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RESEARCH ARTICLE

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# Filarial infections in lemurs: Evidence for a wide geographical distribution and low host specificity among lemur species

Frederik Kiene[1,2,3] | Andrea Springer[2] | Bertrand Andriatsitohaina[4,5] | Malcolm S. Ramsay[1,6] | Romule Rakotondravony[4,5] | Christina Strube[2] | Ute Radespiel[1]

[1]Institute of Zoology, University of Veterinary Medicine Hannover, Hanover, Germany

[2]Centre for Infection Medicine, Institute for Parasitology, University of Veterinary Medicine Hannover, Hanover, Germany

[3]Clinic for Swine and Small Ruminants, Forensic Medicine and Ambulatory Service, University of Veterinary Medicine Hannover, Hannover, Germany

[4]Ecole Doctorale Ecosystèmes Naturels (EDEN), University of Mahajanga, Mahajanga, Madagascar

[5]Faculté des Sciences, de Technologies et de l'Environnement, University of Mahajanga, Mahajanga, Madagascar

[6]Department of Anthropology, University of Toronto, Toronto, Ontario, Canada

**Correspondence**

Ute Radespiel, Institute of Zoology, University of Veterinary Medicine Hannover, Buenteweg 17, Hanover 30559, Germany.
Email: ute.radespiel@tiho-hannover.de

Christina Strube, Centre for Infection Medicine, Institute for Parasitology, University of Veterinary Medicine Hannover, Buenteweg 17, Hanover 30559, Germany.
Email: christina.strube@tiho-hannover.de

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## Abstract

The relevance of emerging infectious diseases continues to grow worldwide as human activities increasingly extend into formerly remote natural areas. This is particularly noticeable on the island of Madagascar. As closest relatives to humans on the island, lemurs are of particular relevance as a potential origin of zoonotic pathogen spillover. Knowledge of pathogens circulating in lemur populations is, however, very poor. Particularly little is known about lemur hemoparasites. To infer host range, ecological and geographic spread of the recently described hemoparasitic nematode *Lemurfilaria lemuris* in northwestern Madagascar, a total of 942 individuals of two mouse lemur species (*Microcebus murinus* [*n* = 207] and *Microcebus ravelobensis* [*n* = 433]) and two rodent species (the endemic *Eliurus myoxinus* [*n* = 118] and the invasive *Rattus rattus* [*n* = 184]) were captured in two fragmented forest landscapes (Ankarafantsika National Park and Mariarano Classified Forest) in northwestern Madagascar for blood sample examination. No protozoan hemoparasites were detected by microscopic blood smear screening. Microfilaria were present in 1.0% (2/207) of *M. murinus* and 2.1% (9/433) of *M. ravelobensis* blood samples but not in rodent samples. Internal transcribed spacer 1 (ITS-1) sequences were identical to an unnamed Onchocercidae species previously described to infect a larger lemur species, *Propithecus verreauxi*, about 650 km further south. In contrast to expectations, *L. lemuris* was not detected. The finding of a pathogen in a distantly related host species, at a considerable geographic distance from the location of its original detection, instead of a microfilaria species previously described for one of the studied host species in the same region, illustrates our low level of knowledge of lemur hemoparasites, their host ranges, distribution, modes of transmission, and

**Abbreviations:** bp, base pairs; COI, cytochrome C oxidase 1; DNA, deoxyribonucleic acid; ITS, internal transcribed spacer; LB, lysogeny broth; NCBI, National Center for Biotechnology Information; PCR, polymerase chain reaction; rDNA, ribosomal deoxyribonucleic acid.

Ute Radespiel and Christina Strube share senior authorship.

their zoonotic potential. Our findings shall stimulate new research that will be of relevance for both conservation medicine and human epidemiology.

KEYWORDS
*Eliurus*, filaria, hemoparasites, Madagascar, *Microcebus*, *Rattus*

## 1 | INTRODUCTION

Due to augmented accessibility of remote areas by growing human infrastructure and anthropogenic fragmentation of natural habitats, rates of direct interaction between humans and wildlife are increasing. This facilitates the introduction of alien and invasive pathogens to native ecosystems and, in reverse, the spillover of zoonotic pathogens from wild animals to humans (Lymbery et al., 2014; Watsa, 2020; Wells et al., 2015). With a fast growing human population expanding into formerly remote areas, Madagascar is considered a hotspot for emerging infectious diseases (Brito et al., 2012; Jones et al., 2008). The island is known for its highly endemic and unique biodiversity (Myers et al., 2000) that includes more than 100 species of lemurs (Schwitzer et al., 2014). As members of the order primates, they are prone to cause zoonotic pathogen spillover to humans (Nozais, 2003). Introduction of several pathogens into wild lemur populations has been previously shown, including several diarrhea-associated viruses (Zohdy et al., 2015) and gastrointestinal protozoa from the genera *Cryptosporidium* and *Entamoeba* (Ragazzo et al., 2018; Rasambainarivo et al., 2013; Villers et al., 2008). The detection of as yet uncharacterized pathogens of the genera *Babesia*, *Borrelia*, *Plasmodium*, *Trypanosoma*, and a potentially new genus of the family Ehrlichiaceae in populations of wild lemurs (*Indri indri*, *Propithecus diadema*, *Avahi laniger*, *Lepilemur mustelinus*, and *Propithecus verreauxi*) may also indicate the presence of potentially zoonotic blood-borne pathogens (Larsen et al., 2016; Qurollo et al., 2018; Springer, Fichtel et al., 2015). A clinical relevance for the presence of these infectious agents in lemurs was not investigated in most studies. However, Zohdy et al. (2015) explicitly reported that diarrhea-associated pathogens had no effect on the fecal texture of the studied individuals and Springer, Fichtel et al. (2015) could not detect any alterations in packed cell volume associated with blood parasite species richness. Ragazzo et al. (2018), however, reported clinical diarrhea associated with *Enamoeba* infections in *Microcebus rufus* and *Eulemur rubriventer*. Similar to primates, rodents, which often live in close proximity to humans, are frequent carriers of zoonotic agents (Han et al., 2015). The most prominent example for Madagascar is probably the plague pathogen *Yersinia pestis*, for which introduced rodents of the genus *Rattus* represent an important reservoir (Chanteau et al., 1998; Hastriter & Dick, 2009; Tollenaere et al., 2010). Additionally, gastrointestinal parasites such as *Giardia* and *Cryptosporidium* (Bodager et al., 2015; Spencer & Irwin, 2020), water-borne *Leptospira* (Herrera et al., 2020) and blood-borne pathogens such as *Bartonella* (Brook et al., 2017) and *Trypanosoma* (Laakkonen et al., 2003) were also detected in endemic (*Trypanosoma* in *Nesomys rufus*) as well as introduced (mostly *R. rattus*) rodents in Madagascar. Within the framework of one health that acknowledges a close link of human health to the health of animals and the shared environment (Buttke et al., 2015; Cunningham et al., 2017; Jenkins et al., 2015; Thompson, 2013), ongoing efforts to expand our knowledge of primate and rodent pathogens are of critical importance. Knowledge of hemoparasites of lemurs is so far very limited and mostly based on incidental reports or small scale studies. Blood protozoa of the genera *Plasmodium*, *Babesia*, and *Trypanosoma* have been mostly detected in larger lemur species (Garnham & Uilenberg, 1975; Larsen et al., 2016; Springer, 2015). Among filaria, the genera *Dipetalonema*, *Paulianfilaria*, *Courduriella*, and *Protofilaria* are known to occur in wild lemurs (reviewed in Irwin & Raharison, 2009; Klein, 2019). However, except for the genus *Dipetalonema*, which includes species infecting dogs, rodents, neotropical primates, and also humans, these filarial genera seem to be unique to lemurs (de Argôlo et al., 2018; Casiraghi et al., 2006). To the authors' knowledge, no human case of zoonotic filarial infection has been reported in Madagascar to date. However, it is worth mentioning that lymphatic filariasis caused by *Wuchereria bancrofti* is highly endemic to the island (Gautret & Parola, 2011). Although a connection with lemurs has been suspected (Nelson, 1965), the pathogen has not yet been detected in these primates (Irwin & Raharison, 2009). For a new filarial genus, *Lemurfilaria*, recently described with the species *Lemurfilaria lemuris* infecting a population of gray mouse lemurs (*Microcebus murinus*) and Milne-Edwards sportive lemurs (*Lepilemur edwardsi*) inhabiting the Ankarafantsika National Park (ANK) in northwestern Madagascar (Hokan et al., 2017; Klein, Strube et al., 2019), there is also no evidence for a zoonotic potential yet. These tissue dwelling parasitic nematodes of the superfamily Filarioidea are transmitted by arthropod vectors and their larvae (microfilaria) circulate in the bloodstream. Although Klein, Strube et al. (2019) thoroughly analyzed seasonal dynamics of filarial infections and longitudinal host infection dynamics in mouse lemurs, host spectrum, vector species and geographical distribution of *L. lemuris* still remain unclear. To our knowledge, no comparable study on hemoparasites of the rodent genera *Eliurus* and *Rattus* has yet been conducted on Madagascar. This study therefore aimed to investigate the geographical distribution and host spectrum of vector-borne hemoparasites, in particular of the newly described *L. lemuris*, by determining prevalence microscopically, and to confirm species assignment by molecular methods in two small lemur species (*Microcebus ravelobensis*, *M. murinus)*, one native rodent (*Eliurus myoxinus*) and one invasive rodent (*Rattus rattus*) in two fragmented forest landscapes in northwestern Madagascar. Since *L. lemuris* is most likely transmitted by flying vectors and host specificity appears to be low, a wide geographic distribution and high prevalence were expected, at least in both investigated lemur species.

## 2 | METHODS

### 2.1 | Sample sites and sampling

All fieldwork protocols were reviewed and approved by the Institute of Zoology, University of Veterinary Medicine Hannover, Germany and the University of Toronto, Canada. The protocols were also reviewed by and met the legal requirements of the Direction du Système des Aires Protégées (DSAP) in Madagascar and Madagascar National Parks (MNP) (Research Permit Numbers N°80/17/MEF/SG/DGF/DSAP/SCB.Rc, N°151/17/MEF/SG/DGF/DSAP/SCB.Rc, N°84/18/MEF/SG/DGF/DSAP/SCB.Rc, and N°93/18/MEF/SG/DGF/DSAP/SCB.Rc). The research adhered to the American Society of Primatologists Principles for the Ethical Treatment of Non-Human Primates.

The study was conducted at two locations characterized by fragmented dry deciduous forests, 120 km south (western ANK, Figure 1a) and 70 km northeast (Mariarano Classified Forest [MAR], Figure 1b) of the city of Mahajanga, within the Boeny region in northwestern Madagascar. In the dry seasons (May to October) of the years 2017 and 2018, we captured 942 individuals of two mouse lemur species (*M. murinus* [*n* = 207] and *M. ravelobensis* [*n* = 433]) and two rodent species (native *Eliurus myoxinus* [*n* = 118] and invasive *Rattus rattus* [*n* = 184]) by installing Sherman Traps (*n* = 11,567; Sherman Traps, Inc.) along transects in 40 smaller forest fragments (0.8–114.6 ha) and four larger forest patches (3683–130,390 ha; Figure 1) according to procedures described by Kiene et al. (2020). Captured animals were measured, weighed, sexed, categorized into juvenile or adult, and a blood sample was taken. Examination took place during the early morning and animals were released at their individual capture site in the evening of the capture day.

Blood samples were obtained by puncturing the saphenous vein with a lancet and collecting the emerging blood drop with a pipette. Blood smears were produced using a standard amount of 3 µl blood following the standard procedures described in Klein, Strube et al. (2019). Surplus blood (5–45 µl) was collected and stored in 95% ethanol (samples from 2017) or in RNAlater© (Qiagen; samples from 2018) in a volume relation of 1:5 for molecular analyses. Blood smears were fixed with methanol on-site and Giemsa-stained upon return to Germany. The stained smears were screened for hemoparasites using an Axiostar plus microscope (Carl Zeiss MicroImaging). Morphometric measurements of the microfilariae were performed by using a Colorview IIIu Camera and the cell^B Image Acquisition Software (version 3.1; Olympus Soft Imaging Solutions).

### 2.2 | Molecular analyses

For DNA isolation from microfilaria, the NucleoSpin Blood kit (Macherey-Nagel) was applied to blood samples obtained from animals with microfilaria-positive blood smears. Sequences of the internal transcribed spacer 1 (*ITS1*) rDNA region (~600 bp), suitable for taxonomic identification of filaria (Blouin, 2002), were amplified using the primers NC1R and NC5 (Monti et al., 1998) following the protocol of Klein, Strube et al. (2019). Sequences of the cytochrome C oxidase 1 (*COI*) region (~600 bp) were amplified using the primers COIintF and COIintR following the protocol of Casiraghi et al. (2001). Most likely due to the presence of multiple gene copies, direct sequencing of the *COI* PCR products was not successful. The respective amplified fragments were hence ligated into pCR®4-TOPO® vectors and transformed into One Shot® TOP10 chemically competent *E. coli* (TOPO® TA cloning kit for sequencing; Invitrogen) and plated on LB agar, supplemented with carbenicillin (100 µg/ml). Ten colonies from each sample (one from a *M. ravelobensis* and one from a *M. murinus* host) were picked and further propagated in 5 ml

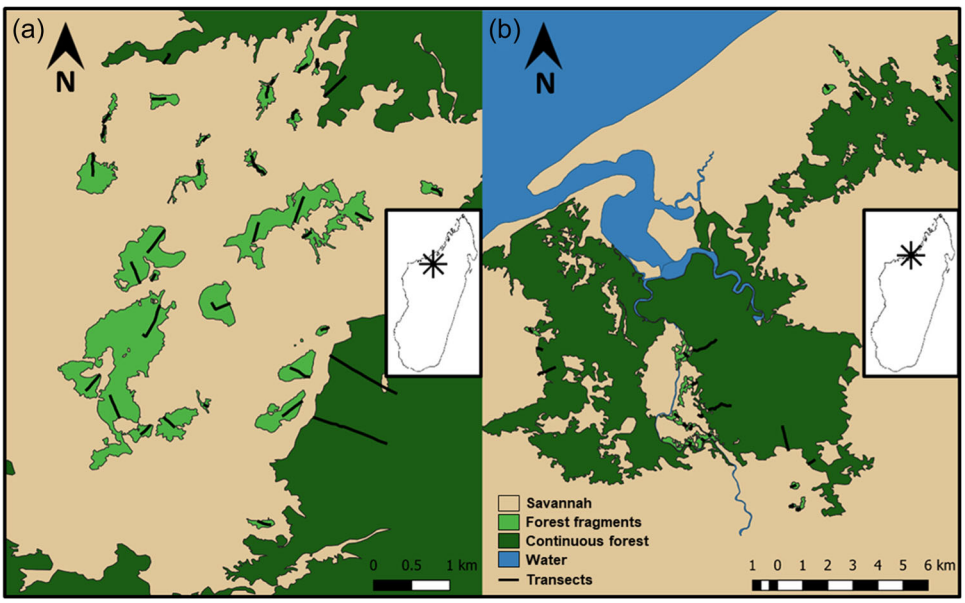


**FIGURE 1** Maps of the two studied areas of fragmented dry deciduous forest in the western Ankarafantsika National Park (ANK, a) and the Mariarano Classified Forest (MAR, b).

LB medium with carbenicillin (100 µg/ml). The NucleoSpin® Plasmid Kit (Macherey-Nagel) was applied to isolate plasmid DNA following the manufacturer's recommendations. Both, PCR products of *ITS* and the isolated plasmid DNA including *COI* sequences were sent for Sanger sequencing (Seqlab Sequence Laboratories Göttingen). Raw sequences were aligned with Geneious 10.2.3 software (https://www.geneious.com) and compared with sequences available in NCBI GenBank using BLAST comparison.

**TABLE 1** Numbers of host individuals positive for microfilaria and prevalence (in brackets) in relation to host species, age class, sampling month, and sampling region

| | Individuals positive for microfilaria | Total individuals |
|---|---|---|
| All individuals | 11 (1.7%) | 640 |
| Host species | | |
| *Microcebus murinus* | 2 (1.0%) | 207 |
| *Microcebus ravelobensis* | 9 (2.1%) | 433 |
| Age class | | |
| Juvenile | 1 (0.9%) | 111 |
| Adult | 10 (1.9%) | 529 |
| Habitat fragmentation | | |
| Continuous forest | 9 (2.3%) | 384 |
| Forest fragments | 2 (0.8%) | 256 |
| Sampling month | | |
| May | 0 (0.0%) | 93 |
| June | 2 (1.5%) | 137 |
| July | 4 (2.5%) | 157 |
| August | 4 (2.7%) | 148 |
| September | 1 (2.3%) | 44 |
| October | 0 (0.0%) | 61 |
| Sampling region | | |
| Ankarafantsika NP | 1 (0.5%) | 219 |
| Mariarano Classified Forest | 10 (2.4%) | 421 |

## 2.3 | Data evaluation

Qualitative comparisons were performed between host species (*M. murinus* vs. *M. ravelobensis*), host age (juvenile vs. adult), habitat fragmentation (continuous forest vs. forest fragment), sampling month (May to October), and sampling region (ANK vs. MAR) regarding the presence/absence of microfilaria, but statistical tests were not possible due to low prevalence.

# 3 | RESULTS

Neither hemoparasitic protozoa nor microfilaria were detected in the blood samples of rodent hosts (*E. myoxinus* and *R. rattus*). Protozoan hemoparasites were also not detected in mouse lemur blood smears. However, blood smears of both mouse lemur species contained microfilaria, although at very low prevalence of 0.97% ($n = 2/207$) in *M. murinus* and 2.08% ($n = 9/433$) in *M. ravelobensis* (Table 1). Filarial counts ranged from 0.33 to 2.00 microfilaria per µl blood. Microfilaria were detected in juvenile as well as adult host animals, between June and September (i.e., not in May or October), in both study regions, and in hosts trapped in continuous forests as well as in forest fragments (Table 1). Microfilaria from both host species appeared morphologically similar (Figure 2). Microfilaria detected in *M. ravelobensis* samples ranged from 203 to 265 µm in length and from 3.8 to 5.5 µm in width ($n = 13$, Figure 2a). Similarly, those detected in *M. murinus* samples ($n = 3$, Figure 2b) varied from 205 to 265 µm in length and from 4.8 to 5.5 µm in width.

The five obtained *ITS1* sequences (one from *M. murinus* and four from *M. ravelobensis* hosts, GenBank accession nos. ON493578-ON493582) were 99.5%–100% identical. BLAST sequence comparison

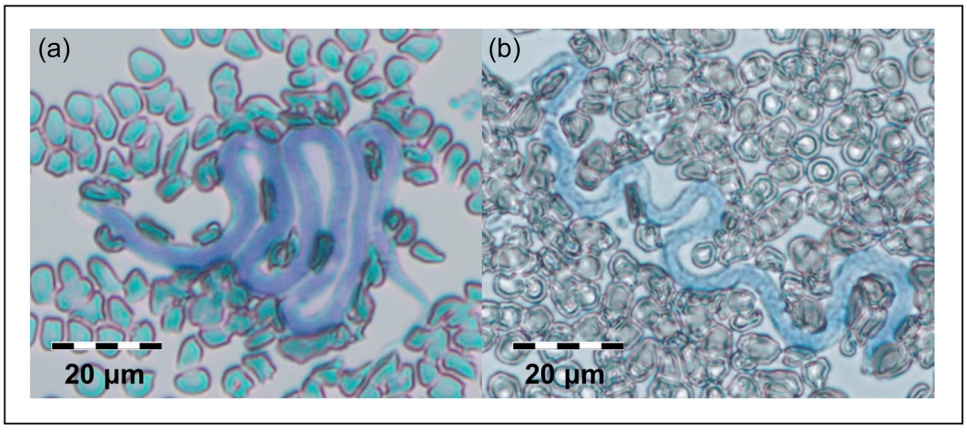


**FIGURE 2** Microfilaria found in blood smears from *Microcebus ravelobensis* (a) and *M. murinus* (b)

revealed 98.9%–100% identity to a published sequence obtained from a yet undescribed Onchocercidae sp. of *P. verreauxi* (GenBank accession no. LN869520; query cover 100%). In contrast, identity to *L. lemuris* ranged only from 95.3% to 96.2% (MK060113; query cover 100%) which falls below the value that characterizes members of the same species (Blouin, 2002). The three obtained *COI* sequences (two from a *M. ravelobensis* and one from a *M. murinus* host, GenBank accession nos. ON468528-ON468530) were 99.8%–100% identical. In this case, the BLAST search revealed highest identity with *L. lemuris* (98.2%–98.4%; GenBank accession no. MN119552; query cover 73%), while *COI* sequences from the filarial nematode found in *P. verrauxi* are not yet available in the database.

## 4 | DISCUSSION

Although all types of blood parasites were considered during blood smear examination, no protozoan hemoparasite stages were detected in any of the host species. Even though studies on protozoan hemoparasites in domestic animals or humans have not been carried out in the study region, such parasites have been detected in wild lemurs in other parts of Madagascar at high prevalence. A study from eastern Madagascar, for example, revealed 12 out of 76 examined lemur individuals from different species to be positive for *Borrelia* spp. and even 73 for *Babesia* spp. (Qurollo et al., 2018). In theory, at least *Babesia* should also be detectable by microscopic examination, which was, however, not compared in the cited study. While studies on rodent hemoparasites in northwestern Madagascar are also still lacking, our results are congruent with the findings of previous lemur studies from the same geographic region. Klein, Strube et al. (2019) investigated blood smears of gray (*M. murinus*) and golden-brown mouse lemurs (*M. ravelobensis*) and Hokan et al. (2017) scanned blood smears of western woolly lemurs (*Avahi occidentalis*) and Milne-Edward's sportive lemurs (*Lepilemur edwardsi*) from Ampijoroa in the ANK, and both studies did not detect any protozoan hemoparasites. Another study investigating blood samples of 36 Verreaux's sifakas (*P. verreauxi*) from Kirindy forest in western Madagascar detected a 36% prevalence of *Plasmodium* spp. by molecular screening, although all samples appeared negative by microscopy (Springer, Fichtel et al., 2015). Parasitaemia in that study was concluded to be too low for microscopic detection, while PCR assay sensitivity was sufficient to detect such very low infection intensities. This may also explain the negative results of this and other microscopic studies (Hokan et al., 2017; Klein, Strube et al., 2019). Systematic molecular screening of all blood samples was, however, beyond the scope of our study.

Microscopic scanning of blood smears was successful for the detection of microfilaria in the two mouse lemur species in both study regions. Klein, Strube et al. (2019) recently described the filarial species *L. lemuris* parasitizing *M. murinus* and *M. ravelobensis* from one forest site (Jardin Botanique A, JBA) close to Lake Ravelobe in the ANK. Since one of the two investigated areas in the present study (ANK) was located in close proximity (10 km) to the sampling site of Klein, Strube et al. (2019) and the other was only about 90 km away (MAR), we expected to find *L. lemuris* in our study but this was not the case. Instead, DNA sequences suggested the presence of an undescribed Onchocercidae species, which was previously detected in Verreaux's sifakas (*P. verreauxi*), lemurs of the family Indriidae, in Kirindy Forest in the Menabe region (Springer, Fichtel et al., 2015), approximately 650 km further south. This species assignment was unambiguously supported by the very high (98.9% –100%) *ITS1* sequence identity to the published reference sequence (GenBank accession no. LN869520). However, *L. lemuris* and the here detected species are closely related and may even belong to the same genus as shown in phylogenetic analyses performed by Klein, Strube et al. (2019).

According to the apparent lack of congruence regarding the filarial species found by Klein, Strube et al. (2019) and in the present study, it has to be taken into account that only a few samples were successfully sequenced in both studies. Thus, both filarial species may circulate in the region, but the simultaneous detection may have been missed so far. The occurrence of multiple filarial species in lemurs was already shown by Springer, Fichtel et al. (2015), who identified two species of Onchocercidae in *P. verreauxi* with a prevalence of 50.0% and 33.3%, respectively, the former being the same species as detected in this study.

The results of our study also differed in another important aspect from other published lemur hemoparasite studies, namely in the very low prevalence of microfilaria (0.97% in *M. murinus* and 2.08% in *M. ravelobensis*). Klein, Strube et al. (2019) described an *L. lemuris* prevalence of 30.4% in *M. murinus* and of 6.6% in *M. ravelobensis*, and Hokan et al. (2017) found microfilariae (retrospectively identified as *L. lemuris* by Klein, Strube et al., 2019) in even 66.7% of the *A. occidentalis* and in 41.7% of *L. edwardsi*. Differences in the individual identification ability of the examiner as a cause for this discrepancy were excluded by comparing the examination results of an independent sample between examiners. However, the results of our study are only partly comparable, since prevalence in those previous studies was either based on cumulative results from repeated examinations of individual hosts across a longer time-span (Hokan et al., 2017; Klein, Strube et al., 2019) or on the combination of microscopy with molecular screening (Springer, Fichtel et al., 2015). In contrast, prevalence values in our study are based on the microscopic examination of one blood smear per host individual only, which may explain the lower values at least partially. A systematic molecular screening of all available blood samples would most likely reveal additional filarial infections that could not be detected by microscopic examination due to low filarial concentrations in the blood. Additionally, it was shown for other filarial species that microfilaremia in the blood varies according to daytime with high levels in the afternoon and at night and lower levels during the day (Paily et al., 2009). Therefore, the timing of our sampling in the early morning hours may also explain the low prevalence observed in our study. Circadian fluctuations in microfilaremia are, however, highly under debate (Lovis et al., 2017), and neither Klein, Strube et al. (2019) nor Hokan et al. (2017) could find any diurnal variations in microfilaremia for *L. lemuris*.

Due to the low prevalence, no clear difference in prevalence between the two host species, between juvenile and adult individuals, between larger forests or forest fragments, between sampling regions or between sampling months was apparent. Similarly, no seasonal prevalence dynamics were observed in the studies by Hokan et al. (2017), Klein, Strube et al. (2019), Springer, Fichtel et al. (2015).

The finding that the filarial nematodes detected during this study belong to an undescribed Onchocercidae species, which has so far only been detected in *P. verreauxi* in the Kirindy Forest (Springer, Fichtel et al., 2015) that is in 650 km distance to our sampling sites, was unexpected. It suggests that (1) this filarial species has a much larger geographical distribution than its host species, and (2) host specificity of this hemoparasite species is not very high. Whereas *P. verreauxi* only occurs in southern and western Madagascar and *M. ravelobensis* is confined to one inter-river-system in northwestern Madagascar, their joint filaria species spans several biogeographic regions that are separated ecologically and by several large rivers (Wilmé et al., 2006). Onchocercidae, as most hemoparasites, require arthropod vectors for transmission (Otranto et al., 2013). In contrast to wingless vectors like fleas, lice and ticks, flying insects of the order Diptera can mediate transmission of filaria over longer distances (Klein, Strube et al., 2019; R. N. Sehgal, 2015). Within the order of Diptera, mosquitos (Culicidae), blackflies (Simuliidae), and midges (Ceratopogonidae) are known to transmit Onchocercidae nematodes (Shelley & Coscarón, 2001). Among these, mosquitos are known to cover distances of several kilometers by themselves (Service, 1997) and might also be carried to distant regions by wind (North & Godfray, 2018). Mosquito larvae can even be spread with human aid by incidental transport within small quantities of water in vehicles and ships (Medley et al., 2015). Nevertheless, populations of susceptible hosts and suitable breeding habitats are constantly needed to naturally spread blood parasites over such long distances.

One of the investigated host species, *M. murinus*, occurs both in northwestern (Ankarafantsika, Mariarano) and southwestern Madagascar (Kirindy Forest) (Mittermeier et al., 2010). Data on filarial infections of *M. murinus* from Kirindy Forest are, however, not available, whereas the sifaka species occurring at the sampling sites of the present study, *Propithecus coquereli*, has also not yet been investigated for filarial infections. If such data were available, a possible preference of the Onchocercidae species for hosts of the genus *Propithecus* could be elucidated, which might be responsible for the enormous differences in prevalence observed between mouse lemurs in the current study and *P. verrauxi* in Kirindy Forest. This would, however, still not explain the lack of *L. lemuris* in the sample of the present work. Further factors, such as vector abundance, may be responsible, but this could not be clarified by the available data.

Filarial nematodes are generally known to exhibit low levels of host specificity (R. N. M. Sehgal et al., 2005). The species *Cardiofilaria pavlovskyi*, for example, was reported to infect birds across the three orders of Falconiformes, Charadriiformes, and Passeriformes (Anderson, 2000). For the Onchocercidae species detected in the present study and also *L. lemuris*, the host spectrum might include the entire infraorder of Lemuriformes, as both species have so far been detected in two different lemur families (Cheirogaleidae and Lepilemuridae, and Cheirogaleidae as well as Indriidae, respectively). Similar patterns were observed regarding filaria of the genus *Dipetalonema* in new world primates. Particularly, *D. gracile* was found to parasitize primates beyond family level all over the continent of South America (Notarnicola et al., 2008). Noteworthy, *D. yatesi* is in contrast only known to infect black-faced spider monkeys (*Ateles chamek*) and has a much smaller distribution area (Zárate-Rendón et al., 2022). The absence of reported spillover events of these nematodes to human hosts, which are the closest relatives of lemurs on Madagascar, and the negative results of blood smear examinations of sympatric rodents in this study, may indicate lemur host-specificity of these Onchocercidae species. However, a larger study on Onchocercidae, their intermediate and definitive hosts, covering a larger wider geographic area and a broader taxonomic representation of lemur and other mammal hosts including humans will be needed to systematically test this prediction.

Taken together, our expectation of detecting the newly described filaria species *L. lemuris* was not met. This study rather raises new questions about the general situation of filaria infections in mammals all over Madagascar: How broad is the geographical distribution and host specificity of different lemur hemoparasites in the different ecoregions (e.g., dry vs. humid forests) of the island and how are they transmitted? What are the main hosts and what is their true prevalence, since the determined prevalence in mouse lemurs would most likely be too low for permanently maintaining a parasite population? Future research efforts should also include a comprehensive molecular screening to detect even cases of low infection intensity. Such studies will be of relevance for the emerging discipline of conservation medicine, as many lemur species are nowadays only found in small populations and have marginal distribution areas, making entire species vulnerable to infectious diseases. Furthermore, intensified research efforts will also be important for preventing future transmission of zoonoses to humans, since pathogen spill-over events cannot be excluded to happen in the future.

## AUTHOR CONTRIBUTIONS

**Frederik Kiene**: conceptualization (equal); data curation (equal); formal analysis (lead); investigation (lead); visualization (lead); writing – original draft (lead); writing – review & editing (equal). **Andrea Springer**: investigation (supporting); methodology (equal); supervision (equal); validation (equal); writing – review & editing (equal). **Bertrand Andriatsitohaina**: investigation (supporting); writing – review & editing (supporting). **Malcolm S. Ramsay**: investigation (supporting); writing – review & editing (supporting). **Romule Rakotondravony**: methodology (supporting); resources (supporting); supervision (supporting); writing – review & editing (supporting). **Christina Strube**: conceptualization (equal); data curation (equal); funding acquisition (equal); methodology (equal); resources (equal); supervision (equal); validation (equal); writing – review & editing (equal). **Ute Radespiel**: conceptualization (equal); data curation (equal); funding acquisition (lead); methodology (equal); project

administration (lead); resources (equal); supervision (equal); validation (equal); writing – review & editing (equal).

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

All prevalence and morphological data are presented directly in the manuscript. All newly generated sequences were deposited at GenBank under accession nos. ON468528-ON468530 and ON493578-ON493582.

## ORCID

*Frederik Kiene* http://orcid.org/0000-0003-2517-8553
*Andrea Springer* http://orcid.org/0000-0002-0051-9086
*Bertrand Andriatsitohaina* http://orcid.org/0000-0001-7222-0289
*Malcolm S. Ramsay* http://orcid.org/0000-0002-5807-4649
*Romule Rakotondravony* http://orcid.org/0000-0003-0454-7743
*Christina Strube* https://orcid.org/0000-0002-9093-1006
*Ute Radespiel* https://orcid.org/0000-0002-0814-2404

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