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Ecology of ecto-parasites of some cave-dwelling microchiropterans of Sri Lanka.

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Abstract

The population and community characteristics of ecto-parasites of bats in Sri Lanka have not been previously examined. The aim of the present study was to investigate these attributes based on incidence, density, prevalence and species composition of ecto-parasites harboured by 3 species of cave-dwelling microchiropteran hosts, *Hipposideros lankadiva, H. speoris* and *Rhinolophus rouxi*. These bats were captured by hand or mist nets at four previously selected sites, distributed in the wet, dry and intermediate zones of Sri Lanka. Parasites were both hand-picked and brushed off the pelage from slightly anaesthetised bats. Five species of bat flies including 2 nycteribiid flies (*Phthiridium ceylonicum* and *P. phillipsi*), 3 streblid bat flies, *Brachytarsina modesta, B. pygialis* and *Raymondia pagodarum* and mites of families laelapidae, spinturnicidae and trombiculidae were recorded on these hosts. With the exception of the two streblid flies and individuals of the three mite families, the other ecto-parasites were host specific. The highest incidence and density were recorded for laelapid and spinturnicid mites on *H. lankadiva*, while lowest values for these indices were also for the same mites on *R. rouxi* in the intermediate zone. No significant difference (2-way ANOVA; $P > 0.05$) in the prevalence of ecto-parasites could be attributed either to the sex or the climatic zone of the host with respect to *R. rouxi* and *H. speoris*. The ecto-parasite species diversity on *H. speoris* in the dry zone was observed to be the highest and that on *H. lankadiva* in the wet zone the lowest. A significant difference in the ecto-parasite assemblage of all hosts were observed except between the populations on *H. lankadiva* and *H. speoris* occurring sympatrically in the wet zone. It is concluded that the prevalence of ecto-parasites is neither dependant on the gender of the host nor the climatic zones of their roosting sites.

Key words: bats, microchiroptera, *Hipposideros lankadiva, Hipposideros speoris, Rhinolophus rouxi,* ecto-parasites, bat flies, bat mites, parasite ecology, sex of host, climatic zone of roost, Sri Lanka.

1. Introduction

Scientific data on parasites of Sri Lankan bats is fragmentary. Previous studies based on taxonomic descriptions of bat ecto-parasites in the island were recorded mostly on few specimens of preserved bats (Scott, 1908, 1914,1925; Phillips, 1924, 1980; Jobling, 1934). However, no work has been documented on the population and community characteristics of either ecto-or endo-parasites of Sri Lankan bats. Therefore, the present study was undertaken as a preliminary investigation on bat ecto-parasite ecology. In addition, an attempt was made to study species composition and diversity based on three selected species of cave-dwelling microchiropterans representing different climatic zones of the island.

2. Materials and methods

Sri Lanka is a small island situated in the Indian ocean below the Indian subcontinent (6^0 4'-6^0 45' North: 81^0 00'- 81^0 05' East). The island is divided into six main climatic zones; viz. low and mid country wet, dry, low and mid country intermediate, montane wet, montane intermediate and arid (Wijesinghe *et al*, 1993) depending on the annual rainfall each zone receives.

The present study was conducted from August to September 1996, at four previously selected caves. These sites were selected based on easy accessibility, type of host species, climatic zones and ease of capture of bats.

Site 1, was an abandoned graphite mine in Bogala, Kegalle (wet zone). Site 2, was the abandoned graphite mine in Siddhamulla, Kegalle (wet zone). Ridiviharaya cave, Ridigama, Kurunegala (intermediate zone) was the 3rd site selected. The 4th site was the Hasthikuchchi cave, Kurunegala (dry zone).

The colony size of bats in each cave was determined by direct counting of bats within the cave while they were roosting and during outflight (i.e. as they emerged to forage after sunset). Hand nets were used to capture bats within caves and mist nets were used to capture bats during their outflight from the caves. From a single sampling site, a total of 30 animals from each sex representing a single bat species were caught (where small colonies were concerned the maximum possible number of bats were caught). Upon capture, each bat was held in a separate cloth sack until inspected for the presence of ecto-parasites.

Ecto-parasites were collected according to Whitaker (1988). Briefly, the fur, base to tip, patagium, ears, head, neck, skin of the abdominal region and dorsal areas of the body of slightly anaesthetised bats were thoroughly examined for ecto-parasites. When present, they were either hand picked using a fine pair of forceps, a paint brush or an arrow pointer or brushed off on to a clean white cloth using a fine bristled toothbrush. Finally, the cloth sack that held the host was examined for ecto-parasites.

All parasites collected off a single host were stored in a single appropriately labelled glass vial containing 70% ethyl alcohol. The number of each type of parasite and its location on the body of the host were also recorded. Identification of parasites was based on keys relevant to the parasite type (Jobling, 1930,1934 & 1936; Maa, 1971; Theodor, 1967; Deane & Catts,1982; Notes on general acaralogy, Institute for Medical Research, Malaysia). The identifications of bat flies were authenticated by Dr D. Kock, Forschungsinstitut und Naturmuseum Senckenberg, Germany and that of the mite families by Prof. P. Amerasinghe, Department of Zoology, University of Peradeniya, Sri Lanka.

Species, sex and maturity of each host were recorded prior to their release. Bats were identified using keys provided by Cobert and Hill (1992). In order to avoid recapture, bats were marked prior to their release with a necklace made of coloured beads, each colour representing a number (Mose cord).

For each of the bats examined, several host- ecto-parasite parameters were estimated, including incidence (the percentage of infested bats; [Number of bats with parasites /Number of bats examined) X100]), prevalence (mean number of ecto-parasites per bat; [Total number of parasites/ Number of bats examined]) and density (mean number of ecto-parasites per infested bat; [Number of parasites per infested host/Number of infested hosts examined]) (Gannon and Willig, 1995).

Where possible data is presented as percentages and as means ± standard error of the mean (SEM). Statistical analysis was made using a pure model 1 2- way analysis of variance (ANOVA). The significant level was set at $P<0.05$.

The Shannon index of diversity with respect to ecto-parasites present on each bat species was calculated (where the sample size was insufficient a correction was made using the Jack knife method) and a *t* test was conducted to test for significant differences between ecto-parasite communities (Magurran, 1988).

The Shannon index of diversity (*H'*) is a measure of the variety and relative measure of abundance of species. It is calculated from the equation, $H' = -\sum p_i \ln p_i$, where p_i is the proportion of individuals found in the i^{th} species. $p_i = n_i/N$, where n_i=number of individuals of the i^{th} species and N = total number of individuals.

Evenness is the measure of how evenly the species is distributed and a greater evenness makes a population more diverse. Evenness is calculated from the equation, E=H' /ln S, where S= number of species. To test for significant differences between samples a *t* value can be calcuated using the equation, $t = (H_1' + H_2') / (\text{Var } H_1' + \text{Var} H_2')^{1/2}$, where Var *H'* is the variance of *H'* and is calculated as,

$$\text{Var } H' = ([\sum p_i(\ln p_i)^2 - (\sum p_i \ln p_i)^2 / N] + [(S-1)/2N^2]).$$

3. Results

The 3 different microchiropteran hosts, *Hipposideros lankadiva, Hipposideros speoris* (both of family Hipposideridae) and *Rhinolophus rouxi* (family Rhinolophidae) were seen to variously occupy the four sampling sites. Site 1 in the wet zone was occupied by a colony of *H. lankadiva* (n=100) and a small colony of *H. speoris* (n=30). Site 2 in the wet zone (n=75) and Site 3 in the intermediate zone (n=5500) were occupied by two colonies of *R. rouxi* while site 3 was also occupied by a small colony of *H. lankadiva* (n=5). Approximately 1000 *H. speoris* occupied Site 4 in the dry zone.

In this study *H. lankadiva* was restricted to a single site in the wet zone. Although about 5 bats were included in the Ridigama colony it was not possible to capture them. Of the 40 bats captured from site 1, only 4 were females. *H. speoris* occurred both in the wet and the dry zones. All 10 bats of which 2 were females examined from the wet zone and 53 (24 males and 29 females) out of 60 bats (29 males and 31 females) examined from the dry zone harboured at least one species of ecto-parasite. *R. rouxi* occurred both in the wet and intermediate zones. 37 (9 males and 28 females) out of a total of 40 bats (9 males and 31 females) examined from the wet zone, and 60 (28 males and 32 females) of all 67 bats (31males and 36 females) examined from the intermediate zone harboured at least one type of ecto-parasite.

Five species of bat flies were collected from the 3 species of bats examined. These included nycteribiid bat flies *Phthiridium ceylonicum* and *P. phillipsi*, streblid bat flies *Brachytarsina modesta, B. pygialis and Raymondia pagodarum.* Adult mites belonging to families spinturnicidae

and laelapidae, and mite larvae of family trombiculidae were also gathered from these bats. The spinturnicid and laelapid mites were counted as a single group as identification of the individual mites was not possible to the naked eye. Of these species *P. ceylonicum, P. phillipsi* and *B. pygialis* were found to be host-specific.

The type of ecto-parasites harboured by *H. lankadiva, H.speoris* and *R.rouxi* and their incidence, prevalence and density are summarised in tables 1, 2a & 2b and 3a & 3b, respectively.

These indices were calculated for the entire sample of *H.lankadiva* irrespective of sex since the number of females was low (table 1). The highest incidence, prevalence and density for this host were recorded for the laelapid and spinturnicid mites. The lowest incidence was for both streblid *R. pagodarum* and for the trombiculid mites while the lowest prevalence was also for *R. pagodarum*. The lowest density was recoded for *P. ceylonicum.*

In the case of *H. speoris,* these indices are listed separately for the wet and the dry climatic zones (table 2a) and for male and female hosts in the dry zone (table 2b). In both wet and dry zones, the highest values for the three indices were recorded for the laelapid and spinturnicid mites with the exception of the highest incidence being recorded for *B. modesta* in the dry zone. The lowest values for these parameters for both zones were calculated for *R. pagodarum* with the exception of the lowest density recorded for *B.modesta* in the dry zone. The highest incidence, prevalence and density for male *H. speoris* in the dry zone were recorded for the laelapid and spinturnicid mites. While in the females, though the highest prevalence and density were for these mites the highest incidence was for streblid *B. modesta*. The lowest incidence and prevalence of ecto-parasites for both sexes of the host in the dry zone were for *R. pagodarum*. However, the lowest density in both males and females was for *B. modesta.*

The highest incidence, prevalence and density for *R. rouxi* in both the wet and the intermediate zones were for *B. modesta* (table 3a), the only exception being laelapid and spinturnicid mites recording the highest density for the intermediate zone. The lowest values for the three indices were recorded for trombiculid mites in both these zones, specially so, as these mites were absent on *R. rouxi* in the intermediate zone. The highest figures for these indices for both males and females in the intermediate zone (table 3b) were for *B. modesta*. The exception was the highest density for laelapid and spinturnicid mites recorded for males. On the other hand, the lowest incidence and prevalence in the male *R.rouxi* were for these mites, while they were completely absent on the female bats. The lowest density in male bats were for *P.phillipsi.*

The nycteribiid bat flies of *Phthiridium* species were observed to be inhabiting the host pelage. The streblid flies, *Brachytarsina* species and *R. pagodarum* were most common in the folds of the patagium while they also occurred on the pelage. The micro-habitats of the mites of families laelapidae and spinturnicidae were the dorsal and ventral surfaces of the patagium, mostly concentrated at the folds and when occurring in large numbers they were also seen on the feet of the bat and between the digits. The trombiculid mites occurred singly or as colonies in a variety of locations on the body. These mites occurred singly along the margins of the patagium, pinnae and attached to the skin on the ventral surface of the body. The colonies were present in the tragus, area surrounding the genital organs and at the fur edge of the ventral surface.

Singnificance levels from a series of pure model 1 2-way ANOVA was used to evaluate the effects of host climate and the sex of the host on the prevalence of ecto-parasite types on *R. rouxi* and *H. speoris*. For this analysis, ecto-parasites of *H. speoris* in the two climatic zones, wet and dry and on the separate sexes in the dry zone were analysed with respect to 4 types of parasites; *B. modesta*, *R. pagodarum*, laelapid and spinturnicid mites as a single group and trombiculid mites. The ecto-parasites of *R.rouxi* were analysed with respect to *P. phillipsi*, *B.modesta* and *R.pagodarum*. No significant differences in the level of prevalence of ecto-parasites could be attributed to either the sex or the climatic zone of the hosts ($P>0.05$).

Since *H. lankadiva* occurred only in the wet zone and the number of females in the sample was small the above mentioned analysis was not performed for this species.

The Shannon species diversity index and the evenness for the five types of parasite populations are summarised in table 4. There was a sigificant difference (*t* test; $P= 0.05$) between the species diversity in all parasite populations except for the two parasite populations harboured by *H. lankadiva* and *H. speoris* occurring sympatrically at site 3.

4. Discussion

Five species of bat flies and individuals belonging to three mite families were identified from the three microchiropteran host species examined. The occurrence of the nycteribiid and streblid flies on *H. lankadiva, H. speoris* and *R.rouxi* confirm previous records from the island (Jobling, 1934; Phillips, 1980). Presence of mites were previously recorded on Sri Lankan bats (Phillips, 1924). However, this is the first study to identify these mites up to the family level; spinturnicidae, laelapidae and trombiculidae.

Some of these ecto-parasites were species specific. The bat flies *P. ceylonicum* and *B. pygialis* occurred only on *H. lankadiva* (table 2) while *P. phillipsi* occurred only on *R. rouxi*. The streblid bat flies *R. pagodarum* and *B. modesta* and individuals of the three families of mites occurred on all three bat species studied.

The denseness of the fur is moderate on *H. speoris* as compared to that of *H. lankadiva* and R. rouxi (Phillips,1933). Therefore, this could be a possible explanation as to why nycteriibid bat flies which were found only within the pelage were absent on *H. speoris*. The trombiculid mites were absent only on *R. rouxi* in the intermediate zone. According to Nadchatram (1970), trombiculids (chiggers) are sensitive to fluctuating environmental conditions.Therefore, the effect of environmental factors could be one of the possible reasons as to why trombiculids were absent on *R. rouxi* in the intermediate zone. Whitaker(1988) observed that chiggers are generally habitat-specific rather than host-specific ecto-parasites and any vertebrate entering a chigger-infested habitat stands an almost equal chance of being infested by chiggers.

Bat ecto-parasites which spend their entire life on the body of the host usually exhibit a small, flattened form and are capable of movement over the surface of the host, although most show a disitinct preference for particular locations on the body (Marshall, 1982). The nycteribiids with several combs inhabit the pelage and streblids which are weak flyers are inhabitants of the patagium and "dive" in and out of the fur for feeding. The patagium of the bat is provided with a good supply of blood to assist in the function of flight (Bradsbury,1977). Also, as these blood vessels are superficial easy access is possible. Therefore, it is reasonable to assume that the small mites are mostly concentrated in these regions in order to receive a ready blood supply.

Body populations are defined by two parameters, the incidence and the prevalence. These two parameters together with the number of hosts examined, form a basic description of a body population upon any set of hosts (age, sex, habitat, etc (Marshall, 1982). Incidence and prevalence can be estimated without regard to any taxonomic identity of the parasites and these can be directly compared among host species (Gannon and Willig, 1995). Of the three species of bats examined, *H. lankadiva* and *H. speoris* in the wet zone had the highest overall incidence (100%) and *H. lankadiva* had the highest overall prevalence (60.7 ecto-parasites per bat).The ecto-parasite prevalence on *H. speoris* in the wet zone was much less (16.9). The incidence on *R. rouxi* in the intermediate zone was the second highest (99%) with a prevalence of 4.3 ecto-parasites per bat. 96% of *R. rouxi* in the wet zone were

infested with a greater prevalence of ecto-parasites (7.9) than in the intermediate zone (2.9). *H. speoris* in the dry zone had the least overall incidence (90%) and a pervalence of 2.3. Therefore, it can be surmised that prevalence of ecto-parasites in the wet zone is comparatively higher.

Density, on the other hand, estimates the population size a parasite is maintaining on the host and is calculated for each parasite species (Whitaker, 1988). This value is especially useful in the host-parasite relationship and it is not comparable to values for other species since different numbers of infested hosts are involved. Hence, this value cannot serve as an estimate for community assessment.

In general, the number of individuals per host is negatively correlated with size of the parasite, with highest infestations recorded for the smallest parasites (Marshall,1982). It was evident that when mites, which are smaller in size than bat flies, occurred most often in vast numbers on the bats examined.

Ecological studies on bat parasites addressing their population and community characteristics are meagre (Gannon and Willig, 1995; Kunz,1976). Although it is apparent that host and climate affect the population size of the ecto-parasite densities little is understood concerning the manner in which differences in host characteristics (for example, species, age, sex) affect population densities of ecto-parasites or the composition of species assemblages on individual hosts (Marshall, 1982).

Based upon a 2-way ANOVA no significant difference ($P>0.05$) in the level of prevalence of ecto-parasites could be attributed to either the sex or the climatic zone of the two hosts, *H. speoris* and *R. rouxi.* Consistent host-specific differences in ecto-parasite infestation exist regardless of season (Gannon and Willig, 1995). This may in part explain the lack of a significant association of parasite prevalence and climatic zone of the host in the present study. The level of ecto-parasite infestation has been found to differ because of host age, but not with sex, where juveniles harbour larger numbers of parasites than adults (Gannon and Willig, 1995). In the present study, only 6 sub-adults from the *R. rouxi* colony in the intermediate zone and 5 sub-adults from the *H.speoris* colony in Hasthikuchchi were collected. Since the sample size was small ecto-parasite attribution to host age was not analysed.

Some tropical bat species exhibit sexual segregation at parturition. In most segregating species, males and females occupy different roosts at this time (Sreenivasan *et al*, 1973; Bradbury,1977). This may probably be the cause for the *H. lankadiva* colony in the abandoned Bogala graphite mine, Kegalle and *R. rouxi* colony in the abandoned graphite mine, Siddemulla, Kegalle were dominated by males, while the *H. speoris* colony in the abandoned Bogala graphite mine, Kegalle was dominated by females.

The ecto-parasite species diversity on *H. speoris* in the dry zone was observed to be the highest while that on *H. lankadiva* in the wet zone was the lowest. A significant difference in the five parasite populations (table 4) were observed except between the populations on *H. lankadiva* and *H. speoris* occurring sympatrically in the same cave. This could probably be due to the fact that they share the same roost, the ecto-parasites which are capable of movement occur on both types of hosts, with similar diversities (Gannon and Willig,1995).

The outcome of the present study is very encouraging and this could form a base line to expand the study of ecto-parasites on the same species of bats occurring in more diverse climatic zones of the island.

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Table 1. Host-parasite parameters of ecto-parasites on *H. lankadiva* in the wet zone.

Parameter	Ecto-parasite(s)				
	Phthiridium ceylonicum	*Brachytarsina pygialis*	*Raymondia pagodarum*	Laelapid & spinternicid mites	Trombiculid mite
Incidence	32.50%	57.5%	5%	95%	5%
Prevalence mean±SEM)	0.65±0.22	1.43±0.32	0.15±0.12	58.38±9.56	0.53±0.41
Density (mean±SEM)	2.00±0.47	2.78±0.43	3.00±0.99	58.82±9.55	10.5±3.5

Host sample size=36

Table 2a. Host-parasite parameters of ecto-parasites on *H.speoris* in the Wet and Dry Zones

Zone	Parameter	*Brachytarsina modesta*	*Rayamondia pagodarum*	Laelapid & spinturnicid mites	Trombiculid mites
Wet	Incidence	30%	10%	90%	30%
	Prevalence (mean±SEM)	2.20±0.77	0.30±0.30	17.20±3.50	0.70±0.42
	Density (mean±SEM)	3.67±0.85	1.00±0.00	19.10±3.28	2.33±0.88
Dry	Incidence	53.33%	21.67%	45%	41.67%
	Prevalence (mean±SEM)	0.90±0.14	0.44±0.14	2.20±0.73	1.60±0.45
	Density (mean±SEM)	1.72±0.16	2.08±0.42	4.56±1.54	3.92±0.90

Host sample size; Wet Zone=10, Dry Zone=60

Table 2b. Host ecto-parameters of Male and Female *H.speoris* in the Dry Zone

Sex	Parameter	*Brachytarsina modesta*	*Raymondia pagodarum*	Laelapid & Spinturnicid mites	Trombiculid mites
Male	Incidence	41.38%	24.14%	41.38%	44.83%
	Prevalence (mean± SEM)	0.62±0.16	0.38±0.14	1.34±0.53	2.28±0.81
	Density (mean±SEM)	1.50±0.12	1.57±0.15	3.25±0.69	5.08±0.10
Female	Incidence	67.67%	19.35%	48.39%	38.71%
	Prevalence (mean±SEM)	1.19±0.21	0.52±0.24	2.71±1.33	1.03±0.41
	Density (mean ± SEM)	1.85±0.18	2.67±0.33	5.60±1.80	2.67±0.54

Host sample size; Male=29, Female=31

Table 3a. Host ecto-parasite parameters of *R.rouxi* in the wet and intermediate

Zone	Parameter	*Phthiridium phillipsi*	*Brachytarsina modesta*	*Raymondia pagodarum*	Laelapid & spinturnicid mites	Trombiculid mites
Wet	Incidence	25%	90%	32.50%	5%	2.5%
	Prevalence (mean±SEM)	0.58±0.18	3.52±0.74	3.03±0.81	0.73±0.68	0.035±0.03
	Density (mean±SEM)	2.30±0.33	4.11±0.79	6.85±2.28	14.50±12.50	1.0±0.00
Inter mediat	Incidence	25.37%	79.10%	20.90%	1.5%	0
	Prevalence (mean±SEM)	0.31±0.08	2.03±0.23	0.33±0.10	0.01±0.01	0
	Density (mean±SEM)	1.24±0.33	2.56±0.24	1.57±0.42	1.00±0.00	0

Host sample size; Wet Zone= 40, Intermediate Zone=67

Table 3b. Host ecto-parameters of Male and Female *R.rouxi* in the Intermediate Zone

Sex	Parameter	*Phthiridium phillipsi*	*Brachytarsina modesta*	*Raymondia pagodarum*	Laelapid & Spinturnicid mites
Male	Incidence	22.58%	77.42%	25.81%	3.23%
	Prevalence (mean±SEM)	0.23±0.08	1.74±0.37	0.48±0.20	0.09±0.096
	Density (mean±SEM)	1.00±0.00	2.25±0.92	1.88±0.26	3.00±0.00
Female	Incidence	27.78%	83.33%	16.67%	0
	Prevalence (mean±SEM)	0.39±0.13	2.56±2.56	0.19±0.08	0
	Density (mean±SEM)	1.4±0.16	3.07±0.32	1.17±0.67	0

Host sample size; Male=31, Female=36

Table 4. Shannon species diversity indices and evennes of five different ecto-parasite communities

Parasite community source	Shannon diversity index	Evenness
H. lankadiva , Wet zone	0.1064	0.1522
H. speoris , Wet zone	0.2787	0.3794
R. rouxi , Wet zone	0.4183	0.5985
R. rouxi , Intermediate zone	0.3316	0.5508
H. speoris , Dry zone	0.5739	0.8211