Gastrointestinal parasitic helminths of bats from a cave in Luzon Island, Philippines

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Article info	Summary
	Bats are often associated with several zoonotic diseases, including helminth infections. Despite their potential detrimental effects on both human and bat populations, information on helminth parasites of Philippine bats remains scarce. Here, we identify gastrointestinal helminths parasitizing bats collected from a cave complex in Cavinti, Laguna Province, Philippines, determine their prevalence (P) and mean intensity (MI), and investigate relationship of helminth prevalence and intensity with bat host characteristics including sex, diet, age, and roosting habit. We isolated 581 individuals of helminths representing six taxa (three trematodes, two nematodes, one cestode) from the gastrointestinal tract of sampled bats. Helminths were observed in 82 out of 172 bats captured (P = 47.67%), with *Miniopterus paululus* having the highest infection rate (P = 91.3%) and highest MI (18 ± 4.5/ infected bat). Helminths parasitizing endemic bat species from the Philippines, such as *Rhinolophus rufus*, *Rhinolophus inops*, *Hipposideros pygmaeus*, and *Ptenochirus jagori* were reported here for the first time. We provide new host records for other helminth species and report the presence of two parasites, *Plagiorchis* sp. and *Toxocara* sp., known for their zoonotic potential. These data will contribute to efforts in identifying parasites and diseases harbored by bats in the country and develop additional conservation measures for bats. **Keywords:** caves; Chiroptera; Luzon; parasite; prevalence; zoonosis

Introduction

With over 1450 species described to date, bats (Chiroptera) are one of the most diverse and widespread of all known mammalian taxonomic orders (Simmons & Cirranelo, 2022). They play a crucial role in preserving ecosystem balance as pollinators, seed dispersers, and in controlling insect pest populations (Kunz *et al.*, 2011; Castillo-Figueroa, 2020).

Bats are also recognized for hosting viruses, microorganisms, fungi, and parasites that can affect both human health and bat populations (Frick *et al.*, 2016; Mandl *et al.*, 2018). Recently, bats were associated in zoonotic outbreaks such as rabies, Ebola virus, Nipah, and even SARS-related coronaviruses (Calisher *et al.*, 2006; Hu *et al.*, 2015; El-Sayed & Kamel, 2021). Increasing trend of studies investigating fungi and ectoparasites of bats have also been observed given their adverse effects in many bat populations (Frick *et al.*, 2016; Haelewaters *et al.*, 2017; Tanalgo & Hughes, 2018).

Various gastrointestinal helminths are also carried by bats and have been reported in many regions, raising awareness on public

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health as these diseases have zoonotic potential and can result to rapid spread of infections given the ability of bats to disperse across wide distances (Sawada, 1983; Cuartas-Calle & Munoz-Arango, 1999; Duval *et al.*, 2007; McCallister, 2007; Muñoz *et al.*, 2010; Mühldorfer *et al.*, 2011; Lord *et al.*, 2012; Gay *et al.*, 2014; Eduardo, 2021). Further, helminth infections can adversely affect the immune system and physiology of bats which can negatively influence their overall fitness and behavior (Scott ,1988; Dobson & Carper, 1992; Adhikari *et al.*, 2020).

Although there is an increasing trend in studies of bat-harbored pathogens such as viruses and ectoparasites in the Philippines, very little is known about helminth infections in bats as well as their interactions (Tanalgo & Hughes, 2018). In addition, studies focusing on helminth communities of bats is very rare compared with other mammalian groups (Eduardo, 1997). The pioneering work of Tubangui (1928) on trematode parasites of Philippine vertebrates reported the first record of helminths parasitizing bats. To date, only three studies have been added to the work of Tubangui (Kifune & Sawada, 1986; Prociv, 1987; Eduardo, 2021). From these surveys, only four species of bats (*Rousettus amplexicaudatus, Scotophilus kuhlii*, *Miniopterus eschscholtzi*, and *Myotis* sp.) were examined for helminths, representing just 5 % of the total number of bat species (n = 79) present in the country (Heaney *et al.*, 2010; Tanalgo & Hughes, 2018). Further, as only small samples of hosts and helminths were examined from these studies, there is scarce information on host-parasite associations, transmission dynamics,

and factors affecting parasite prevalence and intensity.

To fill this large gap in helminthological study of bats in the Philippines, the present study was undertaken to determine the helminth fauna of bats collected in Cavinti Underground River and Cave Complex (CURCC) in Luzon Island and expand our knowledge on helminth species distribution in the Philippines. Further, we determined association between helminth prevalence and intensity with host characteristics such as diet, sex, age, and roosting habit.

Materials and Methods

Study sites

Bat sampling was conducted in Cavinti Underground River and Caves Complex (CURCC) (14°16'50.85"N, 121°38'5.68"E) located in Barangay Paowin, Cavinti at the eastern side of Laguna Province in the Philippines (Fig. 1). Bat sampling was conducted on May 22 – 30, 2014. The cave system was discovered by a local logger in 1980 and was opened to tourists in 2013. Bats were collected from three caves, namely, Cathedral, Minalokan, and Kalaw, as well as the surrounding karst forest areas. Cathedral and Minalokan cave entrances are traversed by a river. Vegetation around the caves is mainly secondary growth forest adjacent to agroforests and coconut plantations. Plants belonging to the families Melastomataceae, Euphorbiaceae, and Moraceae (*Ficus* spp.) dominate the surrounding vegetation.
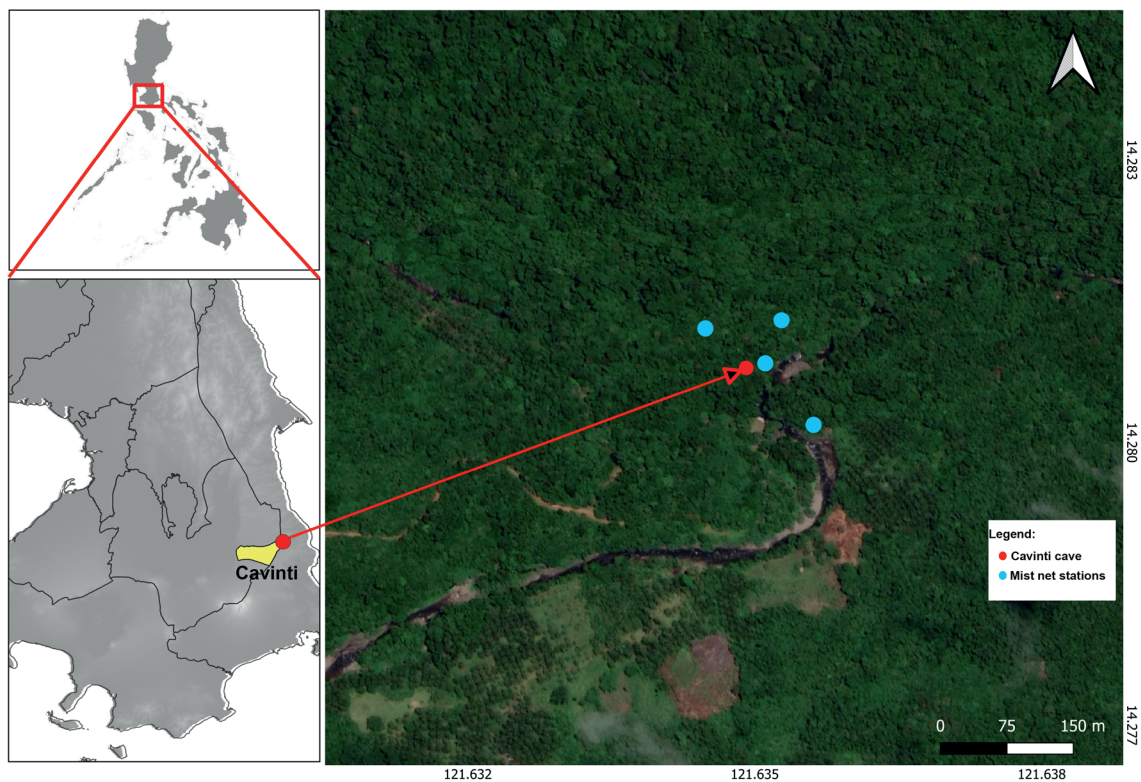

Fig. 1. Location map of Cavinti, Laguna (left) and sampling sites in Cavinti Underground River and Cave Complex (CURCC) (right).

Bat sampling

Bats were captured using mist nets (12 x 2.6 m with 36 mm mesh and four shelves) placed 0 – 3 m above ground, in a series of five nets. A total of 20 nets were set in cave entrances as well as in potential flyways outside the cave (i.e. forest clearings, near streams and rivers). These nets were watched over for an hour after dusk (1800h to 1900h) and were regularly checked every 10 minutes especially during emergence to mitigate potential harm to bat captures. The nets were then left overnight for eight nights then checked early morning the next day to retrieve any captures. Individual bats captured were put separately into clean cloth bags. Bat identification was based on Ingle and Heaney (1992). Morphometric data such as weight, sex, and age category (juvenile, subadult, adult) of the bats captured were identified and recorded. In particular, age classification of bats was determined by illuminating the dorsal side of the wing and assessing the extent of fusion in the epiphyseal plates of the phalanges; bats exhibiting unfused epiphyseal plates were classified as juveniles, whereas those with fused plates were considered adults (Kunz, 1988). Photographs of the species collected were also taken.

Collection and Identification of Helminths

Each bat was sacrificed by placing the cloth bag in a Ziploc with cotton moistened with ethyl acetate. The abdominal cavity was opened, and the digestive tract removed intact and placed in a clean Petri dish. The intestinal tract was gently teased to remove intestinal contents as well as the lining of intestine to remove embedded parasites. Helminths found were picked, counted, and placed in cold saline solution to evert proboscis for identification purposes. The helminths were then placed in tubes filled with 70 % ethanol for further processing in the laboratory.

The helminths were stained and identified using a light microscope. For trematodes and cestodes, isolated samples were directly placed onto depression slides, stained using Acetocarmine stain then de-stained using acid ethanol (1ml HCL: 100 ml 70 % Ethanol) after five minutes to clear some of the red tinge off of the specimen. Total time for clearing of specimen was variable depending on the tinge on the specimen; some might take longer while others have shorter clearing times. Basic ethanol (1ml 10 % NH3: 100ml 70 % Ethanol) was then used to replace the acidic ethanol and add a bluish tinge to some of the parasites' external and internal structures and add a color gradient to the specimen for easier viewing of its structure and organs. The basic ethanol was then pipetted out and the stained specimen was then dehydrated using a series of serial dilutions of ethanol (i.e. 70 %, 80 %, 90 % and 100 % ethanol). The specimen was placed in each dilution for 15 minutes to extract all water from the specimen. The specimens were then cleared in Xylene, then mounted on a slide using Canada balsam. Specimens that were not immediately mounted were stored in either xylene or 100 % ethanol.

For nematodes, collected specimens were subjected to the same serial dilution of ethanol as mentioned above. After dehydration, the nematodes were placed in two washings of clove oil for at least 30 minutes each. These were fixed in a 3:1 ratio of clove oil and Canada balsam, then mounted on a slide using Canada balsam. Identification of isolated helminths were done through the aid of published journals and diagnostic keys by Inglis (1968), Prudhoe and Manger (1969), Mészáros (1973), Fischthal and Kuntz (1975), Lotz and Palmieri (1985), Wong and Anderson (1986), Kifune *et al.* (2001), Okafor *et al.* (2004), Bray *et al.* (2008), and Hechinger (2012).

Data Analysis

Prevalence (P) and mean intensity (MI) of infection for each helminth taxon were calculated based on Bush *et al.* (1997), and values were computed using Quantitative Parasitology (QP) version 3.0 (Rozsa *et al.*, 2000). Chi-square test of independence was used to determine differences in the prevalence of helminths among all the samples while Mann- Whitney (U-test) was used to compare mean intensity between host sex, diet, roosting site, and age; both analyses were done using SPSS version 20 (SPSS Inc., Chicago, IL, USA)

Ethical Approval and/or Informed Consent

Prior to the conduct of the study, the protocol was approved by the research ethical advisory panel of the Institute of Biological Sciences of the University of the Philippines Los Baños in accordance with Department of Agriculture Administrative Order No. 40 series of 1999 otherwise known as "Rules and Regulations on the Conduct of Scientific Procedures Using Animals" pursuant to Republic Act No. 8485 or the "Animal Welfare Act of 1998" and Republic Act No. 9147 or the "Wildlife Resources Conservation and Protection Act". Field sampling and collection of specimens were covered by Gratuitous Permit No. R4AWGP-02-2014-LAG-001.

Results and Discussion

Bat Diversity

A total of 172 bat individuals representing 13 species from four families (Pteropodidae, Hipposideridae, Rhinolophidae and Vespertillionidae) were captured from the study areas (Table 1). Most of the species of bats recorded were observed roosting in the caves visited, while only three species were considered non-cave dwelling: *Cynopterus brachyotis*, *Haplonycteris fischeri*, and *Ptenochirus jagori*. Further, five species were pteropodids (frugivorous/ nectarivores) while eight species were insectivorous bats.

Rousettus amplexicaudatus (19.05 %, n = 32) was the most abundant species captured, followed by *Miniopterus paululus* (13.95 %, n = 24) and *Hipposideros pygmaeus* (12.21 %, n = 21) (Table 1). Of the total captures, 105 individuals were netted in cave entrances while 67 individuals were captured in nets set in a mixture of agricultural areas and secondary growth forest outside the cave. However, 55 % of the captured bats outside the cave are also

Table 1. List of bat species including prevalence (P, %) and mean intensity (MI, mean number of helminths/bat individual) of each helminth group isolated from bats collected in Cavinti Underground River and Cave Complex (CURCC), Cavinti, Laguna.

Bat Species	n	Trematodes		Nematodes		Cestodes		TOTAL	
		P	MI	P	MI	P	MI	P	MI
Rousettus amplexicaudatus	32	0	0	14.29	3	25.71	4	40	4
Cynopterus brachyotis	16	0	0	0	0	6.25	3	6.25	3
Ptenochirus jagori	13	0	0	38.46	1	0	0	38.4	1
Haplonycteris fischerii	2	0	0	0	0	0	0	0	0
Eonycteris robusta	1	0	0	0	0	0	0	0	0
Hipposideros lekaguli	20	0	0	0	0	0	0	0	0
Hipposideros diadema	15	13.33	11	6.67	2	26.67	1	40	5
Hipposideros pygmaeus	21	5	1	15	1	0	0	15	2
Rhinolophus inops	17	64.76	0	0	0	5.88	1	64.71	6
Rhinolophus arcuatus	1	0	0	0	0	0	0	0	0
Rhinolophus rufous	9	33.33	3	22.22	4	44.44	2	66.67	3
Miniopterus paululus	24	75	20	25	2	50	4	91.3	18
Myotis horsefieldii	1	0	0	0	0	0	0	0	0
TOTAL	**172**	**18.97**	**13**	**12.64**	**3**	**12.07**	**2**	**39.66**	**8**

cave-dwelling bat species (i.e. *Rousettus amplexicaudatus*, *Hipposideros diadema* and *Eonycteris robusta*).

Helminth Diversity and Bat Hosts
A total of 581 individuals of gastrointestinal helminths representing six taxa were isolated from the gastrointestinal tract of the 172 bat individuals examined. This includes three trematodes, two nematodes, and one cestode.

Trematodes
Among the three helminth groups, highest prevalence and intensity of infection were observed for trematodes (Table 1). This is in accordance with other studies which reported a higher percentage of bats being infected with trematodes compared with other helminth groups (Nickel & Hansen, 1967; Blankespoor & Ulmer, 1970; Ubelaker, 1970; Coggins, 1988; Hilton & Best, 2000). Further, insectivorous bats were the only positive for trematode infection which is consistent with other studies conducted from other regions (Ubelaker, 1970; Coggins, 1988; García-Vargas *et al*., 1996; Pérez-Ponce de León, 2001). This can be attributed to the fact that insectivorous bats primarily consume insects, which are commonly recognized as intermediate hosts for trematodes (Ubelaker, 1970; García-Vargas *et al*., 1996).

Knight and Pratt (1955) described the first life cycle of a trematode with bat as a final host such that bat feces containing parasite eggs reach water sources, in which the miracidia hatch and infect freshwater snails. Cercariae shed by the latter enter a second aquatic intermediate host, usually insect larvae, and develop into metacercariae, which encyst and are infective to bats as the final host. In some cases, bats can also become infected by trematodes when a free swimming cercaria is ingested when drinking from contaminated water source (Noguiera *et al*., 2004). Thus, considering the typical pattern of digenean life cycles, the more frequently the host comes into contact with water, the higher the likelihood of infection by these helminths (Pérez-Ponce de León, 2001; Niewiadomska & Pojmanska, 2011). As such, the high prevalence of trematodes may also be due to the availability of water sources in the sampling site.

Trematodes isolated from the sampled bats in this study include *Acanthatrium* sp., *Prosthodendrium* sp., and *Plagiorchis* sp. (Fig. 2):
1. *Acanthatrium* sp.
Phylum Platyhelminthes
Class Trematoda
Family Lecithodendriidae
Genus *Acanthatrium* Faust, 1919

This genus was first described by Faust (1919) for all lecithodendriid trematodes having spines in the genital atrium and having pretesticular vittelaria. Spines are visible in the external tegument. The oral sucker is positioned at the anterior terminal end and the acetabulum positioned approximately at the middle portion of the fluke (Fig. 2A). Bulbous pharynx is also visible, leading to the intestinal ceca. Testes are entire and ovoid, located at each side of the fluke and positioned slightly posterior the acetabulum. The ovary is located on the right of the fluke and can usually be seen slightly anterior or posterior to the acetabulum.

In Japan, this trematode was also isolated from the gut of bats such as *Rhinolophus ferrumequinum* and *Myotis pruinosus* (Kifune *et al*., 2001). In Texas, a species of this genus, *Acanthatrium alicatai*, infected cave-dwelling bats such as *Myotis velifer* and *Antrozous*
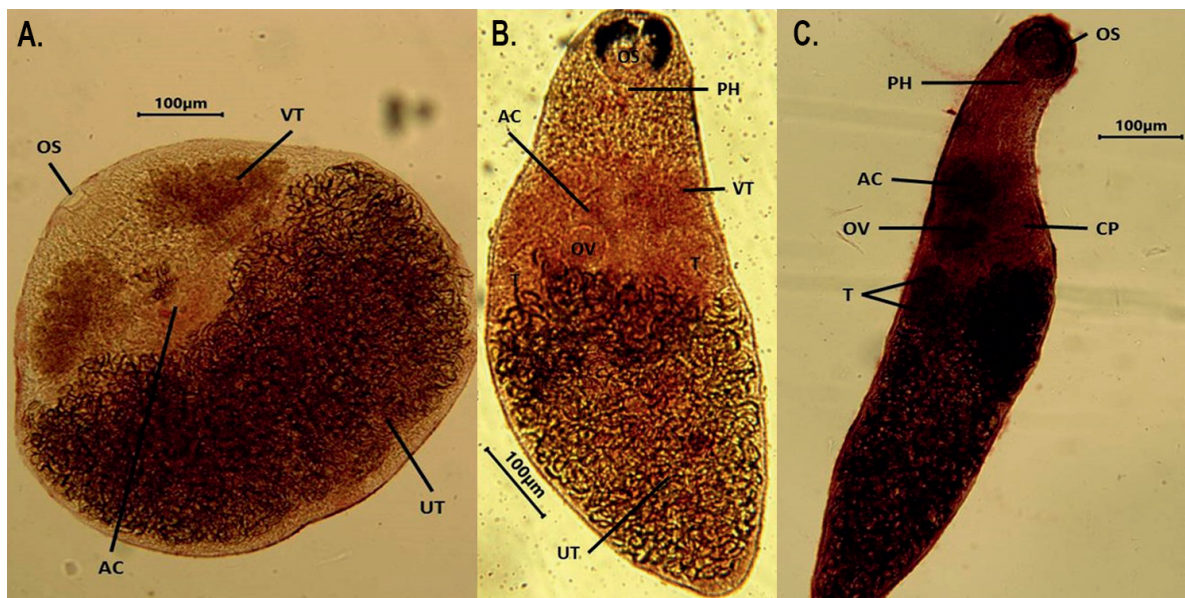
Fig. 2. Trematodes isolated from bats collected in Cavinti Underground River and Cave Complexes (CURCC), Laguna: A. *Prosthodendrium* sp. adult B. *Acanthatrium* sp. adult. C. *Plagiorchis* sp. adult. AC—Acetabulum, CP—cirrus pouch, OS—Oral Sucker, OV—Ovary, PH—Pharynx, T—Testes, UT—Uterus, VT—Vitellaria

pallidus (McAllister *et al*., 2007). In general, *Acanthatrium* transmission to bats involves cercariae developing into sporocyst inside a snail, penetrating caddisfly larvae and bats become infected by eating infected adult caddisflies (Knight & Pratt, 1955).

In this study, this lecithodendrid trematode was isolated from three species of bats sampled namely, *Miniopterus paululus, Rhinolophus rufus*, and *Rhinolophus inops* (Table 2). All three bat host species are new records for the genus *Acanthatrium*.

Sedlock *et al*. (2014) observed species of *Rhinolophus* having insects of order Trichoptera to be a part of their diet. Since Trichopteran and coleopteran insects are known intermediate hosts of *Acanthatrium*, rhinolophids have a high chance of being infected as observed in this study (Ubelaker, 1970). Meanwhile, *Miniopterus* diet consists of a wide range of insects including dipterans, hemipterans, and isopterans and to a lesser extent, lepidopterans and coleopterans which could also have permitted the infection by *Acanthatrium* (Jacobs, 1999; Ubelaker, 1970).

2. *Prosthodendrium* sp.
Phylum Platyhelminthes
Class Trematoda
Family Lecithodendriidae
Genus *Prosthodendrium* Dollfus,1931

The genus *Prosthodendrium* was established for all lecithodendriid flukes having pretesticular vitteline glands. *Prosthodendrium* can be differentiated from *Acantharium* with the former being slightly bigger and more ovoid to circular in shape, sometimes appearing to be wider than they are long. It lacks cuticular spines and the prostate mass does not overlap with the acetabulum. Ovary is located posterolateral on the right side of the acetabulum and slightly below the line of the testis (Fig. 2B).

There were records of this helminth occurring in bats in other countries. In Japan, they were isolated from gut of vespertillionids (i.e. *Myotis ikkonikovi*, *Myotis yanbarensis*, *Myotis pruinosus*, *Myotis natteri* and *Miniopterus fuscus*) and *Rhinolophus ferrumequinum* (Kifune *et al*., 2001). *Prosthodendrium oscidia* and *Prosthodendrium chilostomum* were also isolated from *Taphozous melanopogon and Pipistrellus pulveratus* in Cambodia and were previously recorded in neighboring Asian countries such as Japan, China, Taiwan, Vietnam, Arabia, India, Afghanistan, Iraq, Egypt, and Yemen (Kifune *et al*., 2001).

Hipposiderids such as *Hipposideros armiger* and *H. bicolor* were also known hosts of this trematode (Fischthal & Kuntz, 1975; Lotz, 1985). In this study, this trematode was exclusively isolated from one species, *H. diadema*, making it a new host record of this this trematode species (Table 2). A possible mode of infection of this trematode could have been ingestion of metacercariae encysted in dragon fly larvae larvae (naiads) of the Family Libeluidae (Vajrasthira & Yaemput, 1971).

3. *Plagiorchis* sp.
Phylum Platyhelminthes
Class Trematoda
Family Plagiorchiidae
Genus *Plagiorchis* Lühe, 1901

Plagiorchis is one of the largest families in the order Plagiorchiida. Its taxonomy is currently problematic because of high morphological similarity between different forms and species of this genus. In general, these trematodes have elongated body and a ventral sucker that is slightly larger or at least equal in size to the oral sucker. They also feature a long cirrus-sac along the longitudinal body axis, and the anterior margin of the vitellarium does not

Table 2. Number of bat hosts infected (% prevalence) infected by helminths identified in Cavinti Underground River and Cave Complexes (CURCC), Laguna.

Helminth Taxon	Number of hosts infected (Prevalence %)						
	R. amplexicaudatus	*P. jagori*	*H. diadema*	*H. pygmaeus*	*R. rufus*	*R. inops*	*M. paululus*
Trematodes							
Acanthatrium sp.	-	-	-	-	3 (33%)	11 (64.71%)	18 (75%)
Prosthodendrium sp.	-	-	2 (13.33%)	-	-	-	-
Nematodes							
Nycteridostrongylus	-	-	1 (6.67%)	-	1 (11.11%)	1 (5.88%)	12 (50%)
Toxocara sp.	8 (25%)	-	-	-	-	-	-
Cestodes							
Vampirolepis sp.	6 (18.18%)	5 (38.46%)	1 (6.67%)	3 (15.00%)	4 (44.44%)	-	6 (25.00%)

extend to the posterior margin of the ventral sucker (Sharpilo & Tkack, 1992). Specimens collected from this study have a short esophagus leading to bifurcating ceca that extends almost the entire length of the fluke (Fig. 2C). It has an acetabulum positioned well above the midline of the body. The ovary can be located below the acetabulum and above the two testis that are positioned either in tandem or one on top the other. The uterus when gravid fills almost the entirety of the worm with eggs.

This trematode has been reported infecting species of bats of the genus *Tadarida, Rhinolophus*, *Miniopterus*, *Myotis*, and *Pipistrellus* (Nahhas *et al*., 2005; Horvat *et al*., 2016). One species, *Plagiorchis vespertilionis*, is a common bat trematode but has been reported to infect humans (Guk *et al*., 2007). This was also recently reported for the first time in the Philippines and was isolated from the gut of mouse-eared bat (*Myotis* sp.) (Salcedo, 2021). Thus, this is the second account of this zoonotic parasite in the country. In this study, *Plagiorchis* was isolated from an individual of *Hipposideros pygmaeus*, providing a new host record for this trematode. Transmission of this trematode to bats could be due to ingestion of cercariae encysted in larvae of Ephemeria, Trichoptera, mosquitoes (*Culex*), and dragonfly nymphs (Ubelaker, 1975).

Nematodes
Although studies have shown a high degree of specificity of nematode species in bats of Suborder Microchiroptera, very little is known about their transmission dynamics and life cycle (Ubelaker, 1970; Barus & Rysavy, 1971). These parasites can either have an indirect or direct life cycle. For instance, the nematode *Litosomoides yutajensis*, a vector-transmitted filarial worm from a mormoopid bat was observed to have an indirect life cycle, with filarioids transmitted by haematophagous arthropods when feeding on the host (Anderson, 1992; Guerrero, 2006). Nematode larvae have also been recorded from blood-sucking ticks and insects (Beaver & Burgdorfer, 1984; Bain & Renz, 1993; Spratt & Nicholas, 2002). Meanwhile, *Strongylacantha glycirrhiza* which mostly infects rhinolophid bats has a direct life cycle: eggs from bat's feces are passed into surroundings and infects other intermediate hosts when third-

stage larvae penetrate the skin or are ingested (Anderson, 1992). Some species of nematode larvae also have the ability to escape from the adult female vulva by perforating the cuticle and bats making contact with each other at roosting site allows the infective larvae to penetrate the next host allowing higher intensity of infection (Ubelaker, 1970). Beetles and cockroaches also often serve as intermediate host of nematodes for other mammals (Cram, 1931; Martin, 1976).

Two nematode taxa were isolated from bats in this study:
    1. *Nycteridostrongylus* sp.
    Phylum Nematoda
Class Chromadorea
Family Trichostrongylidae
Genus *Nycteridostrongylus* Baylis, 1930

Isolated worms have a dorsal esophageal tooth at the cephalic extremity (Fig. 3A). The cephalic extremity is somewhat enclosed within a bursa. The samples also have a prominent cervical organ of fixation observed at the anterior extremity of the worms. This structure looks like an expanded flap originating from the anterior and enlarging into a bulbous flap with visible striations.

Nycteridostrongylus was first described by Baylis (1930) to be a common parasite of genus *Miniopterus*. Only three species of *Nycteridostrongylus* were described with one species, *Nycteridostrongylus uncicollis*, restricted to bats of genus *Miniopterus* (Wong and Anderson 1986). Thomas (1959) isolated this nematode in *Miniopterus blepotis* captured in Australia while Gibson *et al*. (2005) and Meszaroz (1973) reported this trematode to infect *Miniopterus schreibersii* in London and *Miniopterus fuligonosus* in Vietnam, respectively. However, no definite life cycle and transmission dynamics involving bats was established.

In this study, 44 individuals of *Nycteridostrongylus* were isolated from *Miniopterus paululus*. In addition, the occurrence of this nematode in other bat species such as *Rhinolophus rufus*, *Rhinolophus inops*, and *Hipposideros diadema* gives additional host record of this nematode aside from *Miniopterus*.
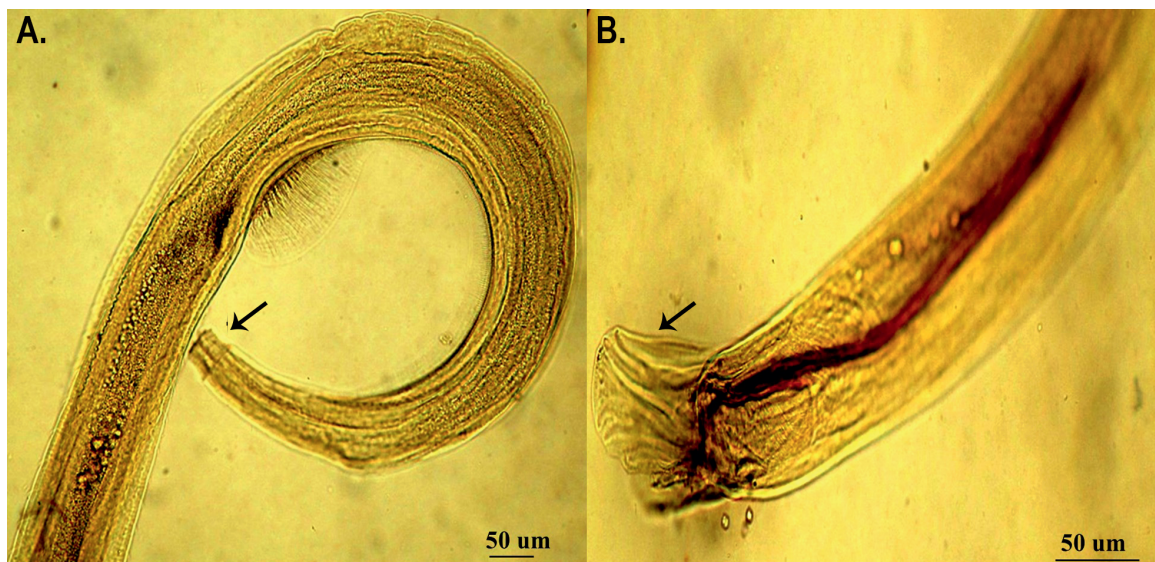
Fig. 3. Nematode *Nycteridostrongylus* sp. (A) anterior part showing cephalic head and (B) copulatory bursa.

2. *Toxocara* sp.
   Phylum Nematoda
Class Chromadorea
Family Toxocaridae
Genus *Toxocara* Werner, 1782

Isolated *Toxocara* samples were milky-white in color. Male and female *Toxocara* were morphologically distinct from each other with males having highly curved tails while the females had none (Fig. 4E). Females were also generally larger and longer with an average length of 106.60 ± 8.68 mm as compared to the males which had average length of 57.00 ± 4.9mm. The worms have three distinct lips which were observed upon close examination of the anterior end (Figs. 4A and 4C). The reproductive organs, copulatory spicule and vulva, are also visible.

Currently, 26 species of *Toxocara* are recognized, most of which are non-transmissible to humans (Ziegler & Macpherson, 2019). Of these, two species are known to infect bats: *Toxocara pearsei* known only from South and Central America with *Natalus tumidirostris* and *Peropteryx macrotis* as known recorded host, while *Toxocara pteropodis* is a known zoonotic nematode infecting flying foxes (*Pteropus* sp.) and has been recorded in Oceania, Australia, India, Indonesia, and Papua New Guinea (Ziegler & Macpherson, 2019).

Prociv (1987) has previously isolated worms similar to *T. pteropodis* from *Rousettus amplexicaudatus* specimens collected from Luzon Island, Philippines. In this study, this nematode was also exclusively isolated from *Rousettus amplexicaudatus*. Average total length measurements of male (57.00 + 4.9mm) and female (106.60 + 8.68mm) *Toxocara* samples isolated in this study were also within the range of sizes obtained by Prociv (1987). A detailed examination of eggs and preserved specimens of *Toxocara* from this study is pending to compare with *T. pteropodis.*

Cestodes
Studies on cestodes parasitizing bats are relatively few compared to other helminth classes. A number of cestode genera have been observed infecting chiropterans including *Hymenolepis*, *Taenia*, and *Oochoristica* (Prudhoe and Manger, 1969; Murai, 1976; Sawada & Harada, 1986). Coleopterans (beetles) are often involved as an intermediate host (Morgan & Hawkins, 1951; Yamaguti, 1961; Ubelaker, 1970). Low occurrence of parasitism by cestodes in bats was observed in some studies (Nogueira *et al*., 2004; Vargas *et al*., 2009; Angoma *et al*., 2020). Similarly, cestode also had the lowest infection rate among the three classes of helminths in this study. This group was represented by only one taxon:

   Vampirolepis sp.
   Phylum Platyhelminthes
Class Cestoda
Family Hymenolepididae
Genus *Vampirolepis* Spassky, 1954

Vampirolepis is closely related to the tapeworms of genus *Hymenolepis* and both belong to the family Hymenolepididae. Isolated *Vampirolepis* samples in this study have scolex with four suckers that are approximately 100 µm in diameter (Fig. 5A). Rostellum was retracted or egested with rows of hooks lining its crown. This cestode was isolated in the small intestine of the following species of bat host: *Hipposideros diadaema*, *Hipposideros pygmaeus*, *Miniopterus paululus*, *Rousettus amplexicaudatus*, *Ptenochirus jagori*, and *Rhinolophus rufus* (Table 2).

Vampirolepis has been reported parasitizing bats in countries like China, Brazil, Chile, Japan, South Africa, and Hungary (Sawada,
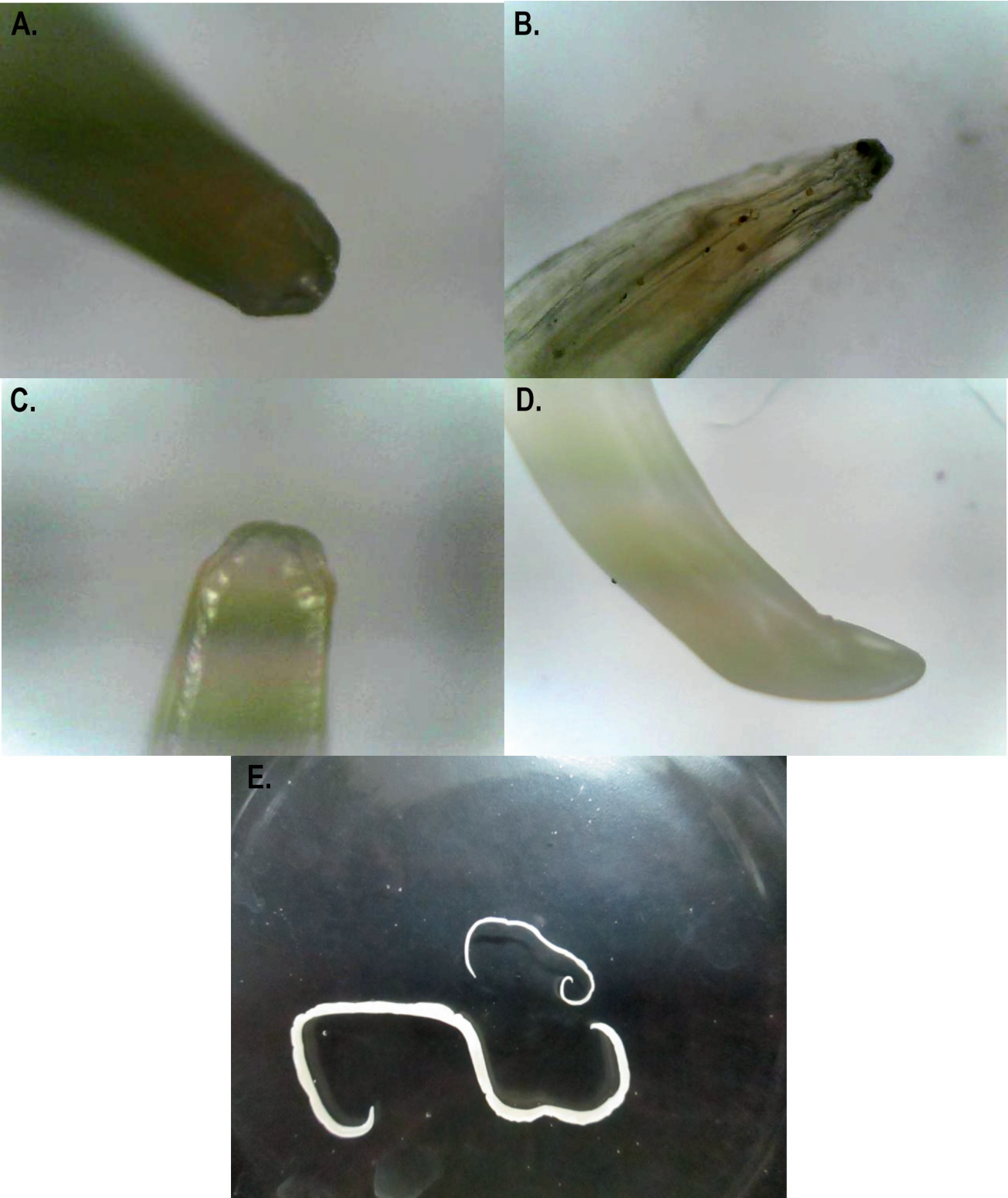
Fig. 4. Male *Toxocara* sp. (A) anterior and (B) posterior part. Female *Toxocara* sp.
(C) anterior and (D) posterior part. (E) *Toxocara* sp. adult male (top) and female (bottom).
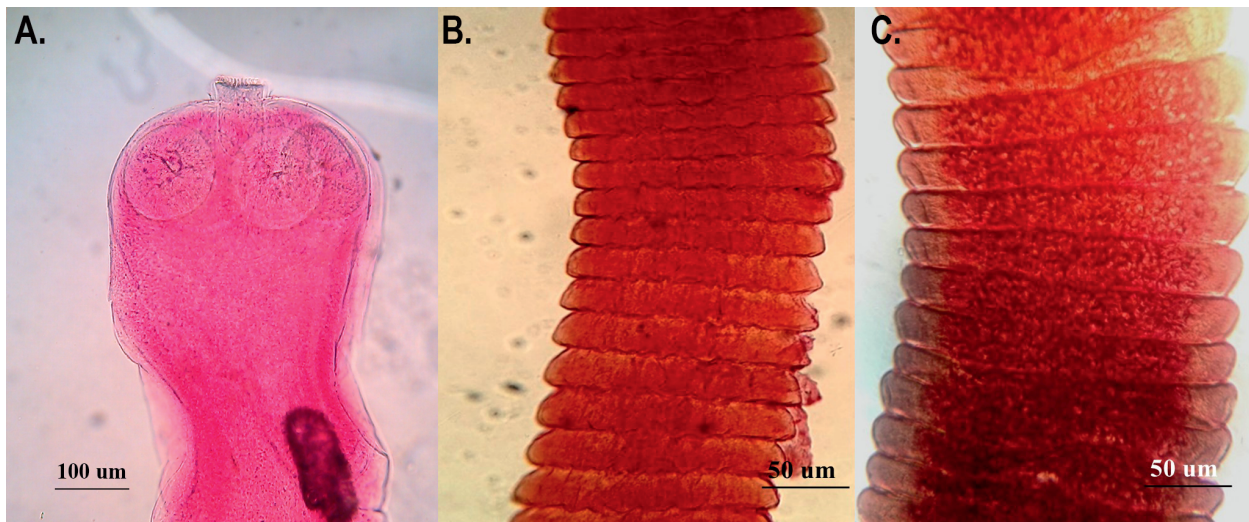
Fig. 5. Cestode *Vampirolepis* sp. scolex (A), mature segment (B), and gravid segment (C).

1970; Sawada *et al*., 1998; Noguiera *et al*., 2004; Junker *et al*., 2008; Muñoz *et al*., 2010) with mean intensity ranging from one to two adult worms per bat and prevalence ranging from one to 30 %, depending on the bat species.

Sawada (1976) hypothesized that based on the ecological standpoint of bats in caves, bat tapeworm life cycle would involve insects from bat guano as intermediate hosts. Bats may be infected by ingesting these insects that have cysticercoids. The cysticercoid will then grow in the bat's small intestine. This may explain the infection of cestodes in insectivorous cave-dwelling bats in this study (i.e. *Hipposideros diadaema*, *Hipposideros pygmaeus*, *Miniopterus paululus*, *Rhinolophus rufus*). However, frugivorous bats, *Ptenochirus jagori* and *Rousettus amplexicaudatus*, were also infected by *Vampirolepis*. Although fruits and nectars are the main dietary component of these species, some studies suggest that fruit bats may add comparatively higher protein foods, mainly insects, to their diets (Morrison, 1980; Giraldo-Martínez *et al*., 2023). For instance, Galorio and Nuñeza (2014) found traces of digested insect parts in stomachs of *Ptenochirus jagori* and *Rousettus amplexicaudatus* in the Philippines. Thus, it is possible that these frugivorous bats ingested infected insects when foraging resulting to cestode infection.

Helminth Prevalence and Intensity

In total, 82 individuals of bats were infected with helminths (P = 47.67 %) (Table 2). Trematodes infected the highest number of bats (P = 18.97 %) and had the highest MI (13 ± 1.7/infected bat), followed by nematodes (MI = 3 ± 0.3/infected bat; P = 12.64 %), while cestodes had the least MI (2 ± 0.4/infected bat; P = 12.07 %) (Table 1).

Prevalence of helminth infection was highest in *Miniopterus paululus* (91.3 %) (x^2 = 31.365, p<0.05) (Table 1). This species also had the highest MI (18 ± 4.5/infected bat), followed by *Rhinolophus inops* (MI = 6 ± 1.0/infected bat) and *H. diadema* (MI = 5 ± 0.5/infect-

ed bat) (Table 1). *Miniopterus paululus '* diet consists of a range of night flying insects such as Diptera (mosquitos and midges), *Lepidoptera, Diptera, Orthoptera*, *Coleoptera, Hemiptera,* and *Hymenoptera* species. It is estimated this bat can consume about 25 % of its 15g average body weight per meal and may eat two or three meals per night (SWIFFT 2007). Therefore, a more prevalent and higher intensity of helminth infection is expected for these bats with a diverse diet, as there is an increased likelihood of ingesting an infected prey item (Holmes 1964; Phillips 1966).

Notably, no helminths were isolated from the 20 individuals of *Hipposideros lekaguli* captured. Further, the following bat species did not harbor any helminth: *Haplonycteris fischerii, Eonycteris robusta, Rhinolophus arcuatus* and *Myotis horsfieldii*; Note however that these species have low sample sizes (1 – 2 individuals), thus, it may not be conclusive to say that they are free of helminth parasites.

Although majority of bats investigated harbored only one helminth taxon, 13 individuals were infected with two or more helminth class: two individuals co-infected by trematode and cestode, seven with trematode and nematode, two with nematode and cestode, and two individuals with nematode, trematode and cestode. This suggests that the helminths observed in these bat hosts can suitably grow, develop, and coexist with other helminths. Multiple infection is often a result of decreased immune response in hosts caused by infection of one species of parasite, which can eventually facilitate infection by other species of parasite (Behnke, 2008). However, most bat species are known to be dietary specialists and thus do not get infected with two or more species or class of helminth (Kunz, 1973; Hilton & Best, 2000).

Helminth prevalence and intensity in relation with host characteristics
Sex
Data showed a significantly higher number of infected male indi-

Table 3. Prevalence and mean intensity of helminths from bats collected in Cavinti Underground River and Cave Complex (CURCC), Cavinti, Laguna with respect to host's sex, diet, age, and roosting site.

Host Characteristic		n	Prevalence (%)	Mean Intensity (# of parasites/bat)	p-value Prevalence	p-value Mean Intensity
Sex	Male	91	49.5	4.0	0.032*	0.231
	Female	81	33.3	16.0		
Diet	Fruit bats	64	26.6	3.3	0.019*	0.006*
	Insectivorous bats	108	44.4	9.9		
Roosting Site	Cave-dwelling	141	41.8	8.9	0.019*	0.009*
	Non-cave dwelling	31	19.4	1.7		
Age	Sub-adult	43	37.2	3.5	0.653	0.171
	Adult	129	41.1	9.1		

* significant value at α=0.05 using Chi-square test (prevalence) and Mann-Whitney U-test

viduals (49.50 %) than female (33.30 %) and that these values are statistically significant ($x^2 = 4.574$, p = 0.032) (Table 3). On the other hand, intensity of infection was higher in female bats (MI = 16±0.1/ infected bat) than males (MI = 4±1.3/ infected bat), although these values were not significantly different between sexes (U = 3149.5, p = 0.231).

Many studies report the influence of host sex on parasite prevalence, intensity, and aggregated distribution (Zuk & McKean, 1996; Poulin, 1996; Lord et al., 2012; Giraldo-Martínez et al., 2023). Male-biased infection of helminths are frequently observed in mammals and other vertebrate groups (Klein, 2004). This is due to sex hormones, mainly testosterone, which may have beneficial effects on helminth development (Haukisalmi et al., 1988). Testosterone could depress both cell mediated and humoral immune responses resulting in higher susceptibility to parasitism in males (Grossman, 1989). Female hosts meanwhile have sex hormone estrogen which was reported to increase hosts' resistance to helminth infection by enhancing humoral immunity and inhibiting cell mediated responses (Klein ,2004).

Helminth intensity has no significant difference between the two sexes which is consistent with previous studies on parasite assemblages of small mammals like bats (Esteban et al., 2001; Lord et al., 2012) and rodents (Feliu et al., 2006). However, some studies report higher intensity and prevalence of helminth infection in other female mammalian hosts: *Trichostryonglyus retortaeformis* in rabbits, *Schistosoma mansoni* and *Taenia crassiceps* in mice, and *Hymenolepis nana* in rats (Klein, 2004).

Diet
Our result showed that insectivorous bats have significantly higher helminth prevalence compared to fruit bats (x^2 = 5.503, p = 0.019) (Table 3). Insectivorous bats also have a significantly higher number of helminths harbored for each infected bat than frugivores (U = 2550.00, p = 0.006).
Since helminths are typically acquired through the food that bats

consume (Holmes, 1964; Phillips, 1966), insectivorous bats may experience more prevalent and higher MI of helminth infections due to their insect diet, which serve as intermediate hosts for many helminth species. Likewise, since fruit bats generally eat fruits and nectar, there is a lower possibility of being infected by helminths. Fruit bats observed in this study only harbored cestodes and nematodes. This suggests that fruit bats may consume insects as a part of their diets or get infected through the incidental ingestion of intermediate hosts. Frugivorous bats have long been observed to consume insects as a source of protein to supplement their diet (Gardner, 1977; Thomas, 1984; Giraldo-Martínez et al., 2023), which could explain infection by helminths.

Roosting behavior
Cave-dwelling bats have a significantly higher number of infected individuals compared to non-cave dwelling bats (x^2 = 5.467, p = 0.019). Mann-Whitney U-test meanwhile showed significant difference in MI between cave-dwelling and non-cave dwelling bats (U = 1505.5, p = 0.009).

This is the first study to compare helminth assemblages between cave and non-cave dwelling bats. Cave-dwelling bats generally have relatively larger population size, higher degree of clumping, and frequently interact with other cave-dwelling bat species compared to non-cave dwelling bats making them more vulnerable to parasite infection. Host colony or group size and roosting behavior of bats have been shown to influence density of infection of ectoparasites, resulting in increased prevalence and intensity of infection with increase in host group size (Ter Hofstede & Fenton, 2005). Grooming behavior might be a potential transmission method for helminths in cave-dwelling bats. Helminth eggs left on their bodies during defecation could be ingested when the bats groom themselves. In addition, since some helminths were also hypothesized to have cave arthropods as intermediate hosts, cave-dwelling bats have a higher chance of being infected with helminths (Sawada, 1976). Lastly, some species of nematode larvae that are

released when eggs from bat roosts hatch, may penetrate the skin of bats when they come into contact, making cave roosting bats more vulnerable to infection (Ubelaker, 1970).

Age

Age class of sampled bats was classified as juvenile, subadult, and adult. However, no juvenile bats were collected in this study. Data showed that helminth prevalence and MI between the two age classes has no significant difference (x^2 = 0.202, p = 0.653 and U = 2339.00, p = 0.171, respectively) It is possible that similarities in the habits and roosting locations of both age class for each species may account for the similarities in their infection rates since there are similar opportunities for exposure to pathogens.

Although not statistically significant, helminth infection was observed to be slightly more prevalent in adult bats (P = 41.1 %, MI = 9±0.9/infected bat) than sub-adult bats (P = 37.2 %, MI = 4±0.4/infected bat. Previous studies have also shown higher infection rates in adult bats compared to younger bats owing to the increased foraging efficiency of older bats (Hamilton & Barclay, 1998; Adams & Pedersen, 2000; Lord *et al*., 2012). Further, adult bats have a wider range of diet including hard-bodied prey items, so a higher diversity of helminth infection is expected, compared to younger bats whose diet is limited to smooth-bodied insects or milk (Aguirre *et al*., 2003). Future studies could increase the sample size for each species to reveal more distinct patterns or variations in infection rates between subadult and adult bats.

Bat Conservation and Public Health Implications

Available information on helminth parasites of bats in the Philippines are very scarce, despite their potential adverse effects to both human and bat populations. Although this study was limited by sample size for some of the bat species, limited locality, and identification of helminths to genus level, our data greatly contributes to efforts in determining helminth fauna parasitizing bats in the Philippines. Based on previous studies, our work increases the overall number of bat species in the Philippines with at least one report of a parasitic helminth from four species to 10 species. This data will help us expand the amount of information available on bat helminth parasites for future research as well as provide additional conservation measures for endemic bat species.

Of the helminths identified in this study, the trematode *Plagiorchis* sp. and nematode *Toxocara* sp. are of public health importance because of their zoonotic potential. This is the second account of both helminths in Luzon Island, Philippines: *Plagiorchis* was recently observed by Salcedo (2021) from individuals of *Myotis* sp. whereas nematodes with close resemblance to the zoonotic *Toxocara pteropodis* was isolated by Prociv (1987) from museum specimens of *Rousettus amplexicaudatus*. As the study site is frequently visited by tourists, it would be advisable to implement precautionary health measures to avoid infection. Further, detailed molecular and epidemiologic studies are needed to ascertain the identity of the collected helminths, evaluate their pathology and

transmission dynamics, and provide additional information on how they influence bat's health and physiology.

Further research on understanding interactions between bats, their helminth community, and their environment and to identify potential impacts of helminths and other parasites on bat health and behavior is crucial for bat conservation. While bats may remain either asymptomatic or unaffected and their parasites may not be fatal, various environmental disturbances may serve as stressors and may pose threats to bat hosts that have weakened immune systems which are more likely to suffer due to severe effects of parasitism (Allen *et al*., 2009; He *et al*., 2021). Anthropogenic impacts such as land use change, biodiversity loss, pollution, etc. could be detrimental to the overall health and behavior of bat populations. These disruptions may also contribute to the transmission of pathogens to other regions or species, a phenomenon known as wildlife disease spillover. Therefore, it is imperative to implement preventive measures aimed at safeguarding not only bats but wildlife species in general. An integrated approach to investigating transmission dynamics in bat host populations and understanding the synergistic effects of disturbances and parasitism could lead to a more in-depth understanding of altered parasite dynamics in a rapidly changing world that may have implications to ecosystem health and public health.

Conflict of Interest

The authors declare that they have no conflict of interest.

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