LISTS OF SPECIES

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Checklist of bat flies (Diptera: Nycteribiidae and Streblidae) and their associated bat hosts in Malaysia

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Abstract: The number of publications on bat flies of Malaysia suggests that this group of parasitic dipterans is understudied. From April 2011 to September 2013, we surveyed 10 localities from seven states in Malaysia with the main objective to compile a checklist of bat flies. As a result, a total of 15 species of bat flies were recorded from 24 species of hosts. Our surveys indicated that there is a correlation between the number of bat species and the number of bat flies species recorded due to the host specific nature of bat flies.

Key words: bats, bat flies, host, Nycteribiidae, Streblidae

INTRODUCTION

Bat flies are parasitic dipterans that are only found on bats. Bat flies from the families Streblidae and Nycteribiidae are part of the superfamily Hippoboscoidea, which includes other obligate sanguivorous families such as Glossonidae and Hippoboscidae (Dittmar et al. 2006). Generally, nycteribiids and streblids can be distinguished easily by the presence (streblids) or absence (nycteribiids) of wings.

Based on a compilation from various sources, there are approximately 30 species of bat flies recorded from Malaysia (Theodor 1967; Maa 1968, 1971). Despite these studies, knowledge of the taxonomy and ecology of bat flies in Malaysia is scarce and there are no recently published literatures on this group from Malaysia since the late 19th century. To remedy this, surveys were conducted to explore the diversity and to compile a checklist of the bat flies and their incidental hosts. Various surveys were carried out between April 2011 and September 2013 to record the host and parasite association of bats and bat flies at several sites in Malaysia. Herein, the results from our surveys are reported.

MATERIALS AND METHODS Study sites

Fieldwork was carried out at 10 different localities in Malaysia. The study sites in Sarawak, Malaysian Borneo include Batang Ai National Park, Gunung Gading National Park, Mulu World Heritage Area and Wind Cave Nature Reserve. The sites in mainland Malaysia include Air Terjun Batu Hampar (Kedah), Gua Musang district (Kelantan), Wang Kelian State Park (Perlis), Pulau Tioman Wildlife Reserve (Pahang), Sekayu Recreational Forest (Terengganu) and Tambun (Perak) (Figure 1).

These study sites have varied habitats, including mixed dipterocarp forest, hill dipterocarp forest, alluvial forest, secondary forest, heath forest, forest near limestone cave areas, and agricultural plots. The details of each trapping site are shown in Table 1.

Data collection

Fieldwork was conducted from April 2011 to September 2013. Different types of sampling methods were used to maximize the representativeness of the bat host species. Two four-bank-harp traps (Francis 1989) and 10 polyester nets were positioned in forest understories, across small streams, and at forest edges. Additionally, hand nets were used to collect roosting bats.

During each sampling day, mist nets and harp traps were set up before the emergent time of bats and were closed at 7:00 h the following day. Mist nets were checked every 15–30 minutes for the first three hours and then every two to three hours before they were closed the next morning. Meanwhile, harp traps were checked three to five times during each sampling night.

Captured bats were placed inside cloth bags. Each bat specimen was identified following Payne et al. (1985), Kingston et al. (2006) and Francis (2008). Each host was scanned with an intensive light source for bat flies



Figure 1. Map showing the sampling locality for the host and bat flies in this study. 1 - Batang Ai National Park; 2 - Gunung Gading National Park; 3 – Air Terjun Batu Hampar; 4 – Pulau Tioman Wildlife Reserve; 5 - Sekayu Recreational Forest; 6 - Gunung Mulu World Heritage Site; 7 - Wind Cave Nature Reserve; 8 – Gua Musang; 9 - Perlis State Park; 10 - Tambun.

Fable 1. Habitat types of the 10 stud	y sites in Malaysian Borneo	and mainland Malaysia
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Locality	Habitat Types	Study Site	Coordinate
1	Alluvial forest, secondary forest, hill dipterocarp	Batang Ai National Park, Sarawak, Malaysian Borneo	01°13'36.6" N, 111°57'32.5" E
2	Mixed dipterocarp forest	Gunung Gading National Park, Sarawak, Malaysian Borneo	01°41'40.6" N, 109°51'25.6" E
3	Lowland dipterocarp forest	Air Terjun Batu Hampar, Kedah, Peninsular Malaysia	05°46'18.2" N, 100°24'14.8" E
4	Heath forest, mixed dipterocarp forest	Pulau Tioman Wildlife Reserve, Pahang, Peninsular Malaysia	02°47'10.1" N, 104°10'17.3" E
5	Lowland dipterocarp forest	Sekayu Recreational Forest, Terengganu, Peninsular Malaysia	04°58'43.3" N, 102°56'21.4" E
6	Alluvial forest, limestone forest, mixed dipterocarp forest, agriculture plots	Gunung Mulu World Heritage Site, Sarawak, Malaysian Borneo	04°02'39.2″ N, 114°49'27.1″ E
7	Limestone forest, secondary forest	Wind Cave Nature Reserve, Sarawak, Malaysian Borneo	01°24'53.2" N, 110°08'07.6" E
8	Limestone forest, Secondary forest	Gua Musang, Kelantan, Peninsular Malaysia	04°50'12.1" N, 101°56'58.9" E
9	Limestone forest	Perlis State Park, Perlis, Peninsular Malaysia	06°30'00" N, 100°15'00" E
10	Limestone forest, caves	Tambun, Perak, Peninsular Malaysia	04°37'19.8" N, 101°09'23.2" E

at the fur area of the body, around the wing, and the uropatagium. Following this, bat flies from each host were stored inside labeled cryovial tubes in 70% ethanol. Hosts were then released after the inspection process. In order to minimize cross-contamination between hosts and bat flies, each cloth bag was cleaned before re-used.

Identification of bat flies

Collected bat flies were first sorted based on morphological characteristics under dissecting microscope. Sorted bat fly specimens were then cleaned using lactophenol solution and mounted on slides with Hoyer's medium. Cover slips were ringed with nail polish to prevent desiccation of medium during storage. Prepared specimens were examined using compound microscope and bat flies were identified following the publications of Jobling (1930), Theodor (1967), and Maa (1968, 1971). The voucher specimens were deposited at the Parasitology Laboratory at Universiti Malaysia Sarawak.

Statistical analysis

To evaluate the relationship between the number of host species collected and their associated bat flies species, a simple linear regression analysis was performed using the statistical package available in Microsoft Excel® 2010. The number of bat species with bat flies (host) captured from each site (10 localities) were used as the independent variable while number of bat fly species collected at each site from different hosts were used as the dependent variable. A scatterplot with a linear trendline (regression) was produced. Trendline was enforced to intercept on Y-axis at zero value, indicating that no bat flies were recorded when there was no host.

RESULTS

There were 15 species of bat flies recorded from 24 species of bats. The bat flies were represented by subfamilies Nycteribiinae (three genera and four species), Archinycteribiinae (one genus and one species), Cyclopodiinae (two genera and five species) and Brachytarsininae (three genera and five species). The hosts were represented by five families, which are Pteropodidae (six genera and ten species), Rhinolophidae (one genus and four species), Hipposideridae (one genus and six species), Vespertilionidae (three genera and four species) and Miniopteridae (one genus and one species) (Table 2). In addition, the correlation graph indicated that there was a moderate correlation between the numbers of host species recorded with the number of bat flies species encountered (Figure 2). The equation for the linear regression is given by y = 1.0719x, $R^2 = 0.4804$.

Subfamily	Species	Host
Nycteribiinae	Nycteribia triangularis	Myotis horsfieldii
		Murina suilla
	Stylidia cf. euxesta	Hipposideros larvatus
	Stylidia cf. caudata	Rhinolophus lepidus
		Rhinolophus affinis
		Rhinolophus stheno
	Basilia hispida	Tylonycteris robustula
		Tylonycteris pachypus
Archinycteribiinae	Archinycteribia octophalma	Penthetor lucasi
Cyclopodiinae	Eucampsipoda penthetoris	Penthetor lucasi
	Eucampsipoda sundaica	Rousettus amplexicaudatus
		Rousettus leschenaultii
		Eonycteris spelaea
	Leptocyclopodia ferrari	Cynopterus brachyotis
		Cynopterus horsfieldii
		Cynopterus sphinx
	Leptocylopodia brachytrinax	Dyacopterus spadiceus
	Leptocylopodia obliqua	Balionycteris maculata
Brachytarsininae	Megastrebla gigantea	Rousettus amplexicaudatus
		Eonycteris spelaea
	Megastrebla limbooliati	Eonycteris spelaea
	Megastrebla nigriceps	Penthetor lucasi
	Raymondia sp.	Hipposideros bicolor
		Hipposideros atrox
		Hipposideros cineraceus
		Hipposideros larvatus
		Hipposideros cervinus
		Hipposideros galeritus
	Brachytarsina sp.	Rhinolophus creaghi
		Rhinolophus lepidus
		Rhinolophus affinis
		Rhinolophus stheno
		Miniopterus medius

Table 2. Taxonomic checklist of bat flies species and their hosts.



Figure 2. Correlation between the number of host species with the number of bat flies species (y = 1.0719x, $R^2 = 0.4804$).

Species account

Subfamily Nycteribiinae Westwood, 1835 Genus *Nycteribia* Latreille, 1796 Pedicularia Group

Nycteribia triangularis Theodor, 1967

Specimen examined (Figure 3) — (1) Gua Musang, Kelantan (GC012); (2) Gunung Jerai, Kedah (GJK005; GJK017).

Host — Myotis horsfieldii, Murina suilla.

Remarks — Eyes are absent. The genitalia have conical phallobase without a dorsal bulge and the aedeagus is slightly curved, with broadly rounded end as wide at the apex as at the base (Theodor 1967). The only previous record



Figure 3. Nycteribia triangularis (A), head (B), and male genitalia (C).

of this particular nycteribiid bat fly was from Selangor, collected from an unidentified host (Theodor 1967).

Genus *Stylidia* Westwood, 1840 Biarticulata Group

Stylidia cf. caudata Theodor, 1967

Specimen examined (Figure 4) — (1) Gunung Gading National Park, Lundu, Sarawak (GGNP029); (2) Gua Batu



Figure 4. Stylidia cf. caudata (A), head (B), and female genitalia (C).



Figure 5. Stylidia cf. euxesta (A), head (B), and male genitalia (C).

Madu, Gua Musang, Kelantan (GBM003; GBM004); (2) Gunung Reng, Jeli, Kelantan (GRK021; GRK022); (1) Sekayu Recreational Forest, Terengganu (SRF031); (1) Gunung Jerai, Kedah (GJK011); (2) Tambun, Perak (LWT019, LWT023).

Host — Rhinolophus lepidus, R. affinis, R. stheno.

Remarks — The individuals of this form resemble the characters presented by *Stylida caudata*. Only female individuals were recorded during sampling. Eyes are absent and labella are short. There is a double genital plate with a basal elliptical cover protruding out from the surface, with three minute hairs on each side at the base and a shield-shaped plate on the surface (Theodor 1967).

Stylidia cf. euxesta Speiser, 1901

Specimen examined (Figure 5) — (1) Tioman Island Wildlife Reserve, Pahang (TWR034); (2) Gua Batu Madu, Gua Musang, Kelantan (GBM017; GBM032); (2) Gunung Reng, Jeli, Kelantan (GRK018; GRK041); (2) Wang Kelian State Park, Perlis (PSP032, PSP033).

Host — *Hipposideros larvatus*.

Remarks — The morphology presented by these individuals are similar to *Stylidia euxesta*. This species is found in Burma and it has been reported that different subspecies can be found in Thailand and Malaya, and possibly in India as well (Theodor 1967). Eyes are absent. The darkly pigmented claspers are straight, with long dorsal setae, which become shorter the lower they are on the tip. The basal arc is large and rounded with a short anterior process (Theodor 1967).

Genus *Basilia* Miranda Ribeiro, 1903 Nattereri Group

Basilia hispida Theodor, 1967

Specimen examined (Figure 6) — (2) Gunung Gading National Park, Lundu, Sarawak (GGNP007; GGNP009).

Host — Tylonycteris pachypus, Tylonycteris robustula.

Remarks — This species can be found in abundance in West Malaysia and is possibly host specific to the bamboo bats, *Tylonycteris pachypus* and *T. robustula* (Marshall 1971). The eyes usually have either two or three unequal-sized lenses with a pigmented base. The triangular shaped basal arc has a long and narrow anterior process. The claspers are slightly curved, with setae present on the dorsal surface (Theodor 1967).

Subfamily Archinycteribiinae Maa, 1975 Genus *Archinycteribia* Speiser, 1901

Archinycteribia octophalma Theodor, 1967

Specimen examined (Figure 7) — (2) Tioman Island Wildlife Reserve, Pahang (TWR013; TWR027); (2) Batang Ai National Park, Sri Aman, Sarawak (BTGA009; BTGA033); (3) Gunung Mulu World Heritage Area, Sarawak (GMWHA077; GMWHA091; GMWHA126); (3) Wind Cave Nature Reserve, Bau, Sarawak (WCNR015; WCNR016; WCNR017).

Host — Penthetor lucasi.

Remarks — This species has the highest number of lenses among the members of Nycteribiidae. The eyes

are a pair of lenses at each side of the two elliptical frames. The short claspers are tapered to a blunt point with a short ventral hook. The basal arc is triangular (Theodor 1967).

Subfamily Cyclopodiinae Maa, 1965 Genus *Eucampsipoda* Kolenati, 1857



Figure 6. Basilia hispida (A) and male genitalia (B).



Figure 7. Archinycteribia octophalma (A), head (B), male genitalia (C), and female genitalia (D).



Figure 8. Eucampsipoda penthetoris (A), E. penthetoris (B), head (C), male genitalia (D) and female genitalia (E).

Eucampsipoda penthetoris Theodor, 1955

Specimen examined (Figure 8) — (3) Tioman Island Wildlife Reserve, Pahang (TWR013; TWR027; TWR034); (1) Batang Ai National Park, Sri Aman, Sarawak (BTGA009); (2) Gunung Mulu World Heritage Area, Sarawak (GMWHA091; GMWHA126); (4) Wind Cave Nature Reserve, Bau, Sarawak (WCNR015; WCNR016; WCNR017; WCNR033).

Host — Penthetor lucasi.

Remarks — This species has a single elliptical lens. The labella are longer than the theca. The claspers are tapered to a long narrow point. There is a row of hairs in the apical half (Theodor 1967).

Eucampsipoda sundaica Theodor, 1955

Specimen examined (Figure 9) — (2) Tioman Island Wildlife Reserve, Pahang (TWR001; TWR003); (3) Gunung Reng, Jeli, Kelantan (GRK004; GRK035:



Figure 9. Eucampsipoda sundaica (A), E. sundaica (B), male genitalia (C) and female genitalia (D).

GRK040); (1) Wang Kelian State Park, Perlis (PSP011).

Host — Rousettus amplexicaudatus, R. leschenaultii, Eonycteris spelaea.

Remarks — The eyes are of single elliptical lenses. The head is compressed and the thorax is pentagonal. The claspers are short and tapered to a point with two rows of pegs in the middle half and hair laterally (Theodor 1967).

Genus *Leptocyclopodia* Theodor, 1959 Ferrari group

Leptocyclopodia ferrari Rondani, 1878

Specimen examined (Figure 10) — (10) Tioman Island Wildlife Reserve, Pahang (TWR005; TWR006; TWR007; TWR008; TWR010; TWR012; TWR019; TWR021; TWR022; TWR029); (2) Batang Ai National Park, Sri Aman, Sarawak (BTGA012; BTGA016); (1) Gunung Gading National Park, Lundu, Sarawak (GGNP002); (3) Gunung Mulu World Heritage Area, Sarawak (GMWHA031; GMWHA033; GMWHA036); (2) Gua Cha, Kelantan (GCK003); (1) Sekayu Recreational Forest, Terengganu (SRF006); (2) Gunung Jerai, Kedah (GJK007; GJK008); (3) Wang Kelian State Park, Perlis (PSP001; PSP002; PSP004); (1) Tambun, Perak (LWT004).

Host — *Cynopterus brachyotis*, *C. horsfieldii*, *C. sphinx*.

Remarks — Members of this species have large eyes with the lenses almost separated. The claspers are long and slender, tapering to a short blunt tip in the apical quarter (Theodor 1967).

Obliqua group

Leptocylopodia brachytrinax Theodor, 1959

Specimen examined (Figure 11) — (1) Batang Ai National Park, Sri Aman, Sarawak (BTGA019).

Host — Dyacopterus spadiceus.

Remarks — Only two individuals were recorded from a single host, *Dyacopterus spadiceus*. The eyes are incomplete divided lenses and the thorax is slightly wider. The clasper is unique in this species because it is strongly spined, with a scantly setose phallobase and a prominent basal process on the inner margin of the anal segment (Maa 1968). The weakly sclerotized dorsal ridges end in two points (Theodor 1967).



Figure 10. Leptocyclopodia ferrari (A and B), head (C), male genitalia (D), and female genitalia (E).

Leptocyclopodia obliqua Theodor, 1959

Specimen examined (Figure 12) — (2) Batang Ai National Park, Sri Aman, Sarawak (BTGA003; BTGA027); (2) Gunung Mulu World Heritage Area, Sarawak (GMWHA006; GMWHA017).

Host — Balionycteris maculata.

Remarks — The eyes are similar with *L. brachytrinax*.

The thorax is triangular with about 15 blunt spines. The slender claspers are tapered to a blunt tip in the apical half (Theodor 1967). The spines at the apex of claspers in *L. ferrari* is absent.

Subfamily Brachytarsininae Speiser, 1900 Genus *Megastrebla* (Maa, 1971)

Megastrebla gigantea Speiser, 1899

Specimen examined (Figure 13) — (1) Gunung Reng, Jeli, Kelantan (GRK013); (1) Wang Kelian State Park, Perlis (PSP020).

Host — Rousettus amplexicaudatus, R. leschenaultii, Eonycteris spelaea.

Remarks — The distribution of this species is restricted to the Oriental region (Maa 1971), although there are



Figure 11. Leptocyclopodia brachytrinax (A), head (B), and male genitalia (C).

Figure 12. Leptocyclopodia obliqua (A), head (B), and male genitalia (C).

several subspecies. Megastrebla gigantea has a very robust body and the wings are very long. The vein R_1 is bent near the base and almost straight at the apical section, and the apex of vein R_{2+3} is more distant than that of vein R_{4+3} (Maa 1971).

Megastrebla limbooliati (Maa, 1971)

Specimen examined (Figure 14) — (1) Gunung Reng, Jeli, Kelantan (GRK011).

Host — *Eonycteris spelaea*.

Remarks — Morphologically, *Megastrebla limbooliati* stands between the *M. bequerti* and *M. wenzeli* of the Ethiopian region and *M. nigriceps* of the Oriental region (Maa 1971). This species has a robust body and long wings. The vein R_1 is distinctly angulate near the base and straight at the apical section, with vein R_{2+3} gently curved to C and the apex more distant from that of vein R_1 than from that of vein R_{4+5} (Maa 1971).

Megastrebla nigriceps Jobling, 1934

Specimen examined (Figure 15) — (1) Batang Ai National Park, Sri Aman, Sarawak (BTGA009); (4) Gunung Mulu World Heritage Area, Sarawak (GMWHA060; GMWHA077; GMWHA091; GMWHA126); (2) Wind Cave Nature Reserve, Bau, Sarawak (WCNR016; WCNR017).

Host — Penthetor lucasi.



Figure 13. Megastrebla gigantea (A) and wing veins (B).



Figure 14. Megastrebla limbooliati (A), head (B), and wing veins (C).

Remarks — This species is the sole representative of a distinct species group. The body is slender compared to *M. gigantea* and *M. limbooliati*. The wings are long, consistent with other members of genus. The vein R_1 has a weak bend near the base, with vein R_{2+3} apically curved to C, the apex is nearly equidistant to R_1 and vein R_{4+5} apices and the first abscissa of vein R_{4+5} is distinctly oblique to vein R_{2+3} (Maa 1971).

Genus Brachytarsina Macquart, 1851

Brachytarsina sp.

Specimen examined (Figure 16) — (2) Tioman Island Wildlife Reserve, Pahang (TWR037; TWR042); (1) Gunung



Figure 15. Megastrebla nigriceps (A), wing veins (B), and head (C).

Gading National Park, Lundu, Sarawak (GGNP029); (1) Gunung Reng, Jeli, Kelantan (GRK022); (1) Gua Batu Madu, Gua Musang, Kelantan (GBM004); (1) Gunung Jerai, Kedah (GJK028).

Host — Rhinolophus creaghi, R. lepidus, R. affinis, R. stheno, Miniopterus medius.

Remarks — All individuals of this species were collected from the wing membranes of bats of the family Rhinolophidae (216 individuals) except for a single one, which was collected from *Miniopterus medius*. This medium-sized unidentified species resembles the morphological description for members of the genus *Brachytarsina*. The head is triangularly rounded with non-prominent eyes. The wing veins M_{1+2} with the first abscissa are much longer than the second abscissa



Figure 16. Brachytarsina sp. (A) and head (B).



Figure 17. Raymondia sp. (A) and head (B).

while vein M_{3+4} is very slightly curved and does not subangulate near the base (Maa 1971). This genus contains about 23 species in the Oriental region and is reported to be parasitic mostly on bats from the families Emballonuridae, Hipposideridae and Rhinolophidae (Maa 1966).

Genus Raymondia Frauenfeld, 1856

Raymondia sp.

Specimen examined (Figure 17) — (2) Tioman Island Wildlife Reserve, Pahang (TWR015; TWR039); (1) Gunung Gading National Park, Lundu, Sarawak (GGNP013); (1) Gunung Mulu World Heritage Area, Sarawak (GMWHA014); (1) Gua Cha, Kelantan (GCK018); (2) Wang Kelian State Park, Perlis (PSP010).

Host — Hipposideros bicolor, H. atrox, H. larvatus, H. cervinus, H. galeritus.

Remarks—Allindividuals of this species were collected from the fur area of bats from family Hipposideridae. This small-sized bat fly possibly belongs to the genus *Raymondia*. Eyes are absent. The head is narrower than the interdistance between the two anterior most major humeral setae and it has a roundish labial theca almost as long as it is broad (Maa 1962). Wings are short and broad, with five longitudinal and two cross veins and a very setose costa (Jobling 1930).

DISCUSSION

There is little published on the ecology, taxonomy and distribution of Malaysian bat flies. Until now, there were at least 30 species of bat flies recorded in Malaysia (Theodor 1967; Maa 1968, 1971). Our study recorded approximately half of the total number of bat fly species previously recorded from Malaysia. Unlike their hosts, the number of bat flies species encountered per survey is highly dependent on the number of host species recorded (Dick and Gettinger 2005). There was a moderate correlation between the numbers of host species recorded with the number of bat flies species encountered in our study. This compliments the statement by Marshall (1980) where the Malaysian nycteribiid and streblid bat flies are highly specialized; more than 50% were limited to a single host species. Thus, we speculate that higher number of host species documented would yield more bat flies species per survey.

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