

Diversity of bat ectoparasites from the caves of selected Key Biodiversity Areas in Central Visayas, Philippines

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Abstract. *Bejec GA, Bucol LA, Ancog AB, Pagente AC, Paneiro JJM, Bejec ALN, Belanizo JD, Tuastomban DJS, Jose RP. 2023. Diversity of bat ectoparasites from the caves of selected Key Biodiversity Areas in Central Visayas, Philippines. Biodiversitas 24(3): 1693-1703.* Bats harbor numerous species of ectoparasites, such as ticks and mites. Many bat ectoparasites are implicated as vectors of pathogens ranging from bacteria to protozoa and viruses. Recent studies verified bats as a natural reservoir of multiple zoonotic viruses, including Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS) coronaviruses. This study assessed the composition, diversity, and prevalence of ectoparasites of cave-dwelling bats in the four Key Biodiversity Areas (KBAs) of Central Visayas. A total of 20 species of bats were recorded in the 30 surveyed caves. Of these, 30 species of ectoparasites belonging to eight families (dipteran flies Streblidae and Nycteribiidae, mites Spinturnicidae, Macronyssidae and Trombiculidae, ticks Argasidae and Ixodidae, and Ischnopsyllidae fleas) were collected and identified. *Leptotrombidium* sp. had the highest number of individuals (n=1,684) harbored in 12 bat host species. The highest prevalence (100%) was noted in four bat host species: Philippine pygmy fruit bat *Haplonycteris fischeri* Lawrence 1939, Philippine forest roundleaf bat *Hipposideros obscurus* Peters 1861, Philippine pygmy roundleaf bat *Hipposideros pygmaeus* Waterhouse 1843, and Common bent-winged bat *Miniopterus schreibersii* Kuhl 1817. The lowest prevalence was noted in the Round-eared tube-nosed bat *Murina cyclotis* Dobson 1872, where no ectoparasite was collected. The highest intensity (n=65) of ectoparasites was observed in the Common Asian ghost bat *Megaderma spasma* Linnaeus 1758. This study provides essential data for future reference in monitoring bat population status and conservation efforts in the region. Given the close relationship between the local human community and bats (e.g., hunting and consumption), more work is needed to address the potential pathogen risks from zoonotic transmission from bats and ectoparasites.

Keywords: Mites, Nycteribiidae, prevalence, Streblidae, Spinturnicidae, vectors, ticks

INTRODUCTION

Bats (Order Chiroptera) are comprised of more than 1,300 species worldwide and are known to be the second largest group (20%) of the extant mammals (Sotero-Caio et al. 2017). In the Philippines, more than 70 species of bats were recorded (Tanalgo and Hughes 2019). Specifically, 12 species were identified from the central west coast and the southern section of the Northwest Panay (Mould 2012). While, 13 species of cave-dwelling bats were recorded in Central Visayas (Siquijor, Negros Oriental, and Bohol), including five Philippine endemics (Philippine forest roundleaf bat *Hipposideros obscurus* Peters 1861, Philippine forest roundleaf bat *Hipposideros pygmaeus* Waterhouse 1843, Greater musky fruit bat *Ptenochirus jabori* Peters 1861, Philippine forest horseshoe bat *Rhinolophus inops* K.Andersen 1905, and Large rufous horseshoe bat *Rhinolophus rufus* Eydoux & Gervais 1836) (Bejec et al. 2021). Furthermore, in a separate study by Jose et al. (2021), a total of 14 bat species belonging to six families were captured and recorded from the selected Key

Biodiversity Areas (KBAs) in Central Visayas (Mt. Bandilaan Natural Park in Siquijor, Balinsasayao Twin Lakes Natural Park in Negros Oriental and Rajah Sikatuna Protected Landscape in Bohol). These surveys revealed that those caves inhabited by bats have high anthropogenic disturbances/activities, including bat hunting, guano extraction, and tourism.

Along with their diversity, bats are also known to be hosts to several ectoparasites, including ticks (Ixodidae and Argasidae), mites (Spinturnicidae and Macronyssidae), and bat flies (Nycteribiidae and Streblidae). These ectoparasites were revealed to be vectors of pathogens ranging from bacteria, protozoa, and viruses (Burazerovic et al. 2018). Recent studies verified bats as a natural reservoir of multiple zoonotic viruses, including MERS and SARS coronaviruses (Chen et al. 2014; Zhou et al. 2020), which are current health issues affecting the global populations. This signifies that bat ectoparasites are of ecological and public health importance. Studies also revealed that even merely spending time inside the caves roosted by bats and the contact between bats and humans increase the possible

risk of interspecies transmission of pathogens (Allocati et al. 2016). Consequently, there is a growing concern about the need for a better understanding of bat and ectoparasite relationships, especially in composition and distribution. In a report by Tanalgo and Hughes (2019), more bat ectoparasite studies in the Philippines focused on new findings and bat hosts' distribution records (Alvarez et al. 2015, 2016; Amarga and Yap 2017; Amarga et al. 2017). In addition, several studies revealed parasite load correlates to the condition of bat health (Sharifi et al. 2019), the decline of the population of specific species of bent-winged bats (Holz et al. 2018), and the increased grooming behavior of bats (Obame-Nkoghe et al. 2016). Much evidence also revealed differences in the relationship between bat sex and parasite load. For example, roosts with hundreds of female bats in maternity colonies resulted in high ectoparasite exposure rates. That could expose more females and juveniles to ectoparasites and ultimately affect females' reproductive success (Vidal et al. 2021). In contrast, aggressive mating behavior in males may increase the chances of exposure and infection to parasites (Sharifi et al. 2019). Furthermore, bat hosts' distribution range and size indicate parasite species wherein new species are documented to hosts distributed in larger areas (Maganga et al. 2014).

Despite this, very little is known about the taxonomic composition and diversity of bat ectoparasites and the variables that affect these factors, specifically in Central Visayas. This comprehensive inventory of ectoparasites on cave-dwelling bats in KBAs would serve as vectors to potential human-health-threatening pathogens. Given the close relationship between the local community and the

bats (e.g., hunting and consumption), a study is needed to address the potential pathogen risks from the zoonotic transmission from bats and the respective ectoparasites. Hence, this study provides important data for future reference in monitoring bat population status and conservation efforts in the region.

MATERIALS AND METHODS

Study sites

Central Visayas is one of the administrative regions in the Philippines, consisting of four provinces, namely Cebu, Bohol, Negros Oriental, and Siquijor. The geographical location of the provinces in Central Visayas contributed to its rich biodiversity, resulting in multiple Key Biodiversity Areas (KBAs) established in the region. Among these areas, four KBAs were surveyed in this study. We surveyed a total of five (5) caves in Mt. Lantoy KBA (Cebu) from 20 to 29 April 2021; ten (10) caves in Mt. Bandilaan Natural Park (Siquijor) from 29 May to 08 June 2021; eight (8) caves in Rajah Sikatuna Protected Landscape (Bohol) from 21 to 30 July 2021, and seven (7) caves in Mabinay, Negros Oriental from 23 May to 31, 2022 as presented in Figure 1 and Table 1. Our team previously assessed the physical and biological characteristics of the caves in Bohol, Siquijor, and Mabinay on the last 2019, thus were selected as the study sites for our current study. Inaccessible caves, especially those heavily affected by the typhoon, were not considered during the survey for safety purposes.

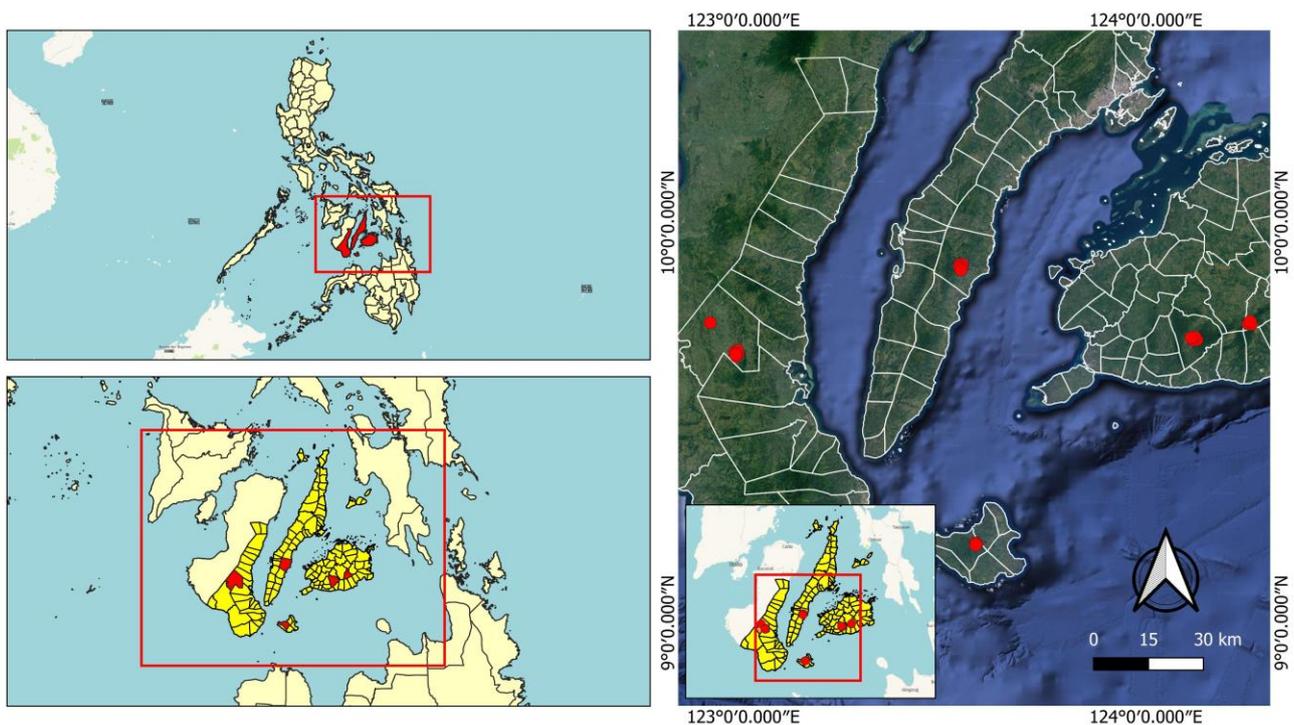


Figure 1. Map of Central Visayas, Philippines highlighting the location of the surveyed sites

Table 1. List of caves surveyed in four Key Biodiversity Areas (KBAs) in Central Visayas, Philippines

Cave sites	Latitude	Longitude	Elevation (m asl.)
Mt. Lantoy KBA-Argao, Cebu			
Agta cave	9.90928	123.53999	208.93
Balay'g colon cave	9.90295	123.54668	131.19
Balay'g halas cave	9.91249	123.54108	274.58
Kasiyay cave	9.9003	123.54537	220.41
Opaw cave	9.9125	123.54102	272.93
Mt. Bandilaan Natural Park in Cantabon, Siquijor, Siquijor			
Ambakag baki	9.19116	123.57931	513
Ambakag baki (Nature Spring)	9.19194	123.5796	498
Bung-aw	9.19191	123.57944	499
Cambulawan	9.18661	123.57332	522
Campong karaan	9.18729	123.57482	522
Cang eskaha	9.18622	123.57165	442
Cang anhao	9.19277	123.57726	440
Cantabon	9.18769	123.56398	497
Tinamnag gabi	9.18651	123.57135	436
Tugok	9.19133	123.57934	511
Rajah Sikatuna Protected Landscape (RSPL) in Bohol			
Canlusong cave 1	9.76209	124.26805	429.73
Duwangon cave 1	9.72228	124.12363	421.28
Duwangon cave 2	9.72228	124.12363	421.28
Hagdán cave 1	9.70337	124.12572	319.66
Hagdán cave 2	9.70327	124.12634	311.8
Kaliguon cave	9.71022	124.13151	455.67
Logarita cave 1	9.70289	124.12229	401.25
Logarita cave 2	9.70395	124.11665	318.63
Mabinay Caves in Mabinay, Negros Oriental			
Cayaso cave	9.73936	122.92033	187.22
Crystal cave	9.65937	122.98424	251.77
Mambajo cave	9.67093	122.98664	272.52
Odloman cave	9.65032	122.99617	268.1
Pandalihan cave	9.65606	122.98549	291.2
Panligawan cave	9.65465	122.98609	271.79
Tubod cave	9.66199	122.98295	270.25

Data gathering

Before actual fieldwork, the team coordinated with the corresponding local government units and the Department of Environment and Natural Resources offices in the respective provinces of Cebu, Siquijor, Bohol, and Negros Oriental. We also obtained our Gratuitous permit to proceed with the study. A reconnaissance survey was performed on each cave before the actual sampling activity. Furthermore, a GARMIN® GPSMAP 64s was used to mark the coordinates of cave entrances. A Mist netting approach was used for capturing bats. In addition, we installed mist nets (3x1m) near cave entrances. EAGLETECH® (Clock/Humidity HTC-2) was used to obtain temperature and relative humidity per cave. Captured bats were then brought back to the base camp for data collection. Each bat individual was screened for the presence of any ectoparasites. The ectoparasites were collected using fine-tip forceps, following their handling procedures and collection. Collected ectoparasites were then preserved in sampling bottles containing 70% ethanol. After each bat was assessed, morphometric measurements for species identification were done using "A Synopsis of the

Mammalian Fauna of the Philippine Islands" by Heaney et al. (1998). Bats were then released back into the wild. Pregnant and lactating female bats were not screened and were released immediately after the capture.

Collected ectoparasites were then brought to the laboratory for identification and analysis using AmScope® B100-5M Digital Compound Binocular Microscope. Morphological and anatomical features for each type of ectoparasite were noted as a reference to their identification. Micrographs were also obtained using AmScope® 5MP Camera with Reduction Lens attached to the microscope. The collected ectoparasites identification and verification based on available guidebooks and the species checklist in the Philippines by Alvarez et al. (2015). Voucher specimens were deposited at the Center for Biodiversity Studies of Cebu Technological University, Argao, Cebu.

Data analysis

Data were presented in tables and graphs, including: ectoparasite prevalence, intensity, relative abundance, diversity, host endemicity, and host conservation status. Prevalence is defined as the number of individuals within a host group infected by at least one ectoparasite divided by the total number of individuals sampled from that host group. The intensity of infection is defined as the number of ectoparasites on one host, which was calculated to characterize differences in the number of ectoparasites infecting a host. Relative abundance refers to the percent composition of the specific species relative to the total number of species in the area. Diversity refers to how varied the species are regarding species richness (the number of species present) and abundance (the number of individuals per species). Therefore, indices such as the Shannon-Wiener Diversity Index were computed to measure ectoparasite diversity in each location. Furthermore, to determine and visualize if there is a trend in ectoparasite abundance and diversity concerning bats' biological (species, age group, sex), caves' environmental variables (location, temperature, humidity), using multivariate statistical analyses such as correlation, multiple linear regression and Canonical Correspondence Analysis (CCA) using the vegan package. In addition, a matrix correlation plot using the corplot package in R software (R Core Team 2017) was performed. Furthermore, to determine the significant effect of the variables tested, the data were subjected to non-parametric tests (significance level set at $p < 0.05$) such as Pillai's trace. Endemicity is described as the state of a single species found in a single geographic location. Host-specificity (defined as the tendency of a parasite to occur on one or a few host species) for each species of ectoparasites was also noted, and two-way ANOVA was used to determine the significance of the variable tested in all the bat hosts collected. There was no specific intended number of bats to be taken per site since we used an opportunistic method to collect the bat hosts.

RESULTS AND DISCUSSION

Results

A total of 20 species of bats belonging to 13 genera with seven endemics were recorded in the four KBAs. The relative abundance, endemism, and International Union for Conservation of Nature (IUCN) status per species are presented in Table 2. Among the 469 bat individuals captured, *Hipposideros diadema* É.Geoffroy Saint-Hilaire 1813 was the most abundant at 24.09%. It was followed by *Miniopterus australis* Tomes 1858 (14.50%), *Rhinolophus arcuatus* Peters 1871 (11.30%), and *Rousettus amplexicaudatus* E.Geoffroy 1810 (10.45%). The least abundant species were: *H. pygmaeus*, *H.s fischeri*, and *M. cyclotis* having only one individual captured.

Differences in prevalence among hosts were also observed. *H. fischeri*, *H. obscurus*, *H. pygmaeus*, and *M. schreibersii* had the highest ectoparasite prevalence (100%). On the contrary, the lowest prevalence was recorded in *M. cyclotis* since no ectoparasite was collected. The highest intensity of ectoparasites was observed on *M. spasma*. At least three of these bats were infested with 195 ectoparasite individuals (Table 3).

The taxonomic classification, relative abundance, and diversity of bat ectoparasites are depicted in Figure 2A total of 30 species ($H'=2.37$) of ectoparasites belonging to four major groups (ticks, mites, fleas, and bat flies) were documented (Figure 3). Two families of bat flies were identified: Nycteribiidae with five genera (*Basilia*, *Nycteribia*, *Eucampsipoda*, *Leptocyclopodia*, and *Penicillida*), and Streblidae with three genera (*Brachytarsina*, *Megastrebla*, and *Raymondia*). Three mites' families were identified: Spinturnicidae with five genera (*Ancystropus*, *Meristapis*, *Paraperiglischrus*, *Periglischrus*, and *Spinturnix*), Trombiculidae with a single genus (*Leptotrombidium*) and Macronyssidae with *Macronyssus* as its representative genus. Two tick families Argasidae and Ixodidae, and one flea family (Ischnopsyllidae) were also documented. Out of the 4,015 sampled ectoparasite individuals, the most abundant Nycteribiid fly was *Leptocyclopodia ferrarii* Rondani 1878 at 5.13%, streblid fly was *Raymondia pseudopagodarum* Jobling 1951 (5.58%), *Carios batuensis* Hirst 1929 tick (3.71%), mite *Spinturnix* sp. (5.16%) and *Thaumapsylla breviceps* Rothschild 1907 flea (1.12%). The population mean of ectoparasites collected is between 24.8 and 243 (95% CI: 133.83±109).

Table 2. Species composition, abundance, endemism, and IUCN status of bats captured from the caves of the four selected Key Biodiversity Areas (KBAs) in Central Visayas, Philippines

Family	Bat species	Common name	Relative abundance	Endemism	IUCN status*
Emballonuridae	<i>Emballonura alecto</i>	Philippine sheath-tailed bat	4.69	Not endemic	Least Concern
Hipposideridae	<i>Hipposideros ater</i>	Dusky roundleaf bat	1.49	Not endemic	Least Concern
	<i>Hipposideros diadema</i>	Diadem round leaf bat	24.09	Not endemic	Least Concern
	<i>Hipposideros lekaguli</i>	Large Asian Roundleaf bat	1.07	Not endemic	Near Threatened
	<i>Hipposideros obscurus</i>	Philippine forest roundleaf bat	1.71	Endemic	Least Concern
	<i>Hipposideros pygmaeus</i>	Philippine pygmy roundleaf bat	0.21	Endemic	Least Concern
Megadermatidae	<i>Megaderma spasma</i>	Lesser false vampire bat	0.85	Not endemic	Least Concern
Molossidae	<i>Chaerephon plicatus</i>	Wrinkle-lipped bat	4.69	Not endemic	Least Concern
Pteropodidae	<i>Cynopterus brachyotis</i>	Common short-nosed fruit bat	4.26	Not endemic	Least Concern
	<i>Eonycteris spelaea</i>	Common nectar bat, Common dawn bat	4.26	Not endemic	Least Concern
Rhinolophidae	<i>Haplonycteris fischeri</i>	Philippine pygmy fruit bat	0.21	Endemic	Least Concern
	<i>Macroglossus minimus</i>	Lesser long-tongued fruit bat	0.85	Not endemic	Least Concern
	<i>Ptenochirus jagori</i>	Greater musky fruit bat	5.76	Endemic	Least Concern
	<i>Rousettus amplexicaudatus</i>	Common rousette bat	10.45	Endemic	Least Concern
	<i>Rhinolophus arcuatus</i>	Arcuate horseshoe bat	11.30	Not endemic	Endangered
Rhinolophidae	<i>Rhinolophus philippinensis</i>	Enormous-eared horseshoe bat	2.77	Not endemic	Least Concern
	<i>Rhinolophus rufus</i>	Large rufous horseshoe bat	2.77	Endemic	Near Threatened
	<i>Miniopterus australis</i>	Little bent-winged bat	14.50	Not endemic	Least Concern
Vespertilionidae	<i>Miniopterus schreibersii</i>	Common bent-winged bat	3.84	Not endemic	Near Threatened
	<i>Murina cyclotis</i>	Round-eared tube-nosed bat	0.21	Endemic	Least Concern

Note: *IUCN status information per bat species based from <https://www.iucnredlist.org/>

Table 3. Prevalence and intensity of ectoparasites from the bats captured in the caves of the four selected Key Biodiversity Areas (KBAs) in Central Visayas, Philippines

Bat Host	Number of bat individuals captured	Number of bat hosts infested with ectoparasite	Total number of ectoparasite extracted	Prevalence ^b	Intensity ^c	Standard deviation
<i>Chaerephon plicatus</i>	22	13	25	59.09	1.92	8.54
<i>Cynopterus brachyotis</i>	20	12	34	60.00	2.83	7.42
<i>Emballonura alecto</i>	22	20	90	90.91	4.50	49.37
<i>Eonycteris spelaea</i>	20	19	211	95.00	11.11	13.95
<i>Haplonycteris fischeri</i>	1	1	2	100.00	2.00	0.00
<i>Hipposideros ater</i>	7	2	4	28.57	2.00	0.00
<i>Hipposideros diadema</i>	113	100	1574	88.50	15.74	320.40
<i>Hipposideros lekaguli</i>	5	3	14	60.00	4.67	4.73
<i>Hipposideros obscurus</i>	8	8	225	100.00	28.13	99.86
<i>Hipposideros pygmaeus</i>	1	1	3	100.00	3.00	0.00
<i>Macroglossus minimus</i>	4	1	1	25.00	1.00	0.00
<i>Megaderma spasma</i>	4	3	195	75.00	65.00	89.57
<i>Miniopterus australis</i>	68	63	312	92.65	4.95	20.96
<i>Miniopterus schreibersii</i>	18	18	139	100.00	7.72	16.07
<i>Murina cyclotis</i>	1	0	0	0.00	0.00	0.00
<i>Ptenochirus jagori</i>	27	24	221	88.89	9.21	13.69
<i>Rhinolophus arcuatus</i>	53	45	238	84.91	5.29	41.81
<i>Rhinolophus philippinensis</i>	13	10	80	76.92	8.00	10.13
<i>Rhinolophus rufus</i>	13	12	73	92.31	6.08	9.95
<i>Rousettus amplexicaudatus</i>	49	45	574	91.84	12.76	36.02

Note: a: Total number of ectoparasites collected from all the captured infested bat hosts. b: The number of individuals within a host group infested by at least one ectoparasite divided by the total number of individuals sampled from that host group. c: Mean number of ectoparasites on one host, calculated to characterize differences in the number of ectoparasites infecting a host

Ectoparasite host specificity was also investigated in this study. Nycteribiid flies were found to be highly host-specific ($p=0.04025$). *Leptocyclopodia simulans* Theodor 1959 were collected only from *P. jagori* hosts, whereas *Basilisa majuscula* Edwards 1919 was collected from the *H. fischeri* host. Among the streblid flies ($p=0.02375$), *Megastrebla gigantea* Edwards 1919 is highly host-specific and collected only from *M. australis*. Mite species *Paraperiglischrus hipposideros* Baker & Delfinado 1964, *Paraperiglischrus strandtmanni* Baker & Delfinado 1963, and *Spinturnix verutus* Delfinado & Baker 1963 were also highly host specific, as they were collected only from *H. diadema*, *E. spelaea*, and *M. australis*, respectively. All the ticks and fleas collected were not host specific as they are harbored and collected from several bat hosts (Table 4).

Correlation of different variables, including the environmental factors (e.g., cave, temperature, and humidity), biological traits (e.g., bat species, age group, sex, morphological features, e.g., total bat body length and weight) to the abundance and diversity of ectoparasites were tested. A matrix correlation plot (using the Pearson method) is presented in Figure 4. Analysis revealed that the abundance and diversity of each specific type of ectoparasites were not highly correlated with the variables tested. Among the environmental variables tested, there is a weak positive correlation between mite abundance and the relative humidity in the cave ($r=0.26$). Weak negative correlations were observed between tick abundance and relative humidity ($r=-0.03$, $p<0.001$) and bat flies'

abundance and relative humidity ($r=-0.07$, $p<0.001$). Weak correlations, ranging from -0.01 to 0.11, were observed between the types of ectoparasites collected in the caves where they were collected. Weak correlations, ranging from -0.02 to 0.14, were observed between the abundance for each type of ectoparasites, the bat age group, and the bat gender. These correlation coefficients indicate an inconsistent correlation pattern between the variables and the types of ectoparasites.

A positive correlation ($r=0.30$) was observed regarding the biological variables tested between bat flies' abundance and the bat host species. Furthermore, a positive correlation was observed between mite abundance and the bats' total body length ($r=0.29$, $p<0.001$), as well as tick and flea' abundance was of positive values, $r=0.10$ ($p<0.001$) and $r=0.07$ ($p<0.001$), respectively. On the contrary, a negative correlation was observed between the bat flies' abundance and the bat' total body length ($r=-0.13$, $p<0.001$) (Figure 5).

Canonical Correspondence Analysis (CCA) supports the results of the correlation analysis performed. Figure 6 presents the CCA biplot for the environmental (caves' temperature and relative humidity) and morphological (bat' total body length and weight) variables tested against the abundance of each type of ectoparasites. Arrows for fleas and ticks indicate a correlation to relative humidity and temperature; however, neither of these two factors (fleas and ticks) is correlated to the bat' total length and weight. On the other hand, bat flies have a weak correlation to total length and weight, as they are on the same axis.

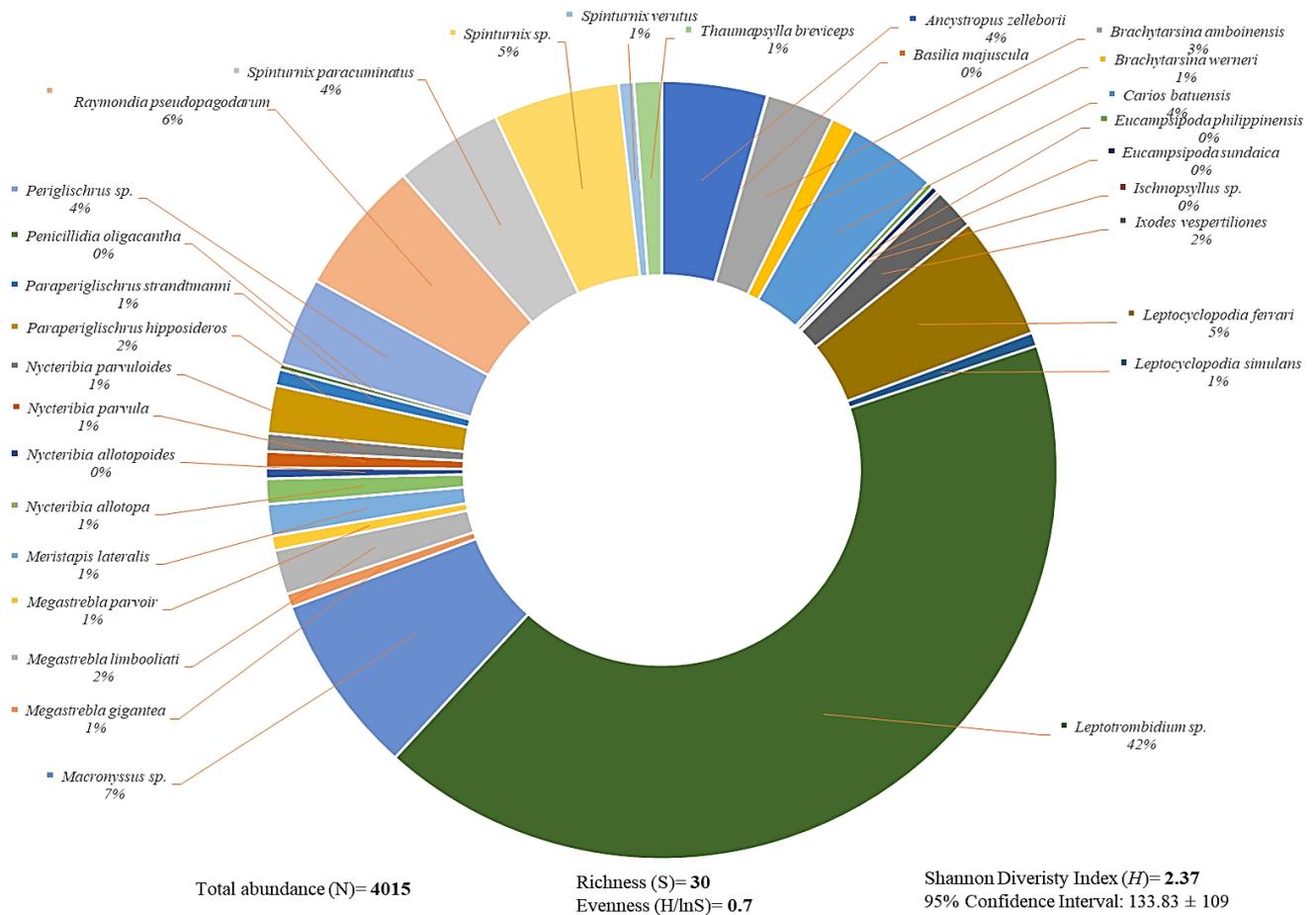


Figure 2. Species composition, diversity, and abundance of ectoparasites from the bats captured in the caves of four selected Key Biodiversity Areas (KBAs) in Central Visayas. With 95% confidence, the population mean of ectoparasites collected is between 24.8 and 243, based on 30 samples

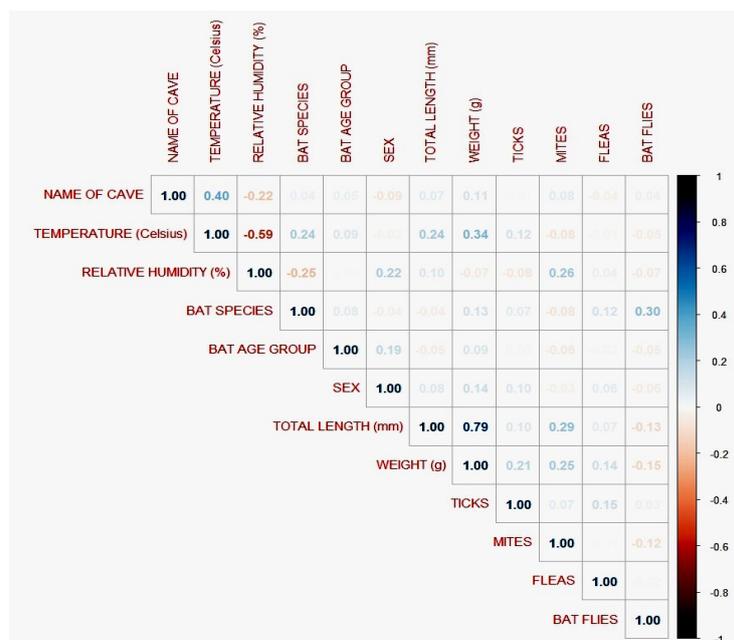


Figure 4. Correlation matrix plot among the tested variables. There is a weak positive correlation between mite abundance and the relative humidity in the cave ($r=0.26, p<0.001$). Weak negative correlations were observed between tick abundance and relative humidity ($r=-0.03, p<0.001$) and bat flies' abundance and relative humidity ($r=-0.07, p<0.001$). Correlation coefficients indicate an inconsistent correlation pattern between the variables and the types of ectoparasites



Figure 3. Bat ectoparasites species collected. 1. *Basilia majuscula*, 2. *Nycteribia allotopa*, 3. *Nycteribia allotopoides*, 4. *Nycteribia parvula*, 5. *Nycteribia parvuloides*, 6. *Eucampsipoda philippinensis*, 7. *Eucampsipoda sunaica*, 8. *Leptocyclopidia ferrarii*, 9. *Leptocyclopidia simulans*, 10. *Penicillidia oligacantha*, 11. *Brachytarsina amboinensis*, 12. *Brachytarsina werneri*, 13. *Megastrebla gigantea*, 14. *Megastrebla limbooliati*, 15. *Megastrebla parvoir*, 16. *Raymondia pseudopagodarum*, 17. *Ancystropus zeleborii*, 18. *Spinturnix* sp., 19. *Spinturnix verutus*, 20. *Spinturnix paracuminatus*, 21. *Meristapis lateralis*, 22. *Periglischrus* sp., 23. *Paraperiglischrus hipposideros*, 24. *Paraperiglischrus strandtmanni*, 25. *Leptotrombidium* sp., 26. *Macronyssus* sp., 27. *Carios batuensis*, 28. *Ixodes vespertiliones*, 29. *Thaumapsylla breviceps*, 30. *Ischnopsyllus* sp.

Table 4. Host-specificity of bat ectoparasites collected from the bats captured in the caves of four selected Key Biodiversity Areas (KBAs) in Central Visayas. Host-specificity was tested with t-Test: Two-Sample Assuming Unequal Variances

Family	Ectoparasite species	Bat host*	P value
Nycteribiidae	<i>Basilia majuscula</i>	HF	0.04025*
	<i>Nycteribia allotopa</i>	MA, MSc	
	<i>Nycteribia allotopoides</i>	MA	
	<i>Nycteribia parvula</i>	MA, RA	
	<i>Nycteribia parvuloides</i>	ES, MSc	
	<i>Eucampsipoda philippinensis</i>	ES, RAM	
	<i>Eucampsipoda sundaica</i>	ES	
	<i>Leptocyclopodia ferrari</i>	CB, ES, MA, PJ, RAM	
	<i>Leptocyclopodia simulans</i>	PJ	
	<i>Penicillidia oligacantha</i>	MA, MSc	
Streblidae	<i>Brachytarsina amboinensis</i>	EA, HD, HO, MS, MA, RA, RP	0.02375*
	<i>Brachytarsina weneri</i>	ES, MA, RAM	
	<i>Megastrebla gigantea</i>	MA	
	<i>Megastrebla limbooliati</i>	ES, HD, MSc, RAM	
	<i>Megastrebla parvoir</i>	HD, HO, RAM	
	<i>Raymondia pseudopagodarum</i>	CP, HF, HA, HD, HL, MA, RA, RP, RR, RAM	
Argasidae	<i>Carios batuensis</i>	ES, HD, HL, HO, MS, MA, RA, RAM	0.11196
Ixodidae	<i>Ixodes vespertilionis</i>	CP, HD, MS, MA, RA, RAM	0.05069
Spinturnicidae	<i>Ancystropus zelleborii</i>	CB, ES, PJ, RAM	
	<i>Meristapis lateralis</i>	CB, ES, RAM	
	<i>Paraperiglischrus hipposideros</i>	HD	
	<i>Paraperiglischrus strandtmanni</i>	ES	
	<i>Periglischrus</i> sp.	CB, MA, PJ, RR	
	<i>Spinturnix</i> sp.	RAM, EA, ES, MM, RA, RP, HD	
	<i>Spinturnix paracuminatus</i>	MA, MSc	
	<i>Spinturnix verutus</i>	MA	
Trombiculidae	<i>Leptotrombidium</i> sp.	CP, EA, HD, HO, MS, MA, MSc, PJ, RA, RP, RR, RAM	
Macronyssidae	<i>Macronyssus</i> sp.	CP, EA, HD, HL, MA, MSc, PJ, RA, RAM	0.21474
Ischnopsyllidae	<i>Ischnopsyllus</i> sp.	HP, RA	
	<i>Thaumapsylla breviceps</i>	ES, RAM	

Note: Bat host (CB: *Cynopterus brachyotis*, CP: *Chaerephon plicatus*, EA: *Emballonura alecto*, ES: *Eonycteris spelaea*, HF: *Haplonycteris fischeri*, HA: *Hipposideros ater*, HD: *Hipposideros diadema*, HL: *Hipposideros lekaguli*, HO: *Hipposideros obscurus*, HP: *Hipposideros pygmaeus*, MA: *Miniopterus australis*, MSc: *Miniopterus schreibersii*, MM: *Macroglossus minimus*, MS: *Megaderma spasma*, RA: *Rhinolophus arcuatus*, RP: *Rhinolophus philippinensis*, RR: *Rhinolophus rufus*, RAM: *Rousettus amplexicaudatus*, PJ: *Ptenochirus jagori*). *Significant

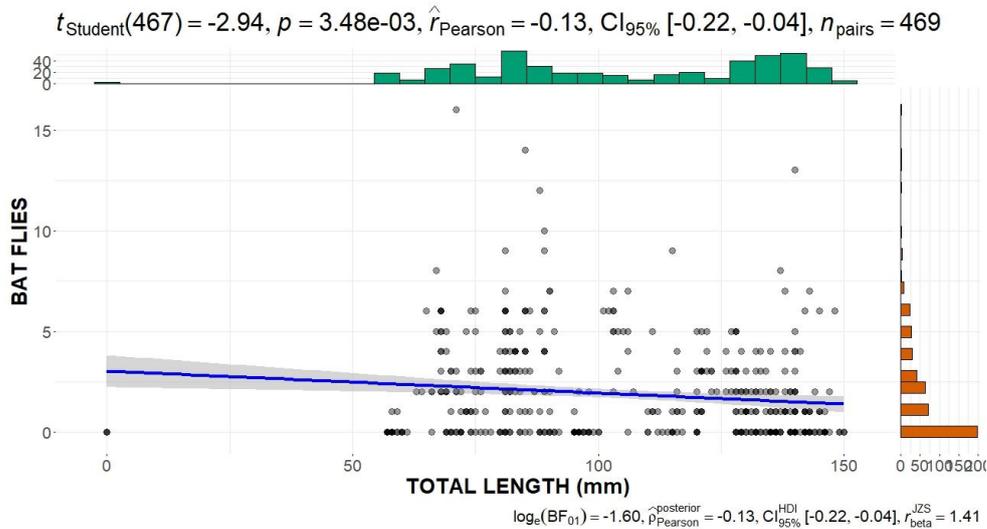


Figure 5. Statistical correlation plot between bat flies' abundance and bat total body length (mm). Pearson's correlation test revealed that across 469 bat hosts examined, each bat host's total body length was negatively correlated ($r = -0.13$) with the number of bat flies collected from each infested host. Furthermore, this effect was statistically significant ($p < 0.001$)

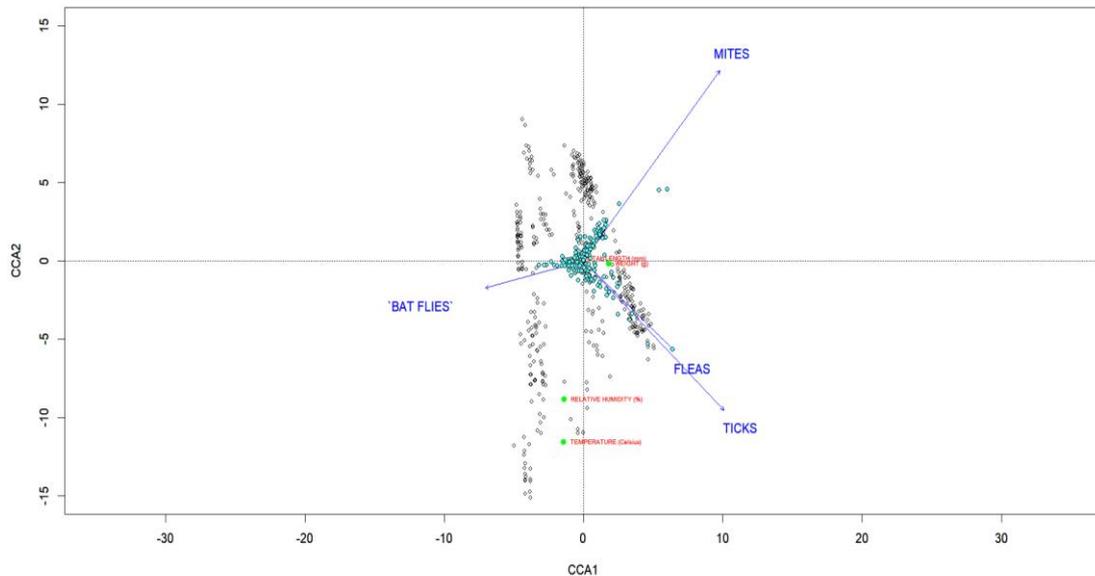


Figure 6. Canonical correspondence analysis (CCA) plot, showing the correspondence of environmental and bat morphological variables (green dots) and the abundance of each type of ectoparasites (bat flies, mites, fleas, and ticks) (blue arrows). The analysis was based on 263 bat individuals infested with mites (blue dots). The eigenvalues of the bat's morphological variables (total body length and weight) are 0.004246 and 0.001199, respectively, which provides a measure of the significance of each variable to the abundance of each type of ectoparasites. Permutation test for CCA under reduced model yielded model 4(Df), 0.005729 (Chi-Square), 15.494 (F), and 0.001 Pr(>F), which is significant

Discussion

In Mt. Lantoy (Cebu), 13 species (123 individuals) of bat hosts were captured from the five caves surveyed; Agta (8 bat species), Opaw (6 bat species), Balay'g Halas (5 bat species), Kolon (1 bat species) and Kasiyay (5 bat species). This number is relatively higher compared to the recent study by Alcazar et al. (2020), in which they reported six species in Agta cave and less than five species in other caves in Cebu. Seven species (125 individuals) of bats were documented in Mt. Bandilaan Natural Park (Siquijor), compared to the five species from the previous survey conducted by our team in 2019. In RSPL (Bohol), 11 bat species (138 individuals) were documented. This relatively higher species diversity observed among the sites can be attributed to the lower anthropogenic activities' exposure to the caves due to the effect of the pandemic. The local government supposedly promoted these caves for tourism; however, due to the restrictions imposed during the onset of the pandemic, tourism and other anthropogenic activities in the caves were halted. Accordingly, biodiversity, habitat loss, and ecological fragmentation are often directly linked to anthropogenic disturbances. Higher anthropogenic disturbance levels reduce species diversity and richness (Yuan et al. 2016). On the other hand, in Mabinay caves (Negros Oriental), only seven bat species (83 individuals) were documented, comparatively lower than the 11 species documented from the other survey by our team in 2019. This lower number in diversity and abundance is probably because most of the caves were damaged by Typhoon Odette. It was very apparent during the actual sampling periods that most of the caves were not accessible anymore and were heavily damaged (debris scattered within the

caves' chambers). Additionally, interviews from the local inhabitants also hinted to the team that there are high bat hunting activities within these caves as locals consume bats.

A total of 30 species of ectoparasites belonging to 4 major groups (ticks, mites, fleas, and bat flies) were documented in this study. This number contributes to the new findings and records of the host and distribution of bat ectoparasites in Central Visayas. Results show that bat flies of the family Nycteribiidae and Streblidae dominated (16 species), followed by mites (10 species). That could be attributed to the morphological adaptations that allow bat flies to attach to their hosts (Reeves and Lloyd 2019). Numerous studies also revealed that bat flies and mites are among the most abundant bat ectoparasites, whereas ticks and fleas are rarely observed (Amarga and Fornesa 2020; Fajri and Armiani 2021).

Ectoparasite intensity and prevalence are different among host species. It was observed that insect-eating bats *H. obscurus* and *M. schreibersii* have the highest prevalence and intensity of ectoparasites. It opposed the assumption that insect-eating bats have lower ectoparasite intensity since they would consume the insects around them. However, there is very little support for this assumption. Instead, factors such as roosting structures and bat behavior affect infestation rates in bats. Higher infestation rates could result from increased contact between bats in larger roosts (Lim et al. 2020). Moreover, frugivorous bats, such as *C. brachyotis*, *E. spelaea*, and *R. amplexicaudatus*, also show a high prevalence and intensity of ectoparasites, which may be attributed to the host roosting behavior. Especially since these hosts used the

cavities of the caves as primary roost, which have higher densities of ectoparasites than those hosts roost on foliage (Obdianela et al. 2021); however, these factors are not investigated within the scope of our study.

Host specificity among ectoparasite species was also documented in this study. Host specificity refers to the degree of restriction of a particular ectoparasite species to a specific host species. While contributing to their morphological adaptations, bat flies are revealed to be highly host specific among the ectoparasites collected. It was presumed that bat flies, specifically of the family Streblidae (winged flies), have lesser host specificity since they can easily move from one host to another in large roost areas. Whereas flightless species of the family Nycteribiidae are more host specific since their movement is limited (Dick and Dittmar 2014). Furthermore, factors such as climate (Pilosof et al. 2012), competition, predation, and physiological adaptations may also affect the degree of host specificity (Dick and Dittmar 2014). *Leptotrombidium* sp. is the most abundant (n=1,684) ectoparasite collected and infests the greatest number of host species (12). This genus is highly recorded from the hosts in Mt. Bandilaan (Siquijor) co-roosting within the caves. However, information on the specificity of *Leptotrombidium* sp. to its primary hosts is very scarce and fragmented.

The presence and absence of ectoparasites have been revealed to be owed to the coevolution of their respective hosts. However, several factors must also be considered in understanding the parasitism dynamics of an organism. According to Wu et al. (2019), both biotic and environmental attributes affect ectoparasitic infestation. Factors including temperature, relative humidity, altitude, vegetation structure, and topography affect the parasitism to hosts. It has already been revealed that mite prevalence increases as temperature decreases. Accordingly, biotic factors such as host sex and age group also affect the host preference of ectoparasites. For example, adult bats are more highly infested with ectoparasites than sub-adult/vulnerable bats (Vidal et al. 2021). Furthermore, ectoparasites prefer female bat host due to their lesser grooming activities (Obame-Nkoghe et al. 2016). However, the results of the correlation analysis contradict this pattern. Each type of ectoparasite has a very weak association with temperature and relative humidity. This pattern is also observed in the other variables tested, irrespective of their sex, age group, size, and weight. This indicates that ectoparasites' parasitism to bat hosts could be because of their adaptation to host conditions, morphological and physiological attributes.

In conclusion, environmental or biological factors do not mediate the preference and rate of infestation of ectoparasites (ticks, mites, fleas, and bat flies). However, given the close relationship between the local human community and bats (e.g., hunting and consumption), the study provides important information regarding ectoparasite awareness to address the potential pathogen risks from zoonotic transmission and bat population conservation efforts in the region.

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