

Diversity of fruit bats (Pteropodidae) and their ectoparasites in Batuputih Nature Tourism Park, Sulawesi, Indonesia

MEIS NANGOY^{1,✉}, TILTJE RANSALELEH¹, HANDRY LENGKONG², RONI KONERI², ALICE LATINNE^{3,4}, RANDALL C. KYES⁵

¹Faculty of Animal Science, Universitas Sam Ratulangi. Jl. Bahu, Manglayang, Manado 95115, North Sulawesi, Indonesia.

✉email: mnangoy@unsrat.ac.id

²Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sam Ratulangi. Jl. Bahu, Manglayang, Manado 95115, North Sulawesi, Indonesia

³Wildlife Conservation Society, Viet Nam Country Program. 106, D Building, No. 3 Thanh Cong Street, Thanh Cong Ward, Ba Dinh District, Ha Noi, Viet Nam

⁴Wildlife Conservation Society, Health Program. 2300 Southern Boulevard Bronx, New York 10460, USA

⁵Departments of Psychology, Global Health, and Anthropology, Center for Global Field Study; Washington National Primate Research Center, University of Washington, Seattle, Washington, USA

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Abstract. Nangoy M, Ransaleleh T, Lengkong H, Koneri R, Latinne A, Kyes RC. 2021. Diversity of fruit bats (Pteropodidae) and their ectoparasites in Batuputih Nature Tourism Park, Sulawesi, Indonesia. *Biodiversitas* 22: 3075-3082. Bats play an important role in the ecosystem as pollinators, seed dispersers, and predators, therefore, this study aims to identify the diversity of fruit bat species and ectoparasites at Batuputih Nature Tourism Park, Sulawesi, Indonesia. The study was conducted from May to July 2019, and carried out in three different habitats, namely primary and secondary forest, as well as agricultural land. Besides, the bats were caught using a mist net while the ectoparasites were collected and identified using morphological criteria. A total of 253 bats were sampled representing 10 species (all belonging to the family Pteropodidae) namely *Cynopterus brachyotis* (24.90%), *C. luzoniensis* (9.88%), *Dobsonia exoleta* (1.19%), *Macroglossus minimus* (3.16%), *Nictymene cephalotes* (4.75%), *N. minutus* (0.79%), *Rousettus amplexicaudatus* (17%), *R. celebensis* (20.95%), *Thoopterus nigrescens* (17%), and *Thoopterus* sp. (0.4%). *Cynopterus brachyotis* was the most abundant species (n = 63). Meanwhile, a total of 479 ectoparasites were collected and identified as belonging to three families, namely Nycteribiidae, Streblidae, and Spinturnicidae. Nycteribiidae (genus *Leptocyclopodia*) was the most abundant ectoparasite taxa (n= 475) while the highest mean abundance and intensity were observed for the genus *Thoopterus* and *Rousettus*. This study provides important baseline data for future reference in monitoring bat population status and conservation efforts in the region. Given the close relationship between the local people and bats (e.g. hunting and consumption), more work is needed to address the potential pathogen risks from zoonotic transmission, both from bats and the respective ectoparasites.

Keywords: Nycteribiidae, parasitism, Pteropodidae, Spinturnicidae, Streblidae

INTRODUCTION

Bats play several important roles in the ecosystem including pollination, seed dispersal, and insect predation. Despite the important contributions to the function and sustainability of the ecosystem, bats are exposed to numerous anthropogenic threats that affect survival, such as hunting (Sheherazade and Tsang 2015; Mildenstein et al. 2016). In certain regions of Sulawesi, Indonesia, fruit bats are used for human consumption (Ransaleleh et al. 2020) and as traditional medicine by local communities living near the forest. Furthermore, bats are also part of wildlife trade extending throughout majority of the island's provinces, as part of a well-organized, dynamic, and easily accessible network involving many actors (Latinne et al. 2020).

Aside from anthropogenic threats such as hunting, parasitism poses another serious threat to the health of bats. Ectoparasites in particular are known to affect the physical conditions and therefore impact the long-term viability of the population (Webber and Willis 2016). Although no

direct effect of ectoparasites on bat mortality has been observed, some studies have shown that ectoparasites tend to decrease bats' fecundity. Ticks, mites, chiggers, bugs, fleas, and flies are among the types of ectoparasites discovered on bats (Almeida et al. 2011; Holz et al. 2018). Some of the ectoparasites are associated with pathogens that might cause disease in humans or animals both wild and domestic (Reeves et al. 2016). Therefore, studies on bat ectoparasites are important to determine the potential role as vectors of zoonotic pathogens (Kim et al. 2012). Moreover, flies belonging to the families Nycteribiidae and Streblidae (Hiller et al. 2020), and mites belonging to the family Spinturnicidae (Almeida et al. 2016) are hematophagous organisms, and are commonly found on bats in tropical regions.

In the last decade, a number of studies have been conducted on bat ectoparasites in several countries namely Brazil (Almeida et al. 2011), Canada (Czenze and Broders 2011), Australia (Holtz et al. 2018), Philippines (Pader et al. 2018), Singapore (Lee et al. 2018; Lim et al. 2020), and South East Asia region (Gay et al. 2014). According to

Luguterah and Lawer (2015), frugivorous bats were more infested by ectoparasites than insectivorous bats. Some fruit-eating bats only eat part of the fruit (Dumont and O'neal 2004) while the remaining portion on the tree or which falls to the ground is then consumed by other wild animals or livestock on agricultural land. This in turn presents a potential source of pathogen transmission from bats to livestock or humans (Mikail et al. 2017). Moreover, Yang et al. (2017) reported that the bat genus, *Rousettus* is a reservoir for filoviruses. *Rousettus* and *Cynopterus* were also reported as reservoirs for the Marburg virus, while *Cynopterus brachyotis* and *Macroglossus sobrinus* were reservoirs for Leptospirosis (Mulyono et al. 2018).

In Indonesia, the knowledge regarding bats and ectoparasites is limited, hence, there is a need for a detailed study on bats and the potential effects of ectoparasites. This study aims to identify the diversity of fruit bats and the ectoparasites at Batuputih Nature Tourism Park (BNTP) in North Sulawesi, Indonesia, and to obtain baseline data about the status of bats in the region as a foundation for future assessment of potential zoonotic pathogen risk posed by both bats and the ectoparasites.

MATERIALS AND METHODS

Study area

This research was conducted in Batuputih Nature Tourism Park (BNTP), North Sulawesi, Indonesia from May to July 2019 (Figure 1). The BNTP is located within the Tangkoko Nature Reserve system (renamed "Tangkoko Conservation Forest Management Unit" in 2016) between latitude 1°33'29.7" N and longitude 125°9'34.5" E. It includes the Batuputih village in the North Bitung Sub-district, Bitung City, North Sulawesi Province, Indonesia. The park is located at an altitude of 0-200 m above sea level with flat and slightly hilly terrain (O'Brien and Kinnaird 1996; Kyes et al. 2013).

The BNTP climate conditions (based on the Schmidt and Ferguson classification) (Arrijani and Rizki 2020) include climate type B with an average annual rainfall ranging from 2,279 mm and a daily average temperature between 23-24°C. Furthermore, the vegetation is dominated by coastal and secondary forest vegetation including species from Anacardiaceae, Burseraceae, Combretaceae, Moraceae, Sapotaceae, Thymeleaceae, Vitaceae, and others. The park has a large camping ground used for recreation and conservation education, as well as a station for field research.

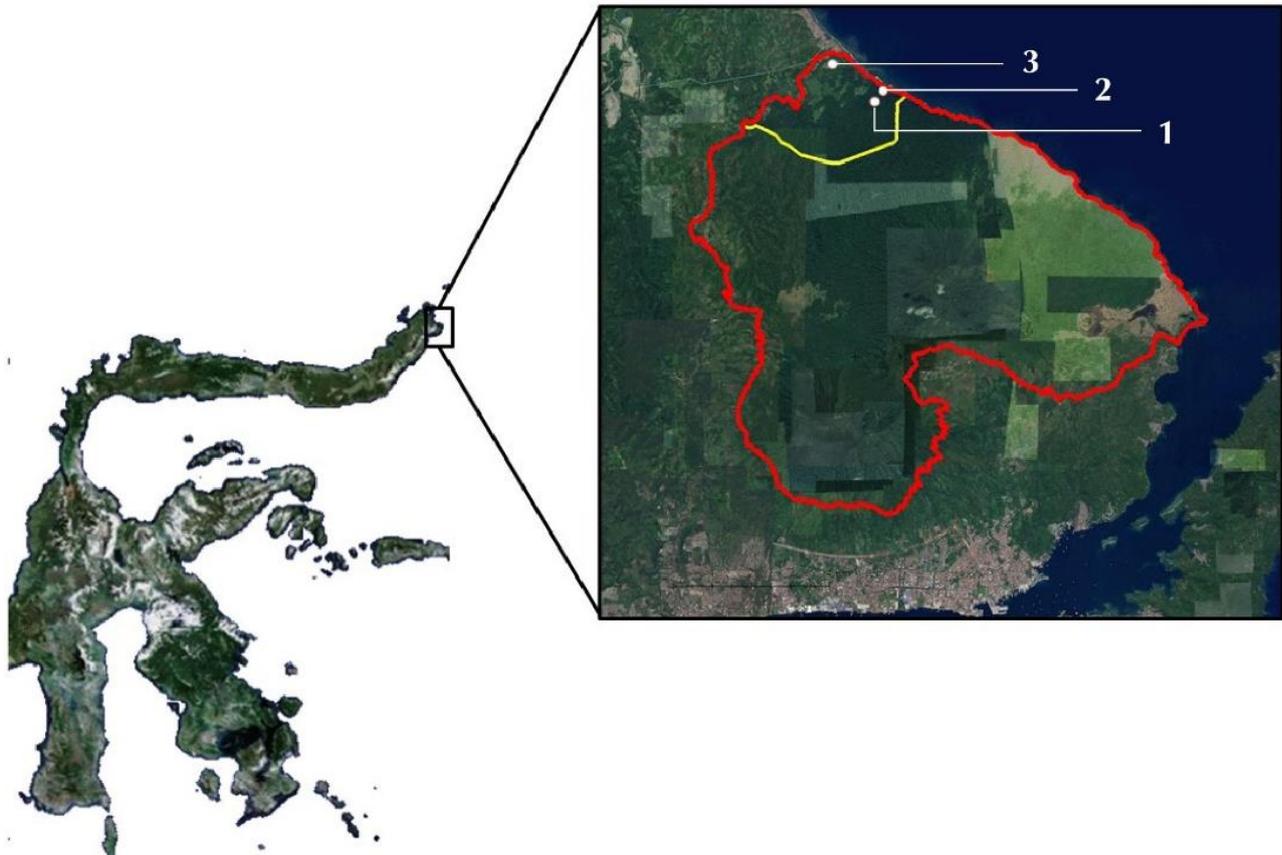


Figure 1. Map showing location of the Tangkoko Nature Reserve system (Tangkoko Conservation Forest Management Unit, outlined in red) and the Batuputih Nature Tourism Park (located at the northern tip of the reserve, within the yellow line). The three bat sampling locations are indicated by the numbers white dots: 1. Primary Forest; 2. Secondary Forest; and 3. Agricultural Land). (Courtesy: Google Earth 2020; Maxar Technologies 2020)

Sampling locations

Three types of habitats were selected as sampling locations: primary and secondary forest, reserve, and agricultural land around the reserve. The primary forest was defined as an undisturbed habitat with trees belonging to the Anacardiaceae (*Dracontomelon dao*), Annonaceae (*Cananga odorata*), Lamiaceae (*Vitex quinata*), Sapotaceae (*Palaquium obovatum*), and Anacardiaceae (*Koordersiodedron pinnatum*). Meanwhile, the secondary forest consisted of regrown forest in disturbed habitat (e.g., following forest fires) and included trees belonging to the Moraceae (*Ficus* sp.), and Combretaceae (*Terminalia catappa*). Agricultural land was located at the forest fringe area, where local communities planted crops such as Anacardiaceae (*Mangifera* sp.), Caricaceae (*Carica papaya*), and Musaceae (*Musa* sp.). A reconnaissance survey was conducted in each habitat before the main study to identify the location of fruit bat foraging trees (bearing fruit or flowering) and flight paths as well as to determine the appropriate netting locations.

In the tropical region, fruit bats avoid foraging behavior during full moon to avoid predators (Lima and O'Keefe 2013). Therefore, netting was carried out during a new moon phase, one night per month, in each habitat for three months (May, June, and July 2019). This resulted in a total of three sampling nights in each habitat. The netting started at 06.00 PM and continued until 06.00 AM the next morning, meanwhile, the mist net was 12 m in length and 2.5 m in height, with a 15x15 mm² mesh (Shijiemesh) and was placed approximately 3 m above the ground. Given that fruit bats are highly dependent on fruits as a source of food, the mist nets were placed near fruit trees that are potential paths. The net was monitored every 30-45 minutes through the night and bats were collected as detected.

Sampling techniques collection of bat and ectoparasite samples

All procedures involving mist-netting, handling, and subsequent release followed the guidelines established by the Indonesian Ministry of Health Research and Development Agency (2015) to ensure the health and safety of both bats and researchers. The study protocol also was approved by the committee Research and Community Service Institutions, Sam Ratulangi University, Manado, Indonesia (1937/UN12.13/LT/2018). Captured bats were removed from the net with a gloved hand (following Mikael et al. 2017) and transferred into a cotton cloth bag for transport to the nearby processing station for morphometric measurements and identification using morphological criteria by Bergmans and Rozendaal (1988) and species of bats in Sulawesi by Yuliadi et al. (2014).

The manual collection of ectoparasites from the bat's body was carried out sequentially starting from the head, ears, neck, wing bases, base of the feet, and toes using tweezers. In addition, bats were combed carefully to collect any additional ectoparasites left in the fur. The samples were then placed in collection tubes containing 70% alcohol, meanwhile, ectoparasites from different body parts were placed in separate tubes. Before release, bats were

marked on the head with a permanent marker to avoid resampling. All collected ectoparasites were subsequently sorted, identified, counted, mounted on microscope slide, and deposited for further study in the Animal Wildlife Laboratory, Faculty of Animal Science, Sam Ratulangi University. Specimens were taxonomically identified using the taxonomic keys by Baker and Delfinado (1964) and Maa (1975) under a binocular microscope. Moreover, the author (M.N.) took photographs under binocular microscope using a digital Samsung Galaxi Note 3 camera and processed with Adobe Photoshop CS4.

Data analysis

Ectoparasite abundance (i.e., mean number of parasites per host), mean intensity (i.e., mean number of parasites per infested host), and prevalence (i.e., number of infested hosts divided by total hosts sampled) (see Bush et al. 1997) were calculated using Quantitative Parasitology Software 3.0. Statistical analyses were performed to assess habitat preference of the bats and the proportion of ectoparasite infestation by gender using Bootstrap Method (SPSS version 23) due to the data not normal distribution and limit sample (Reiczigel et al. 2019). The differences between females and males were not examined for bat species with a small sample size of infested individuals (< 10).

RESULTS AND DISCUSSION

Species and composition of bats sampled

A total of 253 fruit bats were captured in three different habitats in BNTP. The bats were all from the Pteropodidae family, and were further identified as representing 10 species namely *Cynopterus brachyotis*, *C. luzoniensis*, *Dobsonia exoleta*, *Macroglossus minimus*, *Nictymene cephalotes*, *N. minutus*, *Rousettus amplexicaudatus*, *R. celebensis*, *Thoopterus nigrescens*, and *Thoopterus* sp. Moreover, the highest bat abundance was observed in the secondary forest, where 39.53% of the bats were captured, followed by the agricultural land with 38.34% and then primary forest with 22.13%. *Cynopterus brachyotis* was the most abundant species captured, 63 (24.90%), followed by *Rousettus celebensis* (22.57%). The least abundant species was *Thoopterus* sp, with only one sample captured in the primary forest (0.40%) (Table 1). Besides, the Bootstrap Test showed that there is no significant difference in bat abundance in each habitat ($p=0.634$).

Diversity of ectoparasites

A total of 479 ectoparasites representing three families, namely Nycteribiidae, Streblidae (bat flies), and Spinturnicidae (mites) were collected. Majority of these ectoparasites ($n = 475$) belonged to the family Nycteribiidae genus *Leptocyclopodia* and were characterized by the following morphological characteristics; body size 1.11-1.90mm, spider-like, dorsoventrally flat, hairy body, possess several ctenidia or combs, claws at the tips of the feet, and head attached to the thorax. The tibia was characterized by three white rings (Figure 2). A one-winged sample, discovered on the head

of a Dobsonia bat, was identified as a member of the family Streblidae. Three members of the family Spinturnicidae were also reported on the wing of a Dobsonia bat. Meanwhile, due to lack of specimens, Streblidae and Spinturnicidae were not identified at the genus and species level.

Ectoparasites and hosts

Nycteribiidae ectoparasites were discovered on eight bat species, i.e. *Cynopterus brachyotis*, *C. luzoniensis*, *Dobsonia exoleta*, *Macroglossus minimus*, *Rousettus amplexicaudatus*, *R. celebensis*, *T. nigrescens*, and *Thoopterus* sp.

The highest mean abundance (2.00-3.51) and intensity (2.00-3.97) were observed in the genus *Thoopterus*. Meanwhile, parasite from the Spinturnicidae and Streblidae families were found only on *D. exoleta*. No ectoparasites were found on *N. cephalotes* and *N. minutus* (Table 2). The prevalence of Nycteribiide infestation in bats was highly variable among species (20.6-100%).

Ectoparasites (Nycteribiid) based on the bat gender

The results showed that the total number of Nycteribiid ectoparasites was higher in female bats compared to males for all species identified (Table 3). However, the Bootstrap

test showed that the mean intensity of ectoparasite infestation found on female bats was not significantly higher than males ($p > 0.05$).

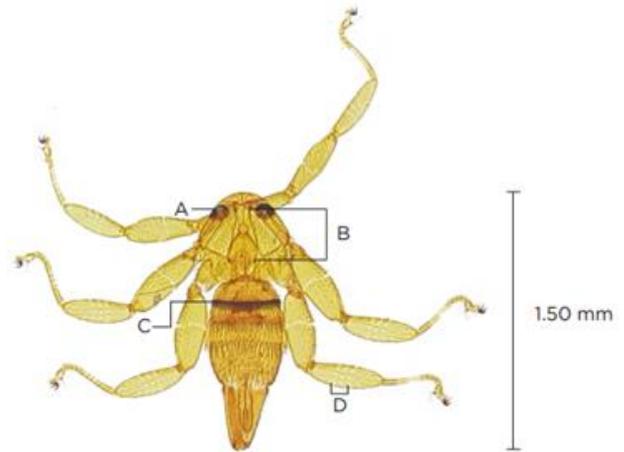


Figure 2. *Leptocyclopodia ferrarii* collected from *Cynopterus brachyotis* at Batuputih Nature Tourism Park, Sulawesi, Indonesia (2018) A (Thorax ctenidia), B (Head), C (Abdomen ctenidia), D (Tibial rings) (Photographs by M.N.)

Table 1. Species and composition of bats sampled in the three habitat types in Batuputih Nature Tourism Park, North Sulawesi, Indonesia

Species	PF		SF		AL		Total			%
	M	F	M	F	M	F	M	F	Total	
<i>Cynopterus brachyotis</i>	3	3	13	5	20	19	36	27	63	24.90
<i>Cynopterus luzoniensis</i>	0	0	4	7	8	6	12	13	25	9.88
<i>Dobsonia exoleta</i>	0	0	1	2	0	0	1	2	3	1.19
<i>Macroglossus minimus</i>	1	1	2	2	1	1	4	4	8	3.16
<i>Nictymene cephalotes</i>	1	1	5	3	1	1	7	5	12	4.74
<i>Nyctimene minutus</i>	0	0	0	0	1	1	1	1	2	0.79
<i>Rousettus amplexicaudatus</i>	7	11	6	8	5	6	18	25	43	17.00
<i>Rousettus celebensis</i>	6	11	7	7	13	9	26	27	53	20.95
<i>Thoopterus nigrescens</i>	3	7	18	10	3	2	24	19	43	17.00
<i>Thoopterus</i> sp.	0	1	0	0	0	0	0	1	1	0.40
Total	21	35	56	44	52	45	129	124	253	100
Total	56		100		97					
%	22.13		39.53		38.34					

Note: PF: Primary Forest; SF: Secondary Forest; AL: Agricultural Land; M: Male; F: Female

Table 2. Ectoparasites, bat species, prevalence, mean abundance, and mean intensity

Ectoparasite (Family)	Bat Species	N	Infected	Prevalence %	Mean Abundance	Mean Intensity
Nycteribiidae	<i>Cynopterus brachyotis</i>	63	13	20.6	0.36	1.77
	<i>Cynopterus luzoniensis</i>	23	11	47.8	0.52	1.18
	<i>Dobsonia exoleta</i>	3	2	66.7	2.00	3.00
	<i>Macroglossus minimus</i>	8	3	37.5	0.5	1.33
	<i>Rousettus amplexicaudatus</i>	43	39	90.7	3.42	3.77
	<i>Rousettus celebensis</i>	53	40	75.5	2.43	3.23
	<i>Thoopterus nigrescens</i>	43	38	88.4	3.51	3.97
	<i>Thoopterus</i> sp.	1	1	100.0	2.00	2.00
Streblidae	<i>Dobsonia exoleta</i>	3	1	33.3	0.33	1.00
Spinturnicidae	<i>Dobsonia exoleta</i>	3	1	33.3	1.00	3.00

Table 3. Number of Nycteribiid ectoparasites based on the bat gender

Bat Species	Total infected bats/bats		Total number of nycteribiid on bats		Mean intensity of nycteribiid		P-value
	M	F	M	F	M	F	
<i>Rousettus amplexicaudatus</i>	16/18	23/25	53	94	3.31±1.74	4.08±2.46	0.29
<i>Rousettus celebensis</i>	18/26	22/27	58	71	3.22±3.52	2.63±3.23	0.99
<i>Thoopterus nigrescens</i>	17/19	21/24	63	88	3.71±2.59	4.19±2.71	0.58

Note: M: male; F: female

Discussion

All the bats captured and sampled were small fruit bats belonging to the family Pteropodidae and are often hunted, sold, and consumed by the local community (Ransaleleh et al. 2020). According to the checklist Mammals of Indonesia (Maryanto et al. 2019), four of the species are endemic to Sulawesi, namely *D.exoleta*, *R. celebensis*, and *Nyctimene minutus* with a conservation status of least concern, and *N. minutus* considered as being vulnerable. *N. cephalotes* is found in East Indonesia and the conservation status is least concern, meanwhile, *Cynopterus minutus* and *Macroglossus minimus* are spread throughout Indonesia and are also considered least concern. Furthermore, *C. luzoniensis* is distributed in Sulawesi and Philippines with a conservation status of least concern. *Cynopterus brachyotis* and *R. amplexicaudatus* are spread throughout South East Asia and are also considered least concern. Eight species were infested with nycteribiids (genus Leptocyclopodia) while Streblidae and Spinturnidae were only found on *Dobsonia exoleta*. Due to lack of specimen, these two families were not identified at the species level.

Composition and ecology of bat species communities in the surveyed habitats

Six species namely *C. brachyotis*, *R. amplexicaudatus*, *R. celebensis*, *T. nigrescens*, *Nyctimene cephalotes*, and *Macroglossus minimus*, were observed in the three surveyed habitat. *Cynopterus luzoniensis* was found in both secondary forest and agricultural land but not in the primary forest. *Dobsonia exoleta* was found only in the secondary forest, *Nyctimene minutus* was only found in the agricultural land. while *Thoopterus* sp. was found only in the primary forest habitat. Furthermore, the species *C. brachyotis*, *R. Celebensis*, and *R. Amplexicaudatus*, *Thoopterus nigrescens* live in groups in large tree holes such as *Ficus* sp., *Rotudivolia* sp., *Livistonia* sp., *Octomeles sumatrana*, *Dracontomelon dao*, *Tetrameles rudiflora* and in coastal cliff caves. *Cynopterus brachyotis* was the most common species observed in the three habitats. Sheherazade et al. (2017) reported that *Cynopterus brachyotis* constitute one of the most common fruit-eating bats in Southeast Asia. This species occupies a wide variety of habitats including primary, secondary, burnt and mangrove forest, as well as agricultural land areas, and urban (Sheherazade et al. 2017; Lee et al. 2018). The ability to successfully adapt to disturbed environments is one of the key factors that make this species thrive in various types of habitats.

The genera *Nyctimene*, and *Macroglossus* reportedly lives in trees with large fronds such as woka (*Livistonia rotundifolia*) and banana leaves (*Musa* sp.). These bats roost under large tree fronds to hide from predators (Chaverri and Kuntz 2010; Lima and O'Keefe 2013), and consume nectar from flowering trees, especially flowering plants such as banana (*Musa* sp.). Besides, the *Dobsonia* genus is usually found in cave along the coast (Fatem et al. 2006). Coconut (*Cocos nucifera*) and palm trees (*Arenga pinnata*), are very common on agricultural land and provide feeding for the genus *Cynopterus* and *Rousettus* bats. The results confirmed the study of Suropto et al. (2006), which reported that *Cynopterus* and *Rousettus* frequently visited *Arenga pinnata* trees to suck the sap. *Macroglossus* sp. and *Nyctimene* sp. are solitary bats and nectar feeders, while *Thoopterus* sp. and *Dobsonia* feed on fruits, meanwhile, *M. minutus* frequently visits flowering plants to feed on the nectar (Fukuda et al. 2009).

Ectoparasites identified

Majority of the sampled species (8 of 10) were infested by ectoparasites of the family Nycteribiidae genus Leptocyclopodia, an obligate hematophagous ectoparasite, reported in bats of the Pteropodidae family (Maa 1975; Rajemison et al. 2017; Lee et al. 2018). In Singapore, *Leptocyclopodia ferrarii* was recorded as monoxenous (parasitizes one host species) (Lim et al. 2020), however, it was categorized as oligoxenous (parasitizes more than one host of the same genus) in Malaysia (Azhar et al. 2015). The difference in host specificity between these studies is related to host diversity in the ecosystem and co-roosting opportunities and interaction among host species. In BTNP dominated by coastal forest with small caves, secondary, and primary forest vegetation are suitable habitats for a diverse range of small fruit bats compared to Singapore.

In this study, ectoparasites were mostly found on the bats' body (87.69%), while ectoparasites on wings and head were less common, 8.28%, and 4.03% respectively. Also, species with dense fur had larger number of ectoparasites compared to others with thin fur. The abundance, intensity, and prevalence of ectoparasites in bats are influenced by a range of variables including, morphology, habitat (Bush et al. 2013), gender, nesting, grooming (Ramanantsalama et al. 2018) social behavior (Czenze and Broders 2011; Hiller et al. 2020), diet (Luguterah and Lawer 2015) and body size (Rajemison et al. 2017). This also was observed in the results. Furthermore, species that live in groups in tree holes and caves such as *Thoopterus* sp., *Rousettus* sp., *Cynopterus* sp.,

and *C. brachyotis*, had a higher number of ectoparasites compared to *Macroglossus minimus*, which lives in tree canopy.

The abundance and intensity of ectoparasite infestation varied widely among the studied species. *Thoopterus nigrescens* had the highest abundance (3.51) and intensity (3.97). This species exhibit roosting communally and use the same locations as permanent roosting sites or for a long time. Nycteribiidae pupate lives in the walls of roost sites (Dick and Dittmar 2014), therefore, permanent roosting site provides stable micro-environmental conditions that favor successful development of parasites (Dube et al. 2018). Moreover, the prevalence of ectoparasite infestation also varied widely, meanwhile, among the bat species with a prevalence value greater than 50%, three species (i.e., *R. celebensis*, *R. amplexicaudatus*, and *Thoopterus nigrescens*) had severe infestations with values ranging from 75.5-90.7%. The sharing of roosting sites among individual bats and others of different species is the likely factor affecting the prevalence of ectoparasite infestation (Hiller et al 2020). This value was found to be 90.7% in *R. amplexicaudatus* as also reported by Alvarez et al. (2016) in a research conducted at Lake Naujan National Park, Mindoro Oriental Philippine Province. In addition, the value also indicated that the bat species *R. amplexicaudatus* had heavy infestation, which is applicable as a health indicator (Bush et al. 2013).

Statistical analysis using Bootstrap methods showed that there was no significant gender difference among the bat species regarding ectoparasite infestation (p -value > 0.05), although female bats had higher total number of infestation compared to males (Table 3). The vulnerability of female bats to ectoparasites might be due to fluctuations in the reproductive cycle such as pregnancy and lactation, which causes reduced immunity and therefore greater tendency of parasite infestation. Furthermore, female bats spend more time at roosting sites during these periods, increasing the duration and intensity of contact with ectoparasites (Webber et al. 2015). In Singapore, the prevalence rate in male *C. brachyotis* was higher than females (Lim et al. 2020) because mature males participate in roost defense (Archarya et al. 2015). Grooming rates related to the average number of ectoparasites consumed per day did not differ between adult males and females (Ramanantsalama et al. 2018). Meanwhile, given the variation in the literature regarding gender differences in infestation, more research is needed to investigate parasite load related to gender.

Based on the literature search, this study represents the first work to document the fruit bat diversity and the ectoparasites in the boundary areas of the Tangkoko Nature Reserve, specifically Batuputih Nature Tourism Park, hence, it provides important data line base for future reference in monitoring bat population status and conservation efforts in the region. Furthermore, given the close relationship between the local people and bats (e.g., hunting and consumption), more work is needed to address the potential pathogen risks from zoonotic transmission-both from the bats and the ectoparasites. For example, several studies have reported that the nycteribiid fly (*Nycteribia kolenatii*) is suspected to play a role in the

development of malaria parasites in Chiroptera (*Polychromophilus murinus*) (Gardner and Molyneux 1988; Witsenburg 2014). In addition, Bartonella was detected in bat ectoparasites such as Streblidae, Nycteribiidae, and Spinturnicidae (Morse et al. 2012; Brook et al. 2014), while arboviruses have been detected in Nycteribiidae by Aznar-Lopez et al. (2013). These examples illustrate the need for further study and continued surveillance to better understand the potential risks that exist and be more proactive in mitigating zoonotic pathogen transmission in Indonesia.

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REFERENCES

- Almeida J, Silva SSP, Serra-Freire NM, Valim MP. 2011. Ectoparasites (Insecta and Acari) associated with bats in Southeastern Brazil. *J Med Entomol* 48 (4): 753-757. DOI: 10.1603/ME09133.
- Almeida JC, Martins MA, Guedes PC, Peracchi AL, Serra-Freire NM. 2016. New records of mites (Acari: Spinturnicidae) associated with bats (Mammalia, Chiroptera) in two Brazilian biomes: Pantanal and Caatinga. *Braz J Vet Parasitol* 2 (51): 18-23 DOI: 10.1590/S1984-29612016005.
- Alvarez JDV, Lit IL, Alviola PA, Cosico EA, Eres EG. 2016. A contribution to the ectoparasite fauna of bats (Mammalia: Chiroptera) in Mindoro Island, Philippines: I. Bloodsucking Diptera (Nycteribiidae, Streblidae) and Siphonaptera (Ischnopsyllidae). *International Intl J Trop Insect Sci* 36 (4): 188-194 DOI:10.1017/S1742758416000187.
- Archarya PR, Racey PR, McNeil D, Sotthibandhu S, Bumrungsri S. 2015. Timing of cave emergence and return in the dawn bat (*Eonycteris spelaea*, Chiroptera: Pteropodidae) in Southern Thailand. *Mammal Stud* 40: 47-52. DOI: 10.3106/041.040.0108.
- Arrijani, Rizki M. 2020. Vegetation analysis and population of tarsier (*Tarsius spectrumgurskyae*) at Batuputih Nature Tourism Park, North Sulawesi, Indonesia. *Biodiversitas* 20 (2): 530-537. DOI: 10.13057/biodiv/d210214.
- Azhar I, Khan FAA, Ismail N, Abdullah MT. 2015. Checklist of bat flies (Diptera: Nycteribiidae and Streblidae) and their associated bat hosts in Malaysia. *Check List* 11 (5): 1777. DOI: DOI: 10.15560/11.5.1777.
- Aznar-Lopez C, Vazquez-Moron S, Marston DA, Juste J, Ibanez C, Jose Miguel Berciano JM, Salsamendi E, Aihartza J, Banyard AC, McElhinney L, Fooks AR, Echevarria J. 2013. Detection of Rhabdovirus viral RNA in oropharyngeal swabs and ectoparasites of Spanish bats. *J Gen Virol* 94: 69-75. DOI: 10.1099/vir.0.046490-0.
- Baker EW, Delfinado M D. 1964. Spinturnicidae of South East Asia and the Pacific regioll. *Pacific Ins* 6: 571-591.

- Bergmans W, Rozendaal FG. 1988. Notes on collections of fruit bats from Sulawesi and some off lying island (Mammalia, Megachiroptera). *Zool Verh* 248: 1-72.
- Brook CE, Bai Y, Dobson AP, Osikowicz LM, Ranaivoson HC, Zhu Q, Kosoy MY, Dittmar K. 2015. *Bartonella* spp. in fruit bats and blood-feeding ectoparasites in Madagascar. *PLoS Negl Trop Dis* 10(2):e0003532. DOI: 10.1371/journal.pntd.0003532.
- Bush AO, Lafferty KD, Lotz JM, Shostak AW. 1997. Parasitology meets ecology on its own terms: Mardolis et al. *Revised*. *J Parasitol* 83(4): 573-583. DOI: 10.2307/3284227.
- Bush SE, Michelle R, Maher S. 2013. Impact of forest size on parasite biodiversity: Implications for conservation of hosts and parasites. *Biodivers Conserv* 22 (6): 1391-1404. DOI 10.1007/s10531-013-0480-x.
- Chaverri G, Kunz T. 2010. Ecological determinants of social systems: Perspectives on the functional role of roosting. *Adv Stud Behav* 42: 275-318. DOI: 10.1016/S0065-3454(10)42009-4.
- Czenze ZJ, Broders HG. 2011. Ectoparasite community structure of two bats (*Myotis lucifugus* and *M. septentrionalis*) from the Maritimes of Canada. *J Parasitol Res* 2011 (2): 341535. DOI: 10.1155/2011/34153.
- Dick CW, Dittmar K. 2014. Parasitic bat flies (Diptera: Streblidae and Nycteribiidae): Host specificity and potential as vectors. In: Klimpel S, Mehlhorn H (eds). *Bats (Chiroptera) as Vectors of Diseases and Parasites*. *Parasitol Res Monog* 5: 131-155. Springer, Berlin. DOI: 10.1007/978-3-642-39333-4_6.
- Dube WC, Hund AK, Turbek SP, Safran RJ. 2018. Microclimate and host body condition influence mite population growth in a wild bird-ectoparasite system. *Intl J Parasitol Parasites Wildl* 7: 301-308 DOI: 10.1016/j.ijppaw.2018.07.007.
- Dumont ER, O'neal R. 2004. Food hardness and feeding behavior in Old World fruit bats (*Pteropodidae*). *J Mammal* 85 (1): 8-14. DOI: 10.1644/BOS-107.
- Fatem SM, Bumbut PI, Ungirwalu A. 2006. Habitat of Fruit bats (*Dobsonia minor*) in Nuni tropical lowland forest of Northern area in Manokwari. *Media Konservasi* 9 (1): 17-20. [Indonesian]
- Fukuda D, Tisen OB, Momose K, Sakai S. 2009. Bat diversity in the vegetation mosaic around a lowland dipterocarp forest of Borneo. *Raffles Bull Zool* 57(1): 213-221.
- Gardner RA, Molyneux DH. 1988. *Polychromophilus murinus*: A malarial parasite of bats: Life-history and ultrastructural studies. *Parasitology* 3: 591-605. DOI: 10.1017/s0031182000080215.
- Gay N, Olival KJ, Bumrungsri S, Siriaronrat B, Bourgarel M, Morand S. 2014. Parasite and viral species richness of Southeast Asian bats: Fragmentation of area distribution matters. *Intl J Parasitol Parasites Wildl* 3: 161-170. DOI: 10.1016/j.ijppaw.2014.06.003.
- Hiller T, Brandel SD, Honner B, Page RA, Tschapka M. 2020. Parasitization of bats by bat flies (Streblidae) in fragmented habitats. *Biotropica* 52 (3): 488-501. DOI:10.1111/btp.12757.
- Holz PH, Lumsden LF, Hufschmid J. 2018. Ectoparasites are unlikely to be a primary cause of population declines of bent-winged bats in South-eastern Australia. *Intl J Parasitol Parasites Wildl* 7: 423-428. DOI: 10.1016/j.ijppaw.2018.10.006.
- Indonesian Ministry of Health Research and Development Agency. 2015. Guidelines for Collecting Reservoir (Bat) Data in the Field. Publisher Indonesian Ministry of Health Research and Development Agency, Jakarta. [Indonesian]
- Kim HC, Han SH, Dick CW, Choi YG, Chong ST, Klein TA, Rueda, LM. 2012. Geographical distribution of bat flies (Diptera: Nycteribiidae and Streblidae), including two new records, *Nycteribia allotopa* and *N. formosana*, collected from bats (Chiroptera: Rhinolophidae and Vespertilionidae) in the Republic of Korea. *J Vector Ecol* 37: 333-337. DOI: 10.1111/j.1948-7134.2012.00235.
- Kyes RC, Iskandar E, Papatungan U, Onibala J, Laatung S, Huettman F. 2013. Long-term population survey of the Sulawesi black macaques (*Macaca nigra*) at Tangkoko Nature Reserve, North Sulawesi, Indonesia. *Am J Primatol* 75 (1): 88-94. DOI: 10.1002/ajp.22088.
- Latinne A, Suryo S, Kalengkongan J et al. 2020. Characterizing and quantifying the wildlife trade network in Sulawesi, Indonesia. *Glob Ecol Conserv* 21: e00887. DOI: 10.1016/j.gecco.2019.e00887.
- Lee VN, Mendehall IA, Lee BPHY, Posa MRC. 2018. Parasitism by bat flies on an urban population of *Cynopterus brachyotis* in Singapore. *Acta Chiropt* 20 (1): 177-185. DOI: 10.3161/15081109ACC2018.20.1.013.
- Lim ZX, Hitch AT, Lee BPHY, Low DHW, Neves ES, Borthwick, Smith GJD, Mendenhall. 2020. Ecology of bat flies in Singapore: A study on the diversity, infestation bias and host specificity (Diptera: Nycteribiidae). *Intl J Parasitol Parasites Wildl* 12 (2020): 29-33 DOI: 10.1016/j.ijppaw.2020.04.010.
- Lima SL, O'Keefe JM. 2013. Do predators influence the behaviour of bats? *Biol Rev* 88: 626-644. DOI: 10.1111/brv.12021.
- Luguterah A, Lawer EA. 2015. Effect of dietary guild (frugivory and insectivory) and other host characteristics on ectoparasite abundance (mite and nycteribiid) of chiropterans. *Folia Parasitol* 62: 021 DOI: 10.14411/fp.2015.021.
- Maa TC. 1975. On new Diptera Pupipara from the Oriental Region. *Pacific Insect* 16 (4): 465-486.
- Maryanto I, Maharadatunkamsi, Achmadi AS, Wiantoro S, Sulistyadi E, Yoneda M, Suyanto A, Sugardjito J. 2019. Checklist of the mammals of Indonesia. Puslit Biologi, Bogor. [Indonesian]
- Mikail M, Putra TATR, Suri AS, Hezmee MNM, Marina MT. 2017. Preliminary study of Malaysian fruit bats species diversity in Lenggong Livestock Breeding Center, Perak: Potential risk of spillover infection. *Vet World* 10 (11): 1297-1300. DOI: 10.14202/vetworld.2017.1297-1300.
- Mildenstein TL, Tanshi I, Racey PA. 2016. Exploitation of bats for bushmeat and medicine. In: Voigt C, Kingston T (eds.) *Bats in the Anthropocene — Conservation of Bats in a Changing World*. Springer, New York. DOI: 10.1007/978-3-319-25220-9_12.
- Morse SF, Olival KJ, Kosoy M, Billeter S, Pattersen BD, Dick CW, Dittmar K. 2012. Global distribution and genetic diversity of *Bartonella* in bat flies (Hippoboscoidea, Streblidae, Nycteribiidae). *Infect Genet Evol* 12 (8): 1717-1723. DOI: 10.1016/j.meegid.2012.06.009.
- Mulyono A, Ristiyanto A, Piyiyanti AS, Joharina, Putro DBW. 2018. A new record on fruit bats (*Macroglossus sobrinus*) as a Leptospirosis reservoir from Indonesia. *Vektora* 10 (2): 103-110. DOI: 10.22435/vk.v10i2 OKT.581. [Indonesian]
- O'Brien TG, Kinnaird MF. 1996. Changing populations of birds and mammals in North Sulawesi. *Oryx* 30: 150-156. DOI: 10.1017/S0030605300021530.
- Pader LMDG, Fiegalan ER, Cruz KGJ. 2018. Chiroptera-associated dipteran ectoparasites (Nycteribiidae, Streblidae) in Minalungga National Park, Nueva Ecija Philippines. *J Biodivers Environ Sci* 13 (4): 29-37.
- Rajemison FI, Lalarivoniaina OSN, Goodman SM. 2017. Bat flies (Diptera: Nycteribiidae, Streblidae) parasitising *Rousettus madagascariensis* (Chiroptera: Pteropodidae) in the Parc National d'Ankarana, Madagascar: species diversity, rates of parasitism and sex ratios. *Afr Entomol* 25 (1): 72-85. DOI: 10.4001/003.025.0072.
- Ramanantsalama RV, Andrianarimisa A, Raselimanana AP, Goodman SM. 2018. Rates of hematophagous ectoparasite consumption during grooming by an endemic Madagascar fruit bat. *Parasites Vectors* 11: 330. DOI: 10.1186/s13071-018-2918-1.
- Ransaleh TA, Nangoy MJ, Wahyuni I, Lomboan A, Koneri R, Saputro S, Pamungkas J, Latinne A. 2020. Identification of bats on traditional market in Dumoga district, North Sulawesi. *IOP Conf Ser Earth Environ Sci* 473 (2020): 012067 DOI: 10.1088/1755-1315/473/1/012067.
- Reeves WK, Beck J, Orlova MV, Daly JL, Pippin K, Revan F, Loftis AD. 2016. Ecology of bats, their ectoparasites, and associated pathogens on Saint Kitts Island. *J Med Entomol* 53 (5): 1218-1225. DOI: 10.1093/jme/tjw078.
- Reiczigel J, Marozzi M, Fabian I, Rozsa L. 2019. Biostatistics for Parasitologists—a primer to quantitative Parasitology. *Trends Parasitol* 35 (4): 277-281. DOI: 10.1016/j.pt.2019.01.003.
- Sheherazade, Tsang SM. 2015. Quantifying the bat bushmeat trade in North Sulawesi, Indonesia, with suggestions for conservation action. *Glob Ecol Conserv* 3: 324-330. DOI: 10.1016/j.gecco.2015.01.003.
- Sheherazade, Yasman, Dimas H, Pradana DH, Tsang SM. 2017. The role of fruit bats in plant community changes in an urban forest in Indonesia. *Raffles Bull Zool* 65: 497-505.
- Suripto BA, Sumaryati, Budi C. 2006. The species of fruit bats and their impact on the Coconut "Nira" production in Kokap, Kulon Progo. *J Plant Prot Indones* 12 (1): 13-24. [Indonesian]
- Webber QMR, McGuire LP, Steven B, et al. 2015. Host behaviour, age and sex correlate with ectoparasite prevalence and intensity in a colonial mammal, the little brown bat. *Behaviour* 152 (1): 83-105. DOI: 10.1163/1568539X-00003233.
- Webber QMR, Willis CKR. 2016. Sociality, parasites, and pathogens in bats. In: Ortega J (eds). *Sociality in Bats*. Springer International Publishing, Switzerland. DOI: 10.1007/978-3-3-38953-0-5.

- Witsenburg F. 2014. The Role of Bat Flies (Nycteribiidae) in the Ecology and Ecology of the Blood Parasite Polychromophilus (Apicomplexa: Haemosporida). [Dissertation]. University of Lausanne, Lausanne, Switzerland.
- Yang XL, ZhangYZ, Jiang RD, Guo H, Wang BLN, Wang L, Waruhiu C, Zhou JH, Li SY, Daszak P, Wang LF, Shi, ZL. 2017. Genetically diverse Filoviruses in *Rousettus* and *Eonycteris* spp. Bats China 2009 and 2015. *Emerg Infect Dis* 23 (3): 482-486. DOI: 10.3201/eid2303.161119.
- Yuliadi B, Sari TF, Handayani FD. 2014. Species of Bats Sulawesi and Their Role in Health. Publishing Institute for Health Research and Development, Ministry of Health of the Republic of Indonesia, Jakarta. [Indonesian].