SHORT NOTE

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Multiple ectoparasites infest *Microcebus griseorufus* at Beza Mahafaly Special Reserve, Madagascar

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ABSTRACT

The mouse lemur *Microcebus griseorufus* at the Beza Mahafaly Special Reserve and general vicinity in southwestern Madagascar were surveyed for ectoparasites as part of a year-long behavioral and ecological study. Of 249 individual mouse lemurs examined, 74 were positively identified as hosting ectoparasites. Ticks from 20 mouse lemurs and lice in a subset of two individuals captured in a 90 ha gallery forest (Parcel 1) were preserved in 70% ethanol or EDTA and stored for analysis and for identification. Two species of *Haemaphysalis* ticks are represented in the sample, *H. lemuris* and possibly *H. simplex*, a tick previously reported on tenrecs, birds and rats. Synchronous development of ticks may pose a risk for vector-borne diseases at the reserve especially during the dry season. The louse represented in the sample belongs to the order Anoplura (sucking lice), and resembles *Lemurpediculus verruculosus*, previously reported on *Microcebus rufus* in eastern Madagascar.

RÉSUMÉ

Un inventaire d'ectoparasites a été réalisé dans le cadre d'une étude de longue durée portant sur le comportement et l'écologie de *Microcebus griseorufus* dans trois parcelles forestières de Beza Mahafaly et ses environs. Sur les 249 microcèbes observés, 74 individus étaient infestés d'ectoparasites. La majorité de ces individus infestés, soit 97,3%, provenait de la parcelle 1 de la réserve. Des poux et des tiques recensés sur ces animaux ont été immédiatement retirés puis préservés dans l'éthanol 70% ou dans l'EDTA à des fins d'analyse et d'identification. Deux espèces de tiques ont été identifiées, *Haemaphysalis lemuris* et probablement *H. simplex,* cette dernière n'était préalablement connue que pour infester les tenrecs, les oiseaux et les rats.

Les rongeurs jouent un rôle significatif en tant qu'hôtes pour près de la moitié des larves et des nymphes d'ixodes de tiques du monde, y compris *H. simplex*. Les rats sont connus pour être des porteurs d'une population importante de différentes tiques. Ils sont ainsi les hôtes avec la plus grande tolérance. Une nouvelle forme de transmission a été identifiée à Beza Mahafaly dans laquelle *H. simplex* et d'autres espèces de tiques ont simultanément infesté des tenrecs. Ce chevauchement de plusieurs parasites forme une sorte de réserve et favorise la transmission des agents pathogènes à travers les espèces hôtes. Les tiques immatures jouent un rôle significatif dans la transmission des maladies véhiculées par les hôtes car les nymphes ainsi infectées peuvent transmettre des pathogènes aux hôtes et aux larves engorgées. À Beza Mahafaly, le microcèbe pourrait être l'hôte des formes immatures des tiques de *H. lemuris* et *H. simplex* chez lesquelles le stade larvaire et le stade nymphal sont synchrones. Ces formes immatures de tiques se nourrissent ensemble sur des individus de microcèbes. Les poux appartiennent à l'ordre des Anoploures (poux suceurs) et ressemblent à *Lemurpediculus verruculosus* qui n'était connu que pour infester *Microcebus rufus* des forêts humides de Ranomafana. Il est possible que *M. griseorufus* accueille sa propre espèce de *Lemurpediculus*, si ce n'est le cas, il faudra admettre que la spécificité de cet arthropode serait moins limitée qu'elle ne le semblait. Pour comprendre la dynamique des parasites de la réserve, des études sur l'écologie des tiques et des poux sont recommandées afin de pouvoir contribuer effectivement à l'amélioration de la protection de la réserve.

INTRODUCTION

Lemurs in the wild are known to carry lice, ticks, mites (Ward 1951, Takahata et al. 1998, Sauther et al. 2002, Durden et al. 2010), leeches and flies (Wright et al. 2009). The most prevalent species of ticks parasitizing lemurs are *Ixodes lemuris* and *Haemaphysalis lemuris* (Hoogstraal and Theiler 1959). At Ranomafana National Park, *Microcebus rufus* are parasitized by several ixodid tick species including *Ixodes lemuris* (Blanco et al. submitted), *H. lemuris* and an unidentified *Haemaphysalis* species (Durden et al. 2010).

Sucking lice of the genus *Lemurpediculus* have been found on *M. rufus* at Ranomafana (Durden et al. 2010) and cheirogalids at Fort Dauphin (Ward 1951). Chewing lice have also been found on lemurs. *Trichophilopterus babakotophilus* have been reported from *Indri indri* at Betampona Strict Nature Reserve (Junge et al. 2011) and *Lemur catta* and *Propithecus verreauxi* at the Beza Mahafaly Special Reserve (BMSR) are infested by *Trichophyloterus babakotus* (Loudon 2009).

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Four species of lemurs are found at the BMSR in southwest Madagascar; *Lemur catta, Propithecus verreauxi, Lepilemur petteri* and *Microcebus griseorufus*. BMSR is comprised of two forested areas, a 90 ha gallery forest (Parcel 1) and a larger 520 ha spiny forest (Parcel 2). A third forested area, Ihazoara, is located near the reserve although it is not part of the reserve itself. The parasite ecology of the diurnal lemurs, *L. catta* and *P. verreauxi*, has been described by Loudon (2009), but much less is known about the parasites harbored by the smaller-bodied species of nocturnal lemurs, *L. petteri* and *M. griseorufus*. Baseline data on the intestinal parasites of *M. griseorufus* at Beza Mahafaly has been documented by Rodriguez (2006); however data on ectoparasite infestations of this species is completely lacking from published literature. An opportunity arose to collect ectoparasites as part of a larger study on the behavior and ecology of *M. griseorufus* at BMSR. This study is the first to describe the ectoparasites infesting this species.

METHODS

Rasoazanabary (2011) used traps baited with banana to capture mouse lemurs during a year-long behavioral and ecological study (October 2006 to September 2007) at BMSR (E44° 34′ 20″, S23° 41′ 20″). Captured individuals were anesthetized, marked by ear clipping and had microchips inserted for easy identification using a transponder. The total number of trap nights during the year at each site was 33,120, for a total of 99,360 traps set in all three forests over the entire year. Traps were set in trees and on the ground. A full description of the trapping schedule is provided by Youssouf Jacky and Rasoazanabary (2008).

Captured individuals were examined for ectoparasites. When present, the number of ticks and their locations on the host were recorded and ectoparasites were removed before the animal was released. Ticks and lice were collected from a subsample of *Microcebus* and were preserved in 70% ethanol or EDTA for future analysis and species identification. Images and measurements of ticks and lice were made microscopically (X 4–5) using SPOT basic imagining software. Positive identification of *Haemaphysalis lemuris* and *H. simplex* ticks was made by comparing the nymphal ticks collected with those previously described (Hoogstraal 1953, Uilenberg et al. 1979, Takahata et al. 1998). Identification of *Lemurpediculus* sp. was made by comparing nymphs in this study's sample with previous descriptions of this louse (Ward 1951, Durden et al. 2010). No voucher specimens of tick or lice were deposited in collections because all samples were needed for genetic analysis and were therefore destroyed during the DNA extraction process.

Three distinct habitats were sampled at Beza: (1) Parcel 1, a protected gallery forest adjacent to the Sakamena river, (2) Parcel 2, a protected spiny forest, and (3) Ihazoara, an unprotected dry forest (botanically similar to Parcel 2) near a village by the same name (for site map see Youssouf Jacky and Rasoazanabary (2008)).

RESULTS

A total of 249 mouse lemurs were live-trapped and examined for ectoparasites of which 74 animals were positively identified as hosting ticks. *Microcebus* were captured year-round but infested captures occurred only during the dry season (May to October) and only at Parcel 1. Ticks were primarily removed from the ears of *Microcebus* and very few were found traveling through the pelage. A subsample of 73 ticks from 20 mouse lemur individuals (including 33 larvae, 38 nymphs, and 2 males) was preserved. Six *Microcebus* presented *Haemaphysalis lemuris* nymphs and eight animals presented nymphs of a second haemaphysaline morphotype. Mean intensity for tick burden in the preserved samples was 2.7 nymphs per individual and 3.3 larvae per individual. Nymphs from both species of *Haemaphysalis* were found throughout the dry season (Figure 1); however, we did not find nymphs of both species co-feeding on single mouse lemurs. Larvae were more common during the early part of the dry season and were generally heavily engorged. Five individual mouse lemurs presented co-feeding nymphs and larvae. With the exception of three male adult ticks taken from one mouse lemur, no other adults were found in our preserved samples. Eight louse nymphs, taken from the head, under the fur, of two mouse lemurs were also preserved. The lice came from individuals that were also infested with ticks.

Insufficient DNA was obtained from ectoparasites and genetic analysis of samples was not possible. Only nymphs were used for morphological identification of tick species, as measurements on larvae were difficult due to the degree of engorgement. Many morphological characteristics distinguish the second tick morphotype from *Haemaphysalis lemuris*, most notably the shape of the mouthparts. Article one of the palps is highly reduced or absent. Ventrally, the basal concavity of the anterior margin of the basis capituli is more pronounced, as is the space between the medial edges of the palps and the chelicera and hypostome. Article three of the palpi is more rounded anteriorly and does not extend as far laterally as article two. This second tick morphotype most closely matches the accounts of Hoogstraal (1953) and Uilenberg et al. (1979) of *H. simplex* nymphs. Diagnostic characteristics from Hoogstral (1953) and Uilenberg et al. (1979) include: palpi that are wider than they are long and are broadly salient laterally; highly reduced, or absent, cornua on the basis capituli; and reduced spurs on trochanters (Figure 2). The morphology of the male ticks in our sample is also consistent with *H. simplex*. The characteristic mouthparts of the second morphotype also resemble those of the *Elongiphysalis* group; however, this group tends to have distinctly longer and sharper spurs on trochanters than do the specimen in our sample.

Total body length of the louse nymphs ranged from 1.1–1.8 mm (n=8). Their morphology is consistent with that of sucking lice belonging to the genus *Lemurpediculus*. Indeed, it closely resembles *L. verruculosus*, which previously has not been reported on western or southern mouse lemurs.

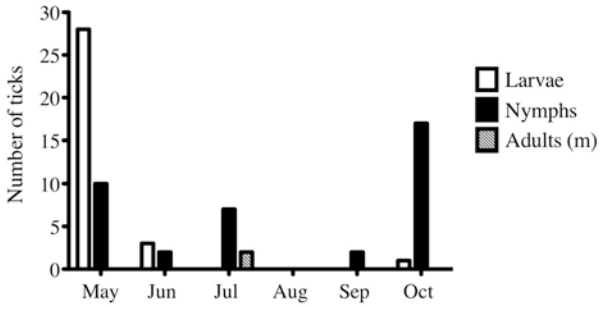

FIGURE 1. Number of preserved larval, nymphal and adult ticks removed from mouse lemurs during the winter months.

FIGURE 2. A. Dorsal view of the mouthparts of the second haemaphysaline in our sample (*Haemaphysalis* cf. *simplex*). B. Ventral view of the mouthparts. C. Ventral view of the body. D. Dorsal view of both haemaphysaline species infesting mouse lemurs at BMSR. *H. lemuris* is pictured at the top.

DISCUSSION

Two species of haemaphysaline ticks, *Haemaphysalis lemuris* and likely *H. simplex*, parasitize mouse lemurs at Beza Mahafaly Special Reserve. *H. lemuris* has also been described parasitizing both sifaka (*Propithecus verreauxi*) and ring-tailed lemurs (*Lemur catta*) at BMSR (Junge and Sauther 2002, Sauther et al. 2002, Loudon 2009). No other tick species has been associated with the lemurs at BMSR.

A combination of diagnostic characteristics of mouthparts and the tick body suggest the second tick species parasitizing mouse lemurs is *Haemaphysalis simplex*. More detailed comparisons with voucher specimen or genetic analysis can verify our tentative species assignment of this tick. The preferred hosts of *H. simplex* are *Setifer setosus* and *Echinops telfairi* but this tick species has also been found on other tenrec species (*Tenrec ecaudatus)*, rodents (*Macrotarsomys bastardi*, *Rattus rattus*) and birds (*Lophotibis c. cristata*) (Uilenberg et al. 1979). Uilenberg et al. (1979) also reported that *H. simplex* was found on the sifaka *Propithecus verreauxi*, but suggested the sifaka may have been an accidental host. The host preference of *H. simplex* may be less specific than for other tenrec tick species (Hoogstraal 1953, Hoogstraal et al. 1974, Uilenberg et al. 1979).

Haemaphysalis simplex feeding on *Microcebus* could be explained by the lack of host-specificity of this tick. At Parcel 1, mouse lemurs live in sympatry with *Echinops telfairi*, *Macrotarsomys bastardi* and *Rattus rattus*, each of which could support *H. simplex* populations and could be a source for transmission. Additionally, immature stages of ticks, as found on *Microcebus*, tend to have a wider host repertoire than do adult ticks. The presence of rats in Parcel 1 could be another factor influencing tick infestations. Rodents play a significant role as hosts to approximately half of the world's larval and nymphal ixodid ticks (Hoogstraal and Kim 1985), including *H. simplex*. Rats at the reserve are known to carry ticks (Rasoazanabary et al. 2009), although the tick species has not yet been identified. It is possible to conceive that rats can serve as vehicles for transmission of ticks between mammalian host species at BMSR. Identification of rat ticks at Beza Mahafaly is essential to elucidate tick infestation patterns.

Parasitism of *Microcebus* by multiple tick species could have important implications for vector-born diseases at the reserve. Arthropod-borne disease agents have been reported in lemurs (Uilenberg 1970, Uilenberg et al. 1972). *Haemaphysalis lemuris* may be a vector of *Babesia cheirogalei* and *B. propitheci* and *H. simplex* may serve as a vector for *B. brygooi* (Uilenberg et al. 1979). *H. simplex* can co-parasitize tenrecs with other tenrec tick species such as *H. elongata* and *H. theilerae* (Hoogstraal et al. 1974), and now possibly with *H. lemuris* on mouse lemurs. Overlapping parasitism may increase the potential for transmission of pathogens among host species. While the current study did not find nymphs of *H. lemuris* and *H. simplex* co-feeding on single mouse lemurs, this possibility cannot definitively be excluded. Additionally, immature ticks are known to play a significant role in the epidemiology of vector-borne diseases. Infected nymphs can transmit pathogens to healthy hosts and also to co-feeding larvae. At BMSR, nymph and larva are active during the dry season and their life cycles appear to be synchronous. Both stages of ticks were observed co-feeding on individual *Microcebus*. More studies of the ticks and their ecology are needed to elucidate these dynamics of parasitism at the reserve.

At BMSR, louse infestations have been reported on diurnal lemurs (Sauther et al. 2002, 2006, Loudon 2009). Loudon (2009) describes the chewing louse *Trichophyloterus babakotus* (Phthiraptera: Trichodectidae) parasitizing both ring-tailed lemurs and sifakas. The same louse species has been found on sifakas from Kirindy Mitea National Park (R. J. Lewis and D. Cailloud, pers. comm.). Different species of lice have been collected on nocturnal lemurs. Sucking lice, genus *Lemurpediculus* (Phthiraptera: Polyplacidae), have been recovered from *Lepilemur* and cheirogaleids in eastern Madagascar (Durden and Musser 1994). *Lemurpediculus petterorum* infests Lepilemur, while *L. verruculosus* parasitizes brown mouse lemurs (*Microcebus rufus*) at Ranomafana (Durden et al. 2010) and other mouse lemurs (possibly also *M. rufus*) at Bemangidy, in the region of Fort Dauphin (Ward 1951). Morphologically, the louse we found on mouse lemurs at BMSR closely resembles *L. verruculosus*. However, the latter louse species may be specific to the brown mouse lemur (Durden et al. 2010). It is possible that *M. griseorufus* has its own species of *Lemurpediculus*; if not, then the specificity of this arthropod is less restricted than previously thought. More research is therefore required to understand louse infestations in lemurs at BMSR.

CONCLUSION

Microcebus griseorufus at BMSR are parasitized by multiple species of ectoparasites, including sucking lice and a species of tick that may be acquired from sympatric species of tenrecs. Synchronous development of ticks may pose a risk for vector-borne diseases, especially during the dry season and possibly across different species of hosts. The ectoparasite profile of mouse lemurs is different from that of sympatric, larger-bodied, diurnal lemurs. The results presented here contribute to the growing compendium of knowledge on parasitism of the lemurs at BMSR and highlight the need for a comprehensive study on the dynamics of parasitism in order to fully understand the parasite ecology of the community. The ecology of parasites has implications for host health, and monitoring changes in the parasite communities can also inform researchers about the effects of altered environments on the ecosystem.

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SUPPLEMENTARY MATERIAL.

AVAILABLE ONLINE ONLY.

FIGURE S1. Ticks found on the ears of *Microcebus* at BMSR.

FIGURE S2. A. Dorsal view of *Haemaphysalis lemuris*. B. Ventral view of *H. lemuris*.

FIGURE S3. *Haemaphysalis* larvae collected on mouse lemurs at BMSR.

FIGURE S4. Ventral view of louse (*Lemurpediculus* cf. *verruculosus*) collected from *Microcebus griseorufus* at BMSR.