

Chapter 4.10 Gastrointestinal Parasites of Small Mammals in the Littoral Forest of Mandena

Brigitte M. Raharivololona^{1,2}, Rakotondravao³ and Jörg U. Ganzhorn²

Abstract

We screened the fecal samples of 179 individuals of small mammal species (Primates: *Cheirogaleus major*, *C. medius*, *Microcebus murinus*, Rodentia: *Eliurus webbi*, *Rattus rattus*, and Afrosoricida: *Setifer setosus*) for gastrointestinal parasites. Nematodes with direct life cycles were most abundant. They included ascarids (*Ascaris* sp. and probably *Ascaris petiti*), strongylids (*Strongyloides* spp. and probably *Oesophagostomum* spp., *Ankylostoma* sp., and *Chabertia* sp.), and trichurids (*Trichuris* sp. and *Capillaria* sp.). We also found trichostrongylids, tentatively identified as *Trichostrongylus* sp., *Nematodirus* sp., and *Cooperia* sp., oxyurids as *Enterobius* sp. and probably *Heterakis* sp., *Lemuricola* sp., and *Syphacia* sp.; spirurids. Some helminths with indirect life cycles were also observed such as the cyclophyllidean cestodes *Hymenolepis* spp., and one non-identified form. Trematode and acanthocephalan were rare and found only in *Microcebus murinus*. Coccidia (Protozoa) occurred frequently. Rats shared many parasites with the native small mammal species. Since rats also use non-forested areas, they can spread parasites from one forest fragment to another. In the large fragments, the number of different helminthes morphotypes per individual (HMI), and the number of eggs found per gram of feces per individual fecal sample (EPG) of *M. murinus* and *Rattus rattus* increased with the level of degradation. The pair-living *Cheirogaleus medius* did not show sex related differences in any measure of parasite loads (HMI and EPG). Among the two promiscuous species, male *M. murinus* tended to have more different types of parasites than females, and male *R. rattus* had higher EPG than females.

Résumé

Parasites gastro-intestinaux des petits mammifères de la forêt littorale de Mandena, Madagascar. Nous avons examiné des échantillons de fèces de 179 individus de petits mammifères (Primata : *Cheirogaleus major*, *C. medius*, *Microcebus murinus* ; Rodentia: *Eliurus webbi*, *Rattus rattus*, et Afrosoricida: *Setifer setosus*) pour rechercher des parasites gastro-intestinaux. Les nématodes avec des cycles de vie directs étaient les plus abondants. Ils incluaient des ascarides avec *Ascaris* sp. et vraisemblablement *Ascaris petiti* ; des strongylides avec *Strongyloides* spp. (probablement *Oesophagostomum* spp., *Ankylostoma* sp. et *Chabertia* sp.) et des trichurides (*Trichuris* sp. et *Capillaria* sp.). Nous avons également trouvé des trichostrongylides que nous avons provisoirement attribués à *Trichostrongylus* sp., *Nematodirus* sp. et *Cooperia* sp. ; des oxyurides tels *Enterobius* sp., probablement *Heterakis* sp., *Lemuricola* sp. et *Syphacia* sp. ; des spirurides. Quelques helminthes avec des cycles de vie indirects ont également été observés comme les cestodes cyclophyllides *Hymenolepis* spp. et une forme non identifiée. Les trématodes et acanthocéphales étaient rares et n'ont été trouvés que chez *Microcebus murinus*. Les Coccidies (Protozoa) existaient avec une très forte

¹ Département de Paléontologie et d'Anthropologie Biologique, BP 906, Université d'Antananarivo, Antananarivo 101, Madagascar. Email: raharivololona@brigitte@yahoo.fr

² Department Animal Ecology and Conservation, University of Hamburg, Martin-Luther-King Platz 3, 20146 Hamburg, Germany. Email: ganzhorn@zoologie.uni-hamburg.de

³ Département de Recherches Zootechniques et Vétérinaires/FOFIFA, BP 4, Antananarivo 101, Madagascar.

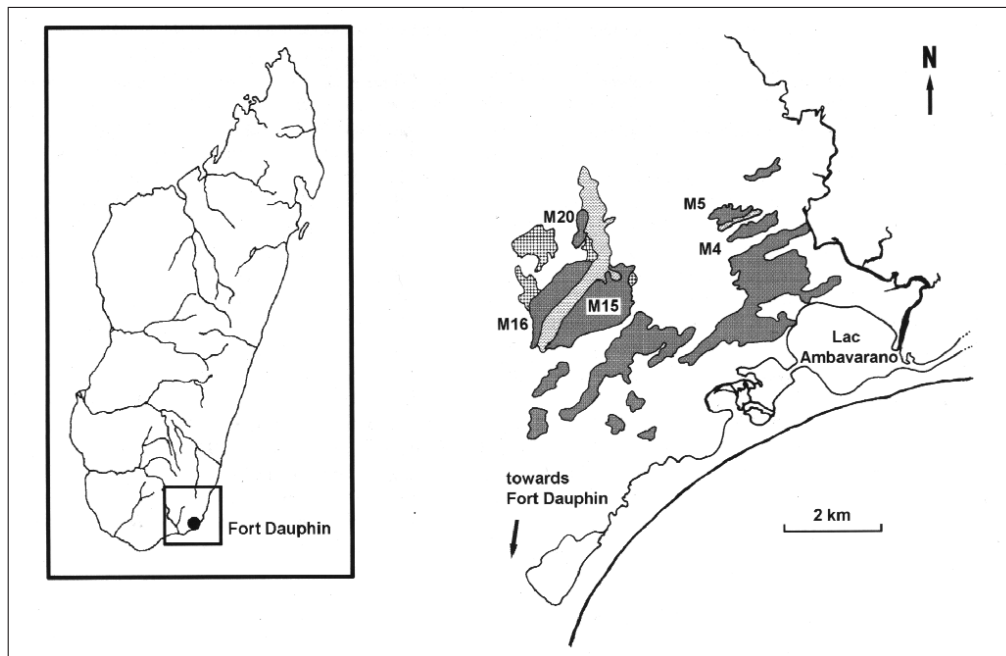


Figure 1. Location of study areas in the Tolagnaro region. The forest remnants are numbered and shown as dark shading. *Eucalyptus* plantations are crosshatched. Swamps with bordering *Melaleuca* (introduced) are marked with curved lines (modified from Ramanamanjato and Ganzhorn 2001).

prévalence. Les rats partageaient de nombreux parasites avec les espèces de petits mammifères indigènes. Comme les rats utilisent aussi des zones non forestières, ils peuvent disséminer des parasites sur des zones ouvertes d'un fragment forestier à un autre. Dans les grands fragments, le nombre de morphotypes d'helminthes différents par individu (HMI) et le nombre d'œufs trouvé par gramme de fèces (EPG) par échantillon fécal de *M. murinus* et *Rattus rattus* étaient plus élevés dans les fragments forestiers dégradés que dans les fragments moins dégradés. Le lémurien vivant en couples, *Cheirogaleus medius*, n'a pas montré de différences entre les sexes dans l'une des mesures de la charge en parasites (HMI et EPG). Pour les deux espèces vivant en promiscuité, les mâles *M. murinus* tendaient à avoir plus de types de parasites différents que les femelles et les mâles *R. rattus* avaient des EPG supérieurs à ceux des femelles.

Introduction

From a conservation point of view, parasitological studies are important to understand ways of infection, and the potential transmission of parasites

between species, both native and introduced (Goodman 1995, Begon *et al.* 1999). In addition, the degree of parasitic infections and the type of parasites are measures used to assess the health of a population (e.g., Randriamiadamanana 1998, Junge and Louis 2002, 2005, Dutton *et al.* 2003, Randrianjafy 2003), and can be linked to dramatic changes in populations (e.g., Dobson and Grenfell 1995, Packer *et al.* 1999, Daszak *et al.* 2000).

Within the framework of QIT Madagascar Minerals' (QMM) environmental and conservation activities, it is important to understand whether parasites can be spread between forest fragments by introduced rats, which would represent a potential threat to the survival of endemic species in isolated conservation zones. It is also of interest to know whether some of the native or introduced species are infected by the same parasites as humans (e.g., wild baboons have died from tuberculosis as a result of contact with human settlements (Sapolsky and Else 1987), *Spirocerca lupi* (Nematoda: Spiruridae), transmitted by dogs, has killed lemurs by aneurysm in the Parc Zoologique et Botanique de Tsimbazaza, Madagascar (Randriamiadamanana 1998), and apes and humans share intestinal parasites (Lilly *et al.*

2002)). This might represent a mutual threat if parasites can be exchanged between humans, their domestic animals, and wild animals. If so, humans would represent a reservoir of parasites, which could be spread to the forest, infecting populations of wild animals. Alternatively, wild animals could be a reservoir of parasites representing a potential danger to humans and their domestic animals.

Apart from studies on livestock diseases, which represent an immediate threat to humans, parasitological studies on native and introduced mammals are still in their infancies in Madagascar, and there is no comprehensive, standard literature that can be used for parasite identification without sophisticated molecular methods (e.g., Chabaud *et al.* 1964, 1965, Euzeby 1981). Gastrointestinal parasites represent one functional group of parasites, which can be used for monitoring the health of a population, and to address the questions mentioned above. However, with respect to parasites of the digestive tract, it is difficult to assign recovered eggs to a specific taxon.

In this study, we investigated gastrointestinal parasites of small mammals in the Mandena Forest. The goal was to provide a baseline for future monitoring. More specifically, we addressed which gastrointestinal parasites are present in small, non-volant mammals of the Mandena Forest? Which of these parasites occur in more than one species, making interspecific transmission possible? Is there any relationship between forest degradation and the size of fragments, and parasite infections?

Methods

Study site

The study was carried out in fragments M5, M13, M15/M16, and M20 of the littoral forest of Mandena, some 12 km northeast of Tolagnaro, at sites ranging in altitude from 0 to 20 m. The forest fragments differed in size and degree of degradation (see Vincelette *et al.* Chapter 2.4). Fragments were classified as: small and degraded (M20); large and degraded (M13); small and less degraded than M20 (M5); and large and less degraded than M13 (M15/M16).

Small mammals were captured with Tomahawk and Sherman live traps using standard procedures (Ramanamanjato and Ganzhorn 2001) at permanent study sites set up in different forest fragments of Mandena. Traps baited with banana were set 4 nights

per month at 80 - 100 localities per fragment between April and October 2003. Additional samples were provided by Petra Lahann, Nina Rüdell, Jörg Schüller, and Björn Siemers. The samples collected by Petra Lahann had been collected in M15/M16 between November 2003 and March 2004. Trapped animals were sexed, measured, weighed, and marked permanently with sub-dermal transponders. Animals were released at the locality where they were captured.

Study species

Cheirogaleus major, the Greater Dwarf Lemur, ranges in weight from 350 to 600 g. This species is arboreal, nocturnal, and omnivorous, feeding on fruit, flowers, young leaves, and insects. They forage alone, but sleep in small groups during the day. This species is found across a broad altitudinal range in the Malagasy eastern rain forests (Mittermeier *et al.* 2006, Lahann 2007b).

Cheirogaleus medius, the Fat-tailed Dwarf Lemur, is also arboreal and nocturnal. This species hibernates during the dry or cool season. Its weight varies seasonally, ranging from an average of 142 g at the beginning of the rainy season, to 217 g at the end (before hibernation). They have a diverse diet comprised mainly of fruit and flowers (nectar), but also feed on insects and small vertebrates (Hladik *et al.* 1980, Fietz and Ganzhorn 1999, Dausmann *et al.* 2005). This lemur is a solitary forager, but sleeps in groups in tree holes during the day. It occurs in the dry deciduous forests of the west, but extends into the evergreen littoral forests around Tolagnaro (Mittermeier *et al.* 2006).

Microcebus murinus, the Gray Mouse Lemur, is a small (average 60 g), nocturnal, and omnivorous lemur. It has a wide dietary spectrum that consists primarily of insects, fruit, flowers, and leaves. They also eat sap, gum, secretions from homopteran larvae, and small vertebrates such as frogs, geckos, and chameleons. This lemur forages alone, but congregates at daytime sleeping sites. It is arboreal, polygynous, and distributed throughout western, southern, and southeastern Madagascar. They occur in primary, secondary, and disturbed forest habitats (Martin 1972, Petter *et al.* 1977, Lahann *et al.* 2006).

Rattus rattus, the Black Rat, has an average adult weight of about 100 g in the study area (Ramanamanjato and Ganzhorn 2001). They are omnivorous, live in groups without a hierarchical structure, and are mate binding (Mohr 1950). *Rattus*

rattus may have been introduced to Madagascar as a human commensal as early as the 11th century. Since then, it has spread into the most remote parts of the native forests. *R. rattus* and its ecto-parasites carry a variety of diseases such as bubonic plague, which can be transmitted to both native species and humans (Goodman 1995, Goodman *et al.* 1997, Duplantier and Duchemin 2003, Duplantier *et al.* 2003, Laakkonen *et al.* 2003, Rousset and Andrianarivelo 2003).

Eliurus webbi ranges in weight from 55 to 95 g. It seems that this endemic rodent nests and forages in the low branches of trees, and at ground level, on lianas, and the lower branches of trees. It feeds on seeds, fruits, and possibly on invertebrates. This species probably lives in pairs in ground burrows up to 1 m in depth. It is found in undisturbed lowland and mid-altitude rainforests from Montagne d'Ambre in the north to the region of Tolagnaro in the southeast (Goodman *et al.* 2003).

Setifer setosus is a nocturnal animal with an average adult weight of about 225 g. It is omnivorous, eating a wide range of fruits, insects, grubs, and other invertebrates, as well as scavenging. It mainly forages at ground level, but is known to climb. Depending on the seasonal environmental conditions, this species can be active throughout the year or become torpid for short periods. The male and female are temporarily together during the breeding season, but adults seem to usually live solitarily. *Setifer setosus* occurs throughout the island, even in some urban areas, and occasionally near rubbish (Goodman *et al.* 2003).

Fecal sampling and parasite identification

Gastrointestinal parasites were identified from feces. For this, fresh feces were collected from handling bags or traps and stored in vials containing 4% formaldehyde. A modification of the McMaster flotation egg counting technique (Sloss *et al.* 1994) was applied to analyze egg shedding, which has been considered a valid method of evaluating worm burdens in several studies (e.g., Gulland *et al.* 1993, Paterson *et al.* 1998, Coltman *et al.* 1999, Cassinello *et al.* 2001). For this and for quantitative analyses, 300 mg of feces were triturated in a beaker with 3 ml of a saturated potassium iodide solution (KI) with a specific weight of 1.5 g/ml (Meyer-Lucht and Sommer 2005). The high specific density improves the detectability of eggs with high specific density, such as those of some nematodes and trematodes (Thienpont *et al.* 1996, in

Harf and Sommer 2005). The mixture was poured through a tea strainer to eliminate large, non-digested particles. The residue in the tea strainer was washed with 1 ml of KI. The suspension was filled to a volume of 4.5 ml with the KI solution, stirred again, and transferred to both chambers of a McMaster counting chamber with a pipette. The slide was transferred to a microscope and left still for 5 minutes before examination. During this time, eggs and larvae floated to the surface of the McMaster counting chambers. The slide was then examined with 100x, 400x, and 600x magnification to identify and count all eggs and larva inside the ruled squares. This procedure has been applied successfully in a number of other studies (e.g., Meyer-Lucht and Sommer 2005, Schad *et al.* 2005). Parasites were measured with a calibrated ocular micrometer, and photographed with a digital camera.

Parasite identification was based on the size, shape, and internal structure of eggs, oocyst, and larvae (Chabaud *et al.* 1964, 1965, Euzeby 1981). The identifications and data presented here for the three lemur species have also been summarized, and their parasites have been illustrated by Raharivololona (2006). The taxonomic identifications still have to be considered preliminary. Pictures of the parasites and the names assigned to the different forms are displayed at <http://www.biologie.uni-hamburg.de/zim/oeko>.

Statistical analyses

For the statistical analyses, each captured mammal was used only once. For recaptures, we normally used the fecal sample taken at the first capture. Some individuals were recaptured throughout the year. Only parasites from adults were used in the quantitative analyses. Juveniles were excluded because they are likely to have different parasite loads than adults. The total number of eggs per gram of fecal material was log₁₀ transformed to facilitate illustrations. The number of parasite species was not transformed. Significance levels are two-tailed, and based on non-parametric tests. Statistical tests were run with SPSS 9.0 (SPSS 1999).

Results

Parasite diversity

In total, 350 fecal samples were collected from 179 individuals, including 2 *Cheirogaleus major*, 26 *C. medius*, 105 *Microcebus murinus*, 36 *Rattus rattus*, 8 *Eliurus webbi*, and 3 *Setifer setosus*. The prevalence

rates of different parasites are listed in Table 1. Individuals with parasites varied from 73% to 100% in the six species.

Cheirogaleus major

The feces of both individuals contained coccidia (Protozoa), and one non-identified species of nematode. The coccidian is likely to be *Eimeria*.

Cheirogaleus medius

Parasites belonged to: Nematodea — *Strongyloides*, strongylids, *Trichuris*, oxyurids, and 3 unidentified morphotypes. Based on morphological structures, strongylids could be *Oesophagostomum*, *Ankylostoma*, and *Chabertia*; one form of strongylid could not be identified. Oxyurids found in the feces resemble *Heterakis*; Cestodea - *Hymenolepis* and other cyclophyllidean species; Protozoa — Coccidia (*Eimeria* ?). Intestinal parasites with the highest prevalence rates were *Trichuris* (26.9%), *Hymenolepis* (26.9%), and coccidia (26.9%, Table 1).

Microcebus murinus

Parasites belonged to: Nematodea — *Ascaris* sp. found in only one individual; one non identified species of ascarid; *Strongyloides*, strongylids (several forms, including probably *Oesophagostomum* and *Ankylostoma*), *Trichuris*, *Capillaria*; Trichostrongylids (several forms, including probably *Trichostrongylus* and *Nematodirus*); Oxyurids — *Enterobius*, two other species seem to be *Lemuricola* and *Syphacia*. Three forms could not identified further; Cestodea — *Hymenolepis* and other morphotypes of cyclophyllidean; Trematodea and Acanthocephala — found in two individuals; Protozoa — Coccidia (*Eimeria*) is the most common intestinal parasite with a prevalence rate of 68.3%. *Capillaria*, *Enterobius*, trichostrongylids, trematodes, and acanthocephala were very rare. Apart from Coccidia infections, many animals were also infected by *Hymenolepis* (33.7%; Table 1).

Eliurus webbi

Only five species of nematodes were found in the feces of this species. They were mainly strongylids, which seem to be *Oesophagostomum* and *Ankylostoma* (prevalence = 75%), *Strongyloides*, possibly *Trichostrongylus*, and one unknown form of nematodes.

Rattus rattus

Parasites belonged to: Nematodea — *Ascaris*, one non identified species of ascarids, strongylids (morphologically similar to *Oesophagostomum* or *Ankylostoma*, *Strongyloides*), trichurids (*Trichuris*, *Capillaria*), trichostrongylids (probably *Trichostrongylus*, *Cooperia*), oxyurids (possibly *Syphacia*), one species of spirurids and four nematodes could not be identified further. According to this preliminary result, *R. rattus* is the only species infected by spirurids; Cestodea — only *Hymenolepis* spp.; Protozoa — Coccidia (*Eimeria*?). Rats were highly infected by *Strongyloides* and strongylids (63.9% and 61.1%, respectively; Table 1).

Setifer setosus

Parasites belonged to: Nematodea — strongylids (probably *Oesophagostomum* and *Ankylostoma*), *Strongyloides*, *Trichuris*, *Capillaria*, and three unknown forms; Cestodea — *Hymenolepis*; Acanthocephala; Protozoa — Coccidia (*Eimeria* ?).

Parasite load in relation to forest degradation and fragment size

The number of eggs found per gram of feces per individual (FEC: fecal egg count), and the number of different helminthes morphotypes per individual (HMI) were used as indicators for the intensity of parasitism (Table 2). Quantitative analyses were not available for all fecal samples, therefore, sample sizes given in Table 2 are slightly lower than those in Table 1. Only *Microcebus murinus* and *Rattus rattus* were caught in sufficient numbers at all the various study sites to allow statistical analyses of possible effects of fragment size and forest degradation on parasite loads.

***Microcebus murinus*: Effect of degradation**

Since the data deviated significantly from a normal distribution, only non-parametric tests could be used. Therefore, we analyzed large and small fragments separately. For large fragments (M13 (degraded) – M15/M16 (less degraded)), the number of parasite species found in the feces differed significantly between the animals caught in M13 and those caught in M15/M16 (Mann Whitney U test: $z = 1.94$, $p = 0.05$). The difference, with respect to the fecal egg count, was not significant even though more eggs

Table 1. Habitat specificity of host species and prevalence of gastrointestinal parasites found in feces of *Cheirogaleus major*, *C. medius*, *Microcebus murinus*, *Eliurus webbi*, *Rattus rattus*, and *Setifer setosus* in the littoral forest of Mandena. Prevalence = Number of infected individuals divided by the number of examined individuals (%). Total number of parasite morphotypes = Number of morphotypes recorded for the different host species. Parasite morphotypes per sample = Number of different parasite morphotypes found per fecal sample. Values are medians and ranges; for sample sizes less than three all values are listed.

	Restricted to forest habitats			Forest and non-forest habitats		
	Primates			Rodentia		Afrosoricida
	<i>C. major</i>	<i>C. medius</i>	<i>M. murinus</i>	<i>E. webbi</i>	<i>R. rattus</i>	<i>S. setosus</i>
	N = 2	N = 26	N = 105	N = 8	N = 36	N = 3
Nematodea						
<i>Ascaris</i> sp.			1.0		13.9	
other ascarids			25.7		8.3	
<i>Strongyloides</i> spp.		3.9	11.9	12.5	63.9	33.3
strongylids		11.5	4.0	75.0	61.1	33.3
<i>Trichuris</i> sp.		26.9	8.9		11.1	33.3
<i>Capillaria</i> sp.			1.0		8.3	33.3
trichostrongylids			2.0	12.5	13.9	
<i>Enterobius</i> sp.			1.0			
other oxyurids		3.9	15.8		5.6	
spirurids					8.3	
Non identified						
nematodes	50.0	26.9	59.4	25.0	11.1	66.6
Cestodea						
<i>Hymenolepis</i> spp.		26.9	33.7		5.6	33.3
other Cyclophyllidean		3.9	5.0			
Trematodea						
			2.0			
Acanthocephala						
			2.0			
Protozoa: Coccidia	100.0	26.9	68.3		8.3	100.0
Total prevalence	100.0	73.1	90.1	87.5	86.1	100.0
Parasite morphotypes / sample	1 / 2	1	2	2	2	3 / 3 / 7
Total number of parasite morphotypes	3	13	≥ 20	5	≥ 17	≥ 10

were found in the feces of animals in the more degraded fragment ($z = 1.86$, $p = 0.06$). For the small fragments (M20 (degraded) - M5 (less degraded)), the number of parasite morphotypes did not differ ($z = 0.74$, n.s.), but the number of eggs found in the feces was significantly higher in M5 than in M20 ($z = 2.03$, $p = 0.04$).

***Microcebus murinus*: Effect of fragment size**

In degraded fragments (M13 (large) - M5 (small)), and less degraded fragments (M15/M16 (large) - M20 (small)), neither the number of parasite species, nor the number of eggs found in the feces differed between fragments of different size ($z < 1.67$ for all comparisons, n.s.).

***Rattus rattus*: Effect of degradation**

Since no rats were captured in M20, analysis of the effect of degradation had to be restricted to the comparison between the large fragments (M13 (degraded) - M15/M16 (less degraded)). Animals caught in the degraded fragment M13 had significantly more parasite varieties, and many more parasite eggs in their feces ($z = 2.66$, $p = 0.008$; $z = 2.53$, $p = 0.01$, respectively).

***Rattus rattus*: Effect of fragment size**

Due to the lack of captures in M20, analysis of the effect of the size of fragments had to be restricted to the comparison between the degraded fragments (M13 (large) - M5 (small)). In this case, animals caught in the large fragment had more different par-

asite forms, and excreted more parasite eggs than the animals in the small fragment ($z = 2.10$, $p = 0.04$; $z = 2.39$, $p = 0.015$, respectively).

Sex differences in parasite loads

Cheirogaleus medius did not show differences in any measure of parasite loads (HMI and EPG) between the sexes (Figs. 2, 3). Male *Microcebus murinus* tended to have more different types of parasites than females (HMI: males - median = 2, quartiles = 1-3, $n = 45$; females - median = 1, quartiles = 0-2, $n = 52$, Mann Whitney U test: $z = 1.88$, $p = 0.06$). EPG did not differ significantly between the sexes of this species. Male *Rattus rattus* also had a non-significant tendency for greater HMI than females (HMI: males - median = 2, quartiles = 2-3, $n = 20$; females - median = 2, quartiles = 0-3, $n = 15$, Mann Whitney U test: $z = 1.64$, $p = 0.10$). Male rats did, however, have significantly higher EPG than females (EPG: males - median = 2.68, quartiles = 2.48–2.99, $n = 20$; females - median = 2.18, quartiles = 0–2.70, $n = 15$, Mann Whitney U test: $z = 2.29$, $p = 0.02$).

Discussion

Only preliminary taxonomic identifications are presented here. During a visit to the Muséum national d'Histoire naturelle, Paris, France, no parasite egg samples, only larva and adult worms, could be located in their important reference collection. Therefore, an open access database was created to advance towards more comprehensive means of identification of Malagasy small mammal parasites (Raharivololona 2006). In addition, future work will include larval cultures and experimental transmission of parasites to laboratory rats in order to obtain larva and adult worms for comparison. This is a long-term project and the taxonomic interpretations presented here are likely to need revision sometime in the future.

In the present survey, all small mammal species had high prevalence infection rates (> 73%) and were hosts for 3 to 20+ gastrointestinal parasite forms. For lemurs Chabaud *et al.* (1965) listed some intestinal nematode parasites as: strongylids: *Lemurostrongylus residuus*; trichostrongylids:

Table 2. Number of different helminthes morphotypes per individual (HMI), and number of eggs found per gram of feces per individual (EPG). Values are medians and quartiles. N = sample size. For a sample size of 2, both values are listed.

	M5		M13		M15/M16		M20	
	HMI	EPG	HMI	EPG	HMI	EPG	HMI	EPG
Size	28 ha		150 ha		230 ha		20 ha	
Degradation	low		high		low		high	
<i>Cheirogaleus major</i>					1 / 2	1.71 / 3.51		
					N = 2			
<i>Cheirogaleus medius</i>	1	2.30	1	1.71	1	2.00		
					0-2	0-2.54		
	N = 1		N = 1		N = 23			
<i>Microcebus murinus</i>	2	3.79	2	3.02	1	2.18	2	2.95
	1.5-5	3.01-4.18	1-3	2.18-3.46	0-2	0–3.26	1-3	2.30-3.57
	N = 9		N = 14		N = 63		N = 15	
<i>Eliurus webbi</i>					2	2.48	2	2.40
					1-3	2.18-2.89		
					N = 6		N = 1	
<i>Rattus rattus</i>	2	2.51	3	2.93	2	2.30		
	1.75-3	1.93-2.78	2-5	2.54-3.92	1-3	1.71-2.70		
	N = 14		N = 11		N = 11			
<i>Setifer setosus</i>			3 / 7	3.41/5.18	3	4.16		
			N = 2		N = 1			

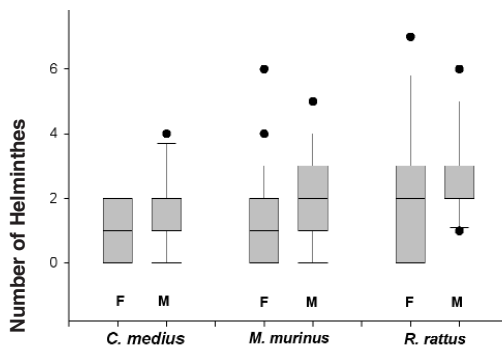


Figure 2. Number of helminthes morphotypes in female (F) and male (M) *Cheirogaleus medius*, *Microcebus murinus*, and *Rattus rattus*. Values are medians, 10, 25, 75 and 90% percentiles and values outside the 10 / 90% percentiles.

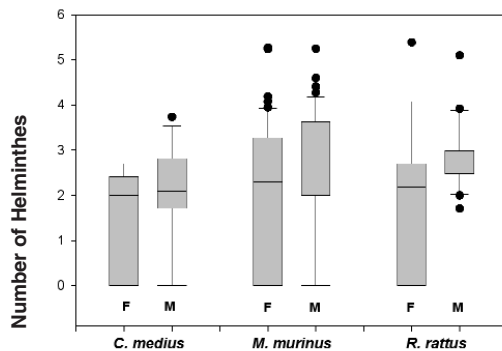


Figure 3. Number of helminthes eggs per gram of fecal sample in female (F) and male (M) *Cheirogaleus medius*, *Microcebus murinus*, and *Rattus rattus*; for details see Fig. 2.

Pararhabdonema longistriata; oxyurids: *Callistoura* spp., *Lemuricola* spp., *Biguetius trichuroides* and *Enterobius lemuri*; subulurids: *Subulura baeri*; ascarids: *Ascaris petiti*; spirurids: *Spirura diplocyphos* and *Rictularia lemuri*; and trichurids: *Trichuris lemuri*. In the present study, strongylids were identified as *Oesophagostomum* sp., *Ankylostoma* sp., and *Chabertia* sp. *Oesophagostomum* sp. is also known to infect lemurs in captivity in the Tsimbazaza Zoo, Antananarivo (Randriamiadamanana 1998). Trichostrongylids in our data set are likely to be *Trichostrongylus* sp. Oxyurids that we have identified are probably *Enterobius* sp., *Heterakis* sp., *Lemuricola* sp., and

Syphacia sp. One non-identified nematode of *Cheirogaleus medius* and *Microcebus murinus* could be a subulurid, probably *Subulura* sp.

The *Ascaris* sp. observed in the present samples of *Microcebus murinus* and *Rattus rattus* could be *A. lumbricoïdes*. Its occurrence in *M. murinus* can be explained by the fact that *M. murinus* descends to the ground to catch invertebrates, and thus, is exposed to human intestinal parasites since people frequently enter the Mandena Forest. It would be most important to follow-up on possible pathways of gut infections between humans, rats, and lemurs. One non-identified nematode of *Cheirogaleus medius* and *M. murinus* is likely to be a spirurid, such as *Physaloptera* sp. The trichurid (*Trichuris lemuri*) described by Chabaud *et al.* (1965) seems to be the same species as that found in the present study. *Capillaria* sp. has also been found in fecal samples of *M. murinus*.

The study presented here expounds upon considerably the information provided by Chabaud *et al.* (1965). Those authors identified four species of intestinal parasites present in *M. murinus*, while in the current work, at least 20 different forms were collected. Chabaud *et al.* (1965) examined two parasite species in *Cheirogaleus major*, *Lemuricola contagiosus* and *Trichuris lemuri*. We did not find these taxa, but rather coccidian, and one species of nematode. Further, Chabaud *et al.* (1965) reported only *Subulura baeri* and *Spirura diplocyphos* as the intestinal parasites of *C. medius*, as compared to at least 13 different forms found in this lemur from the Mandena sample. Finally, the fecal samples from Mandena contained cestodes (*Hymenolepis* and cyclophyllidean) for *C. medius* and *M. murinus*, a trematode and acanthocephalan for *M. murinus*, and coccidia in all three lemur species studied.

Apart from food composition and resource utilization (e.g., Dobson and Pacala 1992, Guegan and Kennedy 1993), the degree of parasitism can be influenced by social interactions. Parasites such as ascarids, strongylids, trichurids, and trichostrongylids are transmitted directly through feces. Inter-individual exchange of parasites is possible, for example, among animals of the same sleeping group. These social interactions generate a network of contacts within populations through which many parasites can spread (Anderson and May 1979, 1991). Hosts living at high density or with frequent intra-specific contacts are expected to accumulate

more parasite species (Morand 2000, Roberts *et al.* 2002). This might explain why parasite prevalence is higher in *M. murinus* than in the other two lemur species. In *C. medius*, which lives in pairs, males and females share the same home range (Lahann 2006, 2007a), and the two sexes do not differ in the intensity of their infections. In contrast, males of the promiscuous species *M. murinus* and *R. rattus* have more different parasites and higher parasite infections than females. This might be because males range over larger areas than females (*M. murinus* in M15/M16: males - about 3 ha, females - 0.5 ha; Lahann *et al.* 2006).

Oxyurids, cestodes, trematodes, and acanthocephalan have an indirect life cycle with arthropods as intermediate hosts (Flynn 1973; Owen 1992). Since all three lemur species considered here eat invertebrates (Mittermeier *et al.* 2006), they can all be infected by these parasites simply through their standard dietary regime.

Parasites of Malagasy nesomyine rodents are less studied than parasites of other groups. One species of trichostrongylids (*Heligmonina tanala*) has been recently identified in *Eliurus tanala* from Parc National de Ranomafana (Durette-Desset *et al.* 2002). *Rattus rattus* is infected by almost all groups of parasites found in the endemic host species except for trematodes and Acanthocephala. Since rats move freely between forest fragments and human habitations, they are the most likely candidates for parasite transmission.

Chabaud *et al.* (1964) described at least five forms of gastrointestinal parasites in *Setifer setosus*, *Uncinaria bauchoti* (strongylids), *Biocastrostrongylus bioccai* (strongylids), *Molineus odgeni* (trichostrongylids), *Physaloptera coelebs coelebs* (spirurids), *Physaloptera coelebs bluntschlii* (spirurids), and ascarids. In the present study, we identified strongylids, which seem to be *Oesophagostomum* sp. and *Ankylostoma* sp., *Strongyloides* sp., *Trichuris* sp., *Capillaria* sp., three unknown species of nematodes, *Hymenolepis* sp., Acanthocephala, and Coccidia. Being insectivorous, it is not surprising to find cestodes and Acanthocephala in the feces of *Setifer setosus*, as these parasites have indirect life cycles with arthropods as intermediate hosts.

The intensity of parasitism varied between species and between forest fragments. Parasitism was elevated in more degraded forests. For the time

being, it is not known how environmental conditions affect parasite loads. Recently, parasite resistance and susceptibility was linked to specific properties of genes involved in the immune responses of individual *M. murinus* and *C. medius* (Schad *et al.* 2005, see Sommer Chapter 4.11, N. Schwensow, unpubl. data). In the case of *M. murinus*, the allele that was linked to susceptibility was more frequent in the degraded forest fragment (M5) than in fragments M13, M15/M16, and M20. In the context of this work, *C. medius* was studied in a different region of Madagascar. Thus, it is not known whether high parasite loads on the population level are due to the environmental situation, population densities, or the genetic constitution of the animals.

In summary, a diverse gastrointestinal parasite fauna was found in the small mammals of the Mandena Forest. Parasite load increased with the increasing degradation of the forest. The possible parasite transmission from humans to wildlife and vice versa deserves special attention (i.e. the occurrence of *Ascaris* sp. in *M. murinus*, *R. rattus*, and humans).

Acknowledgments

The study was conducted under the Accord de Collaboration between the Université d'Antananarivo (Département de Biologie Animale and Département d'Anthropologie et de Biologie Evolutive) and Hamburg University. It is part of the biodiversity assessment and environmental impact studies of the littoral forest fragments initiated by QIT Madagascar Minerals (QMM). We thank Refaly Ernest for assistance with the fieldwork; Manon Vincelette, Jean-Baptiste Ramanamanjato, and their environmental and conservation team, as well as other staff of QMM, for technical assistance and help with logistics; Simone Sommer, her students, and staff at the Department of Animal Ecology and Conservation, Hamburg University; Dr. Frédéric Arieu and the Malaria Department of the Institut Pasteur de Madagascar; Gervais Randrianarivo and the Département de Recherches Zootechniques et Vétérinaires / FOFIFA for help and assistance in the laboratory analyses. We also thank Prof. D.W. Büttner (Bernhard Nocht Institute, Hamburg), Prof. Alain G. Chabaud, Dr. Odile Bain, and Dr. Marie-Claude Durette-Desset (Muséum national d'Histoire naturelle, Paris), and Dr. David Modry (Department

of Parasitology, University of Veterinary and Pharmaceutical Sciences, Czech Republic) for their advice. Nina Schwensow provided very helpful comments on previous drafts of the manuscript. This study was funded by the Margot Marsh Biodiversity Foundation, QMM, German Research Foundation (DFG), and the German Academic Exchange Service (DAAD).

References

- Anderson, R.M. and R.M. May. 1979. Population biology of infectious diseases. I. *Nature* 280:361-367.
- Anderson, R.M. and R.M. May. 1991. *Infectious Diseases of Humans: Dynamics and Control*. Oxford University Press, Oxford.
- Begon, M., S.M. Hazel, D. Baxby, K. Brown, Cavanagh, R. Chantrey, J. Jones, T. and M. Bennett. 1999. Transmission dynamics of a zoonotic pathogen within and between wildlife host species. *Proceedings of the Royal Society of London B, Biological Sciences* 266:1-7.
- Cassinello, J., M. Gomendio and E.R.S. Roldan. 2001. Relationship between coefficient of inbreeding and parasite burden in endangered gazelles. *Conservation Biology* 15:1171-1174.
- Chabaud, A.G., E.R. Brygoo and R. Tchérakoff. 1964. Nématodes parasites d'insectivores malgaches. *Bulletin du Muséum national d'Histoire naturelle*, Paris 36:245-261.
- Chabaud, A.G., E.R. Brygoo and A.-J. Petter. 1965. Les nématodes parasites de lémuriers malgaches. Description de six espèces nouvelles et conclusions générales. *Annales de Parasitologie* (Paris) 40:181-214.
- Coltman, D.W., J.G. Pilkington, J.A. Smith and J.M. Pemberton. 1999. Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution* 53:1259-1267.
- Dausmann, K.H., J. Glos, J.U. Ganzhorn and G. Heldmaier. 2005. Hibernation in the tropics: lessons from a primate. *Journal of Comparative Physiology B* 175:147-155.
- Daszak, P., A.A. Cunningham and A.D. Hyatt. 2000. Emerging infectious diseases of wildlife: threats to biodiversity and human health. *Science* 287:443-449.
- Dobson, A.P. and B. Grenfell, eds. 1995. *Ecology of Infectious Disease in Natural Populations*. Cambridge University Press, Cambridge.
- Dobson, A.P. and S.W. Pacala. 1992. The parasites of *Anolis* lizards in the northern Lesser Antilles. II. Models of parasite community structure. *Oecologia* 91:118-125.
- Duplantier, J.-M. and J.-B. Duchemin. 2003. Human diseases and introduced small mammals. Pp. 158-16 in: Goodman, S.M. and J.P. Benstead, eds., *The Natural History of Madagascar*. University of Chicago Press, Chicago.
- Duplantier, J.-M., J. Catalan, A. Orth, B. Grolleau and J. Britton-Davidian. 2003. Systematics of the black rat in Madagascar: consequences for the transmission and distribution of plague. *Biological Journal of the Linnean Society* 78:335-341.
- Durette-Desset, M.C., Lehtonen, J.T. and V. Haukisalmi. 2002. *Trichostrongylina* (Nematoda) from Malagasy muridae. Description of two new species of *Heligmonina* (Heligmonellidae) in *Nesomys rufus* and *Eliurus tanala*. *Parasite* 9:127-133.
- Dutton, C.J., R.E. Junge and E.E. Louis. 2003. Biomedical evaluation of free-ranging ring-tailed lemurs (*Lemur catta*) in Tsimanampetsotsa Strict Nature Reserve. *Journal of Zoo and Wildlife Medicine* 34:16-24.
- Euzeby, J. 1981. *Diagnostic expérimental des Helminthoses animales. Tome 1*. Edition Informations Techniques des Services Vétérinaires, Ministère de l'Agriculture, Paris, France.
- Fietz, J. and J.U. Ganzhorn. 1999. Feeding ecology of a hibernating primate *Cheirogaleus medius*: or how do they get so fat. *Oecologia* 121:157-164.
- Flynn, R.J. 1973. *Parasites of Laboratory Animals*. Iowa State University Press, Ames.
- Goodman, S.M. 1995. *Rattus* on Madagascar and the dilemma of protecting the endemic rodent fauna. *Conservation Biology* 9:450-453.
- Goodman, S.M., J.U. Ganzhorn and D. Rakotondravony. 2003. Introduction to the mammals. Pp. 1159-1186 in: Goodman, S.M. and J.P. Benstead, eds., *The Natural History of Madagascar*. University of Chicago Press, Chicago.
- Goodman, S.M., J.U. Ganzhorn, L.E. Olson, M. Pidgeon and V. Soarimalala. 1997. Annual variation in species diversity and relative density of rodents and insectivores in the Parc National de la Montagne d'Ambre, Madagascar. *Ecotropica* 3:109-118.
- Guegan, J.F. and C.R. Kennedy. 1993. Maximum local helminth community richness in British freshwater

- fish: a test of the colonization time hypothesis. *Parasitology* 106:61-100.
- Gulland, F.M.D., S.D. Albon, J.M. Pemberton, P.R. Moorcroft and T.H. Clutton-Brock. 1993. Parasite associated polymorphism in a cyclic ungulate population. *Proceedings of the Royal Society London B* 254:7-13.
- Harf, R. and S. Sommer. 2005. Association between major histocompatibility complex class II DRB alleles and parasite load in the hairy-footed gerbil, *Gerbillurus paeba*, in the southern Kalahari. *Molecular Ecology* 14:85-91.
- Hladik, C.M., P. Charles-Dominique and J.-J. Petter. 1980. Feeding strategies of five nocturnal prosimians in the dry forest of the west coast of Madagascar. Pp. 41-73 in: Charles-Dominique, P., H.M. Cooper, A. Hladik, C.M. Hladik, E. Pages, G.F. Pariente, A. Petter-Rousseaux, J.-J. Petter and A. Schilling, eds., *Nocturnal Malagasy Primates: Ecology, Physiology and Behavior*. Academic Press, New York.
- Junge, R.E. and E.E. Louis. 2002. Medical evaluation of free-ranging primates in Betampona Reserve, Madagascar. *Lemur News* 7:23-25.
- Junge, R.E. and E.E. Louis. 2005. Preliminary biomedical evaluation of wild Ruffed Lemurs (*Varecia variegata* and *V. rubra*). *American Journal of Primatology* 66:85-94.
- Laakkonen, J., S.M. Goodman, J.-B. Duchemin and J.-M. Duplantier. 2003. Trypomastigotes and potential flea vectors of the endemic rodents and the introduced *Rattus rattus* in the rainforests of Madagascar. *Biodiversity and Conservation* 12:1775-1783.
- Lahann, P. 2006. Koexistenz dreier Lemurenarten der Familie Cheirogaleidae (Gray, 1873) in einem Küstenregenwald im Südosten Madagaskars. Dissertation, Hamburg University.
- Lahann, P. 2007a. Feeding ecology and seed dispersal of sympatric cheirogaleid lemurs (*Microcebus murinus*, *Cheirogaleus medius*, *Cheirogaleus major*) in the littoral rainforest of south-east Madagascar. *Journal of Zoology, London* 271:88-98.
- Lahann, P. 2007b. Biology of *Cheirogaleus major* in a littoral rainforest in southeast Madagascar. *International Journal of Primatology* 28:895-905.
- Lahann, P., J. Schmid and J.U. Ganzhorn. 2006. Geographic variation in life history traits of *Microcebus murinus* in Madagascar. *International Journal of Primatology* 27:983-999.
- Lilly, A.A., P.T. Mehlman and D.M. Doran. 2002. Intestinal parasites in gorillas, chimpanzees, and humans at Mondika research site, Dzanga-Ndoki National Park, Central African Republic. *International Journal of Primatology* 23:555-573.
- Martin, R.D. 1972. A preliminary field study of the lesser mouse lemur (*Microcebus murinus* J.F. Miller 1777). *Zeitschrift für Tierpsychologie*, Supplement 9:43-89.
- Meyer-Lucht, Y. and S. Sommer. 2005. MHC diversity and the association to nematode parasitism in the yellow-necked mouse (*Apodemus flavicollis*). *Molecular Ecology* 14:2233-2243.
- Mittermeier, R.A., W.R. Konstant, A.F.A. Hawkins, E.E. Louis, O. Langrand, H.J. Ratsimbazafy, M.R. Rasoloarison, J.U. Ganzhorn, S. Rajaobelina, I. Tattersall and D.M. Meyers. 2006. *Lemurs of Madagascar*. 2nd ed. Conservation International, Washington D.C.
- Mohr, E. 1950. *Die freilebenden Nagetiere Deutschlands*. Gustav Fischer Verlag, Jena.
- Morand, S. 2000. Wormy world: comparative tests of theoretical hypotheses on parasite species richness. Pp. 63-79 in: Poulin, R., S. Morand and A. Skorping, eds., *Evolutionary Biology of Host-Parasite Relationships*. Elsevier, Amsterdam.
- Owen, D.G. 1992. *Parasites of Laboratory Animals*. Laboratory handbooks No 12. Laboratory Animal Ltd by Royal Society of Medicine Services, London.
- Packer, C., S. Altizer, M. Appel, E. Brown, J. Martenson, S.J. O'Brien, M. Roelke-Parker, R. Hofmann-Lehmann and H. Lutz. 1999. Viruses of the Serengeti: patterns of infection and mortality in African lions. *Journal of Animal Ecology* 68:1161-1178.
- Paterson, S., K. Wilson and J. M. Pemberton. 1998. Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population (*Ovis aries* L.). *Evolution* 95:3714-3719.
- Petter, J.-J., R. Alagnac and Y. Rumpler. 1977. *Faune de Madagascar: Mammifères Lémuériens*. ORSTOM-CNRS, Paris.
- Raharivololona, B.M. 2006. Gastrointestinal parasites of *Cheirogaleus* spp. and *Microcebus murinus* in the littoral forest of Mandena, Madagascar. *Lemur News* 11:31-35.
- Ramanamanjato, J.-B. and J. U. Ganzhorn. 2001. Effects of forest fragmentation, introduced *Rattus*

- rattus* and the role of exotic tree plantations and secondary vegetation for the conservation of an endemic rodent and a small lemur in littoral forests of southeastern Madagascar. *Animal Conservation* 4:175-183.
- Randriamiadamanana, M. 1998. Contribution au diagnostic coprologique du parasite *Spirocerca lupi* chez les lémuriers du Parc Botanique et Zoologique de Tsimbazaza. Mémoire d'ingénieur, Ecole Supérieure des Sciences Agronomiques, Université d'Antananarivo, Madagascar.
- Randrianjafy, V.N.R. 2003. Contribution à la biologie de conservation de la communauté micromammalienne d'Ankarafantsika. Thèse de Doctorat de 3ème cycle, Département de Biologie Animale, Université d'Antananarivo.
- Roberts, M.G., A.P. Dobson, P. Arneberg, G.A. De Leo, R.C. Krecek, M.T. Manfredi, P. Lanfranchi and E. Zaffaroni. 2002. Parasite community ecology and biodiversity. Pp. 63-82 in: Hudson, P.J., A. Rizzoli, B.T. Grenfell, H. Heesterbeek and A.P. Dobson, eds., *The Ecology of Wildlife Disease*. Oxford University Press, Oxford.
- Rousset, D. and M. R. Andrianarivelo. 2003. Viruses. Pp. 165-170 in: Goodman, S.M. and J.P. Benstead, eds., *The Natural History of Madagascar*. University of Chicago Press, Chicago.
- Sapolsky, R.M. and J.G. Else. 1987. Bovine tuberculosis in a wild baboon population: Epidemiological aspects. *Journal of Medical Primatology* 16:229-235.
- Schad, J., J.U. Ganzhorn and S. Sommer. 2005. MHC constitution and parasite burden in the Malagasy mouse lemur, *Microcebus murinus*. *Evolution* 59:439-450.
- Sloss, M.W., R.L. Kemp and A. Zajak. 1994. *Veterinary Clinical Parasitology*. Iowa State Univ. Press, Ames.
- SPSS. 1999. *SPSS Base 9.0 User's Guide*. SPSS Inc., Chicago.