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

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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. lucionensis (9.88%), Dobsonia exoleta (1.19%), Macroglossus minimus (3.16%), Nictymene cephalotes (4.75%), N. minuta (0.79%), Rousettus aegyptiacus (17%), R. celebensis (20.95%), Thoosterus nigrescens (17%), and Thoosterus 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 Thoosterus 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 (Holz 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 (Dracaenostemon dao), Annonaceae (Camanga odorata), Lamiales (Vitex quinata), Sapotaceae (Palauqium ovobatum), and Anacardiaceae (Koordersiodendron 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 Development Agency (2015) to ensure the health and safety of both bats. All procedures involving mist netting, handling, and resampling 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 Galaxy 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 (Reitzigel 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, Magroglossus 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 amplicaudatus*, *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 Nycteribiidae 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).

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**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     |      |
| *Cynopterus brachyotis*  | 3  | 3   | 13  | 5     | 24.90|
| *Cynopterus luzoniensis* | 0  | 0   | 4   | 7     | 9.88 |
| *Dobsonia exoleta*       | 0  | 0   | 1   | 2     | 1.19 |
| *Macroglossus minimus*   | 1  | 1   | 2   | 2     | 3.16 |
| *Nictymene cephalotes*   | 1  | 1   | 5   | 3     | 4.74 |
| *Nyctimene minutus*      | 0  | 0   | 0   | 1     | 0.79 |
| *Rousettus amplicaudatus*| 7  | 11  | 6   | 8     | 17.00|
| *Rousettus celebensis*    | 6  | 11  | 7   | 7     | 20.95|
| *Thoopterus nigrescens*  | 3  | 7   | 18  | 10    | 17.00|
| *Thoopterus* sp.         | 0  | 1   | 0   | 0     | 0.40 |

Total: 63 56 44 52 45 129 124 253 100

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*       | *Cynopterus*      | 63    | 13       | 20.6         | 0.36           | 1.77          |
|                       | *Cynopterus*      | 23    | 11       | 47.8         | 0.52           | 1.18          |
|                       | *Macroglossus*    | 3     | 2        | 66.7         | 2.00           | 3.00          |
|                       | *Rousettus*       | 8     | 3        | 37.5         | 0.5            | 1.33          |
|                       | *Rousettus*       | 43    | 39       | 90.7         | 3.42           | 3.77          |
|                       | *Thoopterus*      | 53    | 40       | 75.5         | 2.43           | 3.23          |
|                       | *Thoopterus*      | 43    | 38       | 88.4         | 3.51           | 3.97          |
| *Streblidae*          | *Dobsonia*        | 3     | 1        | 33.3         | 0.33           | 1.00          |
| *Spinturnicidae*      | *Dobsonia*        | 3     | 1        | 33.3         | 1.00           | 3.00          |
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 minutas 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 brachyotis 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 minutas 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, Daccontomelon 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.

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 |                              |         |
| Rousettus amplexicaudatus    | 16/18 | 23/25 | 53 | 94 | 3.31±1.74 | 4.08±2.46 | 0.29 |
| Rousettus celebensis         | 18/26 | 22/27 | 58 | 71 | 3.22±3.52 | 2.63±3.23 | 0.99 |
| Thoopterus nigrescens        | 17/19 | 21/24 | 63 | 88 | 3.71±2.59 | 4.19±2.71 | 0.58 |

Note: M: male; F: female

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 Macroglossus sp. 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.

The genera Nyctimene, and Macroglossus reportedly lives in trees with large fronds such as woka (Livistona rotundifolia) and banana leaves (Musas sp.). These bats roost under large tree fronds to hide from predators (Chaverri and Kunz 2010; Lima and O’Keeffe 2013), and consume nectar from flowering trees, especially flowering plants such as banana (Musas 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 Suripto 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).
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. *Thooterus 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 pupae 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. amplicaudatus*, and *Thooterus 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 (Miller et al. 2020). This value was found to be 90.7% in *R. amplicaudatus* as also reported by Alvarez et al. (2016) in a research conducted at Lake Natuna National Park, Mindoro Oriental Philippine Province. In addition, the value also indicated that the bat species *R. amplicaudatus* 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 Batuputh 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 marinus*) (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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