

How Long Are Daddy Longlegs' Legs? Investigating Leg Length Variation in Harvestmen (Opiliones) Across Microhabitats

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ABSTRACT

Despite being the third most biodiverse group of arachnids, little is known about harvestmen (Opiliones). The appendages of harvestmen, specifically their legs, play a crucial role in their survival among the dense neotropical rain forest. Many studies have hypothesized that harvestmen have adapted long legs for prey capture and defense. Studies conducted in temperate regions have also found a connection between leg length and habitat. However, this relationship has not been thoroughly observed in neotropical regions. The purpose of this study is to see if there is a relationship between microhabitat and length harvestmen's legs. The prediction being that longer legs would be associated with microhabitats in the understory while shorter legs associated with microhabitats in the forest floor. Our team collected 47 different specimens, encompassing 12 different species at La Selva Biological Station along the Sendero Tres Rios trail. The understory was divided between shrubs, understory leaf litter, and trunks while the forest floor was divided between forest floor leaf litter and decaying logs. Leg length and body length were measured, and relative leg length was analyzed. We found that longer leg length is typically a trait found in harvestmen that live in the understory habitat while shorter leg length was typically found in the forest floor habitat. However, differences in leg length within the forest floor, i.e. between leaf litter and decaying logs, proved insignificant. Further studies

are needed in this field to determine why habitat and leg morphology are related and what potential implications this finding may have for neotropical harvestmen ecology.

Key words: Leg morphology, understory, forest floor, La Selva, neotropical.

INTRODUCTION

Despite their prevalence, the scientific knowledge on the order Opiliones (harvestmen) in the neotropics is greatly lacking in detail. Few studies have been conducted on a broad range of harvestman species in the neotropics, and there are even fewer recent studies. Despite the lack of research on neotropical harvestmen's behavior and species interactions, the taxonomy and natural history of Opiliones across the world is well understood. Harvestmen belong to the arachnid class. They have a diverse number of defense strategies that help them flourish in neotropical and temperate forests. According to a study that looked at predation among armored arachnids, harvestmen have been observed bouncing up and down to hide their location and using fallen leaves on the forest floor to escape observation (Albin & Toscano-Gadea 2015). Harvestmen are omnivorous predators themselves, and their foraging and hunting strategy includes nightly migrations from their diurnal hideouts to the forest floor. During the day they tend to stay hidden and relatively inactive in their habitats (Proud et al. 2012).

The appendages of harvestmen, specifically their legs, play important roles in all aspects of their lives. The legs are attached to the cephalothorax and numbered from I to IV (Acosta & Machado 2007). In terms of foraging, “the long or very long legs of harvestmen have probably evolved to meet the needs of prey-capture and defense in the particular types of habitats that they occupy” (Hillyard & Sankey 1989). Three pairs of legs are predominantly used for walking and gracefully maneuvering across different substrates while the second pair is always the longest

and used for sensory purposes and to test the surrounding area for resources like water (Hillyard & Sankey 1989). The length of the second pair of legs determines mating strategies as well. Males with longer second legs fight for females while males with shorter second legs rely on invasion tactics and secret mating (Machado & Burns 2022). In some species of harvestmen “the male guards the female wrapping one of her legs with his first pair of legs and following her as she selects potential oviposition sites with her ovipositor” (Machado & Burns 2022). However, many of these leg uses have only been studied in specific species.

The current knowledge about leg morphology in harvestmen is primarily from research conducted in temperate regions. Furthermore, most studies have only examined one specific species and have neglected to compare their results to a broader range of species in a broader range of habitats. Information on possible connections between habitat and leg morphology specifically is less concrete for neotropical communities. Besides the use of legs in harvestmen's defense and mating strategies, their leg length may be related to diurnal hideout preference. According to one of the few books written about them, “long-legged climbing species have more tarsal segments than short-legged and soil dwelling species.” (Shultz & Pinto-da-Rocha 2007). This is one of the few relationships between morphology and habitat that has been observed in both temperate and neotropical populations.

In 2012 another study conducted at La Selva Biological Station in Costa Rica observed that diurnal harvestmen hideouts can be categorized in two specific habitats, the ground/litter layer and the shrub/tree layer. The species richness and abundance between these two types of conditions was different enough to suggest a possible relationship between leg length and preferred habitat especially considering that leg length is related to climbing ability in temperate harvestmen (Proud et al. 2012). In this project, we studied the possible relationship between

habitat, microhabitat, species morphology, and average leg length within species and across communities. By comparing average leg length of 12 species and examining differences in the habitats each species was found in, we tested the hypothesis that microhabitat predicts harvestmen leg length. Specifically, harvestmen with longer legs will be found in the understory habitat and harvestmen with shorter legs will be found in the forest floor habitat.

MATERIALS AND METHODS

We conducted our study at La Selva Biological Station, on the Tres Rios Trail (STR), on July 26, 2025 from 9:00 A.M. - 10:30 A.M.

Preparation

We did a forest walk to garner information on potential harvestmen whereabouts. Having seen numerous harvestmen on both the forest floor and understory, we decided to study two main habitats and divide each habitat into microhabitats. The two habitats we studied were the understory and forest floor. The understory microhabitats were leaf litter, tree trunks, and shrubs. The forest microhabitats were leaf litter and decaying logs.

Field Sampling

We found our harvestmen specimens by intentionally looking for harvestmen in each of the microhabitats we had listed. Furthermore, some group members elected to use a light during this process to ensure maximum clarity.

Finding harvestmen in the forest floor microhabitats was trickier because they tended to be smaller and could hide among the leaf litter. We occasionally used a small stick to poke around the shrubbery and leaf litter, which made the process easier as the Harvestmen would run

from their hiding spots. From there, we called out what area we found these harvestmen, and someone brought the correct container to put them in. We worked in 50 meters of the forest for a duration of 90 minutes at the 150-meter mark along the STR trail.

Leg Analysis

After collecting our harvestmen, we brought them to the lab for further analysis. We tentatively classified them into species based on the locations where they were found. This classification was uncertain, as it was done by eye. The goal of this step was to help streamline the next phase of our research. It is important to note that we did not separate or alter the original groups, but we organized the specimens according to the species we believed were present in each area.

Once again, we split into two groups. The first group took the harvestmen and put them under the microscope to see them more closely. We had to hold the harvestmen down using their legs for a clear view. Next, we took a picture of them through the microscope using our iPhones. The purpose of this was to make sure everyone in the group could easily see the harvestmen and confirm we were classifying them correctly, and to make a record of the species we saw and digitized the data. Using the species pictures included in the 2012 paper from Proud et. al., we classified each harvestman into their species, we did this for each specimen, sending them to the other group when done.

The other group used calipers to measure the harvestmen's leg length and body size. We measured the right fourth leg every time. If they didn't have a right fourth leg, we measured the left fourth leg. We recorded this information as well.

Data Analysis

After compiling the data, we used several statistical tests to examine differences in leg morphology across habitats. We used a two-sided t-test, which evaluates whether the means of two independent groups are significantly different. Next, we conducted a t-test to explore variation across different subgroups of forest floor microhabitats. One-way analysis of variance (ANOVA) was used to compare relative leg length across multiple understory microhabitats. We also calculated t-values and p-values whenever necessary to determine statistical significance.

RESULTS

We caught 47 specimens, which were identified to belong to 12 different species (Table 1). Each microhabitat had different assortments and abundances of each species. In the decaying logs microhabitat, we found four different species, with Zalmoxidae A being the most abundant. In the forest floor leaf litter, we found the highest number of species (eight), with *Glysterus sp.2* being the most plentiful. We found four species in the shrub habitat, and *Prionostemma sp.2* was the most common. The understory leaf litter had three species, and Sclerosomatidae A had the most specimens. Finally, we found three species on tree trunks, and *Prionostemma sp.2* was the most common. Overall, we found the most *Prionostemma sp.2* (Fig 1).

Table 1. Table of harvestman species collected. Each column is a different family. NOTE: sp. 1, sp. 2, etc, are unidentified species within their genus.

Sclerosomatidae

Cosmetidae

Zalmoxidae

Gonyleptidae

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Prionostemma sp. 1



Cynorta marginalis



Zalmoxidae A



Glysterus sp. 1



Prionostemma sp. 2



Eucynorta sp. 1



Zalmoxidae B



Glysterus sp. 2



Prionostemma sp. 5



Eucynorta sp. 5



Zalmoxiadae C



Sclerosomatidae A

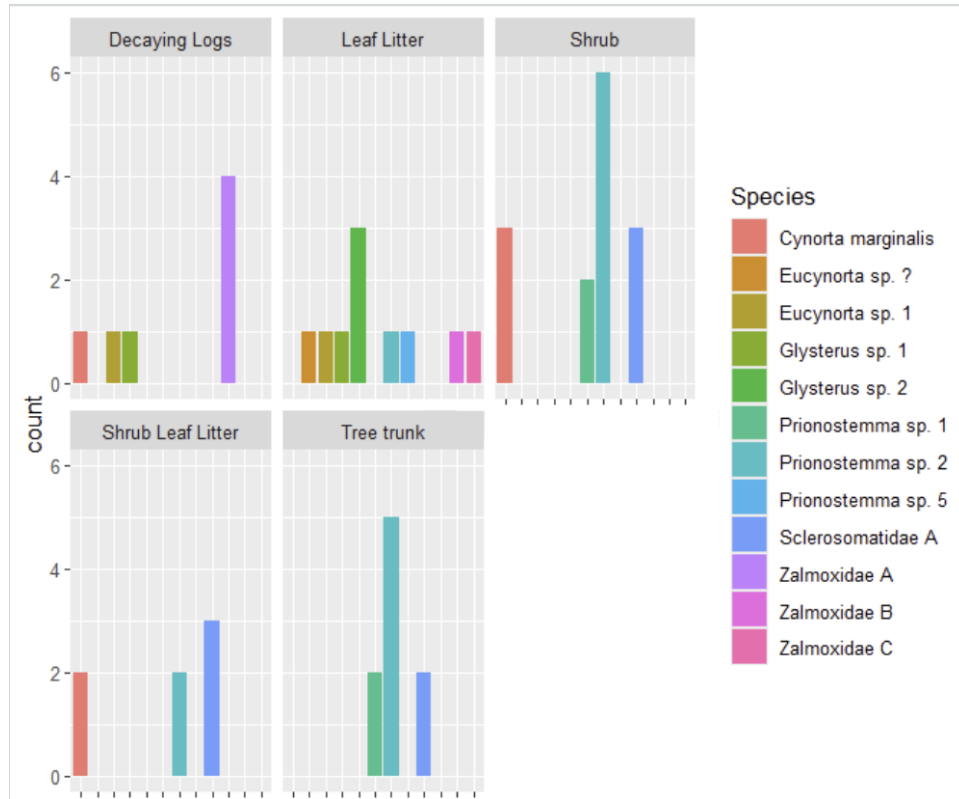


Figure 1. Number of specimens of each species found in each microhabitat surveyed. Legend shows the species that correspond to the colors on the graphs. Decaying Logs and Leaf Litter are in the forest floor habitat. Shrub, understory leaf litter, and tree trunk are in the understory habitat.

We found that the relative leg length was longer for harvestmen in the understory than on the forest floor (Fig 2). We performed a t-test and had a t-value of -5.8303 and a p-value of $8.866e-7$ for 39.058 degrees of freedom. This means that there was a significant difference between the relative leg lengths for the two habitats.

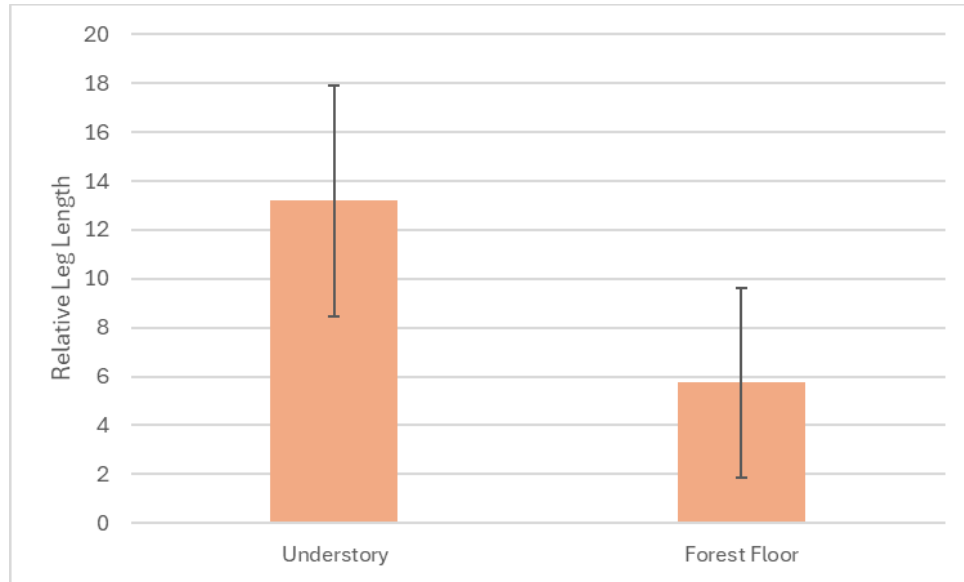


Figure 2. Relative leg lengths for harvestmen in the understory and forest floor habitats. Relative leg length was calculated by taking the average leg length to body length ratio for each habitat.

We compared the relative leg lengths between the microhabitats within each habitat as well. The relative leg lengths for harvestmen in the forest floor microhabitats (leaf litter and decaying logs) were similar. We performed a t-test and got a t-value of 1.3673 with 14.023 degrees of freedom and a p-value of 0.1931. This means that there is no difference between the relative leg lengths in the forest floor microhabitats.

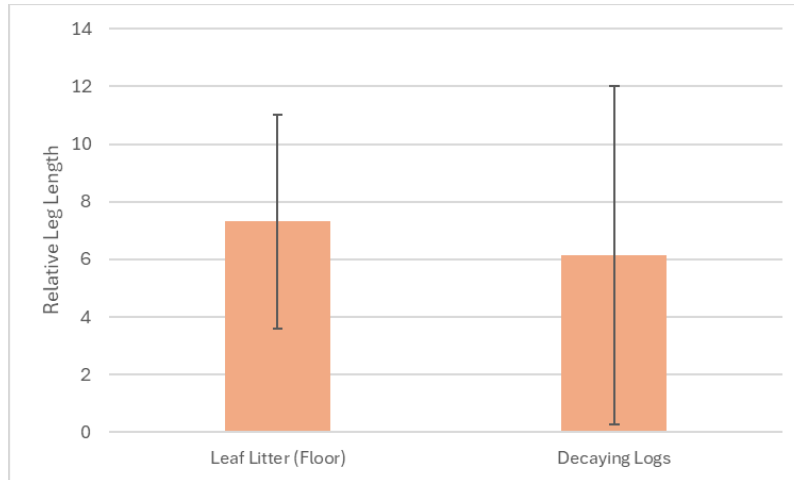


Figure 3. Relative leg lengths of harvestmen in the forest floor habitat.

For the understory habitat the relative leg lengths were similar as well. (Fig 5). We used an analysis of variance (ANOVA) test and got a f-value of 3.3541 with 29 degrees of freedom and a p-value of 0.5185. This result means that there was a slight difference between the relative leg lengths in the understory microhabitats.

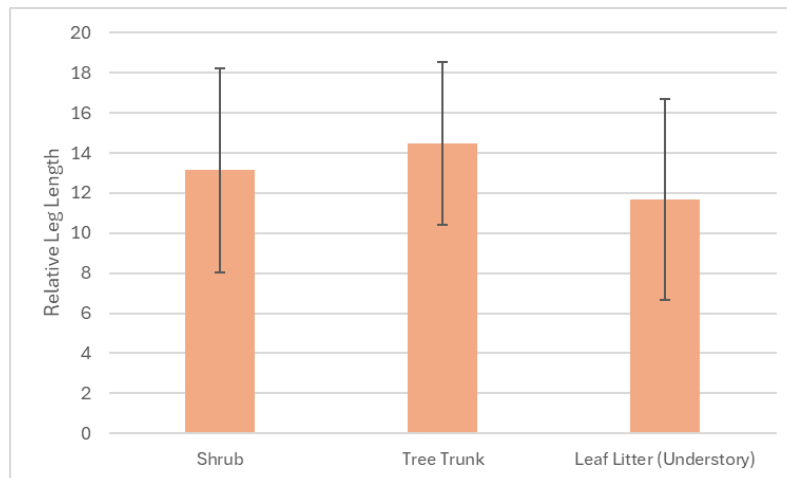


Figure 4. Relative leg lengths of harvestmen in the understory habitat.

DISCUSSION

Through the course of our research, we aimed to learn whether there was a relationship between the relative length of Opiliones' legs and the five microhabitats they inhabited within two general habitats, forest floor and understory. Our findings expanded on a point made in a 2012 study conducted at La Selva where research did not find enough empirical evidence to determine the existence of a relationship between leg length and habitat preference (Proud et al. 2012). We were able to address this knowledge gap by exploring the pattern of leg length and habitats of harvestmen which was proved to be related in temperate regions (J. Adams 1984). However, we utilized data collected during the day to research the harvestmen rather than collecting the individuals at night. This was a purposeful decision due to the order Opiliones' being nocturnal foragers (Acosta & Machado. 2007) and so during the day, they would return to their original location (Silva et al., 2018). By collecting the subjects during the day, we had more accurate knowledge of the true microhabitats each species inhabited.

57.5% of the collected 47 Opiliones fell into the Sclerosomatidae family, 19.1% were members of the Cosmetidae family, 12.8% were from the Zalmoxidae family, and the last 10.6% were a part of the Gonyleptidae family. Considering over half of our collected specimens were Sclerosomatidae, there is some concern that our data could be skewed. What is most interesting about the Sclerosomatidae we collected was that aside from only two individuals who we believe to be outliers; all were collected in the understory habitat. This finding supports our hypothesis that leg length is correlated to the habitats harvestmen inhabit considering Sclerosomatidae have the longest average leg length. The Cosmetidae family, our second most abundant group, was located nearly in equal proportion across the microhabitats which was a unique attribute not seen in the other families. A potential explanation for this that aligns with

our hypothesis is that their intermediate average leg length allows them to be successful in all the microhabitats. While their numbers were small, each of the three individuals we classified as members of the Zalmoxidae family could not be identified to their species or genus. While we were aware from the beginning about the significant gaps in our knowledge about the Opiliones order, being unable to fully classify the Zalmoxidae makes this glaringly obvious. In agreement with our hypothesis, both the individuals of the Zalmoxidae family and the Gonyleptidae family were found solely in the forest floor microhabitats. Both these groups had the shortest average leg lengths and were located only through moving leaf-litter around.

Through statistical analysis, we compared the average leg-length of Opiliones of each microhabitat to the others that fell under the same habitat umbrella. A T-test was used to compare the forest floor microhabitats to one another (Fig. 3) and it was concluded that there was no difference between the two. Similarly, in the ANOVA test that was run for the microhabitats in the understory (Fig. 4), there was only a slight difference. Considering both results did not support our hypothesis fully, we tested to see if there was a statistically significant difference between the two overarching habitats. Running a T-test to compare the understory habitat to the forest-floor habitat (represented by Fig. 2) we learned that there was a difference between the relative leg lengths of Opiliones located in these two different habitats. This indicates there to be a stronger correlation of relative leg-length to general habitats of harvestmen rather than their microhabitats. There is a possibility of a stronger correlation to microhabitats than the one observed in our study due to our small sample size. If we were to replicate this experiment exactly, it would be essential to collect individuals for more than one day and in other locations than the 50m section used.

For future exploration into the relationship between the relative length of Opiliones' legs and their microhabitats, it is essential for more field sessions to occur. Due to our limited

timeline, we only surveyed a single location along the selected trail, resulting in potentially biased data. Additionally, there is the possibility of overlooked species that would disprove our hypothesis located in other microhabitats that have not been researched (i.e. forest canopy and epiphytes). To better understand the extent of Opiliones' diversity within the tropics, studies should also be conducted in other locations, not only at La Selva Biological Research Station. If this further research does in fact disprove our reformed hypothesis that average leg length is correlated to the general habitats of Opiliones families, the possibility of a correlation to courtship rituals should be considered. No research has yet been done to address this possibility within the tropics and could potentially provide insight into the diversification of Opiliones populations.

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