The Fleas of Endemic and Introduced Small Mammals in Central Highland Forests of Madagascar: Faunistics, Species Diversity, and Absence of Host Specificity

STEVEN M. GOODMAN,[1,2,3] H. RICO RANDRENJARISON ANDRINIAINA,[4] VOAHANGY SOARIMALALA[2] AND JEAN-CLAUDE BEAUCOURNU[5]

ABSTRACT Data are presented on the flea species of the genera *Paractenopsyllus* (Ceratophyllidae, Leptopsyllinae) and *Synopsyllus* (Pulicidae, Xenopsyllinae) obtained from small mammals during two 2014 seasonal surveys at a montane humid forest site (Ambohitantely) in the Central Highlands of Madagascar. The mammal groups included the endemic family Tenrecidae (tenrecs) and subfamily Nesomyinae (rodents) and two introduced families Muridae (rodents) and Soricidae (shrews); no fleas were recovered from the latter family. The surveys were conducted at the end of the wet and dry seasons with 288 individual small mammals captured, including 12 endemic and four introduced species. These animals yielded 344 fleas, representing nine species endemic to Madagascar; no introduced species was collected. Some seasonal variation was found in the number of trapped small mammals, but no marked difference was found in species richness. For flea species represented by sufficient samples, no parasite–host specificity was found, and there is evidence for considerable lateral exchange in the local flea fauna between species of tenrecs and the two rodent families (endemic and introduced). The implications of these results are discussed with regards to small mammal species richness and community structure, as well as a possible mechanism for the maintenance of sylvatic cycles of bubonic plague in the montane forests of Madagascar.

KEY WORDS fleas, species richness, seasonality, Ambohitantely, small mammal

While considerable advances have been made in the past few decades on the taxonomy of fleas parasitizing native and introduced small mammals of Madagascar (Duchemin 2003a, b, 2004; Duchemin and Ratovonjato 2004; Hastriter and Dick 2009; Beaucournu and Goodman 2014), little is known about their ecology and levels of host specificity. Such information is important from a medical entomology perspective, as portions of Madagascar, particularly the Central Highlands, have reoccurring epidemics of bubonic plague in humans transmitted by fleas (Duplantier et al. 2005; Chanteau 2006; Andrianaivoarimanana et al. 2013), as well as providing insights into the evolutionary history of these ectoparasites and their mammalian hosts.

The native nonvolant small mammals of the island are placed in the endemic family Tenrecidae, composed of 32 different species of shrew-like or hedgehog-like animals that are part of the Afrotheria radiation (Springer et al. 1997; Stanhope et al. 1998) and the endemic rodent subfamily Nesomyinae, with 27 currently recognized species placed in the family Nesomyidae (Musser and Carleton 2005; Soarimalala and Goodman 2011). Within the Tenrecidae is the speciose genus *Microgale*, often referred to as shrew-tenrecs, with 23 species and, among the Nesomyinae, the genus *Eliurus* with 12 species (Soarimalala and Goodman 2011). These two mammalian groups are respectively monophyletic and each can be explained by a single colonization event of the island and subsequent adaptive radiations (Poux et al. 2008). In Malagasy humid forests, considerable small mammal species diversity occurs at the same site and many taxa live in near sympatry. For example, in the montane forest at Tsinjoarivo in the Central Highlands, 17 species of Tenrecidae, including 13 *Microgale*, have been documented in close proximity (Goodman et al. 2000).

A few small mammal species have been introduced to Madagascar and include three Muridae rodents, *Mus musculus*, *Rattus rattus*, and *R. norvegicus*; the second taxon has broadly invaded native forest formations and occurs in sympatry with tenrecs and nesomyine rodents (Soarimalala and Goodman 2011). The second introduced group includes two species of Soricidae shrews, *Suncus etruscus* and *S. murinus*, both of

[1] Field Museum of Natural History, 1400 South Lake Shore Drive, Chicago, IL 60605.
[2] Association Vahatra, BP 3972, Antananarivo 101, Madagascar.
[3] Corresponding author, e-mail: sgoodman@fieldmuseum.org.
[4] Département de Biologie Animale, Université d'Antananarivo, BP 906, Antananarivo 101, Madagascar.
[5] Laboratoire de Parasitologie et Zoologie appliquée, Faculté de Médecine, et Institut de Parasitologie de l'Ouest, 2 Ave., du Professeur Léon Bernard, F-35043 Rennes cedex, France.

which occur in urban, rural, and native forest habitats (Hutterer and Tranier 1990; Omar et al. 2011; Soarimalala and Goodman 2011). The island's endemic flea fauna is relatively rich, only a few introduced species are present (Duchemin et al. 2003), and new species to science continue to be described (Hastriter and Dick 2009; Beaucournu and Goodman 2014).

The notable level of simplicity of this system, with regard to the origin of the native small mammal fauna, two colonization events followed by extensive speciation, relatively high sympatric species diversity, and many taxa having broad geographical distributions, provides a natural experiment in testing patterns of specificity between ectoparasites and their small mammal hosts. These patterns overlaid on the fleas of introduced small mammals, specifically those that occur in sympatry with the native fauna, provide the means to contrast cospeciation between fleas and their hosts, as compared to lateral transfer across different small mammal families. Further, these patterns might provide insight into the circulation of bubonic plague, particularly what has been referred to as the "wild" or "sylvatic" cycle in natural forest areas of highland Madagascar (Chanteau 2006).

On the basis of flea collections from small mammal hosts made at a montane humid forest in the Central Highlands of Madagascar, during dry and wet season visits, we examine herein the following five aspects: 1) document the species of fleas occurring on the native and introduced small mammal fauna; 2) investigate the level of specificity of different flea species with regards to their small mammal hosts to examine possible parasite–host coevolution; 3) determine possible seasonal aspects of parasitism; 4) ascertain possible ectoparasite transfers between native and introduced small mammals; and 5) consider different life history traits of the island's small mammal community to explain some observed patterns. Aspects concerning the flea fauna of Ambohitantely, specifically taxonomic considerations associated with this collection, are presented elsewhere (Beaucournu et al. 2015).

Materials and Methods

Study Site. Small mammal surveys were conducted during two different visits in 2014 to the Ambohitantely Forest (Fig. 1). The inventoried site, composed of partially disturbed montane humid forest, was located at: Madagascar: ex-Province d'Antananarivo, Région d'Analamanga, Réserve Spéciale d'Ambohitantely, along trail to sentier botanique, 18° 11′45.5″ S, 47° 17′14.0″ E, 1,600 m. The site was surveyed at the end of the wet season from 31 March to 9 April 2014 and at the end of dry season from 15 to 23 October 2014. For further information on the fauna, flora, and ecology of the reserve see Ratsirarson and Goodman (2000) and Langrand (2003). All trapping was conducted in the montane forest, with the exception of the marsh line, which was placed in low-lying open grassland marsh with a small permanent stream and within 100 m of the natural forest where the other trapping devices were installed.

Small Mammal Collection. Different trap types were used to capture small mammals: 1) two styles of live traps, Sherman (LFA folding trap, H. B. Sherman Traps, Tallahassee, FL) and National (201 collapsible trap, Tomahawk Live Trap, Hazelhurst, WI), intended to capture Nesomyinae and Muridae rodents and to a lesser extent Tenrecidae; and 2) pit-fall buckets used to trap Tenrecidae and to a lesser extent rodents. This latter type of trap was composed of 11 plastic buckets, each with a capacity of 15 liter, placed 10 m apart, buried in the earth, so that the upper rim was flush with the ground surface, and with a 100-m-long 0.75-m plastic drift fence stapled to vertical posts and bisecting each bucket. For further information on these different trapping techniques, see Raxworthy and Nussbaum (1994) and Goodman and Carleton (1996).

A live trap in place for a 24-h period, from dawn to dawn, is considered one "trap night" and a pit-fall bucket in place during the same period is a referred to as "pit-fall trap night". Research involving live animals followed guidelines for the capture, handling, and care of mammals approved by the American Society of Mammalogists (Sikes et al. 2011). Research permits for the capture and collection of small mammals were issued by Direction du Système des Aires Protégées, Direction Générale de l'Environnement et des Forêts, and Madagascar National Parks (N° 077/14/MEEF/SG/DGF/DCB.SAP/SCB and 238/14/MEEF/SG/DGF/DCB.SAP/SCB) and following regulations of Malagasy national authorities. Captured mammals were identified based on the long-term experience of two of the coauthors (S.M.G. and V.S.), as well as the published literature (e.g., Carleton 2003; Jenkins 2003; Olson et al. 2004; Soarimalala and Goodman 2011). On one occasion during the March–April survey, too many small mammals were captured during the night that could be processed the following morning and, in this case, 8 of 25 *R. rattus* were not examined for their ectoparasites. However, this should not have biased any interpretation of the fleas occurring on these rodents, as in total 112 of the 122 *R. rattus* captured were inspected for fleas (see below). Small mammal voucher specimens were deposited in the Field Museum of Natural History (FMNH), Chicago, and the Université d'Antananarivo, Département de Biologie Animale (UADBA), Antananarivo. These collections have yet to be cataloged, and specimens cited herein are referred to by field numbers.

Flea Collection and Identification. Immediately after each small mammal specimen was dispatched, it was placed in a large and smooth-sided, light-colored plastic basin with high vertical sides, and the animals were rigorously swept with a stiff-haired brush. Detached fleas were readily visible in the basin; these were collected and placed in tubes with 90% ETOH with a label bearing the mammal specimen field number. Cloth bags used to transport individual live animals were carefully checked for remaining ectoparasites and, after each utilization and before being reused, were turned inside out, left for several hours in direct sunshine, and at least 500 m from trapping stations.
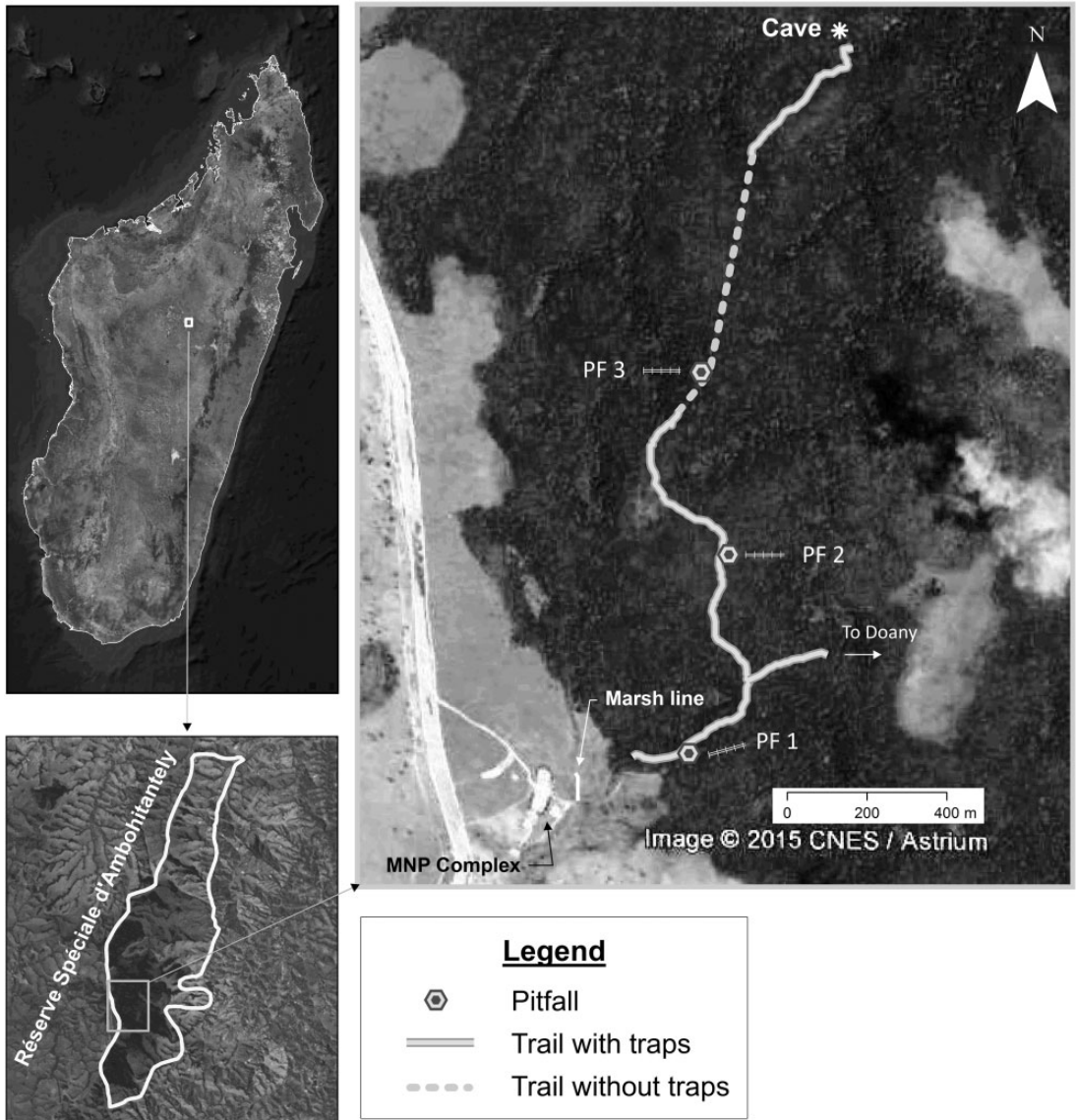
Fig. 1. Map showing the placement of the Réserve Spéciale d'Ambohitantely on Madagascar (upper left), the area of the reserve and zone of small mammal trapping (lower left), and the specific position of the different trapping devices (right). MNP = Madagascar National Parks.

Traps were also inspected for either dead or living fleas.

The complete flea collection was sent to the laboratory of J.C.B. at l'Université de Rennes, France. The fleas were first examined under a dissection scope to identify fragile or poorly sclerotized specimens and the phase of sexual maturity. During this step, hyperparasites (mites) or endoparasites (cysticercoids, microfilaria, tylenchids, hepatozoon cysts, etc.) were searched for, as such parasites would not withstand the procedure outlined below; in no case was a flea parasite found. The fleas were placed in tubes and cleared with a solution of 10% NaOH at ambient temperature for 12–24 h and then transferred to 70% ETOH, which

was refreshed twice. The fleas were then passed through 90% ETOH for 6–12 h, which was renewed one or two times. The next step was to place the fleas in clove oil at either 40 or 50°C; these relatively high temperatures help to accelerate impregnation of the insects. The final preparation steps included placing the fleas in toluene or xylol for a few minutes and then mounting them on slides in a small drop of Canada balsam. Under a binocular scope, the insects were orientated with the head capsule to the right and the legs in an upper position, following procedures adopted by flea specialists. The slides were then allowed to dry. The flea collection has been deposited at the FMNH, except for a portion retained at the Laboratoire de

Table 1. Number of installed traps (live traps and pit-fall traps) and trap capture rates of small mammals in the Réserve Spéciale d'Ambohitantely during two different inventories

	Wet season 31 Mar.–9 Apr. 2014	Dry season 15–23 Oct. 2014
Live traps		
Forest line 1[a]	250/39	250/7
Forest line 2	50/40	250/23
Forest line 3	250/33	250/6
Forest line 4	250/38	250/11
Marsh line	60/10	280/18
Total[b]	1060/160	1280/65
Pit-fall traps[c]		
Line 1	110/10	110/8
Line 2	110/7	110/10
Line 3	110/16	110/12
Total[d]	330/33	330/30

Figures are presented as total "trap nights" or "pit-fall trap nights"/ number of captured animals.

[a] The traps making up the four different forest lines were approximately in the same position during the two different visits to the site. Each line, composed of 50 traps, was in place for five consecutive nights. The total number of captured animals in the live traps does not necessarily match the figures for trap captures in Table 2. See methods for an explanation why a limited number of *R. rattus* were discarded and not processed for their ectoparasites.

[b] Results of Pearson chi-square test, $t = 71.3$, df = 8, $P < 0.001$

[c] All pit-fall lines were set in precisely the same place during the two visits. Each line, composed of 11 buckets, was in placed for 10 consecutive nights.

[d] Results of Pearson chi-square test, $t = 13.4$, df = 8, not significant.

Parasitologie et Zoologie appliquée, Université de Rennes (collection J.C.B.), which will ultimately be deposited in the Muséum national d'Histoire naturelle (MNHN), Paris.

Results

Small Mammals—Capture Rates, Species Richness, and Seasonality

The total accrued trap effort during the wet- and dry-season small mammal inventories at Ambohitantely, whether for live traps or pit-fall traps, was similar (Table 1). Hence, any possible difference in the number of trapped small mammals and their associated fleas between the two treatments is not related to the trapping effort. The main difference in accrued trap-nights between the two field visits was the marsh line, where almost exclusively introduced small mammals were captured (*Mus musculus*, *R. rattus*, and *Suncus murinus*).

In total, 15 species of small mammals were trapped during the two visits to Ambohitantely (Table 2), comprising nine Tenrecidae (including seven species of *Microgale*), two Nesomyinae (*Eliurus minor* and *E. majori*), and two introduced Muridae (*Mus musculus* and *R. rattus*) and Soricidae (*S. etruscus* and *S. murinus*). Hence, 11 endemic species of small mammals were captured. On the basis of other intensive small mammal work conducted in another area in the reserve, the following species are known from the area and not captured during the 2014 fieldwork: *Microgale pusilla* and *Oryzorictes hova* (Goodman and

Rakotondravony 2000). As no agricultural fields or rural villages occur within at least 5 km of the forest site we captured small mammals and that the intervening habitat matrix is sterile anthropogenic grassland, it is assumed that the trapped introduced mammal species represent populations that have successfully invaded the forest and are not in direct contact with populations living as human commensals.

Even though trap effort was largely equivalent during the two visits to the site, small mammal capture rates in the live traps during the wet season were nearly double those during the dry season, while the number of animals in the pit-fall traps was largely identical. The seasonal difference in trap success for the live traps was statistically significant, but not for the pit-fall traps (Table 1). Measures of species richness between the two surveys were essentially equivalent—wet season figures comprised 13 species and dry season figures comprised 10 species; in both cases, these figures include introduced taxa. The principal difference in measures of species richness between the two visits was the three species of *Microgale* captured during the wet season, each represented by a single specimen, and not during the dry season.

Fleas—Number Collected, Species Diversity, and Seasonality. In the context of this study, 344 fleas were collected, 201 individuals during the March–April 2014 wet season visit and 143 individuals during the October 2014 dry season visit. This seasonal difference was statistically significant (Pearson chi-square, $t = 43.6$, df = 10, $P < 0.001$). The total documented flea fauna at the site was nine species, all endemic to the island. During the wet season visit, seven flea species were found, and during the dry season visit, nine species were found. The difference in species richness between the two different seasonal visits was the absence of *Paractenopsyllus kerguisteli* and *P. rouxi* during the wet season and both notably rare during the dry season. No introduced species of flea was found during the survey on nonnative small mammals, specifically *Xenopsylla cheopis*.

Of the 288 small mammals examined in the context of this study for ectoparasites, 187 (65%) had at least one collected flea. The rate of flea parasitism for different small mammal species was variable. In certain cases when fleas were either rare or absent on a given species, this is probably associated with small sample sizes of potential host taxa (e.g., *Tenrec ecaudatus*, *Microgale gymnorhyncha*, *M. majori*, and *M. thomasi*, for which only a single individual was examined for each species [Table 2]). In contrast, high levels of flea parasitism were found for endemic species, such as *M. cowani*, *M. dobsoni*, and *E. majori*, and introduced taxa such as *R. rattus.*

Some seasonal differences were apparent in parasitism rates of certain flea species. In cases of taxa that are poorly represented in the Ambohitantely collection, for example, *P. kerguisteli* and *P. rouxi*, data associated with seasonal differences are inconclusive. For fleas such as *P. duplantieri*, the number of identified individuals was virtually identical during the wet- and dry-season inventories. In general, for flea taxa well

Table 2. Species of small mammals captured during small mammal inventories in the Réserve Spéciale d'Ambohitantely, Madagascar, and their associated flea ectoparasites, including the genera *Paractenopsyllus* (Ceratophyllidae, Leptopsyllinae) and *Synopsyllus* (Pulicidae, Xenopsyllinae)

	Number captured	P. duplantieri	P. grandidieri	P. kerguisteli	P. petiti	P. rouxi	P. vauceli	P. viettei	S. fonquerniei	S. estradei	Flea species richness per host
Family Tenrecidae											
Setifer setosus (Schreber, 1777)	5/21	0/1	–	–	–	–	–	–	0/10	0/3	3
Tenrec ecaudatus (Schreber, 1777)	0/1	–	–	–	–	–	–	–	–	–	0
Microgale cowani Thomas, 1882	22/45	4/15	–	0/3	0/3	0/1	1/9	0/1	–	0/3	7
Microgale dobsoni Thomas, 1884	19/5	15/5	0/2	–	4/2	–	5/1	–	–	0/4	5
Microgale gymnorhyncha Jenkins, Goodman & Raxworthy, 1996	3/0	–	–	–	1/0	–	–	–	–	–	1
Microgale longicaudata Thomas, 1882	1/0	1/0	–	–	–	–	–	–	–	1/0	2
Microgale majori Thomas, 1918	3/3	0/1	–	–	–	–	–	–	–	–	1
Microgale parvula G. Grandidier, 1934	1/0	–	–	–	–	–	–	–	–	–	0
Microgale thomasi Major, 1896	1/0	–	–	–	–	–	–	1/0	–	–	1
Subfamily Nesomyinae											
Eliurus minor Major, 1896	4/2	–	0/1	–	–	–	–	–	–	–	1
Eliurus majori Thomas, 1895	5/8	–	1/4	–	–	–	–	–	0/1	1/1	3
Family Muridae											
Rattus rattus (Linné, 1758)	108/11	1/0	27/4	–	–	0/1	–	–	20/3	23/2	4
Mus musculus Linné, 1758	4/14	–	–	–	–	–	–	0/1	0/1	–	2
Family Soricidae											
Suncus etruscus (Savi, 1822)	0/1	–	–	–	–	–	–	–	–	–	0
Suncus murinus (Linné, 1766)	2/0	–	–	–	–	–	–	–	–	–	0
Total trap captures (first figure) and total number of individual small mammals with a given flea species (second figure)	178/111	21/22	28/11	0/3	5/5	0/1	6/10	1/1	20/15	25/13	
Total number of host species, with endemic host species in parentheses		6 (5)	4 (3)	1 (1)	3 (3)	1 (1)	2 (2)	3 (2)	4 (1)	6 (5)	

Mammal species introduced to Madagascar are marked with an asterisk. Information on "number captured" of different small mammals is separated by season: wet season (31 March–9 April 2014) and dry season (15–23 October 2014). Following the same system, data on the presence of fleas under each species are the number of hosts from which a given flea species was collected and divided by season: wet season and dry season. All of the flea species identified in the collection are endemic to Madagascar.

represented in our collection and showing seasonal differences in the number of parasitized hosts, it is the wet season that had more parasitized small mammals (e.g., *P. grandidieri*, *Synopsyllus fonquerniei*, and *S. estradei*).

The number of flea species on different small mammals showed considerable variation. Several taxa with low capture rates did not have any fleas and little can be ascertained as to whether they are parasitized or not (e.g., *M. parvula* and the two introduced shrews, *S. etruscus* and *S. murinus*). In the case of *Suncus* spp., in the Mediterranean Basin, *S. etruscus*, with its distinctly short and fine fur, has been rarely found with fleas, while *S. murinus*, with notably longer fur, shows more typical levels of parasitism (Beaucournu and Lorvelec 2014). For host species, for which at least 20 individuals were handled, flea species diversity varied from a low of three in *Setifer setosus*, a hedgehog-like Tenrecidae to a high of seven in *M. cowani*, a shrew-tenrec. In cases when a flea species was found on more than 10 different small mammals, we have no example of host specificity. The number of host taxa of a given flea species varied from two in *P. vauceli* to six in *P. duplantieri* and *S. estradei*.

Multiple Flea Species on the Same Individual Host. Of the 130 individual small mammals that yielded fleas, 77 (59%) had at least two flea specimens, either the same or different species. When divided by season, 46 of 75 (61%) examined small mammals during the wet season, and 31 of 55 (56%) during the dry season had at least two fleas. Of these 77 small mammals, infestation rates for those with two different flea species (in some cases, in different genera) were 30 (39%), for three different flea species (in some cases in different genera) were 10 (13%), and for four different flea species (in some cases, in different genera) were 4 (5%). We have several cases of *S. fonquerniei* occurring with *S. estradei* on the same host individual and these two species in different combinations with *Paractenopsyllus* spp.; these data have important implications for the sylvatic cycle of bubonic plague, which is known to be transmitted by *S. fonquerniei* (see Discussion).

The highest flea burden on captured small mammals occurred on the following three individuals: 1) *R. rattus* (UADBA SMG-18620) with *S. estradei* ($n = 7$), *S. fonquerniei* ($n = 2$), and *P. grandidieri* ($n = 3$); 2) *M. dobsoni* (FMNH SMG-18658) with *P. duplantieri* ($n = 5$), *Paractenopsyllus petiti* ($n = 3$), *P. vauceli* ($n = 2$), and *S. estradei* ($n = 1$); and 3) *M. cowani* (FMNH SMG-18662) with *P. duplantieri* ($n = 4$), *P. petiti* ($n = 2$), and *P. vauceli* ($n = 4$). There was also a case of four congeneric species occurring on *M. cowani* (UADBA SMG-18646), and these included *P. rouxi* ($n = 1$), *P. petiti* ($n = 1$), *P. vauceli* ($n = 1$), and tentatively identified as *P. kerguisteli* ($n = 1$).

Discussion

Historical Biogeography and Phylogenetic History. Given the separation and subsequent isolation of Madagascar in deep geological time, having detached from the balance of Gondwana approximately 160 MYA (de Wit 2003), notably before the evolution of modern groups of mammals, the colonization history of the island by ancestors of what are now endemic small mammal groups and their associated ectoparasites is an intriguing question. On the basis of molecular clock inference, Poux et al. (2005) proposed that the ancestor that lead to the Tenrecidae arrived on the island sometime in the mid-Eocene, about 40 MYA, and that for the Nesomyinae at the Oligocene–Miocene boundary, about 22 MYA. In both cases, these families represent monophyletic groups and excellent examples of adaptive radiations, demonstrating considerable interspecific and intergeneric variation in life history traits and morphological variation. Both tenrecids and nesomyines have a diverse flea fauna (Duchemin 2003a).

A recent study based on estimated dates of divergence in the ancestors of what are presumed to have evolved into ectoparasitic Siphonaptera indicates deep lineage splitting in the early Cretaceous, basal lineages diversifying in the Late Cretaceous, and intraordinal divergence at the Cretaceous–Paleogene boundary, about 66 MYA (Zhu et al. 2015). (For an assessment of this proposed scenario, see Beaucournu [2015].) Hence, using these time estimates, there is the possibility that when the mammalian ancestors of the Tenrecidae and Nesomyinae colonized Madagascar, they were carrying fleas. While phylogenies have been published on the evolutionary history of fleas (e.g., Whiting et al. 2008), these do not include endemic Malagasy taxa, and it is not possible to ascertain at this point if certain of the island's flea genera or even subfamilies represent monophyletic lineages or multiple colonization events.

The Lack of Cospeciation Between Fleas and Their Small Mammal Hosts. It is notable that after tens of millions of years of possible cospeciation on Madagascar between fleas and their small mammal hosts, at least for the eastern humid forests, in this case, specifically Ambohitantely, we found no example of a flea taxon parasitizing a single small mammal species host. Another aspect that is remarkable, the flea fauna list for Ambohitantely based on our collections does not include any introduced flea species, such as *Xenopsylla cheopis*, often common on *R. rattus* on Madagascar (Duchemin 2003a). Even though four species of introduced small mammals occur at Ambohitantely, our data suggest replacement of their naturally occurring flea fauna with Malagasy taxa and regular lateral transfer of flea species between hosts representing three different groups of small mammals: the endemic Tenrecidae and Nesomyinae, as well as the introduced Muridae. It has been previously noted that above 800 m elevation, *X. cheopis* is replaced by *S. fonquerniei* as the principal *R. rattus* flea, and this flea also shows greater plague transmission frequency than *X. cheopis* (Andrianaivoarimanana et al. 2013). A possible explanation can be offered for this pattern.

As mentioned above, the native small mammal fauna of montane humid forest areas of Madagascar are species-rich and often with a considerable number of congeneric species of *Microgale* and *Eliurus*, and these taxa generally have broad geographical distributions

across the eastern portion of the island (Goodman et al. 2013). In many cases, at a given site, even though these congeneric species have different dietary regimes (Dammhahn et al. 2013) and phenotypic or morphological attributes (Carleton 2003; Jenkins 2003), they border on being syntopic in the physical ecological setting they occupy. Perhaps of considerable importance is that these different small mammal taxa, whether they are terrestrial or partially scansorial, use common burrow systems or those in close proximity, providing the means for flea transfer.

This broad host spectrum is not limited to Ambohitantely fleas. Over the past few decades, several new flea species have been described from different areas of the eastern humid forest and, in cases when these taxa are well represented by comparative material, they occur on a variety of hosts belonging to the Tenrecidae and Nesomyinae, and most fleas have relatively broad geographical ranges (e.g., Duchemin 2003b, 2004; Hastriter and Dick 2009). Hence, there appears to be some constraint within this flea–small mammal community in the evolution of host specificity. The flea species diversity identified from Ambohitantely is not exceptional, as, for example, in the humid forests of Southeast Asia, the abundance and diversity at the generic and specific levels of fleas is notably higher (Durden and Traub 1990).

In contrast to rather high species diversity and fine-level small mammal species packing in the eastern humid forests of Madagascar, the western dry forest formations show distinctly lower levels of species diversity, density, and number of sympatric congeneric taxa (e.g., Soarimalala 2008; Soarimalala et al. 2013). For genera such as *Microgale*, it is rare to find two species coexisting in the same forest block and most regional members of this genus have distinctly more limited geographical ranges as compared to the eastern humid forests (Soarimalala and Goodman 2011; Goodman et al. 2013). Hence, given this configuration, the possibility in the west of cospeciation or even coevolution between fleas and their small mammals, resulting in host specificity, seems notably higher. As compared to the eastern humid forests, distinctly less research has been conducted on the flea fauna of this area of Madagascar, particularly based on extensive modern collections. Nonetheless, there seems to be some indication of a higher rate of host specificity between fleas and their small mammal hosts: *Synopsyllus smiti* and *Xenopsylla petteri* are only known to occur on *Macrotarsomys ingens* and *Hypogeomys antimena*, respectively (Duchemin 2003a), two rodent species with very limited western geographical ranges (Goodman et al. 2013). Further research on small mammal fleas from the western dry forests is needed to test this possible pattern of cospeciation.

Seasonal Variation. Some notable seasonal differences were found in the level of flea parasitism between the different seasonal periods the field surveys were conducted. Two different aspects can explain this pattern. First, most tenrecids and nesomyines occurring at Ambohitantely breed at the start of the rainy season, falling between November and December, and

the late March–April inventory would have coincided with the high end of annual population cycling and possibly the dispersal period of first-year animals. Few details are known about life history traits of endemic Malagasy flea species, such as if they are principally occurring in host fur or host nests, and if their reproductive cycles coincide with those of their hosts (Klein 1966; Klein and Uilenberg 1966). Elsewhere in the world, male adult small mammal hosts show higher levels of parasitism than immature males, but the presumed hormonal basis for this observation has yet to be elucidated (Launay 1980; Beaucournu 1981). Second, the month of October is normally a cold and dry period of the year, and these environmental factors might impede activity in certain flea species, where, for example, they might find refuge in small mammal burrows and not attach to their hosts when they are actively foraging. However, the fact that two flea species rare in our Ambohitantely collections, *P. kerguisteli* and *P. rouxi*, were only obtained during the dry season, contradicts this second explanation. Further, in a parallel study conducted in northeastern Tanzania, fleas were distinctly more common on small mammals in the dry season, as compared to the rainy season (Laudisoit et al. 2009).

Insight into Cycles of Sylvatic Bubonic Plague. The Ambohitantely Forest and the greater Ankazobe District, in which this forest block is found, have been known for decades as a zone of bubonic plague outbreaks (Chanteau 2006). This disease is associated with the bacteria *Yersinia pestis*, which is transmitted by small mammal fleas. On Madagascar, the fleas of *R. rattus* have been implicated, particularly the introduced flea *X. cheopis*, but transmission has also been demonstrated for endemic taxa, such as *S. fonquerniei*, particularly at higher elevations (Duplantier et al. 2005; Andrianaivoarimanana et al. 2013). This disease is established in portions of the Central Highlands above 800 m, including forested areas, to such an extent it is now considered in epidemiological terms "endemic" (Duplantier and Duchemin 2003; Andrianaivoarimanana et al. 2013). How the cycle is maintained, particularly in forested areas, a habitat *R. rattus* has successfully colonized, remains partially unclear. Although we found no evidence of *X. cheopis* in the Ambohitantely Forest, *S. fonquerniei* was relatively common on *R. rattus*, and we also found on another introduced rodent species (*Mus musculus*), an endemic rodent (*E. majori*), and endemic tenrec (*S. setosus*) (Table 2). The congeneric species *S. estradei* was distinctly common and co-occurred on several individual small mammals with *S. fonquerniei*, as well as an assortment of *Paractenopsyllus* spp. To our knowledge, *Paractenopsyllus* spp. have not been tested as vectors for *Yersinia*. Given the pattern found at Ambohitantely of different fleas occurring on introduced and endemic small mammals, these different parasites might be important vectors and maintain the sylvatic cycle of bubonic plague. Future work needs to focus on testing these different flea species for *Yersinia* and, if the results are positive and they show moderate to relatively high infection rates, they would be implicated in the maintenance of the wild plague cycle. Further,

possible genetic differences in *Yersinia* possibly occurring in flea hosts should be inspected, as this bacterium shows considerable genotypic variability on Madagascar (Riehm et al. 2015).

In conclusion, here, we specifically address the different points mentioned in the introduction concerning the fleas and their small mammal hosts in the Ambohitantely Forest.

1. Document the flea fauna of native and introduced small mammals—nine different endemic species of fleas were found parasitizing the local small mammal fauna, composed of 12 endemic members of the family Tenrecidae and Nesomyinae and two introduced Muridae rodents. Although sample sizes are small, no flea was found on two species of introduced Soricidae shrews.
2. Evidence of coevolution between specific flea species and small mammals—no evidence of cospeciation was found between fleas and their mammal hosts. In contrast, the flea species that were well represented in the Ambohitantely sample occur on a variety of small mammal species, including endemic and introduced taxa, and lateral transfer between these different hosts is the apparent pattern.
3. Possible aspects of seasonality in parasitism—in general, fleas tend to be more common on small mammal hosts at the end of the rainy season than at the end of the dry season. This pattern is also correlated with higher trapping success during the former period.
4. Ectoparasite transfer between native and introduced small mammals—the flea fauna of Ambohitantely is composed only of endemic taxa. No evidence of introduced fleas, such as *X. cheopis*, was found, and all introduced small mammal species from which fleas were recovered only had endemic taxa.
5. Role of small mammal life history traits to explain certain observed patterns—it is proposed that the lack of host–parasite specificity, at least in humid forest formations, may be associated with high diversity of sympatrically occurring small mammal species with broad geographical distributions. Further, many of these host species occupy the same or nearby burrows, allowing for lateral transfer of fleas. These aspects might be important in maintaining the sylvatic cycle of bubonic plague in the humid montane forests of the island.

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