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Ectoparasites associated with bats in tropical forest of northeastern Thailand

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The ectoparasites of bats in Sakaerat Biosphere Reserve, northeastern Thailand were studied during June 2013 to May 2014. Altogether,66 bats were captured using mist nets and investigated for their ectoparasites. Of these, 21 individuals of 4 bat species were infested with ectoparasites (31.82%) i.e. *Hipposideros larvatus*, *Rhinolophus affinis*, *Myotis muricola and Cynopterus sphinx.* A total of 64 ectoparasites were collected from bats belonging to 4 families, 7 genera and 7 species i.e. *Brachytarsina* sp. (n = 28), *Raymondia* sp. (n = 21), *Nycteribia* sp. (n = 2), *Phthiridium* sp. (n =4), *Cyclopodia* sp. (n = 2), *Spinturnix myoti* (n = 6) and *Ixodessimplex* (n = 1). Number of parasite loads among bat species was not different (H = 1.45, df = 3, p = 0.69). Mean intensity of infestation of ectoparasites on bats ranged from 1 to 4.2. The highest Sorenson's similarity index of ectoparasites was found between *Hipposideros larvatus* and *Rhinolophus affinis* (0.67).The Brillouin diversity index of ectoparasites on bats ranged from 0 to0.56 whereas the Brillouin evenness index ranged from 0.71 to 1.

Keywords: Ectoparasite, bat, tropical, Sakaerat Biosphere Reserve, Thailand

Introduction

Ectoparasitesare organisms which live and feed on external surfaces of other organisms. They feed on various animal hosts; both domestic

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animals and wildlife (Hopla *et al*., 1994). Most mammals are well known harborers of ectoparasites, especially small mammals and bats (Ritzi and Whitaker, 2003).

Bats are high mobile animalsand have broad distribution. Several bat species can live together with thousands of individuals in one cave (Findley, 1993). These make them natural reservoirs of many ectoparasites such as ticks, mites, chiggers, bugs, fleas and flies (Dick *et al*., 2003). Many ectoparasites of bats are potential vector-borne which transmit numerous diseases to other animals both wildlife and domestic animals.

Bats are excellent models for studyinghost-parasite relationshipbecause their taxonomy and behaviours are extremely diverse (Kurta*et al*., 2007). In Southeast Asia, about 30% of total bat species in the world were documented (320 species) (Kingston, 2010). However, there is a lack of data on ectoparasites associated with bats in tropical regions, particularly in Southeast Asia (Krichbaum *et al*., 2009).Hence, studying host-parasite relationship between bats and their ectoparasites is important information in biology, systematics and phylogeny of their hosts (Fritz, 1983). Moreover, data on ectoparasites of bats are needed to determine their role as vectors of zoonotic pathogens (Kim *et al*., 2012).

Objectives: The objectives of this study were to identify and quantify rates of infestation of ectoparasites related to bat species in Sakaerat Biosphere Reserve, northeastern Thailand.

Materials and methods

Study area

Sakaerat Biosphere Reserve (SBR) is located in Nakhon Ratchasima province, northeastern Thailand (14° 30′ N, 101° 51′ E) (Fig 1). The approximate area is 80 km^2. The elevation ranges from 250 to 762 m above sea level. Average maximum temperature was 35°C, average minimum temperature was 16°C andthe annual precipitation was 1,200 mm. SBR has a tropical climate and three distinct seasons; the summer (March-May), the rainy season (June-October) and the winter (November-February). The main vegetation types in SBRare dry evergreen and dry dipterocarp forests. Dry evergreen forest covers 60% and**d**ry dipterocarp forest covers 18% of the station area. Other vegetation types in the station include bamboo forest, plantation forest and grassland(Sakaerat Biosphere Reserve, 2013).

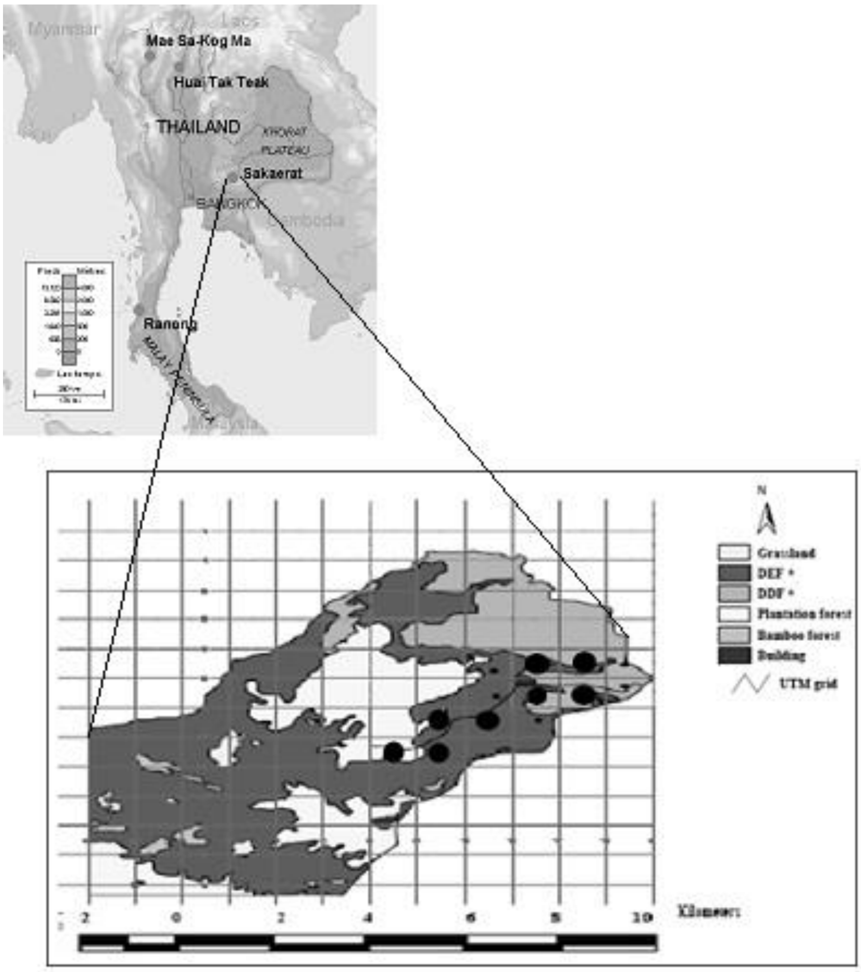

Fig. 1. Sampling plots and location of Sakaerat Biosphere Reserve (SBR), northeastern Thailand; (●) study plots: DEF = dry evergreen forest, DDF = dry dipterocarp forest

Data collection

Bats were captured at eight sampling sites in SBR. At each sampling site, two mist nets (9.4 m long x 2.5 m high, 25 mm mesh size, 4 shelves) were set up at ground level (2.5 m high) and two more others were set up at a higher level (5 m high). Nets were placed at flyways of bats such as ponds, open trails and gaps, operated for six hours from 18.00 to 24.00 and checked every 30 minutes.Bats were captured from June 2013 to May 2014. The total trapping effort, calculated according to Aguirre (2002), was 81,216 net meter hours (nmh).

Captured bats were kept in individual cloth bags and sent to field laboratory in SBR. Subsequently, bats were identified to species level, followingFrancis (2008).Ectoparasites were directly collected from captured bats using forceps and a fine toothbrush. In addition, the cloth bags were examined for ectoparasites that moved off the bats. The collected ectoparasites were preserved in vials containing 70% ethyl alcohol. Ectoparasites were mounted on glass slides in polyvinyl alcohol (PVA)(Millar *et al*., 1999). These, ectoparasites were identified following the method put forward byArthur (1956), Maa(1962) and Uchikawa *et al*. (1994).After processing, bats were released at the capture sites. The study was performed under the approval of the Suranaree University of Technology Animal Care and Use Committee.

Data analysis

Brillouin diversity index (*HB*)was used to quantify diversity of ectoparasites among hosts. Brillouin evenness index (*E*) was used to quantify equitability of ectoparasites among hosts (Magurran, 1988).Similarity in ectoparasites assemblages among hosts was compared using Sorenson's coefficient (*CC*) (Krebs, 1998).Kruskal-Wallis *H*-test was used to examine differences in parasite loads among bat species. The prevalenceof infestation by ectoparasites was calculated as number of infested hosts per number of examined hosts and was expressed as a percentage.The mean intensity of parasitism was examined asnumber of parasites per number of infested hosts(Bush *et al*., 1997).The ecology indices and statistical analyses were performed using PAST 3.02a (Hammer *et al*., 2001).The parasitism parameters were calculated using The Quantitative Parasitology 3.0 software (Rózsa *et al*., 2000).

Results

Altogether 66 individuals of 9 bat species were investigated for ectoparasites. Of these, 21 individuals of 4 bat species were infested with ectoparasites (31.82% of total captured bats), including *Hipposideros larvatus*, *Rhinolophus affinis*, *Myotis muricola and Cynopterus sphinx.Noectoparasite was found on Hipposideros diadema,Megaderma spasma*, *Chaerephon plicata,Cynopterus brachyotis andMegaerops niphanae (Table 1).*

Table1 Number of infested hosts and prevalence of *ectoparasites of captured bats*in SBR, northeastern Thailand

Host species	*No. captured bats*	*No. infested hosts*	Prevalence (%)
Hipposideros larvatus	*23*	*11*	*47.83*
Hipposideros diadema	*4*	-	-
Megaderma spasma	*6*	-	-
Chaerephon plicata	*3*	-	-
Rhinolophus affinis	*5*	*5*	*100*
Myotis muricola	*4*	*3*	*75*
Cynopterus sphinx	*10*	*2*	*20*
Cynopterus brachyotis	*2*	-	-
Megaerops niphanae	*9*	-	-
Total	*66*	*21*	*31.82*

A total of 64 individuals of ectoparasites were collected from batsin SBR, representing 3orders i.e. orders Diptera, Mesostigmata and Parasitiformes. The order Diptera comprised of *Brachytarsina* sp. and *Raymondia* sp. of family Streblidae and *Nycteribia* sp., *Phthiridium* sp. and *Cyclopodia* sp. of family Nycteribiidae. The order Mesostigmata contained *Spinturnix myoti*of family Spinturnicidae and the order Parasitiformes contained *Ixodessimplex*of familyIxodidae*(Table 2).*

Table 2 Prevalence, mean intensity, Brillouin diversity index (*HB*) and Brillouin evenness index (*E*) of ectoparasites collected on bats in SBR, northeastern Thailand

Host species	**n**	**Ectoparasite taxon**	**n**	**Host infested**	**Mean intensity**	**Prevalence (%)**	***HB***	***E***
Hipposideros larvatus	23	*Brachytarsina sp.*	22	11	2	47.83	0.56	0.71
		Nycteribia sp.	2	2	1	8.7		
		Phthiridium sp.	4	4	1	17.39		
Rhinolophus affinis	5	*Brachytarsina sp.*	6	2	3	40	0.47	0.79
		Raymondia sp.	21	5	4.2	100		
Myotis muricola	4	*Spinturnix myoti*	6	3	2	75	0	1
Cynopterus sphinx	10	*Cyclopodia sp.*	2	2	1	20	0.37	1
		Ixodes simplex	1	1	1	10		
Total	42		64	30	-	-	-	-

Mean intensity of infestation of ectoparasites on bats ranged from 0.75 to 2.7. However, numbers of parasite loads among bat species were not different (H = 1.45, df = 3, p = 0.69). The similarity of ectoparasites of *Hipposideros larvatus* and *Rhinolophus affinis* was 0.67. The sample sizes of other bat species did not allow for investigation of the similarity index.The diversity of ectoparasites on bats ranged from 0 to0.56, whereas the evenness of ectoparasites ranged from 0.71 to 1 (Table 2).

Discussions

Ectoparasite community and parasitism parameters

In total, 64 individuals in 7 species of ectoparasites were found on 66 bats in SBR, comprising bat flies (n = 57), mites (n = 6) and tick (n = 1). Only one ixodid tick recorded in this study can be regarded as a contaminant. There were no fleas, chiggers and bat bugs which were reported previously from bats in Thailand (Nadchatram and Mitchell, 1965; Hill and McNeely, 1975;Uchikawa and Kobayashi, 1979). Some ectoparasites were not found in this study may be because of the low number of hosts captured. According toChangbunjong *et al*. (2010)who surveyed ectoparasites of bats in Kanchanaburi, Thailand, only 2 individuals of ectoparsites from 23 bats were found.

Bat flies are the most common ectoparasites of bats in this area, followed by mites. This data corroborate with the study of Autino*et al*. (2011) who reported that mites and bat flies were common ectoparasites on bats, whereas fleas are rarely observed on bats. Many studies also showed that bat flies and mites were the most abundant ectoparasites of bats. For example, Moras*et al*. (2013) found 14 bat fly species and 9 mite species from bats in southern Minas Gerais, Brazil. Almeida *et al*. (2011) sampled 10 bat fly species and 11 mite species from bats in southeastern Brazil. Bertola *et al*. (2005) recorded 19 bat fly species from bats in São Paulo, Brazil. These data confirm that bat flies and mites are the primary ectoparasites of several bat species.

Although bats are notorious ectoparasite reservoirs, few bats in this study were infested by ectoparasites (31.82% of total captured bats).Similarly, other studies in Southeast Asia also showed that there were low ectoparasites prevalence on bats. For instance, Ahamad *et al*. (2013) found that 25.6% of bats in Kelantan, Malaysia were infested with acarines. Mariana *et al*. (2008) reported that 7.32% of bats were infested by ectoparasites in Kedah, Malaysia.Mean intensity of ectoparasites on bats in this study varied from 1 to 4.2. There is no data on quantitative parasitism of ectoparasites on bats in Thailand.However, Camilotti *et al*. (2010) hypothesized that mean intensity increases when competition of ectoparasites for hosts decreases. Moreover, variations in parasitism rates of ectoparasites on bats can be influenced by many factors such as temperature (Marshall, 1982),host shelter (Lewis, 1995;Patterson *et al*., 2007), roosting biology (Whiteman and Parker, 2004;ter Hofstede and Fenton, 2005) and grooming behaviour (Hart, 1994).

Ectoparasites associated with each bat species

Hipposideros larvatus

In the present study, *H. larvatus* were infested by three species of ectoparasites i.e. *Brachytarsina* sp.,*Phthiridium* sp. and *Nycteribia* sp.. This bat species was also found to be associated with many ectoparasite groups. For example, Bush and Robbins (2012) reported that *H. larvatus* were infested by *Ixodes vespertilionis* in southern China. Mariana *et al*. (2005) found that *H. larvatus* were infested by chiggers in *Malaysia*. Moreover, Gay *et al*. (2014) showed that *H. larvatus* were infested by 15 ectoparasite species. These data confirm that *H. larvatus* were harbours of numerous ectoparasite groups.

Rhinolophus affinis

R. affinis in this study were infested by *Brachytarsina* sp. and *Raymondia* sp.. Several studies also recorded that this bat species is associated with various ectoparasites groups. For example, Kolonin (2003) recorded that *R. affinis* was infested by *Ixodes simplex* in Vietnam. Mariana *et al*. (2008) found that *R. affinis* was infested by chiggers in Malaysia. Dahal and Thapa (2009) found fleas in family Ischnopsyllidae and bat flies in families Nycteribiidae and Streblidae fed on *R. affinis* in Nepal.Gay *et al*. (2014) showed that eight ectoparasite species fed on *R. affinis*. These data indicate that *R. affinis* also harbours many ectoparasites groups.

Myotis muricola

*There were no records of ectoparasites associated with M. muricola (*Bush and Robbins, 2012;Gay *et al*., 2014*).However,Spinturnix myoti* was found on *M. muricola in this study.This is the first record of ectoparasite associated with Myotis muricola.*

Cynopterus sphinx

In this study, C. sphinxis associated withCyclopodia sp. and *Ixodessimplex*. Theodor (1955) reported that bat flies of the genus *Eucampsipoda fed on C. sphinx in India.*Rajasekar *et al*. (2006) reported that bat flies of the genus *Basilia* were common ectoparasites of *C. sphinx.*These data support that many bat flies of family Nycteribiidae prefer Old World fruit bats (Dick and Patterson, 2006).

Other remaining bat species

There were no recorded ectoparasites on *Hipposideros diadema,Megaderma spasma, Chaerephon plicata,Cynopterus brachyotis* and *Megaerops niphanae in this study. However,*Gay *et al*. (2014) showed that *H. diadema was associated with 7 ectoparasite species,M. spasmawas associated with 7 ectoparasite species, C. plicata was associated with 11 ectoparasite species and C. brachyotis was associated with 11 ectoparasite species.None of these ectoparasites were found on these bats probably because of the low sample size of hosts.* It should be noted that the number of hosts is important for quantitative study on ectoparasites of bats.

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