

Sampling effort in mite communities associated with cave bat guano

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Guano deposits in caves play an important role as sources of nutrients and as shelters for an extensive set of unique communities of organisms. They have been studied for decades, and there are many works related to species composition and to new taxa description^{1,2,3}. These guano communities include several invertebrate species ranging from decomposers, such as mites, to larger predators, such as spiders that are part of complex trophic webs¹⁰. Recent works also investigated ecological questions such as changes in the community due to differences in guano deposition time, seasonal variation, inter- and intraspecific interactions, and determination of trophic web communities⁴⁻¹¹. Such studies suggest that guano deposits are heterogeneous, being characterized by a high variability of micro-habitats differing in pH, humidity and percentage composition of organic matter. Heterogeneity of the guano deposits may largely reflect a variation in age. Fresh guano tends to be alkaline and humid, but as it ages it becomes more acidic and dry^{4,6}. The resulting substrata shelter diverse zoological communities at different stages of succession².

Implementation of ecological studies can be hindered by some characteristics of guano communities. Many of the organisms are of very small size. Furthermore, many species (mainly mites) can form large populations of hundreds of thousands of individuals in very extensive deposits, making exhaustive collections in those systems unfeasible. For example, the deposits present in Morrinho cave (Campo Formoso, Bahia, Brazil) yielded 12,256 individual mites captured by the Berlese-Tullgren funnel method¹⁰. In this study, the guano piles were divided into sub-samples of 400 cm² that, together, corresponded to only 10% of the total area of each guano pile. Hence more sub-samples were taken from larger guano piles, potentially introducing a source of bias. The huge number of mites in many guano piles makes the total account and taxonomic identification very difficult and, in some cases, unfeasible.

Another work, conducted in Australia, reached incredible numbers of 378,354 collected individuals, of which more than 95% belonged to only one species of mite, *Uroobovella coprophila*¹¹. Such data support the need to optimize the collection methods of arthropods in guano deposits. The objective of our study was to evaluate the efficiency of sampling of the richness and population sizes of mites associated with bat guano in a cave.

The large bat guano deposit we studied is located at the Lapa Nova Cave [Vazante, Minas Gerais, Brazil – (Lat -73°53'31.7"S/Lon 50°50'46.5"W)]¹², in one inner chamber situated at 110 meters from one entrance to the cave. It is characterized as mixed guano of frugivorous and insectivorous bats that are still present in the cave. Unfortunately it was impossible to determine the bat species identity, because the colonies were sheltered in a fissure in the chamber's ceiling.

The sampling was conducted on the 15th of September 2009. We sampled at four equidistant points on a linear transect laid on the major axis (20 meters) of the deposit. At each of the sampling points we collected 2 liters of guano (400 cm² square with 5 cm depth). Each sample was put separately in a plastic pot, sealed, labeled and taken to the laboratory. It was put in Berlese-Tullgren funnels for one week, with saline solutions to collect the invertebrates. Each solution was mixed and separated into subsamples in plastic 1.5 ml tubes. A total of 48 sub-samples were obtained each from points 1-3, and 40 sub-samples from point 4. Point 4 yielded fewer sub-samples because fewer individuals were extracted when compared with the other samples. We carefully checked each of the sub-samples with the aid of a stereomicroscope, and separated the organisms according to morphotypes. Samples were later identified further under a regular microscope (Leica MDLS phase-contrast microscope). We here only consider mites, as they were the most abundant arthropod group recovered from the guano deposit.

We used the *EstimateS* program to construct the species accumulation curves and their confidence intervals, implementing 100 randomization of the accumulated richness. The same curves were used for the calculation of the number of necessary samples to obtain 75% and 50% of the expected richness for each one of the points sampled. This was obtained through the *Jackknife* 1 estimator, also calculated with *EstimateS*. Once having the data, it was possible to establish the number of sub-samples necessary to obtain a good estimate of the richness and abundance of mites in guano samples. A cluster analysis (*Past* program) of the Bray-Curtis similarity index among the four points was used to examine their relationship in terms of species composition. We then examined differences in the community composition among the sampling points to verify if the mite community was distributed regularly throughout the entire guano pile (i.e., all species were distributed along all points).

A total of 46,775 mites belonging to 32 species were found at all four collection points together. At points 1, 2, 3 and 4 abundances of 13,495, 17,286,

10,034 and 5,960 individuals and 22, 14, 17 and 11 species were found, respectively.

The species accumulation curves showed different tendencies among the four sampling points. The curves for points 1, 2 and 3 started to stabilize more quickly, reaching at least 85% of the expected richness. These three curves also showed a tendency to an asymptote. On the other hand, the curve for point 4, which was divided in 40 sub-samples, did not show a tendency to an asymptote, ultimately reaching only 74% of the expected richness. The curve for point 4 is still rising, implying more species are expected to be present.

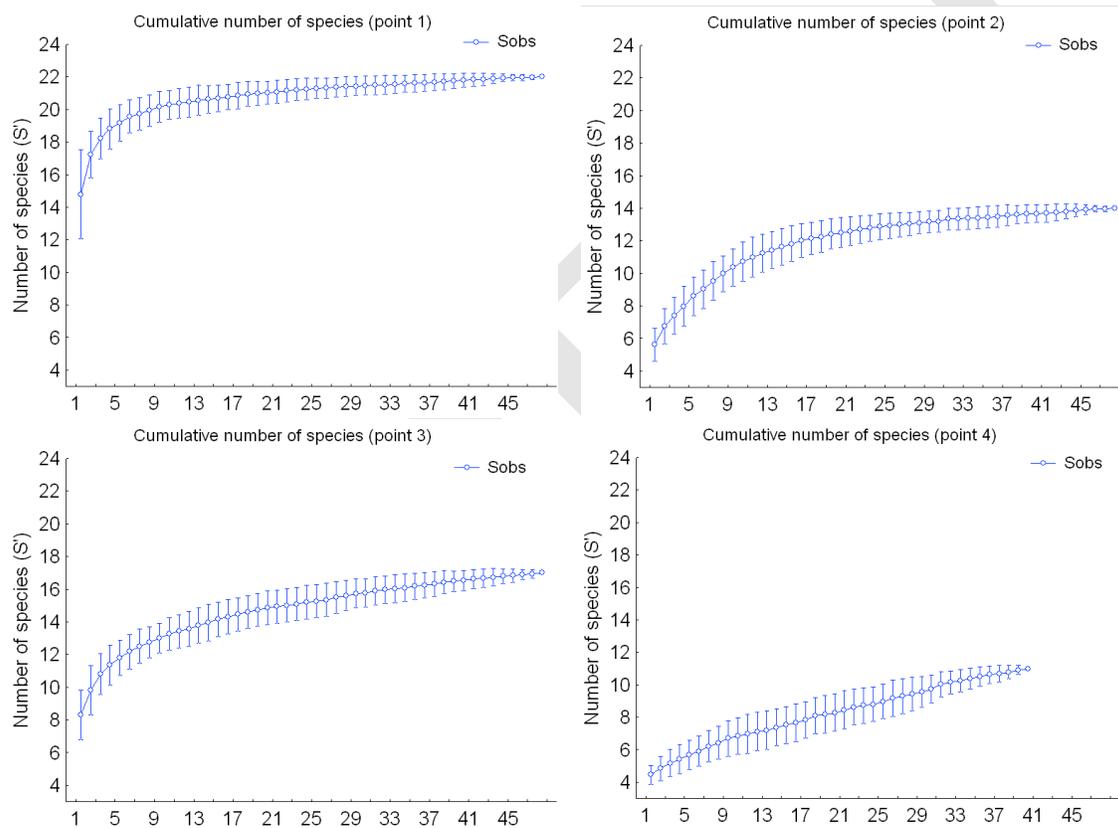


Figure 1. Species-accumulation curves with upper and lower 95% confidence intervals in the Lapa Nova Cave guano deposit for each of the four sample points. The cumulative number of species is plotted against the number of sub-samples.

The present data suggest that less effort is needed to get the expected richness in richer samples. The sampling effort in samples of high richness and abundance, like those observed at point 1, can be smaller than the effort spent in samples with less richness and abundance, like point 4 (Table 1). Furthermore, a large dissimilarity in community composition among the four points was observed (Figure 2). Such differences can reflect the heterogeneity of the guano deposits. However, it is important to note that, we opted for collection of guano samples at

different deposition stages, thus emphasizing these differences. Point 1 represented a fresh guano area, while the others were older and drier. This is especially true for point 4, which was very distant from the recent deposition areas. The physical and chemical features of guano patches vary in time, as previously mentioned. Fresh guano tends to be more alkaline and moist, but becomes more acidic and drier when older^{4, 13}. Several authors have shown that the physiochemical composition of an ephemeral resource is an important determinant of the richness and abundance of the associated communities^{14,11,10,15}. Therefore, variation in richness and abundance can result from ecological succession during the guano desiccation process². Winchester et al. (2008) conducted a study with oribatid mites on a mountain located on Vancouver Island, Canada, and also observed a significant difference in the species accumulative curve according to the change of the micro-habitat¹⁶.

Table 1. Total abundance of individuals and species richness at each point sampled in the Lapa Nova Cave guano deposit. Expected richness was estimated using the Jackknife 1 estimator in *EstimateS*.

	Point 1	Point 2	Point 3	Point 4
Total abundance of individuals	13,495	17,286	10,034	5,960
Observed Richness	22	14	17	11
Expected Richness	22.98	15.96	19.94	14.9
% of expected observed	96%	88%	85%	74%

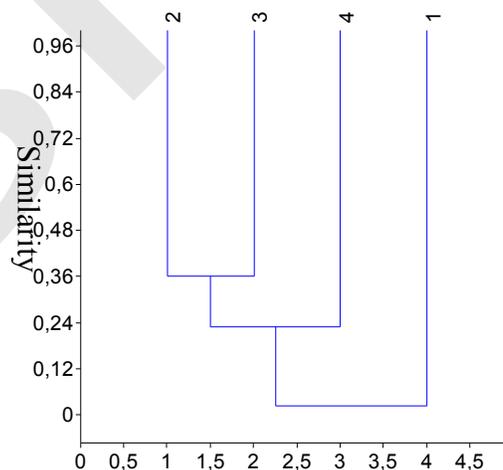


Figure 2. Similarity cluster analyses for the four collected points in the Lapa Nova Cave guano deposit (similarity based on the Bray-Curtis index).

According to the graphs generated in the singleton and doubleton simulations it is possible to infer that at points 1 and 2, there is a high probability

that rare species could appear with a small number of sub-samples. At points 3 and 4 the curve did not stabilize. Therefore, a large number of sub-samples would be necessary to increase the probability of finding rare species (Figure 3).

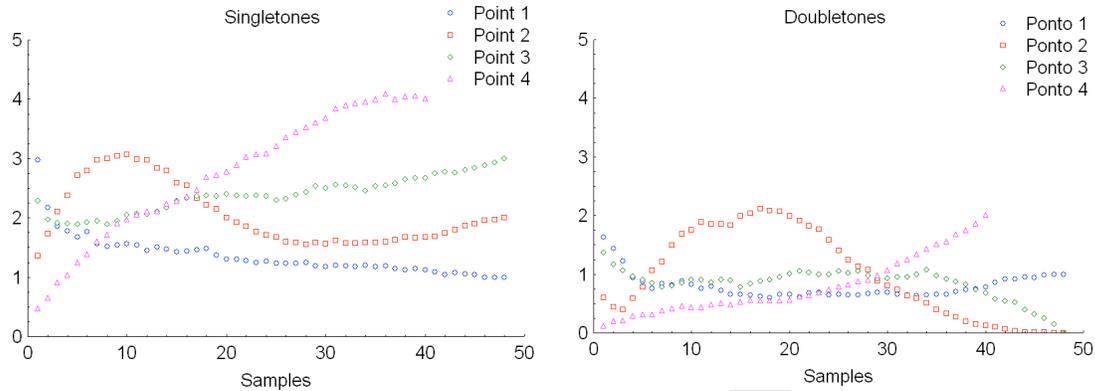


Figure 3. Simulation of the occurrence of rare species for the four guano points collected in the Lapa Nova Cave guano deposit.

Our data suggest that the sampling was adequate to estimate the richness and abundance for points 1-3, but not for point 4. However, the variability observed among the points suggests that a larger number of sub-samples probably would reveal more species, at least for point 4.

In conclusion, it is important to conduct preliminary studies such as this prior to the sampling of the whole community associated with a guano pile. These studies provide an empirical basis for estimating the volume (sub-samples) and the sampling effort necessary to obtain a good species sampling of the mite community. Furthermore, it is also possible to target areas to meet the goals of the study (e.g., optimizing total richness, numbers of rare species, numbers of individuals), considering that huge piles frequently have heterogeneous physicochemical conditions. Some mite species have preferences for specific microhabitats, thus considering such variations are crucial to provide a reliable sampling of a guano pile. More homogeneous deposits allow good richness and abundance estimates starting from the selection of just a fraction of the collected material (sub-samples) in the Berlese-Tullgren funnels. On the other hand, heterogeneous deposits need the selection of the entire biological material to guarantee a thorough sampling, especially in areas with decreased abundance and richness. Unfortunately, based on this work it is not possible to provide the number of samples (points) necessary to obtain a good sampling of the mite community. This number is directly dependent on the heterogeneity (and area) of the guano pile. Ferreira *et al* have proposed the collection of 10% of the total guano pile area¹⁰. Such an idea could be applied, but would be still necessary to obtain the species accumulation curves to verify if the sampling effort was enough for each sample.

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