





Morphological Characterization of Ant Species Found Within the Tropical Rainforest Zone of Calabar, Nigeria

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Abstract	Article History
<p>Aim: This study was carried out to unravel the morphological variations that exist among ant species in the Rainforest zone of the University of Calabar Community.</p> <p>Method: Ants were collected from three locations (Botanical Garden, Cameroon and Unical Farms) within the University of Calabar. Direct hand-picking method and the pitfall traps method were utilized for sample collection. Thereafter, different morphological parameters including total length, head width, eye length, etc. were measured and analysed.</p> <p>Results: The results obtained showed a dominance of Formicinae with 3 species (35 %), followed by Myrmicinae with 2 species (30 %), and lastly Ponerinae and Dolichoderinae and Dorylinae with one species (5 %) each. A total of 7, 4 and 2 ant species were collected from the University of Calabar Botanical Garden, <i>Cameroon</i> and University of Calabar Farm sites respectively. Out of the 7 ant species, 3 species (50 %) were common to all the locations on campus, while another 2 species (45 %) were found exclusive to the Botanical Garden site.</p> <p>Conclusions: This work established that the morphology of ant faunas found in various areas of the campus varied significantly. It also concluded that the morphological space occupied by the ant fauna of the Botanical Garden was significantly larger than those collected from the other locations.</p> <p>Keywords: <i>Ants, morphological, Rain forests, Calabar, Diversity</i></p>	<p>Received: 05 Aug 2023 Accepted: 18 Sept 2023 Published: 07 Oct 2023</p> <p>Scan QR code to view*</p>  <p>License: CC BY 4.0*</p>  <p>Open Access article.</p>
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Introduction

Ants are among the most abundant groups of invertebrates in terrestrial ecosystems, presenting a wide variety of feeding habits, nesting sites, and interactions with organisms from all trophic levels (Kaspari, 2000). Ants are mostly the small insects we find in our homes or gardens. They are ubiquitous, highly diverse and ecologically dominant faunal group of organisms, which represents up to half of the total insect biomass globally (Anderson, 2021). Insects constitute the largest class of arthropods, and arthropods are the largest and most divers animal phylum (Gonzalo and Gregory, 2019). An ant is an insect; it is one of the social animals that takes up to

or occupies about 15% of living terrestrial organisms in the planet. Rosumek (2017) opines that human understanding of the natural history of Neotropical ants is still limited, both due to a lack of detailed descriptive efforts and the widespread use of morph species in community studies.

Ants in general are regarded as omnivorous, feeding on a combination of live prey, dead animals, seeds and plant exudates, with some notorious specialized behaviors such as fungus cultivation and pollen consumption (Kaspari, 2000; Blüthgen and Feldhaar, 2010).

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Ants are deeply connected within terrestrial ecosystems (Anderson, 2021) and play a great role in our ecosystem. Some may include their roles as soil engineers, predators and recyclers of nutrients. They have particularly important interactions with plants as defenders against herbivores, as seed dispersers and as seed predators; as such they have had a significant influence on plant evolution and diversification. The deep connections of ants with ecosystems means that ant-species composition can provide important insights into ecosystem health (Anderson, 2021)

They are adaptive insects that are found in abundance and diversity in the terrestrial environment. Many species of ants are aggressive in behavior such as the red imported fire ant *Solenopsis invicta*, which is recognized as one of the world's most destructive invasive species (Epperson, Allen and Hogan, 2020). Invasive species adapts quickly to several types of habitats, such as the urban and agricultural areas (Ross *et al.*, 2009), and their activities quickly disperse other species of fauna.

The roles of ants in the ecosystem is of great importance, such that their extinction can lead to serious imbalance in the ecosystem. Ants help in decomposition of organic waste by so doing, they keep the environment clean, they also help in distributing of fruits seeds from one place to another. Ants also help in plants pollination. They are among the most ecologically diverse and successful animals on our planet. They have colonized most terrestrial environments they occur, they occupy major positions and have a strong ecological impact. This is made positive because as their important role as scavengers, predators, granivores, herbivores, and mutualists (Libbrech *et al.*, 2013).

A study by Rao and Vinson (2009) showed that male ants are easy to discount because they do not seem to do too much around the colony. To date, scientists have spent very little time studying male ants, but these mysterious and weird-looking creatures, invite a closer look. Male ants have relatively tiny heads and large eyes, and often look like wasps. Oxley (2014) reported that all workers are females, so pretty much every ant you see walking around is a girl, and pretty much every job ants do is done by workers. While queens get the colony rolling and keep it strong by laying eggs, workers get the groceries, keep intruders out, take out the trash, feed the babies, repair the house and more. This thus identifies the social roles of ants as queens, soldiers, and workers. In most of the species, workers can also differ in size and morphology (such as minors, majors and soldiers) (Oxley, 2014).

The morphological characterization of ants having to do with traits like shape, height, colour may reflect a combination of ecological and evolutionary responses by organism. The morphological study of ants is important because it will help to quantify the effects of land use on morphological diversity and maybe it will speed up the use of ants as biological indicators.

Despite their ecological dominance and their role as model organisms for ecological research, ants remain remarkably understudied (Anderson, 2021). In all tropical regions, the

biology of most species is virtually unknown, due to a combination of high richness, taxonomic uncertainty, lack of descriptive studies and widespread use of morphospecies in literature (Krell, 2004; Greene, 2005 cited in Rosumek, 2017). Taylor and Adedoyin (1978) identified *Oecophylla longinoda* and *Tetramorium aculeatum* as dominant species in Nigeria, and *Pheidole megacephala* as a co-dominant species. Further research in the area however proved abortive due to a significant lack in literature. This lack represents a void in the knowledge of ants in Nigeria.

Anderson (2021) rightly stated, ant-species composition can provide important insights into ecosystem health because of their deep connections with ants. Combined with their ubiquity and ease of sampling, ants serve as effective bio-indicators. As humans are part of the ecosystem in the University of Calabar, it would be an inconclusive assertion to claim that the ecosystem in the area is known without a study of the ant species. Ants are rarely studied in Nigeria generally and there is no published research article on ants covering the area around the University of Calabar. This therefore calls for research to be undertaken, to fill the knowledge void in the study of ants generally, and replace the narrative in Nigeria specifically. This study thus is expedient, as it seeks to study the ant species within the University of Calabar, Cross Rivers State Nigeria.

The populations of ants are greatly influenced by climatic factors, land management different soil-management strategies and the availability of resources. As time evolves, these factors can lead to morphological, physiological changes and behavioral traits in organisms from each population (Yales and Andrew, 2011) Because of the different strategies of land and soil management, several and important species of ants are going extinct and this is of great problem to the yield in agriculture. The level of the naturally decomposed soils are gradually being reduced, resulting gradually in an adverse effect in the environment, when these tiny species are not available, we no longer have traits that grow on their own because these animals are no longer present to carry them from one place to another. Natural predators of the ants like the spiders are forced to feed on other insects, thereby causing an imbalance in our ecosystem. Their extinction means they will no longer be there to act as biological indicators (Yates and Andrew, 2011). Therefore, this study was conducted to characterize ants species in the rainforest zone of the University of Calabar, Cross River State, Nigeria.

Aim of the study

The aim of this study was to evaluate the morphological characteristics of ant species found in the rain forest zone of the University of Calabar.

Materials and Methods

2.1 Study location and sample collection

The study was conducted within the University of Calabar Community. The University of Calabar (Unical) is situated in Calabar, Cross River State, South-eastern Nigeria. It is located approximately at latitude 4.8296°N and longitude 6.9671°E. It is one of Nigeria's second-generation universities and was up

till 1975 a campus of the University of Nigeria, Nsukka (Pascal, 2022).

2.2 Collection of samples

Samples were collected from ant species based on availability from three locations within University of Calabar. The locations were: Botanical Garden (a forested, undisturbed area), Cameroun (a highly busy part of the institution with human activities), Unical Farms (an agricultural area). Two sets of methods were employed in sample collection. The direct hand picking method and the pitfall traps method.

Two pitfall traps were deployed in different portions of each sampling location (near a tree and under a dead leaves heap). At each site, the two traps were arranged in a 3 × 3-m grid (Wang, Stranzanac and Butler, 2000). The design of the pitfall trap was similar to that introduced by Wang, Stranzanac and Butler (2000) with some improvements. Each pitfall trap contained an outer liner, a funnel, and an inner storage cup. The outer liner was a 454-ml plastic cup with a top diameter of 92 mm, bottom diameter of 60 mm, and a depth of 105 mm. The inner cup was a 100-ml capacity plastic cup, with a top diameter of 58 mm, bottom diameter of 48 mm, and a depth of 55 mm. The funnel rested in the top of the outer liner and extended down into the inner storage cup. Ants crawling into the trap fell through the funnel into the inner cup. The inner cup contained propylene glycol, which was a killing agent and preservative. This preservative is not known to either attract or repel ants, and it poses little threat to mammals if swallowed. For each trap, the whole set of cups was hung from the center hole of a wood board ring with 21.5 cm diameter. The wood ring was put flush with the ground, and its surface was covered with a thin layer of sand, which made its color and texture similar to that of the ground. The wood ring greatly reduced the amount of soil falling into the pitfall trap and made the sorting of the specimens much easier. The traps were covered by a clear plastic cover as a rain shield that was supported by metal wires. A large fence (1.5 m diameter, 80 cm high) was established around each pitfall trap to deter large animals. Specimens were collected by removing the rain shield and the funnel, lifting out the inner container, pouring the solution and specimens into a plastic bottle, adding new solution, and reassembling the parts. The outer liner was not disturbed during this procedure.

2.3 Measurements and indices

Measurements and indices were performed as previously modified by Sharaf *et al.* (2020). All measurements were in millimeters. Trait measurements were standardised by head length. Head length was used to standardise the traits (trait value / head length) as this was missing from the dataset the least frequently and is highly correlated with overall body size (Weiser and Kaspari 2006). This was for all traits except relative mandible size, which was standardised instead by head width. This was done in line with previously published work (Bishop *et al.*, 2015) and is intended to express the size of the mandible relative to what feasibly may fit inside the mouthparts. Head width was deemed a better measure than head length in this instance.

2.4 Statistical analysis

Morphological parameters were measured using a micrometer ruler coupled to a stereoscopic microscope. Ten traits (Table 1) with functional significance were measured in the 50 samples following the method of Silva and Brandão (2010). Traits were analyzed according to the different levels of variation using both single-trait (linear mixed models) and multitrait analyses (principal component analyses).

In the context of ant morphology, PCA can be used to identify which morphological traits are the most informative in distinguishing between different species or groups of species. By analyzing the correlations among the different variables (e.g. body size, antennal length, eye size), PCA can identify which variables are the most important in accounting for the variation in the data.

Table 1: Measurements and indices

Indices	Description
TL Total Length;	The outstretched length of the ant from the mandibular apex to the gastral apex.
HW Head width	The maximum width of the head behind eyes in full-face view.
HL Head length	The maximum length of the head, excluding the mandibles.
SL Scape length	CI Cephalic Index (HW×100/HL). Excluding basal neck. SI Scape Index (SL×100/HW).
EL Eye Length	the maximum diameter of the eye.
ML Mesosoma length	the length of the mesosoma in lateral view, from the point at which the pronotum meets the cervical shield to the posterior base of the propodeal lobes or teeth.
PL Petiole length	the maximum length measured in dorsal view, from the anterior margin to the posterior margin.
PW Petiole width	maximum width measured in dorsal view
PPL Postpetiole length	maximum length measured in dorsal view
PPW Postpetiole width	maximum width measured in dorsal view.

Source: Sharaf *et al.* (2020).

Results

3.1.1 Ants collection and identification

In the present study areas, a total of 7 ant species (from 357 individuals) with genera from five subfamilies were reported. The distribution of species in the different subfamilies showed a dominance of Formicinae with 3 species (35 %), followed by Myrmicinae with 2 species (30 %), and lastly Ponerinae and Dolichoderinae and Dorylinae with one species (5 %) each. A total 7, 4 and 2 ant species were collected from Unical Botanical Garden, Cameroon and Unical Farms sites respectively. Out of 7 ant species almost 3 species (50 %) were common to all the locations on campus, while another 2 species (45 %) were found exclusive to the Botanical Garden site. From total 7 ant species the black carpenter ant (*Camponotus pennsylvanicus*, *Odentoponera denticulate*,

Tapinoma sessile and *Paratrechina longicornis*) were not reported from Unical Farms whereas, *Eciton burchellii* and *Camponotus pennsylvanicus* were found in Unical Farms sites. The number of ants collected from Botanical Garden sites (2198) were more as compared to the Unical Farms (302) and Cameroun sites (1027) (Fig. 1). In Botanical Garden subfamily Formicinae (3 species) was more diverse then followed Myrmicinae, Ponerinae, Dolichoderinae, Dorylinae comprising only one species each. In Cameroun all subfamilies, except Dorylinae were represented with at least one species; Whereas Unical Farms had one species from Formicinae and Dorylinae.

species and 5 specimens per species on average for Cameroon; then 2 species and 5 specimens (Table 2).

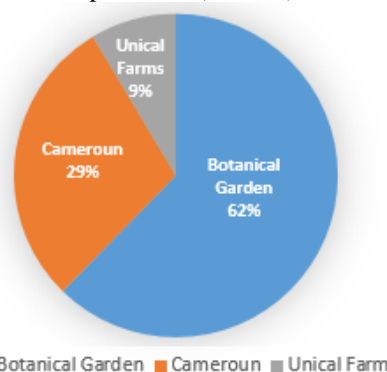


Figure 1: Distribution of ants sampled in three locations in University of Calabar

3.1.2 Morphological characterization

Functional trait measurements were taken from the available species in each list used. This amounted to 6 species and 18 specimens per species on average for Botanical Garden and 4

Table 2: Selected morphological traits measured in each ant assemblage, traits analysed and their suggested functional significance

Characteristic	Trait measured	Trait analysed	Functional significance
Head size	Head width	Head width	Head size may vary allometrically with body size, head size may also be linked to predatory strategies, with wider heads allowing for larger mandibles and therefore larger prey (Kaspari 1993; Sarty <i>et al.</i> , 2006)
	Head length	Head length	
Eye position	Interocular distance	Relative interocular distance; Relative eye position = (Head width – Interocular distance) / Head length	More dorsally positioned eyes (bigger interocular distance) are characteristic of visual predators (Fowler <i>et al.</i> , 1991).
Eye size	Eye width	Relative eye width	Wider eyes may be found in more predatory species, or alternatively may depend on foraging period, with nocturnal or low light level foragers having bigger eyes (Weiser & Kaspari 2006; Narendra <i>et al.</i> , 2013).

In Table 3, the morphometric measurements of ants' assemblage in three locations in the University of Calabar were presented.

Table 3: Morphometric measurements of ants' assemblage in three locations in the University of Calabar

Location	Ant species	Total Length	Head Length	Head Width	Scape Length	Petiolar Length	Petiolar Width	Cephalic Index	Scape Index	Petiolar Index
	<i>P. longicornis</i>	1.69	0.46	0.34	0.25	0.25	0.12	74.67	54.15	205.23
	<i>Messor barbarus</i>	1.79	0.47	0.33	0.29	0.27	0.12	68.99	60.89	220.29
	<i>C. pennsylvanicus</i>	1.81	0.52	0.40	0.28	0.27	0.14	77.84	53.28	200.59
	<i>O. denticulate</i>	2.15	0.30	0.35	0.35	0.30	0.25	116.67	100.00	120.00
	<i>Tapinoma sessile</i>	1.95	0.35	0.32	0.31	0.26	0.24	90.68	86.72	105.35
Cameroun	<i>P. longicornis</i>	1.85	0.51	0.38	0.28	0.26	0.13	75.29	55.28	202.92
	<i>C. pennsylvanicus</i>	1.82	0.51	0.40	0.26	0.27	0.13	78.52	51.56	203.03
	<i>S. globularia</i>	1.82	0.52	0.34	0.27	0.26	0.13	74.12	51.53	202.53
	<i>O. denticulate</i>	2.01	0.51	0.44	0.28	0.27	0.14	83.20	54.29	198.81
Unical Farms	<i>Eciton burchellii</i>	1.87	0.52	0.41	0.34	0.29	0.13	77.33	64.50	225.98
	<i>C. pennsylvanicus</i>	0.55	0.25	0.10	0.20	0.13	0.10	50.00	80.00	130.00

Cephalic index = Head width/Head length x 100

Scape index = Scape length/ Head length x 100

Petiolar index = Petiole length/Petiole width x 100

Univariate analysis using t-tests showed significant differences between the three locations for five traits: head size, relative eye position and relative eye width (Table 4). Trait values for Cameroon were consistently smaller than those in Unical Farms and Botanical Garden.

Botanical Garden ants have relatively larger heads, eyes and overall body size compared to Cameroun. Multivariate analysis of the first two PCoA axes represented ~ 60% of the morphological trait variation in the dataset, with 40% of the variation being described by axis 1 (Table 4).

Table 4: Estimated differences of traits between locations including standard deviation of the difference, 95% confidence intervals, the test statistic Z and permuted p- values

Trait	Estimated difference between locations	SD	95% Lower CI	95% Upper CI	Z	P
Head size	-0.71	0.23	-1.16	-0.27	-3.61	< 0.010
Relative eye width	-0.06	0.01	-0.07	-0.04	-4.71	< 0.010
Relative eye position	-0.03	0.02	-0.07	0.00	-1.48	0.138
Lightness index (v)	0.23	0.05	0.14	0.33	3.93	< 0.01

Statistically significant results are shown in bold for head size and relative eye width, indicated by the lightness v. ($P < 0.5$)

3.1.3. Single-factor analysis

Head size, Relative eye width, and lightness index were the three attributes for which the bootstrapped t-tests revealed significant variations among the three locations (Table 5).

Table 5: Estimated differences of traits between locations including standard deviation of the difference, 95% confidence intervals, the test statistic Z and permuted p- values

Trait	Estimated difference between locations	SD	95% Lower CI	95% Upper CI	Z	P
Head size	-0.71	0.23	-1.16	-0.27	-3.61	< 0.010
Relative eye width	-0.06	0.01	-0.07	-0.04	-4.71	< 0.010
Relative eye position	-0.03	0.02	-0.07	0.00	-1.48	0.138
Lightness index (v)	0.23	0.05	0.14	0.33	3.93	< 0.01

Statistically significant results are shown in bold for head size and relative eye width, indicated by the lightness v. ($P < 0.5$)

The trait ratings for Botanical Garden were consistently higher than those for the other two locations. Botanical Garden ants are generally larger in terms of their Heads, eyes, and total size. Botanical Garden ants were darker in color than those from the other locations. For relative eye position, there were no discernible changes among the locations.

3.1.4. Multivariate analysis

Around 60% of the diversity in morphological traits in the sample was represented by the first two PCA axes, with axis 1 accounting for 40% of the variation (Table 6).

Table 6: Eigenvalues and trait loadings of a PCoA, representing the morphology of all locations of ant communities

Eigenvalues	Botanical Garden	Cameroun	Unical Farms
Eigenvalue	711.67	381.34	254.67
Relative eigenvalue	0.39	0.21	0.14
Cumulative eigenvalue	0.39	0.63	0.74
Trait loadings			
Head length	-0.42	-0.49	- 0.29
Relative eye position	-0.48	-0.06	-0.42
Relative eye width	-0.43	- 0.39	-0.10
Lightness index (v)	0.41	-0.17	-0.76

We can interpret axis 1 as a gradient of ecologies related to habitat structure and openness, temperature regime, and possibly predation based on the trait loadings. Species with negative scores on this axis had large heads, relatively long legs, darker coloration, and eyes that were dorsally positioned. Foraging in flat, open, and less complex habitats is correlated with features such as leg length and head size (Bishop *et al.* 2015). Eyes that are positioned dorsally are frequently linked to predatory behavior (Silva and Brandao, 2010). The reverse

was true for characteristics that scored positive on this axis, indicating individual had lighter skin, smaller, more laterally positioned eyes, and smaller sizes: traits mostly related to foraging in more crowded and complicated environments, reflecting heat in hotter environments, and possibly exhibiting fewer predatory behaviors (Gibb and Parr, 2013).

Negative scores include species with small heads, relatively short legs, and relatively short mandibles—characteristics that

mostly relate to more generalized foraging—on axis 2, which reflects a gradient of predatory specialization. Larger mandibles, longer legs, and larger heads are features that, for the most part, seem to connect with predatory specialization, and they are represented by species with negative scores on axis 2 (Silva and Brandao, 2010).

Discussion

This work has shown that the morphology of ant faunas found in various locations varies significantly. We discover that, on average, Botanical Garden ants are larger than those of Cameroon and Unical farm ants, with higher total length, head length and scape index (Table 3). We may also see that the morphological space occupied by the ant fauna of Botanical Garden is significantly larger than those collected from the other locations. This may be due to limited or no human activities like farming that take place in the Botanical gardens as when compared to the other two locations. More so, this may be as a result of the topography of the Botanical Garden. This findings agrees with earlier research that has shown that ants would be larger and have longer relative legs when foraging in more flat habitats and with its effects on elements like competition, predation, and foraging behavior, habitat complexity can mediate important processes that shape local assemblages (Gibb and Parr, 2010, Wiescher *et al.*, 2012, Gibb and Parr, 2013).

Generally speaking, tooth ants in the Botanical garden were darker than the one obtained in Cameroon, this species was not sighted in the Unical farm. According to Sophie *et al.* (2016), the differences in the thermal environment may be related to changes in ant color (pigmentation) through a thermoregulatory mechanism like that postulated by the theorizing about thermal melanism (Clusella-trullas *et al.*, 2007). Darker pigmentation may be advantageous in colder climates due to rapid heating caused by less body surface reflectance, according to the thermal melanism. In this study location, the coldest of the three studied location was the Botanical garden, hence may be associated to the darker colouration. Yet, it is unclear that this theory can account for the lighter colouring of the ants from Cameroon and Unical farm. The thermal melanism theory assumes that sun radiation reaches the bodies of the organisms. Sunlight will be limited under the Botanical Garden canopy. Hence, we propose that the Botanical Garden ants are using the thermoregulatory benefits of darkness.

According to the findings, since the study was conducted within the same geographical location, the morphological traits measured across the different species were identical. While very minor specialization may be predicted in the Unical farm and Cameroon, some specialization may be anticipated in the species of the Botanical Garden. It is possible that specialization is not necessary where rainforest animals do use liquid resources since those resources are plentiful or simple to get (Sophie *et al.*, 2016).

The morphological characterization of ants has revealed a great deal of information about these insects. This includes the various body parts, their sizes, and the shapes that make up the ant's anatomy. Morphological characterization has also

highlighted some of the differences between the species, such as their coloration, the sizes of their heads, and the shapes of their antennae. This information can be used to identify and separate species of ants, as well as to better understand their biology and behavior. Morphological characterization has become an important tool in the field of entomology and has helped to advance the study of ants. Based on the study, it was concluded that all ants species found in the studied locations would perform better if the environment is stable without the interference of human activities. We discovered that, on average, Botanical Garden ants are larger and darker than those of Cameroon and Unical farm ants.

Conclusion

The morphological characterization of ten species of ants has revealed a great deal of information about these insects. This includes the various body parts, their sizes, and the shapes that make up the ant's anatomy. Morphological characterization has also highlighted some of the differences between the species, such as their coloration, the sizes of their heads, and the shapes of their antennae. This information can be used to identify and separate species of ants, as well as to better understand their biology and behavior. Morphological characterization has become an important tool in the field of entomology and has helped to advance the study of ants. Based on the study, it was concluded that all ants species found in the studied locations would perform better if the environment was stable without the interference of human activities. We discovered that, on average, Botanical Garden ants were larger and darker than those of Cameroon and Unical farm ants.

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Authors' Contributions

JOP conceptualized and designed the study. IOP participated in the design of the study. JE and LA undertook the field work. JE performed the statistical analysis of the data and IOP interpreted the results. JOP, IOP and LA drafted the manuscript. IOP proof-read the manuscript. JOP supervised the work. All authors read and approved the final manuscript.

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