

# ECTOPARASITES ON MURID RODENTS CAUGHT IN MTS. PALAY-PALAY/MATAAS NA GULOD NATIONAL PARK, LUZON ISLAND, PHILIPPINES

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**Abstract.** Rodents in lowland, midland and upland areas of the forest of Mts. Palay-palay/Mataas na Gulod National Park (MPMGNP), Luzon Island, Philippines were trapped using a live capture method. All rodents trapped belonged to genus *Rattus* namely *R. norvegicus* (35.9%), *R. everetti* (27.8%), *R. tanezumi* (20.5%) and *R. argentiventer* (15.8%). Ectoparasites were recovered from the rodents through scraping of host's skin, hair and nails, and collection of dead ectoparasites after insecticide dusting. Among the ectoparasites identified based on their morphological characteristics were *Polyplax spinulosa* (68.0%), *Chirodiscooides caviae* (13.2%), *Laelaps nuttali* (11.5%), *Ornithonyssius bacoti* (4.5%) and *Xenopsyllia cheopis* (2.6%). The infestation rate of the parasite varied based on the rat species, with *P. spinulosa* parasitizing all the rodents caught, and *R. norvegicus* having the highest infestation rate. *Chirodiscooides caviae* parasitized predominantly *R. norvegicus*, while *L. nuttali* was found mainly on *R. tanezumi*. *Ornithonyssius bacoti* was found on both *R. tanezumi* and *R. norvegicus*, while *X. cheopis* was only recovered on *R. argentiventer*. Ectoparasite infestation was also influenced by the gender of the host, with male rats (71.43%) manifesting a significantly higher ( $p < 0.05$ ) infestation rate than female rats (28.57%). All recovered ectoparasites were common parasites of rats. Infested rats near human habitations in the area warrant possible rodent-borne diseases among the residents thus, an investigation of the occurrence of rodent-borne diseases among the dwellers may provide epidemiologic pattern related to such diseases.

## INTRODUCTION

Parasitism of vertebrates, including rats, by terrestrial arthropods has been most widespread in lairs, nests and other host habitations (Harwood and James, 1979). The diversity and infestation variations in ectoparasites among wild rodents may indicate the prevalence of representative parasites on their hosts and may reflect their host specificity required for their survival and proliferation (Soliman *et al.*, 2001b). They also noted that environmental conditions, such as season, topography and vegetation can affect rodent hosts and their ectoparasites (Soliman *et al.*, 2001a).

Pestiferous species of rats and mice in the Philippines are known to cause damage to property and crops (Sanchez *et al.*, 1985; Salibay

and Claveria, 2005). Human interaction with rats potentially exposes the former to the risk of contracting zoonotic diseases caused by viruses, bacteria, protozoa, or invertebrates (Sanchez *et al.*, 1985; Harkness and Wagner; 1989; Stoffolano and Romoser, 1998). One of the areas where murid rodents thrive is tropical forests (Heaney and Regalado, 1998).

The presence of humans as disturbance gradients in natural forests is associated with the presence of wild rodents in natural habitats (Sanchez *et al.*, 1985; Heaney *et al.*, 1999). Arthropologic activities have greatly altered the surrounding environment for habitation, such as forests. The incidence of rodent-borne diseases transmittable to humans has increased and becomes unavoidable because of the close association between rodents and man (Harwood and James, 1979; Sanchez *et al.*, 1985). In addition, environmental manipulation, such as agricultural conversion, industrialization, use of pesticides for crop control and management, established in the host's habitat may increase arthropod populations. This is because destruction of the natural habitats of hosts results in limiting their

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spatial dimension, hence hosts become congested in a “smaller” habitat, giving way to easier transmission of ectoparasites from one host to another.

Taking into account the role of rodents as carriers/reservoir hosts of disease-carrying agents (Harkness and Wagner, 1989) and their close proximity to human habitations (Gratz, 1988; Nowack, 1991; Salibay and Claveria, 2005), especially in rural areas (Soliman *et al*, 2001a,b), and as inhabitants of forests (Nava *et al*, 2003), this study was conducted to determine the rodent host preference of ectoparasites in terms of host species and gender collected in habitats at different area elevations.

## MATERIALS AND METHODS

### The study site

Mts. Palay-palay/Mataas na Gulod National Park (MPMGNP) is a 4,000-hectare mountain range situated within the municipality of Ternate and Maragondon in Cavite, and Nasugbu, Batangas, provinces in Southern Luzon, Philippines (DENR, 1992). The range includes three main peaks which were considered as the forest habitats in this study, Palaypalay, Mataas na Gulod, and Pico de Loro, with elevations of 595 meters above sea level (masl), 622 masl and 648 masl, respectively. The collection sites at the MPMGNP were determined using stratified random sampling with reference to the forest trail. Each habitat was divided into low elevation (LE) (0-207 masl), middle elevation (ME) (208-414 masl), and high elevation (HE) (415 and above). The sampling site was approximately 1 km from human habitation, agricultural, and infrastructural sites. Likewise, within the selected habitat, the point of reference was 15 meters from a human trail.

### Host capture

The technique for capture of wild rodents was adopted from the guidelines and works of PCAARD (1985); Sanchez *et al* (1985); Walker (1994); Heaney *et al* (1999); Soliman *et al* (2001b); ASM ACUC Guidelines (2003); and Salibay (2004) with slight modifications. The survey of rodents along the study site used the elevational transect method. This method was set

in trails along an elevational transect at elevation areas of the three habitats. The study also adopted the 100 meter-transect line live capture method. In each sampling station, 20 spring door wire traps with food bait, which included bananas, grilled coconut meat, dried fish and earthworms, were deployed with an interval of 5 meters for each collection site. The sites were further subdivided into subplots to avoid collection of rats at exactly the same plot per collection site. The traps were set before dusk and checked in the early morning. In the absence of a rodent, traps with fresh bait were left in place for two to three days, then transferred to another location. The captured rats were transported to the DLSU-D Natural History Laboratory for processing.

### Host euthanasia and preservation (ASM ACUC Guidelines, 2003)

Since some ectoparasites leave the body of the host shortly after death, the captured rodents were transferred from the cages to closed containers before the euthanization process to ensure the collection of the ectoparasites present on the body of the euthanized hosts. Under open-air field conditions or well-ventilated areas, chloroform was appropriated for euthanization since it also kills ectoparasites. Collected rodents were processed with formalin and preserved in glass jars with 70-80% ethanol. After the experiment, the rodents were disposed of properly.

### Rodent identification

The rodents were identified using several references (Sanchez *et al*, 1985; Heaney *et al*, 1999; Salibay, 2004). Individual rodent autopsy for host identification was performed assessing for morphological differences according to physical characteristics and external measurements, which included weight and head, hind foot, tail and body lengths. The physical characteristics and morphometrics of the murid rodents recorded for identification were *R. norvegicus* and *R. tanezumi* (Sanchez *et al*, 1985; Salibay, 2004), *R. everetti* (Heaney *et al*, 1999) and *R. argentiventer* (Sanchez *et al*, 1985). Authentication of pre-identified species was done at the Mammalogy Section, Zoology Division of the National Museum, Manila, Philippines.

### Ectoparasite collection

The ectoparasite collection followed the protocol of Soliman *et al* (2001b), and Walker (1994) using the host search method. The ectoparasites were rendered inactive on the body of the host using chloroform. The individual rodents were dry-combed with a toothbrush in a postero-anterior direction to collect parasites. The snout, ears, limbs, and axillary regions of rodents were combed. Wet-combing in a postero-anterior (PA) direction was followed by placing the dead rodent immersed in collecting tray with 70% ethanol. The parasites were then filtered through filter paper and transferred into a glass vial (3 cc) by forcefully flushing them with 70% ethanol using a pipette.

Fleas and lice were collected by parting the hair and picking up the lice with forceps or using a fine comb. Dusting the host with an insecticide and collecting the dead parasites as they fell onto a collecting tray was done. Mites were collected by skin scraping using as improvised plastic scraper with thin, flat edges, or a scalpel held at 90° to the skin. The scraper was wetted with oil or glycerol making a temporary mount on a microscope slide for examination. Spots of blood were drawn from epidermis to check for sarcoptic mites. Affected host skin with dermatitis was incised over nodules to check for demodectic mange mites.

### Ectoparasite preservation

The preservation and identification of ectoparasites was performed following the methods of Walker (1994) and Soulsby (1982). The specimens collected were submerged in 75% ethanol and additional water for liquid preservation. Glycerol was added as one-tenth part of the total volume of ethanol using 5 to 25 ml thick-walled, wide-mouth glass containers. Dry and wet-combed collections were processed for temporary mounting, and preservation (permanent mounting) on glass slides. Temporary mounting was used for counting the ectoparasites and permanent mounting was used for photomicroscopy and identification.

For temporary mounting, fresh specimens were mounted in glycerol directly on a microscope

slide, a cover slip was placed, and the specimen was directly examined under a dissecting microscope. For permanent mounting, the specimens were fixed by immersion in glacial acetic acid with formalin for 5 hours. After fixation, specimens were dehydrated by soaking them in 10% potassium hydroxide solution for one day, then washed with water for a few minutes, followed by a 30 minute-soaking in 10% acetic acid and washed with water before starting the dehydration procedure with ethanol. The specimens were dehydrated by soaking in several mixtures of ethanol and water from 75% to 95% ethanol at one hour for each mixture and were cleared by soaking the specimen with xylene. The specimen was transferred from the xylene to a glass slide, a drop of Canada balsam was placed on the slide and a cover slip was placed.

### Ectoparasite identification

Mounted specimens were examined microscopically using dissecting and binocular microscopes to study their morphological characteristics for identification. The identity of the ectoparasites was established using identification guides by Walker (1994), and the works of Harwood and James (1979), and Salazar and Cabrera (1969).

### Ectoparasite counting

The visual examination or indirect counting method adopted from the work of Clayton and Walther (1997) was used in this study. Counting of ectoparasites was done using a counter while ectoparasites were rendered inactive in a Petri dish or on a dissecting microscope.

### Data gathering and analysis

Infestation rate of recovered ectoparasites from their rat hosts was based on Soliman *et al* (2001b) using the formula:

$$\frac{N1 \times 100}{N2}$$

Where N1 = Number of ectoparasite recovered from a particular individual host species  
N2 = Total number of ectoparasites recovered

Ectoparasite burden per rat host is calculated as:

$$\frac{R1 \times 100}{R2}$$

Where R1 = Number of individual rat infested according to gender

R2 = Total number of rats infested

**Statistical analysis**

Influence of the elevation to the number of caught rodents was determined using *t*-test and regression analysis model. The rodent species collected in terms of forest area habitats of the MPMGNP was tested using univariate and two-way ANOVA at  $p \leq 0.05$ . Ectoparasites per rat hosts were tested using two-way ANOVA at  $p \leq 0.05$ . Chi-square ( $\chi^2$  test) was applied to compare the ectoparasite burden on male and female host groups.

**RESULTS**

**Murine rodent species collected**

Four species of rodents, all from genus *Rattus* were collected from MPMGNP, namely: *R. norvegicus* (Norway rat), *R. everetti* (common

Philippine forest rat), *R. tanezumi* (Oriental house rat) and *R. argentiventer* (ricefield rat). Of the species collected, *R. norvegicus* (39.3%), was significantly ( $p \leq 0.05$ ) the most prevalent, followed by *R. everetti* (28.6%), *R. tanezumi* (21.4%) and *R. argentiventer* (10.7%). The number of wild rats caught in terms of elevation was significantly ( $p \leq 0.05$ ) the highest at low elevation (55.4%), followed by middle (28.6%), then high (16.1%) elevations (Fig 1). As the elevation increased, the frequency of rats decreased.

**Ectoparasites of rodents**

The number and species of ectoparasites recovered from the rats are presented in Table 1. The results indicate all rats collected were parasitized by ectoparasites, with *R. norvegicus* having the highest number of parasites recovered (35.9%). This was followed by *R. everetti* (27.8%), *R. tanezumi* (20.5%), and *R. argentiventer* (15.8%). Although *R. tanezumi* did not show a high infestation rate compared to *R. norvegicus* or *R. everetti*, it harbored four species of parasites. *Rattus everetti* and *R. argentiventer* had the least number of types of ectoparasites recovered.

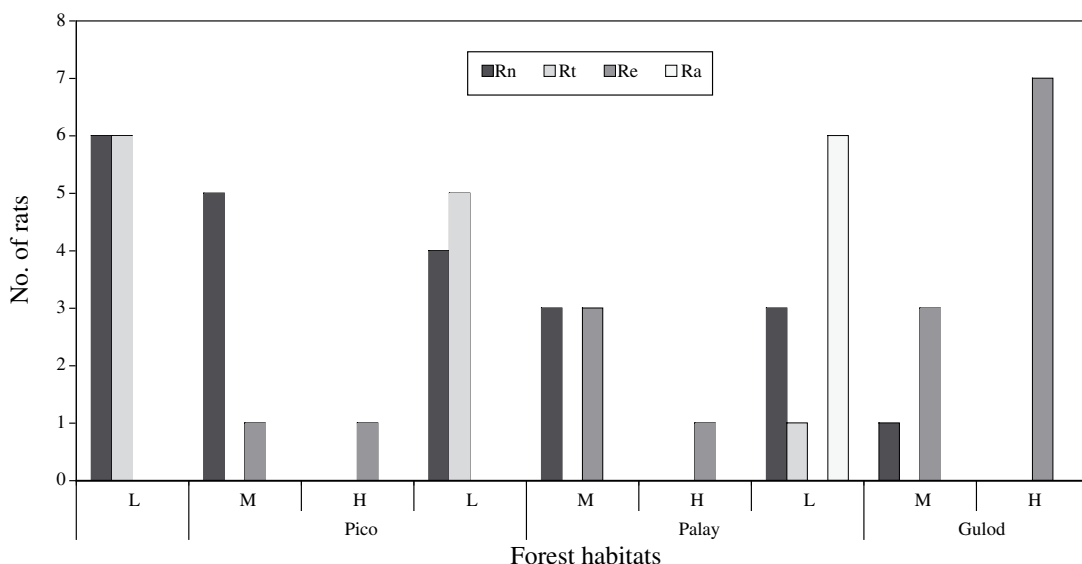


Fig 1- The number of rats collected at different elevations in forest habitats. R.n. (*R. norvegicus*); R.t. (*R. tanezumi*); R.e. (*R. everetti*); R.a. (*R. argentiventer*).

Table 1  
Summary of ectoparasite infestation rates (IR) on murine rodents.

<i>Rattus</i> species (total number collected)	Number of Ectoparasite present (%IR)						Total per rodent species	Infestation rate (%)
	<i>X. cheopis</i>	<i>P. spinulosa</i>	<i>C. caviae</i>	<i>L. nuttali</i>	<i>O. bacoti</i>	<i>Thrips</i> sp		
<i>R. norvegicus</i> (22)	0 (0)	116 (24.8)	26 (5.6)	18 (3.8)	7 (1.5)	1 (0.2)	168	35.9
<i>R. tanezumi</i> (12)	0 (0)	50 (10.7)	12 (2.5)	20 (4.3)	14 (3)	0 (0)	96	20.5
<i>R. everetti</i> (16)	0 (0)	90 (19.2)	24 (5.1)	16 (3.4)	0 (0.)	0 (0)	130	27.8
<i>R. argentiventer</i> (6)	12 (2.6)	62 (13.2)	0 (0)	0 (0)	0 (0)	0 (0)	74	15.8
Total per ectoparasite (%)	12 (2.6)	318 (68.0)	62 (13.2)	54 (11.5)	21 (4.5)	1 (0.2)	468	

Two species of ectoparasites of the class Insecta and three species of the class Arachnida were collected from the rodents (Fig 2). In the class insecta, the spined rat louse, *Polyplax spinulosa* (68.0%), dominated the infestation of all rodents. However, the Oriental rat flea, *Xenopsylla cheopis* (2.6%) was recovered only on *R. argentiventer*, and with a minimal infestation rate. Of the arachnids recovered, the scab mite, *Chirodiscoides caviae* constituted 13.2% of the collected rats, with the highest infestation rate being in *R. everetti*, followed by *R. tanezumi* and *R. norvegicus*, while none were found on *R. argentiventer*. The common rat mite, *Laelaps nuttali* had on 11.5% infestation rate, which was recovered from the three hosts: most frequently in *R. tanezumi*, followed by *R. norvegicus* then *R. everetti*. The tropical rat mite, *Ornithonyssus bacoti* (4.5%) infested both *R. tanezumi* and *R. norvegicus*.

The presence of *Thrips* sp, a plant-sap sucking insect, which belongs to *Thysanoptera*, found on *R. norvegicus*, was found least commonly in this study.

Ectoparasite infestation relative to host gender was significantly higher ( $p < 0.05$ ) in male rats than in females (Table 2). This may be due to the fact that male rats are bigger in size and are more active so that they have high chances of being infested.

## DISCUSSION

The present study found two ectoparasites from the class Insecta and 3 from the class Arachnida, all of which are ectoparasites on *Rattus* species (Harwood and James, 1979; Eduardo and Mercado, 1981; Gratz, 1988). Similar to earlier surveys (Salazar, 1977; Durden and Page, 1991; Soliman *et al*, 2001a,b) of ectoparasites on commensal murid rats, these are known to be found on *Rattus* spp, and are not classified as being host specific (Salazar, 1977; Soulsby, 1982; Walker, 1994). This was evident with *P. spinulosa* found in all rat species collected, which may be indicative of rat-to-rat transmission within and among the different species of hosts. The findings of this study are similar to those in studies by Durden and Page (1991) and Soliman *et al* (2001b) of the presence of mites on rodents. They collected *L. echidnina*, *L. nuttali* and *O. bacoti*; *X. cheopis*; and *Hoplopleura pacifica* and *P. spinulosa* from *R.r.palelae*, *R. argentiventer*, *R. exulans* and *M.m. castaneus*, in Sulawesi Utara, Indonesia (Durden and Page, 1991), and *R. norvegicus* and *R. rattus* from rural Egypt (Soliman *et al*, 2001b). Both studies indicate that *L. nuttali*, *O. bacoti*, *X. cheopis* and *P. spinulosa* were associated with murid rodents, especially *Rattus* species.

Of the *Rattus* species, *R. argentiventer* is

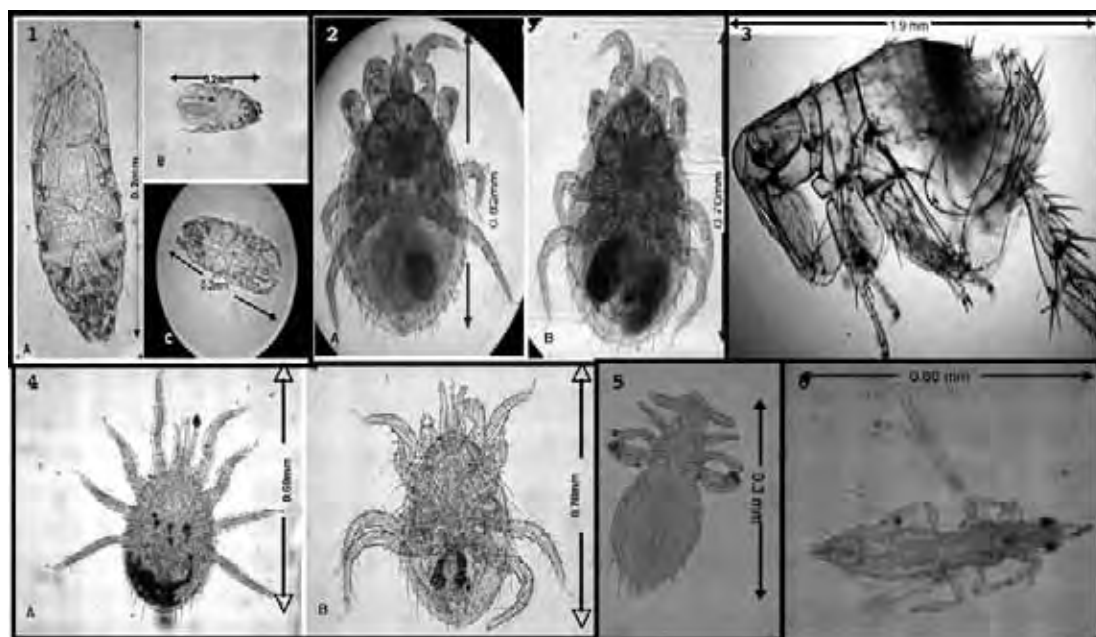


Fig 2- Recovered ectoparasites from wild *Rattus* spp. 1. *Chirodiscoides caviae* adult, 1A and 1C. Male; 1B. Female. 2. *Laelaps nuttali* adult, 2A. Male; 2B. Female. 3. *Xenopsylla cheopis*, adult. 4. *Ornithonyssius bacoti*, 4A. Nymph 4B. Adult. 5. *Polyplax spinulosa*, adult. 6. *Thrips* sp, adult.

Table 2  
Ectoparasite burden relative to host gender.

Host	Number of ectoparasites on rodents (%)	
	Male	Female
<i>R. norvegicus</i>	12 (21.4)	10 (17.9)
<i>R. tanezumi</i>	8 (14.3)	4 (7.1)
<i>R. everetti</i>	16 (28.6)	0
<i>R. argentiventer</i>	4 (7.1)	2 (3.6)
Total	40 (71.4)	16 (28.6)

considered important in the maintenance of campestral or sylvatic plague caused by fleas associated with field rodents. This may cause such disease in rural areas even if there is no outbreak of plague cases (Harwood and James, 1979; Liat *et al*, 1980). In the present study, the presence of *X. cheopis* solely in *R. argentiventer* may indicate a close association between the flea and this rodent species.

A study done by Gratz (1988) proved that the chances of encountering *Rattus* species varied

depending on the rat's habitat preference. *Rattus norvegicus* has a broad habitat, while *R. tanezumi* thrives in trees or on vine-covered fences and landscaped residential or industrial sites, as well as in the vegetation of riverbanks and streams. Their preferences may be influenced by their food sources. This preference may lessen the chances of encountering other rat species in natural forests, such as *R. argentiventer*, which prefers agricultural areas, thereby influencing the transmission of ectoparasites within the same species or among different species.

According to Harwood and James (1979), the majority of siphonapterans, such as *X. cheopis*, are known to leave their host and may transfer to hosts of the same or different species. However, in the present study, it was recovered only from *R. argentiventer*, which may indicate this rodent may not be as mobile as other *Rattus* species.

Notably, *O. bacoti*, which parasitized *R. tanezumi* and *R. norvegicus*, has a strong association with non-native rats in suburban areas of Manila, Philippines (Salazar, 1977). The present study also shows that *O. bacoti* were recovered more often from *R. tanezumi*

and *R. norvegicus* than from *R. everetti* and *R. argentiventer*, which are rodents of forest and agricultural areas, respectively. Non-native murid rats may be more adaptable to disturbed areas (Sanchez *et al*, 1985; Heaney *et al*, 1999; Soliman *et al*, 2001a), and tend to be more susceptible to ectoparasitism compared to native species. In addition to becoming more adaptable, Soliman *et al* (2001b) found a higher infestation rate with ectoparasites with greater body size of the host. This finding is in congruence with the results of the present study, where *R. norvegicus* and *R. everetti* had significantly higher infestation rates and interestingly, were larger rats than *R. tanezumi* and *R. argentiventer*. The high infestation rate of parasite in *R. norvegicus* may be attributable to the greater number of such species in the habitat; thus, changes in rat-to-rat transmission with the ectoparasite is also high within the species (Salazar, 1977; Harwood and James, 1979; Sanchez *et al*, 1985; Gratz, 1988).

Relative to host gender, the highest percentage of ectoparasites recovered was from male rats. This may be attributable to the fact that male rats are more active and can travel significantly farther than females; their broad diet makes them adaptive to travel longer distances (Sanchez *et al*, 1985; Heaney *et al*, 1999; Soliman *et al*, 2001b), making them more susceptible to ectoparasitic infestation. The age of the commensal rat hosts, as well as the species of the ectoparasite, are also important factors that influence the infestation rate (Soliman *et al*, 2001b); however, such factors were not considered in this study.

Environmental modification, such as forest clearing and subsequent conversion into agricultural land may influence the presence of pestiferous vectors, such as *R. norvegicus* and *R. tanezumi*, as well as their ectoparasites, as was reported by Harwood and James (1979); Sanchez *et al* (1985). The high infestation rate of ectoparasite species in *Rattus norvegicus* and *R. tanezumi* can be attributed to these species preference for more congested areas where houses and other settlements are located (Sanchez *et al*, 1985) compared to *R. everetti*, which dwells only in areas near its habitat with less disturbance caused by the presence of human settlements, and *R. argentiventer*, which thrives in agricultural

areas (Sanchez *et al*, 1985; Heaney *et al*, 1999). This results in a greater chance for a physical encounter among rat species in congested areas, and the transfer of ectoparasites from one host to another, as in the case of *R. tanezumi* and *R. norvegicus* in this study.

In natural forest conditions, rats in the wild are considered to be cleaner (Heaney and Regalado, 1998) compared to those that are found in urban areas (Salazar, 1977). This is because rat species dwelling in forests consume fruits or crops, while those dwelling in urban areas are found in poorly sanitized areas and in nearby garbage dumpsites. Salazar (1977) also noted rat species caught in urban areas had poorer hygiene. In this study, some rats recovered in the forest areas were of the same species as those urban dwellers, indicative of the infiltration of urban rats in forest areas especially at lower elevations.

In conclusion, although, the degree of infestation of ectoparasite varies among the host species, the elevation and habitat where the species were collected did not show direct influence as to the infestation rate of the ectoparasites.

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