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Morphological analysis of population samples of termite soldier castes in mid-hills of Meghalaya

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ABSTRACT

Many insects during one or more of their life stages live inside the soil and some in the tunnels made by them for life cycle completion and consumption of food materials. One such group of insects are Termites (Blattodea: Insecta), commonly known as White ants. The random survey made in mid-hills of Meghalaya depicted a total ten species of termites, in that two of them were newly identified from Meghalaya *viz*., *Odontotermes singsiti* Bose and *Odontotermes hainanensis* Wasmann. Macrotermitinae was the most presiding subfamily representing fungus growing termites in mid-hills of Meghalaya. To promote the morphometric variation of soldiers, eight morphometric parameters were selected and measured under stereozoom binocular microscope with a built-in magnification changer. The co-efficient of variation became larger in pronotum length and least in head width and concluded that more variation of termite soldiers were occurred in pronotum length. In addition, principal component analysis of termite soldiers were worked out on the data collected in a species wise manner. PCA was performed using above mentioned parameters, which revealed that soldiers differed distinctly from each species.

1. Introduction

Highest level of organization of enormous insect species that live in a particular community or colony and manifest three distinct categories *viz*., division of labor, overlap of generations and group integration with other individuals of same species is considered as social insect. One of the most important social insects are Termites with many synonyms Termitida, Termitina and Socialia in use, belonging to an orthopteroid group of insects which are found with greater diversity. They are hemimetabolous and look like smaller in size with white to brown body living in highly organized and integrated societies that exhibit ancient polymorphic eusociality life. Morphometry defined the study of measurement and quantitative analysis of form. Morphometry is not a coherent discipline. It was practising in some major fields such as anthropology, entomology, cytology, nematology, geology and paleobiology. These methods are powerful research tools when used in the context of biological knowledge (Daly, 1985). The morphological features of termites are much more important in systematic studies. The basic knowledge of morphogenesis is necessary for understanding the biological occurrence during soldier differentiation (Miura *et al.,* 1999). The morphological diversity of the soldier caste was conspicuous, and therefore major taxonomic characters were concentrated in soldiers. In other castes, such as workers, however, there was no such diversification of morphology (Krishna, 1970). Generally castes like workers, pseudergates and immature reproductives in some genera lack specialized morphology of the mandibles for identification (Hausberger *et al.,* 2011). The morphology of termite soldiers with their larger heads and elongated mandibles thought to be more adaptive for defense against enemies like predators, indicates the close relationship between the morphology and function (Koshikawa et al. 2002). Some studies also reported based on termite morphological changes in conformity with soldier differentiation. Considering the scope and importance of termites in ecosystem building and also the severity and magnitude of termite depredation, a detailed study is a must

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on its variation of different species of soldier castes. The objective of this work was to contribute towards taxonomic knowledge of termite species by means of study of intracolonial variations in the soldier caste.

2. Materials And Methods

2.1 Study area

The investigation was carried out in Ri-Bhoi district of Meghalaya and experimental site was situated at 25^{0}40'N latitude to 91^{0}54'E longitude and at an altitude of 1000 m above the mean sea level (MSL). The experiment was started during July 2019 and continued up to March 2020. At each of these sites, sampling was carried out as per standard procedure.

Map of Ri Bhoi District

2.2 Determination of termite species

Foraging soldiers and workers of all termite species were collected and preserved in 80% ethanol. The species identifications were made by using Chhotani, (1997). Specimens from the samples were selected at random and measured under stereozoom binocular microscope with a built-in magnification changer. Measurements were taken with the aid of a calibrated ocular and stage micrometer. The following characteristics were observed for morphometric variation of termite soldiers such as Head length (HL), Head width (HW), Left mandible length (LML), Pronotum length (PL), Pronotum width (PW), Postmentum length (PoL), Postmentum width (PoW) and Antennal segment (AS). We studied PCA to evaluate the morphological changes between the species of termite soldiers. The first and second principal components were explained by component eigenvectors.

2.3 Analysis

The data pertaining to morphometric variation of termite soldiers were statistically analysed by using Fisher's method of ANOVA in completely randomized design (CRD) (Snedecor and Cochran, 1967). PCA was done by using OPSTAT software.

3. Results

Observations on the external anatomy of termite specimens in the laboratory showed the existence of ten species, which are joined in three sub-families (Macrotermitinae, Termitinae, Amitermitinae) and six genera (*Odontotermes*, *Hypotermes*, *Microtermes*, *Macrotermes*, *Pseudocapritermes*, *Microcerotermes*). To see the morphometric measurements of each species of termite that is found are described as follows:

3.1 Genus *Odontotermes*

The genus *Odontotermes* belongs to the family Termitidae with subfamily Macrotermitinae. In this study, there were 5 types of *Odontotermes*, namely *Odontotermes parvidens*, *Odontotermes horai*, *Odontotermes singsiti*, *Odontotermes feae* and *Odontotermes hainanensis*.

Odontotermes parvidens

Odontotermes parvidens

Diagnostics: Head-capsule large with yellowish brown; Body creamy yellow, moderately pilose; Head sparsely pilose; Left mandible with a small tooth at proximal third.
Measurements (mm): HL: 1.93-2.21, HW: 1.57-1.79, LML: 1.22-1.38, PL: 0.69-0.78, PW: 1.35-1.42, PoL: 1.39-1.49, PoW: 0.58-0.66, AS: 17
Type of feeders: Fungus growing wood/litter feeders (Type-2 feeders)

Odontotermes horai

Odontotermes feae

Odontotermes horai
Diagnostics: Head-capsule brownish yellow, sub-rectangular from above, sparsely pilose; Left mandible with a small tooth on inner region, rudimentary; Labrum sharp but rounded tip.

Measurements (mm): HL: 1.65-1.93, HW: 1.29-1.1.48, LML: 1.02-1.21, PL: 0.57-0.69, PW: 0.91-1.06, PoL: 0.95-1.08, PoW: 0.46-0.56, AS: 16
Type of feeders: Fungus growing wood/litter feeders (Type-2 feeders)

Odontotermes feae
Diagnostics: Head capsule larger; Left mandible with a large tooth and prominent stout; Labrum narrowed in front to a pointed tip.

Measurements (mm): HL: 1.15-2.31, HW: 1.75-1.99, LML: 1.37-1.58, PL: 0.80-0.96, PW: 1.51-1.63, PoL: 1.67-1.81, PoW: 0.76-0.89, AS: 17
Type of feeders: Fungus growing wood/litter feeders (Type-2 feeders)

Odontotermes singsiti

Odontotermes hainanensis

Odontotermes singsiti
Diagnostics: Head weakly convex, converging in front; Labrum triangular with pointed tip; Mandible finely tapering in front and incurved at tip; Left mandible prominent and situated at middle with a large tooth.

Measurements (mm): HL: 1.91-2.19, HW: 1.55-1.74, LML: 1.12-1.35, PL: 0.60-0.72, PW: 1.04-1.19, PoL: 1.20-1.35, PoW: 0.58-0.71, AS: 17
Type of feeders: Fungus growing wood/litter feeders (Type-2 feeders)

Odontotermes hainanensis
Diagnostics: Labrum strongly incurved; Mandibles asymmetrical; Left mandible with larger tooth, weakly to strongly twisted and crenulations in basal half.

Measurements (mm): HL: 1.02-1.35, HW: 0.99-1.21, LML: 0.62-0.81, PL: 0.43-0.57, PW: 0.67-0.80, PoL: 0.72-0.79, PoW: 0.27-0.34, AS: 16
Type of feeders: Fungus growing wood/litter feeders (Type-2 feeders)

3.2 Genus *Hypotermes*

The genus *Hypotermes* belongs to the family Termitidae with subfamily Macrotermitinae. In this study, there is only one type of *Hypotermes*, namely *Hypotermes xenotermitis*.

Hypotermes xenotermitis

Hypotermes xenotermitis

Diagnostics: Labrum strongly incurved at anterior region; Mandibles asymmetrical; Left mandible with larger tooth, weakly to strongly twisted and crenulations in basal half.

Measurements (mm): HL: 1.18-1.36, HW: 1.04-1.22, LML: 0.50-0.72, PL: 0.39-0.45, PW: 0.71-0.82, PoL: 0.83-0.90, PoW: 0.41-0.53, AS: 16

Type of feeders: Fungus growing wood/litter feeders (Type-2 feeders)

3.3 Genus *Microtermes*

The genus *Microtermes* belongs to the family Termitidae with subfamily Macrotermitinae. In this study, there is only one type of *Microtermes*, namely *Microtermes obesi*.

Microtermes obesi

Microtermes obesi

Diagnostics: Head yellowish, moderately hairy; Body whitish, moderately hairy; Head-capsule broadly oval; Mandibles thin, weakly incurved distally.

Measurements (mm): HL: 0.63-0.84, HW: 0.57-0.76, LML: 0.42-0.61, PL: 0.28-0.35, PW: 0.460.60, PoL: 0.42-0.53, PoW: 0.31-0.37, AS: 14

Type of feeders: Fungus growing wood/litter feeders (Type-2 feeders)

3.4 Genus *Macrotermes*

The genus *Macrotermes* belongs to the family Termitidae with subfamily Macrotermitinae. In this study, there is only one type of *Macrotermes*, namely *Macrotermes khajuriai*.

Macrotermes khajuriai

Macrotermes khajuriai

Diagnostics: Head capsule brownish yellow dorsally with two brown longitudinal streaks in middle; Labrum brown with whitish hyaline distal tip; Mandibles moderately incurved; Left mandible serrated in proximal two-thirds of inner margin.

Measurements (mm): HL: 3.59-3.81, HW: 2.74-2.99, LML: 1.58-1.75, PL: 0.96-0.1.15, PW: 1.77-1.93, PoL: 2.59-2.75, PoW: 0.73-0.84, AS: 16

Type of feeders: Fungus growing wood/litter feeders (Type-2 feeders)

3.5 Genus *Pseudocapritermes*

The genus *Pseudocapritermes* belongs to the family Termitidae with subfamily Amitermitinae. In this study, there is only one type of *Pseudocapritermes*, namely *Pseudocapritermes tikadari*.

Pseudocapritermes tikadari

Pseudocapritermes tikadari

Diagnostics: Head capsule straw yellow with body fairly hairy; Labrum asymmetrical at anterior margin; Left mandible curved and twisted, tip weakly bent inwards like a blunt hook.

Measurements (mm): HL: 1.89-2.10, HW: 1.15-1.38, LML: 1.72-1.85, PL: 0.25-0.30, PW: 0.70-0.79, PoL: 1.19-1.30, PoW: 0.41-0.46, AS: 13

Type of feeders: Organic rich soil (or) humus feeders (Type-3 feeders)

3.6 Genus *Microcerotermes*

The genus *Microcerotermes* belongs to the family Termitidae with subfamily Termitinae. In this study, there is only one type of *Microcerotermes*, namely *Microcerotermes pakistanicus*.

Microcerotermes pakistanicus

Microcerotermes pakistanicus

Diagnostics: Head elongate, almost parallel; Labrum broadly rounded with few long and several short hairs; Mandibles sickle shaped and faintly to strongly serrated.

Measurements (mm): HL: 0.77-0.98, HW: 0.78-99, LML: 0.57-0.71, PL: 0.33-0.50, PW: 0.55-0.72, PoL: 0.62-0.75, PoW: 0.35-0.41, AS: 14

Type of feeders: Fungus growing wood/litter feeders (Type-2 feeders)

3.7 Measurement comparison with observed species

The co-efficient of variation became larger in pronotum length and least in head width and based on CV we concluded that more variation of termite soldiers were occurred in pronotum length (Table 1.).

Head length

The mean value of head length for ten observed species varied from 0.78-3.68 mm. The coefficient of variability is 2.491. In general, any pair of species with different alphabets is significantly different at 5% ($P \leq 0.05$) level of significance. As comparison with calculated critical difference value (CD=0.06) of head length from all observed species, *Odontotermes hainanensis* and *Hypotermes xenotermitis* showed similar character of head length and such two species are not significant with each other, rest are highly significant.

Head width

The mean value of head width for ten observed species varied from 0.78-2.89 mm. The coefficient of variability is 1.819. As comparison with calculated critical difference value (CD=0.034) of head width from all observed species, *Odontotermes hainanensis* and *Hypotermes xenotermitis* showed similar character of head width and such two species are not significant with each other, rest are highly significant.

Left mandible length

The mean value of left mandible length for ten observed species varied from 0.50-1.82 mm. The coefficient of variability is 2.983. As comparison with calculated critical difference value (CD=0.042) of left mandible length from all observed species, *Hypotermes xenotermitis* and *Microcerotermes pakistanicus* showed similar character of left mandible length and such two species are not significant with each other, rest are highly significant.

Pronotum length

The mean value of pronotum length for ten observed species varied from 0.25-1.05 mm. The coefficient of variability is 4.769. As comparison with calculated critical difference value (CD=0.036) of pronotum length from all observed species, *Hypotermes xenotermitis* and *Microcerotermes pakistanicus* showed similar character of pronotum length and such two species are not significant with each other, rest are highly significant.

Pronotum width

The mean value of pronotum width of ten observed species varied from 0.53-1.86 mm. The coefficient of variability is 2.387. As comparison with calculated critical difference value (CD=0.032) of pronotum width from all observed species, *Odontotermes hainanensis, Hypotermes xenotermitis* and *Pseudocapritermes tikadari* showed similar character of pronotum width and such three species are not significant with each other, rest are highly significant.

Postmentum length

The mean value of postmentum length for ten observed species varied from 0.48-2.77 mm. The coefficient of variability is 2.686. As comparison with calculated critical difference value (CD=0.042) of postmentum length from all observed species, *Pseudocapritermes tikadari* and *Odontotermes singsiti* showed similar character of postmentum length and such two species are not significant with each other, rest are highly significant.

Postmentum width

The mean value of postmentum width for ten observed species varied from 0.30-1.00 mm. The coefficient of variability is 4.289. As comparison with calculated critical difference value (CD=0.031) of postmentum width from all observed species, *Odontotermes parvidens* and *Odontotermes singsiti* showed similar character of postmentum width and such two species are not significant with each other, rest are highly significant (Table 1.).

3.8 Principal component analysis

PCA defined the summarization of information in larger data tables by means of smaller set of summary indices that can be easily visualized and analysed. To elucidate the morphometric change during differentiation of soldiers in detail, the measurements of each body parts were compared among different observed species. The maximum Eigenvalues were obtained in first principal component proportion. The first principal component accounted for 93.6% of total variance and the second principal component accounted for 4.9% of total variance (Table 9.). In general, the first principal component (PC1) expressed the remaining information and result showed morphometric differences of termite soldiers. The first principal component (PC1) positively correlated with head width, head length and postmentum length; and negatively correlated with left mandible length, pronotum length, pronotum width and postmentum width (Table 10.).

Table 1. Morphometric variation from observed species of termite soldiers

Species	Head length	Head width	Left mandible length	Pronotum length	Pronotum width	Postmentum length	Postmentum width
Odontotermes horai	1.80^{f} (0.007)	1.42^{e} (0.009)	1.11^{f} (0.006)	0.61^{e} (0.004)	0.99^{e} (0.003)	1.02^{e} (0.008)	0.53^{d} (0.007)
Odontotermes parvidens	2.16^{c} (0.039)	1.69^{c} (0.016)	1.31^{d} (0.026)	0.72^{c} (0.024)	1.40^{c} (0.012)	1.45^{c} (0.017)	0.62^{c} (0.008)
Odontotermes hainanensis	1.23^{g} (0.01)	1.10^{g} (0.007)	0.70^{g} (0.017)	0.49^{f} (0.009)	0.75^{f} (0.016)	0.76^{g} (0.01)	0.30^{i} (0.012)
Hypotermes xenotermitis	1.25^{g} (0.02)	1.11^{g} (0.014)	0.62^{h} (0.012)	0.43^{g} (0.011)	0.77^{f} (0.013)	0.87^{f} (0.014)	0.48^{e} (0.009)
Pseudocapritermes tikadari	2.03^{c} (0.009)	1.27^{c} (0.006)	1.82^{a} (0.01)	0.28^{i} (0.009)	0.74^{f} (0.004)	1.25^{d} (0.015)	0.43^{f} (0.02)
Microcerotermes pakistanicus	0.93^{h} (0.008)	0.90^{h} (0.007)	0.64^{h} (0.004)	0.42^{g} (0.008)	0.65^{g} (0.004)	0.69^{h} (0.018)	0.38^{g} (0.012)
Microtermes obesi	0.78i (0.02)	0.68^{i} (0.013)	0.50^{i} (0.014)	0.32^{h} (0.006)	0.53^{h} (0.011)	0.48^{i} (0.02)	0.34^{h} (0.007)
Odontotermes feae	2.24^{b} (0.029)	1.88^{b} (0.016)	1.47^{c} (0.015)	0.89^{b} (0.016)	1.58^{b} (0.013)	1.74^{b} (0.012)	0.83^{b} (0.009)
Odontotermes singsiti	2.09^{d} (0.021)	1.61^{d} (0.014)	1.22^{e} (0.016)	0.65^{d} (0.012)	1.11^{d} (0.014)	1.27^{d} (0.012)	0.64^{c} (0.006)
Macrotermes khajuriai	3.68^{a} (0.022)	2.89^{a} (0.01)	1.66^{b} (0.017)	1.05^{a} (0.013)	1.86^{a} (0.011)	2.68^{a} (0.015)	0.79^{a} (0.013)
C.D.	0.06	0.034	0.042	0.036	0.032	0.042	0.031
C.V.	2.491	2.983	2.983	4.769	2.387	2.686	4.289
SE(m)	0.021	0.012	0.015	0.012	0.011	0.015	0.011

*Mean in a column followed by standard error

Different small letters indicates significant differences

ANOVA, P≤0.05, (n=5)

Table 2. Eigenvalues of Covariance Matrix

PCA	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalues	2.007	0.105	0.024	0.005	0.003	0.001	0
Proportion	0.936	0.049	0.011	0.002	0.001	0	0
Cumulative Proportion	0.936	0.985	0.996	0.998	1	1	1

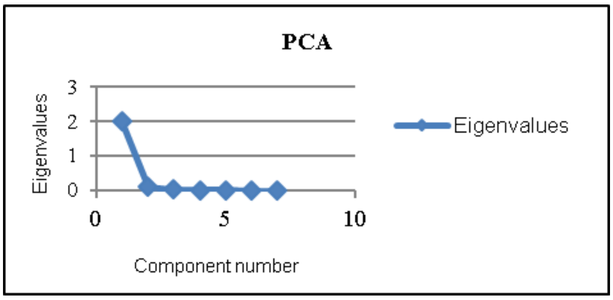

Fig 1. Eigenvalues of Covariance Matrix

Table 3. Loadings (Eigenvectors) of Covariance Matrix

Parameters	PC1
Head length	0.63*
Head width	0.42*
Left mandible length	0.27
Pronotum length	0.15
Pronotum width	0.30
Postmentum length	0.46*
Postmentum width	0.15
Parameters	PC1

3 Discussion

In the present study, we conducted a morphometric analysis of different species of termite soldiers. The determination of species based on morphological characteristics was very difficult in termites due to the high variability of the size. In some publications, the morphometric data of the same species also found a very diverse variety of sizes, from one region to another within a country as well as between countries (Yeap *et al.*, 2009). The present studies showed that all termite species which consist of highly variable population. These morphological changes during differentiation of soldiers are associated with changes in their roles within the colony. The morphology of termite soldiers with their larger heads and elongated mandibles thought to be more adaptive for defense against enemies like predators, indicates the closest relationship between the morphology and function. Through differentiation of soldiers, the morphology of this caste of termites becomes functionally suited for attacking predators and unsuitable for feeding on wood using their mandibles. The main aim of this research was to develop a basis for morphogenesis, in relation with differentiation of soldier termites (Koshikawa *et al.*, 2002).

When focusing on differentiation of soldier was noted, similar to that observed by Manzoor and Akhtar (2006a) in *O. obesus* reported the following variations in the species and this study was based on nine different parameters with highest variation in pronotum length and least in head width. Manzoor and Akhtar (2006b) in *O. walloensis* studied the following measurements regarding the morphological characters of ten parameters and concluded the results with less variation of termite soldiers in head width. The morphological taxonomy of termite soldier castes is based on few minor differences that often difficult to appreciate, therefore taxonomy remains more problematic than that of higher taxa. Most termite species were different in behavior, biology and ecology; which is least studied and analyzed so far in India. Hence, all those aspects need a careful investigation for the backdrop of systematic studies (Saha *et al.*, 2016).

Such resultant parameters were compared with eminent scientists already working on termites was mentioned below.

Table 4. Comparison of measurements (mm) of *O. horai*

Parameters	Manzoor (2002)	Present
Head length	1.64	1.80
Head width	1.28	1.42
Left mandible length	1.00	1.11
Pronotum length	0.57	0.61
Pronotum width	0.96	0.99
Postmentum length	0.95	1.02
Postmentum width	0.51	0.53

Table 5. Comparison of measurements (mm) of *O. parvidens*

Parameters	Manzoor (2002)	Present
Head length	2.19	2.16
Head width	1.64	1.68
Left mandible length	1.36	1.31
Pronotum length	0.71	0.72
Pronotum width	1.35	1.40
Postmentum length	1.41	1.45
Postmentum width	0.66	0.62

Table 6. Comparison of measurements (mm) of *O. feae*

Parameters	Manzoor (2002)	Present
Head length	2.39	2.24
Head width	1.67	1.88
Left mandible length	1.39	1.47
Pronotum length	0.77	0.89
Pronotum width	1.46	1.58
Postmentum length	1.61	1.74
Postmentum width	0.73	0.83

Table 7. Comparison of measurements (mm) of *M. obesi*		
Parameters	Roonwal and Chhotani (1962)	Present
Head length	0.81	0.78
Head width	0.75	0.68
Left mandible length	0.49	0.50
Pronotum length	0.31	0.32
Pronotum width	0.51	0.53
Postmentum length	0.46	0.48
Postmentum width	0.36	0.34

Table 8. Comparison of measurements (mm) of *M. khajuriai*		
Parameters	Roonwal and Chhotani (1962)	Present
Head length	3.75	3.68
Head width	2.90	2.89
Left mandible length	1.68	1.66
Pronotum length	1.05	1.05
Pronotum width	1.88	1.86
Postmentum length	2.65	2.68
Postmentum width	0.80	0.79

Table 9. Comparison of measurements (mm) of *H. xenotermitis*		
Parameters	Roonwal and Chhotani (1962)	Present
Head length	1.38	1.25
Head width	1.20	1.11
Left mandible length	0.72	0.62
Pronotum length	0.55	0.43
Pronotum width	0.80	0.77
Postmentum length	0.84	0.87
Postmentum width	0.46	0.48

Table 10. Comparison of measurements (mm) of *P. tikadari*		
Parameters	Roonwal and Chhotani (1962)	Present
Head length	2.13	2.03
Head width	1.23	1.27
Left mandible length	1.93	1.82
Pronotum length	0.33	0.28
Pronotum width	0.70	0.74
Postmentum length	1.19	1.25
Postmentum width	0.40	0.43

PCA clearly revealed differential pathway of soldiers explained by first and second principal components. A termite morphometric variation study in *Hodotermopsis japonica*, the results concluded which was very close finding done by Koshikawa *et al.* (2002) reported from the first principal component was accounted for 91.7% of total variance and second principal component accounted for 5.4% of total variance. Another finding by Haifig and Costa-Leonardo (2016) who also reported that total variance value was obtained maximum in PC1 as compared to PC2. Under these kinds of morphological changes, there might be some morphogenetic regulations that known in developmental biology. Any morphogenetic factors might be important for differentiation of soldiers and environment conditions in individuals probably that triggers the expression of such soldier-specific factors (Miura, 2001).

5. Conclusion

It is hereby concluded that termite surveillance programme used for understanding the morphological variations between the termite species. The results of our study is highly useful for taxonomic importance. This study provides the base line information about termites and also useful data for future comparison of samples of the termite species. The morphometric study of the termites is much more important for greater understanding of role and functioning of the different population samples of soldier caste. Therefore, proper identification and having knowledge of the termite species are the initial steps which is very crucial for developing environment compatible/sustainable IPM strategies for termite.

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