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Microplastic Contamination in Amazon Vampire Bats (Desmodontinae: Phyllostomidae)

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Abstract: Microplastics (MPs) are polymers smaller than five millimeters, are easily dispersed, and are considered a contaminant of emerging concern. The objective was to quantify and characterize, both by color and shape, the presence of MPs in the stomach, intestine, and lungs of vampire bats, analyzing the difference between organs and their morphometric measurements in relation to the abundance of MPs found in each organ. The bats were collected between 2017 and 2021. In the laboratory, the specimens were euthanized, and the organs of interest were extracted. After removal, the organs were chemically dissolved and then filtered through a suitable membrane. The membranes were inspected through visual analysis of the samples. Our result was significant, we found at least one MP in each organ analyzed, with all individuals being contaminated with particles only in fiber format, mostly white/transparent. The gastrointestinal system had greater contamination compared to the respiratory system. It is not yet known for sure how the ingestion of plastic waste has affected this population of bats, but the presence of plastic waste in the gastrointestinal tract of hematophagous individuals proves the effectiveness of the contagion of these particles.

Keywords: Chiroptera; fibers; plastic; hematophagy

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1. Introduction

Plastic pollution poses significant threats to ecosystems and is considered one of the foremost environmental challenges of the 21st century [1]. Plastics have become integral to the global economy, present in nearly every economic sector [2]. Despite their utility, the most widely produced and consumed plastics are "single-use" or disposable items [3]. Improper disposal of these materials leads to their accumulation in the environment, where they can persist for decades [4].

Environmental plastic debris exists in various sizes, including macro-, meso-, and microplastics [5]. Particles smaller than five millimeters are classified as microplastics

(MPs) [6] and are considered one of the most concerning forms of plastic in recent years [7]. MPs are categorized as either primary or secondary, based on their production type [8], but regardless of classification, these particles disperse widely, reaching even the most remote ecosystems and contaminating local fauna and flora [9].

Microplastic contamination commonly occurs through inhalation or accidental ingestion [10]. Once in the body, these microparticles accumulate in vital organs, such as the lungs, stomach, and intestines, and can even enter the bloodstream, reaching other target organs like the liver, kidneys, and nervous system [11]. This contamination can lead to inflammation, respiratory diseases, digestive issues like intestinal blockage, and even neurobehavioral disorders [12–14].

Although MPs can travel through the atmosphere over distances of up to 95 km [15], areas impacted by human activity are particularly susceptible to contamination [16]. In the Neotropics, livestock farming significantly alters ecosystem functions and increases anthropogenic impact [17]. Some species, such as *Desmodus rotundus* (the common vampire bat), adapt well to these human-modified landscapes [18].

The common vampire bat, *Desmodus rotundus* (É. Geoffroy, 1810), belongs to the Phyllostomidae family, alongside *Diaemus youngii* (Jentnik, 1893) and *Diphylla ecaudata* Spix, 1823 [19]. These vampire bats, part of the Desmodontinae subfamily, have specialized gastrointestinal systems adapted for blood consumption [20]. While *Diaemus youngii* and *Diphylla ecaudata* are less studied, they tend to inhabit preserved, forested environments, using tree hollows as their primary shelters and primarily feeding on bird blood, specifically targeting areas like the legs and cloacal region [21,22]. *Desmodus rotundus* exhibits a unique anticoagulant in its saliva, known as draculin, which has medical applications, and is well-suited to human-modified landscapes [23,24].

Human activities such as deforestation to create pastures for cattle, horses, deer, and pigs [25] have provided vampire bats with easier access to these domesticated animals, often leading them to favor these prey over wild species [26]. Predatory vampire bats tend to feed on both domestic and wild birds, particularly those perching on trees with sparse foliage for easier access [22].

When predators shift to more anthropogenic environments for food, they are exposed to higher levels of contamination [27]. Bats, in particular, may absorb MPs through incidental ingestion or inhalation [10]. Our study examined the contamination levels between bats' digestive and respiratory systems and assessed the relationship between organ size (length and weight) and MP abundance. We hypothesize that, given the bats' feeding habits, their respiratory systems will show higher contamination than their digestive systems [28]. We also expect that MP contamination will be directly proportional to organ dimensions, as larger organs may retain more particles [29].

2. Material and Methods

2.1. Study Area

Sampling was conducted at eight rural sites across the municipalities of Altamira, Medicilândia, and Vitória do Xingu, all located in the state of Pará (Figure 1). This region has a tropical climate characterized by a short dry season and significant rainfall throughout most of the year, with an average temperature of 26.1 °C. According to the Köppen climate classification, it is classified as "Am" [30].

Since the 1970s, the state of Pará has experienced significant anthropogenic pressure, with the construction of the Trans-Amazonian Highway being a major driver of increased deforestation and fire incidence in the area [31]. The municipalities where sampling took place are situated within the "arc of deforestation", a zone marked by intense land-use

change and environmental degradation. Here, land use and cover are largely focused on vegetable and mineral extraction, agriculture, and livestock farming [32].

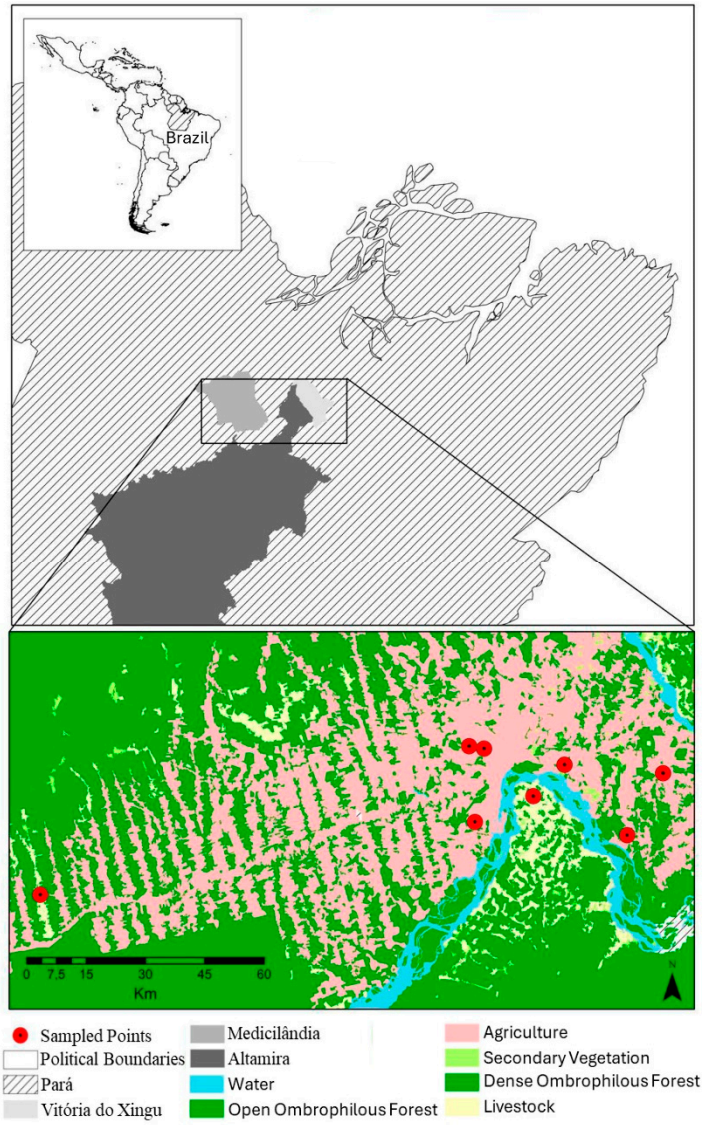

Figure 1. Geographic distribution of sampled areas for the collection of vampire bats (Phyllostomidae: Desmodontinae) in municipalities within the state of Pará, Brazil.

2.2. Sampling Procedure

Collections were conducted from April 2017 to May 2021. Bats were sampled using ten mist nets (9 m × 2.5 m) set up at sunset and left open for six hours, with inspections every 30 min. After capture, bats were placed in 100% cotton bags and transported to the Ecology Laboratory at the Federal University of Pará. The bats were euthanized by cervical dislocation, and all morphometric data were recorded. Specimens were then sorted, fixed in 10% formaldehyde, and preserved in 70% ethanol in glass jars.

These specimens are housed in the bat collection of the Center for Ecology and Conservation of Chiropterans (ChiroXingu) at the Altamira campus of the Federal University of Pará. The study was authorized by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (Chico Mendes Institute—SISBIO permit No. 57294-2) and approved by the Ethics Committee on the Use of Animals at the Federal University of Goiás (CEUA UFG protocol No. 004-21).

2.3. Extraction and Digestion of Biological Material

Using forceps, the digestive and respiratory systems were completely removed. For the digestive system, the esophagus to the anus was removed, with the stomach separated from the intestine to assess retention within each section. The respiratory system was removed from the trachea to the lungs. All biological samples were measured and weighed on a semi-analytical digital balance (accuracy of 0.0001 g).

Following extraction, the organs were individually stored in 10 mL glass bottles that had been cleaned with distilled and filtered water. Approximately 5 mL of 10% Potassium Hydroxide (KOH; 10% V/V) was added to each bottle to dissolve the tissues. The bottles were sealed with metal lids and incubated at a constant temperature of 50 °C for 24 h to expedite tissue digestion [33].

Once the tissues were completely digested, the samples were filtered through a 0.2 µm porosity ME24 membrane (composed of Cellulose Ester Mixture composed of Cellulose Nitrate + Cellulose Acetate), using a filtration device and vacuum pump. The membranes were then placed in glass Petri dishes, wrapped in aluminum foil, and heated in an oven at 50 °C for one hour before being stored for visual analysis. Aluminum foil serves as a light barrier, preventing color fading, drying, and breakage of plastic particles, as well as minimizing contamination from microplastics in the work environment.

2.4. Visual Analysis of Plastic Waste

The samples were analyzed under a stereoscopic microscope (Digilab-microscope Stereo Trinocular DI-106T zoom, Digilab, Piracicaba, Brazil) with a 120× magnification. Each membrane was meticulously examined by scanning systematically from left to right and top to bottom. Each microplastic particle encountered was measured (1 to 5 mm in size) [34] and classified by color (white, blue, yellow, red, green, or black) and shape (fiber, film, spherule, or fragment).

To confirm plastic composition, the hot needle test was applied [35]. In this test, a heated needle is placed on the suspected plastic; a change in shape or burning reaction confirms its plastic nature.

2.5. Quality Control and Quality Assurance

All materials used were non-plastic, with glass or aluminum preferred whenever possible. Throughout each stage of the work, we used distilled and filtered water, including for washing glassware, preparing potassium hydroxide (KOH), and cleaning the filtration apparatus. All reagents were filtered prior to contact with the organs. Due to the risk of plastic fiber contamination from clothing, only lab coats and clothing made from 100% cotton fabric were worn.

Specimen handling took place inside a sanitized fume hood, with thorough cleaning performed before each activity. An environmental blank sample was collected at each stage [36] to monitor potential contamination. This blank control consisted of an open bottle containing 10 mL of KOH with no specimen inside, which was analyzed alongside the samples. If microplastics detected in the blank matched those in the samples analyzed on the same day, these were subtracted from the primary sample results to account for potential environmental contamination [36].

2.6. Statistical Analyzes

We conducted a one-sample t-test to determine if the number of plastic fibers found in the organs was significantly different from zero. To compare contamination levels across organs, we used a Kruskal–Wallis test, treating the organ as the grouping variable and the number of fibers as the dependent variable. Additionally, we applied the Kruskal–Wallis

test to compare contamination levels between the digestive and respiratory systems, with the system as the grouping variable and the number of fibers as the dependent variable.

These analyses focused exclusively on fiber-type microplastics (MPs), as no other types of particles were detected in the samples. Fiber color characterization was illustrated with a bar graph; however, due to the low count of non-white fibers, statistical analysis by color was not feasible. Lastly, a Spearman correlation was conducted to explore the relationship between organ length and weight and the abundance of MPs. All analyses were performed using the R programming environment version 4.4.2 (R Core Team, 2020) [37] and the Jamovi software version 2.3 (Jamovi project, 2021) [38], with a significance level set at $\alpha = 0.05$.

3. Results

We analyzed 12 vampire bats, including ten *Desmodus rotundus*, one *Diaemus youngii*, and one *Diphylla ecaudata*. Microplastics were detected in at least one organ (stomach, intestine, or lung) in each individual, resulting in a 100% contamination rate across all bats (Table 1). In total, 110 particles (35%) were observed in the stomach (mean 7.42 $\pm$ 6.20), 138 particles (43%) in the intestines (mean 10.17 $\pm$ 8.27), and 69 particles (22%) in the lungs (mean 4.92 $\pm$ 6.04) (Figure 2).

Table 1. The table lists the number of individuals analyzed, their specific species, and the abundance of microplastics in each organ examined.

Individuals	Species	Stomach	Intestine	Lung
1	*Desmodus rotundus*	-	11	20
2	*Desmodus rotundus*	3	19	-
3	*Desmodus rotundus*	10	16	-
4	*Desmodus rotundus*	17	6	-
5	*Desmodus rotundus*	-	16	6
6	*Desmodus rotundus*	9	1	1
7	*Desmodus rotundus*	-	-	5
8	*Desmodus rotundus*	9	4	6
9	*Desmodus rotundus*	19	9	13
10	*Desmodus rotundus*	7	28	2
11	*Diaemus youngii*	7	4	2
12	*Diphylla ecaudata*	8	8	4

All particles found were fibers, predominantly white or transparent, although a smaller number of blue, black, and red fibers were also present (Figure 2).

The number of microplastics (MPs) found in the organs was significantly different from zero: stomach (t = 4.14; df = 11; $p = 0.002$), intestine (t = 4.26; df = 11; $p = 0.001$), and lung (t = 2.28; df = 11; $p = 0.017$). However, the average degree of contamination did not differ significantly among the organs ($F_{(2, 21.7)} = 1.57$; $p = 0.230$). Similarly, there was no significant difference in contamination levels between the respiratory and digestive tracts ($H(2, N = 36) = 2.998$; $p = 0.223$). Finally, we found no correlation between the size of the organs and the number of fibers present (Table 2).

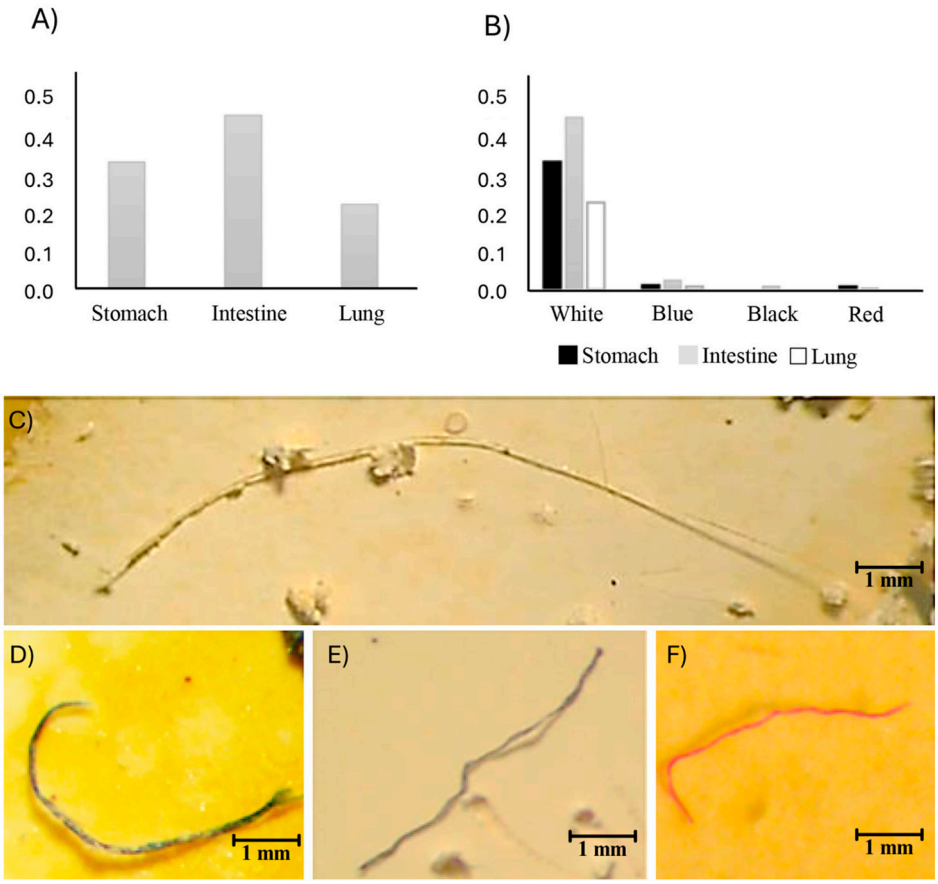

Figure 2. Percentage of fibers found (**A**) by organ and (**B**) categorized by color and by organ in hematophagous bats sampled during the period 2017 to 2021. White/Transparent (**C**), Blue (**D**), Black (**E**), and Red (**F**) Fiber.

Table 2. Spearman correlation test between the length and weight of the organs and the number of fibers found.

Organ	Variable	Spearman r	*p*-Value
Stomach	length	−0.467	0.126
	weight	0.040	0.901
Intestine	length	−0.195	0.543
	weight	0.325	0.303
Lung	length	0.203	0.528
	weight	−0.131	0.685

4. Discussion

In this study, we documented microplastic (MP) contamination in at least one organ—stomach, intestine, or lung—in all analyzed vampire bats (*Desmodus rotundus*). Contrary to our initial expectations that the digestive system would exhibit minimal contamination due to the bats' hematophagous feeding habits, our findings reveal significant levels of MPs. One likely contributing factor may be the bats' feeding behavior, which involves cutting into prey and licking blood [39]. This behavior may expose bats to MPs present on the surface of prey animals, particularly in anthropized environments where contamination is more prevalent.

The location where bats choose to feed on prey is also critical. For instance, bats may come into contact with MPs attached to the prey's fur, feathers, or skin, especially in areas exposed to high levels of contamination, such as limbs or necks [40]. In agricultural

settings, the use of fertilizers and pesticides, which can contain MPs, may further contribute to soil contamination. These particles can persist in soil and transfer to plants, which are subsequently consumed by livestock, thus introducing MPs into the food chain that ultimately reaches vampire bats [41]. Therefore, it is plausible that the contamination observed in the bats' digestive systems originates from MPs present on the fur, neck, and paws of their prey. Another important point is the bats' ability to echolocate. During this process, bats keep their mouths open as they emit sound waves through their mouths or noses, which return as echoes when they encounter objects or prey in their path [42]. In this way, we believe that this could be another possible form of contamination by microplastics present in the atmospheric air.

With increasing environmental degradation and the encroachment of domesticated animals into previously undisturbed habitats, vampire bats have begun foraging in more anthropized areas, leading to potential exposure to airborne MPs [43]. While agricultural regions generally exhibit lower contamination levels compared to urban areas [44], they still contribute significantly to overall MP contamination due to atmospheric dispersion. This likely explains the relatively low contamination levels observed in the bats' respiratory systems, as fewer particles were detected in these organs compared to the digestive tract. This observation aligns with findings in other bat guilds where MPs' presence in respiratory systems was also noted, though at lower levels [45].

Our statistical analysis confirmed that the number of MPs detected in the bats' organs was significantly different from zero, affirming contamination. However, we found no significant differences in MP counts between different organs, a result that did not support our initial hypothesis that contamination would be unevenly distributed between the digestive and respiratory systems. The absence of a correlation between organ size and MP levels suggests that environmental exposure, rather than anatomical factors, plays a dominant role in contamination levels.

Of the various MP shapes (films, spheres, fragments, etc.) present in the environment, fibers were the most frequently detected form in our samples. This may be due to fibers' lightweight nature, ease of dispersion, and high potential for ingestion [46]. The prevalence of white and translucent fibers suggests that they originate from synthetic materials commonly used in human activities, such as clothing and cleaning products [47]. Additionally, fibers can come from objects used in animal handling, like ropes, saddles, and whips. These materials adhere readily to fur, feathers, and skin, making them likely vectors for MP transmission [48]. Since fibers are the most common form of MPs in the atmosphere, they are more prone to adhere to the surfaces of prey animals, thereby increasing the likelihood of contamination in predatory species like vampire bats.

Our findings underscore the urgent need for measures to mitigate MP pollution, given its potential to infiltrate biological systems and negatively impact organism health. Bats, due to their ecological roles and sensitivity to environmental changes, serve as effective bioindicators for assessing MP contamination in terrestrial ecosystems. The implications of our study raise serious concerns for the food chain, as vampire bats consume blood from domesticated animals that humans also consume. MPs can enter animals through ingestion or dermal absorption, eventually accumulating in the bloodstream and potentially transferring up the food web [49]. This raises the possibility of human exposure to MPs through the consumption of contaminated livestock.

Future research should focus on identifying specific sources of MPs in natural habitats and evaluating the long-term impacts of MP exposure on bat health, behavior, and reproduction. In conclusion, our study provides compelling evidence that microplastic contamination poses a significant threat to vampire bats and, by extension, other organisms

in their ecosystem. Addressing this pollution should be a priority to safeguard biodiversity and protect ecosystem health.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee on the Use of Animals of the Federal University of Goiás—CEUA UFG N°. 004-21.

Data Availability Statement: Data is contained within the article.

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Conflicts of Interest: The authors declare no conflicts of interest.

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