Lice and Ticks of the Eastern Rufous Mouse Lemur, *Microcebus rufus*, with Descriptions of the Male and Third Instar Nymph of *Lemurpediculus verruculosus* (Phthiraptera: Anoplura)

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LICE AND TICKS OF THE EASTERN RUFOUS MOUSE LEMUR, *MICROCEBUS RUFUS*, WITH DESCRIPTIONS OF THE MALE AND THIRD INSTAR NYMPH OF *LEMURPEDICULUS VERRUCULOSUS* (PHTHIRAPTERA: ANOPLURA)

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ABSTRACT: Sucking lice and ticks were collected from live-trapped eastern rufous mouse lemurs, *Microcebus rufus* Geoffroy, in and around the periphery of Ranomafana National Park, southeastern Madagascar, from 2007 to 2009. Samples of 53 sucking lice (Insecta: Phthiraptera: Anoplura) and 28 hard ticks (Acari: Ixodidae) were collected from 36 lemur captures representing 26 different host individuals. All of the lice were *Lemurpediculus verruculosus* (Ward) (6 males, 46 females, 1 third instar nymph). Only the holotype female was known previously for this louse and the host was stated to be a ''mouse lemur.'' Therefore, we describe the male and third instar nymph of *L. verruculosus* and confirm *M. rufus* as a host (possibly the only host) of this louse. All of the ticks were nymphs and consisted of 16 *Haemaphysalis lemuris* Hoogstraal, 11 *Haemaphysalis* sp., and 1 *Ixodes* sp. The last 2 ticks listed did not morphologically match any of the Madagascar *Haemaphysalis* or *Ixodes* ticks for which nymphal stages have been described.

Little is known about the ectoparasites of lemurs in Madagascar, and it seems that nothing has been published specifically on ectoparasites of the eastern rufous mouse lemur, *Microcebus rufus* Geoffroy, a denizen of rain forests in parts of eastern and northern Madagascar (Groves, 2005). Ward (1951) described the sucking louse, *Lemurphthirus verruculosus* (Ward), from a ''mouse lemur'' based on a single female louse specimen recovered in 1948 during one of Harry Hoogstraal's collecting expeditions, and it is possible that the host was *M. rufus*. Later, Paulian (1958) reassigned this louse to a new genus, *Lemurpediculus*. Three species of ticks, *Haemaphysalis lemuris* Hoogstraal, *Haemaphysalis simplex* Neumann, and *Ixodes lemuris* Arthur, have been recorded from lemurs in Madagascar (Uilenberg et al., 1979; Hoogstraal and Kim, 1985), but none of these ticks has been recorded from *M. rufus*. An opportunity to collect lice and ticks from *M. rufus* arose in connection with fieldwork one of us (S.Z.) has undertaken in Madagascar that involved live-trapping and recapturing individuals of this small primate.

MATERIALS AND METHODS

Lemur identification in this article follows Groves (2005). Eastern rufous mouse lemurs were captured at three study sites in or around Ranomafana National Park (RNP) (47°18′–47°37′E, 21°02′–21°25′S), in southeastern Madagascar during 3 field seasons; the first from October to December 2007, the second from September to December 2008, and the third from August to November 2009. Ectoparasite records are from the 2007 and 2009 seasons. RNP includes 43,500 ha of continuous rain forest from lowland to montane regions (Wright, 1992; Atsalis, 2000). The first study site is within the Talatakely Trail System, a section of RNP that was selectively logged in the mid-1980s and is now heavily visited by tourists (Wright and Andriamihaja, 2002). The second study site, Campsite, is immediately outside RNP, on the opposite side of the Namorona River from the Talatakely Trail System and was used previously for agricultural purposes but is currently in the process of regrowth. The third site, Ambatolahy Dimy, is 400 m away from, and parallel to, the Campsite transect. Thirty-four Sherman traps (XLR, Sherman Traps Inc., Tallahassee, Florida) were set in pairs at each site along selected trails at approximately 20–50-m intervals. To maximize trapping effort, traps were set on consecutive nights during the study period. Traps were baited with fresh banana and set at 1600 hr; they were then checked a few hours later, between 1930 and 2000 hr. Non-primate captures were identified and released onsite. Captured eastern rufous mouse lemurs were taken to the Centre ValBio Research Station, where they were handled by a trained research technician, individually scanned for a microchip (AVID Powertracker VI, South Chailey, U.K.), sexed, and weighed. In addition, the ears were examined for ectoparasites. Specimens were collected using sterile forceps and stored in 90% ethanol for preservation. Lemurs were returned to the forest and released at the site of capture immediately after data collection to minimize disturbance. Ectoparasite specimens were later identified or described as detailed below. Field research was completed under permits 110/09 MEFT/SG/DGEF/DSAP/SLRSE and 215/08 MEFT/SG/DGEF/DSAP/SSE, project ID 2009-1608.

Selected sucking louse specimens (2 males, 3 females, 1 third instar nymph) were cleared in 10% potassium hydroxide, dehydrated in an ethanol series of ascending concentrations (up to 100%), further cleared in xylene, slide-mounted in Canada balsam, and dried in a slide oven. Slide-mounted louse specimens were examined at high power (×100–600) by using a phase contrast Olympus BH-2 compound binocular microscope, measured using a calibrated eyepiece graticule, and then drawn and recorded following standard techniques (Durden and Rausch, 2007). Measurements are in millimeters unless stated otherwise. Ticks were air-dried for a few minutes and then identified using a low-power (×10–60) binocular microscope and consultation of appropriate literature sources (Hoogstraal, 1953; Arthur, 1958; Uilenberg et al., 1979).

RESULTS

In total, 53 *L. verruculosus* (6 males, 46 females, 1 third instar nymph) and 28 nymphal ticks (16 *H. lemuris*, 11 *Haemaphysalis* sp., 1 *Ixodes* sp.) were collected during 36 eastern rufous mouse lemur captures, representing 26 different host individuals.

In 2007, 29 specimens of *L. verruculosus* (4 males, 24 females, 1 third instar nymph) were collected from 5 individual lemurs (all males), giving a mean intensity (mean per infested host) of 5.8, although total infestations were probably much higher (Fig. 5). In 2009, 24 specimens of *L. verruculosus* (2 males, 22 females) were collected from 14 lemur captures (10 males, 1 female) representing 11 individual lemurs for a mean intensity of 1.7 lice per infested lemur capture. The external ears of *M. rufus* were a frequent host attachment site for *L. verruculosus* (Fig. 5).

In 2007, 17 ticks were collected during 9 lemur captures, representing 8 lemur individuals (5 males, 3 females). Fourteen *H. lemuris* nymphs were recovered from 6 lemurs (3 males, 3 females), giving a mean intensity of 2.3 for this tick. Two additional *Haemaphysalis* sp. nymphs were collected (both belonging to the same species), and an additional *Ixodes* sp.

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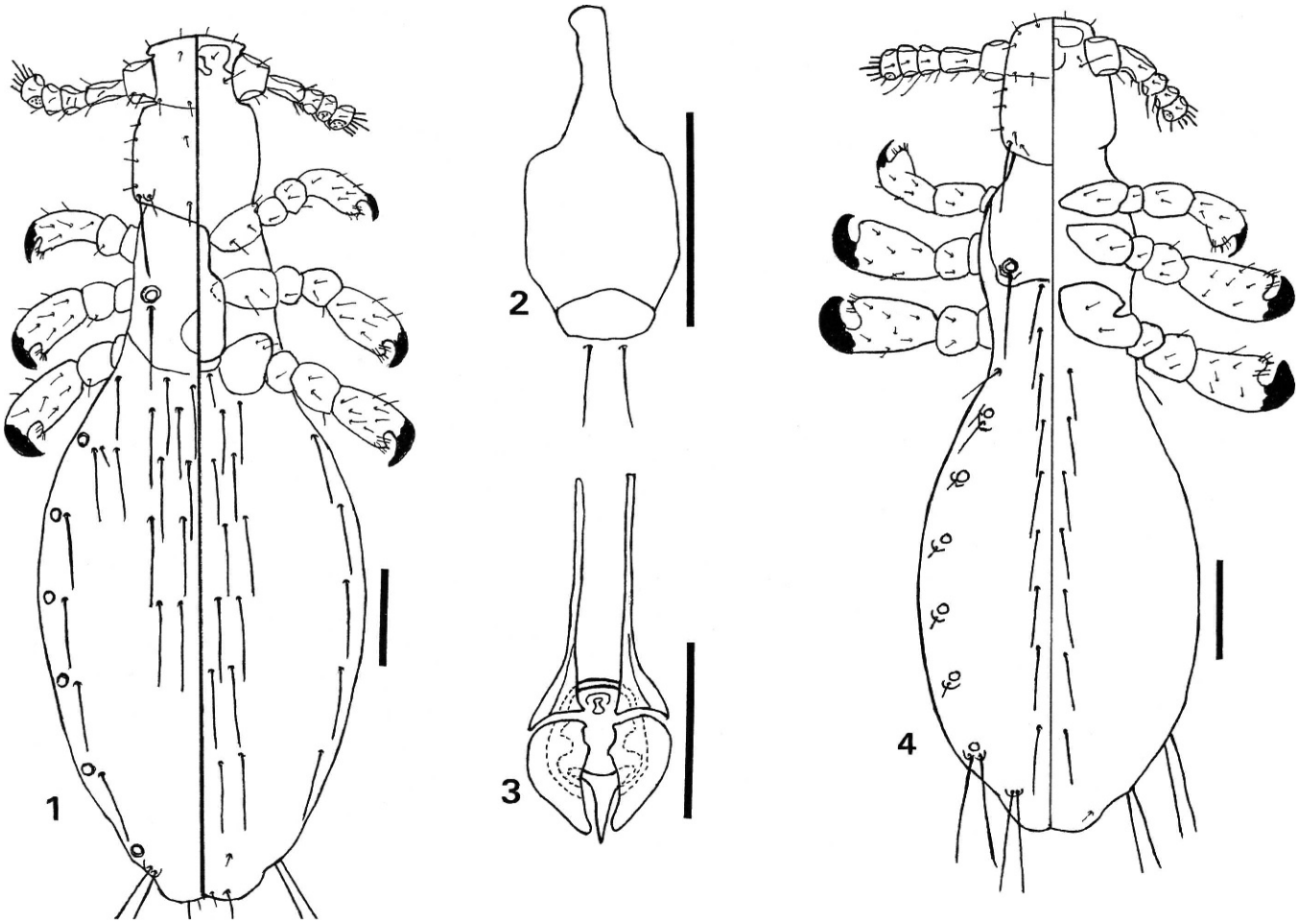

FIGURES 1–4. (**1**) Dorsoventral view of adult male *Lemurpediculus verruculosus*, depicting ventral features to the right of the midline and dorsal features to the left. (**2**) Thoracic sternal plate of adult male *L. verruculosus*. (**3**) Genitalia of adult male *L. verruculosus*. (**4**) Dorsoventral view of third instar nymph of *L. verruculosus*. All scale bars = 0.1 mm.

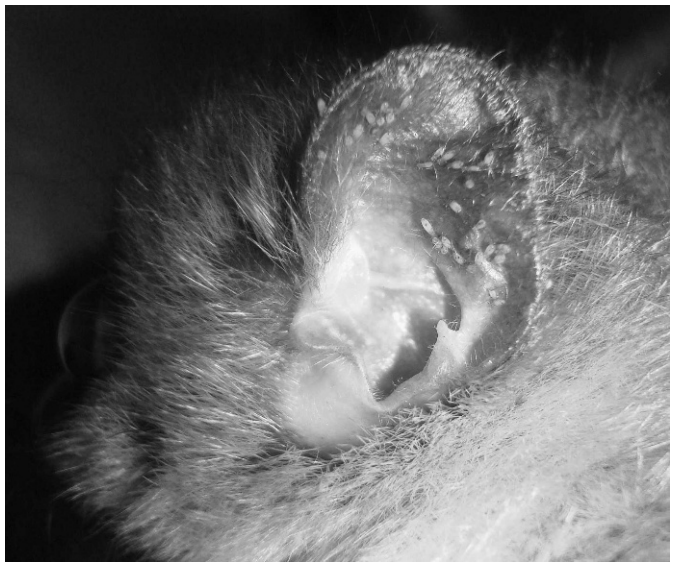

FIGURE 5. Cluster of *Lemurpediculus verruculosus* on an outer ear pinna of a live *Microcebus rufus*. The characteristic body shape of *Lemurpediculus* and the presence of digesting host blood (visible as dark spots near the middle of the abdomen) can be distinguished in some specimens.

nymph did not match any published nymphal tick descriptions for Madagascar. In 2009, 2 *H. lemuris* nymphs were collected from 2 individual lemurs (both males) and 9 *Haemaphysalis* sp. (all belonging to the same species collected in 2007) were collected from 6 lemur captures (5 males, 1 female), representing 5 lemurs. The overall mean intensity for ticks in 2009 was 1.8.

DESCRIPTION

Lemurpediculus verruculosus (Ward, 1951)

(Figs. 1–4)

Male (Figs. 1–3): Total body length of illustrated specimen (slide-mounted), 0.945; mean, 0.948; range, 0.945–0.950 (n = 2). Maximum head width of illustrated specimen, 0.14; mean, 0.15, range, 0.14–0.16 (n = 2). Head well sclerotized anteriorly with broadly curved anterior margin and, mostly ventral, curved band of tanned exoskeleton; band notched antero-laterally on each side to connect with anterio-medial margin of first antennal segment. Lateral margins of head gently convex, but almost parallel. Antennae 5-segmented with first segment much larger than other segments and slightly broader than long; second segment narrow and elongate; segments 3–5 each about as long as wide. Two sutural head setae (SHS), 4 dorsal marginal head setae (DMHS), 2 apical head setae (ApHS), 1 dorsal anterior head seta (DAnHS), 1 dorsal anterior central head seta (DAnCHS), 1 dorsal posterior central head seta (DPoCHS), 1 dorsal principal head seta (DPTS), 1 dorsal accessory head seta (DAcHS), 1

ventral principal head seta (VPHS), and 1 ventral preantennal head seta (VPaHS) on each side; DAcHS medial to DPTS and borne on small protuberance.

Thorax barely wider than head and then only in posterior region of thorax. Thoracic sternal plate (Fig. 2) shield-shaped, but with elongate anterior projection and more heavily sclerotized distinct posterior region; 2 setae of intermediate length immediately posterior to thoracic sternal plate. Mesothoracic spiracle diameter, 0.024. Dorsal principal thoracic seta (DPTS) 0.11 mm in length; no dorsal mesothoracic setae (DMsS) present. Small subcircular dorsal plate present posteriorly. Forelegs smaller than mid and hindlegs, which are approximately equal in size, each leg terminating in an acuminate claw; leg coxae widely separated; coxae I and II subtriangular; coxa I with small antero-lateral extension bearing small seta.

Abdomen wider than thorax, with broadly rounded lateral margins and 6 spiracles on each side; paratergal plates absent; 1 dorsal lateral abdominal seta (DLAS) inserted anterior to spiracle I on each side, 3 DLAS inserted postero-medial to spiracle I on each side (consisting of 2 long outer setae and 1 short seta between them); 1 long DLAS inserted medial to spiracles II–V; small lateral protuberance bearing 2 long outwardly directed DLAS posterior to spiracle VI. Five anterior rows of 4 long dorsal central abdominal setae (DCAS) ending at approximately the level of spiracle 3; 5 rows of 2 long ventral lateral abdominal seta (VLAS); 6 rows of 4 long ventral central abdominal setae (VCAS), followed by 1 row of 2 tiny VCAS, and then by 4 terminal setae (2 fairly long, curved outer setae and 2 small setae between them).

Genitalia (Fig. 3) with basal apodeme approximately 2.5 times length of parameres; basal apodeme flared posteriorly and differentially sclerotized; parameres broad with wide anterior margins, convex lateral margins, notched antero-medial margins and broadly acuminate posterior apex; subeliptical endomere present with medial extension on each side; pseudopenis barely extending beyond posterior margins of parameres.

Female: No differences noted from the original description by Ward (1951) who reported the total length of the holotype female to be 1.25. The 3 slide-mounted female specimens from this study measured 1.11, 1.24, and 1.28 (mean, 1.21).

Third instar nymph (Fig. 4): Total body length (slide-mounted), 0.870. Head, thorax, and abdomen as in male unless stated otherwise. Maximum head width, 0.125. Head with broadly rounded anterior margin and lacking lateral notch anterior to antennae. Two SHS, 3 DMHS, 2 ApHS, 1 DPTS, 2 DAcHS, 1 VPHS, 1 VPaHS, and 1 supraantennal head seta (SpAtHS) on each side, with the 2 DAcHS medial and slightly anterior, respectively, to DPTS.

Thorax slightly wider than head, with gently convex lateral margins. Thoracic sternal plate lacking, but broad dorsal plate present and bearing mesothoracic spiracle (0.024 in diameter) and DPTS (0.093 long) on each side. All leg coxae subtriangular, but coxa I lacking anterior protuberance and coxa III with antero-lateral notch.

Abdomen with 9 rows of 2 DCAS; 1 fairly long DLAS anterior to spiracle I on each side; other DLAS all borne on small protuberances, 6 of which are immediately postero-lateral to spiracles on each side; spiracle I with 2 associated DLAS on each side (1 short, 1 of intermediate length); spiracles II–V each with 1 associated short DLAS on each side; spiracle VI with 2 associated long DLAS on each side followed by 2 equally long DLAS borne on a small protuberance, but not associated with spiracle. Seven rows of 2 long VCAS, followed by 1 small subterminal seta on each side.

Taxonomic summary

Host: Microcebus rufus Geoffroy (eastern rufous mouse lemur), males, ID nos. 081871020 (for the 2 male lice), 081869865 (for the nymphal louse), and 071810305 (for the 3 female lice); all lice removed from external ears of hosts.

Locality: All hosts were live-trapped in Madagascar: Ranomafana National Park (21.2567°S, 47.4218°E) by S. Zohdy. Host 081871020 was captured on 3 October 2007 at Ambatolahy Dimy and hosts 081869865 and 071810305 were both live-trapped at nearby Campsite on 29 October 2007 and 5 November 2007, respectively.

Specimens deposited: One male, 1 female, and the third instar nymph are deposited in the U.S. National Parasite Collection under accession numbers USNPC 102805, USNPC 102806, and USNPC 102807, respectively. The remaining specimens are in the collections of L.A.D. or S.Z.

Remarks

The lice described here undoubtedly represent the previously undescribed adult male and third instar nymph, respectively, of *L. verruculosus*. These stages were collected from the same host individuals that harbored females of *L. verruculosus*, which were compared with the illustrations of the female provided by Ward (1951). A unique morphological character, the sclerotized posterior section of the thoracic sternal plate, shared by the male and female, but unknown in other sucking lice (Ward, 1951), further attest to the conspecific nature of these lice. The general body shape, lack of paratergal plates, sternal and tergal abdominal plates, and wide separation of the leg coxae, also are shared by both sexes and the nymph. Both sexes also have a dorsal thoracic plate. Paulian (1958) reassigned *L. verruculosus* from *Lemurphthirus* to his new genus *Lemurpediculus* based mainly on these characters, which this species shares with both sexes of *Lemurpediculus petterorum* Paulian, the only other known representative of this unusual louse genus (Durden and Musser, 1994). However, *L. verruculosus* and *L. petterorum* also are extremely distinct morphologically. Although there are several morphological differences (compare illustrations of *L. petterorum* in Paulian [1958] with drawings of *L. verruculosus* presented in Ward [1951] and this study), the easiest way to separate both sexes of these 2 species is by examining features associated with the genitalia. For females, the subgenital plate of *L. verruculosus* is bulbous anteriorly with an anchor-shaped section posteriorly, whereas in *L. petterorum* this structure has 2 anterior lacunae and an undulating semicircular posterior margin. For males, the basal apodeme is approximately 2.5 times the length of the parameres in *L. verruculosus* (Fig. 3), but the parameres are slightly longer than the basal apodeme in *L. petterorum*. The sclerotized posterior section of the thoracic sternal plate and its anterior extension in both sexes of *L. verruculosus* also distinguish this species from *L. petterorum*, both sexes of which lack these characters (Paulian, 1958). These are only examples of some of the distinct character differences between these 2 species; there are many additional differences.

DISCUSSION

We have documented and described, for the first time, the previously unknown male and third instar nymph of *L. verruculosus*, a taxon known previously only by the holotype female. We also confirm *M. rufus* as a host for this louse. In the original description, Ward (1951) listed the host as a "mouse lemur." Because many species of sucking lice are host-specific (Durden and Musser, 1994), *M. rufus* could be the only host for this louse. We also show that the external ears of *M. rufus* are a prime host location for *L. verruculosus* (and for ticks). External ears are often preferred host attachment sites by ectoparasites of mammals, mainly because of their rich peripheral blood supply and sparse fur; in addition, the host cannot self-groom these sites with its teeth (Nilsson, 1981; Spears et al., 1999).

Because lemurs were not subjected to intense whole-body searches, the mean intensities recorded in this study should be considered as minimal mean intensities. As shown in Figure 5, infestations of *L. verruculosus* on individual lemurs can be quite large, especially on the ears.

Despite the relatively rich extant fauna of lemurs in Madagascar (Groves, 2005), only 5 species of sucking lice are currently known from this group of primates: *L. verruculosus* from *M. rufus*, *L. petterorum* from *Lepilemur mustelinus* Geoffroy (weasel lemur), *Phthirpediculus avahidis* Paulian from *Avahi laniger* (Gmelin) (eastern woolly lemur), *Phthirpediculus brygooi* Clay from *Eulemur mongoz* (L.) (mongoose lemur), and *Phthirpediculus*

propitheci Ewing from *Propithecus edwardsi* Grandidier (Milne-Edwards's sifaka) (Ewing, 1923; Ferris, 1932; Ward, 1951; Paulian, 1958, 1960; Clay, 1977; Durden and Musser, 1994). This suggests that additional species of sucking lice may await discovery on other species of lemurs.

Before this study, there were no published records of identified ticks from *M. rufus*. Perhaps, not surprisingly, all 16 of the nymphal ticks we could identify to species were *H. lemuris*. The higher proportion of nymphal ticks that were *H. lemuris* compared with *Haemaphysalis* sp. in 2007 (14 of 16) than in 2009 (2 of 11) could have resulted from small sample sizes or from seasonality. In 2007, ectoparasites were collected from 16 October through 19 November, whereas in 2009, collection dates ranged from 14 August to 11 October.

Haemaphysalis lemuris seems to be the most common tick associated with lemurs, and it has been recorded previously as an ectoparasite of *Lemur catta* L. (ring-tailed lemur), *Lepilemur ruficaudatus* Grandidier (red-tailed sportive lemur), *Lepilemur* sp., *Propithecus verreauxi* Grandidier (Verreaux's Sifaka), and *Varecia variegata* (Kerr) (black and white ruffed lemur) (Hoogstraal, 1953; Uilenberg et al., 1979). Uilenberg et al. (1979) stated that *H. lemuris* is a potential vector of both *Babesia cheirogalei* Uilenberg and *Babesia propitheci* Uilenberg, Blancou and Andrianjafy, 2 piroplasms known to parasitize lemurs.

The remaining 11 *Haemaphysalis* sp. nymphs that we recovered did not match any of the 6 *Haemaphysalis* species (of 13 known *Haemaphysalis* spp. for Madagascar) for which nymphs have been described from Madagascar. Excluding *H. lemuris*, the *Haemaphysalis* tick fauna of Madagascar is mainly associated with native tenrecs, rodents, and carnivores (Uilenberg et al., 1979; Hoogstraal and Kim, 1985).

The other tick species that seems to be associated with lemurs is *Ixodes lemuris*, which has been recorded from *Eulemur rufus* (Audebert) (red-fronted lemur) and *Rattus rattus* (L.) (black rat) (Arthur, 1958; Uilenberg et al., 1979); the latter host association is assumed to be accidental, although very few host records exist for *I. lemuris*. Nymphal stages have been described for 2 of the 7 known species of *Ixodes* from Madagascar, but our nymph matched neither of those species. Although the nymph of *I. lemuris* has not been described, the general body shape and that of the auriculae and coxal spurs in our nymph seem to correlate best with those same structures in the adult female of this species than with corresponding structures of any other adult *Ixodes* spp. from Madagascar. Therefore, we suspect our nymph is *I. lemuris*. If *I. lemuris* nymphs can be reared from identified *I. lemuris* females or if sequenced DNA from identified adults becomes available in the future, then it should be possible to confirm (or refute) the identity of this nymphal tick specimen as *I. lemuris*.

For many ixodid tick species, smaller (and earlier) life stages (larvae and nymphs) parasitize smaller hosts, whereas conspecific (larger) adults parasitize larger hosts (Durden, 2006). Because *M. rufus* is a small lemur, this could explain why we only collected nymphal ticks from this host. Perhaps adults of *H. lemuris* more commonly parasitize larger species of lemurs sharing the same general habitat as *M. rufus* and perhaps adults of the other 2 tick species we collected similarly parasitize larger mammals.

Clearly, much needs to be learned about the ectoparasites associated with lemurs, and future fieldwork in the remaining indigenous forests of Madagascar has the potential to produce a wealth of data on this topic.

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