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Research Article

Bat Diversity in Imbak Canyon Conservation Area: Note on their Echolocation Calls and Ectoparasites

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Abstract
Imbak Canyon Conservation Area (ICCA) is one of the conservation areas managed by the Sabah Foundation, which comprise of mixed vegetation forest landscape. A bat survey was conducted at ICCA from August 16th to 26th, 2017. A total of 141 individuals of bats representing 17 species were recorded from the eight nights of mist netting and harp trapping at various sites within the conservation area. Echolocation calls from 120 individuals of insectivorous bats representing 13 species were recorded, with 90% accuracy in relative amount. The captured bats were screened for ectoparasites from Order Diptera (91%), Mesostigmata (5%) and Ixodida (1%), and indicate that there is 66.7% prevalence. The results from the survey are paramount in enhancing information and knowledge on Bornean bats and their obligate ectoparasites.

Keywords: Bats, Conservation, Diversity, Echolocation, Ectoparasites

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Introduction

Bats are the second most species-rich mammalian Order in the world with over 1,400 described species (Burgin et al., 2018; Simmons & Cirranello, 2020). Regionally, Southeast Asia hosts about 378 species of bats (see Görföl et al., 2013; Soisook et al., 2013; Douangboubpha et al., 2014; Douangboubpha et al., 2016; Ith et al., 2015; Tu et al., 2015; Soisook et al., 2015; Soisook et al., 2016; Kuo et al., 2017; Tu et al., 2017; Soisook et al., 2017), of which 32% of the Southeast Asian bats are found in Malaysia, and at least 26% of these are found in Borneo (Payne et al., 1985; Francis, 2008; Phillipps & Phillipps, 2018). These figures indicate the significance of Malaysia as one of the centres for Old World bat diversity. However, more than a quarter of these bats are red listed by IUCN and many more have a declining population trend (IUCN, 2018). Bat populations have declined over the past decades, globally and regionally, largely due to habitat loss (Mickleburgh et al., 2002; Frick et al., 2019).

Regarded as the most abundant mammal in the tropics and sub-tropical forests (Findley, 1993; Patterson et al., 2003), bats are a vital component of an ecosystem. They are important seed dispersers, pollinators, and suppressors of arthropod populations, including agricultural pests (Fleming, 1998; Kunz et al., 2011). Regardless of their diversity and significance towards ecosystems, bats are still considered as a marginalised component of biodiversity, subsequently underestimating their role in ecosystems. Various species of these nocturnal flying mammals are highly dependent on forest covers. Their presence correlate with availability and suitability of roosting sites, signifying the impact of habitat loss toward species that exploit tree cavities and foliage as roost (Schulze et al., 2000).

In Borneo, more than half of the insectivorous bats are dominant in the forest interior. Their wings and echolocation calls are designed for manoeuvrability and detection in a cluttered environment (Schnitzler & Kalko, 1998; Kingston et al., 2003), contributing to their superiority in a cluttered environment, however, energetically costly in open spaces. Besides exploring the eco-morphology of bats, another component that offers important information to understand the biology, systematics and phylogenetics aspects of bats is the bat obligate ectoparasites (Fritz,1983). Like other mammals, bats are susceptible to parasitism and information on bat-obligate parasites are scarce in certain regions including Malaysia (e.g. Azhar et al., 2015). Hence, understanding the host-parasite association might provide better insights to further understand the complex ecology of bats.

Like most part of Southeast Asia, the continuous loss of forest landscape in Borneo have gained growing attention among biologists and conservationists in exploring the values of secondary and regenerated forest as a potential ground to facilitate the recovery of a local biodiversity. These prospective areas may provide substantial areas and resources to support viable bat populations, eventually promoting population recovery. ICCA is one of the optimal areas portentous for such capacity. The heterogeneous landscape within this conservation area could provide refuge, and potentially contribute to population recovery of various faunal species. Several bat surveys have been conducted at various sites in ICCA with the most recent one conducted in August 2017, in conjunction with the scientific expedition at Batu Timbang Research Station organised by Yayasan Sabah. The results from this survey will complement results from previous bat surveys in ICCA. Herein, results from the recent survey and the compilation of bat species, echolocation calls, and ectoparasites recorded from ICCA are reported.

Methodology

Study sites

ICCA is among prominent forest areas in Sabah managed by Yayasan Sabah. The area is covered by various types of vegetation including dipterocarp forest, tropical heath forest, and montane forest. This area was gazetted in 2009 as a Class I Forest Reserve to protect the vigorous ecosystem and its content. Bat trapping was done at various trails around the Imbak Canyon Study Centre (05°05.623'N, 117°02.371'E) and Batu Timbang Research Station (05°00.197'N, 117°04.762'E). Among the trails covered during the survey were Big Belian Trail, Nepenthes Trail and Waterfall Trail at Imbak Canyon Study Centre, and Rafflesia Trail and Lanap Trail at Batu Timbang Research Station (Figure 1).

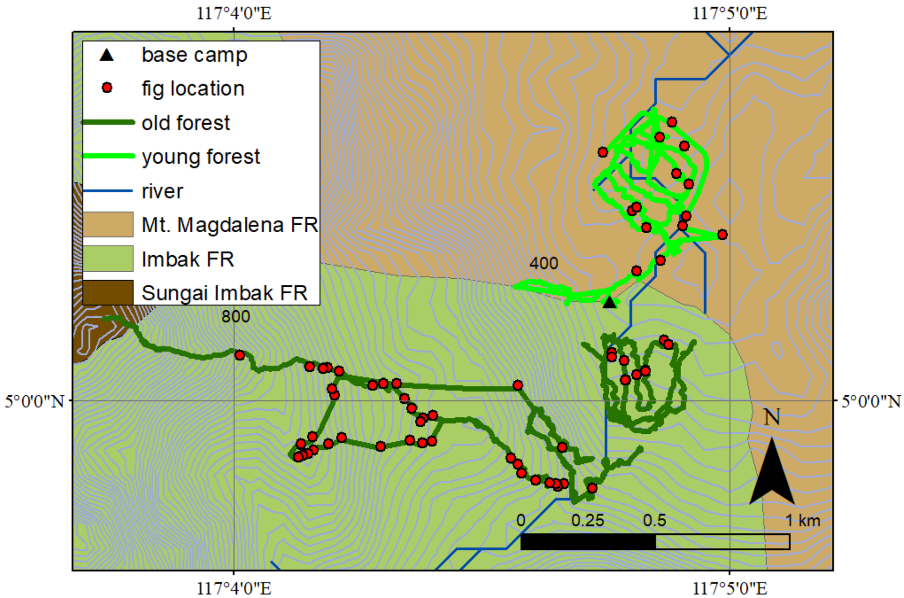

Figure 1. The trapping sites composed of primary forest, riverine forest, kerangas and regenerated forest.

Trapping and Sample Processing

Bat trappings were done using four four-bank harp traps (Francis, 1989) and an average of eight mist nets (four shelves; 36 mm mesh size). Both nets and traps were positioned in the forest understorey, across jungle trails and streams. The nets and traps were moved to a new location each night to avoid habituation by the bats, which may cause the capture rate to dwindle (Kunz et al., 2009). Mist nets and harp traps were set at dusk before the first bat emerged and were checked every 15-30 minutes until 2200 hours, depending on the intensity of capture. During the day, each trail was explored to locate active roosts. Roosting bats were captured using mist nets and hand nets. Captured bats were held inside individual cloth bags and identified following Payne et al., (1985) and Phillips & Phillips (2016). Biometric measures such as body mass (g) and forearm length (mm) were taken for each captured individual. In addition to this, sex and reproduction stage for each captured bat was recorded. Echolocation calls of the insectivorous bats were recorded using Pettersson M500 USB Ultrasound Microphone. Calls were recorded by releasing the bats in a flight tent. Captured bats were released at the capture point once measurements and recordings were completed.

Ectoparasite screening was done by examining each bat host under an intensive light source specifically at the fur area of the body, wing membranes and uropatagium. All visible ectoparasites were removed and placed in vials containing 70% ethyl alcohol, using separate vials for each bat host. Each vial was labelled with field number and host information. Meanwhile, taxonomic assignment for the ectoparasites was done following Jobling (1930), Theodor (1967), Maa (1962; 1971) and Azhar et al. (2015). Voucher specimens for the host and ectoparasites were stored at the Institute for Tropical Biology and Conservation. Ectoparasite prevalence was calculated in percentage, for each species and later to be total up for the sampled population, using given formulation (**P**):

$$\mathbf{P} = \frac{\sum I}{\sum F} \text{ x } 100\%; \text{ where}$$

Σ^I is the number of infected individual from each species
Σ^F is the total number of bats captured in this study

Results

Bat species richness of Imbak Canyon Conservation Area

In total, 141 bat individuals were captured during this survey. The bats accumulated represent 17 species from 5 families, Pteropodidae (three genera; three species), Emballonuridae (one genus; two species), Rhinolophidae (one genus; seven species), Hipposideridae (one genus; two species) and Vespertilionidae (two genera; four species). To date, the results for the accumulated number of bat species from the current survey and previous surveys is 27 species (Table 1).

Table 1. Number of individuals for each bat species captured using various sampling methods from the current study.

Family	Species	Common Name	Number of Individuals
Pteropodidae	*Cynopterus brachyotis*	Lesser Dog-faced Fruit Bat	1
	Penthetor lucasi	Lucas's Short-nosed Fruit Bat	1
	Macroglossus minimus	Dagger-toothed Long-nosed Fruit Bat	2
Emballonuridae	*Emballonura alecto*	Small Asian Sheath-tailed Bat	10
	Emballonura monticola	Lesser Sheath-tailed Bat	4
Rhinolophidae	*Rhinolophus acuminatus*	Acuminate Horseshoe Bat	1
	Rhinolophus creaghi	Creagh's Horseshoe Bat	10
	Rhinolophus borneensis	Bornean Horseshoe Bat	3
	Rhinolophus sedulus	Lesser Woolly Horseshoe Bat	2
	Rhinolophus trifoliatus	Trefoil Horseshoe Bat	1
Hipposideros	*Hipposideros ater*	Dusky Leaf-nosed Bat	3
	Hipposiders cervinus	Fawn Leaf-nosed Bat	74
Vespertilionidae	*Myotis muricola*	Nepalese Whiskered Myotis	1
	Myotis horsfieldii	Horsfield's Myotis	2
	Kerivoula papillosa	Papillose Woolly Bat	20
	Kerivoula pelucida	Clear-winged Woolly Bat	1
	Kerivoula minuta	Least Woolly Bat	5

Echolocation Calls of Insectivorous Bats

From the total number of individuals captured in the survey, echolocation calls for 120 individuals of insectivorous bats from 13 species were successfully recorded. The discriminant function analysis indicated that out of 120 individuals, 108 were grouped into the call ranges of the 13 species of bats with 90% accuracy in relative amount. Table 2 shows the analysed calls for bats of ICCA.

Table 2. Echolocation calls for the insectivorous bats recorded from this survey at Imbak Canyon Conservation Area.

Species	Call Structure	SF (kHz)	EF (kHz)	FmaxE (kHz)	D (ms)	IPI (ms)	Duty Cycle (%)	n
EMBALLONURIDAE								
Emballonura alecto	QCF-FM	48.23 ± 1.18 (47.05-49.41)	43.07 ± 2.62 (40.45-45.69)	46.14 ± 0.09 (46.05-46.23)	3.09 ± 0.56 (2.53-3.65)	26.97 ± 4.85 (22.12-31.82)	11.80 ± 2.97 (8.83-14.77)	9
Emballonura monticola	QCF-FM	48.00 ± 0 48.00	48.00 ± 3.29 (44.71-51.29)	48.63 ± 1.10 (47.53-49.73)	2.90 ± 0.26 (2.64-3.16)	16.73 ± 1.03 (15.70-17.76)	17.33 ± 1.55 (15.78-18.88)	3
HIPPOSIDERIDAE								
Hipposideros ater	CF-FM	142.10 ± 0.55 (141.55-142.65)	118.40 ± 1.74 (116.66-120.14)	142.12 ± 0.50 (141.62-142.62)	5.24 ± 0.61 (4.63-5.85)	22.18 ± 1.80 (20.38-23.98)	23.71 ± 2.96 (20.75-26.67)	6
Hipposideros cervinus	CF-FM	119.53 ± 2.64 (116.89-122.17)	108.93 ± 7.13 (101.80-116.06)	119.54 ± 2.63 (116.91-122.17)	4.28 ± 0.89 (3.39-5.17)	22.19 ± 2.68 (19.51-24.87)	19.52 ± 4.49 (15.03-24.01)	27
RHINOLOPHIDAE								
Rhinolophus borneensis	FM-CF-FM	76.66 ± 2.72 (73.94-79.38)	76.84 ± 7.33 (69.51-84.17)	84.37 ± 2.09 (82.28-86.46)	51.67 ± 7.73 (43.94-59.40)	123.64 ± 18.90 (104.74-142.54)	42.60 ± 8.80 (33.80-51.40)	9
Rhinolophus creaghi	FM-CF-FM	59.93 ± 1.95 (57.98-61.88)	64.85 ± 4.20 (60.65-69.05)	66.69 ± 1.94 (64.75-68.63)	44.10 ± 4.12 (39.98-48.22)	103.98 ± 10.77 (93.21-114.75)	42.76 ± 5.22 (37.54-47.98)	21
Rhinolophus sedulus	FM-CF-FM	57.45 ± 1.14 (56.31-58.59)	59.48 ± 2.20 (57.28-61.68)	60.75 ± 0.60 (60.15-61.35)	48.98 ± 8.38 (40.60-57.36)	94.22 ± 5.54 (88.68-99.76)	52.47 ± 10.80 (41.67-63.27)	6
Rhinolophus trifoliatus	FM-CF-FM	47.93 ± 0.58 (47.35-48.51)	50.80 ± 0.52 (50.28-51.32)	51.40 ± 0.00 51.40	39.67 ± 1.45 (38.22-41.12)	118.67 ± 4.61 (114.06-123.28)	33.49 ± 2.53 (30.96-36.02)	3
VESPERTILIONIDAE								
Kerivoula minuta	FM	162.02 ± 15.57 (146.45-177.59)	85.52 ± 8.23 (77.29-93.75)	115.59 ± 8.04 (107.55-123.63)	3.27 ± 1.56 (1.71-4.83)	22.99 ± 6.31 (16.68-29.27)	13.63 ± 3.41 (10.22-17.04)	9
Kerivoula papillosa	FM	175.16 ± 12.01 (163.15-187.17)	74.31 ± 5.63 (68.68-79.94)	107.15 ± 11.37 (95.78-118.52)	2.92 ± 0.55 (2.37-3.47)	15.81 ± 3.11 (12.70-18.92)	18.92 ± 4.28 (14.64-23.20)	15
Kerivoula pellucida	FM	227.03 ± 8.97 (218.06-236.00)	81.20 ± 0.00 81.20	154.23 ± 22.46 (131.77-176.69)	2.41 ± 0.15 (2.26-2.56)	8.68 ± 0.11 (8.57-8.79)	27.82 ± 2.05 (25.77-29.87)	3
Myotis muricola	FM	82.57 ± 4.56 (78.01-87.13)	59.17 ± 1.10 (58.07-60.27)	64.87 ± 3.96 (60.91-68.83)	1.56 ± 0.36 (1.20-1.92)	25.33 ± 2.65 (22.68-27.98)	6.19 ± 1.59 (4.60-7.78)	3
Myotis horsfieldii	FM	98.47 ± 11.44 (87.03-109.91)	52.25 ± 3.58 (48.67-55.83)	63.67 ± 2.77 (60.90-66.44)	2.11 ± 0.51 (1.60-2.62)	18.58 ± 2.13 (16.45-20.71)	11.30 ± 2.33 (8.97-13.63)	6

Sf – Start Frequency; EF – End Frequency; FmxE (kHz) – Frequency at Max Energy; D (ms) – Duration; IPI (ms) – Pulse Interval; n – number of individuals

Host-ectoparasite association in ICCA Bats Community

In total, 61 and 32 individuals of bats were recorded with ectoparasites from Imbak Canyon Study Centre and Batu Timbang Research Station, respectively. In total, 13 species of ectoparasites from six families were recorded from the 93 infested individuals, with 66.7% prevalence. Specifically, four out of ten species of bats recorded from the Imbak Canyon Study Centre come up with a total of 54 incidence of bat flies, 4 incidence of mites, and 1 incident of tick infestation. Meanwhile, seven out of 10 species recorded from Batu Timbang Research Centre came up with 32 incidence of bat flies and one incident of mite and tick infestation. For both areas where trappings were conducted, *Hipposideros cervinus* have the highest incidence of ectoparasite, followed by *Kerivoula papillosa* (Table 3). The colour plates represent each species of ectoparasites collected from the survey (Figure 2 and Figure 3).

Table 3. Taxonomic checklist of ectoparasites and their hosts recorded from this study.

Host Species	Ectoparasite Species										
	Brachytarsina sp.	*Raymondia* sp.	*Stylidia* sp.	*Eucampsipoda penthetoris*	Nycteribiidae sp. 1	Nycteribidae sp. 2	*Meristaspis* sp.	*Spinturnix* sp.	Unidentified sp. 1	Unidentified sp. 2	Unidentified sp. 3
Cynopterus brachyotis	-	-	-	-	-	-	-	-	-	-	-
Penthetor lucasi	-	-	-	1	-	-	1	-	-	-	-
Macroglossus minimus	-	-	-	-	-	-	-	-	-	-	-
Emballonura Alecto	1	-	1	-	-	-	-	-	-	-	1
Emballonura monticola	-	-	-	-	-	-	-	-	-	-	-
Rhinolophus acuminatus	-	-	-	-	-	-	-	-	-	-	-
Rhinolophus creaghi	3	-	3	-	-	-	-	-	-	-	-
Rhinolophus borneensis	1	-	-	-	-	-	-	-	-	-	-
Rhinolophus sedulus	-	1	-	-	-	-	-	-	-	-	-
Rhinolophus trifoliatus	-	-	-	-	-	-	-	-	-	-	-
Hipposideros ater	-	1	1	-	-	-	-	-	-	-	-
Hipposiders cervinus	7	44	11	-	2	1	-	-	-	1	1
Myotis muricola	-	-	-	-	-	-	-	-	-	-	-
Myotis horsfieldii	-	-	2	-	-	-	-	-	-	-	-
Kerivoula papillosa	-	-	3	-	-	-	-	4	-	-	-
Kerivoula pelucida	-	-	-	-	-	-	-	-	1	-	-
Kerivoula intermedia	-	-	-	-	-	-	-	-	-	-	-
Total Incidence						92					

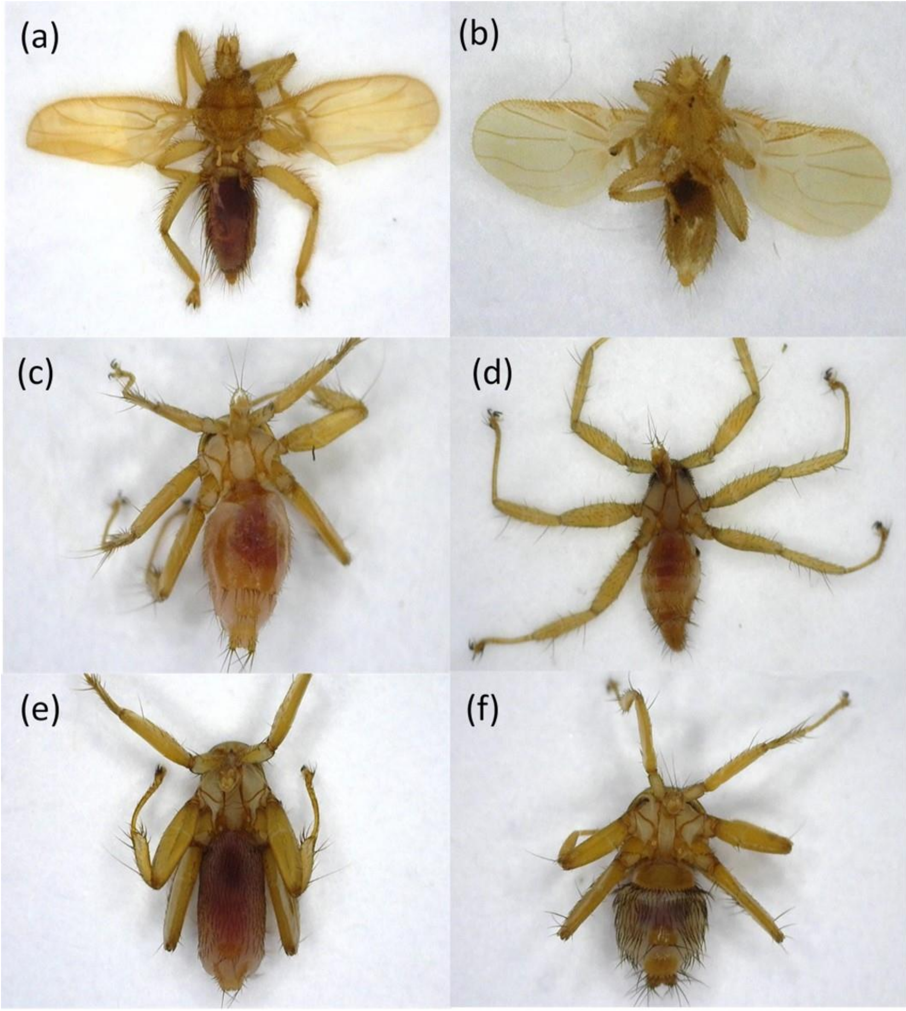

Figure 2. (a) Dorsal view of *Brachytarsina* sp._ (b) Dorsal view of *Raymondia* sp._ (c) Dorsal view of female *Stylidia* sp._ (d) Dorsal view of *Eucampsipoda penthetoris* (e) Dorsal view of Nycteribiid species 1. (f) Dorsal view of Nycteribiid species 2.

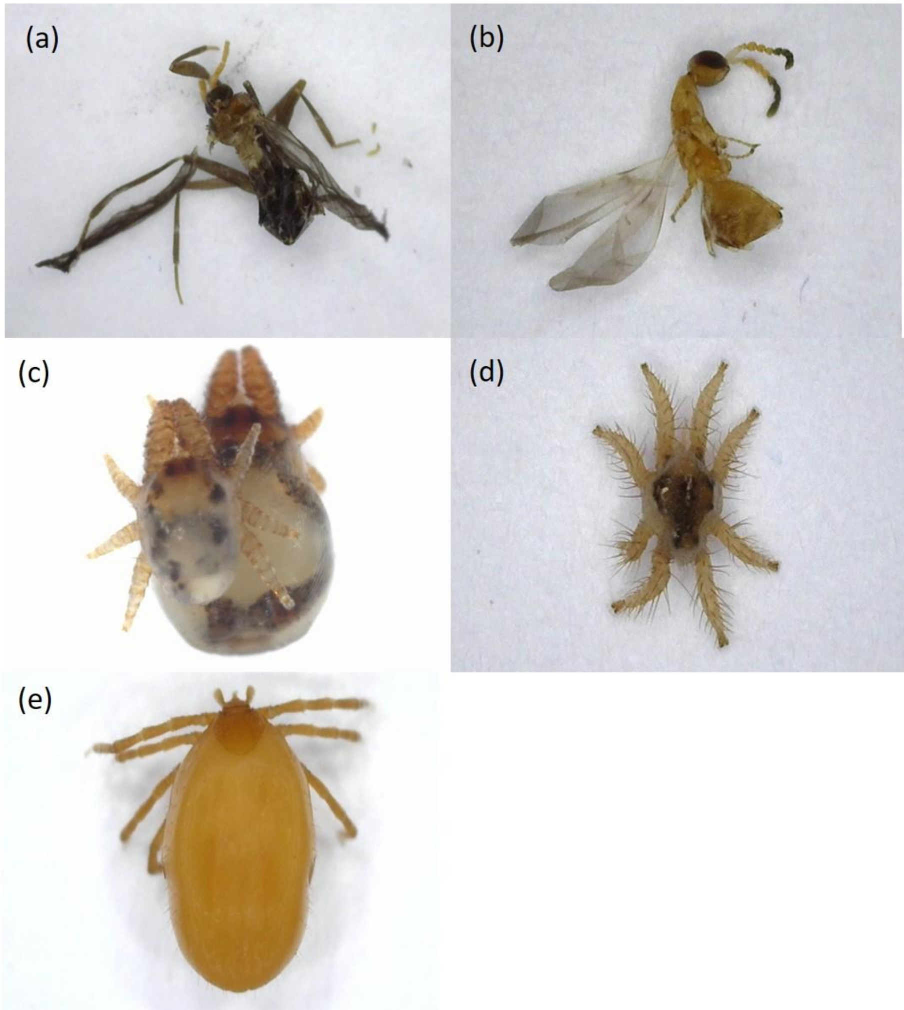

Figure 3. (a) Dorsal view of Unknown sp. 3 (b) Side view of Unknown sp. 4 (c) Dorsal view of adult and juvenile *Meristaspis* sp. (d) Dorsal view of *Spinturnix* sp. (e) Dorsal view of Unknown sp. 5.

Discussion

This study recorded a total of 17 species of bats from the eight nights of mist-netting and harp trapping. The most abundant species was *Hipposiders cervinus*, followed by *Kerivoula papillosa*. There were six species of bats recorded in singleton, namely, *Cynopterus brachyotis*, *Penthetor lucasi*, *Rhinolophus acuminatus*, *R. trifoliatus*, *Myotis muricola*, and *Kerivoula pellucida*. It is speculated that the low capture of pteropodid bats was influenced by the availability of fruiting trees and the placing of mist nets which cover only forest understorey. Hodgkison et al. (2004) mentioned that temporal variation affects the presence of pteropodids where these bats often travel to areas where food can be found in abundance. Meanwhile, the latter may also have effect on the capture rate of pteropodids where some pteropodids are more abundant in the higher forest strata (Francis, 1990; Francis, 1994). The abundance of potential roost sites found in ICCA reckon the suitability of the forest in providing resources for the bats. There were numerous roosting sites identified during the survey at ICCA, from which *Emballonura alecto*, *E. monticola* and *R. borneensis* were captured. Furthermore, ginger plants were found abundant particularly at Batu Timbang Field Station. These plants are known as one of the preferred roosts for *Kerivoula hardwickii*. However, there were no individuals of *K. hardwickii* recorded roosting in the unfurled leaves of ginger plants.

Based on the IUCN Red List of Threatened Species, six out of 27 species of bats recorded from ICCA are listed as Near Threatened, while the others were Least Concern. Conversely, these evaluations are not consistent with the protection list at local level. Based on the Sabah Wildlife Conservation Enactment 1997, none of the bats in the ICCA are listed in the protected species list except for the large flying fox, for which the hunting and selling of this species is allowed with the acquirement of a license. Bats alongside with other small mammals are often deemed as a less charismatic animal, negating their significant ecosystem services both in the natural environment and commercial industries. With growing environmental awareness and conservation driven direction of Sabah, the state government should consider taking an immediate action to plan and strategize on how to increase environmental awareness and sustainable practices among the locals. Furthermore, it is vital for the State Government to revise and remap the conservation status in the Sabah Wildlife Conservation Enactment 1997 to enhance and expand their conservation and management plans (Azhar et al., 2018).

Insectivorous bats made up 77.8% of the total bats recorded at ICCA. One of the important features of the insectivorous bats is their ability to produce echolocation, which empower their flight capacity and manoeuvrability (Schnitzler et al., 2003). Information on echolocation calls of bats in Sabah is very limited hence, accentuating the significance of the calls collected from this survey towards bat research in Sabah. The call data generated from this study is fundamental to the construction of call libraries to facilitate monitoring for this elusive taxon. On top of all, echolocation is one of the significant components derived from the multitude of selections and radiation occurring throughout millennia within the chiropterans (see Schnitzler & Kalko, 2001; Kingston & Rossiter, 2004; Jung et al., 2014).

The survey confirmed a monoxenous association between the bat fly, *Eucampsipoda penthetoris*, and the Dusky fruit bat, *P. lucasi*, indicating an evidence of host specificity and supporting previous report in Malaysia (Azhar et al., 2015). However, there were no specific information on the degree of association between bats and their ectoparasites acquired from this survey. Furthermore, *H. cervinus* was recorded with the highest infestation rate. This species is known to roost in large colony, which may have resulted in the higher infestation rate (Matthee & Krasnov, 2009). Noteworthy, there was an incidence of ixodid ticks recorded from one of the bat species captured during this survey. It is an unusual encounter because most of the hard ticks are known to infest the non-volant small mammals. However, bats have diverse roost-site selection which could have resulted this accidental incidence. Future studies should be designed to understand roosting dynamics in bats and how it influences the host-parasite association.

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Appendix 1. List of all bat species recorded from Imbak Canyon Conservation Area and their conservation status following IUCN Red List of Threatened Species and Sabah Wildlife Conservation Enactment 1997.

Family	Species	Common Name	Conservation Status	
			IUCN Red List	WCE 1997
Pteropodidae	*Pteropus vampyrus*	Large Flying Fox	NT	Schedule 3
	Cynopterus brachyotis	Lesser Dog-faced Fruit Bat	LC	-
	Penthetor lucasi	Lucas's Short-nosed Fruit Bat	LC	-
	Balionycteris maculata	Spotted-winged Fruit Bat	LC	-
	Aethalops aequalis	Borneo Fruit Bat	LC	-
	Macroglossus minimus	Dagger-toothed Long-nosed Fruit Bat	LC	-
Emballonuridae	*Emballonura alecto*	Small Asian Sheath-tailed Bat	LC	-
	Emballonura monticola	Lesser Sheath-tailed Bat	LC	-
Rhinolophidae	*Rhinolophus acuminatus*	Acuminate Horseshoe Bat	LC	-
	Rhinolophus creaghi	Creagh's Horseshoe Bat	LC	-
	Rhinolophus borneensis	Bornean Horseshoe Bat	LC	-
	Rhinolophus affinis	Intermediate Horseshoe Bat	LC	-
	Rhinolophus sedulus	Lesser Woolly Horseshoe Bat	NT	-
	Rhinolophus trifoliatus	Trefoil Horseshoe Bat	LC	-
Hipposideros	*Hipposideros bicolor*	Bicolored Leaf-nosed Bat	LC	-
	Hipposideros ater	Dusky Leaf-nosed Bat	LC	-
	Hipposideros dyacorum	Dayak Leaf-nosed Bat	LC	Schedule 2
	Hipposiders cervinus	Fawn Leaf-nosed Bat	LC	-
	Hipposideros diadema	Diadem Leaf-nosed Bat	LC	-
Nycteridae	*Nycteris tragata*	Malayan Slit-faced Bat	NT	-
Vespertilionidae	*Myotis muricola*	Nepalese Whiskered Myotis	LC	-
	Myotis ater	Peter's Myotis	LC	-
	Myotis horsfieldii	Horsfield's Myotis	LC	-
	Kerivoula papillosa	Papillose Woolly Bat	LC	-
	Kerivoula pelucida	Clear-winged Woolly Bat	NT	-
	Kerivoula intermedia	Small Woolly Bat	NT	-
	Kerivoula minuta	Least Woolly Bat	NT	-