MORPHOLOGICAL POLYMORPHISM AND ABUNDANCE OF *Camponotus compressus* IN SELECTED HABITATS OF JAFFNA, SRI LANKA, WITH MOLECULAR CONFIRMATION

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Abstract: Ants (Hymenoptera: Formicidae) are ecologically important insects whose success is largely attributed to social organization and caste-based morphological polymorphism. Among them, the carpenter ant *Camponotus compressus* is one of the most widespread species observed in both natural and human-modified habitats. Despite its abundance in Sri Lanka, detailed studies on its caste-specific morphology and molecular confirmation remain limited, particularly in the Jaffna Peninsula. This study examined the abundance, morphological diversity, and molecular identity of *Camponotus compressus* across selected sites in Jaffna from May to September 2024. Three ant species were collected from five distinct habitats, with 100 individuals per species (n = 100) sampled. Specimens were identified using standard taxonomic keys and dissected for caste-specific morphometric analysis of mouthparts. Statistical analyses were performed using one-way ANOVA. DNA was extracted, and the mitochondrial COI gene sequenced for molecular confirmation. Three species were recorded: *C. compressus*, *Solenopsis geminata*, and *Oecophylla smaragdina*, of which *C. compressus* was dominant, especially on host plants such as *Cocos nucifera* and *Vitex negundo*. Morphological analysis revealed significant caste polymorphism, with major workers showing broader mandibles for defense, minor workers exhibiting slender mandibles for foraging, queens with intermediate traits, and males with narrower mandibles reflecting reduced roles; mandible posterior width differed significantly ($P<0.01$). Molecular results confirmed species identity, with COI sequences showing > 99% similarity to *C. compressus* reference sequences. These findings highlight *C. compressus* as the most abundant and morphologically diverse ant species in Jaffna, with pronounced caste polymorphism enhancing ecological versatility. They also demonstrate the value of integrating morphological and molecular approaches for robust species identification and understanding adaptive specialization in carpenter ants.

Keywords: ***Camponotus compressus*****, Morphological diversity, Jaffna.**

I. INTRODUCTION

Ants (Hymenoptera: Formicidae) are among the most ecologically significant and evolutionarily successful insect groups, with an estimated 12,000 species formally described and total global diversity projected to exceed 20,000 species [1]. Occupying virtually every terrestrial ecosystem, ants exert a profound influence on ecological processes through their roles as scavengers, predators, seed dispersers, and ecosystem engineers. Their contributions to nutrient cycling, soil aeration, and organic matter decomposition have made them integral to maintaining soil fertility and ecosystem stability [1]. Furthermore, ants function as bio indicators of ecosystem health and environmental change, given their sensitivity to habitat alteration and their central position in food webs [1].

The evolutionary success of ants is largely attributed to their social organization and division of labour, mediated by the presence of distinct reproductive and worker castes [2]. Ant colonies function as highly integrated superorganisms in which individuals adopt specialized roles, ranging from brood care to foraging and defence [2]. A striking feature of this social organization is morphological polymorphism among castes, particularly in polymorphic genera such as *Camponotus* [2]. Worker polymorphism, in which individuals within the same caste exhibit size-based morphological differences, enables colonies to perform diverse ecological tasks with efficiency [4].

Within the genus *Camponotus*, comprising more than 1,000 described species globally, *Camponotus* compressus stands out as one of the most widespread carpenter ants in Asia. This species is distributed across South and Southeast Asia, including India, Sri Lanka, Bangladesh, and extending to parts of Southeast Asia [3]. In Sri Lanka, it is commonly observed in both natural and anthropogenically altered habitats, nesting in soil, rotting wood, and crevices within human structures. Its adaptability to diverse ecological conditions, ranging from forests to urban landscapes, underscores its ecological versatility.

Camponotus compressus is particularly noted for its large body size relative to other ants in Sri Lanka and its ability to form conspicuous colonies. These colonies often interact closely with human activities: they forage on food scraps, invade stored products, and occasionally damage wooden structures. At the same time, their foraging behavior contributes to ecological functions such as scavenging and soil modification [3]. Their ubiquity and ecological relevance make them an ideal model organism for investigating abundance and morphological diversity across different habitats.

Female ants exhibit three principal castes: queens, workers, and intermediate “soldier-like” forms in polymorphic species [2]. Males, in contrast, are short-lived and morphologically distinct, with adaptations geared primarily toward reproduction. In Camponotus compressus, as in other carpenter ants, worker polymorphism is well developed, with colonies exhibiting major workers (often termed “soldiers”) and minor workers, each with distinct morphologies and functional roles [2].

Major workers are characterized by disproportionately large heads and robust mandibles, suited for colony defence and processing tough food materials. Minor workers, being smaller and more agile, perform tasks such as brood care, nest maintenance, and foraging. Such division of labor enhances colony efficiency and resilience, allowing *C. compressus* colonies to exploit diverse food resources and thrive across multiple habitat types. Investigating this morphological diversity within and between populations provides insights into how environmental factors shape caste-specific adaptations [1].

In Sri Lanka, despite a rich body of work on ant taxonomy and diversity [3], detailed studies focusing on caste-based morphological variation in *C. compressus* remain scarce. Moreover, the northern dry-zone region of Jaffna is ecologically unique, characterized by seasonal drought, high soil salinity in certain areas, and extensive human modification of habitats for agriculture and urbanization. These factors make Jaffna an ideal natural laboratory for examining how abundance and morphological diversity vary across ecological gradients.

Ant morphology, particularly mouthparts, provides valuable clues about their ecological roles and adaptive strategies [4]. The mouthparts of ants are composed of five primary components: mandibles, maxillae, labium, labrum, and hypopharynx. Among these, the mandibles are the most functionally diverse, serving roles in food processing, brood care, defense, and nest construction [4]. In *C. compressus*, the robust mandibles of major workers reflect adaptations for defense and handling coarse food items, while minor workers exhibit mandibles better suited for delicate tasks.

The maxillae and labium, forming the maxillolabial complex, facilitate food handling and sensory interaction, whereas the labrum functions as a cover and assists in manipulating food during ingestion. Such morphological diversity within a single species emphasizes the importance of caste-specific adaptations for colony survival. By quantifying and comparing these morphological traits among castes in Jaffna populations, this study aims to reveal patterns of intra-specific diversity shaped by local ecological pressures.

Although morphological identification remains the cornerstone of ant taxonomy, molecular techniques have become indispensable for species confirmation, population genetics, and evolutionary studies. Ants often display cryptic diversity, where morphologically similar species can only be distinguished using DNA-based methods.

DNA extraction from ant tissues, followed by polymerase chain reaction (PCR) amplification and sequencing of standard genetic markers such as mitochondrial COI (“DNA barcoding”), allows for accurate species-level identification and the detection of genetic divergence within populations [5]. Next-generation sequencing (NGS) has further advanced this field, enabling large-scale genomic studies that shed light on ant social evolution, chemoreception, and ecological adaptation.

Despite the ecological and taxonomic importance of *C. compressus*, there is limited research on its abundance patterns, caste-specific morphological variation, and molecular confirmation in Sri Lanka, particularly in the Jaffna Peninsula. Given the region’s unique environmental conditions and the ecological significance of ants, studying *C. compressus* populations in this context addresses several key gaps:

- Abundance patterns
- Morphological diversity among castes
- Molecular confirmation

II. MATERIALS AND METHODS

The study was conducted from May 2024 to September 2024 at the Zoology Special Laboratory and the Molecular Biology Laboratory, Faculty of Science, University of Jaffna, Sri Lanka. Additional laboratory procedures, particularly molecular confirmation, were carried out at the JICA Biotechnology Laboratory and Plant Protection Laboratory, Faculty of Agriculture, University of Jaffna, during the last week of September 2024.

A. Sample Collection

Ant specimens were collected from five selected sites across the Jaffna District: Kopay, Thirunelveli, Chavakachcheri, Neervely, and Urumpirai, representing diverse ecological habitats, including agricultural fields, home gardens, and semi-urban areas. These sites were selected based on accessibility and the limited scope of the present study, while also providing a representative range of habitats for assessing ant abundance, morphological diversity, and mouthpart polymorphism under varying environmental conditions. At each site, 20 individuals per species were collected through multiple sampling events, yielding a total of 100 specimens per species. Sampling was conducted during daylight hours to coincide with peak ant foraging activity. At each site, multiple sampling events were carried out, with approximately 1 hour spent per sampling session. Each site was visited on several occasions between May and September 2024 to account for temporal variation in ant activity and ensure representative sampling of the species. Collection was performed using aspirators, forceps, and paint brushes, and specimens were stored in labeled vials containing information on sample code, collection site, GPS coordinates, host plant, and date of collection.

Collected specimens were maintained at ambient room temperature (~25 °C) in shaded conditions for 3–5 hours to minimize stress and degradation. They were subsequently transferred to vials containing 70% ethanol for temporary preservation during transport to the laboratory, where they were stored at –20 °C until morphological and molecular analyses were performed, ensuring the preservation of specimen integrity.

B. Climatic Parameters of Sampling Sites

Temperature and relative humidity were recorded during sampling using the AccuWeather mobile application. GPS-enabled smartphones were used to capture real-time climatic data from each site at consistent time intervals.

C. Morphological Identification

Initial morphological identification of ants was performed using a stereo dissection microscope (Olympus, Tokyo). Taxonomic confirmation followed the published keys of Dias [3], focusing on external features such as body coloration, petiole structure, mandible dentition, and antennal segmentation.

Specimens were placed in boiling tubes containing 10% KOH and boiled for 15–20 minutes to clear tissues. After boiling specimens were thoroughly rinsed with distilled water to remove residual alkali. To ensure complete neutralization, a few drops of 1% acetic acid were added before proceeding the dissection. Following cooling, the head was dissected, and mouthparts were carefully removed under a stereo dissection microscope.

Permanent slide preparation involved serial dehydration using ethanol concentrations (25%, 50%, 70%, and 100%), followed by immersion in xylene for fixation. Mouthparts were mounted in an albumin–glycerin medium, dried at 55 °C for 48 hours, and sealed using DPX (Distyrene, Plasticizer, and Xylene) mountant. Slides were cured at 65 °C for one hour to ensure durability. Albumin was separated from fresh chicken eggs and mixed with glycerin in equal proportions (15 ml each). The mixture was stirred for 5 minutes, adjusted with distilled water to achieve proper viscosity, and stored at 4 °C for later use.

Mouthparts (mandibles, maxillae, and labium) (Figure 1) were measured using a calibrated ocular micrometer at 40× magnification. Parameters recorded included:

- Mandible total length (left and right)
- Mandible posterior width
- Mandible anterior width
- Maxilla length and width
- Maxillary palp length
- Labium total length
- Labial palp length

Measurements were replicated across different castes (major worker, minor worker, queen, and male). A total of 100 individuals were selected for morphometric measurements, comprising 45 major workers, 42 minor workers, 10 queens, and 3 males. Measurements were performed separately for each caste to capture caste-specific morphological variation, with each individual measured once to ensure consistency.

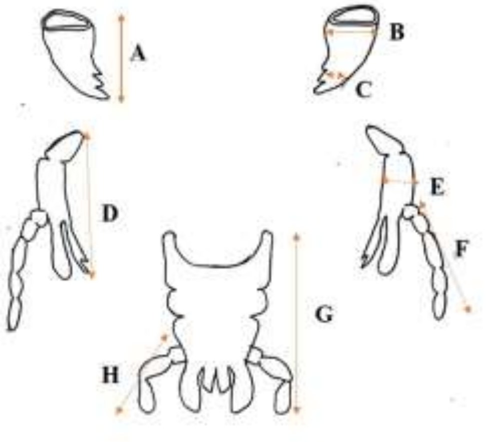

Figure 1. Schematic representation of measured parameters in ant mouthparts.

A- Maximum Length of Mandible
B- Mandible Posterior Width
C- Mandible Anterior Width
D- Maxillae Length
E- Maxillae Width
F- Maxillary Palp Length
G- Labium Length
H- Labial Palp Length

D. Molecular identification

DNA was extracted using the ethanol precipitation method [5]. Heads, thoraces, and abdomens were separately homogenized in the lysis buffer (1 M Tris, 4 M NaCl, 0.5 M EDTA, spermidine, sucrose, dH_2O). Following incubation at 65 °C, samples were treated with potassium acetate, centrifuged, and DNA was precipitated with absolute ethanol. The pellets were washed with 70% ethanol, dried at 50 °C, rehydrated in nuclease-free water, and stored at –20 °C.

The mitochondrial COI gene was amplified using the universal primers LCO1490 (5'-GGT CAA CAA ATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') [5]. PCR was performed with the total of 30 ⌠L reaction mixes with 3.0 µL each primer, 15 ⌠L of 2X master mix, 0.5 ⌠L of *Taq* polymerase, 4.0 ⌠L of DNA, 0.5 ⌠L of $MgCl_2$, and the remaining volume made with nuclease-free water (4.5 ⌠L). PCR amplification was carried out in a 30 µL reaction mixture following a thermal profile that included an initial denaturation step at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 40 seconds, annealing at 45 °C for 1 minute, and extension at 72 °C for 12 seconds. A final extension was performed at 72 °C for 7 minutes to ensure complete amplification.

The amplified PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Germany). DNA was subsequently eluted in Tris buffer and electrophoresed on 1.5% agarose gel stained with ethidium bromide to confirm amplification success. Gel documentation revealed clear banding patterns of the expected size. Purified PCR products were then outsourced to Macrogen Inc. (Korea) for Sanger sequencing to obtain high-quality sequence data.

Resulted sequences were examined using FinchTV to ensure accuracy and quality of base calls. Multiple sequence alignment was performed in MEGA 11. where pairwise genetic distances were calculated to assess sequence variation. Phylogenetic trees were constructed using the Neighbor-Joining method to visualize genetic relationships among samples. Finally, BLASTn searches were conducted against the NCBI GenBank database to validate species identity and confirm the molecular characterization of the samples.

E. Statistical Analysis of the mouthpart measurements

To assess the significance of differences in mouthpart measurements across species, a One-way ANOVA and Tukey's HSD post-hoc test were employed. Before conducting the statistical tests, the data were carefully reviewed and entered into SPSS (Version 22), with each variable appropriately labelled. Missing values and outliers were handled diligently to ensure the integrity of the dataset. Descriptive statistics, including means and standard deviations, were calculated to provide an overview of the data distribution and variability for each species. A One-way ANOVA was performed to evaluate whether there were statistically significant differences in mouthpart measurements between the ant species under investigation. Following the ANOVA, Tukey's HSD post-hoc test was applied to identify specific species pairs that exhibited significant differences in their mouthpart measurements. The results from the ANOVA and post-hoc analyses were interpreted in line with the study's objectives, focusing on the functional adaptations of ant mouthparts.

III. Results and Discussion

Three ant species (Figure 2) were identified from the study locations in Jaffna District: *Camponotus compressus*, *Solenopsis geminata,* and *Oecophylla smaragdina*.

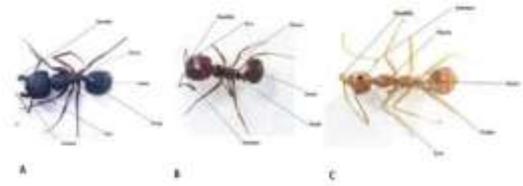

Figure 2. Ant species identified from the study 2.1a. *Camponotus compressus*, 2.1b. *Solenopsis geminata,* and 2.1c. *Oecophylla smaragdina*

Among the three ant species, *Camponotus compressus* was the most frequently encountered, recorded across diverse habitats and host plants, particularly *Cocos nucifera* and *Vitex negundo*. *Oecophylla smaragdina* and *Solenopsis geminata* were less frequently observed, occurring in 30% and 20% of sampling events, respectively, and were associated with a narrower range of habitats and host plants. Overall, the percentage occurrence of the three species in the total sample was 33.3% each, reflecting uniform sampling effort, while *C. compressus* dominated in terms of frequency across habitats. Its abundance compared to the other species is summarized in Table 1, which illustrates the distribution of ant species across host plants in the study area. The observed dominance of *C. compressus* highlights its ecological adaptability, consistent with its generalist foraging behavior and wide nesting preferences reported in previous studies [3].

Table 1. Distribution of *C. compressus* on different host plants in Jaffna District.

Location	Host Plant
Kopay	*Cocos nucifera*
Kopay	*Vitex negundo*
Thirunelvely	*Syzygium samarangense*
Chavakachcheri	*Gliricidia sepium*
Neervely	*Acalypha wilkesiana*

Morphological examinations revealed distinct caste-based polymorphism in *C. compressus*. Minor workers displayed slender mandibles suited for foraging, while major workers possessed significantly larger and broader mandibles optimized for defense and heavy labor. Queens showed intermediate morphologies, while males had comparatively narrow mandibles, reflecting reduced functional demand (Figure 3).

Figure 3. Stereo dissection microscopic images of *C.compressus*, 3.1a. Different sizes mandibles, 3.2b. Mandible of Major worker, 3.3c. Maxillae of Major worker

The statistical comparison of mouthpart dimensions across castes using one-way ANOVA (see Table 2) confirmed that mandible posterior width differed significantly ($P<0.01$).

Table 2. The statistical comparison of caste-specific mouthpart dimensions in *C. compressus.*

Parameter	ANOVA p-value
Mandible Total Length	0.442

Parameter	ANOVA p-value
Mandible Posterior Width	**0.006**
Mandible Anterior Width	0.903
Maxillae width	0.398
Maxillary Palp Length	0.176
Maxillae Total Length	0.873
Labium Total Length	0.176
Labial Palp Length	0.105

Tukey test was performed to assess the level of variance among different casts and the results are tabulated in table 3.

Table 3. Tukey's HSD post-hoc test comparing mandible posterior width among castes of *C.compressus*

Comparison	Mean Difference	Std. Error	Sig.	95% Confidence Interval
Major Workers vs. Minor Workers	0.0040	0.0465	0.988	(-0.0908, 0.0988)
Major Workers vs. Queen	0.0035	0.0665	0.999	(-0.1380, 0.1450)
Major Workers vs. Male	0.0230	0.0550	0.992	(-0.0877, 0.1337)
Minor Worker vs. Queen	-0.0025	0.0500	1.000	(-0.0960, 0.0910)
Minor Worker vs. Male	0.0190	0.0457	0.997	(-0.0847, 0.1227)
Queen vs. Male	0.0215	0.0555	0.992	(-0.0865, 0.1295)

Table 4. Tukey's HSD post-hoc test comparing mandible posterior width among castes of *O.smaragdina*

Comparison	Mean Difference	Std. Error	Sig.	95% Confidence Interval
Major Worker vs. Minor Worker	-0.0080	0.0625	0.944	(-0.1431, 0.1271)
Major Worker vs. Queen	0.0450	0.0450	0.836	(-0.0712, 0.1612)
Major Worker vs. Male	0.0030	0.0525	0.993	(-0.0889, 0.0959)
Minor Worker vs. Queen	0.0530	0.0575	0.923	(-0.0695, 0.1755)
Minor Worker vs. Male	-0.0120	0.0550	0.999	(-0.1249, 0.1009)
Queen vs. Male	-0.0250	0.0635	0.984	(-0.1495, 0.0995)

Table 5. Tukey's HSD post-hoc test comparing mandible posterior width among castes of *S. geminata*

Comparison	Mean Difference	Std. Error	Sig.	95% Confidence Interval
Major Workers vs. Minor Worker	-0.0320	0.0660	0.780	(-0.1344, 0.0704)
Major Workers vs. Queen	0.0500	0.0830	0.915	(-0.1552, 0.2552)
Major Workers vs. Male	0.0250	0.0600	0.975	(-0.0934, 0.1434)
Minor Worker vs. Queen	0.0150	0.0620	0.995	(-0.1079, 0.1379)
Minor Worker vs. Male	-0.0070	0.0590	0.999	(-0.1125, 0.0985)
Queen vs. Male	-0.0220	0.0720	0.932	(-0.1733, 0.1293)

All pairwise comparisons of mandible posterior width were non-significant ($p > 0.05$), indicating functional similarity among castes in all three species. *C. compressus* showed slightly more variation among castes compared to *S. geminata* and *O. smaragdina*, which displayed relatively uniform mandible morphology. This suggests that while caste-based differentiation in mandible structure exists to some extent in *C. compressus*, it is minimal in the other two species. Their relative uniformity across castes suggests reliance on behavioral rather than morphological specialization for task allocation. This interspecific difference demonstrates the diverse evolutionary pathways ants employ to achieve colony efficiency.

Average site temperatures ranged from 28 to 32 °C with relative humidity between 68 and 84%. These stable dry-zone conditions provide favorable environmental settings for *C. compressus*, explaining its dominance across study sites.

Molecular confirmation further validated the morphological results. PCR amplification of the mitochondrial *COI* gene successfully yielded fragments of ~700 bp. Sequencing results demonstrated > 99% similarity with *C. compressus* reference sequences in GenBank, confirming accurate species identification.

IV. Conclusions

These findings confirm that *C. compressus* is the most abundant and the most morphologically diverse ant species in the Jaffna region. The pronounced caste polymorphism, particularly in mandible architecture, reflects its adaptive strategy to maximize efficiency in defense, foraging, and resource processing. Unlike *S. geminata* and *O. smaragdina*, where caste differences were less marked, *C. compressus* exhibits strong structural specialization, providing a clear evolutionary advantage in heterogeneous environments. By integrating morphological and molecular approaches, this study not only provides robust confirmation of species identity but also emphasizes the ecological and evolutionary significance of caste polymorphism in carpenter ants.

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