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Research Article

Ultrastructure and morphometric characteristics of *Oecophylla smaragdina* (Fabricius, 1775) (Hymenoptera: Formicidae)

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ABSTRACT: In this study, we explored various aspects of *Oecophylla smaragdina*, a potential biocontrol agent, evolution by examining its morphometric characteristics. We employed an intensive All-out search method (AOSM) to gather specimens from multiple locations in Vadakku Karaseri, Tamil Nadu. Our analysis involved measuring 16 morphological traits and indices, including Total Length, Head Width, Head Length, Eye Length and more, using specimens from five colonies in each locality. By calculating various morphometric indices, we assessed specific features and conducted a Principal Component Analysis (PCA) to determine the significance of head-related variables, antennae, and body size in shaping morphometric variation. Our study also revealed positive and negative associations between different morphometric variables, as highlighted by Pearson correlation coefficients. Furthermore, we used a Scanning Electron Microscope to examine the ultrastructure of the abdomen, revealing distinct features such as a one-jointed pedicel and modifications of the poison gland and stings. This comprehensive research provides valuable insights into *O. smaragdina* morphometric characteristics, enhancing our understanding of its variability and potential adaptations in various habitats.

KEYWORDS: Morphometric characteristics, *Oecophylla smaragdina*, Principal Component Analysis (PCA), taxonomic investigations

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INTRODUCTION

Ants are highly prominent creatures in both terrestrial and arboreal ecosystems found in tropical and subtropical regions, serving as significant scavengers and predators of various arthropods, including other ants (Hölldobler & Wilson, 1990; Tobin, 1991). *Oecophylla smaragdina* Fab belongs to subfamily Formicinae of Formicidae one of the largest families of order Hymenoptera. It is found in Southern Asia, Southeast Asia, Northern Australia, and Melanesia (Crozier *et al*., 2010; Verghese *et al*., 2013). In India, deep forests, tropical rainforests, coastal regions, and mangrove ecosystems are typical habitats for *O. smaragdina*. These locations are especially rich in plant species diversity, affording a variety of food sources and an abundance of nesting sites for this species (Crozier *et al*., 2010).

The castes of the weaver ant species demonstrate size dimorphism, with distinctly small and large workers easily discernible, in addition to differences observed between males and females. In the weaver ant, *O. smaragdina*, major worker adults measured 8 mm long compared to 5 mm for minor workers, while their respective pupae were 4 mm and 3 mm in length (Bharti & Silla, 2011).

An important biological control agent against economically important agricultural pests is *O. smaragdina*, which is well known for its aggressive predatory behavior. This species has proven effective in controlling various pests in different horticultural crops, as reported by Ambika and Nalini, 2019. Furthermore, Lim *et al*. (2008) demonstrated *O. smaragdina* superior larval control over chemical insecticides in preventing shoot damage. Additionally, Peng and Christian (2004) explored the economic aspects of weaver ants as biocontrol agents. Notably, their pheromones, as demonstrated by Peng and Reilly in 2012, can effectively deter insect herbivores and fruit flies. Worker ants are essential to the survival of their nest mates throughout the life history of *O. smaragdina* because they feed the queen, who then produces the next generation (Divyangi & Nikunj, 2020).

Morphology alone does not suffice, as it represents only one aspect of an animal's characteristics, which is an important consideration in taxonomy (Bahder, 2009; Jarman & Elliott, 2000; Blaxter, & Floyd, 2003). Early evolutionists initially relied on morphology for the confident identification of ants based on their physical traits, as emphasized. (Afzal, 2013). In their study, Queiroz (2015) measured three characteristics (head width, body and femur length) of ants collected from each vegetation type. They then used the average Head Width (HW) of the ants functioning in the capacity of measure for complete body size. Allometry of leg length was noted in every type of vegetation. Head width is a precise indicator of body size, as noted by Hölldobler and Wilson (1990) and Kaspari and Weiser (1999), while femur length reliably proxies total leg length.

A principal component analysis of a covariance matrix between morphological traits primarily reflects an overall multivariate body size measure. Principal component analysis was useful in describing patterns of allometric variation in some populations of *Camponotus cruentatus* and *Aphaenogaster senillis* from Morocco and the western Mediterranean basin (Cagniant *et al.*, 1991). Barr *et al*. (1985) used an equivalent approach as principal component analysis in connection with non-metric multidimensional scaling.

Our study focuses on collections and morphological analysis of *O. smaragdina* ants and their morphometrics, including whole body and ultrastructural variations of the abdomen using scanning electron microscopy.

MATERIALS AND METHODS

Collection site

Collections of ant species were carried out in Vadakku Karaseri, Thoothukudi District, Tamil Nadu, employing an intensive All-out Search Method (AOSM), as described in Gadagkar *et al*. (1993) and Raj *et al*. (2017). The specimens captured in each trap were counted and subsequently identified. *O. smaragdina* (Fabricius, 1775) ants were collected using hand-picking method during January and May 2022. We selected sampling locations (Figure 1) and collected ant specimens for morphometric measurements from five colonies within an anthill at each of the following localities: Dheivaseyalpuram (8° 44´16´´N; 77° 55´10´´E), Vadakku Karaseri (8° 45´24´´N; 77° 53´12´´E), Periyakulam (8° 45´53´´N; 77° 53´26´´E), Amman Kovil (8°45´19´´N; 77° 53´49´´E), Emmanuel Church (8°45´28´´N; 77°53´43´´E), and Kasalingapuram (8°46´35´´N; 77°53´59´´E). Geomapping coordinates were obtained from Google Earth Pro Software and subsequently analyzed to create a map using QGIS software (Version 3.16).

Morphometric measurements and anatomical indices

Measurements represented adaptations from earlier morphological approaches (Seifert 2002; Seifert *et al*. (2017)

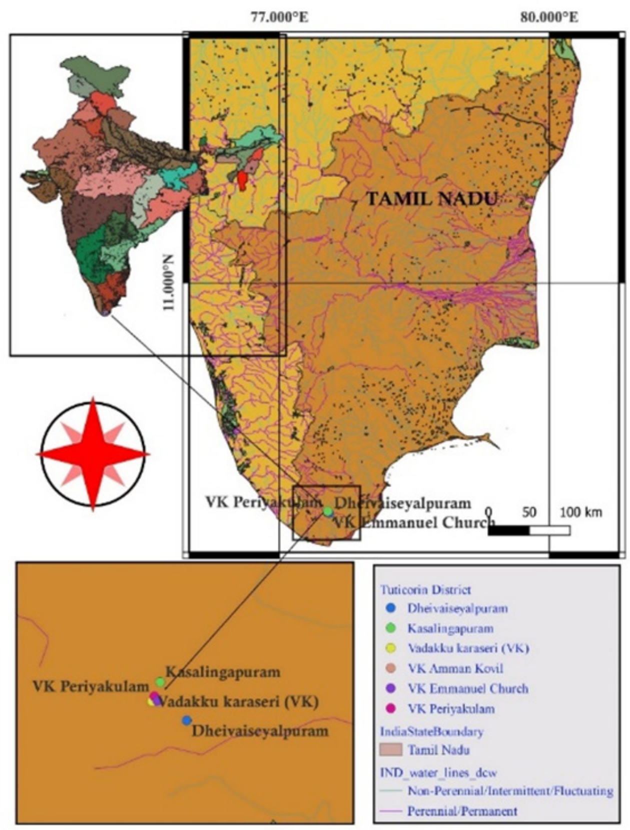

Figure 1. Illustrates the mapping of the sampling area.

as the morphometric quantification methodology built upon prior protocols (Brendon *et al*., 2013; Branstetter, 2013). The dehydrated specimens were fixed using a pin-holding stage. For each specimen, we measured 10 morphological characters and indices as distinct. (Seifert, 2002; Seifert *et al*., 2017; Gratiashvili *et al*., 2020).

Scanning Electron Microscopy (SEM)

For the SEM study, the dissected abdomens were thoroughly washed with distilled water and fixed in 10% formalin for 12 hours. The specimens were then dehydrated in an ascending series of alcohol grades 95%, cleared in acetone, and dried at room temperature. Subsequently, these appendages were mounted on carbon-coated metallic stubs at various angles (Parag & Deepak, 2021). The specimens were observed using a CAREL ZEISS: EVO 18 SEM at magnifications ranging from 25X to 5500X at the Archbishop Casimir Instrumentation Centre, St. Joseph's College (Autonomous), Tiruchirappalli, India.

Statistical analysis

The mean value, range, and Standard Deviation (SD) for each morphological character were calculated. Pearson's simple correlation analyses were conducted between morphometric variables, and significance was tested at 1% and 5% probability levels. Patterns of morphometric variation were investigated through Principal Component Analysis (PCA) using the software package Past v.4.03 (Hammer *et al*., 2001).

RESULTS AND DISCUSSION

The present study represents the first-ever attempt to investigate the morphometric structures of *O. smaragdina* ant colonies, a weaver ant species. These ants were collected from nests located on *Mangifera indica* and *Morinda citrifolia* trees in Vadakku Karaseri regions (Figure 2). The nests are constructed by binding leaves together with a fine whitish membranous tissue paper or synthetic substance (Tiwari, 1999), the ant larvae generate silk, which the worker ants use to suture foliage (Patel & Bhatt, 2020). Known as the aggressive 'Red Ant' of India, these ants inhabit trees and build their nests in leaves. After gathering samples, various measurements for different characteristics and indices were analyzed to confirm the morphometric characteristics of *O. smaragdina* species.

These morphometric measurements provide valuable data on the physical characteristics of *Oecophylla smaragdina* workers from Karaseri, including the size and dimensions of various body parts (Figure 3). The mean values represent the

Figure 2. Habitat of *O. smaragdina* on the trees of *Mangifera indica* and *Morinda citrifolia*.

Table 1. Morphometric measurements of workers of *O. smaragdina* from Karaseri (n=50)

Variable	Mean±SD	[Range]
HW	1.580±0.252	[1.200,2.100]
HL	2.614±0.278	[2.100,3.000]
ML	0.862±0.171	[0.500,1.100]
AL	0.868±0.166	[0.500,1.100]
POL	0.900±0.136	[0.700,1.100]
EL	0.500±0.000	[0.500,0.500]
SL	2.382±0.126	[2.200,2.600]
PW	0.832±0.160	[0.600,1.100]
PePL	0.968±0.180	[0.700,1.300]
TL	9.124±0.625	[8.100,10.200]
PPL	0.492±0.078	[0.400,0.600]
PPW	0.548±0.109	[0.400,0.700]
PL	0.604±0.165	[0.400,1.100]
PW	0.680±0.101	[0.500,0.800]
MFL	3.452±0.115	[3.300,3.600]
MTL	2.938±0.107	[2.800,3.100]

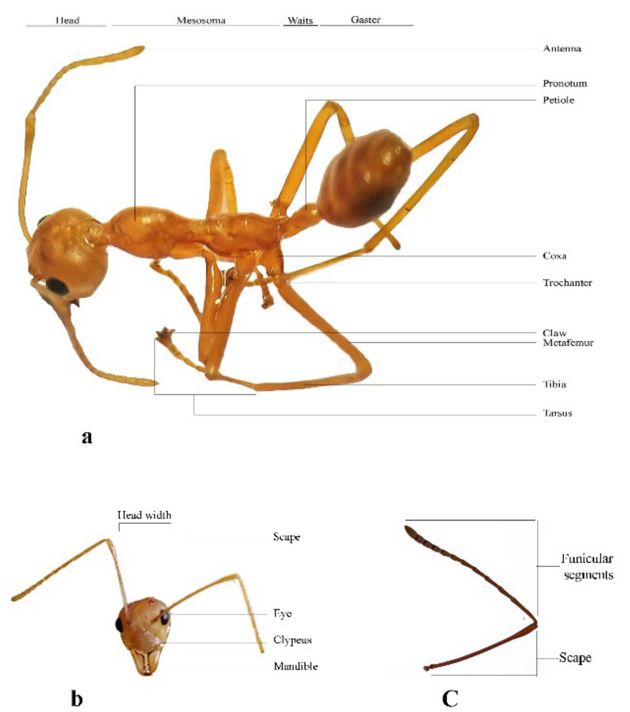

Figure 3. Depicts the morphology of *O. smaragdina*, including **(a)** the body profile, **(b)** head, **(c)** antenna.

average measurements, while the Standard Deviations (SD) indicate the variability around the mean. The documented range encompasses the lowest through highest measurements gathered from the sample population of 50 workers. (Table 1).

The results for the morphometric measurements of *O. smaragdina* presented in Table 2 and Figure 4 demonstrate quantified indices for head ratio (Cephalic Index) of 59.84; Eye Index of 32.25; proportional ocular ratio (Relative Eye Index) of 32.25; mandible ratio of 34.36; antenna ratio (Scape Index) of 137.4; Ocular Index of 19.30; Petiole Index of 68.08; postpetiole width comparison (Petiole Width Index) of 117.02; and metafemur ratio (Meta Femur Index) of 44.92. Additionally, the overall cranial size (Head size) was 104.8. These quantified indices offer valuable insight into various morphological and proportional characteristics of *O. smaragdina*, including cranial, ocular, mandibular, antennal, petiolar, leg, and body metrics.

Table 2. Morphometric indices of *O. smaragdina*

S. No	Morphometric indices	Result
1	Cephalic Index	59.84
2	Eye Index	32.25
3	Relative Eye Index	32.25
4	Mandible	34.36
5	Scape Index	137.41
6	Ocular Index	19.30
7	Petiole Index	68.08
8	Petiole Width Index	117.02
9	Meta Femur Index	44.92
10	Head size	104.85

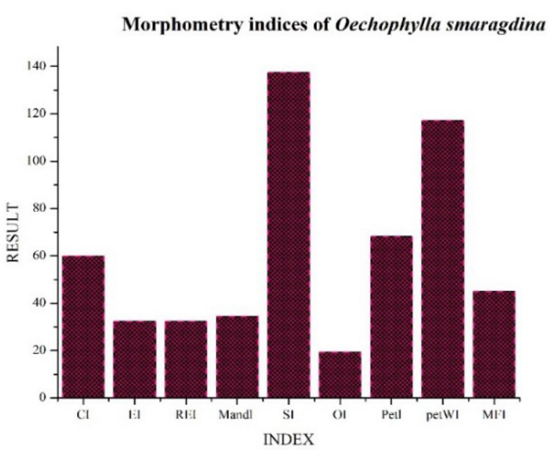

Figure 4. Morphometric indices of *O. smaragdina*.

The Principal Component Analysis (PCA) was conducted on 16 morphometric variables from 50 specimens (both male and female) from Vadakku Karaseri (Table 3, Figure 6) and its surrounding areas. The Principal Component Analysis (PCA) was conducted on morphometric characteristics of *O. smaragdina* to explore the proportion of contribution and variable coefficients of the first two eigenvectors (Principal components). PC 1: The first principal component (PC 1) showed the highest eigenvalue of 0.405, explaining 53.25% of the total variance in the dataset. Among the variables, Head Width (HW) had the highest positive coefficient of 0.129, contributing significantly to PC 1. Eye Length (EL) also exhibited a notable positive coefficient of 3.40, contributing substantially to the component. Other variables such as Mesosoma Length (ML), Antenna Length (AL), Posterior Length (PoL), and Total Length (TL) also made meaningful contributions to PC 1 (Figure 6).

PC 2: The second principal component (PC 2) had an eigenvalue of 0.087, explaining 11.52% of the total variance. The variable with the highest absolute coefficient in PC 2 was Head Length (HL) with a value of -0.0721, indicating a significant contribution to the component. Scape Length (SL) showed a negative coefficient of -0.00551, contributing modestly to PC 2. Other variables like Pronotum Width (PnW), Petiole Length (PpL), Petiole Width (PW), and Maximal Femur Length (MFL) also had non-negligible contributions to PC 2. In summary, the PCA revealed that the morphometric characteristics of *O. smaragdina* are mainly influenced by variables related to the head, antennae, and

Table 3. PCA Eigenvector proportion and variable contribution

Variables	PC 1	Eigenvalue	% Variance
HW	0.12919	0.405084	53.254
HL	-0.07211	0.087628	11.52
ML	0.067396	0.061789	8.123
AL	0.044624	0.046105	6.0611
PoL	0.077165	0.037819	4.9718
EL	3.40	0.029642	3.8969
SL	-0.00551	0.019643	2.5824
PnW	-0.03417	0.01633	2.1468
PpL	0.015725	0.014298	1.8796
TL	0.98014	0.012854	1.6898
PPL	-0.01053	0.009831	1.2924
PPW	-0.01372	0.007374	0.96936
PL	0.0491	0.004883	0.64192
PW	-0.00039	0.004355	0.57255
MFL	-0.01305	0.003034	0.39883
MTL	0.024894	1.05	1.38

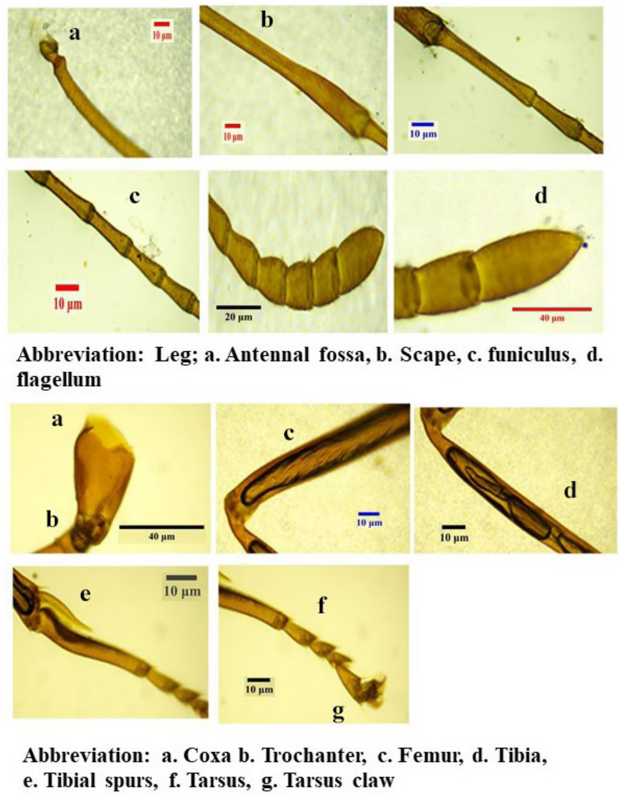

Figure 5. morphology of *O. smaragdina*, highlighting the antennal fossa and the leg structure.

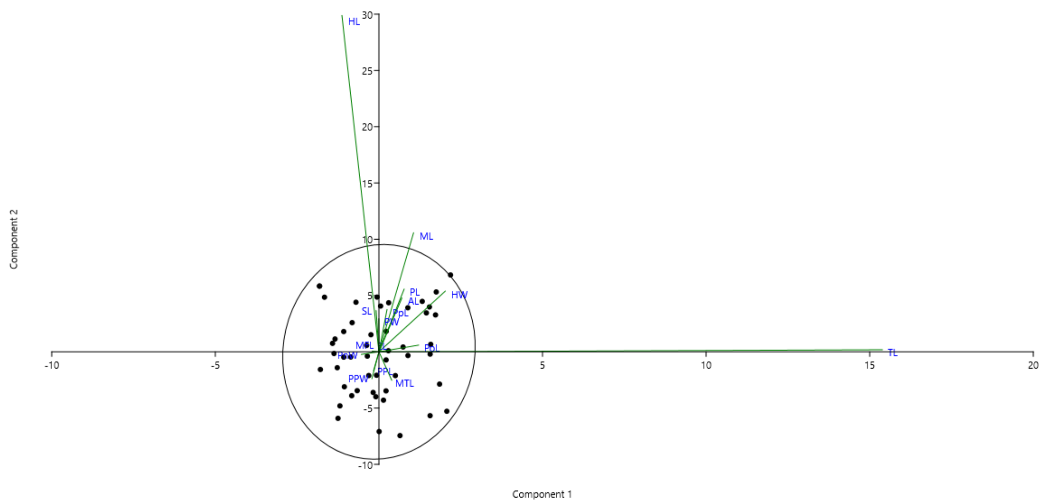

Figure 6. Scatter diagram based on PCA of significant morphometric variables *of O. smaragdina*.

body size (e.g., HW, HL, ML, AL, PoL, and TL), which together account for the majority of the variation observed in the dataset. The second principal component (PC 2) primarily captures variation in variables related to head size (HL) and scape length (SL). Gratiashvili *et al*. (2020) performed a PCA analysis, where they observed that PC1, which accounted for 68% of the total variation, was primarily influenced by CW, PEW, PoOc, in contrast to EYE W. Additionally, PC2, explaining 31% of the total variation, was primarily influenced by EYE L, in contrast to SL. The subsequent plotting of PC1 against PC2 unveiled three distinct clusters: one separating *C. koshewnikovi* and *C. stambuloffii*, and the other differentiating the samples from Georgia from the two other taxa.

Table 4. Pearson correlation coefficient of *O. smaragdina* (n=50)

Correlation Matrix																	
		HW	**HL**	**ML**	**AL**	**PoL**	**EL**	**SL**	**PnW**	**PepL**	**PPL**	**PPW**	**PL**	**PW**	**MFL**	**MTL**	**TL**
Correlation	HW	1.000	.013	-.042	.218	.090		-.050	-.171	.035	.023	-.224	.256	.016	-.006	.127	.276
	HL	.013	1.000	.346	.059	-.054		.218	-.056	.111	-.070	-.090	.132	.236	.047	-.211	-.148
	ML	-.042	.346	1.000	.481	.141		.043	.053	.006	-.314	-.195	.236	.050	-.095	-.065	.243
	AL	.218	.059	.481	1.000	.300		.138	-.161	-.158	-.194	-.172	.027	-.063	-.361	.001	.139
	PoL	.090	-.054	.141	.300	1.000		.204	-.075	.075	-.174	.110	-.091	.194	.039	.056	.347
	EL						1.000										
	SL	-.050	.218	.043	.138	.204		1.000	.009	.154	-.140	.020	.004	.212	-.075	.128	-.026
	PnW	-.171	-.056	.053	-.161	-.075		.009	1.000	.264	-.291	-.289	.320	.040	.063	-.229	-.128
	PepL	.035	.111	.006	-.158	.075		.154	.264	1.000	-.310	-.180	.121	.121	.280	.054	.056
	PPL	.023	-.070	-.314	-.194	-.174		-.140	-.291	-.310	1.000	.166	-.140	-.021	-.021	.087	-.080
	PPW	-.224	-.090	-.195	-.172	.110		.020	-.289	-.180	.166	1.000	-.079	.163	-.171	-.142	-.068
	PL	.256	.132	.236	.027	-.091		.004	.320	.121	-.140	-.079	1.000	-.068	-.076	-.124	.173
	PW	.016	.236	.050	-.063	.194		.212	.040	.121	-.021	.163	-.068	1.000	.056	-.496	.005
	MFL	-.006	.047	-.095	-.361	.039		-.075	.063	.280	-.021	-.171	-.076	.056	1.000	.102	-.066
	MTL	.127	-.211	-.065	.001	.056		.128	-.229	.054	.087	-.142	-.124	-.496	.102	1.000	.136
	TL	.276	-.148	.243	.139	.347		-.026	-.128	.056	-.080	-.068	.173	.005	-.066	.136	1.000

Abbreviations: Head width (HW), Head length (HL), mandible length (ML), antenna length (AL), post orbital length (PoL), eye length (EL), scape length (SL), Pronotal width (PnW), propodeal length (PepL), Postpetiole length (PPL), post petiole width (PPW), petiole length (PL), petiole width (PW), Metafemur length (MFL), Metatibial length (MTL), total length (TL)

Table 4 presents the Pearson correlation coefficients of various morphometric variables of *O. smaragdina*. Head Width (HW) positively correlates with Head Length (HL) (r = 0.013), Antenna Length (AL) (r = 0.218), Post Orbital Length (PoL) (r = 0.090), and Petiole Length (PL) (r = 0.256). Conversely, it negatively correlates with Mandible Length (ML) (r = -0.042), Pronotal Width (PnW) (r = -0.171), Postpetiole Width (PPW) (r = -0.224), and Metafemur Length (MFL) (r = -0.006). HW also shows a weak positive correlation with Scape Length (SL) (r = -0.050) and a moderate positive correlation with Metatibial Length (MTL) (r = 0.127) and Total Length (TL) (r = 0.276).

Head Length (HL) positively correlates with Mandible Length (ML) (r = 0.346), Postpetiole Length (PepL) (r = 0.111), and Petiole Width (PW) (r = 0.236). It is negatively correlated with Post Orbital Length (PoL) (r = -0.054), Postpetiole Length (PPL) (r = -0.070), Postpetiole Width (PPW) (r = -0.090), and Mesosoma Width (MTL) (r = -0.211). HL also shows a moderate positive correlation with Scape Length (SL) (r = 0.218) and a weak negative correlation with Total Length (TL) (r = -0.148).

Mesosoma Length (ML) is positively correlated with Antenna Length (AL) (r = 0.481), Petiole Length (PL) (r = 0.236), and Total Length (TL) (r = 0.243). It is negatively correlated with Postpetiole Length (PPL) (r = -0.314), Postpetiole Width (PPW) (r = -0.195), and Mesosoma Width (MTL) (r = -0.065).

Antenna Length (AL) is positively correlated with Post Orbital Length (PoL) (r = 0.300) and Petiole Length (PL) (r = 0.027). It is negatively correlated with Pronotal Width (PnW) (r = -0.161), Postpetiole Length (PPL) (r = -0.158), and Postpetiole Width (PPW) (r = -0.174). AL also shows a weak positive correlation with Metafemur Length (MFL) (r = 0.039) and a negligible correlation with Total Length (TL) (r = 0.139).

Post Orbital Length (PoL) is positively correlated with Postpetiole Width (PPW) (r = 0.110) and Petiole Width (PW) (r = 0.194). It is negatively correlated with Pronotal Width (PnW) (r = -0.075), Postpetiole Length (PPL) (r = -0.174), and Petiole Length (PL) (r = -0.091). PoL also shows a weak positive correlation with Metafemur Length (MFL) (r = 0.039) and a moderate positive correlation with Total Length (TL) (r = 0.347).

Based on our morphometric analysis, a notable morphological variation in leg size was observed in polymorphic individuals. The infertile members of the colony exhibited two types of sensillae, sensilla trichodea, and sensilla trichodea curvata, on their leg segments. In contrast, the queen's leg segments showed the presence of sensilla basiconica, in addition to the previously mentioned sensillae, confirming the sensory roles of legs in addition to their locomotion functions in *O. smaragdina* polymorphs (Parag & Deepak, 2021).

The weaver ant *Oecophylla smaragdina* displays specialized morphological adaptations like the pronounced antennal fossa on their head capsule (Figure 5) which houses antennae used for sensory reception and communication (Saarinen, 2006). This allows effective exchange of chemical and tactile signals important for complex social coordination. Additionally, their distinctly elbowed and thin hind legs (Figure 5) allow them to grasp and manipulate leaves with silk produced by larvae to sew them together into the intricate arboreal nests that this species is known for (Saarinen, 2006).

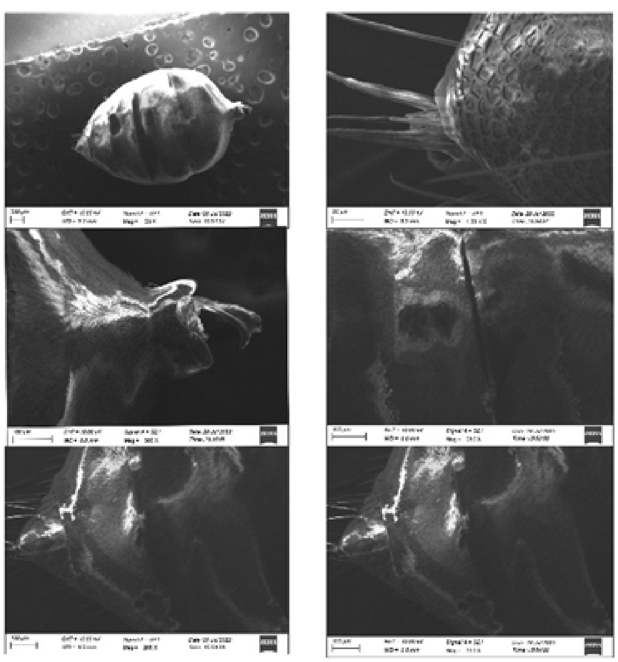

Figure 7. Ultrastructure of *O. smaragdina* (abdomen).

The hind legs work seamlessly with the mandibles to construct protective enclosed nest spaces ideal for thriving arboreal ant colonies. Thus the antennal fossa and leg jointly facilitate complex group behaviors like nest building fundamental to the ecology of *O. smaragdina* as a weaver ant species.

In this study, we employed a Scanning Electron Microscope to examine the abdominal ultrastructure of *O. smaragdina*, leading to the discovery of distinct features. Notably, the abdomen exhibited a one-jointed pedicel with no constriction between the two basal abdominal segments (Figure 7). Furthermore, significant modifications of the poison gland and stings were observed, providing valuable insights into the unique anatomical characteristics of this ant species.

CONCLUSIONS

The results of our study provide essential knowledge about the morphological characteristics of *O. smaragdina*, enhancing our understanding of this remarkable ant species and its ecological significance. These findings pave the way for future studies on the ecological and behavioral adaptations of *O. smaragdina*, further contributing to our understanding of evolution.

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