# Host specificity, prevalence and intensity of infestation of fleas (Order Siphonaptera) of small mammals at selected sites in the city of Windhoek, Namibia

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#### Abstract

Small mammals host diverse communities of parasites including fleas. There is considerable research interest in effects of parasites on their hosts. Host specificity, prevalence and intensity of infestation of fleas on small mammals were studied at selected sites in the city of Windhoek, Namibia from April to July 2005. Small mammals were live-trapped using Sherman traps and autopsied before collection of fleas. Fleas were processed using standard parasitological procedures and were mounted permanently onto slides using Canada balsam. Small mammal hosts and fleas were identified to species level. A total of sixty one (61) small mammals belonging to four rodent species, i.e. bushveld gerbil Gerbilliscus leucogaster, hairy-footed gerbil Gerbillurus paeba, black-tailed tree rat Thallomys nigricauda and the four-stripped mouse Rhabdomys pumilio and one insectivore, bushveld sengi Elephantulus intufi, were captured. One hundred and thirty six (136) fleas belonging to eight species, i.e. Xenopsylla brasiliensis, Xenopsylla cheopis, Xenopsylla hirsuta, Xenopsylla trispinis, Dinopsyllus ellobius, Dinopsyllus zuluensis, Epirimia aganipes and Listropsylla aricinae were collected from infested hosts. Dinopsyllus ellobius and X. trispinis and L. aricinae were host specific, being collected only from G. leucogaster and G. paeba, respectively. No fleas were collected from E. intifi and R. pumilio. The prevalence of fleas ranged from zero in E. entufi and R. pumilio through 50 % in T. nigricauda, 55.1% in G. leucogaster to 61.1% in G. paeba. High species richness of fleas was recorded in G. leucogaster (seven out of eight flea species) and in G. paeba (six out of eight flea species). The overall prevalence of fleas was higher in male (54.3%) than in female (34.6%) hosts. There was no association between the body mass of small mammal hosts and the intensity of flea infestation. The intensity of infestation of fleas did not vary significantly by host species and sex of hosts. .

Keywords: Fleas, prevalence, intensity of infestation, small mammals, Namibia.

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## **1** Introduction

Small mammals host diverse communities of parasites including fleas. There is considerable research interest in the effects of parasitic infections (endoparasites) and infestations (ectoparasites) on their hosts (Tavassoli et al. 2010; Rahman et al. 2009; Case et al. 2006; Akucewich et al. 2002; Begon et al. 1996 and Freeland 1983) because of the medical and veterinary importance of parasites. For example, ectoparasites including fleas (Order Siphonaptera), are important vectors of pathogens. The *Murine typhus* is transmitted to humans by fleas (Goddard 1998). The bubonic plague, caused by the bacterium *Yersinia pestis* is commonly transmitted from wild and commensal rodents to humans by the flea *Xenopsylla cheopis* (Borror & De Long 1964; Perry and Fetherston 1997). Plague outbreaks have been reported in Namibia between 1983 and 1997 (Eiseb 2002). Parasite-induced host mortality is common in mammals including humans. For example, malaria, which is caused by *Plasmodium falciparum*, is one of the most important parasitic diseases of man (WHO 1997). A study by Scott (1987) showed that high parasitic loads of the intestinal nematode *Heligmosomoides polygyrus* caused 10% mortality of laboratory mice. Endoparasites such as nematodes have also been reported to alter the behaviour of parasitized wood mouse *Apodemus sylvaticus* (Brown et al. 1994)

Fleas are amongst the most common ectoparasites of small mammals. A wide range of studies have been undertaken on flea-host interactions. Some studies have documented the flea host range, abundance and species diversity (Ritz et al. 2012; Rahman et al. 2009; Presley and Willig 2008; Lareschi et al. 2004; Oguge et al. 1997). Other studies reported the prevalence of infestation of fleas on small mammal hosts (Oguge et al. 2009; Eiseb 2002). More recently interest has shifted towards investigating the effects or influence of various ecological parameters on the flea-host interactions such as density of the host species (Krasnov et al. 2004a), evolution of flea-host specificity (Wellis et al. 2011; Poulin et al. 2006; Krasnov et al. 2004b), the influence of body size of the host (Presley 2008; Freeland 1983; Kuris et al. 1980), age and sex of the host (Presley 2008; Krasnov et al. 2005) and season (Krasnov et al. 2005; Makundi and Kilonzo 1994).

In Namibia, the prevalence, intensity of infestation and species diversity of ectoparasites especially fleas, are largely understudied despite their potential as vectors of diseases. Although up to 8 families (*Pulicidae, Chimaeropsyllidae, Tungidae, Rophalopsyllidae, Ischnopsyllidae, Leptopsyllidae, Ceratophyllidae, Histrichopsyllidae*) (Holm & Schotz 1985) and approximately 110 species of fleas representing 34 genera, occur in southern Africa (Segerman 1995), little has been documented about fleas of mammal and bird hosts Namibia. A study by Eiseb (2002) on seasonal variation in species composition of fleas inhabiting small mammals at a heavily degraded farmland (Nebaos) and a relatively non degraded farmland (Gella-Ost) in southern Namibia revealed that habitat degradation led to reduction of densities of small mammals and associated flea communities. The composition of fleas varied seasonally, being higher during the hot dry season than during the hot wet season (Eiseb 2002). A study by Amutenya (2004) revealed that differences in vegetation structure especially grass cover influenced densities of small mammal hosts and associated prevalence, species diversity and intensity of infestation of fleas on small mammals at selected sites in Gross Hertzog farm, Gamsberg and a habitat in Pioneers Park in Windhoek.

The study was therefore carried out in selected habitat sites in the city of Windhoek, Namibia with the following objectives:- to determine the proportion (or prevalence) of small mammal hosts that were infested with fleas, to estimate the abundance (or intensity of infestation) of different species of fleas of small mammal hosts, to compare the species diversity of fleas on small mammals by species and sex of the host and to infer host specificity of fleas.

## **2** Materials and Methods

### 2.1 Study area

The study was conducted at four different sites, all within the city of Windhoek i.e. a habitat near the Kupferberg dump site, Olympia suburb, wooded habitats on western side of University of Namibia main Campus and at Avis Dam, from April to July 2005. The Kupferberg habitat had dense and breast-high grass along the river and was dominated by *Catophractes alexandrii* shrubs. The Olympia site was dominated by tall *Acacia* trees (approximately 3-5m) and *Ziziphus macronata* trees and had a low grass cover. The habitat near the University of Namibia was characterized by a thick, short and diverse grass cover while shrubs of *Acacia mellifera* and *C. alexandrii* made up the dominant woody vegetation. The habitat at Avis Dam mainly consisted of *A. erioloba* trees, the alien *Datura* species, *C. alexandrii* shrubs and dense grass cover of *Stipagrostis* species.

#### 2.2 Field trapping of small mammal hosts

At each trapping site, fifty Sherman-live traps, baited with rolled peanut butter mixed with oats, were set in a line transect. Traps were placed 10 metres apart. They were set before sunset and inspected at dawn or early morning around 08h00. Traps were set for 4 consecutive nights at each site. All captured small mammals were taken in the traps while still alive, to the laboratory for processing and collection of fleas.

## 2.3 Collection of fleas from hosts in the laboratory

All trapped small mammal hosts were individually euthanized using cotton wool soaked in chloroform and placed in plastic Ziploc bags. This was done to avoid mixing ectoparasites amongst host specimens and to ensure that all ectoparasites were dead prior to collection from the host. Use of chloroform to euthanize small mammals is a standard human method commonly used for such studies. All dead small mammal hosts were removed from the Ziploc bags and brushed vigorously with a fine toothbrush while holding the animal above a white tray to dislodge and remove ectoparasites. All ectoparasites from each host were placed in a petri dish and inspected for fleas under a Olympus SZ51 (Model SZ-ILST) dissecting microscope. Fleas (along with other ectoparasites) were picked from the petri dish with a pair of fine forceps. Ziploc bags, in which hosts were euthanized, were searched thoroughly to collect any remaining ectoparasites. All ectoparasites were stored in labelled vials containing 70% alcohol.

All individual hosts were identified to species level using the identification keys developed by Skinner and Smithers (1990) and Mills and Hes (1997) for all terrestrial mammalian species occurring in the southern African sub-region. Species identifications were confirmed at the National Museum of Namibia. The following were also recorded for each host:- body mass (to the nearest gram), measured using a Pesola spring balance, sex and standard measurements including:- tail length, ear length, hind foot length and head body length. These standard measurements aid in the identification of small mammals to species level (Gurnell and Flowerdew 1989). Habitat site, date of collection and a specific identification number for each host were also recorded. Host specimens were refrigerated and stored.

#### 2.4 Processing and identification of flea specimens

Fleas were prepared using the following standard procedure (Peterson 1981). Fleas were removed from storage vials in 70% alcohol and were paced in distilled water for one hour to rinse alcohol off the specimens. Flea specimens were then transferred into petri dishes containing 15% Potassium hydroxide (KOH) and incubated at room temperature for 4 days to clear/dissolve endodermal and mesodermal tissues, leaving only the exo-skeleton which is required for the identification of fleas. Flea specimens were placed in distilled water for one hour to remove KOH and neutralized using 10% acetic acid for 30 minutes. Rinsing of KOH in distilled water was repeated once.

The specimens were subsequently dehydrated using different strengths of alcohol:- 70% for 30minutes, 80% for 30 minutes, 96% for 30 minutes, and absolute alcohol for an hour. Flea specimens were placed in oil of cloves (Peterson 1981) and mounted permanently onto microscope glass slides using Canada balsam. The slides were air dried, ready for identification of fleas to species. Specimens of other ectoparasites including ticks, mites and lice were put in labelled vials and stored in 70

The fleas were identified using a standard key developed by Segerman (1995) for fleas known to occur in the sub-region of Southern Africa including Namibia. The flea specimens were examined under a compound binocular microscope and identified to species level. Identifications were confirmed at the National Museum of Namibia. All fleas on each small mammal host were counted and recorded.

#### 2.5 Data analysis

In the present study, only data for fleas were processed and analyzed. The number of small mammal hosts, by species and sex, that harbored or were infested by fleas was calculated and expressed as percentage (%). This proportion is referred to as the prevalence of infestation of small mammals by fleas. Intensity of infestation of small mammals by fleas was calculated as the total number of fleas per infested host. This was done for each host species and for male and female hosts. The prevalence and intensity of infestation were recorded.

Species diversity of fleas was calculated for each infested host species and for male and female small mammals following the Shannon-Wiener index measure of biological diversity. The index is given by  $H = -\sum p_i \ln p_i$ , where H is diversity index,  $p_i$  is the proportion of individuals belonging to the *i*th species and ln is the natural log (Krebs, 1994).

The intensity of infestation and the Shannon-Wiener index of diversity data were tested for normality following the Kolmogorov-Smirnov normality test using the Minitab 12.1 statistical software. The intensity of infestation data was non-normally distributed (D = 0.336, n = 61, p < 0.01) hence non-parametric statistical tests were used in the analysis of the data.

## **3** Results

## 3.1 Host and flea species

A total of 61 small mammals belonging to four rodent and one insectivore species were captured in the study (Table 1). The rodent species included the bushveld gerbil *Gerbilliscus leucogaster*, hairy-footed gerbil *Gerbillurus paeba*, black-tailed tree rat *Thallomys nigricauda* and the fourstriped mouse *Rhabdomys pumilio*. The bushveld sengi *Elephantulus intufi* was the only insectivore trapped in the study.

Table 1: The median intensity of fleas (median number per infected host), irrespective of species of fleas for *G. leucogaster* and *G. paeba* captured during this study. *n*=sample size of hosts.

Flea species	Small mammal host species					Total
	G. leucogaster	G. paeba	T. nigricauda	E. intufi	R. pumilio	
	( <i>n</i> = 29)	( <i>n</i> = 18)	( <i>n</i> = 2)	( <i>n</i> = 11)	( <i>n</i> = 1)	( <i>n</i> = 61)
Xenopsylla brasiliensis	16	23	14	0	0	53
Xenopsylla cheopis	14	11	31	0	0	56
Xenopsylla hirsuta	5	1	0	0	0	6
Xenopsylla trispinis	1	0	0	0	0	1
Dinopsyllus ellobius	2	0	0	0	0	2
Dinopsyllus zuluensis	11	3	0	0	0	14
Epirimia aganipes	1	1	0	0	0	2
Listropsylla aricinae	0	2	0	0	0	2
Totals	50	41	45	0	0	136

A total of 136 fleas belonging to eight species were collected from small mammals that were trapped (Table1). Seven out of eight flea species were recovered from *G. leucogaster* and six out of 8 species of fleas were collected from *G. paeba*. No fleas were recovered from *R. pumilio* and *E. intufi*. Despite the small sample size, *T. nigricauda* (n = 2) harboured comparatively high abundance of fleas (n = 45) from only two species namely *Xenopsylla brasiliensis* (n = 14) and *Xenopsylla cheopis* (n = 31) indicating heavy flea infestation. Two flea species *X. brasiliensis* (n = 53) and *X. cheopis* (n = 56) were collected from three different host species and made up about 80% of all fleas collected in the present study. The flea *Listropsylla aricinae* was only collected from *G. paeba* whereas *X. trispinis* and *D. ellobius* only occurred on *G. leucogaster*.

#### 3.2 Prevalence

The prevalence of fleas on small mammal hosts was highest in *G. paeba*, followed by *G. leucogaster* and *T. nigricauda* (Figure 1). The prevalence of fleas was higher in males than in female hosts (Figure 2).

#### 3.3 Intensity of infestation of fleas

A Mann-Whitney U test (W = 160, p = 0.7773) revealed that there was no significant difference in the intensity of infestation of fleas between G. *leucogaster* and G. *paeba*, the two species that had

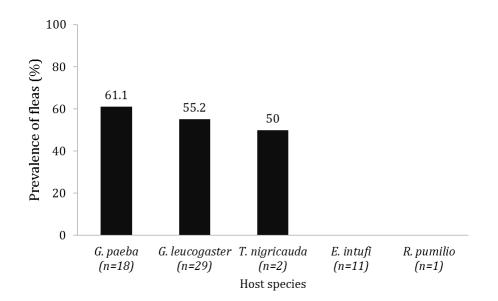


Figure 1: The prevalence (%) of fleas (considering all flea together, irrespective of species) on different species of small mammals captured during the study period in the city of Windhoek. No fleas were collected from *E. intufi* and *R. pumilio.* n= sample size of small mammal hosts.

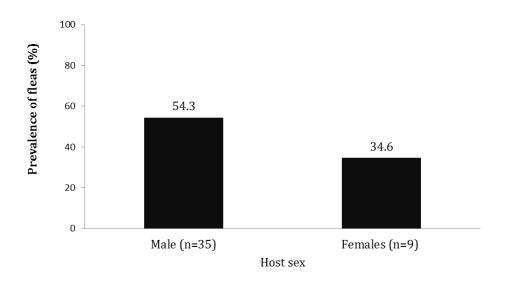


Figure 2: The prevalence (%) of fleas (considering all fleas together, irrespective of species) between male and female hosts captured during the study in the city of Windhoek. n = sample size of hosts.

the highest number of infested hosts in the study area (Figure 3). It was not possible to compute the median intensity for *T. nigricauda* because only one host was infested while *E. intufi* and *R. pumilio* were not infested by fleas in the present study. Hence these are not included in the discussion of the intensity of infestation of fleas in the present study.

Figure 4 revealed that there was no significantly difference (Mann-Whitney U test: W = 245, p = 0.2567) in the median intensity of infestation of fleas between male and female infested hosts.

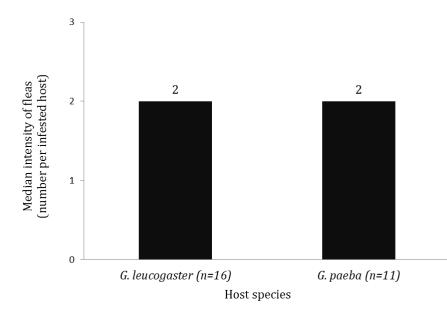


Figure 3: The median intensity of fleas (median number per infected host), irrespective of species of fleas for *G. leucogaster* and *G. paeba* captured during this study. *n*=sample size of hosts.

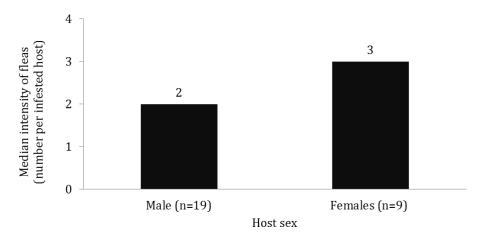


Figure 4: The median intensity of fleas (median number per infected host), irrespective of species of fleas, for male and female hosts captured during this study. n= sample size of infested host species.

#### **3.4** Host body mass and intensity of fleas

Body mass is commonly used as a surrogate for age. It is assumed that hosts with larger body mass are older than those that have smaller body mass within a species (Gurnell and Flowerdew, 1989). We investigated association between body mass and the number of infested fleas on each host. Pearsons coefficient revealed a weak positive correlation (r = 0.3679) between host body mass and the intensity of fleas per host (Figure 5).

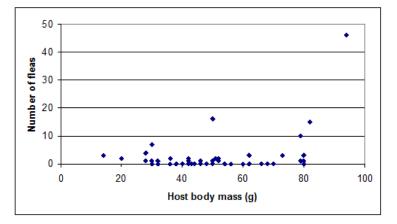


Figure 5: A scatter plot showing the relationship between host body mass (g) and the total number of fleas per small mammal host that were captured.

#### 3.5 Species diversity of fleas on hosts

The Shannon-Wiener index of diversity was used to compare the diversity of flea species in small mammal hosts captured in the study. The species diversity and richness of fleas did not vary significantly between different species of infested small mammal hosts (Table 2). The species diversity of fleas did not vary significantly between male and female small mammal hosts captured in this study (Figure 3).

Table 2: The Shannon-Wiener species diversity and species richness of fleas on different species of small mammals captured from the sites surveyed during this study. *n*=sample size of host species.

Host species	Species richness of fleas	Species diversity of fleas
<i>G.leucogaster</i> $(n = 16)$	7	1.5697
<i>G. paeba</i> $(n = 11)$	6	1.1971

Table 3: The species diversity of fleas (Shannon-Wiener) for male and female hosts captured from the study sites surveyed. *n*=sample size of host species.

Host sex	Species richness of fleas	Species diversity of fleas
Male $(n = 19)$	7	1.6966
Female $(n = 9)$	5	1.0641

## 4 Discussion

## 4.1 Host specificity

The flea species *D. ellobius* and *X. trispinis* exclusively infested the rodent *G. leucogaster* while *L. aricinae* was only recorded from *G. paeba* (Table 1). This pattern suggests that these flea species either prefer these hosts or are host specific. An ectoparasite is considered to be host specific when it is associated with a single species of host (monoxemy) or a group of host species that are closely related phylogenetically (poeixemy) (Esberard et al. 2005). Parasite-host specificity has been recorded in different species. For example the flea *Tarsopsylla octodicemdentata* exclusively exploits the red squirrel *Sciurus vulgaris* (Krasnov et al. 2008), *Nosopyllus elegans puerensis* only infests *Rattus flavipectus* (Guo et al. 1999) and mites of genus *Laelaps Koch* are host specific on small mammals in Brazil (Gettinger 1992). Host specificity is believed to have evolved to reduce the cost of adaptations against multiple host defence systems (Krasnov et al. 2004c) and to reduce competition to enhance survival and co-existence (Rosenweig 1996). There is need to extensively sample for fleas on different small mammals hosts, in different habitats in Namibia in order to ascertain flea host specificity.

The flea species X. brasiliensis and X. cheopis were recorded on three out of five host species (Table 1), and in high abundance in each case, suggesting no host preference or host specificity. When parasites like X. brasiliensis and X. cheopis, are not host specific, they infest many different hosts and thus increase the chance to acquire and transmit pathogens among other rodent hosts (Eiseb 2002; Trpis 1994). The flea species X. cheopis is the principal vector of bubonic plague from rodents to man (Borror & De Long 1964: Perry and Fetherston 1997) and it is probably a successful vector because it is exhibits no host specificity. Similarly, X. brasiliensis is widely distributed in the Afrotropical region and is considered to be an important transmitter of plague in the rural environments (Braack et al. 1996). The observed high infestation of G. leucogaster and G. paeba suggests that these host species may be more susceptible to infestation by fleas. The bushveld gerbil does not generally excavate their own holes instead they live in abandoned rodent burrows (Skinner and Smithers 1990). This behaviour may expose G. leucogaster to fleas of other animals and consequently lead to infestation by different species of fleas. The rodent species R. pumilio, Mastomys species, and G. leucogaster have been found in the plague focal areas in Namibia (Eiseb 2002). The most common flea species associated with these hosts were X. philoxera, X. versuta and X. brasiliensis (Eiseb 2002). Hence X. brasiliensis and X. cheopis have potential to transmit plague in Namibia.

#### 4.2 Prevalence, intensity of infestation and body mass of hosts

In this study, prevalence of infestation was calculated as the number of hosts that were infested by fleas while intensity of infestation was calculated as the number of fleas per infested small mammal host. No fleas were collected from the sengi, *E. intufi* and the rodent *R. pumilio* (Figure 1). Absence of fleas on *R. pumilio* may be attributed to small sample size since fleas have been recovered from *R. pumilio* elsewhere (Matthee et al. 2007). Failure to collect fleas on *E. intufi* despite catching 11 individuals can be attributed to many factors. Perhaps there were no fleas that parasitize the host in the study site, or *E. intufi* has good resistance against ectoparasitic infestations, or because fleas at the study site did not select *R. pumilio* as a host. In the present study, it was noted that *E. intufi* were dirty and had a distinctively strong smelling odor compared to other small mammals captured.

Some fleas may have avoided the sengi on account of the odor. However in a study in the Nama karoo biome in southern Namibia, Eiseb (2002) collected flea species *Macroscelidopsylla albertyni* from *E. intufi*.

The high prevalence of fleas on *G. leucogaster* and *G. paeba* indicates that they were more susceptible to infestation. In ecological terms, the greater the variety of habitats, the greater the species diversity of organisms that inhabit them and this may correspond to the amount of niches available within that particular habitat (Rosenzweig, 1996). Fleas may have found these two rodent hosts more suitable as habitats.

The high prevalence of fleas in male than female rodent hosts in this study (Figure 2) has been commonly reported in many host-parasite prevalence studies (Presley and Willig 2008; Krasnov et al. 2005; Morand et al. 2004). Males are more active and have larger home ranges and hence disperse further than females and have higher chances of being infested by fleas (Morand et al. 2004). In addition, males have higher androgen levels which suppress their immunity leading to reduced immunocompetence in males than females (Forstad and Karter 1992).

A Mann-Whitney U test (W = 160, p = 0.7773) showed that there was no significant difference in the median number of fleas that infested G. *leucogaster* and G. *paeba* (Table 2). Similarly, there was no significant difference in the intensity of infestation of fleas between males and females of the two species (Mann-Whitney U test: W = 245, p = 0.2567) (Table 3). This may be attributed to small sample size of hosts. It is commonly known that in most host-parasite systems, the parasite load exhibits an over-dispersed frequency distribution pattern in which the majority of hosts harbour very few parasites while the majority of parasites are found on very few hosts (Krebs 1989). This highlights the need to aim for large sample size in order to capture both hosts that may have high parasite load and those with few parasites. Although it is not clear why we did not observe a significant difference in the intensity of infestation of fleas between male and female hosts in this study, Presley and Willig (2008) reported that in their study on ectopasites of bats, males harboured fewer ectoparasites than female hosts.

The weak association between host body mass and the number of fleas per infested host (Figure 5) suggests that host body mass (a surrogate for body size) did not significantly influence the intensity of fleas on small mammal hosts. In a study on mammal density and patterns of ectoparasite species richness and abundance, Stanko et al. (2002) reported lack of correlation between host body size and parasite species richness. Similarly, Presley and Willig (2008) did not find a consistent pattern between body size and abundance of ectoparasites on bats. They observed that ectoparasite abundance increased with body size in 12 instances and decreased with body size in 11 instances. In contrast, Oguge et al. (1997) reported a significant positive correlation between host body mass and intensity of infestation of ectoparasites. More surveys should be carried out in different habitats and geographic areas in Namibia to investigate further the influence of host body size and body mass on the intensity of infestation of ectoparasites.

#### 4.3 Species diversity of fleas on hosts

The species diversity and richness of fleas did not vary significantly between *G. leucogaster* and *G. paeba* and between male and female hosts (Tables 2 and 3). The epidemiological hypothesis (Kuris et al. 1980; Anderson and May 1978) predicts an increase in the species richness of ectoparasites with increase in the density or abundance of hosts. Stanko et al. (2002) reported that richness of

ectoparasite communities are significantly influenced by the density of host species. It is not clear why we did not find significant differences in species diversity amongst small mammal hosts and by host sex in the present study. In a study by Amutenya (2004) in selected habitats in Windhoek, species diversity of fleas on small mammals varied between different sites. Eiseb (2002) reported that habitat degradation led to reduction of densities of small mammals and associated flea communities. He noted that the composition of fleas varied seasonally, being highest during the hot dry season than during the hot wet season (Eiseb 2002). More comparative studies should be carried out on flea species diversity on small mammals in Namibia. Such studies should take into account the effects of habitat type, geographic variation of habitats types, season and densities on both small mammal hosts and fleas. For example, it will be important to compare flea species diversity on moist woodland and dry savannah as suggested by Oguge et al. (2009).

In conclusion, the present study has revealed the following: Gerbilliscus leucogaster and G. paeba were infested with the highest numbers of different species of fleas. This suggests that the two species were the most susceptible to infestation by different species of fleas. No fleas were collected from R. pumilio and E. intufi. Although only two individuals of the rodent T. nigricauda were captured, they harboured comparatively high numbers of X. brasiliensis and X. cheopis indicating heavy flea infestation. The flea species X. brasiliensis and X. cheopis were recorded on three different host species and were the most abundant, making up about 80% of all fleas collected in the present study. The flea species L. aricinae was only collected from G. paeba whereas X. trispinis and D. ellobius, only occurred on G. leucogaster. These three species showed host specificity. The prevalence of fleas on small mammal hosts was highest in G. paeba followed by G. leucogaster and T. nigricauda. The present study showed that the prevalence of fleas was higher in males than in females while there was no significant difference in the intensity of infestation of fleas between male and female hosts. There was no significant difference in the intensity of infestation of fleas between G. leucogaster and G. paeba. There was no association between host body mass and the intensity of infestation of fleas. The species diversity and richness of fleas did not vary significantly amongst the different species of small mammal and by host sex.

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