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DNA BARCODING REVEALS HIGH DIVERSITY AND PHYLOGENETIC RELATIONSHIPS AMONG ANTS (FORMICIDAE) IN SOUTHEASTERN NIGERIA

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Abstract

DNA barcoding is a reliable tool for rapid species identification and assessing biodiversity patterns. Our data set includes 103 ant samples collected from subtropical areas of Enugu State, southeastern, Nigeria, to assess the population distribution, genetic diversity and phylogenetic relationship of ant species using cytochrome c oxidase (COI) gene. The most abundant taxa recorded included *Camponotus sp., Pachychondyla sp., Pheidole sp., Pheidole rugaticeps, Crematogaster sp., Monomorium subopacum, and Paratrechina longicornis.* Sequence analysis of the heatmap grouped the ant samples into four (A–D), and its aligned with subfamily affiliations. A total of five subfamilies were identified with Myrmicinae (39%) having the highest abundance followed by Formicinae (38%), Ponerinae (20%), Dolichoderinae (2%), and Dorylinae (1%). Formicinae exhibited the highest haplotype diversity (Hd = 0.938), while Myrmicinae showed the highest nucleotide diversity ($\pi = 0.203$). Analysis of genetic distance indicated Myrmicinae subfamily is most closely related to the Formicinae (Nei = 0.275; $F_{ST} = 0.039$). Furthermore, the Dolichoderinae had a closer association with the Formicinae, suggesting shared evolutionary lineages. This finding offers the first phylogenetic framework for ants in southeastern Nigeria based on DNA barcoding, and reveals that the Myrmicinae and Formicinae subfamilies possess broader habitat adaptation than other groups in this region. It also establishes the baseline for future conservation strategies targeting ant communities in West African tropical regions.

Keywords: Abundance, Subfamily; Distribution; Genetic distance; Phylogeny analysis, Tropical regions

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INTRODUCTION

Ants (Hymenoptera: Formicidae) represent one of the most diverse and ecologically important groups of insects, with more than 16,000 species described worldwide (AntWeb, 2023). Their widespread distribution and key functional roles in terrestrial ecosystems make them excellent model organisms for ecological, taxonomic, and genetic studies (Tuma et al., 2020; Rongier et al., 2023). In addition, ants contribute significantly to ecosystem processes such as seed dispersal, the persistence of grass species in fire-prone grazing lands, and the enhancement of soil structure through aeration (Siddiqui et al., 2019). Additionally, they are effective biocontrol agents, such as the Asian weaver ants effective in controlling certain field crops (Offenberg 2014). Thus, because of their mutualistic interactions with both plants and animals, ants are regarded as excellent biological indicators.

They have several species with variations in their body form, making it challenging to differentiate. Thus, traditional morphological approaches to ant identification often face challenges due to the subtle morphological differences and cryptic species complexes present within the group (Chen and Zhou, 2017). Also, it demand substantial taxonomic expertise, which is often limited by the scarcity of experienced taxonomists (Engel et al., 2021). Thus, research on ants that primarily relied on morphological features for species identification often lead to taxonomic uncertainties and hinder progress in biodiversity conservation (Wang et al., 2025).

Nigeria, with its diverse ecological landscapes, has a rich understudied ant fauna (Jimoh et al., 2024). In southeastern Nigeria, in particular, the ant biodiversity remains poorly characterized at the molecular level due to limited access to taxonomic tools. A significant proportion of morphospecies remain difficult to identify in Afro-tropical zones (Ashigar and Majid, 2022), and this attributed to several factors, like numerous undescribed species, lack of up-to-date revisionary research, and insufficient identification resources for already known species. Furthermore, Patkar et al. (2014) reported a significant global decline in economically important insect populations, including ants, driven by deforestation, urbanization, and pesticide use. These activities result in adverse effects leading to habitat loss, changing climatic conditions, and degradation of insect natural environments (Outhwaite et al., 2022).

DNA barcoding serves as a valuable complement to conventional taxonomy, delivering a dependable, unbiased, and swift method for species identification that minimizes the necessity for specialized morphological taxonomy knowledge (Dmitrović et al., 2022). Since DNA barcoding proposal by Hebert et al. (2003), its has been applied widely on taxonomy, phylogenetics, biodiversity assessment, and monitoring of organism. In DNA barcoding, species are identified using short, standardized DNA sequences derived from conserved regions of the genome (Abdi et al., 2024). The cytochrome c oxidase subunit I (COI) gene is widely used as the standard DNA barcode for animals and and has shown promise in resolving the genetic diversity of various taxonomic groups, including ants (Bandral et al., 2023). Therefore, this study aims to: (i) assess ant diversity using COI barcoding; (ii) estimate relative abundance of subfamilies; and (iii) infer phylogenetic relationships. This study seeks to establish the first molecular baseline for Formicidae in Enugu southeastern Nigeria, aiding in biodiversity evaluations and guiding upcoming conservation efforts.

MATERIALS AND METHODS

Study Area and Ant Collection

The study was carried out to identify a diverse range of ant species in subtropical areas of Enugu State, southeastern Nigeria, between May 10 and June 30, 2021. Approximately 103 samples were collected from different habitats including tree parts, soil, refuse dump areas, and rocks (Appendix 1). The sampling technique employed was a multi-habitat, opportunistic (active) sampling approach. Collection was done either by baiting, direct handpicking or shifting, following the School of Ants guidelines (http://schoolofants.org). The specimens were put inside a vial and preserved in 100% ethanol and subsequently stored at -20°C. Freezing was done within four hours of collection to reduce the risk of DNA degradation as noted by Marizzi et al. (2018). This procedure preserved the integrity and quality of the ant specimen for subsequent molecular analyses. Each specimen was individually stored in sterile microcentrifuge tubes and labeled with the collection site, habitat, and date to ensure traceability and prevent cross-contamination.

DNA Extraction, PCR Amplification and Sequencing

The study was carried out at the DNA Learning Center, Ugwuomu-Nike, Enugu State, Nigeria. To extract genomic DNA, the leg or whole ant body were used to ensure sufficient tissue availability. The tissues was crushed directly in a sterile microcentrifuge tube using a disposable sterile micropestle in 300-350 µL of lysis buffer (6M guanidine hydrochloride (Sigma-Aldrich, USA) and subjected to a 65°C water bath for 10 minutes. Following tissue lysis, total genomic DNA was extracted using a silica-based extraction protocol as described by Marizzi et al. (2018).

The extracted DNA was stored at −20 °C until further use. Amplification of the mitochondrial cytochrome c oxidase subunit I (COI) barcode region was carried out using Cytiva PuReTaq™ Ready-To-Go PCR Beads (Global Life Sciences Solutions, UK). Each bead contained 2.5 units of Taq DNA polymerase, 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM of each dNTP, and 10 mM Tris-HCl (pH 9.0). PCR amplification was performed using the COI primers LCO1490 (5′-TGTAAAACGACGGCCAGTGGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-CAGGAAACAGCTATGACTAAACTTCAGGGTGACCAAAAAATCA-3′), following the protocol of Folmer et al. (1994). The primers were premixed with a loading dye consisting of 640 µL molecular-grade water, 460 µL cresol red loading dye, 20 µL of 15 pmol/µL forward primer, and 20 µL of 15 pmol/µL reverse primer.

PCR reactions were conducted in a Bio-Rad T100™ thermal cycler in a total volume of 25 µL, comprising 2 µL of template DNA and 23 µL of the primer/loading dye mixture. The cycling conditions included an initial denaturation at 94 °C for 1 minute, followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 54 °C for 15 seconds, and extension at 72 °C for 30 seconds, with a final extension at 72 °C for 5 minutes. PCR products were resolved on a 2% agarose gel stained with ethidium bromide (0.1 µg/mL) and electrophoresed at 100 V for 30 minutes. Amplification success was confirmed under UV illumination using an Accuris UV Transilluminator E-3000-E, with a 100 bp DNA ladder (New England BioLabs

Inc.) used as a molecular size marker. Successfully amplified products were subsequently sent to GENEWIZ (Azenta Life Sciences, South Plainfield, NJ, USA) for DNA sequencing.

Sequence editing, alignment and analysis

The generated sequences were aligned and trimmed to a uniform length using MEGA version 11 (Tamura et al., 2021). The aligned sequences were subsequently translated into amino acids to verify sequence quality and to check for the presence of nuclear mitochondrial pseudogenes, insertions, deletions, or premature stop codons. To determine taxonomic identity, the sequences obtained in this study were compared against reference databases using the Barcode of Life Data Systems (BOLD) and the National Center for Biotechnology Information (NCBI) database through the Basic Local Alignment Search Tool (BLAST). Species assignment was carried out using the Best Match (BM), Best Close Match (BCM), and All Species Barcode (ASB) criteria, in which each query sequence was evaluated against the complete reference dataset. Under the Best Match criterion, sequences were assigned based on the closest barcode match, irrespective of genetic distance. While in BCM a distance below a predetermined threshold, the barcode match that was closest to the query sequence was used to identify it. In ASB, we created a list of all barcodes for every question, arranged according to how similar they were to the query, using the same criterion as for best close match. When every match below the threshold was conspecific, the query sequence was determined. A permutation approach was used to estimate the significance of sequence difference between and within the ant species. This involved the estimation of statistical pairwise nucleotide difference.

Genetic diversity and relative abundance estimates

The DnaSP v. 6 software (Rozas et al., 2017) was used to estimate diversity using the subfamilies whose ant samples are above 5 in the subfamily. This approach was adopted to minimize potential bias associated with a limited sample size, as previously noted by Wakeley (1998). Diversity analyses were subsequently carried out, including the estimation of the number and diversity of mitochondrial haplotypes, nucleotide diversity (Nei, 1987), the mean number of nucleotide differences between sequence pairs (Tajima, 1983; Nei, 1987), as well as the number of singleton sites, parsimony-informative polymorphic sites, and fixed genetic differences between populations (Hey, 1991). The relative abundance of the ant subfamily was calculated according to Manikandan et al. (2018).

Genetic distance and phylogeny analysis

The genetic distance between the subfamilies was performed according to Nei, (1987) and pairwise FST values using the DnaSP v. 6 software (Rozas et al., 2017). Heatmap analysis was conducted in R software (R Core Team, 2023) by visualizing pairwise genetic distance matrices generated from aligned DNA sequences using the pheatmap package. Evolutionary relationships among the sequences were inferred using the Neighbour-Joining method described by Saitou and Nei (1987), and the phylogenetic tree was constructed based on the Maximum Composite Likelihood approach outlined by Tamura et al. (2011) as implemented in MEGA version 11 (Tamura et al., 2021). The robustness of the tree topology was assessed using bootstrap analysis with 1,000 replications.

RESULTS

Ant identification and DNA sequence

The results from sequence validation using the BOLD system and the online BLAST (NCBI) tool indicated that the sequenced samples matched closely to most of the ant species listed in the database. A reference sequence library was established for each species, containing sequences with 85%–100% similarity (Appendix 1). The most frequently recorded taxa were Camponotus sp., Pachychondyla sp., Pheidole sp., Pheidole rugaticeps, Crematogaster sp., Monomorium subopacum, and Paratrechina longicornis. Among the 26 ant species identified from the collected samples, 14 species belonged to the Myrmicinae subfamily, while 9 species were part of the Formicinae subfamily, while the remaining subfamilies are only 1 species each. Figure 1 shows the heatmap analysis of the sequence distances for the ant samples and demonstrated that the majority of ants from the same subfamily formed clusters together. They were categorized into four groups (A to D), with most of the sequences found in group B.

Genetic diversity and abundance estimates

The COI sequences from the ant samples were examined to uncover any genetic diversity within the subfamily (Table 1 and Table 2). The findings indicate that the DNA barcoding datasets allowed for the discrimination of ant species and highlighted the previously unrecognized biodiversity within their subfamily. Analysis of the sequences showed that the Myrmicinae subfamily contained the greatest number of polymorphic sites (168) and parsimony informative sites (156) compared to other evaluated subfamilies (Table 1). The Ponerinae subfamily had 75 polymorphic sites and 56 parsimony informative sites, whereas the Formicinae exhibited 111 polymorphic sites and 95 parsimony informative sites. Moreover, the ant subfamilies demonstrated considerable nucleotide diversity, with the Myrmicinae exhibiting the highest diversity (0.203), followed by the Formicinae (0.183), and the Ponerinae showing the least (0.115). The findings also indicated that the Formicinae subfamily possessed significant gene diversity (0.938), while the Myrmicinae subfamily showed a gene diversity of 0.913, with the Ponerinae subfamily being the lowest at 0.852 (Table 2). Among the 21 available ant subfamilies, only 5 subfamilies (Myrmicinae, Formicinae, Ponerinae, Dolichoderinae, and Dorylinae) were identified from the sampled regions. The Myrmicinae subfamily was the most abundant, comprising 39% of the total, followed closely by the Formicinae at 38%, the Ponerinae at 20%, the Dolichoderinae at 2%, and 1% for Dorylinae as shown in Figure 2.

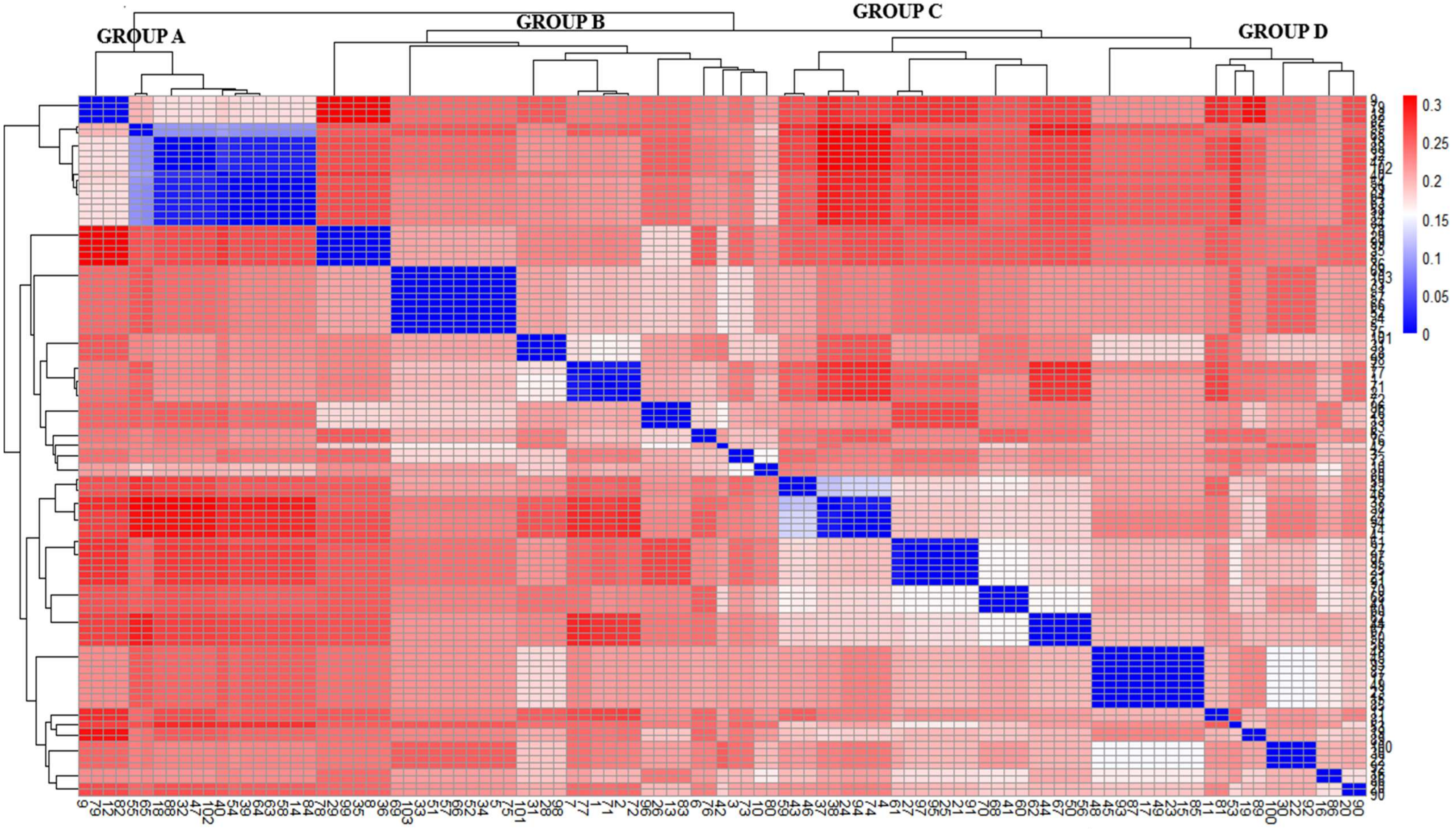

Figure 1: Heatmap of pairwise genetic distances among ant COI barcode sequences with hierarchical clustering. Blue regions indicate low intra-cluster genetic distances, whereas red regions represent higher inter-cluster divergence.

Table 1: Polymorphic sites in COI partial sequences of three ant subfamilies collected in Enugu State, Nigeria

ANF	**NS**	**MS**	**PS**	**SVS**	**PIS**	**TM**
Myrmicinae	40	209	168	12	156	251
Ponerinae	21	138	75	19	56	87
Formicinae	39	154	111	16	95	164
Total	100	78	99	1	98	184

ANF = Ant Families; NS = Number of sequences; MS = Monomorphic sites; PS = Polymorphic site; SVS = Singleton variable sites; PIS = Parsimony informative sites; TM = Total mutation

Table 2: Genetic diversity parameters of the COI partial sequences for three ant subfamilies collected in Enugu State, Nigeria

ANF	NS	NSS	*H*	Hd	π	PiJC	ANN
Myrmicinae	40	87	21	0.913	0.203	0.243	35.955
Ponerinae	21	64	9	0.852	0.100	0.115	17.743
Formicinae	39	80	17	0.938	0.183	0.214	32.426
Total	100	99	48	0.972	0.248	-	43.98

ANF = Ant Families; NS = Number of sequence; NSS = Number of segregating sites; *h* = Number of haplotypes; Hd = Haplotype (gene) diversity; π = Nucleotide diversity; PiJC = Nucleotide diversity with JC, PiJC; ANN = Average number of nucleotide difference

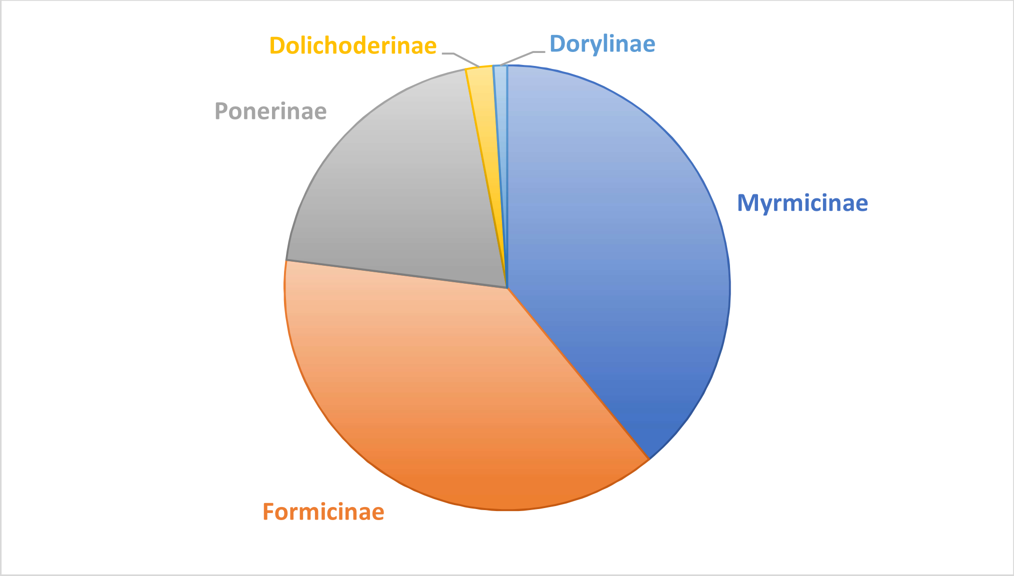

Figure 2: Relative abundance of ant the studied subfamilies from Enugu state Nigeria

Phylogenetic relationship and genetic distance of the ant subfamilies

The phylogenetic analysis demonstrated that the COI sequence is effective in categorizing the ant samples into specific subfamilies, with all sequences forming cohesive and well-defined groups or clades as shown in Figure 3. The clades showed the Formicinae subfamily are clustered closely with the Myrmicinae subfamily than the Ponerinae subfamily. The findings also indicated that most of the nodes within the subfamily exhibit significantly high levels of support, resulting in the establishment of multiple clusters within each subfamily, which reflects substantial variation in the COI gene. The genetic distance calculations among the subfamilies suggested that the Myrmicinae subfamily is most closely related to the Formicinae (Nei = 0.275; FST = 0.039) in comparison to the other subfamilies analyzed (Table 3). Additionally, the Dolichoderinae subfamily (Nei = 0.817; FST = 0.174) shows a more distant relationship with the Ponerinae subfamily when contrasted with the other subfamilies.

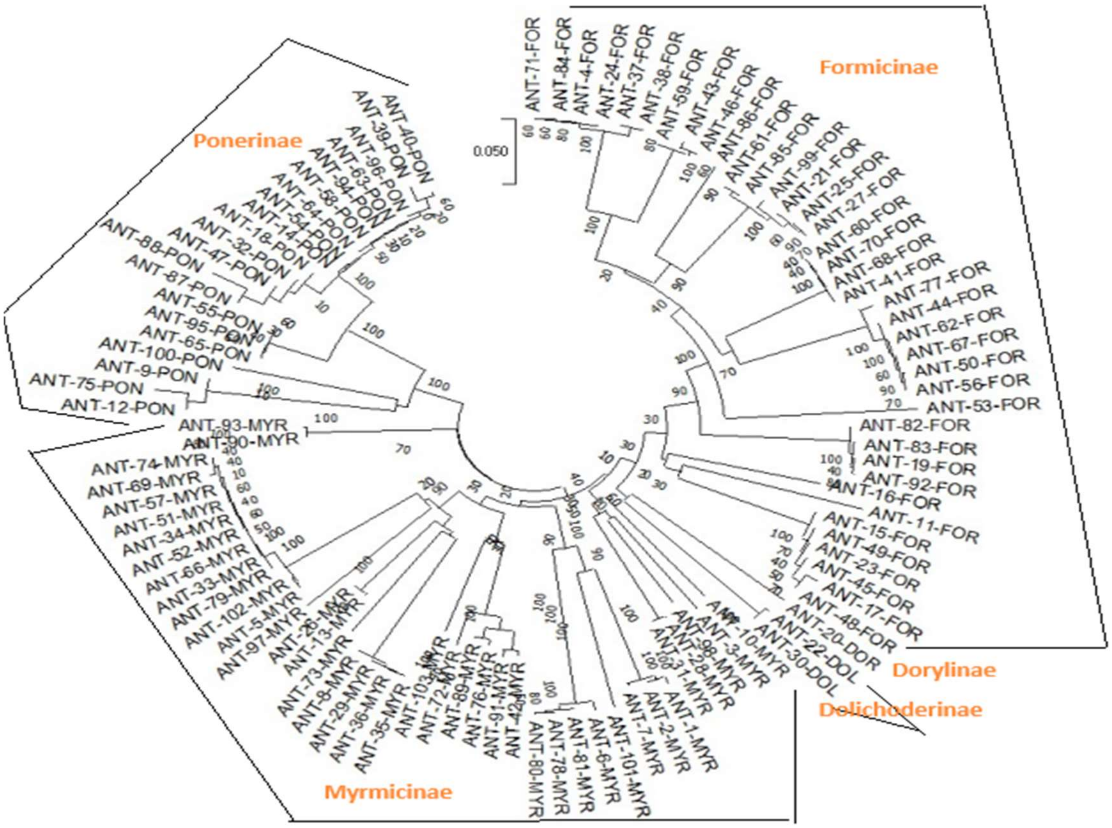

Figure 3: Phylogenetic tree using neighbour-joining analysis of the ant subfamily generated from COI sequence data

Table 3: Genetic divergence between ant subfamilies. Below diagonal is pairwise Nei's genetic distance (D) while above diagonal is pairwise F_{ST} values.

	Myrmicinae	**Ponerinae**	**Formicinae**	**Dolichoderinae**
Myrmicinae	-	0.058	0.039	0.141
Ponerinae	0.477	-	0.051	0.174
Formicinae	0.275	0.541	-	0.138
Dolichoderinae	0.637	0.817	0.607	-

DISCUSSION

The findings of this study provide the first molecular baseline for ants in Enugu, southeastern Nigeria, and generated 103 sequences that could be deposited in a public repository for use in future research. Public deposition of barcode sequences enhances global taxonomic efforts and facilitates future comparative studies (Vasconcelos et al., 2021). Hebert et al. (2003) discovered that identification using COI sequences was 100% successful in the class Hexapoda and 96.4% in the kingdom Animalia. Furthermore, the COI gene marker, when applied to the 103 samples of ants studied was able to assess mitochondrial variation among samples. DNA barcoding enables rapid assessment of biological diversity and provides complementary information that can be easily compared across different research programs and taxonomic groups (Smith and Fisher, 2009). The heatmap analysis of the ant sample sequence distances showed that most of the ants from the same subfamily clustered together. These similarity patterns were consistent with established ant phylogenies, with sequences clustering by subfamily. This result agrees with the previous report of observed patterns of relatedness for sequence in the same subfamily level (Ward, 2014; Lau et al., 2019). The present research created sequence which measured the ant subfamilies diversity and delimitation. Thus, DNA barcoding effectively resolved ant species into distinct subfamilies, supporting its utility for unambiguous taxonomic assignments. Furthermore, based on the resolution obtained through Best Close Match (BCM) analysis, the DNA barcoding methodology is helpful in giving higher objectivity for ant identification and may be used to validate morphological or other molecular methodologies.

The relative abundance of the ant subfamilies samples revealed that among the 21 available ant subfamilies, only 5 subfamilies (Myrmicinae, Formicinae, Ponerinae, Dolichoderinae, and Dorylinae) were identified from the various areas. According to Benoit (2013), Myrmicinae, Formicinae, Ponerinae, and Dolichoderinae are the four primary subfamilies that make up the majority of ant variety, accounting for more than 87% of all documented ant species. Siddiqui et al. (2019) conducted DNA barcoding of ants found in subtropical southern China and identified only three subfamilies (Dolichoderinae, Formicinae, and Myrmicinae). The distribution of ants within an area may be attributed to available materials for the construction of a nest (Holldobler and Wilson, 1990), soil types present within the area, the nature of vegetation, and the nutritional quality of the soil (Majeed, 1998). Myrmicinae (39%) had the highest abundance among the ant subfamilies, followed by Formicinae (38%), Ponerinae (20%), Dolichoderinae (2%), and Dorylinae (1%). Our findings align with Magagula and Hawkes (2023), who reported similar dominance of Myrmicinae and

Formicinae in Southern Africa (Eswatini) while in Central Cameroon, the Myrmicinae ant subfamily is the most abundant comprising of 54% of the total ant species (Masse et al., 2021). Siddiqui et al. (2019) observed in subtropical southern China that Myrmicinae was the dominant ant subfamily, with seven genera and 13 species making up over half (55.48%) of the 301 samples analyzed. The abundance of the Myrmicinae subfamily is because of their global bio-geographic distribution, and availability in every habitat where ants are found (Borowiec et al., 2020). The dominance of Myrmicinae and Formicinae in the present study may reflect environmental conditions favorable to their adaptation. Mohseni and Pashaei (2021) reported that edaphic or environmental factors influence the abundance and distribution of ant genera found in their habitat.

Sequence analysis showed that the Myrmicinae subfamily had the highest numbers of polymorphic (168) and parsimony-informative (156) sites, indicating higher intra-population genetic variability and diversity compared with the other subfamilies. Benoit (2013) reported that Myrmicinae is the most species-rich ant subfamily, comprising more than 6,000 described species across 144 genera, and accounting for nearly half of all known ant species. The predominance of the Myrmicinae subfamily is a result of the group's capacity to adapt to environmental change, broad diversity in feeding, and their ability to occupy different niches (Junir et al., 2017). Furthermore, the result also showed that haplotype diversity for Formicinae (0.938), Myrmicinae (0.913), and Ponerinae (0.852) was high. According to earlier research (Grant and Bowen, 1998; Ueda and Itino, 2017), values larger than 0.70 indicate considerable high haplotype diversity. Mayoke et al. (2020) noted that high haplotype diversity in tsetse fly is an indication of the large population size in the area.

In addition, the ant subfamilies examined exhibited high nucleotide diversity, with values in this study exceeding those previously reported for ants (Ueda and Itino, 2017) and tsetse flies (Mayoke et al., 2020). Both nucleotide diversity and haplotype diversity were high for the studied Myrmicinae, Formicinae, and Ponerinae ant subfamilies. High nucleotide diversity combined with high haplotype diversity generally reflects populations that are large, demographically stable, and have existed for an extended evolutionary period (Grant and Bowen, 1998). The lower diversity of the Ponerinae subfamily when compared with other subfamilies studied may be associated with reduced evolutionary potential that inhibits them to adapt in diverse environments or a very low level of genetic divergence within the subfamily reflecting their low intra-specific polymorphism. Wakeley (1998) revealed that 5 to 10 small samples are sufficient for an accurate estimation of diversity in an organism. Further studies with more Ponerinae samples and detailed genomic scans are needed to clarify this.

The phylogeny analysis revealed that the COI sequence is promising in allocating the ant samples into distinct subfamilies and all the sequences clustered into cohesive and well-differentiated groups or clades. Palasio et al. (2017) reported that cluster analyses based on species-delimitation algorithms showed that substantial genetic divergence within populations can lead to the formation of distinct substructures within clades and clearly differentiated genetic clusters. The results also revealed that the majority of the nodes that comprise the subfamily have notably high levels of nodal support. In this work, we found the creation of multiple clusters within each subfamily, showing considerable variation in the COI gene. The genetic divergence within the clusters was significant enough to cause sub-structuring within clades. Several authors have successfully separated samples of ant species using phylogenic analysis and concluded that molecular data are informative for species discrimination (Smith et al., 2005; Smith and Fisher, 2009; Ng'endo et al., 2013). The ant formed a monophyletic clade for each subfamily, showing that grouping the ant into subfamilies is reliable for characterization. DNA-based identification confirmed the distinctions between Pheidole rugaticeps Emery and Pheidole decarinata Santschi, which was challenging using morphology alone (Ashigar and Majid, 2022).

The genetic distance between the subfamilies revealed that the Myrmicinae subfamily is closely related to the Formicinae (Nei = 0.275; FST = 0.039) when compared to the other investigated subfamilies. This finding is consistent with Ohnishi et al. (2003), who conducted molecular analyses of ant subfamilies and discovered that the Myrmicinae and Formicinae are closely linked when compared to other ant subfamilies analyzed. Furthermore, in the present research among the subfamilies the Dolichoderinae subfamily showed a close relationship with the Formicinae subfamily (Nei = 0.607; FST = 0.138) compared to others. Shattuck (1992) performed a higher classification of ant subfamilies and reported that the Dolichoderinae and Formicinae are closely related taxa belonging to the same sister clade. These results also agree with the report of Dlussky and Fedoseeva (1988), who placed Dolichoderinae and Formicinae as sister groups

CONCLUSION

The DNA barcoding of ants has provided insight into ant species distribution and dispersion by analyzing the genetic diversity and phylogeny of ant subfamilies. The genetic distance revealed that the Myrmicinae and Dolichoderinae subfamilies are closely related to the Formicinae subfamily when compared with other subfamilies investigated. Further studies that involve using large numbers of ant species and conducting genomic scans would help to elucidate the present findings. Thus, DNA barcoding is effective for accurately representing ant species in public databases and identifying them using integrative techniques

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

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APPENDIX 1:
BLAST-BASED SPECIES IDENTIFICATION AND GENBANK ACCESSION NUMBERS OF THE STUDIED ANT SAMPLES

Sample Analysis Code	Sample ID	Species (Closest Blast Hit)	Subfamily	% identity	Closest BLAST Hit Accession Number	New GenBank Record - Novel seq (1 for yes, 0 for no)
ANT-1-MYR	HBK-001	Crematogaster auberti	Myrmicinae	84.24%	FJ824415.1	1
ANT-2-MYR	HBK-002	Crematogaster hova (Crematogaster degeeri)	Myrmicinae	84.20%	HM880631.1	1
ANT-3-MYR	HBK-003	Tetramorium sericeiventre	Myrmicinae	95.53%	JN283586.1	0
ANT-4-FOR	HBK-004	Camponotus sp.	Formicinae	86.33%	MH290664.1	1
ANT-5-MYR	HBK-005	Pheidole rugaticeps	Myrmicinae	100.00%	MW080378.1	0
ANT-6-MYR	HBK-006	Cremogaster sp.	Myrmicinae	98.49%	MF673709.1	0
ANT-7-MYR	HBK-012	Cremogaster sp. (Crematogaster grevei)	Myrmicinae	84.37%	HQ928675.1 (HQ925537.1)	1
ANT-8-MYR	HBK-013	Pheidole sp.	Myrmicinae	91.98%	KJ141928.1	1
ANT-9-MYR	HBK-014	Pachycondyla sp.	Ponerinae	91.59%	LN609160.1	1
ANT-10-MYR	HBK-015	Atopomyrmex mocquerysi	Myrmicinae	93.84%	DQ353339.1	1
ANT-11-MYR	HBK-016	Lepisiota canescens	Formicinae.	90.71%	GU709886.1	1
ANT-12-MYR	HBK-019	Pachycondyla sp.	Ponerinae	91.38%	LN609160.1	1

ANT-13-MYR	HBK-021	Pheidole decarinta	Myrmicinae	100.00%	MW080385.1	0
ANT-14-MYR	HBK-022	Pachycondyla sp.	Ponerinae	98.69%	MF673717.1	0
ANT-15-FOR	HBK-023	Paratrechina longicornis	Formicinae	100.00%	MK207917.1	0
ANT-16-FOR	HBK-024	Oecophylla longinoda	Formicinae	96.86%	MT152291.1	0
ANT-17-FOR	HBK-025	Paratrechina longicornis	Formicinae	100.00%	MK207917.1	0
ANT-18-PON	HBK-028	Pachycondyla sp.	Ponerinae	99.69%	MF673717.1	0
ANT-19-FOR	HBK-029	Camponotus sp.	Formicinae	99.54%	MF673708.1	0
ANT-20-DOR	HBK-030	Dorylus (Rhogmus) sp	Dorylinae	94.70%	EF413831.1	1
ANT-21-FOR	HBK-031	Camponotus sp.	Formicinae	99.31%	KF978700.1	1
ANT-22-DOL	HBK-032	Tapinoma melanocephalum	Dolichoderinae	100.00%	MK768133.1	0
ANT-23-FOR	HBK-033	Paratrechina longicornis	Formicinae	99.85%	MK207917.1	0
ANT-24-FOR	HBK-034	Camponotus sp.	Formicinae	86.30%	MH290664.1	1
ANT-25-FOR	HBK-035	Camponotus sp.	Formicinae	99.10%	KF978700.1	0
ANT-26-MYR	HBK-037	Pheidole decarinata	Myrmicinae	100.00%	MW080385.1	0

ANT-27-FOR	HBK-038	Camponotus sp.	Formicinae	99.21%	KF978700.1	0
ANT-28-MYR	HBK-039	Tetramorium bicarinatum	Myrmicinae	91.47%	KX054987.1	1
ANT-29-MYR	HBK-040	Pheidole sp.	Myrmicinae	92.50%	KJ141928.1	1
ANT-30-DOL	HBK-041	Tapinoma melanocephalum	Dolichoderinae	100.00%	MK768133.1	0
ANT-31-MYR	HBK-042	Tetramorium bicarinatum	Myrmicinae	91.16%	KX054980.1	1
ANT-32-PON	HBK-043	Pachycondyla sp.	Ponerinae	99.69%	MF673717.1	0
ANT-33-MYR	HBK-044	Pheidole rugaticeps	Myrmicinae	100.00%	MW080382.1	0
ANT-34-MYR	HBK-045	Pheidole rugaticeps	Myrmicinae	100.00%	MW080382.1	0
ANT-35-MYR	HBK-046	Pheidole sp.	Myrmicinae	92.51%	KJ141928.1	1
ANT-36-MYR	HBK-047	Phediole sp.	Myrmicinae	91.96%	KJ141928.1	1
ANT-37-FOR	HBK-048	Camponotus sp.	Formicinae	86.86%	KF978753.1 (kf978684.1)	1
ANT-38-FOR	HBK-049	Camponotus sp.	Formicinae	86.52%	KF978753.1	1
ANT-39-PON	HBK-052	Pachycondyla sp.	Ponerinae	97.63%	MF673719.1	0
ANT-40-PON	HBK-053	Pachycondyla sp.	Ponerinae	95.04%	MF673719.1	0
ANT-41-FOR	HBK-057	Camponotus eugeniae	Formicinae	92.21%	HQ925729.1	0

ANT-42-MYR	HBK-060	Monomorium subopacum	Myrmicinae	99.53%	GU710429.1	0
ANT-43-FOR	HBK-061	Camponotus aurosus	Formicinae	87.39%	JN134849.1	1
ANT-44-FOR	HBK-068	Camponotus sp.	Formicinae	87.48%	JN270678.1 (HQ925768.1)	1
ANT-45-FOR	HBK-069	Paratrechina longicornis	Formicinae	100.00%	MK207917.1	0
ANT-46-FOR	HBK-070	Camponitus imitator	Formicinae	87.30%	GU709962.1	1
ANT-47-PON	HBK-072	Pachycondyla sp.	Ponerinae	99.67%	MF673717.1	0
ANT-48-FOR	HBK-074	Paratrechina longicornis	Formicinae	100.00%	MK207918.1	0
ANT-49-FOR	HBK-075	Paratrechina longicornis	Formicinae	99.80%	MK207918.1	0
ANT-50-FOR	HBK-076	Camponotus sp.	Formicinae	87.00%	HQ925763.1	1
ANT-51-MYR	HBK-077	Pheidole rugaticeps	Myrmicinae	100.00%	MW080382.1	0
ANT-52-MYR	HBK-078	Pheidole rugaticeps	Myrmicinae	100.00%	MW080382.1	0
ANT-53-FOR	HBK-079	Camponotus pennsylvanicus	Formicinae	90.70%	MH757091.1	1
ANT-54-PON	HBK-080	Pachycondyla sp.	Ponerinae	98.25%	MF673719.1	1
ANT-55-PON	HBK-081	Pachycondyla sp.	Ponerinae	92.66%	MF673716.1	1

ANT-56-FOR	HBK-082	Camponotus sp.	Formicinae	87.52%	JN270678.1 (KC685012.1)	1
ANT-57-MYR	HBK-083	Pheidole rugaticeps	Myrmicinae	99.81%	MW080382.1	0
ANT-58-PON	HBK-084	Pachycondyla sp.	Ponerinae	98.10%	MF673719.1	0
ANT-59-FOR	HBK-087	Camponotus sp.	Formicinae	87.07%	KF978649.1	1
ANT-60-FOR	HBK-088	Camponotus eugeniae	Formicinae	92.29%	HQ925729.1	1
ANT-61-FOR	HBK-089	Camponotus sp.	Formicinae	97.85%	KF978700.1	1
ANT-62-FOR	HBK-090	Camponotus sp.	Formicinae	87.38%	JN270678.1	1
ANT-63-PON	HBK-091	Pachycondyla sp.	Ponerinae	98.43%	MF673719.1	0
ANT-64-PON	HBK-092	Pachycondyla sp.	Ponerinae	98.14%	MF673719.1	0
ANT-65-PON	HBK-093	Pachycondyla sp.	Ponerinae	92.55%	MF673717.1	0
ANT-66-MYR	HBK-095	Pheidole rugaticeps	Myrmicinae	100.00%	MW080382.1	0
ANT-67-FOR	HBK-096	Camponotus parius	Formicinae	87.16%	KC685012.1	1
ANT-68-FOR	HBK-098	Campotonotus eugeniae	Formicinae	92.37%	HQ925729.1	1
ANT-69-MYR	HBK-099	Pheidole rugaticeps	Myrmicinae	100.00%	MW080382.1	0
ANT-70-FOR	HBK-100	Campotonotus eugeniae	Formicinae	92.45%	HQ925729.1	1

ANT-71-FOR	CAK-001	Camponotus sp.	Formicinae	86.44%	KF978691.1	1
ANT-72-MYR	CAK-002	Monomorium subopacum	Myrmicinae	95.93%	GU710429.1	0
ANT-73-MYR	CAK-003	Pheidole annemariae	Myrmicinae	85.69%	HM419532.1	1
ANT-74-MYR	CAK-004	Pheidole rugaticeps	Myrmicinae	100.00%	MW080382.1	0
ANT-75-PON	CAK-005	Pachycondyla sp.	Ponerinae	90.91%	LN609160.1	1
ANT-76-MYR	CAK-007	Monomorium subopacum	Myrmicinae	97.57%	GU710429.1	0
ANT-77-FOR	CAK-008	Camponotus sp. (Camponotus petersii)	Formicinae	85.78%	JN270678.1 (JN270705.1)	1
ANT-78-MYR	CAK-010	Cremogaster sp.	Myrmicinae	98.29%	MF673709.1	0
ANT-79-MYR	CAK-012	Pheidole rugaticeps	Myrmicinae	100.00%	MW080382.1	0
ANT-80-MYR	CAK-014	Cremogaster sp.	Myrmicinae	98.46%	MF673709.1	0
ANT-81-MYR	CAK-015	Cremogaster sp.	Myrmicinae	98.41%	MF673709.1	0
ANT-82-FOR	CAK-016	Camponotus sp.	Formicinae	99.63%	MF673708.1	0
ANT-83-FOR	CAK-018	Camponotus sp.	Formicinae	99.84%	MF673708.1	0
ANT-84-FOR	CAK-021	Camponotus sp.	Formicinae	86.85%	MF804584.1	1

ANT-85-FOR	CAK-023	Camponotus sp.	Formicinae	97.85%	KF978700.1	0
ANT-86-FOR	CAK-025	Camponotus sp.	Formicinae	87.76%	GU710117.1	1
ANT-87-PON	CAK-026	Pachycondyla sp.	Ponerinae	99.75%	MF673717.1	0
ANT-88-PON	CAK-027	Pachycondyla sp.	Ponerinae	99.22%	MF673717.1	0
ANT-89-MYR	CAK-031	Monomorium subopacum	Myrmicinae	96.97%	MT606357.1	1
ANT-90-MYR	CAK-032	Myramicaria natalensis	Myrmicinae	90.47%	MK591893.1	
ANT-91-MYR	CAK-034	Monomorium subopacum	Myrmicinae	99.15%	GU710429.1	0
ANT-92-FOR	CAK-035	Camponotus sp.	Formicinae	99.51%	MF673708.1	0
ANT-93-MYR	CAK-037	Myrmicaria natalensis	Myrmicinae	90.54%	MK591893.1	1
ANT-94-PON	CAK-039	Pachycondyla sp.	Ponerinae	98.56%	MF673719.1	
ANT-95-PON	CAK-042	Pachycondyla sp.	Ponerinae	93.67%	MF673716.1	0
ANT-96-PON	CAK-043	Pachycondyla sp.	Ponerinae	98.38%	MF673719.1	0
ANT-97-MYR	CAK-052	Pheidole sp.	Myrmicinae	98.10%	KJ141921.1	0
ANT-98-MYR	CAK-053	Tetramorium vorvigi	Myrmicinae	90.65%	HQ547326.1	1
ANT-99-FOR	CAK-062	Camponotus sp.	Formicinae	97.97%	KF978700.1	0

ANT-100-PON	CAK-064	Odontomachus troglodytes	Ponerinae	98.12%	EF611004.1	0
ANT-101-MYR	CAK-066	Crematogaster sp.	Myrmicinae	98.63%	MF673710.1	0
ANT-102-MYR	CAK-068	Pheidole rugaticeps	Myrmicinae	100.00%	MW080378.1	0
ANT-103-MYR	CAK-070	Wasmannia auropunctata	Myrmicinae	100.00%	KX585772.1	0