



Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Co-infection patterns of intestinal parasites in arboreal primates (proboscis monkeys, *Nasalis larvatus*) in Borneo



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ARTICLE INFO

Article history:

Received 13 February 2017

Received in revised form

28 August 2017

Accepted 14 September 2017

Keywords:

Nasalis larvatus

Gastrointestinal parasites

Co-infection

Conservation

South-East Asia

Malaysia

Zoonosis

ABSTRACT

Non-human primates of South-East Asia remain under-studied concerning parasite epidemiology and co-infection patterns. Simultaneously, efforts in conservation demand knowledge of parasite abundance and biodiversity in threatened species. The Endangered proboscis monkey, *Nasalis larvatus*, a primate flagship species for conservation in Borneo, was investigated in the present study. Habitat loss and fragmentation are among the greatest threats to bachelor and harem groups of this folivorous colobine. Designed as a follow-up study, prevalence and co-infection status of intestinal parasites from *N. larvatus* in a protected area in Malaysian Borneo were analyzed from fecal samples using a flotation method. For the first time, the intestinal parasite co-infection patterns were examined using quantitative analyses. Overall, 92.3% of fecal samples (N = 652) were positive for helminth eggs. Five helminth groups were detected: (1) trichurids (82.7% prevalence) including *Trichuris* spp. (82.1%) and *Anatrichosoma* spp. (1.4%), (2) strongyles (58.9%) including *Trichostrongylus* spp. (48.5%) and *Oesophagostomum/Ternidens* spp. (22.8%), (3) *Strongyloides fuelleborni* (32.7%), (4) *Ascaris lumbricoides* (8.6%), and (5) *Enterobius* spp. (5.5%). On average, an individual was co-infected with two different groups. Significant positive associations were found for co-infections of trichurids with strongyles and *S. fuelleborni* as well as *S. fuelleborni* with *A. lumbricoides* and strongyles. This study shows a high prevalence of various gastrointestinal helminths with potential transmission pathways primarily related to soil and with zoonotic relevance in wild proboscis monkeys in their remaining natural habitats. Observed positive associations of trichurids with strongyles and *Strongyloides* spp. may result from the high prevalence of trichurids. Similarly, positive associations between *Strongyloides* and *Ascaris* were found, both of which typically occur predominantly in juvenile hosts. These findings should be considered when proposing conservation actions in altered habitats nearby human settlements and when managing captive populations.

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1. Introduction

The study of wildlife parasites plays a crucial role for conservation efforts of threatened species worldwide (Daszak et al., 2000). To understand the impact of parasitic infections on

wildlife endangerment, comprehensive datasets on parasite abundance, co-infection status, and transmission pathways of potential pathogens in natural systems are required (Thompson et al., 2010). Parasitic infections are among the most common diseases found in non-human primates (hereafter referred to as 'primates') (Strait et al., 2012). Specifically, intestinal parasitic infections are the focus of a large proportion of studies, but their role in influencing ecosystems and population dynamics remains controversial (Marcogliese, 2005; Gillespie and Chapman, 2008; Nguyen et al.,

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2015). In addition, the zoonotic potential of pathogens in wild primates has received considerable attention as contact between domestic communities and local primate species has increased over the past decades.

Mechanisms of interactions among parasitic species were examined for nematodes as well as between helminths and protozoa, but results remain ambiguous (Petney and Andrews, 1998; Murphy et al., 2013). Several mechanisms of direct and indirect interaction between co-infecting parasites have been proposed. Parasites may compete for host resources (e.g. space and food), or they may benefit from immunosuppression by one species favoring host infection with another species (Pedersen and Fenton, 2007). However, few data are available on co-infection patterns in primates. To date, most is known about intestinal helminths and protozoa in wild African ape populations or New World monkey species (e.g. Nizeyi et al., 2002; Michaud et al., 2003; Eckert et al., 2006; Gillespie et al., 2010). In contrast, parasites in wild primate populations of South-East Asia have been little studied, especially in relation to their total population size, geographical distribution and conservation status (Hopkins and Nunn, 2007).

The island of Borneo, South-East Asia, is one of the world's 34 biodiversity hotspots (De Bruyn et al., 2014), suffering from the highest deforestation rate in the tropics (Sodhi et al., 2010) as a result of the growing economy of local human populations, particularly resource extraction. As Borneo also attracts many tourists who visit its endemic wildlife (King et al., 2013), it is important to study the prevalence and diversity of zoonotic parasites in local primate species, that may act as reservoirs for human infections.

The folivorous proboscis monkey, *Nasalis larvatus*, is a colobine Old World monkey endemic to Borneo. Classified as Endangered EN A2cd since 2000 (EN: considered to be facing a very high risk of extinction in the wild, A: through a reduction in population size, 2: of $\geq 50\%$ over the last 10 years or three generations, based on c: a decline in area of occupancy, extent of occurrence and/or quality of habitat and d: actual or potential levels of exploitation) (IUCN, 2016), it acts as a primate flagship species for conservation in Sabah, Malaysian Borneo (Goossens and Ambu, 2012) attracting many tourists (Leasor and Macgregor, 2014). Priority areas of proboscis monkey populations exist near the coast up to the headwaters of major rivers in all provinces of Borneo. Groups have also been recorded in disturbed habitats of secondary forest near human settlements and remnant tidal forest close to agricultural land use (Sha et al., 2008).

To date, data are available from necropsy of a single individual (Hasegawa et al., 2003) and from fecal examinations of captured animals (Hernasari, 2011) and wild proboscis monkeys (Ranglack and Yeager, 1986; Salgado Lynn, 2010). Overall, infection rates were high (62–96% overall prevalence) and infections with soil-transmitted helminth species were most common (Ranglack and Yeager, 1986; Salgado Lynn, 2010; Hernasari, 2011). Salgado Lynn (2010) pointed out that almost 90% of proboscis monkeys from different forest lots of the Lower Kinabatangan Wildlife Sanctuary (LKWS) in Sabah, Malaysian Borneo, were co-infected with multiple parasite species. Analyses of these concomitant parasite infections are not available but are urgently required for drawing conclusions regarding transmission dynamics, as well as for identifying whether this host may act as a potential super-spreader within primate populations.

The present study aimed to examine gastrointestinal parasite co-infection patterns via quantitative analyses in wild proboscis monkeys for the first time. Based on previous findings in 2007/2008 (Salgado Lynn, 2010), in 2012 we conducted a follow-up study to record the infection status of wild proboscis monkey groups after five years. Special attention was given to potentially zoonotic

parasite species. We predicted that 1.) infection rates in wild proboscis monkeys would remain high, and 2.) interactions among intestinal parasite species do not exist, but co-infection patterns in wild proboscis monkeys were shaped by the frequency of the occurrence of intestinal parasite species. Used as a baseline for further investigations in different proboscis monkey habitats, this study could support conservation actions for subpopulations of this tropical flagship species in Borneo. Furthermore, it contributes to assessing the poly-parasite infection risk originating from these primate populations to nearby villagers by identifying potential connections to human disease in the region.

2. Material and methods

2.1. Study site

The Malaysian state of Sabah is located at the North-Eastern tip of Borneo (Fig. 1). The climate throughout the island is tropical with small variations in temperature, usually between 21° and 34° Celsius with annual precipitation being around 3000 mm (Ancrenaz et al., 2004). Over the past century most parts of the dry lowland forests have suffered from human agricultural activities and have been logged for oil palm plantations. In 2005, the State Government declared 27,000 ha of highly disturbed forests along the floodplain of the Lower Kinabatangan River (5°10'–5°50'N; 117°40'–118°30'E) a wildlife sanctuary (Goossens et al., 2005). Divided into 10 riparian forest blocks, called 'Lots', the sanctuary forms a corridor of different forest types including mangrove and riverine forest surrounded by oil palm plantations. Eco-tourism and wildlife tours are popular along the Kinabatangan (Leasor and Macgregor, 2014). Furthermore, Sabah includes many fishing communities along the coastline and river mouths. The majority of the human settlements in rural areas are closely associated with river systems and coincide with major concentrations of proboscis monkeys (Sha et al., 2008).

Lot 6 of the LKWS was the specific sampling area for the present study. Starting from the Danau Girang Field Centre located in Lot 6, sample collection took place 6.1 km upstream (3.7 km linear distance) and 4.7 km downstream (3.0 km linear distance) along the southern bank of the river (Fig. 1).

2.2. Study species

River surveys along the majority of Lots of the LKWS counted 1454 proboscis monkeys in 101 groups in 2008 (Sha et al., 2008). In 2010, 818 individuals in 113 groups with group encounter rates about 1 group/km on average were observed along 30 km of the Kinabatangan River and a nearby tributary of Lot 6 (Stark et al., 2012), which represents around one-fifth of the estimated population in Sabah.

Proboscis monkeys are sexually dimorphic and the largest foregut-fermenting colobines, with males being about twice as heavy (20 kg body mass on average) as females (10 kg body mass on average) (Bennett and Sebastian, 1988). Their social organization consists of either harems (one male-multi female-groups with their offspring) or bachelor (all male) groups of up to 30 individuals, which are closely associated with waterways and forage about 1 km each day before returning to their sleeping sites at the riverbank in the evenings (Boonratana, 2000). Home range areas of groups were estimated to be 80 ha on average (Stark et al., 2017) and can extensively overlap each other (Yeager, 1989).

2.3. Sample collection

A total of 652 fecal samples were collected from June to

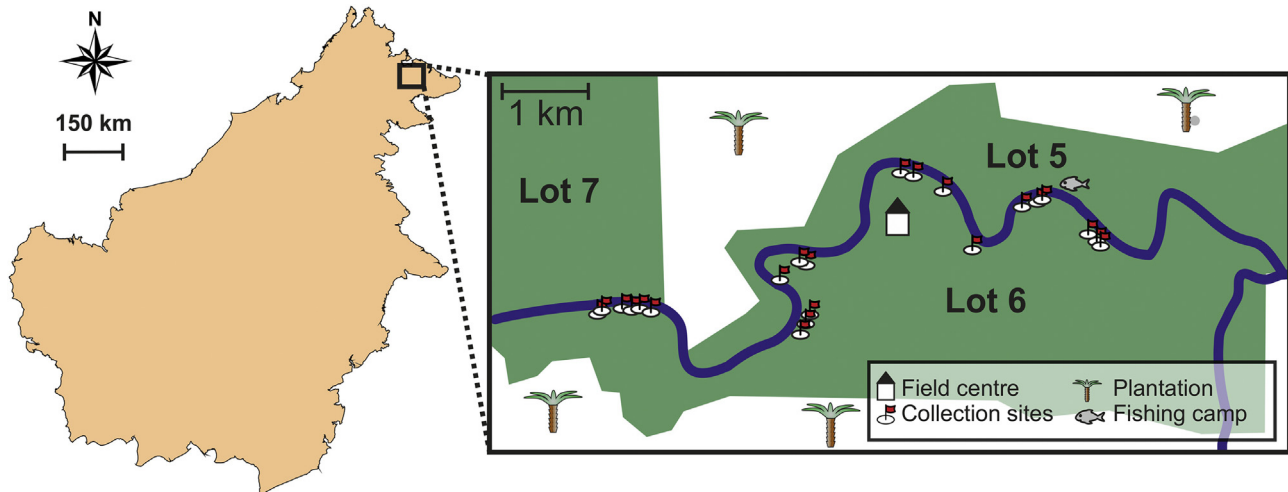


Fig. 1. Sample collection sites along the Kinabatangan River in Borneo. The island of Borneo, South-East Asia, with position of Lot 6 on the southern riverbank in the Lower Kinabatangan Wildlife Sanctuary in Sabah, Malaysian Borneo. Map reproduced according to GPS data points collected and mapped via Garmin Map Source (version 6.16.3).

September 2012. The sampling months constituted the local dry season and the beginning of the wet season. Samples were obtained on 16 occasions from harem groups, on four occasions from bachelor groups, and on two occasions from solitary proboscis monkey males. In the evening before sunset (17:00–19:00 h), groups close to the southern riverbank were approached to record data on group size, group type and GPS coordinates before the animals fell asleep. On the following day, the same group was visited before sunrise (05:00–08:00 h) for collecting fresh fecal samples. The surface of each fecal sample was removed with a spatula to avoid contamination with parasite stages from the ground and samples were put in 15 ml tubes. Collection started as soon as the group had started foraging but not any earlier to avoid disturbing the animals. Assignment of samples to individual groups was uncertain due to the possible overlap of home ranges, joint travel routes and common sleeping trees (Yeager, 1989). Therefore, samples collected on different days were treated as different sampling units. However, based on their distribution along the Kinabatangan River, published estimates of home ranges (Stark et al., 2017), and our own observations of group sizes, we assume that we sampled at least nine different harem groups, three different bachelor groups and one solitary proboscis monkey male. Each fecal sample was weighed and preserved in 10% buffered formalin (for 1L: 900 ml distilled water, 100 ml formaldehyde 37–40%, 6.5 g anhydrous disodium hydrogen phosphate Na_2HPO_4 , 4.0 g sodium dihydrogen phosphate NaH_2PO_4) in a 1:3 ratio.

2.4. Fecal sample analyses

Fecal samples were analyzed using a modified sodium nitrate flotation method suitable for detection of helminth eggs and a variety of protozoan oocysts (Gillespie, 2006). The flotation solution contained 600 g sodium nitrate (NaNO_3) dissolved in 1 L distilled water, resulting in a specific gravity of 1.3, which was continuously verified with a hydrometer (Hydrometer g/ml Tp. 20 °C 55 mN/m ST. N° 0310, ALLA France). Prior to flotation the preservative formalin was largely removed from the feces by centrifugation at 700g for 10 min (Heraeus Multifuge 3 S-R, Thermo Fisher Scientific, Waltham, MA, USA). Supernatant was discharged and feces were washed by homogenization in distilled water and centrifuged again, the supernatant removed, and the remaining feces weighed. Up to 2 g of this material were thoroughly mixed with sodium nitrate up to the 15 ml mark on the tube, centrifuged at 860 g for 10 minutes, and the meniscus transferred onto a slide

and a coverslip applied. The sample was immediately examined microscopically at $\times 10$ and $\times 40$ magnification with an Axiophot microscope (Carl Zeiss MicroImaging, Oberkochen, Germany). All detected parasitic stages were counted to calculate the number per gram feces. Subsequently, the eggs were photographed using an Olympus ColorView Illu digital camera (OLYMPUS Soft Imaging Solutions, Munster, Germany) and measured with the software cell^B (version 3.1, Olympus Soft Imaging Solutions). All samples were analyzed in a randomized order to avoid any bias in evaluation.

2.5. Data analyses

Egg measurements of different trichurid and strongylid morphotypes were compared in the R software environment (version 0.99.489, R Core Team), using nonparametric Kruskal-Wallis rank sum tests to investigate morphotype differences in length and width, respectively. Morphotype T4 was excluded from statistical analyses due to low sample size ($n = 2$). Post hoc pairwise comparisons between measurements were made by pairwise Mann Whitney-U tests. P-values were adjusted via Holm correction (Holm, 1979).

Interactions between parasite infections were investigated in generalized linear mixed models (GLMM) using the binomial presence/absence data of 652 fecal samples for each parasite order separately. To identify the best model of fixed effects for each parasite order, the step-up method was applied, i.e. the consecutive addition of fixed factors (West et al., 2006) and model comparisons and selection via the *anova* function using the packages lme4 (Bates et al., 2015) and effects (Fox, 2003) were performed. Parasite orders except for the tested one were set as fixed effects. In each model, the sampling occasion (= sampled group of proboscis monkeys) was set as a random factor to control for group variations. The size of unidirectional effects and reciprocal associations between co-infecting parasite orders were calculated using R's exponential function for estimating values of each fixed effect inside best models. As a post hoc procedure Pearson's Chi Square tests were conducted in SPSS Statistics (version 23.0, IBM) for each associated pair of parasites.

2.6. Ethics statement

Investigations and sample collection complied with Malaysia's law on foreign research, and project license was approved by the

Sabah Biodiversity Council, the Sabah Wildlife Department and the Danau Girang Field Centre (Licence N° UPE: 40/200/19/2822). Study of the free-ranging proboscis monkeys without interacting with the animals as well as non-invasive fecal sample collection adhered to the Code of Best Practices for Field Primatology of the International Primatological Society (IPS), the ethical guidelines of the German Primate Society (GfP) and the German Animal Protection Act.

3. Results

3.1. Parasite prevalence and taxonomic diversity

Helminth eggs were detected in fecal samples from each sampling occasion (harem groups $n = 16$, bachelor groups $n = 4$, and solitary individuals $n = 2$). Protozoan oocysts or cysts were not detected. In total, 602 samples (92.3%) were positive and only 50 samples (7.7%) were negative for helminth eggs. Five helminth orders were identified based on egg morphology, all being of zoonotic importance: Enoplida, trichurids (prevalence: 82.7%), genera *Trichuris* and *Anatrichosoma*; Strongylida (58.9%), genera *Trichostrongylus* and *Oesophagostomum/Ternidens*; Rhabditida, genus *Strongyloides* (32.7%); Ascaridida, genus *Ascaris* (8.6%); and Oxyurida, genus *Enterobius* (5.5%). Parasite order richness (POR) ranged from 0 to 5 orders per fecal sample. Positive samples most often contained eggs from two different parasite orders (38.0%), followed by one parasite order (27.5%), three orders (22.7%), none (7.7%), four orders (4.0%) and one sample with a POR of 5 (0.1%). On average, samples showed a POR of 1.8 (± 0.9 SD).

3.2. Egg morphotypes within taxonomic groups

Trichurid eggs showed the highest variation in morphology (Fig. 2). Five different morphotypes that differed in egg shape, shell appearance, color and width (Table 1) could be discriminated in a subset of 520 fecal samples positive for trichurids: T1–T4 *Trichuris* spp. and T5 *Anatrichosoma* spp. There were three morphotypes of eggs from the Strongylida that differed in shape, content and length (Fig. 2, Table 1) in 352 positive fecal samples: S1 *Trichostrongylus* spp., S2 *Oesophagostomum/Ternidens* spp. and S3 unknown strongylid. Ascarid eggs showed a smooth surface indicating that

they had lost their rough outer shell layer (Fig. 2), most probably due to sample processing during coproscopical examination.

A comparison of metric details of the family Trichuridae and order Strongylida revealed no significant differences between the morphotypes of Trichuridae in length ($H = 3.938$, $p = 0.268$, $n = 81$) but significant differences in width ($H = 60.268$, $p < 0.0001$, $n = 81$), as well as significant differences in length between the morphotypes of Strongylida ($H = 32.456$, $p < 0.0001$, $n = 77$) but not in width ($H = 5.958$, $p = 0.051$, $n = 77$). Post hoc pairwise comparisons with Holm-corrected p-values (Table 2) still showed significant differences in width for trichurid morphotypes (Fig. 3) and in length for morphotypes of Strongylida (Fig. 4).

3.3. Egg excretion intensity

For trichurid eggs, a minimum of 0.5 and a maximum of 849.8 eggs were counted per gram fecal sample with a mean number of eggs per gram feces (epg) of 47.0 (± 101.1 SD). The mean epg of the four detected trichurid morphotypes was 4.0 (± 6.1 SD) for T1 *Trichuris* spp., 45.5 (± 101.9 SD) for T2 *Trichuris* spp., 2.8 (± 3.9 SD) for T3 *Trichuris* spp. and 1.1 (± 0.4 SD) for T5 *Anatrichosoma* spp., respectively. T4 was excluded from calculations due to low sample size ($n = 2$). Strongylida egg excretion ranged from 0.5 to 53.3 epg with a mean epg of 4.3 (± 6.8 SD). The mean epg of the three different strongylid morphotypes was 3.3 (± 5.4 SD) for S1 *Trichostrongylus* spp., 1.7 (± 1.8 SD) for S2 *Oesophagostomum/Ternidens* spp. and 2.0 (± 1.9 SD) for S3 unknown strongylid. For *Strongyloides*, a minimum of 0.5 and a maximum of 23.6 eggs per gram feces were counted with a mean epg of 2.6 (± 3.2 SD). Excretion of ascarid eggs ranged from 0.5 to 42.1 epg (mean epg 2.6 \pm 6.3 SD), excretion of *Enterobius* eggs from 0.5 to 7.9 epg (mean epg 2.1 \pm 1.8 SD).

3.4. Parasite co-infections

The trichurid morphotype T1 (*Trichuris* spp.) was never shed simultaneously with T4 (*Trichuris* spp.) or T5 (*Anatrichosoma* spp.). Furthermore, unknown strongylid eggs of the morphotype S3 were never shed simultaneously with those of S2 (*Oesophagostomum/Ternidens* spp.). The analysis of co-infections for the five detected parasitic helminth orders only revealed evidence of positive

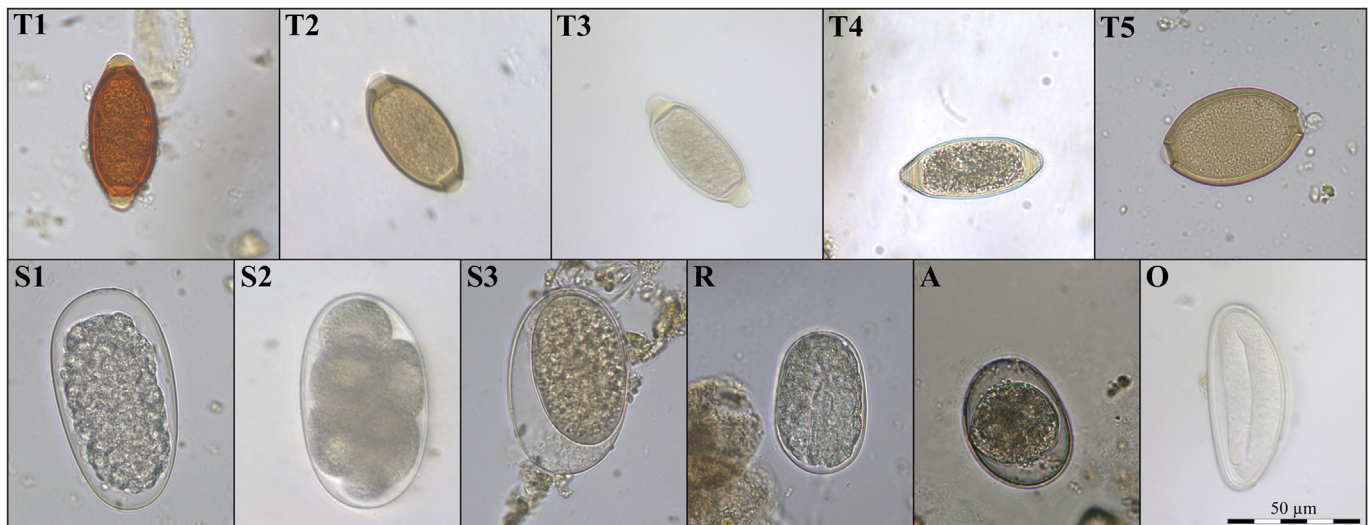


Fig. 2. Taxonomic diversity of helminth parasites found in proboscis monkeys. The five detected helminth orders were: the order Enoplida, trichurids (morphotypes T1–T4 genus *Trichuris*, T5 genus *Anatrichosoma*), the order Strongylida (morphotypes S1 genus *Trichostrongylus*, S2 genus *Oesophagostomum/Ternidens*, S3 unknown strongylid), the order Rhabditida, genus *Strongyloides* (R), the order Ascaridida, genus *Ascaris* (with exfoliated rough brown outer shell layer) (A) and the order Oxyurida, genus *Enterobius* (O). Scale bars = 50 μ m. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 1
Morphology and prevalence of helminth egg morphotypes in proboscis monkeys.

Parasite group	Morphotype	Genus/Species; key references	Prevalence ^{b, c}	Prevalence within order	Mean length (μm) ± SD	Mean width (μm) ± SD	Egg shape	Shell appearance and color	Content
Trichurids (n ^a = 520)	T1	<i>Trichuris</i> spp.	11.0%	13.4%	55.69 ± 2.30 (n = 11)	24.53 ± 1.35 (n = 11)	Lemon	Thick with adhesions, prominent transparent bipolar plugs, dark golden brown	Granulated single cell (zygote)
	T2	<i>Trichuris</i> spp.	79.9%	97.3%	54.54 ± 2.93 (n = 30)	26.44 ± 1.34 (n = 30)	Compact to ellipsoid barrel	Very thick, flat transparent bipolar plugs, brown	Granulated single cell (zygote)
	T3	<i>Trichuris</i> spp.	27.0%	32.8%	54.05 ± 2.57 (n = 30)	23.00 ± 1.08 (n = 30)	Flattened lemon	Thick, prominent transparent bipolar plugs, light brown	Granulated single cell (zygote)
	T4	<i>Trichuris</i> spp.	0.2%	0.2%	56.32 ± 1.55 (n = 2)	23.69 ± 0.36 (n = 2)	Lemon	Thin, tiny ribbed bipolar plugs, light color	Granulated single cell (zygote)
	T5	<i>Anatrichosoma</i> spp. (Allen, 1960)	1.4%	1.7%	53.91 ± 2.67 (n = 10)	32.11 ± 2.01 (n = 10)	Balloon-like barrel	Very thick, flat transparent bipolar plugs, brownish yellow	Granulated single cell (zygote)
Order Strongylida (n ^a = 352)	S1	<i>Trichostrongylus</i> spp. (Jessee et al., 1970), (Ash, 2007)	48.5%	84.1%	82.81 ± 4.31 (n = 30)	45.82 ± 3.16 (n = 30)	Ellipsoid to ovoid, elongated	Thin, light color	Morula with numerous grapelike blastomeres (>12)
	S2	<i>Oesophagostomum</i> / <i>Ternidens</i> spp. (Cogswell, 2007), (Ghai et al., 2014a), (Sandground, 1931)	22.8%	39.5%	80.93 ± 3.06 (n = 30)	46.68 ± 2.21 (n = 30)	Ellipsoid	Thin, light color	Morula with few, countable distinct blastomeres (5–12), in parts light at centre
	S3	Unknown strongylid	3.1%	5.4%	72.19 ± 4.43 (n = 17)	44.59 ± 2.93 (n = 17)	Ellipsoid	Thin, light color	Egg content has a smooth surface (59.42 ± 4.77 × 34.47 ± 2.69, n = 17) Folded larva, not always clearly visible
<i>Strongyloides</i> spp. (n = 213)	R	<i>Strongyloides</i> spp.	32.7%	100%	48.69 ± 4.18 (n = 30)	34.01 ± 2.83 (n = 30)	Ellipsoid	Thin, light color	
<i>Ascaris</i> spp. (n = 56)	A	<i>Ascaris lumbricoides</i> (Strait et al., 2012), (Hernasari, 2011)	8.6%	100%	47.06 ± 4.62 (n = 15)	36.23 ± 3.16 (n = 15)	Globular to oval	Thick shell with brown, rough surface (this outer shell layer may be partially or totally exfoliated)	Granulated single cell (zygote), mostly does not fill the entire egg
Order Oxyurida (n = 36)	O	<i>Enterobius</i> spp. (Hasegawa et al., 2003), (Bolette et al., 2016)	5.5%	100%	72.00 ± 2.83 (n = 16)	27.65 ± 2.33 (n = 16)	Asymmetrical (one flattened side-wall, one more convex)	Thick, light color	Folded larva

SD = standard deviation.

^a considered positive samples.

^b Considered samples for trichurids n = 633, strongylids n = 610 and other parasite groups n = 652.

^c Prevalence *Trichuris* spp. T1-T4 in total 82.1%.

Table 2

Holm-corrected p-values of pairwise conducted Mann Whitney-U tests for widths of trichurid morphotypes^a T1, T2, T3, and T5, and lengths of Strongyloidea morphotypes S1, S2, and S3.

	T1	T2	T3
T2	0.0015	–	–
T3	0.0015	2.8e-09	–
T5	1.7e-05	1.2e-05	1.2e-08

	S1	S2
S2	0.1	–
S3	3.2e-08	1.6e-06

^a The trichurid morphotype T4 was excluded from statistical analysis because of low sample size (n = 2).

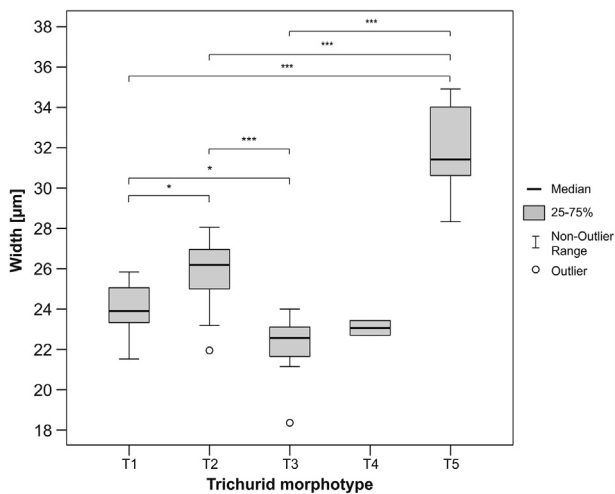


Fig. 3. Differences in width among trichurid egg morphotypes found in proboscis monkey feces. (T1 n = 11, T2 n = 30, T3 n = 30, T4 n = 2, and T5 n = 10). Median, boxes define the 25th and 75th percentiles, whiskers extend to maximum ± 1.5 times the interquartile range (IQR = middle 50% of the records).

*p = 0.05; **p = 0.001; ***p = 0.0001.

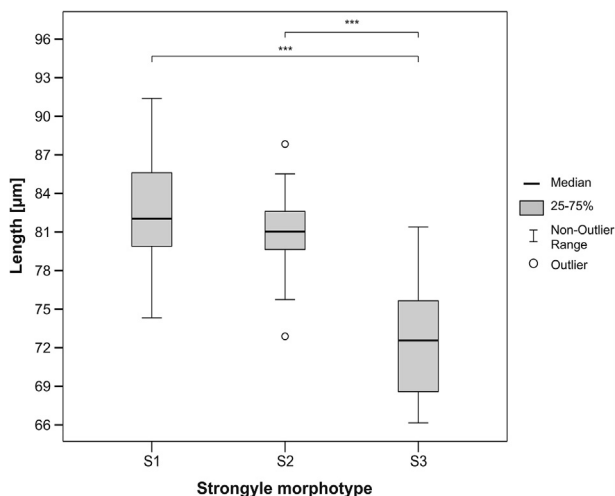


Fig. 4. Differences in length among strongyloid egg morphotypes found in proboscis monkey feces. (S1 n = 30, S2 n = 30, and S3 n = 17). Median, boxes define the 25th and 75th percentiles, whiskers extend to maximum ± 1.5 times the interquartile range (IQR = middle 50% of the records).

*p = 0.05; **p = 0.001; ***p = 0.0001.

associations between different parasite orders but no evidence for negative effects. In total, there were three significant reciprocal

associations and one unidirectional positive effect that was combined with a trend in the other direction (Table 3). The strongest association was found between the Trichuridae and Strongyloidea. The presence of either of these two parasite groups increased the probability of co-infection with the other group almost threefold (factor 2.8). The second reciprocal association appeared between *Strongyloides* and *Ascaris* spp. with an increase in *Strongyloides* infection probability by the factor 2.4 when an *Ascaris* infection was present and vice-versa. Furthermore, there was a positive association between the infection with Trichuridae and *Strongyloides* spp. These two parasitic groups mutually raised the likelihood of their presence in the same host by the factor 1.8. Additionally, the probability of finding strongyle eggs in feces was 1.5 times higher when *Strongyloides* spp. were present. However, the reverse association was only a statistical trend. Finally, there was evidence for one reciprocal positive statistical trend between the orders Strongyloidea and Ascaridida (Table 3). No association with other helminths was found for the order Oxyurida.

4. Discussion

4.1. Parasite prevalence and diversity

As hypothesized, the high overall parasite prevalence of 92.3% in the present study is similar to a prevalence of 96.6% along the Kinabatangan River detected in 2007/2008 (Salgado Lynn, 2010). These data are comparable to investigations on other group-living Old World primates in the wild, e.g. baboons (*Papio anubis*) and vervet monkeys (*Cercopithecus aethiops*) in Ethiopia (Legesse and Erko, 2004). Among others, host sociality could be linked to spillover rates and increased susceptibility to parasite infections in primate colonies (Nunn and Altizer, 2006). In particular, the high prevalence of *Trichuris* spp. (82.1%) is in accordance with previous results on groups of captive (83.3%) (Hernasari, 2011) and wild proboscis monkeys along the Kinabatangan (91.8%) (Salgado Lynn, 2010). Similar figures (79–100% prevalence) were found in African colobines (Gillespie et al., 2005; Teichroeb et al., 2009). In Asian species, *Trichuris* is likewise common, but with generally lower prevalence of up to 30% (Hilser, 2011; Hartmann et al., 2015). Divergent infection patterns may be due to behavioral factors; the majority of colobines tend to be arboreal. However, some species spend more time on the ground than others (Zinner et al., 2013). We observed proboscis monkeys occasionally walking on the ground, which may enhance the potential for parasite transmission, as reported previously by Kawabe and Mano (1972). In African colobines, ground contact was identified as the main nematode transmission mode for Angolan colobus (*Colobus angolensis palliatus*). In contrast, this was not the case for guereza colobus (*Colobus guereza*), which was observed higher above the ground (Okanga, 2005). Similar results were obtained for two Malagasy lemurs (*Propithecus verreauxi* and *Lemur catta*) with varying degrees of arboreality (Muehlenbein et al., 2003; Loudon and Sauter, 2013). Additionally, the habitat along the Kinabatangan River seems to be an ideal environment for the development of *Trichuris* spp. eggs and other soil-transmitted species as its banks are regularly flooded, thus providing the required humidity. Moreover, frequent recurrence at the same locations may facilitate accumulation of infective parasite stages and could result in reinfection (Hausfater and Meade, 1982). Proboscis monkey groups return to sleeping sites (Matsuda et al., 2016) and the surroundings of single trees appeared to be particularly popular as they were occupied regularly. The physical structure and location of both sleeping trees and their surrounding vegetation are important for the sleeping site selection of *N. larvatus* (Thiry et al., 2016). Comparisons of these results with those of Salgado Lynn (2010) revealed similar

Table 3

Best models of nematode co-infections and results of Chi Square tests (H and p-value) for associated pairs among helminth groups found in proboscis monkeys.

Tested order	Fixed effects	Estimate	Std. Error	Z value	Pr (> z)	Chi Square	p value	Factor exp.
Trichuridae	(Intercept)	0.9872	0.2108	4.684	<0.0001	–	–	–
	Strongylida	1.0446	0.2235	4.6750	<0.0001 ***	H = 30.276	<0.0001	2.8
	<i>Strongyloides</i> spp.	0.5670	0.2574	2.2030	0.0276 *	H = 6.424	0.011 *	1.8
Strongylida	(Intercept)	–0.6090	0.2427	–2.510	0.0121	–	–	–
	<i>Ascaris</i> spp.	0.6151	0.3462	1.7770	0.0756.	H = 6.800	0.009 *	–
	Trichuridae	1.0303	0.2258	4.5630	<0.0001 ***	H = 30.276	<0.0001 ***	2.8
	<i>Strongyloides</i> spp.	0.3804	0.1925	1.9760	0.0481 *	H = 9.525	0.002 *	1.5
<i>Strongyloides</i> spp.	(Intercept)	–1.4577	0.3301	–4.416	<0.0001	–	–	–
	Strongylida	0.3260	0.1969	1.6560	0.0978.	H = 9.525	0.002 *	–
	<i>Ascaris</i> spp.	0.8617	0.3233	2.6650	0.0080 *	H = 5.273	0.022 *	2.4
	Trichuridae	0.5908	0.2625	2.2510	0.0244 *	H = 6.424	0.011 *	1.8
<i>Ascaris</i> spp.	(Intercept)	–3.4339	0.4328	–7.935	<0.0001	–	–	–
	Strongylida	0.5915	0.3450	1.7150	0.0864.	H = 6.800	0.009 *	–
	<i>Strongyloides</i> spp.	0.8473	0.3208	2.6410	0.0083 *	H = 5.273	0.022 *	2.4
<i>Enterobius</i> spp.	(Intercept)	–3.5952	0.4718	–7.62	<0.0001	–	–	–

Significant associations are marked with asterisks (*p = 0.05; **p = 0.001; ***p = 0.0001). For the order Oxyurida, genus *Enterobius*, no association with other orders was found.

prevalences for strongyles (58.9% in 2012 vs. 45.2% in 2007/08), *Anatrichosoma* spp. (1.4% vs. 2.0%) and oxyurids (5.5% vs. 4.1%). Differences were found regarding the prevalence of *Strongyloides* spp. (32.7% in 2012 vs. 5.5% in 2007/08) and *Ascaris* spp. (8.6% vs. 67.1%). Furthermore, in 2007/08 but not in the present study, eggs of two cestode genera (*Taenia* sp. 28.8%, *Dipylidium*-like 9.6%), one trematode (dicrocoeliid liver fluke 2.0%) as well as one acanthocephalan parasite species (2.7%) were detected. Variations in prevalence can be explained by seasonal parasitic life cycles or varying infection rates and egg shedding intensities of individuals (Eckert et al., 2006). Also, several Lots (Lots 1–7 and 10) were sampled by Salgado Lynn (2010) at different times (between October 2007; November 2008), whereas sampling in the presented study was performed from June to September 2012 in Lot 6 only. Moreover, the flotation method has a low sensitivity to detect cestode eggs as these are usually shed in proglottids. By using anal swabs, tapeworm prevalence can be estimated more precisely; however, this method is not feasible in wild proboscis monkeys. Furthermore, best detection rates for dicrocoeliid eggs can be achieved through a combined sedimentation flotation method using a flotation solution with a specific gravity of 1.44 (Cringoli et al., 2004).

Examined morphologic and metric details suggest that eggs of different trichurids and strongylids were present in the fecal samples. Variability in *Trichuris* spp. eggs was already shown in chimpanzees (*Pan troglodytes*), olive baboons (*Papio anubis*) and humans (Ghai et al., 2014b) and corresponds to morphotypes T1–T4 in the present investigation. Recent studies confirm that taxonomy of this genus is much more complex and diverse than previously thought, leaving species identification without molecular tools highly unreliable (Cavallero et al., 2015). Based on egg morphology, *Capillaria* was not detected. In addition, different strongyle eggs were detected. Morphotype S2 was shed in an early division stage. Shape and measurements correspond best to *Oesophagostomum* spp. (Cogswell, 2007). However, eggs from different, potentially cryptic *Oesophagostomum* species cannot be conclusively differentiated from one another (Ghai et al., 2014a) or from those of the genus *Ternidens* (Sandground, 1931; Jessee et al., 1970). Morphotype S3 could not be assigned to a certain genus and may belong to a previously undescribed species. Eggs of the genus *Strongyloides* found in the present study presumably represent *S. fuelleborni*, as size and shape differed from those of *S. papillosus* (Basir, 1950), which was also described in Old World monkeys and apes (Strait et al., 2012). In contrast, *S. stercoralis* is detected in feces as already hatched larvae. Ideally, genetic analyses should be

performed for species assignment of the different egg types, but our attempts to perform PCR from different strongylid eggs were not successful, probably because of the long fixation in formalin. Surprisingly, the number of eggs per gram feces (epg) was reported to be much higher in 2007/08 (Salgado Lynn, 2010) than in the present study, i.e., a mean of 690 *Trichuris* egg compared to a mean of 45.7 *Trichuris* spp. epg of morphotypes T1–T3 in the present study, 135 vs. 4.3 strongyle epg and 100 vs. 2.6 ascarid epg. Besides a lower egg shedding intensity due to seasonal variations or differences, the fixation technique has to be taken into account as well. Although formalin is a standard solution for storing feces and its advantages were clearly stated (Gillespie, 2006), fixation can have a negative impact on recovery rates, especially after some time has passed (Foreyt, 1986). Alternative methods for storing feces are available (e.g., different concentrations of ethanol or refrigeration); however, they might likewise affect the detectability of helminth eggs (Crawley et al., 2016).

4.2. Parasite associations

In accordance with previous results (Salgado Lynn, 2010), proboscis individuals in Lot 6 were co-infected on average with species of two different helminth orders. The strong associations between the Trichuridae and strongylids may reflect their high prevalence in the fecal samples. Nevertheless, a parasite-induced immunosuppression caused by infection with one intestinal helminth species may explain the spread of a secondary infection (Cox, 2001). To date, little is known about the quality of co-infection dynamics between helminths in wild primates. The association of *S. fuelleborni* with *A. lumbricoides* as well as strongyles is a common phenomenon. *Strongyloides* as well as *Ascaris* spp. primarily affect juvenile hosts, which additionally show higher prevalence and infection intensity with strongyles during primary infection than adult individuals (Cattadori et al., 2005; Strait et al., 2012). Helminth-naïve infants and juvenile members of proboscis monkey groups may thus serve as important reservoirs for (re)infections with *S. fuelleborni*, *A. lumbricoides*, *Trichostrongylus* spp. and *Oesophagostomum/Ternidens* spp. Cases of infant strongyloidiasis were described for orangutans (*Pongo pygmaeus*) and mentioned as the most significant cause of death of wild-born individuals in zoos (Lowenstine et al., 2008). Missing interactions with *Enterobius* spp. may be due to low prevalence. Future work is needed to investigate the impact of age on infection patterns among group members. Positive relationships were found between helminth infections and shedding intensities of propagules by common enteric

microparasites (e. g., Wilcox et al., 2015) as well as intracellular microparasite richness (Nunn et al., 2014). This supports the idea that an immune-modulation through infection with different helminths may primarily affect the spread of microparasite infections.

4.3. Conservation implications and zoonotic risks

Intact ecosystems naturally include a diversity of parasites; however, disease risk for primates is likely to rise in altered habitats (Chapman et al., 2005a). The present study revealed that wild proboscis monkeys are hosts for different helminths, some of which were highly prevalent. Anthropogenic habitat destruction and infection with *Trichuris* spp., for example, have been reported to contribute to a population decline of black-and-white colobus (*Colobus guereza*) (Chapman et al., 2005b), while individual infestation with trichurids is commonly not recognized (Strait et al., 2012).

Almost all helminth parasites and associated co-infections observed in this study have zoonotic potential. Except for *Anatrichosoma* and *Enterobius*, the identified parasites are soil-mediated. In particular, *Trichuris trichiura* is one of the main soil-transmitted helminths (STHs) causing clinical disorders in people worldwide and is the most prevalent helminth in Malaysia, notably among underprivileged citizens (with regional prevalences up to 98%) (Ahmed et al., 2011). *Trichuris* sp. has been detected in Malaysia's human population (Singh and Cox-Singh, 2001) and in different primate species of the LKWS (*N. larvatus* and *Macaca fascicularis*) (Salgado Lynn, 2010). The extent of *Trichuris* host affiliations was recently discussed and single taxa were found to infect both humans and wild primates (Ghai et al., 2014b). Along the Kinabatangan River, a complex anthro-zoonotic transmission cycle may be maintained. Contamination of soil and water with infective parasite stages needs to be considered by locals, plantation workers and researchers in the field. Uncooked contaminated vegetables and poor sanitary habits may facilitate ingestion of eggs. Likewise, cross-infection with ascarids from animals to humans is possible (Strait et al., 2012). Furthermore, Sabah is rated among the world's areas with highest prevalence of strongyloidiasis (Peters and Pasvol, 2007), a neglected tropical human disease of major medical importance (Olsen et al., 2009). A peculiarity of *Strongyloides* spp. larvae is their ability to penetrate the host's skin (Gholami et al., 2015). South of the Kinabatangan river, in the Danum Valley, *Strongyloides* was also detected in orangutans (*Pongo pygmaeus morio*) (Kuze et al., 2010). Along with *Strongyloides* spp., infective strongylid larvae develop in moist substrate. Yet, cases of human infection with *Oesophagostomum* or *Ternidens* spp. have been rarely reported in South-East Asia. Transmission pathways of zoonotic helminths detected in the present study are primarily related to soil.

To mitigate the risks of zoonotic transmission, anthelmintic baiting is used for wild mammals near urban areas (Hegglin et al., 2003), but it might not be a suitable regime for free-ranging proboscis monkey groups. Deworming of wildlife remains debatable and may have unforeseen consequences (Stringer and Linklater, 2014; Ezenwa and Jolles, 2015). Potential environmental consequences of anthelmintic residues in feces, e.g., impacts on arthropods or vertebrates feeding on dung-associated insects, have to be taken into account (McCracken, 1993). Furthermore, access by people to habitats may cause disturbance in wild primate populations. To help minimize these effects, educational work on-site and behavioral rules for the local population and tourists will be a much more promising tool to protect against zoonotic parasitic infections, for example, maintaining a safe distance from the animals, using sanitary facilities and practising proper waste disposal (Williamson and Macfie, 2010; Muehlenbein and Ancrenaz, 2009).

To prevent the spread of disease in both directions, human pathogens which can lead to population declines in Endangered primate species must be examined in the same way (Leendertz et al., 2006; Köndgen et al., 2008).

5. Conclusions

Infestation with potentially zoonotic helminths in wild proboscis monkeys in a protected area in Borneo frequently visited by tourists is high and co-infections are common. Long-term effects of ongoing habitat destruction on intestinal helminth communities in *N. larvatus* are unclear, but it can be assumed that (1) contamination of soil with infective parasite stages is high, (2) limited habitats are likely to result in frequent re-infections, and (3) parasite load together with pressure of infection by human pathogens could have negative effects on primate population levels. Further studies on parasitic infections in different primate species along the Kinabatangan, in habitats with varying degrees of destruction, and among rural communities or tourists are desirable. Such studies will help to shed light on actual transmission routes and aid in the prevention of zoonotic parasite transmission.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

We wish to thank the Sabah Wildlife Department and the Sabah Biodiversity Centre for giving permission to carry out this research in the Lower Kinabatangan Wildlife Sanctuary. Furthermore, we wish to thank the field assistants from the Danau Girang Field Centre for their help with field work, Sönke von den Berg for excellent technical assistance and Andrea Springer for assisting with preparation of the manuscript. A grant of the German Academic Exchange Service (DAAD) to AK enabling the field work in Borneo is gratefully acknowledged (D/12/40192, grant number 332 4 04 106). This publication was supported by Deutsche Forschungsgemeinschaft (DFG) and University of Veterinary Medicine Hannover, Foundation within the funding programme Open Access Publishing.

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