

Morphological Characteristics of Cave and Surface-dwelling Ants (Hymenoptera: Formicidae) in Puting Buhangin, Prosperidad, Agusan Del Sur

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ARTICLE INFO

Keywords: Ants, Caves, Environment, Formicidae, Morphology

Received : 05, January

Revised : 10, February

Accepted: 15, March

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ABSTRACT

In this study, it was hypothesized that morphological differences were evident between the surface-dwelling and cave ant faunas. There were 9 species of ants found in the study area, namely; *Aenictus* sp., *Anoplolepis gracilipes*, *Aphaenogaster* sp., *Carebara* sp., *Crematogaster* sp., *Hypoponera* sp., *Odontoponera* sp., *Polyrhachis* sp., and *Tetramorium* sp. In this study, limited ant species are present in the cave; in fact, only one species of ant, *Aphaenogaster* sp., was found in the cave entrance. This study identified no morphological changes between surface-dwelling and cave ants. For accurate data, this research recommends more studies on cave ant morphology.

INTRODUCTION

Environment influences species ecology and evolution. If a species is to survive, it must be able to adapt to the local conditions, including the temperature, rainfall, humidity, complexity of the habitat, and disturbance regimes (Weiher *et al.*, 2011). Evolutionary biology requires understanding natural phenotypic variation. Natural selection forces vary due to variations in the spatial and temporal environment, leading to an adaptive phenotypic divergence between populations and species (Endler, 1986). Comparing organisms that inhabit similar environments provides a particularly potent natural setting for establishing the relationship between environment and phenotypic evolution, whether similar adaptations appear repeatedly and independently in different populations of the same species (parallel evolution) or independently and convergently in different taxa (Schluter *et al.*, 2004; Bolnick *et al.*, 2018).

Due to their distinct and constant environments, "island-like" characteristics like simple communities and restricted gene flow, and similar environmental conditions in geographically separate locations creating independent replicates, caves provide a naturally repeated experiment for studying evolution (Mammola, 2019). Troglomorphy is the term Christiansen (1962) gave to the early recognition of repetitive morphological adaptations in the history of cave biology. Later, troglomorphy was expanded to encompass all phenotypic alterations caused by caves. Regressive and progressive adaptations are two categories into which these changes can be divided (Christiansen, 2012). Regressive adaptations reduce the visual system, but progressive troglomorphy increases extra-optic sensory systems (Pipan & Culver, 2012).

Ants, the world's most successful eusocial insects, have occupied most terrestrial habitats and left a worldwide ecological impact since their early Cretaceous origin. Due, in part, to the search for cave-adapted ants, the importance of individual ant colonies utilizing caves has not received adequate attention. Some ant species, particularly hypogaeic foraging species, have a strong affinity for subterranean environments and can quickly enter deep zones of some caves (Pape, 2016) where there is a stable, moisture-saturated atmosphere (Howarth, 1983). These characteristics are conducive to life in underground environments. Ants use pheromones for navigation in addition to secreting antimicrobial substances from a gland in their metapleura. This gives them defense against bacteria and fungi, which are more prevalent in caves, although they exist in all environments. The variability of foraging strategies among ant species often results in their opportunistic resource utilization in both epigeal and hypogean environments (Tinaut & Lopez, 2001; Silva & Silvestre, 2004). The lack of light, excessive moisture, and oligotrophy may be pre-adaptations to underground environments (Mammola, 2019).

Ant morphology, the environment, and life history have been investigated, revealing environmental filters and how ants exploit their morphology (Tinaut & Lopez, 2001; Silva & Silvestre, 2004; Gibb & Parr, 2010, 2013). The size grain hypothesis, for instance, has been the subject of several

studies examining the relationship between body size, leg length, and habitat complexity. According to this theory, ants should have relatively small legs to move around effectively in complex, interstitial habitats. In plain, flat areas, this limitation is eliminated, and rather lengthy legs are favored. Evidence for this has repeatedly been discovered (Kaspari & Weiser; 1999, Sarty et al. 2006; Gibb & Parr, 2010, 2013). Additionally, associations between diet and morphology are predicted. Small, laterally positioned eyes are typical of predatory ants (Weiser & Kaspari, 2006; Gibb *et al.*, 2014).

Given the difference in the habitat conditions between a cave and the surface environments, this study hypothesizes that morphological differences are evident between the ant faunas found in them. Moreover, this research aims to answer the following objectives:

- a. What are the species of ants in the cave and the surrounding areas of Puting Buhangin, Prosperidad, Agusan del Sur?
- b. What morphological distinctions exist between the ants in the cave and those in the surface environment that may be impacted by biotic and abiotic environmental factors?

METHODOLOGY

Study Site

Puting Buhangin Cave, situated at 8°36'12.4 "N 125°54'31.5 "E, at Purok 20 in Barangay Poblacion, is reachable from these intersections on the Daang Maharlika/National Highway: Purok 13 close to the Monato Institute of Technology, and Purok 20, Sitio Os-os in front of the Municipal Hall.

Being a Class II cave, according to the Regional Cave Committee, it is accessible to the general public for tourism purposes, but qualified cave guides must accompany visitors to the cave. It is a network of caves with numerous caverns, underground streams, and unbroken natural tunnels. Fruit bats, rare fish species, cave spiders, crickets, reptiles, and the Philippine swiflets' habitat are all part of its ecology.

The site is selected to establish a recorded study about the ant species and its morphological characteristics in the said area. Being a protected landscape, Puting Buhangin Cave is composed of 3 levels or sections, of which Levels I and II are used for tourism purposes, while Level III is for research purposes. The cave area is transected by streams and a cave river, while the surface and the surrounding areas are covered with vegetation.

Collection of Ants Species

Permission letters addressed to the municipal mayor and other authorities for the collection of specimens were disseminated before the study. The ant specimens were collected from later October to early November 2022. Collection of the specimens was done only during the daytime. Sampling was done only twice for the said time period.

Three 1-meter-square quadrats were placed within and outside the cave (Figueras & Nuñez, 2013). Bait, pitfall traps, and hand-picking were used to

gather ant species. Tuna (protein), peanut butter (carbs), nuts, and dead insects attracted trophic generalists and those with specific diets. Baits were put on paper platforms to attract dominant species, while oil beneath and surrounding the paper attracted less aggressive ants (Agosti *et al.*, 2000). The baiting strategy lasted 60–90 minutes since dominant species may find and recruit baits in this period. Pitfall traps were loaded with propylene glycol in 5.5-cm polypropylene cups. A few drops of unscented detergent weakened surface tension, preventing ants from escaping the traps. Traps were placed with little surface disturbance. Hand-reposition gritty sand, stones, and leaf litter were done. Digging was done with a little hand shovel. To better catch ants, traps were buried with their lips level with dirt or leaf litter. After placing the traps, free-roaming ants were hand-collected for 5 minutes at each location using a trimmed paintbrush. These methods assessed ground-foraging ant composition and richness (Bestelmeyer *et al.*, 2000). According to Pag-ong *et al.*, (2022) ten collecting locations (5 baits and 5 pitfall traps) with a 10-meter minimum spacing for each subpart were used.



Figure 1. Map of Prosperidad, Agusan del Sur with the study site, Puting Buhangin Cave (top photos); cave entrance, stalactites, and the vegetative part on top of the cave (bottom photos, left to right)

Identification of Ant Species

Identification of the ant species were made using the identification key presented by Goulet and Huber (1993) and the synoptic review of the ant genera (Hymenoptera, Formicidae) of the Philippines by General and Alpert (2012). Identification and comparison of species images were made using AntWeb (<https://www.antweb.org>) and Antbase (<https://www.antbase.net>).

Specimens were preserved in a 90% ethyl alcohol for future use. The assistance of ant taxonomists was also asked to identify and verify the ant species until the species level.

Selected Morphological Characteristics

Researchers have investigated the relationship between ant morphology, the environment, and life history, shedding light on potential environmental filters and how ants use their morphology (Gibb & Parr, 2010, 2013; Schofield, Bishop & Parr, 2016). The selected morphological characteristic was described qualitatively from the different ant species in the study area. The following characteristics were observed:

- a. Head size - Head size may vary allometrically with body size and be connected to predatory techniques. Broader heads allow for bigger mandibles and larger prey (Sarty *et al.*, 2006).
- b. Clypeus - Liquid-feeding animals have more modified clypeus (Davidson *et al.*, 2004).
- c. Mandible size - Larger mandibles enable bigger prey during predation (Weiser & Kaspari, 2006).
- d. Eye position - Visual predators have dorsally positioned eyes (greater interocular distance) (Fowler *et al.*, 1991).
- e. Eye size - Predatory species or low-light foragers may have larger eyes (Weiser & Kaspari, 2006; Narendra *et al.*, 2013).
- f. Leg length - In simple habitats, leg length boosts locomotion speed; in complicated ecosystems, it facilitates crevice exploitation and maneuverability (Sarty *et al.*, 2006).
- g. Mesosoma - Mesosoma indicates body size and resource utilization (Kaspari & Weiser, 1999).
- h. Color - Pigmentation may respond to thermoregulation and solar radiation, with lighter individuals in warmer climates (Willmer & Unwin, 1981).



Figure 2. (A) Lateral view and (B) head of *Odontoponera sp.* showing morphological features: (A) C-clypeus, E-eye, H-head, L-leg, Ma-mandible, Me-Mesosoma

RESULTS

This study served as preliminary data on ant species in Puting Buhangin Cave. There were 9 species of ants found in the study area. Eight ant species were found on the ground, the surrounding vegetation, and the mountain on top of the cave.

Table 1. The list of ant fauna recorded in Puting Buhangin

Species No.	Species	Subfamily
1	<i>Aenictus sp.</i>	Aenictinae
2	<i>Anoplolepis gracilipes</i>	Formicinae
3	<i>Polyrhachis sp.</i>	Formicinae
4	<i>Aphaenogaster sp.</i>	Myrmicinae
5	<i>Carebara sp.</i>	Myrmicinae
6	<i>Crematogaster sp.</i>	Myrmicinae
7	<i>Tetramorium sp.</i>	Myrmicinae
8	<i>Hypoponera sp.</i>	Ponerinae
9	<i>Odontoponera sp.</i>	Ponerinae

Only one species of ant, *Aphaenogaster sp.*, was discovered at the entrance to Puting Buhangin Cave. This was consistent with the findings of Wilson (1962) and Tinaut and Lopez (2001), who noticed incidental occurrences of ants at the cave entrance. The majority of species thought to be exclusively cavernicolous have been discovered outside of caves. Even the uncommon *Hypoponera ragusai*, which according to Tinaut (2001) is confined to caves in Europe, has been obtained outside of caves in France (Bernard, 1968) and on the Mediterranean islands of Lampedusa and Linosa (Mei, 1995).

Table 2. List of ants, nesting habitat, and habitat type

Species	Nesting habitat	Habitat type
<i>Aenictus sp.</i>	soil	surrounding forest
<i>Anoplolepis gracilipes</i>	soil, leaf litters, dead logs	surrounding forest
<i>Polyrhachis sp.</i>	soil, dead logs	surrounding forest
<i>Aphaenogaster sp.</i>	soil, dead logs	surrounding forest, specimen found near the cave entrance
<i>Carebara sp.</i>	soil, leaf litters	surrounding forest
<i>Crematogaster sp.</i>	soil, leaf litters, dead logs	surrounding forest
<i>Tetramorium sp.</i>	leaf litters, dead logs	surrounding forest
<i>Hypoponera sp.</i>	soil, rotten wood, leaf litter	surrounding forest
<i>Odontoponera sp.</i>	soil, leaf litters	surrounding forest

Abiotic factors may alter Argentine ant dispersal. Water limits the Argentine ant's range in California's Central Valley (Ward, 1987; Holway, 1995). Size and body color impact heating and desiccation, which may alter an ant's activity and range (Casey, 1976; Tremper, 1976). Behavioral strategies (Marsh, 1988), and nest structure may modulate temperature and humidity (Andrews, 1927; Brian 1973). Argentine ant workers are small, making them more susceptible to desiccation than many native ants (Tremper, 1976).

Silva *et al.* (2011) concluded that ant species density increased with the increasing number of leaves and a number of leaf morphotypes, but was also influenced by plot location factors such as plant species richness, vegetation structure, and environmental conditions. *Anoplolepis gracilipes* dominates the study region even at 125.37 masl. (Figure 3B). Eyes are well-developed and large. Presence of petiole is evident, while post-petiole is absent. It has a lighter orange pigmentation. Acidophore is present.

DISCUSSION

Ants have not given any indisputably troglobitic species, despite their regular appearance in caves. Based on the features of cave life, the theoretical debate of the possibility of a really cavernicolous ant raises an important issue about the ecological constraints on the idiosyncrasies of sociability. The lack of food in caves, even under extraordinary circumstances, would render this crucial deme population unsustainable (Wilson, 1962).

Aphaenogaster species (Figure 2C) has its head severely reduced posteriorly, the mandible has a distinguishing triangular form, the petiole and postpetiole are present, and the leg length is enlarged. Mesosoma has flexible pronotum, mesonotum, and propodeum. The eyes are located dorsally and are black in color. Ortuño *et al.* (2014) found *Aphaenogaster cardenai* in the Iberian Peninsula's Milieu Souterrain Superficiel (MSS), a hypogean environment with a huge network of microscopic gaps and fissures. This environment has been characterized for a long time as an ideal home for troglobitic organisms, but it is also good for other facultative species. However, he found that *A. cardenai* could not be classed as a hypogean species as a whole since the sexual forms execute the nuptial flight outside the subterranean habitat. Nonetheless, it is evident that the bulk of the colony, the workers, are connected to the hypogean environment, particularly the MSS or the shallow or superficial zones of the crack or fissure network, and therefore they use a hypogean approach.

Theoretical evolutionary solutions to this constraint would need a drop in colony size (which would be accompanied by a reduction in body size) and an increase in polygyny and polygamy (related to genetic variability in the

populations, i.e., Crozier & Pamilo, 1996). For a social insect species to exist in caves, it would have to become as similar as possible to a non-social species.

In the study of Figueras and Nuñez (2013), most of the caves have opportunistic ants. It is likely, given the presence of opportunist species, that all of the caves have been subjected to a significant amount of stress and disturbance. Observations of hypogeic army ants in caves imply that they would not go to considerable vertical depths in pursuit of food. However, they can be persistent inhabitants in relatively shallow and horizontal areas of caves, where they can hunt indigenous cave creatures (Pape, 2016).

Certain morphological adaptations of subterranean creatures, such as the absence of body coloration or the elongation of appendages, are notable (Pipan & Culver, 2012). Even these frequent changes are limited by factors other than the absence of light (which is ubiquitous in all caves; see Badino, 2000; Meja-Ortiz *et al.*, 2018), such as a lack of daily and annual rhythmicity and limited food sources (Culver *et al.*, 2010). Moreover, in this study, there are limited ant species present in the cave and, in fact, it was only found in the cave entrance. There is limited morphological data, so comparing morphological distinctions between surface-dwelling and cave ant species would be impossible.

It is crucial to recognize that neither apterism, brachypterism, anophthalmia, eye reduction, depigmentation, or elongation of appendages in reproductive forms or workers indicate adaptation to hypogean habitats in ants. These features help overcome subterranean habitats. Hypogean adaptations of ants should modify the colony's population structure and reproductive strategy (Wilson, 1962). Moreover, in the study site, one particular ant species, *Aenictus sp.* (Figure 3A), exhibits anophthalmia (absence of eyes) and it is found on the surface of the top of the cave site.

The study of parallel evolution (repeated, separate development of comparable traits in similar contexts) is a potent method for gaining an understanding of environment-dependent selection pressures. Surface-dwelling organisms that have frequently and independently invaded caverns provide unique models for such research. The species complex of the predominantly surface-dwelling crustacean *Asellus aquaticus* is an excellent candidate for such study since it has invaded various caves in Europe. Balazs *et al.* (2021) compared 17 functional morphological factors across six cave and nine surface populations of the *A. aquaticus* species complex to examine population divergence and sexual dimorphism.

Most of the ant species were hand-picked from leaf litters, rotten logs and branches, and some were on the leaves and stems of trees. Such species included *Aenictus sp.*, *A. gracilipes*, *Carebara sp.*, *Crematogaster sp.*, *Hypoponera sp.*, *Odontoponera sp.*, *Polyrhachis sp.*, and *Tetramorium sp.* These ant species have

mandibles that are suitable for foraging. Eyes are well-developed among these species, except in *Aenictus sp.*, which are mostly positioned dorsally and wider in size which may be found in more predatory species or may depend on the foraging period (Weiser & Kaspari, 2006; Narendra *et al.*, 2013). However, in this study, it was not noted whether the ant species are diurnal, crepuscular, or nocturnal. Further studies could be done in this area.

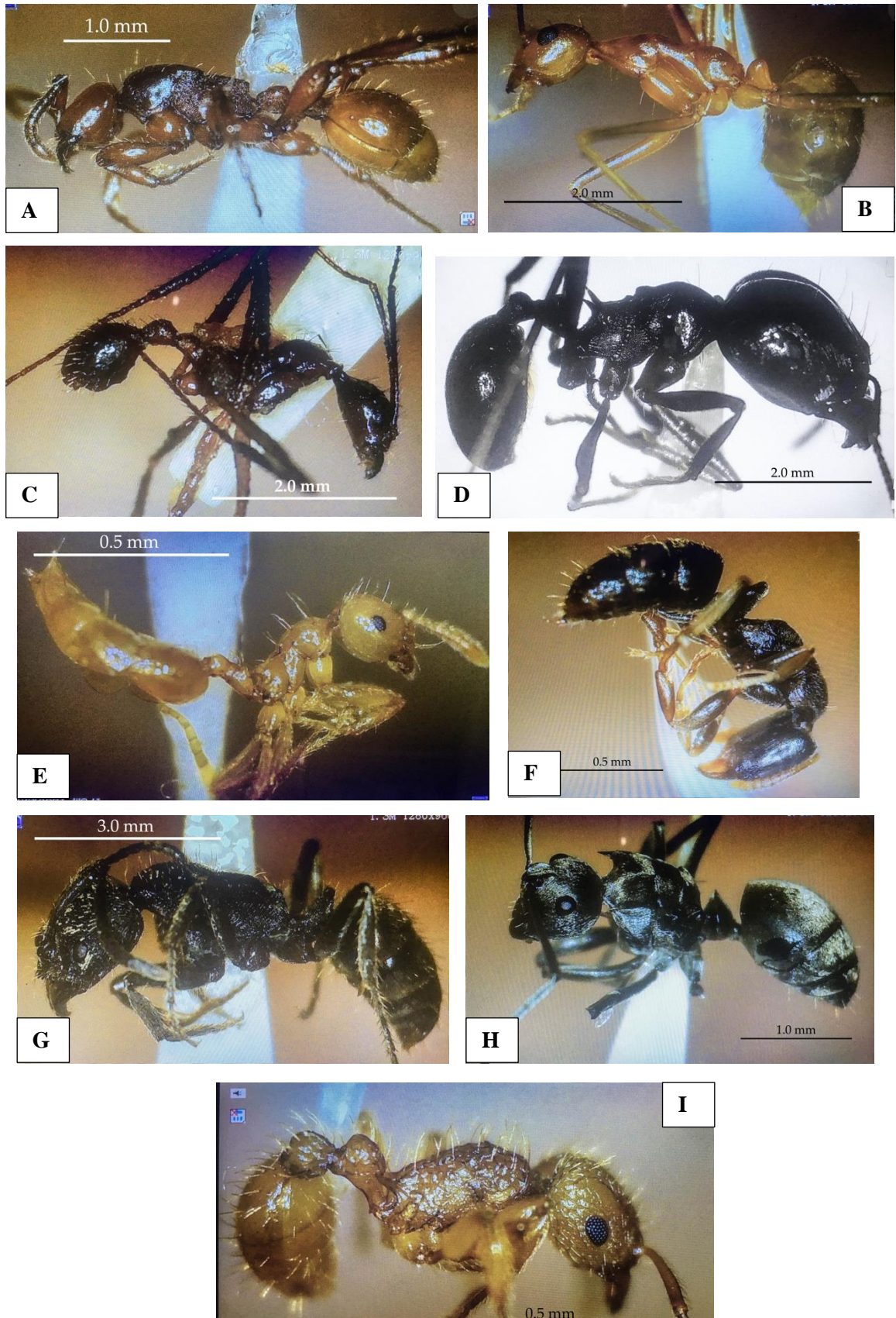


Figure 3. Ant species found in Puting Buhangin site; A-*Aenictus* sp., B-*Anoplolepis gracilipes*, C-*Aphaenogaster* sp., D-*Carebara* sp., E-*Crematogaster* sp., F-*Hypoponera* sp., G-*Odontoponera* sp., H-*Polyrhachis* sp., I-*Tetramorium* sp.

CONCLUSIONS AND RECOMMENDATIONS

Despite the fact that species have a wide variety of characteristics, physical characteristics have the greatest potential to be readily evaluated across a broad sample of species for whom little is known about them. Previous research has shown unequivocally that there are direct connections between the morphology of insects and their functions. The complexity of an environment, that of the ground and the cave environments as such in this study, may have an effect on an organism's morphology. However, in this study, there were no observed morphological distinctions between surface-dwelling and cave ants due to limited cave ant species. This research recommends studying cave ants and noting their morphological traits for accurate data.

ACKNOWLEDGMENT

The author would like to show his gratitude to the wonderful people of Putting Buhangin Cave Hub, and the local government unit of Prosperidad, Agusan del Sur, for allowing him to conduct his study in the said area. He would like also to recognize the assistance of Mrs. Josephine Dumas during the sampling.

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