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Seasonal variation in the abundance and distribution of ticks that parasitize *Microcebus griseorufus* at the Bezà Mahafaly Special Reserve, Madagascar

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A B S T R A C T

At Bezà Mahafaly Special Reserve (BMSR), Madagascar, mouse lemurs (*Microcebus griseorufus*) are parasitized by multiple species of haemaphysaline ticks. At present we know little about the role ticks play in wild lemur populations and how they can alter interspecies relationships within communities or impact host fitness. In order to better understand these dynamics at BMSR, we examined parasite-host interactions as well as the ecology of mouse lemurs and their infesting ticks, *Haemaphysalis lemuris* and *H.* sp. cf. *simplex*. We show that season, host sex, and habitat influence the relative abundance of ticks on mouse lemurs. Specifically, infestations occur only during the dry season (May–October), are higher in males, and are higher at the study site with the most ground cover and with greater density of large-bodied hosts. *Microcebus* likely experience decreased susceptibility to tick infestations during the wet season because at that time they rarely if ever descend to the ground. Similarly, male mouse lemurs have higher infestation rates than females because of the greater time they spend traveling and foraging on the ground. During the dry season, *Microcebus* likely serve as hosts for the tenrec tick, *H.* sp. cf. *simplex*, when tenrecs hibernate. In turn, during the wet season when mouse lemurs rarely descend to the ground, other small mammals at the reserve may serve as maintenance hosts for populations of immature ticks. The synchronous development of larvae and nymphs could present high risk for vector-borne disease in *Microcebus*. This study also provides a preliminary description of the ecology and life cycle of the most common lemur tick, *H. lemuris*.

1. Introduction

Epidemiological studies of ectoparasitism in lemurs have generally focused on diagnostics (Takahata et al., 1998; Junge, 2002; Loudon et al., 2006; Durden et al., 2010). Few have provided significant information regarding parasite-host interactions or the ecology of the parasites. Understanding host-parasite relationships and tick ecology is important for evaluating the hosts' risk of disease from ticks or from microparasites that ticks may carry; this in turn can be critical for conservation management. Wild *Microcebus* (mouse lemurs) live in relatively high densities, often descend to the ground, and engage in social grooming. These characteristics place them at high risk for ectoparasite infestation. In fact, mouse lemurs are parasitized by multiple species of ticks. These small primates primarily present immature tick stages (Durden et al., 2010; Rodriguez et al., 2012; Blanco et al., 2013) and likely serve as maintenance hosts to various three-host tick species, including *Haemaphysalis lemuris*, *Ixodes lemuris* (Blanco et al., 2013), and other *Haemaphysalis* spp. (Durden et al., 2010; Rodriguez et al., 2012).

At the Bezà Mahafaly Special Reserve (BMSR) in southwestern Madagascar *Microcebus griseorufus* are parasitized by *Haemaphysalis lemuris* and another tick, possibly *Haemaphysalis simplex* (Rodriguez et al., 2012), and which we call here conservatively *H.* sp. cf. *simplex*. *Haemaphysalis lemuris* is the most common lemur tick although little is known about its life cycle. This tick has been collected from at least nine lemur species (Hoogstraal and Theiler, 1959; Koyama et al., 2008; Durden et al., 2010; Junge et al., 2011),

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including two larger-bodied lemur species, *Propithecus verreauxi* (Verreaux' sifakas) and *Lemur catta* (ring-tailed lemurs), that live in sympatry with *M. griseorufus* at BMSR (Takahata et al., 1998; Loudon et al., 2006; Loudon, 2009). The second tick species exhibits morphological characteristics similar to the old world *Haemaphysalis* subgenus specialized for parasitizing birds as well as tenrecs, *Ornithophysalis* (e.g., *H.* (*Ornithophysalis*) *simplex* and *H.* (*O.*) *simplicima*) (Hoogstraal, 1953; Hoogstraal et al., 1974). At Ranomafana, Durden et al. (2010) observed haemaphysaline ticks (*Haemaphysalis* sp.) on *Microcebus rufus* that could not be identified. Unfortunately, no description of *Haemaphysalis* sp. was provided and it is therefore not yet possible to confirm the species status of the second haemaphysaline tick found at BMSR. While more work is necessary to verify species identification, nymphs of the tick species collected from mouse lemurs at BMSR have tentatively been identified as *H. simplex* by morphological analysis (Rodriguez et al., 2012).

At BMSR, tick infestations on mouse lemurs are not random; instead, both *Haemaphysalis lemuris* and *H.* sp. cf. *simplex* are found on mouse lemurs exclusively during the austral winter and primarily at one of the reserve's two "parcels," which are non-contiguous forests (Rodriguez et al., 2012). Ticks have been recovered, however, from ring-tailed lemurs and sifaka from both of the reserve's parcels and at varying times of the year (Loudon et al., 2006; Loudon, 2009). Because mouse lemur infestations are restricted temporally and spatially, we believe that patterns of parasitism at the reserve are influenced by the life cycles of parasitizing ticks and the ecology of the hosts. In addition, the presence of *H.* sp. cf. *simplex* on mouse lemurs at BMSR indicates that mouse lemurs serve as alternate hosts to ticks from other mammalian species.

Here we examine tick infestations of *M. griseorufus* in their ecological contexts at and in the vicinity of the Bezà Mahafaly Special Reserve to determine which factors likely control haemaphysaline tick abundance and distribution. We address these questions by exploring infestation rates of ticks on *M. griseorufus* males and females living in different microhabitats and within the same microhabitat at different times of the year. We test three hypotheses (that habitat matters, that sex matters, and that season matters) and examine mouse lemur behavioral characteristics that may affect their tick infestation rates and their potential as hosts to various tick species. Finally, on the basis of this information we present a preliminary description of the ecology and life cycle of *H. lemuris*.

2. Materials and methods

2.1. Study sites

Microcebus living in three non-contiguous forests were studied for tick infestations; two inside the Bezà Mahafaly Special Reserve (Parcels 1 and 2) and one outside of the reserve (Ihazoara forest). Parcel 1 is an 80 ha gallery forest that is protected by a fence and is regularly monitored (Ratsirarson, 2003; Rasoazanabary, 2011). It borders a research camp. This site has considerable understory and thick ground litter. It contains the highest population densities of species of lemurs present at BMSR, *L. catta*, *P. verreauxi*, *Lepilemur petteri* (sportive lemurs) and *M. griseorufus*. Parcel 1 also has the highest density of the introduced rodent, *Rattus rattus* (Youssouf Jacky and Rasoazanabary, 2008). Parcel 2, is a larger 520 ha forest that is characterized by deciduous and Didiereaceae-dominated spiny vegetation (Ratsirarson, 2003; Axel and Maurer, 2011). The ground cover at Parcel 2 is much thinner than at Parcel 1. Ring-tailed lemurs are rare in Parcel 2; sifakas and sportive lemurs are more common. The third study site, in the Ihazoara forest, lies adjacent to Ihazoara village and is the most disturbed of the three sites. Livestock roam regularly through the site along paths created by the villagers. The vegetation is similar to that of Parcel 1, and the forest floor is rocky and virtually devoid of herbaceous vegetation (Rasoazanabary, 2011). At the study site, no ring-tailed lemurs or sifakas were observed. Fieldwork was conducted by ER.

2.2. Mouse lemur trapping

We used Sherman traps baited with banana to capture mouse lemurs during a year-long study (October 2006–September 2007). At each of our three study sites, we conducted intensive sampling in a large main study area (275 m × 225 m) during four months of the year (January, May, September and October). In addition, smaller or "supplementary" areas (20 m × 20 m) near the main study areas were selected for sampling during the other eight months of the year. In Parcel 1, the main study area was regularly used by researchers and a trail grid laid by prior researchers was used for this study. The supplementary study site was more pristine, with tall grass and leaf litter, as it was not regularly used by prior researchers and had no trail grid. A full description of the trapping schedule is provided by Youssouf Jacky and Rasoazanabary (2008).

We set traps in trees and on the ground at night and checked them each morning for captured animals. We marked captured mouse lemurs by clipping the ears, and inserting microchips for easy identification using a transponder. We collected basic data (date, place of capture, sex, and basic morphometrics including body mass) for each captured individual. Animals were released at the location of their capture around sundown, the beginning of their active period. On a daily basis, total rainfall and minimum and maximum temperature at Parcel 1 were also recorded.

2.3. Tick recovery and identification

All captured mouse lemurs were examined for ectoparasites and when present, all ticks were removed from the host and counted. For identification and future analysis, ectoparasites from 20 host animals were preserved in 70% ethanol or EDTA. Identification of *Haemaphysalis lemuris* and *H.* sp. cf. *simplex* ticks was made by comparing the nymphal ticks collected with those described previously (Hoogstraal, 1953; Uilenberg et al., 1979; Takahata et al., 1998), and by consulting with experts in the field. Morphological descriptions and images of both *Haemaphysalis* types are provided in Rodriguez et al. (2012). No voucher specimens of ticks were deposited in collections because all samples were utilized for genetic analysis and samples were destroyed during the DNA extraction process. Insufficient DNA was recovered from samples for amplification.

2.4. Statistical methods

We used the chi-square functions in Graph Pad Prism and the Statistical Package for the Social Sciences (SPSS 22.0) to ascertain the significance of differences in tick infestation rate by season, site and sex. A number of mouse lemur individuals were "trap happy" (captured multiple times – up to 43); some were heavily infested. Because these individuals become overrepresented when the sample comprises total captures and recaptures, comparisons by capture and recapture are useful only when looking at the overall infestation pattern across forest types and habitats. Comparisons by individual give a more accurate measure of infestation rates. For each statistical comparison, we indicate whether the test is based on number of captures or number of individuals in each test category.

3. Results

3.1. Identification of preserved ticks

The subsample of ticks preserved for later analysis contained larvae and nymphs. Larvae could not be identified at the species level because they were heavily engorged, but two morphologically distinct nymph types were observed. Six mouse lemurs presented *H. lemuris* nymphs and eight presented *H.* sp. cf. *simplex* nymphs.

3.2. Infestation rates at BMSR and Ihazoara forest

Of the 1552 mouse lemur captures (including first captures and recaptures of 249 animals), 29.7% (or 74 individuals) were positive for ticks. Infestation rates were significantly higher in Parcel 1 than in the other two sites ($\chi^2 = 141.9$, df = 2, P < 0.0001). Ninety-six percent (71 individuals) of infested individuals occurred in Parcel 1; Parcel 2 had 2.7% (2 individuals) infested captures and Ihazoara forest had 1.3% (1 individual). Within Parcel 1, the percentage of captures positive for ticks was significantly higher in the supplementary sampling area than in the main study area ($\chi^2 = 8.48$, df = 1, P < 0.01). As noted earlier, the grass was markedly denser and taller and leaf litter was thicker in the supplementary study area than in the main study area. These data suggest that the specific location of *Microcebus* and the thickness of the ground cover influence the risk of infestation.

3.3. Infestation rates by season

During the wet season (November–April), there were 21 captures in Parcel 1, 27 in Parcel 2 and 13 in Ihazoara forest but no ticks were found on mouse lemurs. During the dry season (May–October), the number of captures were 733, 296, and 462 in Parcel 1, Parcel 2, and Ihazoara forest respectively. All captures that were positive for ticks occurred during the dry season ($\chi 2 = 5.58$, df = 1, P = 0.02), indicating a seasonal bias in infestation rates at the reserve.

Because tick infestations occurred primarily in Parcel 1, we examined seasonal bias in Parcel 1 only. Of the total 113 individual mouse lemurs (2 individuals were excluded due to incomplete demographic data) captured in Parcel 1, 61.7% (69 individuals) presented ticks during at least one capture (16.9% of total captures and recaptures were positive for ticks). As is common with parasite infestations, approximately 21% of the infested population of mouse lemur individuals carried most of the ectoparasites (58.2% of all ticks). The number of ticks collected from individual hosts ranged from 1 to 25, but only 23.3% of infested mouse lemur captures yielded more than 10 ticks. Mean tick intensity for the dry season was 5.4 per captured lemur, with the highest tick burden occurring during the month of August (8) and lowest occurring in May (2.9) and October (3.2) (Fig. 1a). Peak activity for immature stages of ticks infesting *Microcebus* coincided with the driest periods (Fig. 1b) and the lowest ambient temperatures of the year (Fig. 1c).

3.4. Temporal distribution of tick species

The vast majority of larvae collected from mouse lemurs for preservation came from captures during the early part of the dry season, primarily during the month of May. *Haemaphysalis* sp. cf. *simplex* nymphs were found in the early part of the dry season (May–July) whereas *H. lemuris* nymphs came from captures later in the dry season, and peaked in October. Peak activity for *H. lemuris* and *H.* sp. cf. *simplex* may occur at different times during the dry season.

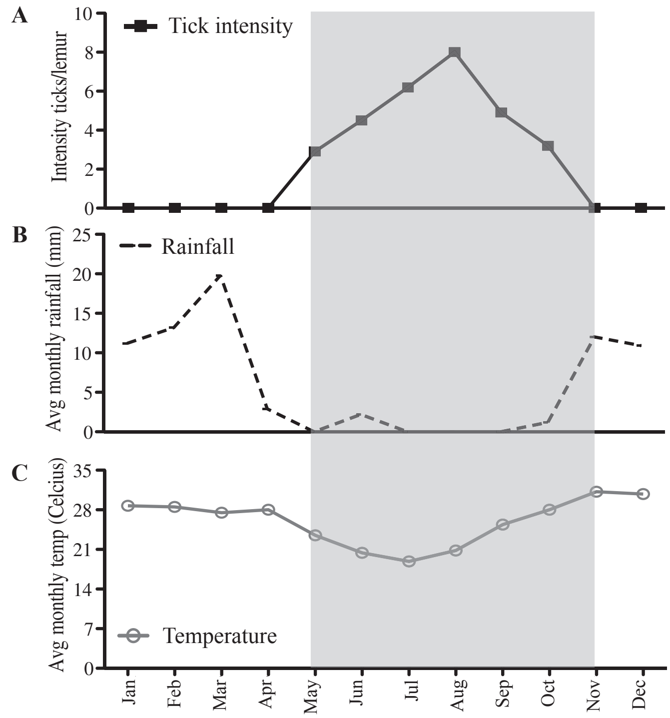

Fig. 1. Monthly averages for A) tick intensity on mouse lemurs as it compares to B) rainfall and C) temperature, during the year-long study season. Shaded area indicates months included in the dry season. Environmental data were collected daily.

3.5. Infestation rate by sex at Parcel 1

Approximately 45% of captured mouse lemur individuals (51 individuals) were male and 55% (62 individuals) were female. Infested males and females had similar mean tick intensity, averaging 5.0 per individual for females and 5.6 for males (t = 0.68,

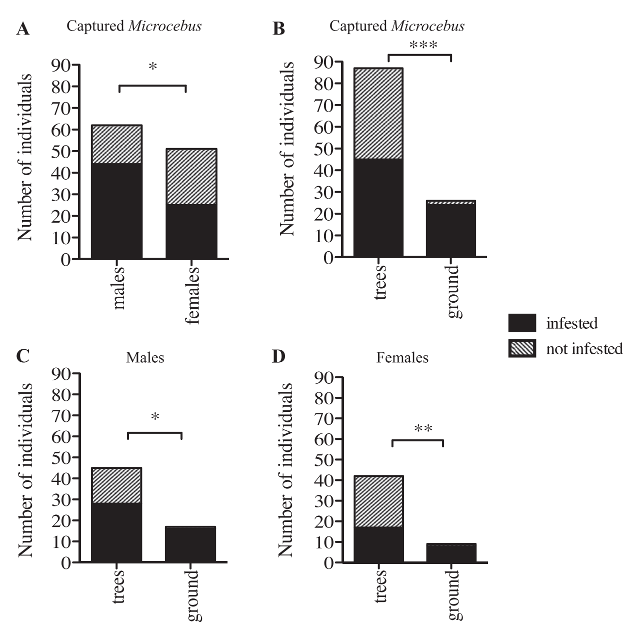

Fig. 2. Differences in infestation rates at Parcel 1 by A) sex B) substrate C) males and substrate and D) females and substrate. * indicates $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$ and compares variables on the *x*-axis.

df = 122, NS). However, males (43 out of 62 individuals or 69.3%) had much higher infestation rates than females (25 out of 51 individuals or 49%) ($\chi^2 = 5.69$, df = 1, P = 0.02) (Fig. 2a).

3.6. Infestation rates by substrate at Parcel 1

The vast majority of traps were set in the trees, but more than three times the infested captures of *Microcebus* came from traps set on the ground. Individuals trapped on the ground were much more likely to carry ticks ($\chi^2 = 63.89$, df = 1, P < 0.0001) than those captured in trees (Fig. 2b). The mean tick intensity for individual lemurs captured on the ground was 7.3 vs. 4.2 in trees.

Both male and female individuals captured in ground traps had significantly higher infestation rates than those captured in trees (males: $\chi^2 = 17.21$, df = 1, P < 0.0001, females: $\chi^2 = 66.61$, df = 1, P < 0.0001) (Fig. 1c). Male *Microcebus* were much more likely to be found on the ground than females ($\chi^2 = 26.11$, df = 1, P < 0.0001). Together, these data suggest that terrestrial behavior influences the risk of tick infestation for *Microcebus*.

3.7. Seasonality of substrate use by small mammals at BMSR and vicinity

Microcebus were captured in traps set on the ground only during the dry season (Table 1), suggesting that mouse lemurs rarely descend to the ground during the wet summer months. Other small mammals at the reserve, including *Rattus rattus*, *Mus musculus* and *Echinops telfairi*, were often captured on the ground during the wet season (Table 1); species differences were highly statistically significant ($\chi^2 = 185.8$, df = 3, P < 0.001). *Echinops*, which go into a state of torpor during the dry season, were absent from all tree traps and found in only four ground traps at this time of year (Table 1). These data suggest that seasonal differences in infestation rates in *Microcebus* are influenced by a shift in substrate utilization by *Microcebus* and also by the disappearance of tenrecs from the pool of potential tick hosts during the dry season.

4. Discussion

Our data demonstrate a distinct seasonal pattern to mouse lemur tick infestation rates at BMSR. Ticks are present on mouse lemurs at BMSR only during the months of May through October, corresponding to the dry austral winter. During the dry season mouse lemurs become more terrestrial and both males and females captured in ground traps have significantly higher infestation rates than males and females captured in trees. In addition, male *Microcebus*, who tend to travel more and forage further away from their nesting sites (and who are more often caught in ground traps) (Rasoazanabary, 2011), are more vulnerable to tick infestations than are females. We propose here that during the wet season, *Microcebus* are protected from tick infestations because they rarely if ever descend to the forest floor, where they are more likely to come into contact with questing ticks. Higher levels of tick parasitism in subadult and adult *Lemur catta* males have been explained as a by-product of lower levels of grooming (Takahata et al., 1998; Sauther et al., 2002; Koyama et al., 2008), as adult females and infants are groomed more frequently than males due to their higher rank. It is possible that similar factors may come into play with *Microcebus*, as females are dominant over males (Rasoazanabary, 2011). Our data do not allow us to test this hypothesis directly, however, our data do suggest that terrestrial behavior contributes significantly to tick infestation rates as both males and females that engage in more terrestrial behaviors have significantly higher infestation rates than those that spend more times in the trees. More research on grooming behavior in *M. griseorufus* is necessary to verify this.

Table 1
Frequency of successful ground captures of four species of small mammals by season.

Species	Rainy season	Dry season	Total
M. griseorufus	0	191	191
R. rattus	35	44	79
M. musculus	29	36	65
E. telfairi	42	4	46
Total	106	275	381

Higher ectoparasitism at Parcel 1 could be explained by thicker ground cover at this site compared to Parcel 2 or Ihazoara, and why within Parcel 1, areas with thicker ground cover yielded more infested animals than areas with thinner understory. Additionally, the ground at Parcel 1 is moist due to the proximity to the riverbed and because trees block the sun's rays from penetrating to the forest floor. Ticks require moisture for survival during off-host periods. A moist, hydrating microhabitat during the dry winter may aid in tick water vapor uptake or may help prevent water loss during the different stages of tick development.

The presence of rats may also contribute to greater ectoparasitism at Parcel 1 than at other sites. Rats occur in forests near human settlements and Parcel 1 borders a research camp (Youssouf Jacky and Rasoazanabary, 2008). Systematic trapping has demonstrated that rat populations are much higher at Parcel 1 than at Parcel 2 or Ihazoara (Youssouf Jacky and Rasoazanabary, 2008). These rats carry ticks, although the species has not yet been identified. Rodents host approximately half of all ixodid tick species (Hoogstraal and Kim, 1985), and larval and nymphal stages have a wider host repertoire than adult stages. *Haemaphysalis simplex*, which has more relaxed specificity than *H. lemuris*, is known to use rats as maintenance hosts (Hoogstraal and Wassef, 1973; Uilenberg et al., 1979). Tenrecs hibernate during the dry season, when both *Microcebus* and rats exhibit peak infestation rates. Rats, and to some extent mouse lemurs, may help maintain the *H.* sp. cf. *simplex* tick population at BMSR during the dry season. Rats may also provide an avenue for transmission of immature ticks between tenrecs (or other animals at the reserve) and mouse lemurs. Studies of tick ecology of the other mammals at BMSR, and identification of their tick species, would elucidate this question.

The higher level of ectoparasitism at Parcel 1 may also be a consequence of the higher population density of larger-bodied lemurs (*P. verreauxi* and *Lemur catta*) at Parcel 1 (Axel and Maurer, 2011; Rasoazanabary, 2011). Larger-bodied hosts can harbor immature and adult stage ticks. However, adult ticks quest higher in the vegetation, require more blood than do immature ticks, and generally feed on larger host species. Because they are small in body size, *Microcebus* are competent hosts to larval and nymphal ticks, but not to adult ticks. At the Berenty Reserve in eastern Madagacar, 98.3% of ticks collected from *L. catta* during the early part of the wet season were adult-stage *H. lemuris* (Takahata et al., 1998). The presence of larger-bodied hosts may be critical for completion of the life cycle of *H. lemuris*. Parcel 1 provides hosts such as *L. catta* and *P. verreauxi* for the reproductive stages of adult ticks, while individuals belonging to these species of lemur are less abundant in Parcel 2.

Fig. 3 presents our model of the life cycle of *Haemaphysalis lemuris*. As with other haemaphysaline species, *H. lemuris* depends on multiple hosts to complete its life cycle. Because *H. lemuris* is generally associated with lemurs (Hoogstraal and Theiler, 1959), immature stages likely feed on *Microcebus* and adult stages on *Lemur* and *Propithecus* (tick infestation data on the other species of lemur at BMSR, *Lepilemur*, is unknown). However, in the wet

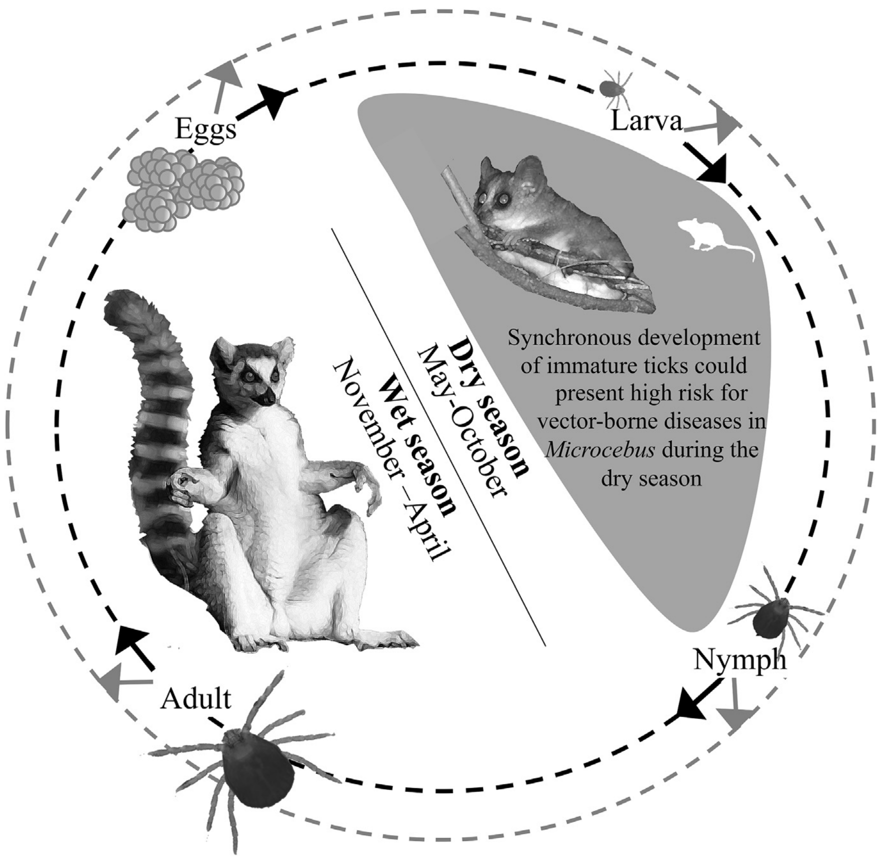

Fig. 3. Possible life cycle of *H. lemuris*. Peak activity for larvae occurs in May, but larvae may be found feeding into June and October. Larvae attach to *Microcebus* hosts and after a blood meal, fall off and molt into nymphs. Nymphs are active and feed on *Microcebus* throughout the dry season and likely feed on other lemurs during part of the wet season. Adult-stage ticks remain active during the wet season, feeding on larger-bodied lemurs, such as *L. catta*, and *P. verreauxi*. Engorged females fall off and lay eggs in leaf litter. It is possible that all four stages can diapause if no suitable hosts or conditions are found (gray dotted line). Mice or rats may also serve as hosts to larvae during the dry season.

season, mouse lemurs do not spend significant amounts of time on the ground and thus have reduced contact with questing ticks. During this season, *H. lemuris* may utilize non-lemur hosts that serve as good maintenance hosts, including rats and mice. Alternately, *H. lemuris* may go into diapause. It is well established that species of haemaphysaline ticks enter diapause under certain environmental conditions (e.g., changing day length, dropping temperature). We do not yet know whether *H. lemuris* enters diapause at any life-history stage. It is plausible that at BMSR, where the climate consists of wet, hot summers and dry, cold winters, or where competent hosts for each life stage may not be available year-round, *H. lemuris* may diapause and have a life-cycle that spans multiple years (Fig. 3; gray arrows).

The mammalian community is very different at BMSR than it was even 1000 years ago. Many endemic large- and small-bodied mammals in the region are now locally extirpated or extinct; these include eight species of giant extinct lemurs. A fossil site, Taolambiby, located only a few kilometers away from the reserve, documents changes in the mammalian community since humans began arriving in the region slightly over 2000 years ago (Burney et al., 2004; Perez et al., 2005; Crowley et al., 2011). Humans introduced mammals from other parts of the world both purposefully and inadvertently. The latter include rats (*R. rattus*) and mice (*M. musculus*), which are replacing endemic nesomyid rodents (e.g., *Eliurus myoxinus*, *Macrotarsomys bastardi*) in parts of Madagascar. Surviving endemic species may be experiencing dramatic population declines and ticks may be establishing new hosts. Interestingly, ixodid ticks that feed on tenrecs also feed on introduced *R. rattus*, but rarely on endemic Malagasy rodents (Hoogstraal and Aeschlimann, 1982).

Finally, most transmissions of microparasites by ticks occur in two stages: first, the ticks acquire the pathogen. Second, after molting, the ticks transmit the pathogen. Haemaphysaline ticks are known to transmit zoonotic agents such as *Borrelia* spp., *Ehrlichia*, *Anaplasma* and *Theileria* (Kim et al., 2003; Lee et al., 2005; Garcia-Sanmartin et al., 2008; Sun et al., 2008) and both *H. lemuris* and *H. simplex* are potential vectors for the piroplasm *Babesia* (Uilenberg et al., 1979). The synchronous development and co-feeding of larvae and nymphs on the same individuals increases the risk of transmission of microparasites in mouse lemur communities, especially during the dry season. In addition, ticks such as *H. simplex* that are less discriminating in host selection could place lemurs and other small mammals at the reserve at increased risk for inter-species transmission of vector-borne parasites. Research, such as presented here, highlights the importance of studying mixed-species communities in order to effectively understand ecological interactions of parasites and their hosts. It also provides a basis for future studies on the biology and vector potential of *Haemaphysalis* spp. that infest lemurs.

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