

## **GUANO ARTHROPOD ASSEMBLAGES IN CAVES OF MABINI, PANGASINAN (PHILIPPINES)<sup>1</sup>**

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### **ABSTRACT**

This study documented the guano arthropod communities inside Cacapangan, Timori, Santo Rosario and Cabalyorisa Caves in Mabini, Pangasinan in terms of taxonomic composition, density, and distribution within guano deposits. Several types of guano with their unique assemblages of associated arthropods were observed. Even the same type of guano supported different assemblages in different caves. A total of 42 morphospecies belonging to at least six classes were recorded, including three possible new cave-restricted species – a tarantula from Timori Cave (*Orphnaecus* sp.), troglomorphic cockroach from Santo Rosario Cave (*Nocticola* sp.), and a troglomorphic millipede found in all four caves (Haplodesmidae). Swiftlet guano deposits were found to contain more arthropod taxa than bat guano. It was also found that the abundance of arthropods decreased with guano depth, but taxon richness did not significantly differ with depth.

**Key words:** arthropod assemblages, guano, limestone caves, Pangasinan

### **INTRODUCTION**

Guano pertains to the accumulation of egested wastes (e.g., feces, urine, or urates) of particular animals, which is rich in inorganic and organic nutrients (Hutchinson, 1950 as cited by Iskali & Zhang, 2015; Gnaspini, 2012). In Philippine caves, guano is usually deposited by roosting bats (order Chiroptera), although nesting swiftlets (family Apodidae) can also deposit significant quantities. Worldwide, bats represent the most important source of guano in caves (Moulds, 2004; Gnaspini, 2012).

According to Gnaspini (2012), guano-dwelling arthropod communities are simple and are composed mainly of two trophic levels: the guanophages (animals that directly feed on guano and/or on microorganisms that grow on guano) and their predators. However, he noted that trophic relations are complex since predators may feed on several guanophages and even on some predators.

Taxonomically, guano communities world-wide would almost always include mites (laelapid, macrochelid, oribatid, and uropodid), springtails (entomobryid, hypogastrurid, and isotomid), moth larvae (tineid), pseudoscorpions (chernetid), beetles (carabid, cholevine, histerid, and staphylinid) and flies (drosophilid, fanniid, milichiid, and phorid); although flatworms, earthworms, other pseudoscorpions, harvestmen, spiders, isopods, millipedes, centipedes, psocopterans, cockroaches, crickets, and other beetles and flies are also frequently observed (Gnaspini, 2012). Of these taxa, mites and springtails are the most species-rich in guano piles (Gnaspini, 2012).

In the Philippines, arthropod taxa so far documented to inhabit guano deposits inside caves include 19 species of mites (see Corpuz-Raros & Lit, 2015). Also, bat guano deposits in the main chamber of Bulalon Cave in Polillo Island are dominated by the guanobitic cockroach *Pycnoscelus striatus* (Kirby) (Lucañas et al., 2015; Lucañas & Lit, 2016). On the other hand, Lagare & Nuñez (2013) reported the raphidophorid cricket, *Macropathus* sp., to be associated with abundant guano deposits in caves in Mindanao.

Whereas each of the studies cited above inventoried only one specific taxonomic group found in caves, this present work describes the guano arthropod communities or assemblages in selected caves, looking into some ecological parameters already studied in other caves in the world but not yet locally.

Several factors influence the abundance, richness, and composition of arthropods in guano deposits in caves. These can be classified into macro-factors and internal guano characteristics. Macro-factors include the distance from the entrance, size of deposits, and depth of deposits. Internal guano characteristics include the type of guano (based on what animal source), age of deposits, moisture content, pH, nutrient content (N, P, and K) and organic content. Because of these factors, several distinct faunal assemblages are known to inhabit different microenvironments within a guano deposit, as well as different guano deposits within the same cave (Ferreira et al., 2000; Gnaspini, 2012). On top of these, different caves in different faunal regions and different parent materials (i.e., cave lithology) usually have distinct faunal assemblages (Culver & Pipan, 2009; Ferreira et al., 2000).

Ferreira et al. (2000) reported that larger contiguous guano patches support more microhabitats and, therefore, higher diversity than smaller patches in Lavoura Cave in Brazil. Also, fresher guano deposits supported greater diversity than older deposits (Ferreira et al., 2000). Iskali & Zhang (2015) found that macroinvertebrate abundance decreased with depth within the guano, and macroinvertebrate richness was significantly higher near the cave entrance in Bracken Cave, Texas.

This present study aimed to describe the types of guano habitats in selected caves in terms of physical characters and taxonomic composition of their supported arthropod assemblages. The effect of depth within guano deposits to inhabiting arthropods was also studied.

## MATERIALS AND METHODS

### Study Sites

Four caves were selected as study sites (Table 1) from the 50 known limestone caves in the town of Mabini in the western district of Pangasinan, Luzon Island. Cacupangan and Santo Rosario caves are known tourist caves

**Table 1.** Summary of the characters of the four caves selected as sites.

Cave	Location (Barangay)	Cave system <sup>a</sup>	Roosting bats <sup>b</sup>	Nesting swiftlets <sup>b</sup>	Number of sites
Cacupangan	Tagudin	CCS	++	-	1
Timori	Villacorta	CCS	++	-	2
Santo Rosario	San Pedro	SRCS	-	+	1
Cabalyorisa	Barlo	SRCS	++	+	2

<sup>a</sup>CCS = Cacupangan Cave System, SRCS = Santo Rosario Cave System

<sup>b</sup>Initial assessment of abundance: (-) absent, (+) present, (++) more than 10 individuals

whereas Cabalyorisa and Timori are described by locals to be seldom visited.

Cabalyorisa and Timori had intact guano deposits. However, not all of these guano deposits were examined in the study to prevent excessive disturbance. Instead, areas covering approximately 2.0 m x 5.0 m were used as sampling sites for the arthropod study.

Two sites each were established within the main chambers of these caves to account for variation within a cave chamber. The sites in Timori have thick patches of bat guano in potholes (bowl-like depressions on the limestone floor). Only dry guano (both from bats and swiftlets) was assessed for arthropod density and taxon density due to the difficulty of sifting wet guano. The second site in Cabalyorisa is like those in Timori with thick patches of bat guano in potholes. The first site in Cabalyorisa, on the other hand, only had a thin layer (~1 cm deep) of bat guano plus patches (20-30 cm in diameter) of swiftlet guano underneath active nests.

Santo Rosario and Cacupangan, on the contrary, only have patches (20-30 cm in diameter) of swiftlet guano and thin (~1.5 cm deep) bat guano deposits, respectively, so only one sampling site each was done in these caves.

### Cabalyorisa Cave (N 16° 00' 23.45", E 119° 56' 24.60")

Spelled also as Caballareza or Cabalyarisa, the cave spans 420 m (Rendon, 2011) and has a wide entrance. The entrance is on the side of a hill with dense vegetation, facing a rice field. It is in Barangay Barlo, about seven km south of the Poblacion of Mabini. The cave is the upstream-most entrance of the Santo Rosario Cave System. The first site was located approximately 110 m into the cave, at the start of the first chamber. The second site was 20 m further at the end of the chamber. The first site had an average air temperature of 26.2°C

whereas the second had 24.9°C (air temperature measured using a thermo-hygrometer: Traceable® 4040. Accuracy:  $\pm 1$  °C. SD = 1.38 °C). Relative humidity in both sites also varied during the six-month field study period, suggesting that both sites were part of the transition zone of the cave characterized by complete darkness but still fluctuating air temperature and relative humidity (Poulson & White, 1969; Culver & Pipan, 2009).

### **Timori Cave (N 16° 02' 44.7", E 119° 58' 19.6")**

Timori Cave, known to locals as Villacorta Cave, has the largest entrance among the four caves, measuring some 10 m high and 11 m wide. It is also located on the side of a karst outcrop with dense vegetation and facing a rice field. The cave has a perennial stream running its length which starts from seven m into the cave. The stream increases and decreases in width and depth, reaching up to 2.5 m (DENR RCC-R1, 2009 as cited by Rendon, 2011). The first site was located 650 m from the entrance, on the right side of the second dry chamber. The second site was located about six m further in, also on the right side of the chamber. These two sites had the highest average air temperatures of all sites (27.6 and 27.4°C, respectively), and had relative humidity measurements >90% during all visits (relative humidity measured using Traceable® 4040. Accuracy:  $\pm 5\%$ ). Considering the distance from the nearest entrance, stable air temperature, and constantly high relative humidity, it can be said that they are part of the dark zone of the cave (Poulson & White, 1969; Culver & Pipan, 2009).

### **Santo Rosario Cave (N 16° 00' 34.2", E 119° 56' 18.4", 99 masl)**

Santo Rosario Cave is located just nine m from a one-lane concrete barangay road. The area around the cave is shaded by tall trees but has already been modified into a grotto (i.e., religious pilgrimage or tourist site) with a concrete rest table for visitors. The cave was already mined of its guano deposits in the 1960s (Rendon, 2011) and is open to local government-regulated cave visitation. It has a small entrance which has been installed with a bat-friendly gate in 2008, the key to which is kept by barangay officials (Rendon, 2011). The site was located just 11 m from the entrance, in a branch to the right of the main passage, called the Bedroom. It had an average air temperature of 25.7°C and fluctuating relative humidity averaging 86.6%, and is part of the transition zone of the cave.

### **Cacupangan Cave (N 16° 03' 10.3", E 119° 57' 33.9")**

Cacupangan Cave is the best known among the caves in Mabini. It is a long cave with a perennial stream running its length. It has easily navigable passages with high ceilings, several large chambers, and interesting formations. These characters make it an ideal tourist cave. The surface landowner has developed the area around the cave entrance into a resort with concrete pathways, cottages, comfort rooms, and a pipe channeling some of the water from the cave

into a shower. The area still has ample shade from tall trees in the resort. The site in Cacupangan Cave was located 600 m from the entrance, in an area called Alcove 2. Average air temperature in the site measured 26.8°C while relative humidity was always above 88%, suggesting that it was also part of the dark zone already.

Santo Rosario and Cabalyorisa are related as they are part of the Santo Rosario Cave System (SRCS), which also include Bual Cave and Tubo-tubo Cave (Rendon, 2011). Cacupangan and Timori, on the other hand, are part of the Cacupangan Cave System (CCS) which also includes Binmatya Cave, Branch 3, and Bintanilya Cave (Rendon, 2011). Cabalyorisa and Timmori, the relatively undisturbed caves, are also similar in that they are the upstream-most ends of their respective cave systems, and their entrances are on hillsides, covered with natural vegetation and facing rice paddies.

Site codes used in succeeding sections of the paper to refer to the cave sites: Cacupangan (Cac), Timori sites 1 and 2 (Tim1 & Tim2), Santo Rosario (Sto), Cabalyorisa sites 1 and 2 (Cab1 & Cab2)

Preliminary visits to the caves were done on August 16 and 31, 2015. Monthly visits were done between 9 a.m. and 4 p.m. on the following dates: October 10 to 12, 2015; November 14 and 15, 2015; December 12 and 13, 2015; January 9 and 10, 2016; February 13 and 14, 2016; and March 12 and 13, 2016. No more insect samples were collected in March.

## **Taxon Richness and Abundance**

Taxon richness refers to the number of distinct taxa of the same taxonomic level in a defined space of habitat at a particular time. This is an important gauge of biological diversity and has conventionally been measured at the level of biological species (Gaston, 2000). However, the reporting of higher-taxa identification further sorted into tentative morphologically distinguishable species (= morphospecies) in place of biological species, still has value especially in cases of poorly-studied taxonomic groups (Oliver & Beattie, 1996; Krell, 2004), and has been recommended for the purpose of rapid biodiversity assessment (Kerr et al., 2000; Encinares & Lit, 2014). In these cases, properly preserved voucher specimens should be deposited to reputable repositories allowing for follow-on taxonomic studies (e.g. Lucañas et al., 2015).

For this study, morphospecies was used to measure species richness instead of biological species for interest of time. The author did the sorting to morphospecies, after which sample specimens or their photographs were forwarded to reputable taxonomists for tentative identification. Hence, the reported species count should be taken with a grain of salt. The list of taxonomists consulted for tentative specimen identification can be found in the Acknowledgement section of this paper. Nonetheless, samples collected in this study were deposited in the Entomology Section of the UPLB Museum of Natural History.

Also, taxon richness was determined per unit volume of guano substrate. Thus, it can also be referred to as Taxon Density. Arthropod abundance, on the

other hand, refers to the cumulative number of all arthropod individuals present in a defined space of habitat. This is also Arthropod Density. Determining the abundance of organisms is important as it identifies taxa that are rare or abundant in a community. The relative abundance of different taxa present in a community is the other variable that defines biological diversity. For this study, arthropod abundance was also measured per unit volume of guano.

To determine taxon richness and arthropod abundance, guano substrate was collected using a hand trowel. Iskali & Zhang (2015) found a decrease in macroinvertebrate (all of which were arthropods) abundance with increasing guano depth in Bracken Cave, in Texas. The present study determined if there are greater macroarthropod abundance and taxon richness in top guano layers in the cave sites.

A plastic frame (10 cm x 5.0 cm) was used as a guide to collect approximately 100 cubic centimeters (cc) of guano at two sampling layers (top 2.0 cm and next 2.0 cm). This 2-cm depth was based on preliminary data collected in August 2015 from Timori Cave that established that guano piles had an average depth of 3.2 cm and a standard deviation of 2.0 cm. Only a top layer was collected in patches not exceeding 2.0 cm, in which case, counts for the bottom layer was considered missing data.

The five-month guano sampling only collected complete sets of samples from Tim1, Tim2, and Cab2. There were six unavailable bottom samples from Cac, 10 from Sto and four from Cab1.

The collected guano were placed in separate Zip-lock® bags and transported to the laboratory for processing and examination. Three guano samples were randomly collected from each cave site per month when possible. However, limited guano deposits in Sto, Cac, and Cab1 prevented the collection of bottom layer samples in some months whereas the fewer swiftlet guano patches in Sto also limited randomization.

For this study, a standard testing sieve (Advantech®, No.18, 1.00 mm) was used to extract macroarthropods while microarthropods were hand-picked from a 2.5 cc subsample of the sifted guano under a dissecting microscope at 20x magnification. Each guano subsample was suspended in 70% ethanol in a Petri plate for the examination. Five minutes were allotted for picking macroarthropods from the sieve while 10 minutes were allotted for picking under the microscope. A piece of paper with 1-cm grids was set at the base of the Petri plate to serve as a guide for the thorough and systematic examination of the guano subsamples under the microscope.

Collected arthropods were sorted into morphospecies, identified to lowest taxonomic rank possible (at least order level for most specimens), counted, and preserved in micro-centrifuge tubes with 70% ethanol. Some specimens were mounted on glass slides using modified Hoyer's medium to allow examination under a microscope and better identification. The count data of the macro- and microarthropods were listed and analyzed separately to get around possible errors if counts are extrapolated and unified to the same volume unit.

The comparison of arthropod abundance and taxon richness between guano layers is only made for Tim1, Tim2, and Cab2, while comparisons between the six sites were made considering only their top guano layers.

### **Supplementary Macroarthropod Collection**

Supplementary visual searching and opportunistic (i.e., manual) collection of cave arthropods (megafauna or macroarthropods) were also done in each cave site every visit to get a more comprehensive listing of arthropod taxa present in the caves. Cave insect collection studies have recommended visual searching and the manual collection as a good supplementary method for a better cave faunal inventory (see Weinstein & Slaney, 1995, and Encinares & Lit, 2014). Organisms present each month were noted and samples of newly observed species were taken for identification purposes. Five minutes were allotted for this procedure at every site per month.

This collection and sample processing procedure was based on the work of Iskali & Zhang (2015).

### **Feeding Relations and other Ecological Observations**

Feeding relations, the occurrence of mating, life stages present, and specific location of different guano-dwelling arthropods were also observed and described during each cave visit. Photo-documentation of cave organisms was also conducted by cave guides, especially Mr. Guillermo R. Rendon, of the Balincaguin Conservancy.

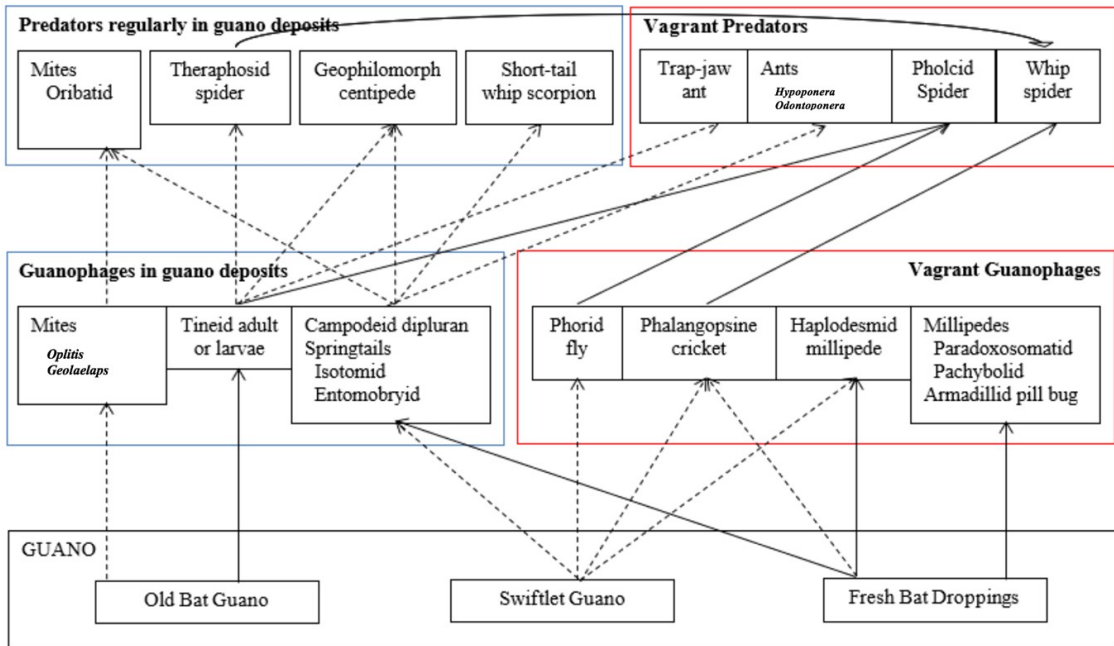
All in situ ecological information were discussed in the text, and a food web (Figure 1) was also constructed to better visualize the trophic interactions inferred in the guano community.

### **Statistical Analyses**

Friedman Rank Sum Test was used to check for any temporal effects on all response variables.

Arthropod taxon richness and abundance were compared statistically between the top 2-cm layer vs. the bottom 2-cm layer from Tim1, Tim2, and Cab2. The data were first tested for normality using the Shapiro-Wilk Test. Separate one-way paired *t*-tests (for normally-distributed data) or Wilcoxon Signed Rank Tests (for non-normal data) were done for the two response variables (i.e., taxon richness and arthropod abundance). This was also done separately for the macro- and microarthropod data, and separately per cave site.

Results were then checked for consistency across all sites. Consistent test results imply a low effect of site on taxon richness and abundance. Differences in the test results between sites for the response variables, on the other hand, may reflect significant site influence on the difference between the top and bottom guano layers.



**Figure 1.** Consolidated guano-based arthropod food web inside Mabini Caves, Pangasinan. Arrows indicate direction of energy flow. Solid lines indicate actual observations while dashed lines indicate assumed trophic interactions based on literature and consultation with taxonomists.

Any occurrence of difference was confirmed using *F*-test (for normally-distributed data) or Kruskal-Wallis Rank Sum Test (for non-normal data). This was done per layer across sites. Furthermore, Friedman Rank Sum Test was also used to check for any site effect on the response variables across all samples.

All statistical tests were performed in Statistical Tool for Agricultural Research (STAR® ver 2.0.1.), a freeware developed by the Biometrics and Breeding Informatics of the International Rice Research Institute (IRRI).  $\alpha$  was always set at 0.05 for all tests performed, as standard in science.

## RESULTS AND DISCUSSION

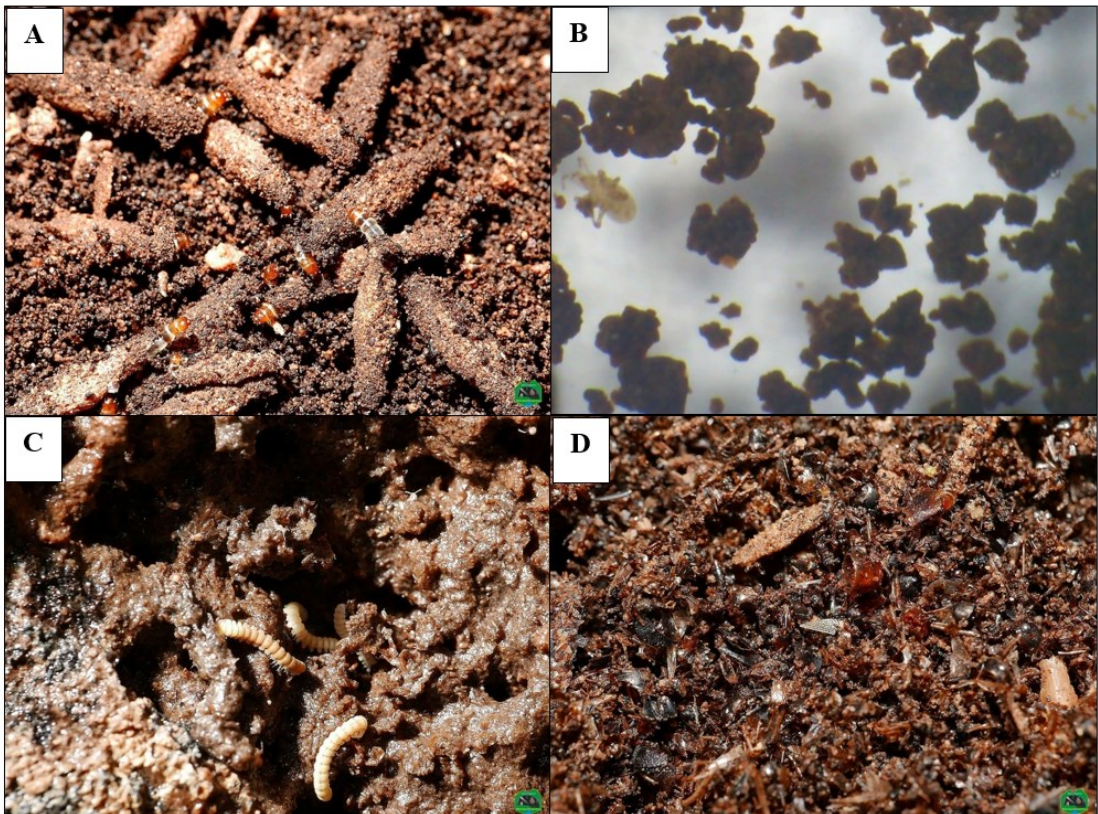
### Types of Guano

Differences were observed in the texture and composition between bat and swiftlet, fresh and old, and dry and wet guano. Fresh insectivorous bat droppings appear as small black pellets. Fresh swiftlet droppings appear as splattered wet black pasty material usually with white portions (uric acid). Swiftlet droppings underneath nests are usually mixed with feathers and plant-based nesting materials also.



Older insectivorous bat guano is finely granulated (Figure 2A), like that described by Gnaspini (2012). It appears as small dark-brown balls or aggregations of organic mud-like material and limestone bits under the microscope (Figure 2B). Old swiftlet guano is made of coarser and more distinguishable materials, i.e., fragments of insect exoskeleton (Figure 2D). Under the microscope, fragments of exoskeleton, insect mandibles, hollow leg segments, ant, hemipteran and beetle head capsules, and beetle elytra were identified. These are dry and hollow materials that are easily blown by a breeze.

Bat guano under dripping water and around its splatter area are clayey (Figure 2C). They are compact, unlike dry bat guano which is loose and powdery. Arthropods were observed to burrow in dry guano (geophilomorph centipede and theraphosid spider) while arthropods were observed only on the surface of wet compact bat guano (haplodesmid millipede and scarabaeid beetle). Aside from being more compact, nutrients are expected to be washed away more easily in



**Figure 2.** Finely granulated old bat guano with several guano moth larvae in their cases (A), view of bat guano particles under the microscope with one laelapid Mite on the left side (B), wet bat guano with haplodesmid millipedes and several sminthurid springtails (C), and fragments of insect exoskeleton making up old swiftlet guano (D). Photos A, C and D by G.R. Rendon.

guano under dripping water compared to dry guano thereby supporting fewer fauna (Gnaspini, 2012).

Dry powdery bat guano was characteristic of Cab2, Tim1 and Tim2, although thin deposits were also present in Cab1 and Cac. In all of these sites, guano moth larvae were always the most dominant in all months by unaided visual inspection. Wet bat guano deposits were observed especially in Cac and Tim1. The most dominant organisms in wet guano were small white haploidesmid millipedes. However, the second most dominant organism varies between sites. In Cac, the haploidesmid is associated with armadillid pill bugs, whereas in Tim1, it is associated with the scarabaeid beetle, *Canthon* sp.

Swiftlet guano was characteristic of Sto and Cab1. They were in patches directly below swiftlet nests, whether active or abandoned. Guano moth larvae were the most dominant in swiftlet guano, followed by haploidesmid millipedes.

Fresh bat droppings were observed in all cave sites. They were unevenly scattered over the cave floor and on rocks but appeared mainly under bat roosts and flyways.

Overall, three different kinds of guano were observed in the cave sites with their distinct assemblages of associated arthropods. These differences were pronounced even when different patches were just meters away from one another. Hence, guano type is an important micro-environmental factor influencing arthropod community composition in Mabini caves.

## **Arthropod Abundance and Taxon Richness in Guano Patches**

### **Macroarthropods**

Appendix Table 1 summarizes the counts of all macroarthropods collected from the guano samples. A total of 4,439 individuals belonging to five classes and 11 macroarthropod morphospecies were collected from the guano samples.

The six sites had different numbers of macroarthropod taxa ranging from two in Tim1 to seven in Cac. However, Friedman Rank Sum Test revealed no significant differences ( $P = 0.5512$ ) among the taxon densities of the six sites. The most abundant taxon is the guano moth larvae (Tineidae), with an average density of 39.5 individuals per 100 cc of guano sample. In Tim1, Tim2 and Cab2, tineid larvae had a density of 56.6 individuals per 100 cc of guano sample. About 94.7% of all macroarthropod individuals collected were tineid larvae. The second and third most abundant taxa are tenebrionid larvae and dipteran larvae, which were observed to occur in four and five cave sites, respectively.

The top 2.0 cm of guano was observed to have more macroarthropod individuals than the succeeding 2.0 cm in Tim1, Tim2 and Cab2 (Wilcoxon Signed Rank Test:  $P_{Tim1}=0.0005$ ,  $P_{Tim2}=0.0004$ ,  $P_{Cab2}=0.0000$ ) similar to the findings of Iskali & Zhang (2015). However, the top layer did not have more macroarthropod taxa (Wilcoxon Signed Rank Test: Tim1 = no difference between layers,  $P_{Tim2}=0.5464$ ,  $P_{Cab2}=0.6817$ ).

## Microarthropods

A total of 336 microarthropod individuals belonging to three classes and 18 morphospecies were collected from the guano sub-samples examined (see Appendix Table 2). Samples from Sto and Cab1 recorded the most microarthropod taxa with 10 morphospecies each, suggesting that swiftlet guano piles may host more microarthropod taxa than bat guano piles. However, Friedman Rank Sum Test did not detect any statistical difference across sites ( $P=0.0586$ ). Cab2 was the poorest site, taxon-wise, with only three morphospecies recorded. Microarthropod richness also did not differ significantly during the five-month study period (Friedman Rank Sum Test:  $P=0.1706$ ).

Comparing the arthropod density between the guano layers in the three guano-rich caves, a greater density on the top layer was present only in Tim2 ( $P=0.0042$ ) as compared to Tim1 ( $P=0.0945$ ) and Cab2 ( $P=0.0837$ ). Kruskal-Wallis Rank Sum Test, however, did not confirm any site-effect on microarthropod density in both the top ( $P=0.1978$ ) and bottom layers ( $P=0.6925$ ), suggesting that the significantly higher micro-arthropod density in Tim2 top guano layer was likely just incidental.

The most abundant microarthropod taxa are isotomid springtail, laelapid mites and *Oplitis* mites, and they were all present in all four caves studied. Unlike the findings of Iskali and Zhang (2015) wherein 95.9% of their guano arthropods consisted mites, only 50.9% of the collected microarthropods were mites while 36.3% were collembolans in the present study. Ten of the 18 morphospecies collected were mites (orders Mesostigmata and Sarcoptiformes). This conforms with global trends that mites are one of the most species-rich groups in guano piles (Gnaspini, 2012).

## Other Macroarthropods

The more conspicuous and mobile macroarthropods collected through opportunistic sampling are also part of the cave guano community since they also are also guanophagous or are predators of guano-dwelling guanophages.

A total of 24 taxa were collected by opportunistic sampling. Among them are possible new species of tarantula from Timori Cave (*Orphnaecus* sp., Barrion-Dupo, pers. comm.), troglomorphic cockroach from Santo Rosario Cave (*Nocticola* sp., Lucañas, pers. comm.), and a troglomorphic millipede found in all four caves (Haplodesmidae, San Juan, pers. comm.). There is also a possible new species of troglomorphic planthopper from Santo Rosario Cave (Meenoplidae, Yap, pers. comm.) which feeds on plant roots present in the study site. Another very interesting specimen is that of the ant *Euprenolepis negrosensis* (Wheeler) collected from Santo Rosario Cave which only has three other specimens known to science (General, pers. comm.).

These findings point to the high biological importance of Mabini caves, even those considered to be historically highly disturbed (e.g., Santo Rosario Cave). This also suggests that some cavernicolous taxa are more resilient to anthropogenic disturbance than commonly thought. Historically disturbed caves may, therefore, still offer opportunities for important scientific discoveries upon

closer study, especially for the “small” and “less noticed” taxa (i.e., arthropods, other invertebrates, and microbes).

The results also corroborate the importance of visual searching and opportunistic collection in complementing other methods of collecting a comprehensive sample of arthropods from caves as it really collects a different set of taxa from other methods (Encinares & Lit, 2014; Weinstein & Slaney, 1995). Opportunistic sampling was especially effective in collecting larger, more mobile taxa. Eighteen morphospecies were collected exclusively from guano samples while the visual searching and opportunistic collection contributed 18 additional taxa. Six morphospecies consciously collected by opportunistic sampling were also collected from guano the samples including the geophilomorph centipede, armadillid pill bug, haplodesmid millipede, campodeid dipluran, tineid moth larva, and the ant *Hypoconer*.

### **Trophic Relations and Miscellaneous Ecological Observations**

In terms of trophic relations, notable interactions were observed although there were a lot of guanophages that were not observed to be preyed upon by any predator. There were also predator taxa whose prey preferences were not observed. As a result, the food web constructed still utilized secondary data collected by other studies from other places (see Figure 2). The following are descriptions of the trophic interactions observed.

#### **Guanophages**

Tineid larvae were always observed to be active in both swiftlet and bat guano patches, possibly indicating guanophagous behavior on both substrates. Aside from old guano, they were also observed to feed on a dead tineid moth and on fresh bat droppings that fall into old guano patches. Fresh and moldy bat droppings were observed to be approached and fed upon by haplodesmid, paradoxosomatid and pachybold millipedes, armadillid pill bugs, campodeid diplurans, mites, and springtails.

Other potentially guanophagous taxa recorded include phalangopsine crickets (Otte & Alexander, 1983, as cited by Zefa, 2006), pedilid and scarabaeid beetles, phorid fly, adult soft tick *Antricola* sp., the troglomorphic cockroach *Nocticola* sp. and the ant *Hypoconer* sp. The millipede *Siphonophora luzonensis* Wang was reported to likely feed on soft fungal tissue and spores (Marek et al., 2012). The pedilid and scarabaeid beetles are general detritivores but are likely to also feed on guano (Adorada, pers. comm.). The pedilids were observed to frequent swiftlet guano piles but were more commonly observed flying around our headlamps. The scarabaeid, on the other hand, was not observed to leave wet bat guano.

Table 2 lists the different guanophages and predators associated with the different kinds of guano observed.

**Table 2.** List of guanophages and predators associated with different types of guano in caves in Mabini, Pangasinan.

<b>Guano type</b>	<b>Guanophages</b>	<b>Predators</b>
Swiftlet Guano	Tineid Larvae Phalangopsine cricket Campodeid dipluran Haplodesmid millipede Phorid fly Pedilid beetle Springtails Mites	Whip spider Short-tailed whip scorpion
Fresh Bat Droppings on cave floor	Paradoxosomatid millipede Haplodesmid millipede Pachybolid millipede Armadillid pill bug Campodeid dipluran Tineid larvae Springtails Mites	Whip spider
Old Bat Guano	Tineid larvae Springtails Mites	Trap-jaw ant Theraphosid spider Whip spider Geophilomorph centipede
Wet Bat Guano	Haplodesmid millipedes Scarabaeid beetle Armadillid pill bug Campodeid dipluran Springtails	Whip spider Theraphosid spider Geophilomorph centipede Short-tailed whip scorpion

### **Predators**

Predatory interactions observed include: whip spider (*Amblypygi*) feeding on phalangopsine cricket, on immature *Orphnaecus* sp. (Araneae: Theraphosidae) and on another whip spider individual; *Orphnaecus* feeding on another *Orphnaecus*; a pholcid spider feeding on phorid fly and on adult guano moths; and worker trap-jaw ant (*Odontomachus infandus* Smith) catching a winged male *O. infandus*. An indirect indicator of predation observed is a pile of empty tineid larval cases below a trap-jaw ant colony entrance in Cab2. It is also likely that *Orphnaecus* sp. also feeds on guano moth larvae since these larvae were the only guanophagous species directly observed in dry guano piles in Tim1 and Tim2 where *Orphnaecus* spiders make their silk-lined burrows. There were no crickets observed in Tim1 and Tim2.

Other predatory taxa recorded from the caves include two morphospecies of soil centipede (Geophilomorpha), *Hypoconera* and *Odontoponera* ants (Formicidae), whip scorpion (Thelyphonida, formerly Uropygi) and short-tailed whip scorpion (Schizomida).

The geophilomorphs were observed crawling on moist cave substrate and

on dry bat guano piles in Cac and Tim1. They were also collected in two bottom guano samples from Tim2, indicating their burrowing behavior on dry guano. These centipedes are known to prey on worms and soft bodied hexapods, and are likely to prey on springtails, diplurans and possibly on tineid larvae in the caves (San Juan, pers. comm.). The two genera of ants, as well as *O. infandus*, are known to be scavengers and generalist predators that feed on soft-bodied insects (General, pers. comm.). The short-tailed whip scorpions were frequently observed in and around swiftlet guano patches but were also observed roaming on moist cave substrate and wet bat guano in Tim1. They likely feed on the campodeid diplurans and springtails also found in these substrates, although no actual predation was observed. No predation was observed for the whip scorpions, although they are known to be nocturnal hunters of insects and other small invertebrates.

## CONCLUSION

A six-month study conducted from October 2015 to March 2016 was able to document a total of 42 morphospecies of arthropods belonging to 19 orders and six classes. Among these are five potential new species and five new records. Surprisingly, most of these come from Santo Rosario Cave in Brgy. San Pedro, which is the most human-disturbed cave among the four studied. This indicates that biologically important taxa may persist even in previously disturbed caves elsewhere.

Aside from the high taxonomic richness, the diversity of Mabini caves was also demonstrated by the high habitat-specific assemblages observed. Distinct assemblages of arthropods were observed to inhabit old bat guano, wet bat guano and swiftlet guano patches. The same type of guano also harbors different assemblages in different caves. For example, Tim1 and Cab2 both have old dry bat guano, but Tim1 has the *Orphnaecus* sp. as the dominant predator in the guano piles while Cab2 has Trap-jaw Ant *O. infandus* instead. This implies that each cave site is unique, and each would be worthy of conservation.

In terms of environmental factors affecting guano arthropods, it was observed that more guano-dwelling arthropod taxa are present in swiftlet guano than in bat guano, but in terms of number of individuals, there are more arthropods in bat guano compared to swiftlet guano. This is mainly due to the abundance of Guano Moth larvae in old bat guano piles. Within bat guano piles, it was further observed that deeper guano layers had fewer macroarthropod individuals compared to surface layers.

The most common arthropod morphospecies in Mabini caves is the tineid larva which was present in all sites studied, in both swiftlet and bat guano. For the microarthropods, the most taxa-rich groups were mites (10 morphospecies) and springtails (three morphospecies) consistent with Gnaspini (2012).

Furthermore, aside from the guano-dwelling taxa, more vagrant cave arthropods were observed to be part also of the guano-based food web inside caves and are also supported by guano subsidy from roosting bats and nesting swiftlets.

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## **REFERENCES CITED**

- ARITA HT. 1996. The conservation of cave-roosting bats in Yucatan, Mexico. *Biological Conservation* 76(1996): 177-185.
- CORPUZ-RAROS LA & LIT ILJR. 2015. List of mites (Acari) inhabiting Philippine caves and cave-dwelling vertebrates. *UPLB Museum Publications in Natural History* 4: 30-54.
- CULVER DC & PIPAN T. 2009. *The Biology of Caves and Other Subterranean Habitats*. New York: Oxford University Press Inc.
- ENCINARES JMA & LIT ILJR. 2014. Evaluation of leaf litter baits for sampling insects in Bulalon Cave, Burdeos, Polillo Island, Quezon Province, Philippines. *Philippine Entomologist* 28(1): 76-89.
- FERREIRA RL, MARTINS RP & YANEGA D. 2000. Ecology of bat guano arthropod communities in a Brazilian dry cave. *Ecotropica* 6(2): 105-116.
- GASTON KJ. 2000. Biodiversity: higher taxon richness. *Progress in Physical Geography* 24(1): 117-127.
- GNASPINI P. 2012. Guano Communities. In Culver DC & White WB. (eds.). *Encyclopedia of Caves*. Elsevier Academic Press. pp. 357-364.

- ISKALI G & ZHANG Y. 2015. Guano Subsidy and the Invertebrate Community in Bracken Cave: The World's Largest Colony of Bats. *Journal of Cave and Karst Studies* 77(1): 28-36.
- KERR JT, SUGAR A & PACKER L. 2000. Indicator taxa, rapid biodiversity assessment, and nestedness in an endangered ecosystem. *Conservation Biology* 14(6): 1726-1734.
- KRELL F. 2004. Parataxonomy vs. taxonomy in biodiversity studies – pitfalls and applicability of ‘morphospecies’ sorting. *Biodiversity and Conservation* 13: 795-812.
- LAGARE NJS & NUÑEZA OM. 2013. The cavernicolous crickets in selected caves in Davao Oriental and northern Mindanao, Philippines. *ELBA Bioflux* 5(2): 130-140
- LUCAÑAS CC, ENCINARES JMA & LIT ILJR. 2015. Addenda and corrigenda to the litter-baited cockroach (Dictyoptera: Blattodea) fauna of Bulalon Cave, Burdeos, Polillo Island, Quezon, Philippines. *UPLB Museum Publications in Natural History* 4: 62-66.
- LUCAÑAS CC & LIT ILJR. 2016. Cockroaches (Insecta, Blattodea) from caves of Polillo Island (Philippines), with description of a new species. *Subterranean Biology* 19: 51-64. doi: 10.3897/subtbiol.19.9804
- MAREK PE, SHAER WA & BOND JE. 2012. A redescription of the leggiest animal, the millipede *Illacme plenipes*, with notes on its natural history and biogeography (Diplopoda, Siphonophorida, Siphonorhinidae). *Zookeys* 2012 (241): 77-112.
- MOULDS T. 2004. Review of Australian cave guano ecosystems with a checklist of guano invertebrates. *Proceedings of the Linnean Society of New South Wales* 125: 1-42.
- OLIVER I & BEATTIE AJ. 1996. Invertebrate morphospecies as surrogates for species: a case study. *Conservation Biology* 10(1):99-109.
- POULSON TL & WHITE WB. 1969. The cave environment: Limestone caves provide unique natural laboratories for studying biological and geological processes. *Science* 165(3897).
- RENDON GR. 2011. Development of the Municipal Cave Management Plan of Mabini, Pangasinan. Municipality of Mabini, Pangasinan, Philippines. Balincaguin Conservancy, Inc. 104 pp. Available at the Municipal Hall, Mabini, Pangasinan.
- SMITH TM & SMITH RL. 2015. *Elements of Ecology*. 9th ed. England: Pearson Education Ltd. p. 482.
- WECKSTEIN JD, VENI G, TAYLOR SJ, TAKIYA DM, REDDELL JR, MURDOCH JD, KREJCA JK & JOHNSON KP. 2007. Phylogeography of cave crickets (*Ceuthophilus* spp.) in central Texas: A keystone taxon for the conservation and management of federally listed endangered cave arthropods. *Environmental Sustainability Publications*. Paper 834.



- WEINSTEIN P & SLANEY D. 1995. Invertebrate survey of Rope Ladder Cave, Northern Queensland: a comparative study of sampling methods. *Journal of the Australian Entomological Society* 34: 233-236.
- ZEFA E. 2006. Comparison of calling songs in three allopatric populations of *Endecous itatibensis* (Orthoptera, Phalangopsinae). *Serie Zoologia* 96(1): 13-16.

**Appendix Table 1.** Cumulative counts of the different macroarthropod morphospecies collected from guano samples from the six caves in Mabini, Pangasinan for all five months of the study.

<i>i</i>	morphospecies	Macroarthropod individuals count per guano layer per cave												Total Count
		Cac		Tim1		Tim2		Sto		Cab1		Cab2		
		top	bot	top	bot	top	bot	top	bot	top	bot	top	bot	
1	Tineid moth larvae	502	25	456	130	970	289	155		230	28	1125	249	4159
2	Tenebrionid beetle larvae	125						18		6	3	8	8	168
3	Dipteran larva			10		38		2		1	7	9		67
4	<i>Hypoponera</i> ant	16												16
5	Campodeid Dipluran	13												13
6	Geophilomorph centipede	1			1		2							4
7	Phorid fly												4	4
8	Springtail sp. 1	3												3
9	Haplodesmid millipede						1	2						3
10	Armadillid pill bug	1												1
11	Fungus midge							1						1
	Cumulative Abundance	661	25	466	131	1008	292	178	0	237	38	1142	261	4439
	Cumulative Taxon Richness	7	1	2	2	2	3	5	0	3	3	3	3	

Notes: Macroarthropods extracted from three 100 cc guano samples per guano layer per site per month, except for Cac, Sto and Cab1 where there were 6, 10 and 4 instances of unavailable bottom guano samples, respectively and 3 instances of unavailable top and bottom guano sample for Sto.

**Appendix Table 2.** Cumulative counts of the different microarthropod morphospecies collected from guano subsamples from the six cave sites in Mabini Pangasinan for all five months of the study.

<i>i</i>	morphospecies	Microarthropod individuals count per guano layer per cave												Total Count	% Occurrence
		Cac		Tim1		Tim2		Sto		Cab1		Cab2			
		top	bot	top	bot	top	bot	top	bot	top	bot	top	bot		
1	Isotomid springtail		14	8	1			29	1	10	10			73	58
2	<i>Geolaelaps</i> mite	12		15	2	16	6	1				14	4	70	67
3	<i>Oplitis</i> mite	39	9	1				3			1			53	42
4	Tenebrionid beetle larvae	11	2			1		11		1		1	3	30	58
5	Springtail sp. 1	12	9		1	2		1		3				28	50
6	Sminthurid springtail									4	17			21	17
7	Oribatida mite sp. 2							15						15	8
8	Oribatida mite sp.1	5			1	2		1		1	2			12	50
9	Uropodina mite sp. 2		1							8	1			10	25
10	Dipteran larva							1		8				9	17
11	Uropodina mite sp. 1	1				1				2	1			5	33
12	White mite sp. 1			2										2	8
13	Oribatida mite sp. 3					2								2	8
14	Tineid moth larvae		2											2	8
15	<i>Deraiphorus</i> mite										1			1	8
16	White mite sp. 4							1						1	8
17	Campodeid dipluran								1					1	8
18	Brown ant											1		1	8
	Cumulative Abundance	80	37	26	5	24	6	63	2	37	33	16	7	336	
	Cumulative Taxon Richness	6	6	4	4	6	1	9	2	8	7	3	2		

Note: Microarthropods extracted from three 2.5 cc guano sub-samples per guano layer per site per month, except for Cac, Sto and Cab1 where there were 6, 10 and 4 instances of unavailable bottom guano samples, respectively and 3 instances of unavailable top and bottom guano sample for Sto.