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Host-Parasite Ecology and Description of the Louse Fly, *Allobosca crassipes*, from Madagascar

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HOST-PARASITE ECOLOGY AND DESCRIPTION OF THE LOUSE FLY, *ALLOBOSCA*
CRASSIPES, FROM MADAGASCAR

A Thesis

Presented to

The Faculty of the Department of Biological Sciences

San José State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Stanley E. Vaughn

August 2010

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The Designated Thesis Committee Approves the Thesis Titled

HOST-PARASITE ECOLOGY AND DESCRIPTION OF THE LOUSE FLY, *ALLOBOSCA*
CRASSIPES, FROM MADAGASCAR

by

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August 2010

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ABSTRACT

HOST-PARASITE ECOLOGY AND DESCRIPTION OF THE LOUSE FLY, *ALLOBOSCA CRASSIPES*, FROM MADAGASCAR

by Stanley E. Vaughn

The louse fly, *Allobosca crassipes* (Diptera: Hippoboscidae), is an obligate ectoparasite exclusive to lemurs in Madagascar. Research in Ranomafana National Park (RNP) in southeastern Madagascar has resulted in the identification of a new association of this fly with the black and white ruffed lemur, *Varecia variegata variegata*, and a confirmed association with Milne-Edwards' sifaka, *Propithecus edwardsi*. Previous descriptions of *A. crassipes* were based on only a few specimens, and the type specimen is presumed destroyed. The specimens of *A. crassipes* collected by SJSU in 2000-2001 provide the largest collection available for the purpose of describing this species.

Habitat disturbance is a major threat to the biodiversity of RNP and is predicted to affect parasite-host interactions. Disturbance has changed the behavior of the lemur hosts, which has indirectly affected the parasites. *A. crassipes* is found on several lemur species that rarely interact, but its low host specificity is an enigma since it is wingless as an adult. Disturbance may, however, indirectly contribute to low host specificity by forcing proximity and increasing interaction between host species. Further evidence of disturbance is seen in the isotopic composition of *A. crassipes*. *A. crassipes*-parasitizing lemurs inhabiting disturbed forests are significantly nitrogen depleted (as are their hosts) vs. those inhabiting undisturbed forests. These data further validate stable isotope ecology as a means of identifying ecosystem-wide habitat disturbance in rainforests.

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Blut ist ein ganz besonderer Saft

Blood is a special juice

– Goethe’s Faust Part I

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INTRODUCTION

Madagascar is the fourth largest island in the world and lies 400 km to the east coast of Mozambique, Africa in the Indian Ocean. This geographic isolation is the result of separation from Africa some 165 million years ago (Rabinowitz 1983). It has been isolated in its present position for over 88 million years (Krause 2003). The effects of this geographic isolation are evident from the 9,703 plant, 771 vertebrate and 2,305 macro invertebrate species that have been described as endemic to the island (Myers et al. 2000; Goodman & Benstead 2003). When considering the numbers of endemic flora and fauna, the rate of endemism and habitat loss, Madagascar ranks the “hottest” of the world’s biodiversity hotspots (Myers et al. 2000). The unique primate fauna of Madagascar comprises more than fifty distinct taxa (Wright 1992), including Milne Edwards’ sifaka (*Propithecus edwardsi*) and the Black and White Ruffed lemur (*Varecia variegata variegata*). Following her discovery in the 1980s of a rare lemur species, the Golden Bamboo Lemur (*Haplemur aureus*), Dr. Patricia Wright convinced the government of Madagascar to establish a national park (Ranomafana National Park, RNP) to protect the flora and fauna of this region. Dr. Wright also established a research station and encouraged cross disciplinary researchers from all over the world, including SJSU, to work in RNP. The work presented here on association of the parasitic Louse-fly *Allobosca crassipes* (Speiser) with *P. edwardsi* and *V. variegata variegata*, and the effects of habitat disturbance on the ecological relationship of these species is the result of this invitation and the subject of this study.

Chapter I describes the physical and biological characteristics of the three study sites in Ranomafana National Park, Madagascar. Chapter II addresses taxonomic inaccuracies and inconsistencies that historically have been problematic in the identification of *Allobosca crassipes*. Chapter III, which was published in *Pan-Pacific Entomologist* in 2009, describes the parasite-host association of *A. crassipes* with the Black and White Ruffed lemur, (*Varecia variegata variegata*), and Milne Edwards' sifaka (*Propithecus edwardsi*), and finally, Chapter IV discusses the effect of habitat disturbance on the stable isotope composition of *A. crassipes* and one of its host species, *P. edwardsi*. The thesis concludes in Chapter V with a summary of this research.

CHAPTER I: Description of Study Sites in Ranomafana National Park

INTRODUCTION

Ranomafana National Park (RNP) is located between 47°18' - 47°37' East and 21°02' – 21°25' South on the east-facing escarpment of the central high plateau in southeastern Madagascar (Figure 1). The park comprises 43,000 ha and ranges in elevation from 500-1500m (Dew & Wright 1998; Balko & Underwood 2005). Although the park is surrounded by a buffer zone of mostly secondary forest, much of this border is agricultural land that is used by several villages. The use of this land by these villages includes the harvesting of timber for construction and the collection of medicinal plants (Wright 1992). In 2000-2001 SJSU conducted research in RNP at three locations: Talatakely, Vatoharanana and Valohoaka. These three sites vary in extent of anthropogenic impact and provide an opportunity to compare the impact of selective commercial logging on this rainforest ecosystem.

Study Sites

Talatakely, a small village from 1935 to 1947, comprises approximately 3 km² and was intensely selectively logged from 1986-90 (Wright 1997). Talatakely is considered the most anthropogenically disturbed habitat within RNP, and is a mixed primary/secondary forest. The location of the first research station in Talatekely from 1986-2003 also contributed to pressure on the local environment. Vatoharanana and Valohoaka, in contrast, are primary forests further to the South. Vatoharanana, which is 6 km southeast of Talatakely, comprises approximately 8 km² and was moderately

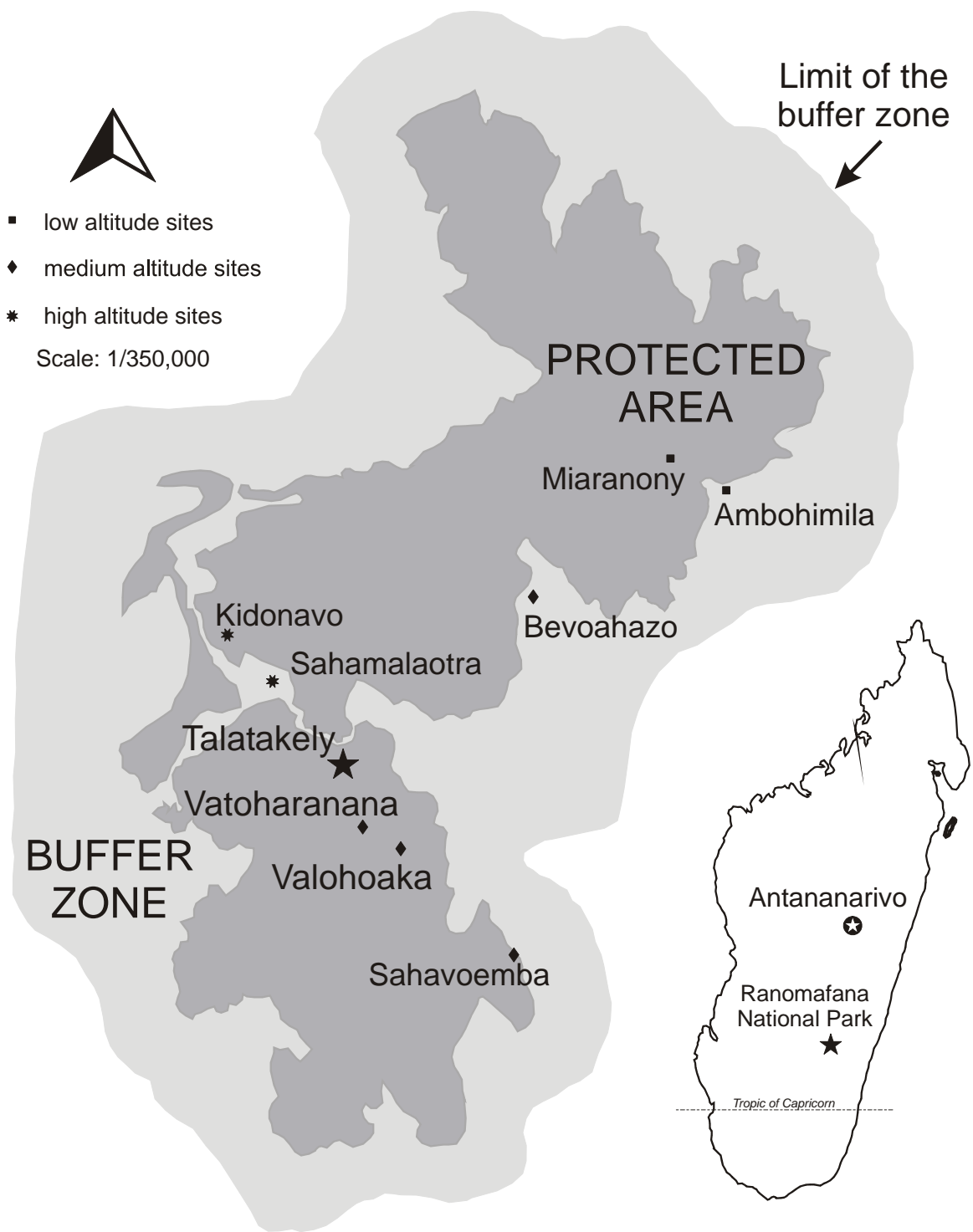


Figure 1: Ranomafana National Park, Madagascar (after Wright 1992, 1997)

selectively logged from 1987-88. Valohoaka is approximately 8.5 km southeast of Talatakely and consists of 9 km². A moratorium on logging in 1988 prohibited commercial operations in Valohoaka thereby ensuring that the forest remained relatively intact (Balko & Underwood 2005). Physical and botanical parameters of the three sites are given in Table 1.

Scope of Research at RNP from 2000-2001

The field research conducted by investigators present in RNP during the period of SJSU's work in 2000-2001 was quite diverse. Because the work of several researchers required the darting and sedation of lemurs, it was important to synchronize and coordinate our efforts to ensure that as much data could be collected by different research teams during a single procedure. For example, Patricia Wright's team collected morphometric data on *P. diadema* as part of a long-term study on the demography of this species (Pochron et al. 2004). Major et al. (2006) collected skin samples for genetic analysis as part of a study on the systematic status of *Propithecus*. McGee et al. (2001, 2002) collected hair samples to study the biogeochemistry of *P. edwardsi*. Other researchers fitted individual lemurs with radio tracking collars to monitor the movements of individuals and groups. For example, Arrigo-Nelson (2006) followed *P. edwardsi* to study the feeding behavior of groups inhabiting disturbed and undisturbed habitats. The collection of ectoparasites for this project from these lemurs was part of this coordinated effort, and the results of this research are described in the following chapters.

Table 1: Physical and Botanical Characteristics of Sites in Ranomafana National Park

	Talatakely	Vatoharanana	Valohoaka
Altitude (m)	900	1125*	1200
Mean tree height (m)†	6.7	9.1	not available
Mean tree height (m)§	13.22	15.07	14.82
Mean tree height (m)‡	10.05	not available	11.76
Mean tree DBH (cm)†	21.5	23.3	not available
Mean tree DBH (cm)‡	11.83	not available	13.31
Mean tree DBH (cm)§	19.76	31.20	29.38
Mean crown volume (m ³)◇	198.52	238.73	259.47
Mean crown volume (m ³)‡	42.56	not available	72.41
Forest type	Mixed primary/secondary	Primary	Primary
History of anthropogenic impact to	Inhabited 1930s-1940s; heavily selectively logged 1986-1990; Research station 1986 - 2003.	Minimal selective logging 1988-1989. Satellite research camp 1988 – 2005; ANGAP satellite camp 2005 – present.	Not logged; satellite research camp 1990 present

Sources of data: *Overdorff 1991, †White et al. 1995, ◇Balko 1998, §Balko and Underwood 2005, ‡Arrigo-Nelson 2006.

CHAPTER II: A Description of *Allobosca crassipes* (Hippoboscidae: Diptera)

INTRODUCTION

Flies of the family Hippoboscidae, commonly known as louse flies or keds, are parasites of a variety of birds and mammals. This family comprises 213 species, with 21 genera divided into 3 subfamilies (Dick 2006). These flies are patchily distributed, with locality records world-wide except from the polar regions (Maa 1969). Hippoboscids exhibit an unusual reproductive strategy with respect to most Diptera in that the females do not lay eggs. Instead, the females hold the larvae in their abdomen until they develop as pupae which are then deposited on the substrate, in proximity of or on the host (Bequaert 1953). Many louse flies are excellent fliers, one example being *Ornithophila gestroi* (Rondani) which has been observed pursuing falcons in flight, even in steep, high-speed dives (Balgooyen et al. 1999). Louse flies that parasitize mammals, such as the sheep ked *Melophagus ovinus* (Linnaeus), are flightless and exhibit vestigial wings so reduced that they are virtually wingless (Ferris & Cole 1922). The hippoboscid that is the subject of this thesis, *Allobosca crassipes* (Speiser), exhibits an intermediate winged state in that it most probably emerges fully winged but loses the basal portion of its wings soon after acquiring a host (Theodor & Oldroyd 1965).

The louse fly *A. crassipes* (Speiser) was first described and named by Paul Speiser in 1899. It is the monospecific representative of the genus *Allobosca*, subfamily Alloboscinae in the family Hippoboscidae, Order Diptera. It is an obligate parasite of lemurs in Madagascar and has traditionally been a little collected species (Maa 1969).

The type description of *A. crassipes* was based on examination of three dried specimens obtained from “C. Hilger in Karlsruhe, Germany” (Speiser 1899:197). Speiser’s (1899) original description of *A. crassipes* does not detail specific host or locality information from the specimens observed, mentioning only that the flies were collected from lemurs in Madagascar.

The type specimen is presumed destroyed during the bombings of Dresden in February of 1945 (Evenhuis, N. 2009. Personal communication). The initial description by Speiser and subsequent descriptions by Ferris & Cole (1922) and Theodor & Oldroyd (1965) were based from observations of nine total specimens which were in relatively poor condition. As no original material exists, the focus of this chapter is to re-describe and designate a neotype specimen for *A. crassipes* using specimens collected during San Jose State University expeditions in 2000-2001. The modern curation methods and the large sample size collected during the SJSU expeditions allows for consideration of intraspecific variation in *A. crassipes* as well.

MATERIALS AND METHODS

Live specimens of *A. crassipes* were collected from two species of lemurs, *Varecia variegata variegata* and *Propithecus edwardsi* in Talatekely (Tala), Vatoharanana (Vato) and Valohoaka (Valo), in Ranomafana National Park (RNP), Madagascar during field expeditions in 2000-2001. Lemur hosts (n = 29) were darted and tranquilized using Telazol®, with all collection methods in accordance with the protocol approved by San Jose State University’s Institutional Animal Care and Use

Committee (SJSU IACUC #728). Fly specimens (n = 101) were collected within 30 minutes of capture of the hosts using forceps and a flea comb, and preserved in 80% ETOH. Precautions to prevent contamination by flies from different prosimian hosts included separation and isolation of lemurs prior and during ectoparasite collection. Cetylclide II was used to cleanse and disinfect all examination surfaces.

Specimen Depository

All specimens studied are deposited in the J. Gordon Edwards Museum of Entomology (JGEME) at San Jose State University, San Jose, California, 95192-0100, USA.

RESULTS

A total of 101 specimens were collected during the SJSU 2000-2001 expeditions to RNP (Table 2). All specimens, including the proposed neotype, are associated with lemurs that have been identified and tracked by lemur researchers since the 1980s (Pochron et al. 2004).

Material Studied

Description

The description of *A. crassipes* below is based on Speiser (1899), Ferris & Cole (1922) and Theodor & Oldroyd (1965), in addition to my own observations. Several anatomical features of *A. crassipes* are distinct. The head is rounded, well developed and easily viewed from the dorsal aspect (Figure 2). It is distinctly separated from the thorax and can move about freely. The eyes are well developed, and the antennae present a

Table 2: List of Flies by Individual Lemur Host

Locality	Lemurs				Parasites		
	Host Species	Host ID	Host Sex	Host Weight	Fly Accession Number	Fly Sex	Number of Flies on Host
Vato	VVV	Blue Green Grp II	male	5.7 kg	2000.14AF	F	3 flies
Vato	VVV	Blue Green Grp II	male	5.7 kg	2000.14BM	M	3 flies
Vato	VVV	Blue Green Grp II	male	5.7 kg	2000.14CM	M	3 flies
Vato	VVV	Red Gold Grp II	male	3.4 kg	2001.21AF	F	3 flies
Vato	VVV	Red Gold Grp II	male	3.4 kg	2001.21BF	F	3 flies
Vato	VVV	Red Gold Grp II	male	3.4 kg	2001.21CM	M	3 flies
Vato	VVV	Yellow Green	male	3.3 kg	2001.31AM	M	2 flies
Vato	VVV	Yellow Green	male	3.3 kg	2001.31BF	F	2 flies
Vato	VVV	Radio I	male	3.0 kg	2001.24AM	M	2 flies
Vato	VVV	Radio I	male	3.0 kg	2001.24BF	F	2 flies
Vato	VVV	Red Blue	female	4.05 kg	2001.27AF	F	2 flies
Vato	VVV	Red Blue	female	4.05 kg	2001.27BF	F	2 flies
Vato	VVV	Blue Green Grp I	male	3.85 kg	2001.28AF	F	2 flies
Vato	VVV	Blue Green Grp I	male	3.85 kg	2001.28BF	F	2 flies
Tala	PE	Turquoise	male	4.8 kg	2000.6AF	F	1 fly
Tala	PE	Blue Blue	female	5.3 kg	2000.4AF	F	7 flies
Tala	PE	Blue Blue	female	5.3 kg	2000.4BM	M	7 flies
Tala	PE	Blue Blue	female	5.3 kg	2000.4CM	M	7 flies
Tala	PE	Blue Blue	female	5.3 kg	2000.4DM	M	7 flies
Tala	PE	Blue Blue	female	5.3 kg	2000.4EM	M	7 flies
Tala	PE	Blue Blue	female	5.3 kg	2000.4FM	M	7 flies
Tala	PE	Blue Blue	female	5.3 kg	2000.4GM	M	7 flies
Tala	PE	Blue Green Grp IV	female	5.2 kg	2000.2AM	M	2 flies
Tala	PE	Blue Green Grp IV	female	5.2 kg	2000.2BM	M	2 flies
Tala	PE	Blue Purple	male	5.3 kg	2000.5AF	F	6 flies
Tala	PE	Blue Purple	male	5.3 kg	2000.5BF	F	6 flies
Tala	PE	Blue Purple	male	5.3 kg	2000.5CF	F	6 flies
Tala	PE	Blue Purple	male	5.3 kg	2000.5DF	F	6 flies
Tala	PE	Blue Purple	male	5.3 kg	2000.5EM	M	6 flies
Tala	PE	Blue Purple	male	5.3 kg	2000.5FM	M	6 flies
Tala	PE	no collar	female	4.5 kg	2000.9AF	F	9 flies
Tala	PE	no collar	female	4.5 kg	2000.9BF	F	9 flies
Tala	PE	no collar	female	4.5 kg	2000.9CF	F	9 flies
Tala	PE	no collar	female	4.5 kg	2000.9DF	F	9 flies
Tala	PE	no collar	female	4.5 kg	2000.9EF	F	9 flies
Tala	PE	no collar	female	4.5 kg	2000.9FM	M	9 flies
Tala	PE	no collar	female	4.5 kg	2000.9GM	M	9 flies
Tala	PE	no collar	female	4.5 kg	2000.9HM	M	9 flies
Tala	PE	no collar	female	4.5 kg	2000.9IM	M	9 flies
Tala	PE	Pink Grp IV	male	5.4 kg	2000.1AF	F	4 flies
Tala	PE	Pink Grp IV	male	5.4 kg	2000.1BM	M	4 flies
Tala	PE	Pink Grp IV	male	5.4 kg	2000.1CM	M	4 flies
Tala	PE	Pink Grp IV	male	5.4 kg	2000.1DM	M	4 flies

Table 2: List of Flies by Individual Lemur Host (Continued)

Locality	Lemurs				Parasites		
	Host Species	Host ID	Host Sex	Host Weight	Fly Accession Number	Fly Sex	Number of Flies on Host
Tala	PE	Purple Grp IV	male	4.9 kg	2000.3AM	M	2 flies
Tala	PE	Purple Grp IV	male	4.9 kg	2000.3BM	M	2 flies
Tala	PE	Blue Grp IV	male	5.3 kg	2001.17AM	M	1 fly
Tala	PE	Green Orange Grp I	male	5.9 kg	2001.16AM	M	4 flies
Tala	PE	Green Orange Grp I	male	5.9 kg	2001.16BF	F	4 flies
Tala	PE	Green Orange Grp I	male	5.9 kg	2001.16CF (type)	F	4 flies
Tala	PE	Green Orange Grp I	male	5.9 kg	2001.16DF	F	4 flies
Tala	PE	no collar Grp I	male	2.9 kg	2001.15AF	F	2 flies
Tala	PE	no collar Grp I	male	2.9 kg	2001.15BF	F	2 flies
Vato	PE	Red Grp I	male	5.3 kg	2000.10AM	M	1 fly
Vato	PE	Green Gold Grp III	female	5.5 kg	2000.11AM	M	2 flies
Vato	PE	Green Gold Grp III	female	5.5 kg	2000.11BF	F	2 flies
Vato	PE	Purple Gold Grp III	male	5.4 kg	2000.12AM	M	2 flies
Vato	PE	Purple Gold Grp III	male	5.4 kg	2000.12BM	M	2 flies
Vato	PE	Yellow Blue Grp III	female	5.6 kg	2000.13AF	F	7 flies
Vato	PE	Yellow Blue Grp III	female	5.6 kg	2000.13BF	F	7 flies
Vato	PE	Yellow Blue Grp III	female	5.6 kg	2000.13CF	F	7 flies
Vato	PE	Yellow Blue Grp III	female	5.6 kg	2000.13DF	F	7 flies
Vato	PE	Yellow Blue Grp III	female	5.6 kg	2000.13EF	F	7 flies
Vato	PE	Yellow Blue Grp III	female	5.6 kg	2000.13FM	M	7 flies
Vato	PE	Yellow Blue Grp III	female	5.6 kg	2000.13GM	M	7 flies
Vato	PE	Brown Blue Grp III	male	5.8 kg	2001.23AF	F	4 flies
Vato	PE	Brown Blue Grp III	male	5.8 kg	2001.23BF	F	4 flies
Vato	PE	Brown Blue Grp III	male	5.8 kg	2001.23CM	M	4 flies
Vato	PE	Brown Blue Grp III	male	5.8 kg	2001.23DM	M	4 flies
Vato	PE	Green Gold III	male	5.0 kg	2001.22AF	F	4 flies
Vato	PE	Green Gold III	male	5.0 kg	2001.22BM	M	4 flies
Vato	PE	Green Gold III	male	5.0 kg	2001.22CM	M	4 flies
Vato	PE	Green Gold III	male	5.0 kg	2001.22DM	M	4 flies
Vato	PE	Green silver I	male	3.5 kg	2001.26AM	M	6 flies
Vato	PE	Green silver I	male	3.5 kg	2001.26BM	M	6 flies
Vato	PE	Green silver I	male	3.5 kg	2001.26CF	F	6 flies
Vato	PE	Green silver I	male	3.5 kg	2001.26DF	F	6 flies
Vato	PE	Green silver I	male	3.5 kg	2001.26EF	F	6 flies
Vato	PE	Green silver I	male	3.5 kg	2001.26FF	F	6 flies
Vato	PE	Green silver red II	male	3.05 kg	2001.20AM	M	1 fly
Vato	PE	Pink silver II	male	4.60 kg	2001.19AM	M	6 flies
Vato	PE	Pink silver II	male	4.60 kg	2001.19BM	M	6 flies
Vato	PE	Pink silver II	male	4.60 kg	2001.19CM	M	6 flies
Vato	PE	Pink silver II	male	4.60 kg	2001.19DM	M	6 flies
Vato	PE	Pink silver II	male	4.60 kg	2001.19EM	M	6 flies
Vato	PE	Pink silver II	male	4.60 kg	2001.19FF	F	6 flies

Table 2: List of Flies by Individual Lemur Host (Continued)

Locality	Lemurs				Parasites		
	Host Species	Host ID	Host Sex	Host Weight	Fly Accession Number	Fly Sex	Number of Flies on Host
Vato	PE	Purple blue I	male	5.4 kg	2001.29AF	F	4 flies
Vato	PE	Purple blue I	male	5.4 kg	2001.29BF	F	4 flies
Vato	PE	Purple blue I	male	5.4 kg	2001.29CF	F	4 flies
Vato	PE	Purple blue I	male	5.4 kg	2001.29DF	F	4 flies
Vato	PE	Radio I	male	5.35 kg	2001.25AF	F	9 flies
Vato	PE	Radio I	male	5.35 kg	2001.25BF	F	9 flies
Vato	PE	Radio I	male	5.35 kg	2001.25CF	F	9 flies
Vato	PE	Radio I	male	5.35 kg	2001.25DM	M	9 flies
Vato	PE	Radio I	male	5.35 kg	2001.25EM	M	9 flies
Vato	PE	Radio I	male	5.35 kg	2001.25FM	M	9 flies
Vato	PE	Radio I	male	5.35 kg	2001.25GM	M	9 flies
Vato	PE	Radio I	male	5.35 kg	2001.25HM	M	9 flies
Vato	PE	Radio I	male	5.35 kg	2001.25IM	M	9 flies
Vato	PE	Radio II	male	5.90 kg	2001.18AM	M	1 fly
Valo	PE	Pink blue IV	female	6.25 kg	2001.30AM	M	2 flies
Valo	PE	Pink blue IV	female	6.25 kg	2001.30BM	M	2 flies

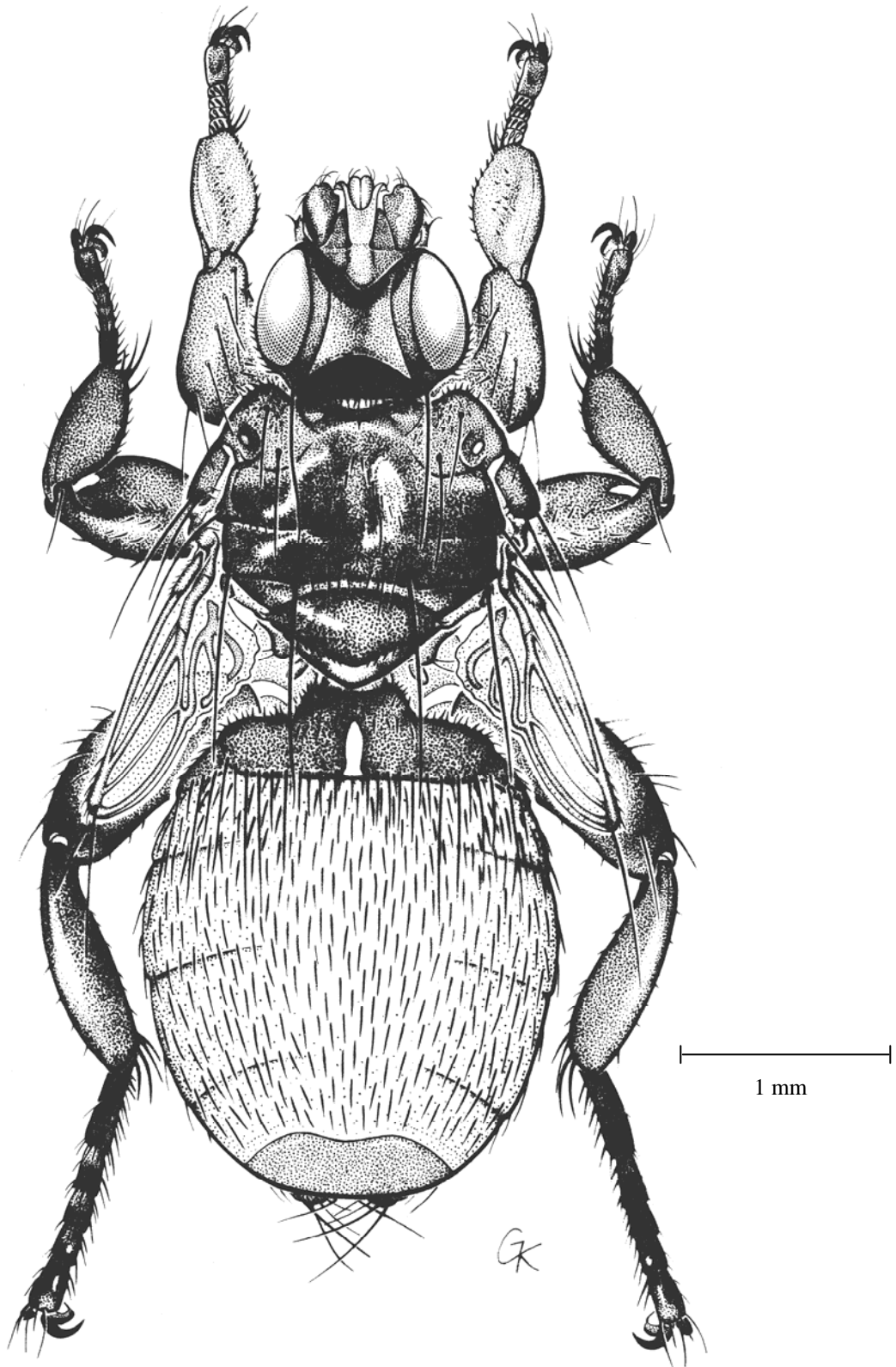


Figure 2. *Allobosca crassipes*, male, dorsal view. (Illustration by Ginny Kirsch.)

dorsal process and a lobed, leaf-shaped arista. The fore femora are strongly swollen basally and the front tarsi differ from the mid and hind legs in exhibiting short, nearly spatulate setae on the first four segments. The wings, when present, usually have seven longitudinal veins, all heavily sclerotized, with three cross veins in line with each other. The veins making up the discal and anal cells may or may not be entire or complete, depending on the extent of the shedding of the basal portion of the wing. The discal and anal cells generally are triangular in shape and nearly equal in size (Figures 3A & 3B). There are no other distinctive features of the thoracic region useful for differentiating *A. crassipes* from any other related species.

Included in Theodor & Oldroyd's (1965) description of *A. crassipes*, was an illustration of a wing with a substantial portion of the basal membrane still present. This is illustrated in Figure 3A. Observations of the specimens collected during the SJSU 2000-2001 expeditions did not reveal such a complete remnant of this membrane. A typical wing of the flies collected from RNP, with the basal portion more completely shed, is shown in Figure 3B for comparison.

Sexing Adult Flies

The external structure and morphology of the adult male and female are essentially the same. The only difference of note is some degree of sexual dimorphism; the male is generally smaller in size and the abdomen is clearly rounded (Figure 4A). The morphology of the female abdomen presents a distinct bilobed terminex as shown in Figure 4B. The specimen proposed as the neotype is an adult female, designated here as

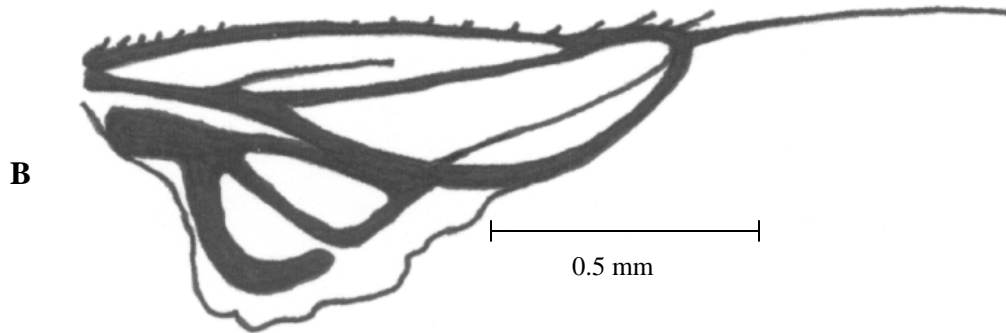
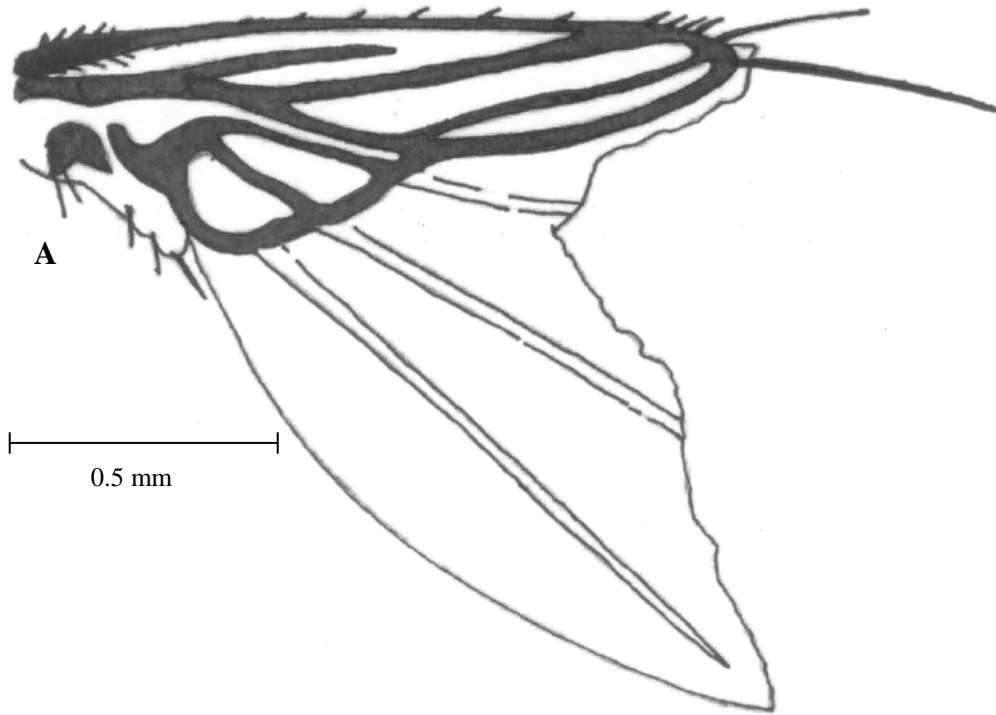


Figure 3. *A. crassipes* wing; (A) with basal portion partially intact (after Theodor & Oldroyd 1965); (B) with basal portion of the wing shed (Specimen 2000.9BF).

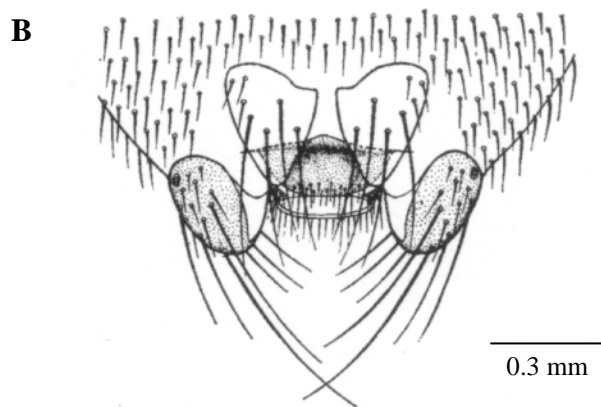
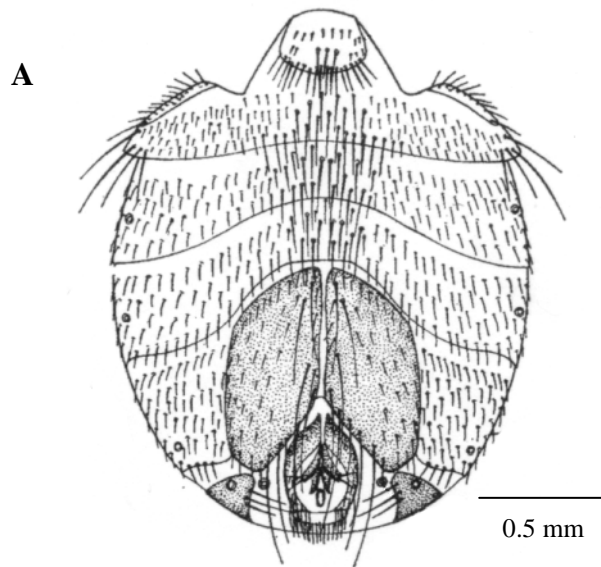


Figure 4. (A) *A. crassipes*, abdomen, male, ventral; (B) *A. crassipes*, posterior part of abdomen, female, ventral. From Theodor & Oldroyd (1965:95-96).

2001.16CF; collected on 7 November 2001 from *P. edwardsi* (individual “Green Orange” group I, Talatekely Trail System), Ranomafana National Park, Madagascar.

DISCUSSION

The description presented above uses the key morphological features discussed by Speiser (1899), Ferris & Cole (1922), Bequaert (1952), and Theodor & Oldroyd (1965). There are some differences, however, with respect to some of the morphological features used by previous researchers and the observation of these features from specimens collected during SJSU expeditions in 2000-2001. For example, Ferris & Cole (1922) incorrectly report that both sexes of *A. crassipes* possess a single, large sclerotized abdominal plate. This is inaccurate as only the female presents a single large sclerotized abdominal plate. The male exhibits two distinct, oval abdominal plates of equal size. This difference, first noted by Theodor & Oldroyd (1965), is consistent with observations of the specimens collected during the SJSU expeditions.

An abdominal feature not previously used in describing the structural differences between male and female flies is the relative density of setae found on the 5th abdominal sternite and the thickness of the individual setae in this region. Observations of the setae found on the 5th abdominal sternite of males is generally denser and individual setal thickness is greater than that observed on the same region in females. Further, some noticeable variation in the density and thickness of these abdominal setae was observed in male specimens. For example, specimens 2000.1BM, 2001.19EM, and 2001.25IM exhibit considerably more densely distributed and thicker setae than specimen 2000.4EM.

All other male specimens examined presented a setal density and thickness somewhere in between these extremes. Comparable variation of setal density and thickness was not observed in the female specimens collected.

Previous descriptions of the wings of *A. crassipes* indicate shedding of the basal portion, leaving a stump with radial veins R_1 to R_{4+5} , the basal sections of medial veins M_{1+2} and M_{3+4} and the anal cell heavily sclerotized (Theodor & Oldroyd 1965).

Although this description is consistent with the observations made of the specimens collected from RNP in 2000-2001, the anal cell was not observed to be entirely closed as had been previously reported (Theodor & Oldroyd 1965). Figure 3 illustrates this observed difference.

The differences in the observations of Speiser (1899), Ferris & Cole (1922) and Theodor & Oldroyd (1965) may be attributed to the limited sample size and the less than ideal condition of the specimens examined. Speiser (1899) reports that the specimens he received were “dried”, and Ferris & Cole (1922) based their descriptions on specimens that were obtained from a single lemur skin housed in the National Museum (Smithsonian Institution) some 60 years after the lemur specimen was collected. In both cases, specific records of host and locality are incomplete. Prior to the SJSU study, the number and condition of the examined specimens were few in number (three by Speiser and six by Ferris & Cole) and were degraded in comparison with the specimens from SJSU reported here. The modern collection and curation of the 101 specimens collected during SJSU field work in 2000-2001 provide the most comprehensive data set thus

established for detailing key morphometric characters of *A. crassipes*, with sufficient sample size for further investigation and discussion of intraspecific variation.

CHAPTER III: Association of *Allobosca crassipes* (Diptera: Hippoboscidae) with the Black and White Ruffed Lemur (*Varecia variegata variegata*) and Milne-Edwards' Sifaka (*Propithecus edwardsi*) in Southeastern Madagascar*

INTRODUCTION

Field research on the relationship between lemurs and their ectoparasites in Ranomafana National Park (RNP, 21°16' S, 47°20' E, elevation 900-1200m) in southeastern Madagascar has resulted in a new association of the hippoboscid *Allobosca crassipes* (Speiser, 1899) with the black and white ruffed lemur (*Varecia variegata variegata* (Kerr, 1792)) and a confirmed association with Milne-Edwards' sifaka (*Propithecus edwardsi* (Grandidier, 1871)). Previously, Maa (1969) noted host records for the lemurids *Eulemur macaco* (Linnaeus, 1766) and *E. rubriventer* (I. Geoffroy, 1850), the lepilemurid *Lepilemur mustelinus* (I. Geoffroy, 1851), and the indriids *Avahi laniger* (Gmelin, 1788) and *Propithecus diadema* (Bennett, 1832). Ferris & Cole (1922) collected six specimens of *A. crassipes* from a skin of *Propithecus edwardsi* housed in the collections of the United States National Museum, but did not provide specific locality or specimen information of the host, and the repository of the six flies is unstated and currently unknown.

MATERIALS AND METHODS

A. crassipes, an obligate ectoparasite restricted to Madagascar, historically has been a little collected species. During San Jose State University expeditions in 2000-2001, individuals of two lemur species (*V. variegata variegata* and *P. edwardsi*) were

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tranquilized as part of a larger study investigating the biogeochemical effects of anthropogenic disturbance in lemur populations within RNP (McGee & Vaughn 2007, McGee et al. 2008). Collection methods are in accordance with the protocol approved by San Jose State University's Institutional Animal Care and Use Committee (SJSU IACUC #728). A total of 101 specimens of *A. crassipes* were obtained from *V. variegata variegata* and *P. edwardsi*. Ectoparasites were collected within 30 minutes of capture using forceps and a flea comb. Specimens were preserved in 80% ETOH. Precautions to prevent host contamination by ectoparasites from different individuals included separation and isolation of lemurs prior, during and after ectoparasite collection. Cetyl chloride II was used to cleanse and disinfect all examination surfaces.

RESULTS

On 7 December 2000, three hippoboscids were collected from an immature male of *V. variegata variegata* (identified by University of Texas banding collar as Blue Green Group II, a male weighing 5.7 kg) from the Vatoharanana trail system in RNP. The following year, 11 additional hippoboscids were collected between 13 November and 17 November 2001 from five individuals of *V. variegata variegata*: Red Gold II, male, 3.4 kg (3 flies); Yellow Green, male, 3.3 kg (2 flies); Radio I, male, 3.0 kg (2 flies); Red Blue, female, 4.05 kg (2 flies); and Blue Green I, male, 3.85 kg (2 flies) also from the Vatoharanana trail system. A total of 5 male and 9 female flies were collected from *V. variegata variegata*.

The 2000-2001 expeditions also yielded 87 specimens of *A. crassipes* from

another species of lemur, *P. edwardsi*, in RNP. These confirm a host record that previously was noted only from a single museum specimen of *P. edwardsi* by Ferris & Cole (1922).

Since Speiser's (1899) initial identification of an association of *A. crassipes* with the diademed sifaka, *P. diadema*, and Ferris & Cole's (1922) subsequent identification of *A. crassipes* with *P. edwardsi*, the taxonomy of *Propithecus* has undergone significant reinterpretation and rearrangement, which may lead to some confusion about host records. *P. diadema* and *P. edwardsi* were initially described as separate species in the 1800s by Bennett (1832) and Grandidier (1871), respectively. Petter et al. (1977) and Tattersall (1982), however, considered the diademed sifaka and Milne-Edwards' sifaka as polytypics of a single species, *P. diadema*. Following Tattersall (1986), four subspecies of *P. diadema* were recognized (*P. diadema diadema*, *P. diadema edwardsi*, *P. diadema candidus* (Grandidier, 1871), and *P. diadema perrieri* (Lavauden, 1931)), each distinct phenotypes with nonoverlapping ranges in eastern and northern Madagascar. The use of subspecific designations, however, predates Petter et al. (1977) and Tattersall (1982, 1986); one particularly noteworthy example comes from Bequaert (1952) who refers to an association of *A. crassipes* with *P. diadema edwardsi*. The subspecies designations of *Propithecus* remained in use until Mayor et al. (2004) gave all subspecies specific status on the basis of morphometric and genetic data. Groves and Helgen (2007) supported this classification with additional morphometric data. With *P. diadema* and *P. edwardsi* once again considered distinct species, the associations of Speiser (1899) and Ferris & Cole (1922) are consistent with current taxonomic usage.

Between 19 November and 29 November 2000, flies were collected from seven individuals of *P. edwardsi* from the Talatakely trail system: Turquoise, male, 4.8 kg (1 fly); Blue Blue, female, 5.3 kg (7 flies); Blue Green IV, female, 5.2 kg (2 flies); Blue Purple, male, 5.3 kg (6 flies); “No collar”, female, 4.5 kg (9 flies); Pink IV, male, 5.4 kg (4 flies); and Purple IV, male, 4.9 kg (2 flies). The following year on 11 November 2001, flies were collected from three individuals of *P. edwardsi* from the Talatakely trail system: Blue IV, male, 5.3 kg (1 fly); Green Orange I, male, 5.9 kg (4 flies); and “No collar I”, male, 2.9 kg (2 flies). A total of 21 male and 17 female flies were collected from *P. edwardsi* at Talatakely.

A. crassipes was also collected from *P. edwardsi* along the Vatoharanana and Valohoaka trail systems in RNP. Between 27 November and 5 December 2000, 12 flies were collected from four individuals of *P. edwardsi* from the Vatoharanana trail system: Red I, male, 5.3 kg (1 fly); Green Gold III, female, 5.5 kg (2 flies); Purple Gold III, male, 5.4 kg (2 flies); and Yellow Blue III, female, 5.6 kg (7 flies). The following year, between 12 November and 16 November 2001, 35 flies were collected from eight individuals of *P. edwardsi* along the Vatoharanana trail system: Brown Blue III, male, 5.8 kg (4 flies); Green Gold III, male, 5.0 kg (4 flies); Green Silver I, male, 3.5 kg (6 flies); Green Silver Red II, male, 3.05 kg (1 fly); Pink Silver II, male, 4.60 kg (6 flies); Purple Blue I, male, 5.4 kg (4 flies); Radio I, male, 5.35 kg (9 flies); Radio II, male, 5.90 (1 fly). A total of 26 male and 21 female flies were collected from *P. edwardsi* at Vatoharanana. Lastly, on 8 November 2001, two specimens of *A. crassipes* were collected from an individual of *P. edwardsi* along the Valohoaka trail system: Pink Blue

IV, female, 6.25 kg (2 flies). A total of two male flies (no females) were collected from *P. edwardsi* at Valohoaka.

DISCUSSION

The type specimens of *A. crassipes* referred to by Speiser (1899) are believed to have been destroyed during the World War II bombings of Dresden (N.L. Evenhuis, personal communication 2009). The 14 specimens taken from *V. variegata variegata* from Vatoharanana represent a new host record in Madagascar. The 87 specimens collected from *P. edwardsi* confirm an association previously identified and add to the record detailed information on localities and host animals. Collected specimens are housed at the J. Gordon Edwards Museum of Entomology at San Jose State University, San Jose, CA 95192-0100.

Maa (1963) commented that host specificity in hippoboscids is moderately high, noting in particular that louse flies are found on unrelated species in similar habitats. However, our documentation of the presence of *A. crassipes* on several species of related lemurs that vary in diet and habitat suggests a different type of host specificity of this parasite. The plasticity of the louse fly is remarkable given the differences between ruffed lemurs and sifakas. Ruffed lemurs and sifakas occur sympatrically in eastern Madagascar but have different niches. Ruffed lemurs are obligate frugivores and are patchily distributed in low densities in mid-latitude and lowland rainforests (Garbutt 1999). They are behaviorally and demographically highly sensitive to the availability of fruit, particularly in the dry season (Balko & Underwood 2005). Sifakas, in contrast, rely

less heavily on fruit, switching to leaves during the dry season (Balko & Underwood 2005). Whereas elsewhere ruffed lemurs and sifakas inhabit primary and secondary forest, in Ranomafana National Park, the black and white ruffed lemur inhabits only primary forest. Milne-Edwards' sifaka is more broadly distributed, inhabiting both primary and disturbed forests. On the other hand, these species have comparable home ranges (i.e., distance traveled for food and cover), and at least half of the fruit species consumed by Milne-Edwards' sifaka are also consumed by species of *Varecia* (Dew & Wright 1998, Arrigo-Nelson, 2006). Both *V. variegata variegata* and *P. edwardsi* have been observed to be geophagous (i.e., deliberately ingesting soil). Otherwise habitually arboreal, these lemurs descend to the ground on a regular basis to consume dirt (White 1989, Arrigo-Nelson et al. in press). *A. crassipes* most likely emerges from the pupa with fully developed wings, with the basal membrane lost after it locates its host (Theodor & Oldroyd 1965). All flies collected exhibited significant wing reduction (i.e., are flightless), and therefore the geophagous behavior of *V. variegata variegata* and *P. edwardsi* affords the flies increased opportunities for contact and feeding on these hosts. Finally, differences due to habitat disturbance are likewise not likely to differentiate the distribution or abundance of *A. crassipes*. Wright et al. (2009) found that there was no significant difference in infection intensity of hippoboscids on *P. edwardsi* inhabiting disturbed and undisturbed forests in Ranomafana National Park: on average, 1.25 to 2.48 flies were found on *P. edwardsi* in Ranomafana. Of the two lemur species, *V. variegata variegata* has the most narrow or specialized dietary and habitat requirements. Future study might then focus on characteristics that are restrictive in the life history of the host

species, i.e., in the case of *V. variegata variagata*, the distribution and phenology of fruiting tropical trees that may have played a role in the ecology and evolution of this host-parasite interaction.

CHAPTER IV: Effect of Habitat Disturbance on Stable Isotope Composition of *Allobosca crassipes* (Diptera: Hippoboscidae) from Southeastern Madagascar

INTRODUCTION

A biodiversity hotspot is defined as a biogeographic region with a significant reservoir of biodiversity that is under threat from humans (Myers et al. 2000). Anthropogenic habitat disturbance in Madagascar is well documented, with over 90% of the native vegetation lost, mostly due to the practice of slash-and-burn agriculture (Wright 1992). This practice, as well as commercial logging, has occurred in areas that are within and around the boundaries of Ranomafana National Park (RNP), as recently as the late 1980s. Within RNP, about 50% of the vegetation has been altered by selective logging and other anthropogenic activities (Wright 1997). International teams have been studying the effects of anthropogenic disturbance on biodiversity in RNP for three decades (see Wright 1997). For example, since 2000, Dr. Elizabeth McGee (San Jose State University) has been looking at the effects of anthropogenic disturbance on the biogeochemistry of two lemurs, *Propithecus edwardsi* and *Varecia variegata variegata*. As part of her analysis, she has used stable isotope analysis to develop a profile detailing carbon and nitrogen composition across habitats and through time. I suggest that anthropogenic disturbance will track biogeochemically between primary consumers (lemurs) and secondary consumers (louse flies). To quantify these effects, I employed the same analysis of stable carbon and nitrogen as was done by McGee & Wright (2001), McGee & Vaughn (2003, 2006, 2007), McGee et al. (2007, 2008).

STABLE ISOTOPE ANALYSIS

Stable isotope analysis covers a broad spectrum of applications that take advantage of the phenomenon of fractionation between isotopes of the same element due to differences in mass. The use of stable isotopes was initially used by geophysicists (Hobson & Wassenaar 1999) to compare differential changes in the ratios of isotopes to established standards to examine physical phenomena such as climate change, etc. (Burk & Stuiver 1981; Yapp & Epstein 1982). The earliest applications in ecology were from DeNiro & Epstein (1978) and DeNiro & Epstein (1981) who examined the influence of diet on the distribution of carbon isotopes and nitrogen isotopes in animals, respectively. This technique has also been used by researchers in archeology to reconstruct the diets of historic and prehistoric human populations (Ambrose & DeNiro 1986) and in paleontology to identify the diets of extinct animals (Koch et al. 1994).

Regarding its ecological application, isotope analysis provides information about energy transfer in an ecosystem, i.e., the isotopic composition of organisms, yields important data that indicate how energy flows through the biosphere. Recent research suggests that the carbon and nitrogen isotope composition of primate hair is an indicator of habitat use and diet, respectively (Schoeninger et al. 1997, 1998). More recently, studies indicate that stable isotope analysis may be useful as a tool to track disturbance in rainforest ecosystems in that changes in isotope composition will track environmental changes (natural and anthropogenic) to the extent that habitat and diet are affected (McGee & Wright 2001; McGee & Vaughn 2003, 2006, 2007; McGee et al. 2007, 2008).

Several elements occur as stable isotopes (Table 2). For the purpose of this study, the stable isotopes of carbon (^{12}C and ^{13}C) and nitrogen (^{14}N and ^{15}N) were used. Average terrestrial abundance (%) for these elements is shown below in Table 3.

Table 3: Average Terrestrial Abundance for Carbon and Nitrogen

Element	Isotope	Average Terrestrial Abundance (%)
Carbon	^{12}C	98.980
	^{13}C	1.110
Nitrogen	^{14}N	99.630
	^{15}N	0.370

Stable Nitrogen Isotopes

Within animals stable nitrogen is an indicator of diet; specifically the relative amount of protein in an animal's diet. It has been observed that each increase in trophic level in the natural food chain results in a 3‰ enrichment in ^{15}N (DeNiro & Epstein 1981; Minagowa & Wada 1984; Macko & Engel 1991).

Isotopic composition is expressed as a ratio using the δ notation in parts per thousand (‰). Nitrogen isotope composition is:

$$\delta^{15}\text{N}(\text{‰}) = \left[\frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}}}{(^{15}\text{N}/^{14}\text{N})_{\text{standard}}} - 1 \right] \times 1000$$

The standard for nitrogen is atmospheric air.

Stable Carbon Isotopes

Stable carbon isotopes have been used to determine where an animal feeds in a rainforest ecosystem (Ambrose & DeNiro 1986). Several researchers have noted the presence of a vertical cline (the “canopy effect”) in the change in ^{13}C in leaves from the forest floor to the top of the canopy in these ecosystems (Vogel 1978; Medina & Minchin 1980; Cormie & Schwarcz 1994, 1996). Thus, in a rainforest ecosystem, ^{13}C is a useful indicator of habitat use. Carbon isotope composition is:

$$\delta^{13}\text{C}(‰) = \left[\frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1 \right] \times 1000$$

The standard for ^{12}C is the Peedee belemnite marine limestone.

HYPOTHESES

Previous research has suggested that habitat modification due to anthropogenic disturbance is reflected biosynthetically in the stable isotope composition key plant and animal species in the Ranomafana National Park ecosystem. Specifically, isotope composition of hair from lemurs inhabiting the disturbed forest (Talatekely) in RNP is depleted in stable nitrogen (^{15}N); stable carbon (^{13}C), however, does not show any biologically significant difference between disturbed versus undisturbed forests (Vatoharanana and Valohoaka) (McGee & Wright 2001; McGee & Vaughn 2003, 2006, 2007; McGee et al. 2007, 2008).

The purpose of this research is to determine how habitat disturbance affects nutrient cycling and energy flow through this rainforest ecosystem as it applies to the host-parasite relationship between the ectoparasitic fly, *A. crassipes* (Diptera:

Hippoboscidae) and its host, the Milne Edwards' sifika, *Propithecus edwardsi* (Primates: Indriidae). For stable nitrogen, the null hypothesis is that there is no difference in stable nitrogen in *A. crassipes* inhabiting disturbed versus undisturbed forests in RNP. The alternate hypothesis is that stable nitrogen in *A. crassipes* inhabiting disturbed forests will show nitrogen depletion. For stable carbon, the null hypothesis is that the patterns observed in carbon isotope composition in *A. crassipes* will be comparable to those seen in *P. edwardsi*, i.e. there will be no biologically meaningful association between carbon isotope composition and forest disturbance. The alternate hypothesis is that there will be a biologically meaningful association between carbon isotope composition and forest disturbance.

MATERIALS AND METHODS

Lemurs were darted using the Pneu-Dart system (Pneu-Dart, Inc., HC 31, Williamsport, PA). This system uses non-barbed, disposable darts with a 3/8-in needle delivered from either a blowgun or from a CO₂ powered rifle. Chemical immobilization of darted lemurs is accomplished by using Telazol, a nonnarcotic anesthetic at the standard dosage of 25 mg/kg of body weight (Schoeninger et al. 1997). All protocols used in animal darting are approved by the Institutional Animal Care and Use Committee (IACUC # 728) at San Jose State University.

After darting, the injected lemurs usually fall from the trees within 2-10 minutes and are caught using 6-ft by 10-ft nylon nets by Malagasy darting teams. These teams carry the animals to the field research station where hair sampling and ectoparasite collection takes place. While the lemurs are anesthetized, a small amount of hair is taken

at the base of the neck and tail for isotope analysis of the hair. The lemurs are then “combed” for ectoparasites using a cat flea comb (PETCO SKU 320951) and forceps.

Table 4 shows the number of flies collected from each locality in RNP.

Table 4: Count of *A. crassipes* by Locality

Location	Number of flies from <i>P. edwardsi</i>	Average number of flies per individual
Talatakely (disturbed)	38	3.8
Vatoharanana (undisturbed)	45	4.09
Valohoaka (undisturbed)	4	2

Specimen Preparation - Hair

Hair specimens were washed in a 1:1 chloroform/methanol solution and then air dried. The resulting product is weighed in an analytical balance and 2-3 mg is placed in Sn foil boats (Costech, 5x9 ml tin capsules). Samples were shipped to the Stable Isotope Ratio Facility for Environmental Research (SIRFER) at the University of Utah for analysis on a dual inlet Finnigan MAT model 252 Isotope Ratio Mass Spectrometer (IRMS) coupled with a Carlo-Erba EA operating at continuous flow (analytical precision was $\pm 0.15\%$).

Specimen Preparation - Parasites

Fly specimens, after being stored in 80% ETOH, were air dried and submerged in liquid nitrogen. Specimens were then weighed and, if less than 2 mg, placed in Sn foil boats (Costech, 5x9ml tin capsules). Specimens over 2 mg were cut and a 2 mg sample

was placed in the Sn capsule. Samples were shipped to the Stable Isotope Ratio Facility for Environmental Research (SIRFER) at the University of Utah for analysis on a dual inlet Finnigan MAT model 252 Isotope Ratio Mass Spectrometer (IRMS) coupled with a Carlo-Erba EA operating at continuous flow (analytical precision was $\pm 0.15\%$).

RESULTS

Results from the study on lemur isotope composition (McGee & Wright 2001; McGee & Vaughn 2003, 2006, 2007; McGee et al. 2007, 2008) show discrete differences in the ratios of ^{13}C and ^{15}N in *P. edwardsi* from disturbed versus undisturbed habitats. The stable carbon values range from approximately -23% to -24% in Talatekely and from -23.3% to -22.5% in Vatoharanana and Valohoaka. The stable nitrogen values range from approximately 1.5% to 2.9% in Talatekely and from 2.9% to 4% in Vatoharanana and Valohoaka. Figure 5 shows stable nitrogen plotted against stable carbon for *P. edwardsi* in disturbed versus undisturbed forests.

Although differences are seen in both stable carbon and nitrogen, only nitrogen could be considered biologically significant. Figure 5 shows a maximum of 4% for lemurs inhabiting undisturbed sites and a minimum of 1.5% for lemurs inhabiting disturbed sites. As noted previously, each trophic level is represented by a 3% difference in stable nitrogen. Therefore, a difference of 2.5% in the same species of lemur inhabiting forests that are comparable except in degree of disturbance is truly exceptional. These differences are verified statistically (Single Factor ANOVA, $p < .001$; $df = 53$). The range of values in lemurs for stable carbon between disturbed and undisturbed forest,

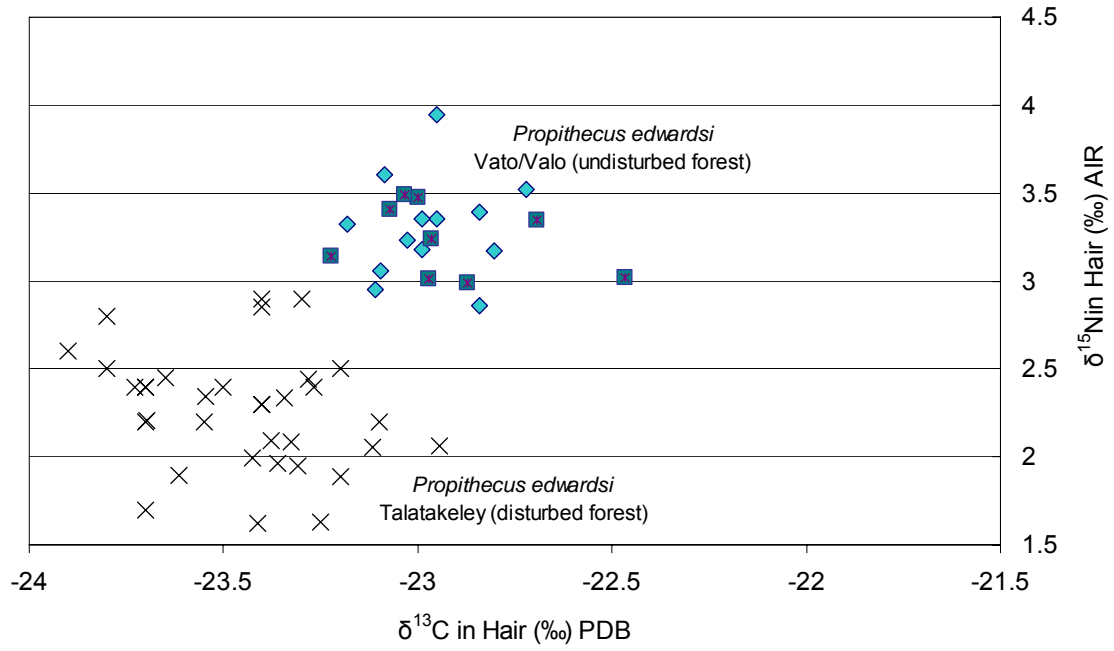


Figure 5: Biogeochemical Profile of *Propithecus edwardsi*

on the other hand is at most 1.5‰ and while is statistically significant (Single Factor ANOVA, $p < .001$; $df = 53$) is unlikely to be a biologically significant difference.

Results from this study on isotope composition in *A. crassipes* also show discrete differences in the ratios of ^{13}C and ^{15}N from disturbed versus undisturbed forests. The stable carbon values range from -23.9‰ to -25.7‰ in Talatekely and from -24.2‰ to -24.8‰ in Vatoharanana. The stable nitrogen values range 7.4‰ to 8.5‰ in Talatekely and from 8.6‰ to 9.7‰ in Vatoharanana (Table 5).

Table 5: Stable isotope values for *A. crassipes*

	n	$\delta^{15}\text{N}$ (AIR) (‰)			$\delta^{13}\text{C}$ (PDB) (‰)		
		x	SD	Range	x	SD	Range
<i>A. crassipes</i> Talatekely	5	8.0	0.43	7.4, 8.5	24.8	0.6	-25.7, -23.9
<i>A. crassipes</i> Vataharanana	7	9.0	0.36	8.6, 9.7	24.5	0.2	-24.8, -24.2

Figure 6 shows stable nitrogen plotted against stable carbon for *A. crassipes* in disturbed versus undisturbed forests. Stable carbon and nitrogen isotopes for the disturbed forest of Talatekely and the undisturbed forest of Vatoharanana form distinct clusters. Stable nitrogen values for *A. crassipes* feeding on lemurs from disturbed versus undisturbed forests are significantly different (Single Factor ANOVA, $p = .001$, $df = 11$).

There is no significant difference in the stable carbon isotope composition of *A. crassipes* feeding on lemurs in disturbed versus undisturbed forests (Single Factor ANOVA, $p = .31$, $df = 11$). The values of ^{13}C of *A. crassipes* is between -24 and -25.5,

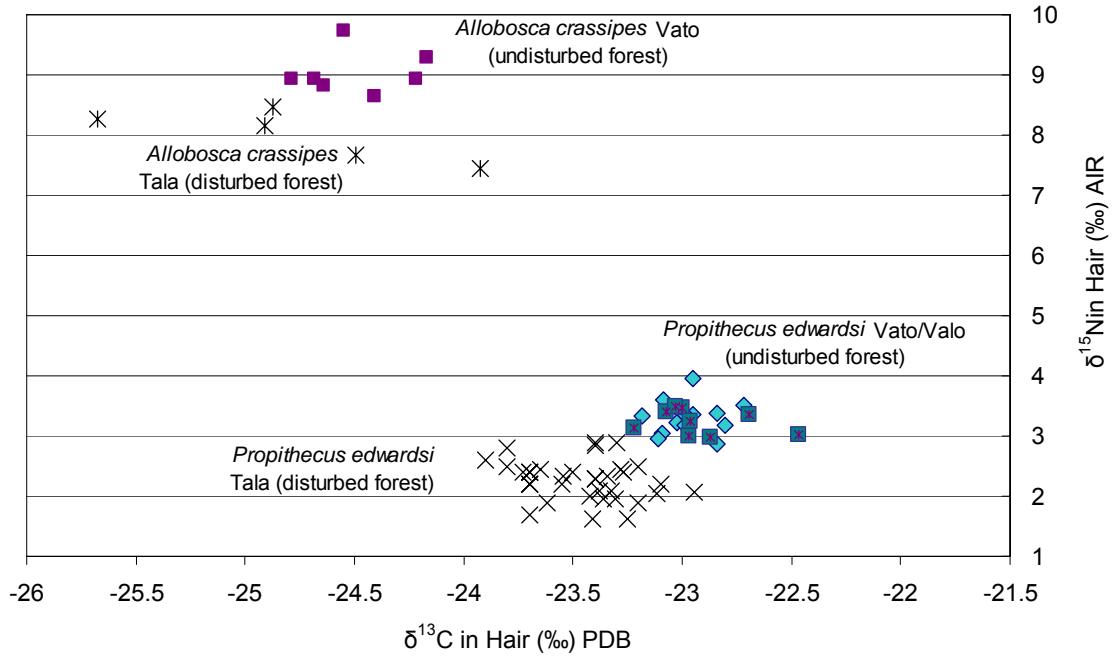


Figure 6: Biogeochemical Profile of *Allobosca crassipes* with *Propithecus edwardsi*

and spans both Talatekely and Vatoharanana. The range of ^{13}C in lemurs is comparable (1.5‰), however, within this range the lemurs from Talatekely are more carbon depleted than the lemurs from Vatoharanana so the differences are statistically different. As noted previously, a difference of 1.5‰ in stable carbon (which in C_3 plants range from -31 to -33) is not likely to be biologically significant.

CONCLUSION

I analyzed flies collected from *P. edwardsi* in Talatekeley, Vatoharanana and Valohoaka to compare their isotope ratios with those of the lemurs. The isotope ratios observed in the flies collected from *P. edwardsi* track the depletion of stable nitrogen observed in lemur hair from previous studies at these sites. The data presented here support the hypothesis that the stable nitrogen composition of *A. crassipes* collected from lemurs inhabiting disturbed forests is depleted compared to flies collected from lemurs in undisturbed forests. Further, the patterns in stable carbon isotopes of *A. crassipes* between disturbed and undisturbed habitats are comparable to those seen in *P. edwardsi*, i.e., there is no association between stable carbon isotopes and habitat disturbance.

On average, the stable nitrogen isotope composition of *A. crassipes* is one trophic level greater than that of *P. edwardsi*, and a distinct difference is seen in the flies and lemurs inhabiting disturbed and undisturbed forests. These data further validate stable isotope ecology as a means of identifying habitat disturbance at the level of ecosystem in rain forests.

CHAPTER V: Conclusions

The limited sample size and the degraded condition of the specimens of *Allobosca crassipes* examined by previous researchers historically have made the identification and the differentiation of species and sex of this fly problematic. These earlier descriptions of key morphological features such as the abdominal and wing characteristics are at best, inconsistent. The collection and curation of the 101 specimens collected in 2000-2001 provide the most comprehensive assemblage of *A. crassipes* yet established for taxonomic use in the description of this species. The observations recorded from my examination of this modern collection provide continuity and clarity in the use of these key characters used in the identification of *A. crassipes*. The 87 specimens housed in the J. Gordon Edwards Museum at San Jose State University represent the largest collection known, for use in study of the taxonomy and systematics of *A. crassipes*.

Host specificity in the family Hippoboscidae has been described as moderately high, with flies being found on unrelated host species in similar habitats Maa (1963). The new host association of *A. crassipes* with the Black-and-White Ruffed lemur, *Varecia variegata variegata* and confirmed host association with the Milne-Edwards sifaka, *Propithecus edwardsi* is further evidence that supports the pleioxyeny of *A. crassipes*, first suggested by Bequaert (1953). The postulated wing-shedding of the louse fly render it flightless, but these non-vagile flies still have opportunity for host acquisition if the lemurs light on the ground. The geophagous behavior of *V. variegata variegata* and *P. edwardsi* present questing flies increased opportunities the parasite to acquire a host and expand its host associations. Clearly, host specificity in *A. crassipes* is higher

than previously thought, and the new association with *V. variegata variegata* invites further investigation into the ecology of this parasite and its hosts.

Regarding ecosystem level effects of habitat disturbance, the depletion of stable nitrogen of *A. crassipes* in disturbed habitats tracks with the depletion seen in the lemurs feeding in disturbed habitats (McGee & Vaughn 2003). The predicted enrichment of stable nitrogen in the flies collected from lemurs in undisturbed forests is further evidence of the use of stable isotope analysis as a tool in quantifying trophic changes in the rainforest ecosystem due to anthropogenic activity.

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