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RESEARCH ARTICLE

Comparative morphological divergence among few common Indian species of the family Termitidae: Isoptera

Pranesh M. K[1] and Harini B. P[2*]

1. Centre for Applied Genetics, Bangalore University, Bangalore-560 056, Karnataka, India
2. Department of Zoology, Bangalore University, Bangalore-560 056, Karnataka, India

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Corresponding Author

Harini B. P

Abstract

Morphometric variations among the soldier samples from thirty three nests of five species namely, *Odontotermes ceylonicus* (Wasmann), *O. horni* (Wasmann), *O. obesus* (Rambur), *O. redemanni* (Wasmann) and *Trinervitermes biformis* (Wasmann) (Isoptera, Termitidae) were statistically analyzed for mean, standard deviation, standard error, coefficient of variability, confidence interval (95%) and analysis of variance (one way ANOVA). The mean values of the Jnanabharathi population samples and the ANOVA values were compared according to Tukey-Kramer test. The measurements play a very important role, especially for identification of species; on the other hand the reliability of the measurements will also depend on the extent of variability of the structures observed between the colonies. Each individual soldier were subjected to eight parameters: i) total body length; ii) maximum width of head; iii) head length without rostrum or mandible; iv) pronotum lenght; v) pronotum width; vi) body index; vii) head index and viii) pronotum index. Further, the five species recorded were clustered based on coding the mean values followed by Manhattan distance. The Tukey-Kramer test values were coded followed by Mcquity distance and the dendrograms obtained were compared to ascertain the extent of morphological relationship among the species.

Introduction:

The termites are group of eusocial insects classified under the taxonomic rank of order Isoptera. Along with ants and some bees and wasps which are all placed in the separate order Hymenoptera, termites divide labor among gender lines, produce overlapping generations and take care of young collectively. Termites of the order Isoptera are classified into seven families of which Termitidae being the largest family, with 14 subfamilies, 280 genera and over 2600 species (Krishna, 1970; Pearce and Waite, 1994; Kambhampati and Eggleton, 2000; Eggleton, 2001, Ohkuma et al., 2004). Termites mostly feed on dead plant material, generally in the form of wood, leaf litter on soil, or animal dung, and about 10% of the estimated 4,000 species (about 2,600 taxonomically known) are considered as pests. When the fungi are eaten, their spores pass undamaged through the intestine of the termites to complete the cycle by germinating in the fresh fecal pellets (Aanen et.al., 2002 and Mueller et.al., 2002).
As eusocial insects, termites live in colonies where adult number varies from several hundred to several million individuals. Typical colony contains nymphs (semi-mature young), workers, soldiers, and reproductive individuals of both genders, sometimes there are few egg-laying queens. Nymphs are prealate forms, which function as reproductive stock for the colony. Larvae are the undifferentiated young of the reproductive stocks, which are hatched from eggs as tiny, immature and incapable of feeding (Howard & Haverty 1980) young ones. The worker castes are always quite easily separated from the young,

undifferentiated, immature forms of the other castes. Often there are different forms of the workers and soldiers. Generally different forms exists either in size, shape or both within the same colony. Workers have a vital role in the colony. Workers feed all the dependents castes; maintain colony atmospheric homeostasis and build and repair the nest. But the workers of the subfamily Macrotermitinae show the extraordinary phenomenon of making fungus combs inside the termitophiles (Hickin 1971). Termites are economically significant as pests that can cause serious structural damage to buildings, crops or plantation forests (Shanbhag and Sundararaj, 2012). Apart from being pests, termites are one of the major detritivores which are particularly in the subtropical and tropical regions, and their recycling of wood and other plant matter is of considerable ecological importance.

Morphological analysis in termites is very crucial for the identification of the species and assessing genetic variability (Manzoor et.al., 2006 “a” and “b”; Manzoor, 2009; Noor and Nashir Uddin, 2010). Similarly by studying the distribution of any species we can address many questions related to management of biodiversity under future climate (Sinclair et.al., 2010). Since environment and termite distribution always complement each other for bringing morphological variation, the study help us understand the reason for its prevalence and also variation. In view of this, the current work is continuation of contribution to the taxonomic understanding of the species in terms of intercolonial variations among the soldier castes of different species. The study is based on the collection of termite samples from different localities of Jnanabharathi (JB), Bangalore University Karnataka, India.

Materials and methodology

Termite samples were collected from different localities of JB in the month of December and January (winter season) during 2013 and 2014. During this season the soil will have 30-40% of humidity and rich with leaf litter and other organic debris. The relative humidity was in the range 29-35% and temperature 12-29^{o}C.

Mount building termites were collected by using rubber-pump manual aspirator Fig.-1. Termites which constructs galleries on tree and those which dwell underground were collected by hand pick using feather light forceps. The collected samples were stored in 50% ethanol for further analysis.

A total of 33 samples were collected from different localities of JB, the details of the collection are mentioned (Table-1). Based on the description given by Roonwal and Chhotani (1989) 5 species were recorded.

Morphometric analysis was done with the aid of Motic microscope attached with 3MP camera. The measurements were taken by using the calibrated Motic Images Plus 2.0v software. The morphological data which was used in the analysis were total body length (BL), maximum head width (BW), head length without mandible or rostrum (HL), pronotum length (PL), pronotum width (PW), body index (BI=BW/BL), head index (HI=BW/HL) and pronotum index (PI=PW/PL).

The data recorded were subjected to statistical analysis for mean, standard deviation, standard error, coefficient of variability and confidence interval (95%) and analysis of variance (one way ANOVA). The mean values of the different population samples were used to construct dendrogram in Fig.3A by following Manhattan distance according to Manzoor and Akhtar (2006). Further, the ANOVA results were subjected to Tukey-Kramer test, the test results were tabulated and assessed for significance similarity between the species. The Tukey-Kramer test was conducted by using the ANOVA values in critical range formula.

$$CR = \underset{n-c}{\downarrow}Q^{\rightarrow c}\left[\sqrt{\frac{MSW}{2}\left(\frac{1}{n_i}+\frac{1}{n_j}\right)}\right]$$

where, CR is critical range;
Q is tabulated value for 'c' number of coloumns and 'n-c' number of rows;
c is total number of groups
n is total number of observations
MSW = mean sum of squares within the groups obtained by ANOVA;
n_i = number of observations in group – i (where i can be 1, 2, 3 etc. the group to be compared with j)
n_j = number of observations in group – j (where j can be any group that has to be compared with i)

The CR values were then compared with differences between the mean values of group-i and group-j. If the CR value was more than the difference of mean value then it was accepted that similarity existed between the groups. If the CR value was less than the difference of mean value then it was not accepted to have similarity between the groups.

The dendrogram in Figure-3B was constructed based on Tukey-kramer test for all the 80 assumptions made and for the entire five groups which are listed in Table-3. According to Tukey-Kramer test each comparison are made in pairs. So group-i which has to be compared is coded as one. Now group-i has to be compared with group-j, here if group-j shows similarity it will be coded one and if no similarity is present between group-i and group-j it will be coded as zero. In an assumption only two groups are compared at a time, those groups which are not considered are coded as zero. Based on this coding method McQuitty linkage was constructed.

Results:

There were 5 different species observed among the collected nest samples. These species can easily be distinguished based on morphological traits. *T. biformis* (Wasmann) show bimorphism in the soldier caste . The head is ovoid and protruded into rostrum at anterior and at posterior head is bulged. *O. obesus* (Rambur) and *O. redemanni* (Wasmann) show high level of similarity. They show difference only in the trait mandibular index. Both have convexly curved head or oval shaped head capsule with weak convergence at anterior.

O. horni (Wasmann) is a larger species with total body length ranging from 7.37mm to 10.05mm. These are the most prevalent species found on tree galleries. They have sub rectangular head with thick strong mandible. Similarly *O. ceylonicus* (Wasmann) has strong rectangular head with strong mandible but the total body is much smaller than *O. horni* (Wasmann) and slightly bigger than other three species.

The statistical analysis for the trait BL± SD (Table-2A) value varies from 0.65 to 1.98 and CV is recorded highest in *O. obesus* (Rambur) with 9.336. The high F-value suggests very high significance in the differences between the mean values of the different species. Trait BW (Table-2B) reveals maximum value in *O. horni* (Wasmann) with 0.0868 which is close enough to resemble *O. obesus* (Rambur) with 0.0791. F-value is recorded to be 693.685 specifying high significance in the differences between the mean values. Trait HL (Table-2C) reveals highest value in *O. horni* (Wasmann) with 0.162 and the value is close to the *T. biformis* (Wasmann) with a value of 0.0952. Lowest value for the trait was recorded by *O. ceylonicus* (Wasmann) with 0.05188.F-value is 693.685 specifying very high significance between the mean values of the trait. Trait PW (Table-2D) reveals high SD values in *O. obesus* (Rambur) 0.649 and *O. horni* (Wasmann) 0.687. F-value was recorded to be 889.596 specifying high significance between the difference of mean values. If PL (Table-2E) trait is also compared with other traits it shows very less SD values among which *O. ceylonicus* (Wasmann) show 0.07 which is the highest among all other groups.

In this study three indices are also briefed in detail like other traits. For the trait BI (Table-2F) highest SD value is recorded by *O. obesus* (Rambur) of about 0.0225 and lowest in *O. ceylonicus* (Wasmann) with 0.0121. F-value is comparatively low but significant enough to specify higher significance in the difference of mean values among the species. Similarly HI (Table-2G) recorded highest SD value in g1 is 0.078 and lowest in *O. ceylonicus* (Wasmann) with 0.0298. The F-value of 59.41 is comparatively low like BI specifying high level of significance. PI (Table-2H) has high SD value in *T. biformis* (Wasmann) and lowest in *O. redemanni* (Wasmann) with 0.11. F-value is recorded 86.132 with similar significance like that of HI and BI.

According to Tukey-Kramer test 80 assumptions were made as mentioned in the Table-3, among which 20 were accepted to show significance similarity among the species specified in green. These 20 accepted assumptions specify that there is a total of 75% dissimilarity among the species. This test shows maximum similarity level of 87.5% between *O. redemanni* (Wasmann) and *O. obesus* (Rambur) and records 0%similarity between *T. biformis* (Wasmann) and *O. redemanni* (Wasmann), *T. biformis* (Wasmann) and *O. obesus* (Rambur) and *T. biformis* (Wasmann) and *O. horni* (Wasmann) where as between *T. biformis* (Wasmann) and *O. ceylonicus* (Wasmann) 25% of similarity was observed.

The mean values were coded according to Manzoor et.al., (2006) and the values were submitted to Minitab (v.16) to plot a dendrogram in (Fig.-3A). This analysis shows 50% of similarity between *T. biformis* (Wasmann) and the rest of the species, thus forming two main clusters. In the second cluster *O. redemanni* (Wasmann) and *O. obesus* (Rambur) shows 100% whereas *O. horni* (Wasmann) and *O. ceylonicus* (Wasmann) shows 60% similarity between them and between *O. redemanni* (Wasmann) and *O. obesus* (Rambur) together.

The dendrogram based on Tukey-Kramer test (Fig. 3B) shows a total of four clusters. Cluster one show 30.75% of similarity between *T. biformis* (Wasmann) and the rest of the species. Cluster two shows 45.53% of similarity between the last two clusters. Cluster three shows 50.31% of similarity between *O. redemanni* (Wasmann) and *O. obesus* (Rambur). Similarly the last cluster shows 58.92% of similarity between *O. horni* (Wasmann) and *O. ceylonicus* (Wasmann).

Table-1: Distribution of different species of termites in different localities of Bangalore

Locality	Different types and No. of species collected	No. nest samples collected
Jnanabharathi, Bangalore University campus	1. *O. ceylonicus* (Wasmann)	4
	2. *O. horni* (Wasmann)	10
	3. *O. obesus* (Rambur)	10
	4. *O. redemanni* (Wasmann)	4
	5. *T. biformis* (Wasmann)	5

Figure-1: Rubber pump aspirator

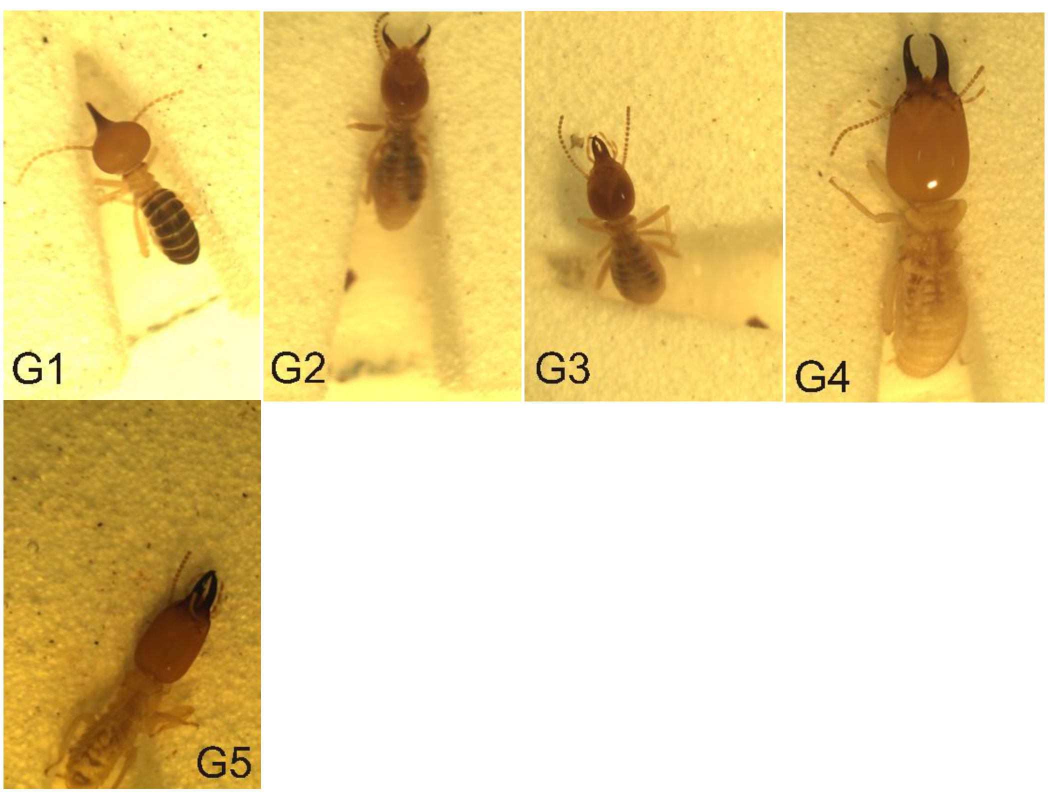


Figure-2: Five different species of termites from G1 to G5.

Table-2A: Mean ±SE of total body length (BL) of five different species of termites

Groups	Species	N*	OR*	BL(mean)	SD*	SE*	CV*	CI_{95}*	**F**
G1	*T. biformis* (Wasmann)	16	3.98-5.1	4.515	0.29	0.073	6.48	4.381-4.661	**302.98**
G2	*O. redemanni* (Wasmann)	18	4.52-5.95	5.055	0.39	0.091	7.65	4.929-5.266	
G3	*O. obesus* (Rambur)	53	4.12-6.16	4.975	0.46	0.063	9.34	4.857-5.137	**P≤0.05 4.71839E-61**
G4	*O. horni* (Wasmann)	32	6.91-10.05	8.426	0.66	0.116	7.79	8.158-8.713	
G5	*O. ceylonicus* (Wasmann)	4	5.8-6.21	6.1	0.19	0.099	3.25	5.948-6.153	

*Note:- N is Number of soldiers observed, OR is Observed range, SD is standard deviation, SE is Standard error, CV is coefficient of variance and CI_{95} is Confidence interval at 95%.

Table2B: Mean ±SE of maximum head width (BW) of five different species of termites

Groups	Species	N*	OR*	BW(mean)	SD*	SE*	CV*	CI_{95}*	**F**
G1	*T. biformis* (Wasmann)	16	1.2-1.4	1.27	0.051	0.013	3.96	1.265-1.315	**693.69**
G2	*O. redemanni* (Wasmann)	18	0.99-1.19	1.1	0.05	0.012	4.55	1.075-1.122	
G3	*O. obesus* (Rambur)	53	0.88-1.4	1.12	0.079	0.011	7.07	1.086-1.158	**P≤0.05 6.08374E-81**
G4	*O. horni* (Wasmann)	32	1.78-2.15	1.95	0.087	0.015	4.47	1.916-1.981	
G5	*O. ceylonicus* (Wasmann)	4	1.28-1.42	1.33	0.061	0.03	4.56	1.306-1.376	

Table-2C: Mean ±SE of Head length at base of mandible/ Head length without rostrum (HL) of five different species of termites

Groups	Species	N*	OR*	HL (mean)	SD*	SE*	CV*	CI_{95}*	**F**
G1	*T. biformis* (Wasmann)	16	1.04-1.39	1.58	0.095	0.024	8.22	1.128-1.216	**837.17**
G2	*O. redemanni* (Wasmann)	18	1.04-1.29	1.2	0.068	0.016	5.62	1.164-1.223	
G3	*O. obesus* (Rambur)	53	1.03-1.28	1.17	0.055	0.008	4.73	1.151-1.186	P≤0.05 1.4108E-85
G4	*O. horni* (Wasmann)	32	1.72-2.55	2.38	0.163	0.029	6.83	2.261-2.408	
G5	*O. ceylonicus* (Wasmann)	4	1.63-1.74	1.71	0.052	0.026	3.04	1.669-1.724	

Table-2D: Mean ±SE of Pronotum width (PW) of five different species of termites

Groups	Species	N*	OR*	PW(mean)	SD*	SE*	CV*	CI_{95}*	**F**
G1	*T. biformis* (Wasmann)	16	0.56-0.69	0.648	0.037	0.009	5.65	0.626-0.658	**889.59**
G2	*O. redemanni* (Wasmann)	18	0.84-0.95	0.899	0.031	0.007	3.39	0.885-0.911	
G3	*O. obesus* (Rambur)	53	0.74-	0.892	0.065	0.008	7.28	0.871-	**P≤0.05**

			1.11					0.922	**4.42002E-87**
G4	*O. horni* (Wasmann)	32	1.37-1.71	1.552	0.069	0.012	4.43	1.52-1.58	
G5	*O. ceylonicus* (Wasmann)	4	0.98-1.09	1.05	0.053	0.027	5.07	1.014-1.069	

Table-2E: Mean ±SE of Pronotum length (PL) of five different species of termites

Groups	**Species**	**N***	**OR***	**PL(mean)**	**SD***	**SE***	**CV***	**CI$_{95}$***	**F**
G1	*T. biformis* (Wasmann)	16	0.19-0.31	0.253	0.031	0.0078	12.39	0.237-0.267	**534.99**
G2	*O. redemanni* (Wasmann)	18	0.51-0.6	0.553	0.036	0.0084	6.41	0.543-0.564	
G3	*O. obesus* (Rambur)	53	0.42-0.61	0.535	0.039	0.0055	7.47	0.519-0.545	**P≤0.05 1.34016E-74**
G4	*O. horni* (Wasmann)	32	0.72-0.94	0.85	0.055	0.0098	6.55	0.827-0.866	
G5	*O. ceylonicus* (Wasmann)	4	0.53-0.69	0.62	0.07	0.035	11.38	0.573-0.653	

Table-2F: Mean ±SE of Body Index (BI = BW/BL) of five different species of termites

Groups	**Species**	**N***	**OR***	**BI (mean)**	**SD***	**SE***	**CV***	**CI$_{95}$***	**F**
G1	*T. biformis* (Wasmann)	16	0.243-0.321	0.286	0.021	0.0052	7.27	0.275-0.295	**29.68**
G2	*O. redemanni* (Wasmann)	18	0.192-0.262	0.219	0.019	0.0044	8.47	0.212-0.229	
G3	*O. obesus* (Rambur)	53	0.164-0.296	0.227	0.022	0.0031	9.93	0.218-0.236	**P≤0.05 4.42094E-17**
G4	*O. horni* (Wasmann)	32	0.192-0.27	0.23	0.019	0.0035	8.47	0.225-0.239	
G5	*O. ceylonicus* (Wasmann)	4	0.206-0.231	0.22	0.012	0.006	5.52	0.213-0.225	

Table-2G: Details Mean ±SE of Head Index (HI = BW/HL) of five different species of termites

Groups	**Species**	**N***	**OR***	**HI (mean)**	**SD***	**SE***	**CV***	**CI$_{95}$***	**F**
G1	*T. biformis* (Wasmann)	16	0.96-1.22	1.12	0.078	0.019	6.99	1.08-1.14	**59.42**
G2	*O. redemanni* (Wasmann)	18	0.85-1.01	0.92	0.047	0.011	5.16	0.90-0.94	
G3	*O. obesus* (Rambur)	53	0.72-1.21	0.96	0.072	0.009	7.49	0.92-0.99	**P≤0.05 2.17068E-27**
G4	*O. horni* (Wasmann)	32	0.72-1.08	0.82	0.065	0.011	7.85	0.8-0.87	
G5	*O. ceylonicus* (Wasmann)	4	0.76-0.82	0.78	0.029	0.015	3.82	0.77-0.8	

Table-2H: Mean ±SE of Pronotum Index (PI=PW/PL) of five different species of termites

Groups	**Species**	**N***	**OR***	**PI (mean)**	**SD***	**SE***	**CV***	**CI$_{95}$***	**F**
G1	*T. biformis*	16	2.185-	2.596	0.31	0.077	11.9	2.494-	**86.13**

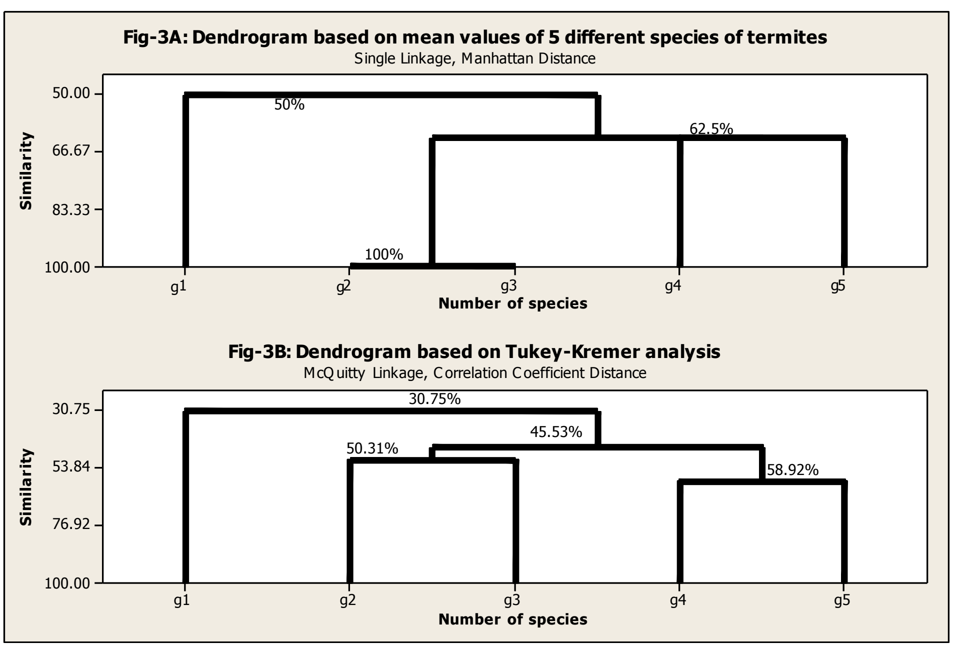
	(Wasmann)		3.316					2.776	
G2	*O. redemanni* (Wasmann)	18	1.483-1.827	1.631	0.11	0.025	6.56	1.596-1.677	
G3	*O. obesus* (Rambur)	53	1.375-2.31	1.675	0.17	0.023	10.17	1.633-1.762	**p≤0.05 4.47305E-34**
G4	*O. horni* (Wasmann)	32	1.522-2.194	1.83	0.15	0.027	8.24	1.779-1.897	
G5	*O. ceylonicus* (Wasmann)	4	1.58-1.943	1.72	0.16	0.079	9.21	1.648-1.829	

Table-3: Tukey-Kramer results showing similarity (highlighted) between 5 different species

Hypothesis	BL	BW	HL	PW	PL	BI	HI	PI
(G1=G2)=A,(G1≠G2)=R	R	R	R	R	R	R	R	R
(G1=G3)=A,(G1≠G3)=R	R	R	R	R	R	R	R	R
(G1=G4)=A,(G1≠G4)=R	R	R	R	R	R	R	R	R
(G1=G5)=A,(G1≠G5)=R	R	A	A	R	R	R	R	R
(G2=G3)=A,(G2≠G3)=R	A	A	A	A	A	A	R	A
(G2=G4)=A,(G2≠G4)=R	R	R	R	R	R	A	R	R
(G2=G5)=A,(G2≠G5)=R	R	R	R	R	A	A	R	A
(G3=G4)=A,(G3≠G4)=R	R	R	R	R	R	A	R	R
(G3=G5)=A,(G3≠G5)=R	R	R	R	R	A	A	R	A
(G4=G5)=A,(G4≠G5)=R	R	R	R	R	R	A	A	A

Note:- The hypothesis is "there is similarity between the groups". 'A' means the hypothesis is accepted and 'R' means hypothesis is rejected.

Figure-3: Dendrograms of morphological data based on Manhattan distance and Tukey-Kramer analysis.



Discussion:

According to Manzoor et.al., (2006 "a"), the coding was done by using mean values of different traits to compare the similarity between the populations of a species. In this study comparisons are made between five different species for 8 variable morphological traits. The dendrogram in Fig.-3A according to Manzoor et.al., (2006 "b"); Manzoor et.al., (2006 "c"); Manzoor F, (2009), Manzoor F, (2010) and Nadeem Sheikh et.al., (2005) show results as expected. Similar results are reported from Coronel and Porcel (2002), in Microcerotermes strunckii. In this analysis O. redemanni (Wasmann) and O. obesus (Rambur) shows 100% similarity because the traits by which the species are distinguished are not considered. The reason behind this is that not all the species in this study contains mandible and left madibular tooth. According to Tukey-Kramer analysis O. redemanni (Wasmann) and O. obesus (Rambur) show 50.31% of similarity which is an actual indication of correlation coefficient value though the trait which distinguishes the species were not considered. Apart from O. redemanni (Wasmann) and O. obesus (Rambur) rest of the clusters also show comparatively less amount of similarity to that of Manhattan method. Due to these reasons for the analysis of intercolonial variation involving post-hoc test followed by correlation coefficient distance method served more accurate result than Manhattan distance method.

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