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Soil Preference and Burrow Characteristics of Two Theraphosidae Species in Penang Island, Malaysia

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Abstract: Tarantulas play a crucial role in maintaining ecological balance by regulating insect populations. However, little is known about the soil preferences and burrow structures of tarantulas in Malaysia. This study aims to determine the soil preference as well as the burrow structure of *Coremiocnemis cunciularia* and *Chilobrachys andersoni* from Penang Island. The soil characteristics of the soil samples collected around the burrows of *Coremiocnemis cunciularia* (n = 30) and *Chilobrachys andersoni* (n = 30) were determined using soil texture analysis. The measurements and burrow structures from adults and juveniles of *Coremiocnemis cunciularia* and *Chilobrachys andersoni* were determined. It was revealed that the moisture content and clay percentage in the soil samples around burrows of *Chilobrachys andersoni* and *Coremiocnemis cunicularia* were significantly different. Meanwhile, there is some variation in the structure and measurements of the burrows of the two tarantula species. The findings in this study could be useful for the conservation works and habitat management of tarantulas on Penang Island.

Keywords: *Arachnid, burrow structure, tarantula, soil characteristics.*

1. Introduction

Tarantulas, which belong to the Theraphosidae family, are known for their imposing size and distinctive appearance. Despite their formidable reputation, tarantulas play a crucial role in maintaining ecological balance by regulating insect populations. Furthermore, Theraphosidae can act as the sentinel or indicator species to determine the health and stability of the ecosystem (Wilson et al., 2012). The presence of these eight-legged marvels is scattered across diverse habitats worldwide. From dense rainforests to arid deserts, tarantulas have adapted to a wide range of environments, demonstrating remarkable resilience and versatility (Bertani, 2013).

Briefly, tarantulas are terrestrial spiders that burrow underground as a temporary or permanent home, providing shelter from adverse weather conditions, protection from predators, a place to ambush prey, and a breeding ground (Hils & Hembree, 2015). Additionally, fossorial tarantulas, such as Cyriopagopus lividus, often require an environment with a deeper layer of substrate when constructing a burrow (Marnell, 2016). Spiders dig their burrows by using both their fangs and pedipalps, which can efficiently create a hole inside the soil (Hils & Hembree, 2015). Furthermore, spiders will create webbing on the wall burrow, depending on the species (Pérez-Miles et al., 2005; Hils & Hembree, 2015). However, the structure of the burrow varies, depending on the species, size, and behaviours of the tarantula (Pérez-Miles et al., 2005; Machkour-M'rabet et al., 2007; Hils & Hembree, 2015).

Malaysia, blessed with diverse ecosystems ranging from dense rainforests to urban pockets of greenery, provides a habitat for several species of tarantulas. To date, three species of Theraphosidae have been previously found living on Penang Island, including Omothymus schioedtei, Coremiocnemis cunicularia, and *Chilobrachys* sp. (Thorell, 1891; Karsch, 1892; Simon, 1892). The *Omothymus schioedtei* is an arboreal living tarantula, while the other two species live terrestrially (Thorell, 1891). Interestingly, *Coremiocnemis cunicularia* is fossorial in nature, preferring its habitat on sloped ground with shaded areas at elevations above 600 m (West & Nunn, 2010). Unfortunately, there have been no in-depth studies on the soil preferences and burrow structures of Malaysian Theraphosidae. Therefore, the present study aims to determine the soil preferences and burrow structures of *Coremiocnemis cunicularia* and *Chilobrachys andersoni* found in Penang Island, Malaysia.

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2. Materials and Methods

Study Sites

This study was done at several selected locations in the Bukit Bendera Biosphere Reserve on Penang Island, Malaysia. The study areas include Bukit Bendera, Penang Hill, Penang National Park, Balik Pulau, Air Itam Dam, and Elvira Hill, as shown in Figure 1.

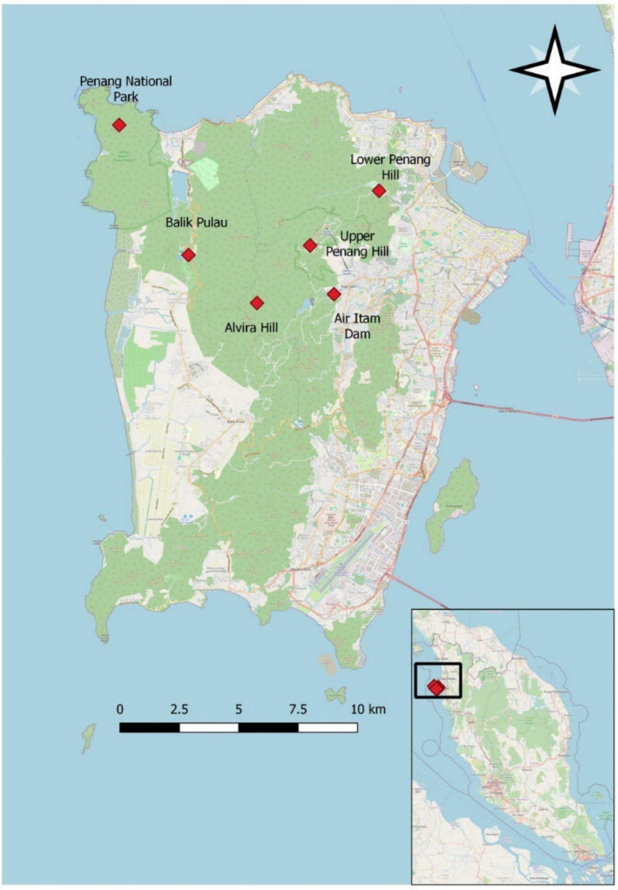

Figure 1. The study sites are on Penang Island.

Identification of Tarantula Species

In this study, various sampling sites inside Penang Island were selected based on the availability of the tarantula burrows. The sampling periods began in May 2022 and continued until September 2022. As the tarantulas are nocturnal animals that stay near their burrows during nighttime, all fieldwork was conducted at night to facilitate the identification of tarantulas (Fukushima et al., 2019). The species can be differentiated by their physical morphology. The *Coremiocnemis cunciularia* has finer hairs and shorter legs, but with stouter and black femurs, a cinnamon-colored carapace, and long hair on its hind legs. (West & Nunn, 2010). The Chilobrachys andersoni generally have long legs, a carapace that is slightly shorter than the protarsus number IV, and coarser hair, with a light brown body coloration (Pocock, 1900). Voucher specimens from the two species were deposited at Universiti Malaysia Terengganu (UMT). The burrow of the tarantula was detected by the presence of silk webbing, covering the burrow entrance and the burrow wall (Machkour-M'rabet et al., 2007).

Soil Characteristic Analysis

In this study, sixty soil samples were collected from the burrows of Chilobrachys andersoni (n = 30) and Coremiocnemis cunciularia (n = 30) to investigate the moisture content, pH value, type of soil, as well as the proportion of sand and clay (%) within the soil samples. The sampling was conducted at night and on dry days to minimize the impact of weather on the soil's moisture content. During sampling, 500 g of soil samples were weighed and collected around 50 cm from the burrow entry of *Chilobrachys andersoni* and *Coremiocnemis cunciularia*. The soil characteristics around the tarantula burrows were determined using a soil texture analysis (jar method), following the United States Department of Agriculture soil triangle, and applying the soil moisture content standard test method of the Australian Department of Sustainable Natural Resources (Canning et al., 2014). Before conducting the soil moisture content analysis, the pH values of the soil samples were determined using a pH meter. For soil moisture content analysis, each soil sample was weighed before and after drying in an oven at 100 ˚C. The differences in weight between the soil before and after drying were recorded as a percentage (%). After that, the soil samples were sieved to remove extraneous soil components, including organic matter, leaves, and stone particles. The sieved soil samples were then placed into a cylindrical jar. Then, the soil samples were mixed with water and a tablespoon of detergent. Then, the mixture of soil and water was thoroughly shaken and left to stand for 24 h. After 24 h, the soil samples were divided into different layers. The length of each layer was measured and compared to the total length of the soil samples to determine the percentages of clay, silt, and sand within the soil samples.

Burrow Characteristics Analysis

In the present study, the burrow characteristics of Chilobrachys andersoni and Coremiocnemis cunciularia were investigated to determine burrow diameter (BD), total burrow length (TBL), and total burrow depth (TBD). As the abundance of two tarantula species is relatively low in the sampling areas, only four burrows from *Chilobrachys andersoni* (n = 4) and *Coremiocnemis cunciularia* (n = 4) were selected to prevent disturbance to the tarantula population. Prior to conducting the experiments, the tarantula was carefully removed from its burrow by luring it out. The tarantula species was identified and released back into the wild. The burrow characteristic was determined by using Plaster of Paris (POP) to know the shape of the burrow (Chakraborty, 2017). First, the aqueous solution of Paris was poured into the burrows with the help of an air pump and syringe until the burrows were filled. It was then allowed to dry for 30 to 60 min. The casts were then carefully dug up with a spade and cleaned to remove any unwanted sediment from their surface. Each cast was then marked. The burrow casts were then brought to the laboratory for further analysis. The BD, TBL, and TBD of the burrows were then measured using the parameters, as shown in Figure 2 (Qureshi & Saher, 2012).

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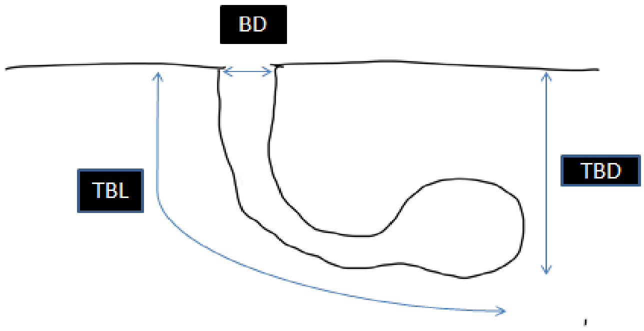

Figure 2. The measurement of the characteristics of the burrow. BD = Burrow diameter; TBL = Total length; TBD = Total burrow depth.

2.5 Data Analysis

The collected data on soil and burrow characteristics were analyzed using descriptive statistical methods. The t-test was used to compare the soil characteristics of the two tarantula species. PCA was applied to continuous data, including soil moisture content, pH value, as well as the sand, silt, and clay content, to determine the most significant parameters contributing to the highest variation in the burrows of *Coremiocnemis cunicularia* and *Chilobrachys andersoni*. The PCA was carried out based on the variance-covariance matrix. All statistical analyses were performed using PAST software version 4.03 (Kędzior & Kosewska, 2022; Mugot, 2021; Viana et al., 2021).

3. Results

Distribution of Tarantula Species

In this study, *Coremiocnemis cunicularia* was found at Bukit Bendara, Penang Hill (above 400 m above sea level), and Elvira Hill. Meanwhile, *Chilobrachi andersoni* was detected at Penang Hill (below 400 m above sea level), Penang National Park, Balik Pulau, and Air Itam Dam, as shown in Table 1.

Table 1. The presence of tarantula species at sampling sites.

No	Localities	GPS	*Coremiocnemis cunicularia*	*Chilobrachi andersoni*
1	Bukit Bendera	5°25'35.8"N, 100°15'08.0"E	Yes	No
2	Penang Hill > 400m a.s.l	5°25'22.0"N, 100°15'54.6"E	Yes	No
3	Penang Hill < 400m a.s.l	5°24'30.7"N, 100°16'37.3"E	No	Yes
4	Penang National Park	5°27'21.4"N, 100°11'50.0"E	No	Yes
5	Balik Pulau	5°25'36.1"N, 100°13'10.6"E	No	Yes
6	Air Itam Dam	5°23'57.2"N, 100°16'28.9"E	No	Yes
7	Elvira Hill	5°23'27.2"N, 100°14'26.6"E	Yes	No

* N = North; E = East; a.s.l = Above sea level.

Soil Characteristics

The average moisture from soil samples close to burrows of *Chilobrachys andersoni* was 11.4%, whereas that of *Coremiocnemis cunicularia* was 22.2%. The average pH value of the soil samples near the burrows of Chilobrachys andersoni and Coremiocnemis cunicularia was 6.31 and 6.25, respectively. Meanwhile, the average percentages of sand, silt, and clay in the soil from Chilobrachys andersoni burrows were 75%, 12%, and 10%, respectively. As for the soil samples from the *Coremiocnemis cunicularia* burrow, the average percentages of sand, silt, and clay were 80%, 14%, and 6%, respectively. The types of soil around the burrows of *Chilobrachys andersoni* were mostly sandy loam (n = 23), followed by loamy sand (n = 6) and sand (n = 1). However, the soils around the burrow of *Coremiocnemis cunicularia* were mostly loamy sand (n = 24), followed by sandy loam (n = 6), as shown in Table 2.

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Table 2. Loadings showing the association between soil characteristics and PC1 and PC2.

No.	Soil characteristics	PC 1	PC 2
1.	Moisture content (%)	0.138	**0.876**
2.	pH value	0.001	-0.004
3.	Sand content (%)	**0.958**	-0.064
4.	Silt content (%)	-0.236	0.363
5.	Clay content (%)	-0.085	-0.312

Boxplot analysis of the moisture content of the soil samples demonstrated that the boxplots of both species appear asymmetric and normally distributed, as shown in Figure 3(A). Nonetheless, both species have different ranges of moisture content in their soil. The value from the boxplot of *Chilobrachys andersoni* was between 10% and 15%, whereas Coremiocnemis cunicularia ranged between 20% and 25%. The pH values for both species were not significantly different from each other. The soil pH values of the soil around *Coremiocnemis cunicularia* burrows ranged from 6.1 to 6.4. Meanwhile, the pH values of the soil around the burrow of Chilobrachys andersoni ranged from 6.1 to 6.4, as shown in Figure 3(B). The soils around the burrow of *Chilobrachys andersoni* have a maximum pH value higher than that of *Coremiocnemis cunicularia*. In addition, the boxplot also revealed that the percentage of the sand range for *Chilobrachys andersoni* was lower compared to *Coremiocnemis cunicularia*, as shown in Figure 3(C). Moreover, the range of sand percentage of soil samples from the burrows of *Coremiocnemis cunicularia* was higher (9% to 17%) compared to soil samples from *Chilobrachys andersoni* burrow soil (7% to 14%), as shown in Figure 3 (D). Lastly, the percentage of silt was different between the two species. Based on Figure 3 (E), the percentage ranges of silt in soil samples surrounding the burrows of *Chilobrachys andersoni* (8% to 12%) were higher as compared to the soils around burrows of *Coremiocnemis cunicularia* (4%-6%).

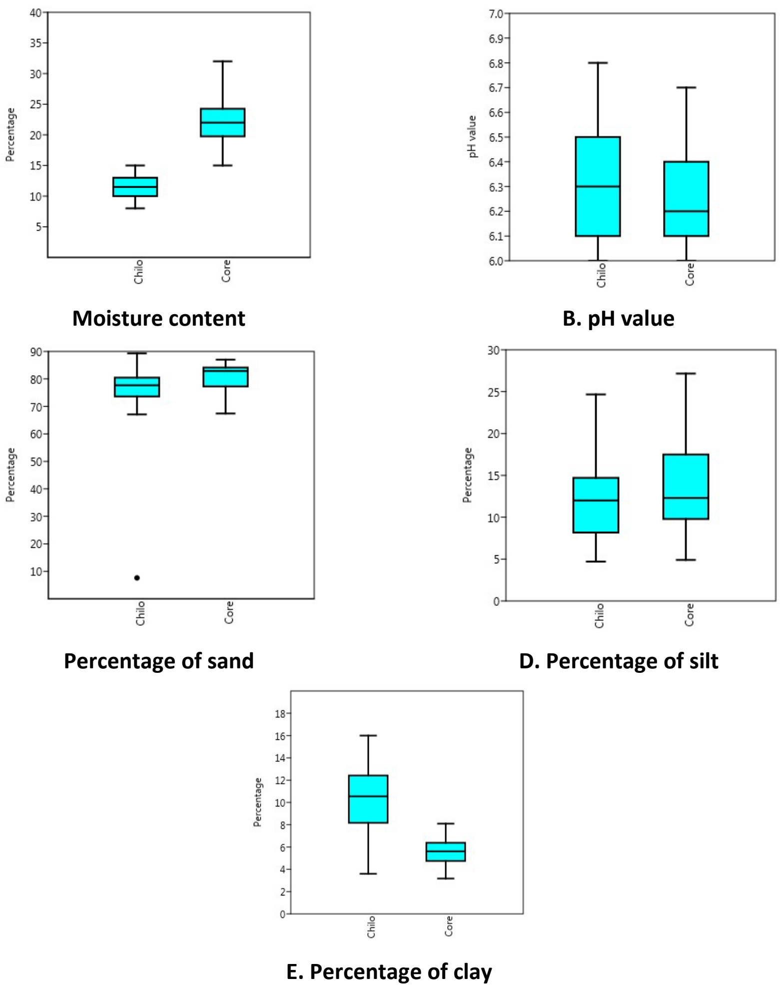

Figure 3. The boxplot for the (A) moisture content of the soil, (B) pH value of the soil, (C) Percentage of sand in the soil, (D) Percentage of silt in the soil, (E) Percentage of clay in the soil. Chilo represents *Chilobrachys andersoni*, while Core stands for *Coremiocnemis cunicularia.*

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The T-test analysis demonstrated that the moisture content of the soil (p-value = 0.0001) and clay percentage in the soil samples (p-value = 0.0001) around burrows of *Chilobrachys andersoni* and *Coremiocnemis cunicularia* were significantly different. Nonetheless, the pH value (p-value = 0.26), sand percentage (p-value = 0.06), and silt percentage (p-value = 0.19) between the two species were not significantly different.

PCA Analysis of Soil Samples

Regarding the PCA test of the continuous microhabitat parameters, principal component 1 (PC1) and principal component 2 (PC2) explain 64.8% and 23.6% of the variance, respectively. It was found that PC1 is strongly associated with the percentage of sand within the soil samples. Hence, the sand content within the soil samples was the most significant parameter contributing to the variation observed in the soil samples of both species. Meanwhile, PC2 showed the strongest association with the moisture content of the soil samples, as shown in Table 2. A scatter plot based on PC1 and PC2 was generated, as shown in Figure 4. It was revealed that the percentages of sand in the soil samples of the species *Coremiocnemis cunicularia* and *Chilobrachys andersoni* showed variation. Furthermore, the moisture content of soil samples from the burrows of *Chilobrachys andersoni* appeared to be lower compared to *Coremiocnemis cunicularia*. Nonetheless, *Coremiocnemis cunicularia* and *Chilobrachys andersoni* can be clustered into main groups, illustrating the differences between these two species based on the soil characteristics around their burrows.

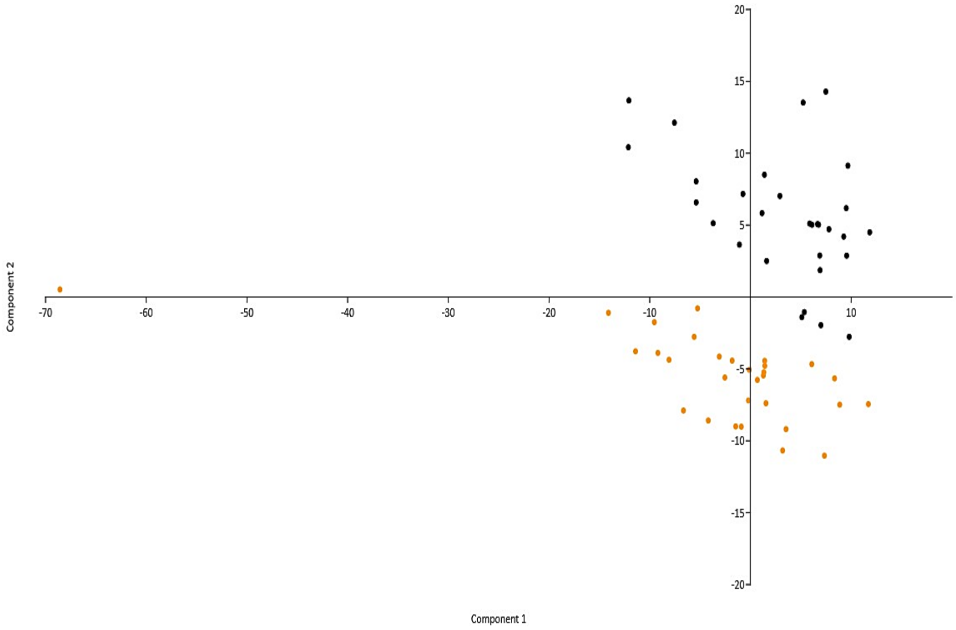

Figure 4. PCA scatter plot (PC1 vs PC2) for the soil characteristics of *Coremiocnemis cunicularia* (black colour dots) and *Chilobrachys andersoni* (orange colour dots).

Burrow Characteristics

In this study, a total of four samples of burrows (three burrows from juvenile and one burrow from adult tarantula) from each species were examined. The burrow of the adult *Coremiocnemis cunicularia* was 'U' shaped with a basal chamber near the end of the burrow as shown in Figures 5 (A) and 5 (B). The BD, TBL, and TBD of the burrow from adult *Coremiocnemis cunicularia* were 4.0, 51.2, and 39.6 cm, as shown in Table 3. The shaft of the burrow is quite long, with a uniform diameter up to the basal chamber. Meanwhile, the burrow of the adult *Chilobrachys andersoni* was 'C' shaped, as shown in Figure 5 (C). The BD, TBL, and TBD of the burrow of adult *Chilobrachys andersoni* were 4.7, 36.5, and 31.0 cm, respectively, as shown in Table 3. The burrow of adult *Coremiocnemis cunicularia* has a well-built, smooth surface. In contrast, the burrow of *Chilobrachys andersoni* is rough and has a small stone effect on its shaft and basal chamber.

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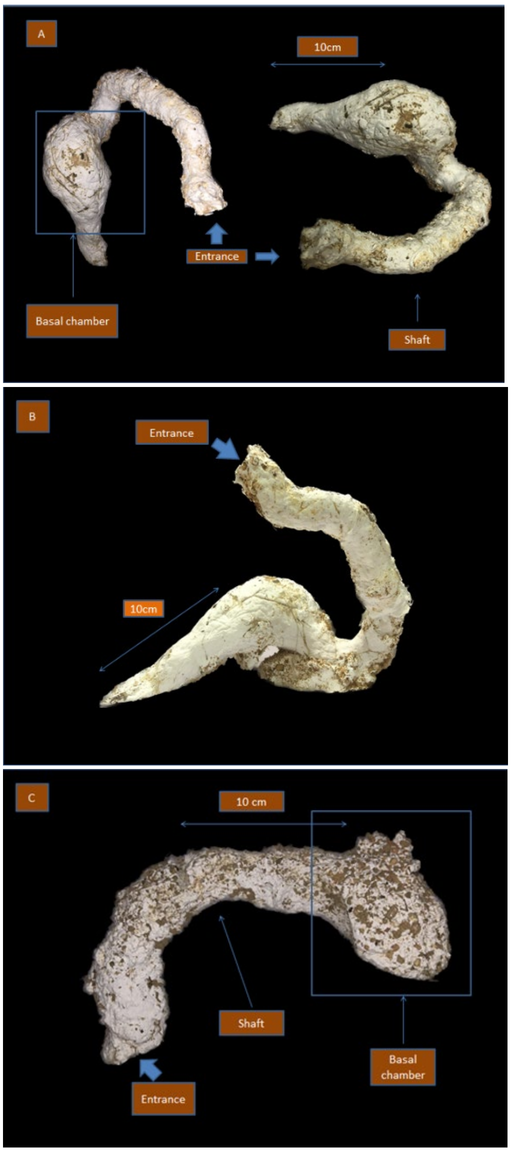

Figure 5. Plaster cast of burrows using POP. (A) and (B) are the burrow of adult *Coremiocnemis cunicularia,* whereas (C) is the burrow of adult *Chilobrachys andersoni*.

Table 3. The burrow diameter (BD), total burrow length (TBL), and total burrow depth (TBD) of the burrows for both *Coremiocnemis cunicularia* (n = 4) and *Chilobrachys andersoni* (n = 4).

	Species	Burrow measurement (cm)		
		BD	TBL	TBD
Adult	Core 1	4.0	51.2	39.6
	Chilo 1	4.7	36.5	31.0
Juvenile	Core 1	1.5	27.5	24.0
	Core 2	1.3	27.1	21.2
	Core 3	1.2	28.7	26.1
	Chilo 1	1.9	27.5	19.5
	Chilo 2	2.0	24.3	20.6
	Chilo 3	2.1	27.3	23.6

* Core = *Coremiocnemis cunicularia*; Chilo = *Chilobrachys andersoni.*

Regarding the structure of burrows from juveniles, the shape of the burrows of *Coremiocnemis cunicularia* was more defined and sophisticated. Moreover, the shaft of the burrows of *Coremiocnemis cunicularia* is better shaped compared to juveniles of *Chilobrachys andersoni,* as shown in Figures 6 and 7. Furthermore, the burrow basal chamber of juveniles *of Coremiocnemis cunicularia is smoother, and its* shape is more visible. Meanwhile, the burrows of juveniles of *Chilobrachys andersoni* have coarse walls, which are compensated for by crevices in the soil. Both species were observed to construct additional tunnels within their burrows, as shown in Figures 6(c) and 7(c). The BD, TBL, and TBD of burrows from juveniles of *Coremiocnemis cunicularia* ranged from 1.2 to 1.5 cm, 27.1 to 28.7 cm, and 24.0 to 26.1 cm, respectively (Table 2). The burrow diameter of juveniles of *Coremiocnemis cunicularia* is smaller than that of *Chilobrachys andersoni,* as shown in Table 2. However, the ranges for TBD and TBL between the juvenile species were not significantly different.

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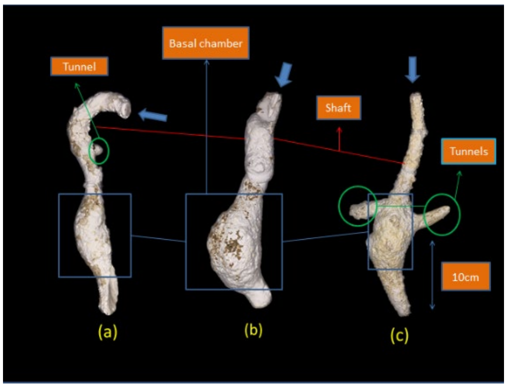

Figure 6. Plaster cast of three burrows of juvenile *Coremiocnemis cunicularia*.

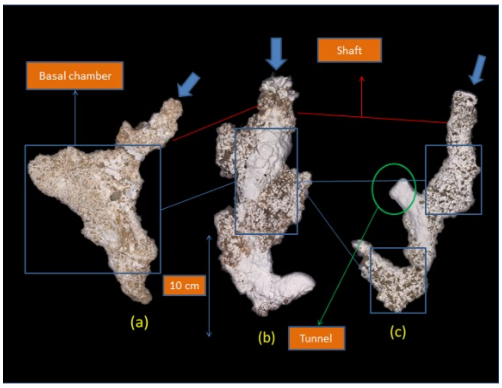

Figure 7. Plaster of cast burrow for juvenile samples of *Chilobrachys andersoni*.

4. Discussion

Soil type is considered one of the most important criteria when it comes to terrestrial Theraphosidae habitat selection. Due to the fossorial lifestyle of tarantulas, it is important to study the soil type preferred by these arachnids on Penang Island to better understand the possible habitat of the tarantula species (West & Nunn, 2010). From the soil samples, it was revealed that both species have different preferences for soil types. *Coremiocnemis cunicularia* prefers loamy sand, whereas Chilobrachys andersoni prefers sandy loam. In the past, several studies have linked soil characteristics with the habitat preference of certain Theraphosidae (Canning et al., 2014; Machkour-M'rabet et al., 2007; Yáñez & Floater, 2000). For example, *Nesiergus insulanus* prefers to build burrows in sandy loam-type soil, while *Brachypelma klaasi* prefers sandy soil (Yáñez & Floater, 2000; Canning et al., 2014). Meanwhile, *Brachypelma vagans* commonly build their burrows on soil with high clay content while avoiding soil with high stone density (Machkour-M'rabet et al., 2007). Furthermore, it was revealed that *Ami bladesi* favor clay soil (Lapinski et al., 2018).

The structure of the burrow is one of the most crucial factors for the tarantula's survival. However, the burrow structure of the tarantula on Penang Island is poorly understood. As the number of tarantulas in the sampling areas is low, only four samples of burrows from each species were taken to minimize the disturbance to the local tarantula population. In this study, the burrow structures of both species of tarantula differed, mainly in terms of the inner surface of the burrows. These differences in burrow structure may be due to different behaviour or stage of life of the tarantula species. In this study, the burrows of juveniles of *Chilobrachys andersoni* have coarse walls, possibly due to the preference of this tarantula species to build its burrows around crevices, without significantly altering the internal structure of the burrow. Another study by Marshall & West (2008) also showed that the burrow characteristics of tarantulas differ according to their stage of life. In addition, the height, depth, and structure of burrows can also vary between species within the same genus. For instance, a study by Machkour-M'rabet et al. (2007) demonstrated that *Tiltocatl vagans* can have four different types of burrow structures, where the adult female constructs a more complex burrow with more chambers. Another recent study showed that the burrows of *Nesiergus insulanus* can be J-shaped, V-shaped, and U-shaped (Canning et al., 2014).

Tarantulas, which belong to the Theraphosidae family, are known for their imposing size and distinctive appearance. Despite their formidable reputation, tarantulas play a crucial role in maintaining ecological balance by regulating insect populations. Furthermore, Theraphosidae can act as the sentinel or indicator species to determine the health and stability of the ecosystem (Wilson et al., 2012). The presence of these eight-legged marvels is scattered across diverse habitats worldwide. From dense rainforests to arid deserts, tarantulas have adapted to a wide range of environments, demonstrating remarkable resilience and versatility (Bertani, 2013).

5. Conclusion

In summary, this study reveals that both species have distinct soil requirements and habitat preferences. Thus, it is essential to conserve the natural habitat of spiders, particularly in hilly areas, to protect and maintain their populations in the wild. This information can serve as a reference for Bukit Bendera and The Habitat Foundation regarding the conservation efforts for tarantulas on Penang Island, Malaysia.

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