampling effort is insufficient, especially if parasite prevalence is low (Walther et al., 1995).
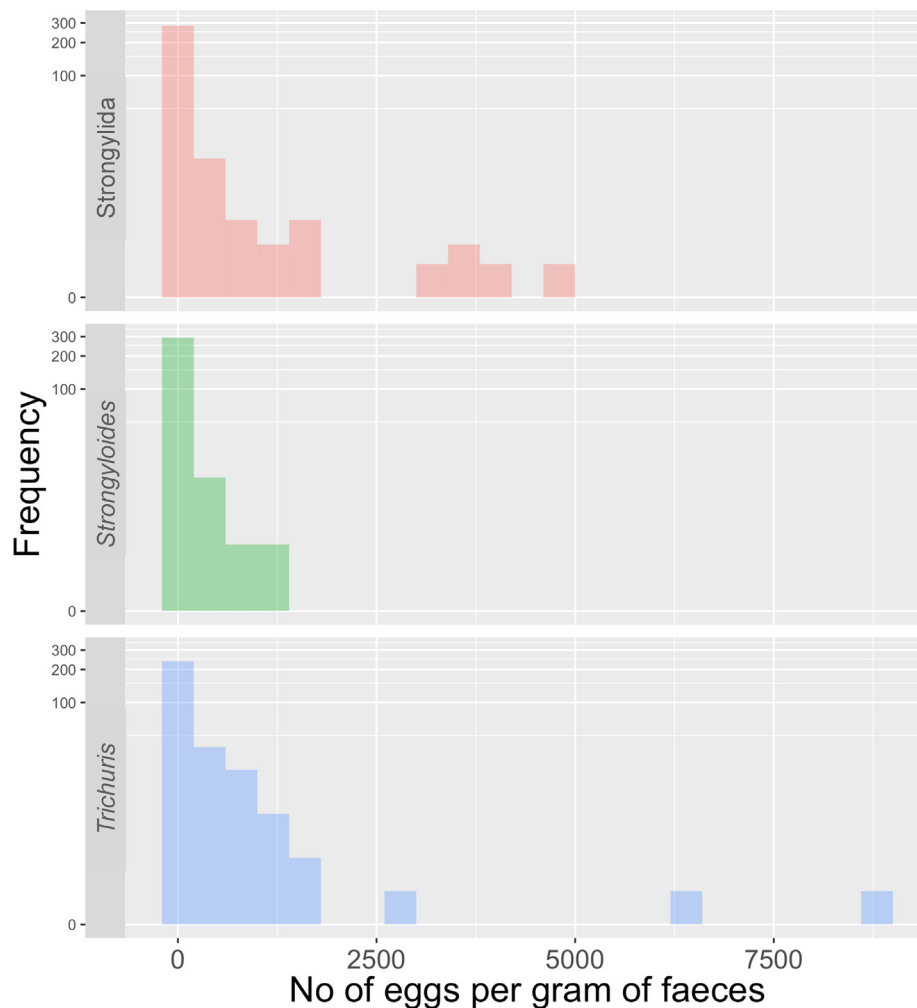
Fig. 4. Observed frequency distribution for the most abundant parasites identified in the primate community (Y axis was log-plus-one transformed).

In this context, species accumulation curves are not only useful to guide sampling efforts and predict richness, but they can inform about patterns of parasite diversity (Dove and Cribb, 2006). Species accumulation curves in this study indicated that, for well-sampled species such as long-tailed macaques and colobines, we were able to capture a more accurate representation of their true parasite species richness (Fig. 2B–F). Within the primate community, colobines showed the lowest parasite diversity; however, even though our sample size for silvered langurs was modest, it was sufficient to capture the majority of their parasite species (Fig. 2E). This suggests that even a small sampling effort can be invaluable for rare colobines, for which parasite data are scarce or absent altogether (Supplementary Table S2). Among those under-sampled primates, slow lorises stand out as having the highest parasite diversity in the whole primate community, even with only 16 samples examined here, which suggests there remain more parasite species to discover in this elusive host.

From the perspective of infracommunities, most parasites seem to infect most primate species, with varying prevalence and egg shedding output across the host community (Supplementary Table S1). Certain parasite taxonomic groups (*Capillaria* spp. and Trematoda) were only identified in nocturnal primates, and one additional parasite species (*Trichuris* sp. 2) was found only in one of them. Apart from the nocturnal lifestyle that differentiates them from the rest of the community, slow lorises were the only strepsirrhine primate, while tarsiers were the only primarily carnivorous primate studied. Such findings suggest that the composition

of parasite infracommunities in nocturnal primates may be constrained by conditions not present in the rest of the host community, i.e. phylogenetic distance and ecological traits, but only molecular analyses will be able to determine whether the apparently shared parasites are in fact the same or not. This is particularly problematic for rare parasites (e.g. spirurids in most primates studied here, or capillariids in slow lorises), where low prevalences make it difficult to differentiate true parasitism from spurious pseudo-infections. On the other hand, parasites that are widespread but shed few eggs in feces are likely to be overlooked in parasitological analysis. In this study, for example, we were not able to detect *S. fuelleborni* eggs from all orangutan feces that produced larvae through coprocultures (Frias et al., 2018), thus inevitably underestimating the parasite's prevalence in this primate species. Finally, this work entailed some degree of useful "stamp collecting" – i.e. cataloguing the players in the host-parasite community investigated – and thereby expands current knowledge of primate parasites in an under-represented area of their range. We also provide the first known parasite records for southern pig-tailed macaques, silvered langurs and Western tarsiers in the wild, while adding to the scant existing literature for the other species in the community

This study is also among the few to consider parasite aggregation in primate hosts (but see Müller-Graf et al., 1996 (baboons, *Papio cynocephalus anubis*), Monteiro et al., 2007 (golden lion tamarins, *Leontopithecus rosalia*) and MacIntosh, 2014 (Japanese macaques, *Macaca fuscata*)). Parasite aggregation has implications

that extend across multiple ecological scales, from individual fitness constraints to host population regulation (Anderson and May, 1978; May and Anderson, 1978). Parasite aggregation may be a relevant feature for parasite ecology as well, influencing intra- and interspecific interactions such as mating and colonisation success. We observed different levels of aggregation for the three most abundant parasites identified in the community (Fig. 4); while the distribution of *Strongyloides* spp. was highly aggregated ($k = 0.1$–0.28), that of *Trichuris* spp. ($k = 0.14$–0.4) and Strongylida ($k = 0.1$–0.54) showed a wider variability. Exploring patterns of parasite aggregation in natural habitats may not only highlight underlying infection processes but, monitored over time, it could give us an idea of the stability of host populations. For example, because highly aggregated distributions translate into fewer heavily infected hosts, a drop in parasite aggregation in a host population over time could be indicative of mortality of highly infected individuals or changes in the demographic structure of the host population as a whole (Wilson et al., 2002; Jolles et al., 2008).

In this study, we observed that while parasite diversity was the lowest in colobines, the prevalence of *Trichuris* sp. 1 in this group was the highest. Similar observations have been reported for other colobines. In Uganda, for example, black-and-white colobus (*Colobus guereza*) and red colobus (*Procolobus rufomitratus*) inhabiting continuous forests exhibited prevalences ranging from 36%–50%, respectively, which increased to 84%–90% in individuals inhabiting fragmented forest (Gillespie and Chapman, 2008). In Asia, proboscis monkeys living in their natural habitat exhibited a prevalence of 82.1% for *Trichuris*, the highest among other parasites detected, which increased to 91.1% in individuals living in a sanctuary environment (Klaus et al., 2018). This suggests that diverse parasite communities may buffer against an overabundance of generalist parasites, which tend to be more resilient to anthropogenic changes and are often associated with species extinctions (Gog et al., 2002). Studies such as this can only offer a starting point and should not be viewed by any means as a complete record of parasite diversity in Bornean primates. However, given the gap in information regarding primate-parasite dynamics in the region, such information should prove useful to future studies of parasite biodiversity and infectious disease ecology in Asia and elsewhere.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2021.03.003.

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