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Ectoparasitism in Black-and-White Ruffed Lemurs (*Varecia variegata*) in Southeastern Madagascar

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ABSTRACT: We documented ectoparasites found on wild black-and-white ruffed lemurs (*Varecia variegata*) in the southeastern rain forests of Madagascar and describe trends in parasitism. In this study, 235 mesostigmatid mites (1 male, 87 females, 147 nymphs) identified as *Liponyssella* sp., in the acarine family Macronyssidae, were collected during 87% (34/39) of lemur examinations (mean number/host=7.9). The only other ectoparasite collected was the louse fly (*Allobosca crassipes*; 3 males, 8 females) in the dipteran family Hippoboscidae, which was collected during 26% (10/39) of lemur examinations (mean number/host=1.1). The lemur most heavily parasitized by mites was an adult female with 29 adult females and 17 nymphs, all collected from the face.

Key words: Louse fly, mite, primate, scanning electron microscopy, SEM.

Madagascar is home to more than 100 lemur species that occupy all of the island's ecotones. Despite being the most threatened group of mammals on earth (Schwitzer et al. 2014), very little is known about the ecology of these nonhuman primates. In particular, very little is known about the ectoparasites of lemurs. Studies of lemur ectoparasites are therefore necessary to better understand their ecology before their host populations drastically decline or disappear. Ectoparasites are a significant group of the Malagasy fauna, and their evolutionary adaptations have the potential to affect the health and well being of their hosts. Because lemurs provide an ecosystem for their ectoparasites, a thorough understanding of lemur ecology should include an understanding of lemur-ectoparasite associations.

Previously, the tick *Haemaphysalis lemuris* and the hippoboscid louse fly *Allobosca crassipes* have been reported from the black-and-white ruffed lemur (*Varecia variegata*) in Madagascar (Junge and Louis 2002, 2005; Vaughn and McGee 2009). Ectoparasites were collected from these lemurs by one of us (A.L.B.) as part of a larger study of black-and-white ruffed lemur molecular ecology that involved live-captures of this arboreal primate (Baden et al. 2008, 2014).

Sampling was conducted from 2006 to 2008 (Tables 1, 2) in Mangevo (21°22′60″S, 47°28′0″E), a bush camp located in undisturbed, midaltitude primary rainforest in Ranomafana National Park, southeastern Madagascar. Adult and subadult black-and-white ruffed lemurs belonging to two social communities were captured by remote injection (Glander 1993). Experienced Malagasy technicians performed all captures, and a veterinarian was always present. Location at the time of capture was recorded with a handheld global positioning system unit before transporting animals back to camp where complete workups were performed.

Lemurs were searched for ectoparasites using forceps and a flea comb. The time it took to examine a lemur varied, but never exceeded 15 min. Collected parasites were preserved in 90% ethanol and stored at ambient temperature until they could be brought to the US. Samples were stored in the Hunter College Primate Molecular Ecology Laboratory (New York, New York, USA) at ambient temperature until they could be sent to Auburn University and Georgia Southern University for analyses.

During lemur processing, we monitored heart rate, respiratory rate, and body temper-

TABLE 1. Thirty-nine black-and-white ruffed lemurs (*Varecia variegata editorum*) were sampled during three capture seasons between 2006 and 2008 in Ranomafana, Madagascar, for ectoparasites. Two ectoparasite species were found on the lemurs (*Allobosca crassipes* and *Liponyssella* sp.) with the number of parasites ranging from 0 to 46 on each individual lemur at the time of capture. NA indicates not applicable.

Year	Collection period	Season	No.	Ectoparasites per individual	
				Min	Max
2006	11–16 August	Cold, wet	18	0	14
2007	23–25 August	Cold, wet	13	0	46
2008	29 March–2 April	Warm, wet	8	3	8
Totals			39	NA	NA

TABLE 2. Between 2006 and 2008, six black-and-white ruffed lemurs (*Varecia variegata editorum*), all adults, were captured during more than one season and sampled for ectoparasites in Ranomafana, Madagascar. Details of sex and intensity of ectoparasite infestation each year are provided, and years in which individuals were not captured (NC) are noted.

Individual ID	Sex	No. of ectoparasites collected		
		2006	2007	2008
RANO5.5	Female	14	1	NC
RANO5.8	Female	1	1	NC
RANO5.10	Female	0	NC	5
RANO6.20	Female	5	0	NC
RANO6.23	Male	13	4	3
BADEN7.3	Male	NC	8	8

ature of the study subjects and administered a balanced electrolyte solution (Lactate Ringer Solution, Aspen Veterinary Resources LTP, Liberty, Missouri, USA) subcutaneously. Before release, captured lemurs were given AVID® microchips (Avid Identifications Systems Inc., Norco, California, USA) and fitted with unique collar-tag combinations to catalog individuals for identification. Animals recovered in breathable cloth bags (at least 3 h) and were released at the site of capture.

Protocols were approved by Stony Brook University (SBU) and Henry Doorly Zoo Institutional Animal Care and Use Committee (IACUC) standards (SBU IACUC 2005-20081449), and research permits were granted by the Tripartite Committee of the Malagasy government (Association Nationale pour la Gestion des Aires Protégées), as well as by the US Fish and Wildlife Service. No injuries or deaths resulted from sampling procedures.

Thirty-nine examinations for ectoparasites were made on 31 individual lemurs (16 males, 15 females) during three capture seasons (Table 1); six lemurs were resampled in two or three sampling years (Table 2). Two species of ectoparasites but no ticks were recorded. Eleven adult specimens of *A. crassipes* (Fig. 1) were collected during 26% (10/39) of lemur captures, with a mean intensity (mean per infested host) of 1.1 flies per lemur. Of the 11 parasites, three were males and the remaining eight were females. Only one lemur was parasitized by more than one fly during the same examination. The hindlimbs and groin of black-and-white ruffed lemurs were a frequent host site for *A. crassipes*.

Two hundred and thirty-five *Liponyssella* mites (one male, 87 females, 147 nymphs) were collected, mostly from the head of lemurs, specifically the chin, mouth, neck, and around the eyes. A total of 87% (34/39) of lemur examinations yielded mites for a mean intensity of 7.9 mites per lemur.

One of the two species of ectoparasites we recorded from black-and-white ruffed lemurs, the louse fly, has been recorded previously from this host and from other species of lemurs (Rahola et al. 2011). The other ectoparasite is a new species of mite belonging to the macronyssid genus *Liponyssella* and will be described separately. In contrast to Junge and Louis (2002, 2005), we did not record *H. lemuris* or any other species of ticks from black-and-white ruffed lemurs in this survey.

Fourteen specimens (five males, nine females) of *A. crassipes* were recorded from six black-and-white ruffed lemurs in Ranomafana National Park (Vaughn and McGee 2009), which represents a mean intensity of 2.3

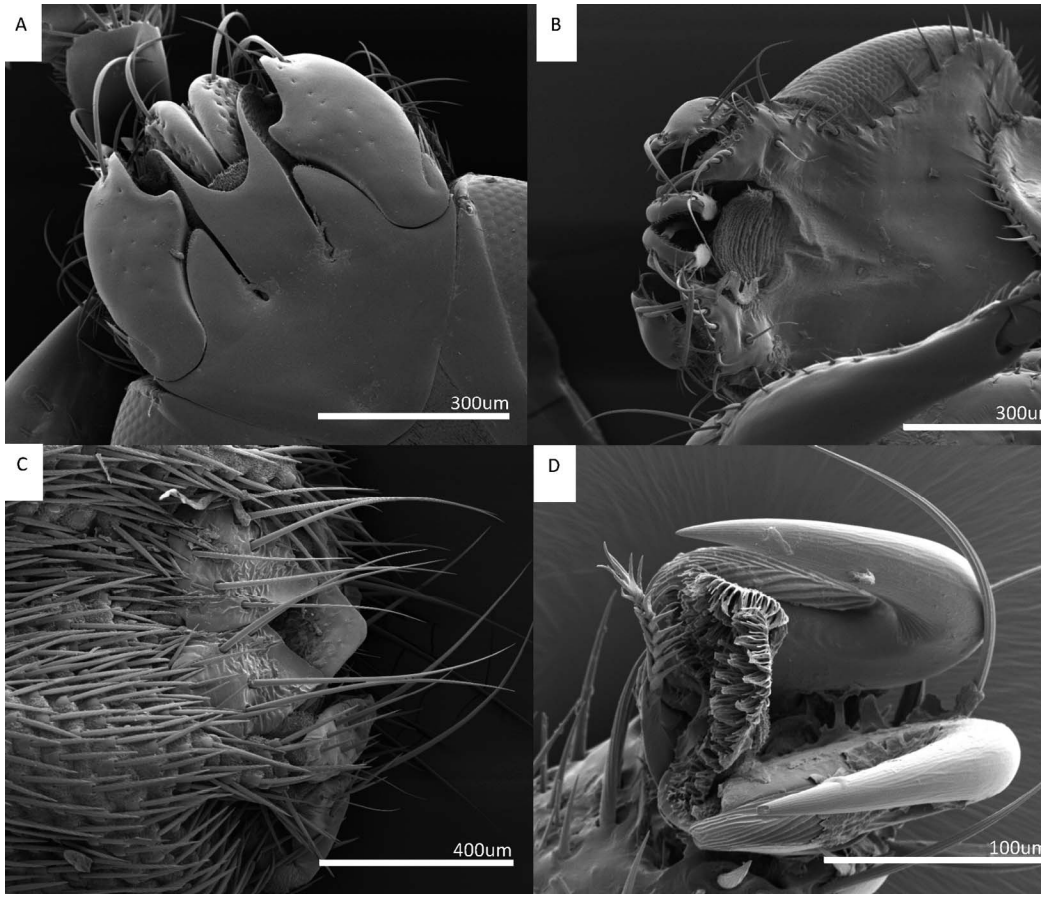

FIGURE 1. Scanning electron microscopy imagery of a female louse fly (*Allobosca crassipes*) collected from a wild black-and-white ruffed lemur (*Varecia variegata*) in Ranomafana, Madagascar, highlights characters used to identify the species, including (A) mouthparts—ventral view, (B) mouthparts—dorsal view, (C) posterior abdomen—ventral view showing bilobed terminex, and (D) front tarsi showing short setae.

compared with 1.1 in our study. Vaughn and McGee (2009) also collected 87 *A. crassipes* (49 males, 38 females) from 23 Milne-Edwards' sifakas (*Propithecus edwardsi*) in the same habitat, a mean intensity of 3.8, suggesting that Milne-Edwards' sifakas may be preferred over black-and-white ruffed lemurs as a host by this ectoparasitic fly. Milne-Edwards' sifakas and black-and-white ruffed lemurs are arboreal lemur species that co-occur in the eastern rainforests of Madagascar, with the former having a slightly larger body mass (5–6.5 kg; King et al. 2012) than the latter (2.6–4.1 kg; Baden et al. 2008), which may influence odorant and carbon dioxide attractants to *A. crassipes* adults. *Allobosca crassipes* has also been recorded from additional lemur species (Maa 1963, 1969; Rahola et al. 2011), which suggests relatively low host specificity by *A. crassipes*. However, all known hosts of this fly are relatively large lemurs (2–8 kg). Perhaps smaller lemurs do not tolerate these large ectoparasites or are more efficient groomers. Future collections of ectoparasites from lemurs should provide additional data on the host specificity of *A. crassipes*. One additional species of Hippoboscidae, *Proparabosca alata,* is known to parasitize lemurs, but it has only been recorded from *Propithecus verreauxi coronatus* (Theodor and Oldroyd 1965; Maa 1969; Rahola et al. 2011).

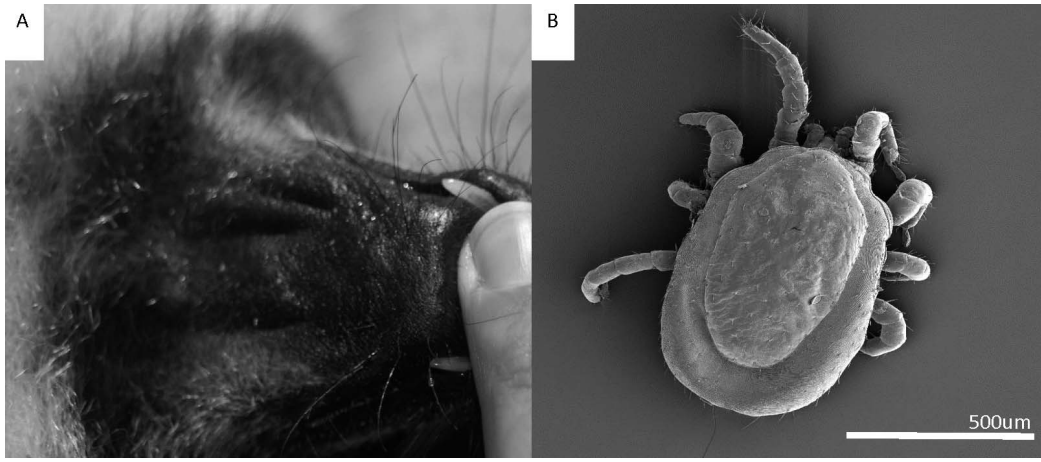

FIGURE 2. *Liponyssella* mites collected from black-and-white ruffed lemurs (*Varecia variegata*) in Ranomafana, Madagascar, represent a new species. These mites are shown around the mouth of a female lemur (A), and a close-up dorsal scanning electron microscopy image (B) depicts a male mite sampled as part of this study.

The new species of *Liponyssella* we recorded could be host-specific to black-and-white ruffed lemurs, because it has not previously been documented from other species. However, the only other congeneric mite species currently known from Madagascar, *Liponyssella madagascariensis*, has been recorded from multiple species of large lemurs (OConnor 2003; Klompen et al. 2015), suggesting this may not be the case. Unlike adult *A. crassipes*, which were large and easily detected, the *Liponyssella* mites were very small, moved quickly, and were difficult to sample. The inner inguinal region and mouth area (Fig. 2) were the most heavily parasitized regions of black-and-white ruffed lemurs by this mite, perhaps because of sparse hair, less efficient host grooming, or the presence of an abundant peripheral blood supply in those regions.

The genus *Varecia* is notable among lemurs for its flexible fission-fusion social dynamics (Baden et al. 2016), as well as its tendency to crèche litters of young communally (e.g., Morland 1990; Baden et al. 2013). Members of social communities exhibit variable affiliation, and inter-community interactions are limited to infrequent territorial disputes (e.g., Morland 1991; Baden 2011). Moreover, the species is known for its strict seasonal reproduction, with mating in July, and giving birth about 90–110 d later (e.g., Boskoff 1977; Rasmussen 1985). As ectoparasite levels are known to vary in accordance with the strength of social interactions (MacIntosh et al. 2012), patterns of nest sharing (Stamp et al. 2002), and hormones (Zohdy et al. 2017), future studies should consider whether and how this potentially host-specific parasite varies in presence and prevalence across these and other variables through space and time.

Allobosca crassipes is not host-specific to black-and-white ruffed lemurs, and both ectoparasites we recorded include free-living stages during their life cycles, emphasizing the importance of interactions between black-and-white ruffed lemurs and the environment for ectoparasite transmission. It is not currently known whether the louse flies and mites are vectors of any pathogens or parasites.

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