ASM Sc. J., 13, 2020
https://doi.org/10.32802/asmscj.2020.404

Morphometric Analysis of *Nasutitermes* (Termitidae: Nasutitermitinae) from Sarawak, Malaysian Borneo

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This study aims to analyse the soldier's morphological variation of *Nasutitermes*. In this study, a total of 107 individuals representing six species were appropriately measured and recorded, in which 12 morphometric measurements were used. The morphological comparison was made among six *Nasutitermes* species used in this study based on the head capsule and shape of rostrum, pronotum and postmentum. Following the comparison between six *Nasutitermes* species, we suggest that *N. regularis* and *N. longinasus* were closely resembled to each other than to the remaining four species; *N. havilandi, N. matangensis, N. matangensiformis* and *N. fuscipennis* based on the external morphological characteristics. The cluster analysis shows by the dendrogram resulted in two major clades; clade 1 grouped almost individuals of *N. longinasus* while clade 2 consists of the remaining five species. Accordingly, this study may serve as a baseline reference for the morphological characteristics that can be used in resolving taxonomic issues for *Nasutitermes* species.

Keywords: nasute termites; morphological variation; Sarawak

I. INTRODUCTION

Soldier caste in termite social system is very important for identification (Miura and Matsumoto 2000) since it is the only caste that highly sclerotised and easily differentiates among the others. *Nasutitermes* species or well known as nasute termites is the largest genus lies under subfamily Nasutitermitinae (Syaukani and Thompson 2011). Subfamily Nasutitermitinae holds a unique characteristic that distinguishes them from other families which is the frontal projection (nasus or rostrum) on the head (Thapa 1981; Tho 1992). The utilisation of morphometric in phylogenetic studies can be one of the contributions to increase the understanding regarding termite taxonomy generally (Lee *et al.* 2005).

According to Syaukani and Thompson (2011), this genus usually possesses the conical to cylindrical shape of nasus which differentiate them from other genera. Besides, rostrum is specialised to secrete protection fluid to avoid predation for termites' colonies (Thapa 1981). Tho (1992) acknowledged that members of this genus are the most difficult to be distinguished when it relies on their morphological characters. Thus, people usually confused with the other genus within the subfamily. However, there are only a few studies conducted for the past years on the morphological variations.

The main problem faced in *Nasutitermes* is the species delimitation, and the taxonomic status among members of *Nasutitermes* is intensely confusing, which always lead to misidentification. Unfortunately, there are limited studies on morphological variation of subfamily Nasutitermitinae notably under genus *Nasutitermes* (Syaukani and Thompson 2011). In addition, there are several studies of morphometric analysis for other species of termites such as in the genus *Odontotermes* (i.e. Manzoor & Akhtar 2006a;

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Manzoor & Akhtar 2006b; Manzoor 2010) and *Coptotermes* (i.e. Norsyarizan & Wan Nurainie 2016; Norsyarizan *et al.* 2018) but not *Nasutitermes*, especially in Sarawak.

The morphometric analysis of soldier caste presented in this paper aims to elucidate the morphometric variation among selected species in the genus *Nasutitermes* aided with cluster analysis. Thus, the diagnostic character(s) resulted then will be useful to enhance the identification process of *Nasutitermes* effectively. Moreover, accurate termites' taxonomy and recognition are crucial to identify pest species especially for applying the effective termite management (Kirton 2005). The study will provide baseline information to taxonomic of this species in terms of intercolonial variation in the soldier caste.

II. MATERIALS AND METHODS

A. Termites Collection

Nasute termites were collected from three different location in Southwest of Sarawak namely Borneo Highland, Kubah National Park and Kampung Temurang, Padawan. Borneo Highland is located on Mount Penrissen, which is isolated from the central highland spine of Borneo. The area is rich in montane vegetation, and the temperature ranged from 18°C to 28°C annually. Kubah National Park, on the other hand, is situated on a small sandstone plateau, which mostly covered by mixed dipterocarp forest. This national park has a wide variety of palm tree species and wild orchids in Borneo (Hazebroek & Abang Kashim 2000). Another termites' locality used in this study is Kampung Temurang, Padawan. Kampung Temurang is about 25 kilometres south of Kuching and one of the tourist spots, famous for the Rafflesia that can be found here. Other than that, Kampung Temurang is surrounded by agricultural activities.

All specimens were collected from the field using a random sampling and standardised protocol by Jones *et al.* (2005) with a modification of sampling transects following Norsyarizan *et al.* (2018) (i.e. An area of 100m long and 2m wide was divided into five small plots with dimension of 20m × 2m one person sampled each and plot for 30 minutes. The samples were preserved in absolute ethanol and brought to the laboratory for identification. All specimens were stored in the Insect Reference Collection, Universiti Malaysia Sarawak (UNIMAS). The identification of termites to the species level was made using taxonomic keys and previous studies published by Ahmad (1965), Thapa (1981) and Tho (1992).

B. Morphological Characterisation and Termites Identification

The morphological characters were photographed using a Motic SMZ-16B series stereomicroscope attached to a Moticam 2000 camera. General morphological terminology used for describing soldiers follows those of Tho (1992) and Gathorne-Hardy (2001). The calibrated measurements were taken using Motic Image Plus 2.0 software. A total of 107 individuals of soldiers from six *Nasutitermes* species namely, *N. longinasus, N. fuscipennis, N. havilandi, N. matangensis, N. matangensiformis* and *N. regularis* (Table 1) were microscopically examined and identified.

Table 1. Checklist of species used in this study with their localities and number of individuals obtained.

Species	Number of soldiers collected			Total individuals of soldier
	Borneo Highlands	Kubah National Park	Kampung Temurang, Padawan	
Nasutitermes fuscipennis Haviland	10	19	-	29
Nasutitermes havilandi Desneux	-	25	-	25
Nasutitermes longinasus Holmgren	10	12	10	32
Nasutitermes matangensis Haviland	-	10	-	10
Nasutitermes matangensiformis Holmgren	-	6	-	6
Nasutitermes regularis Haviland	-	5	-	5
	Total individuals of soldier used in this study			**107**

Note: '-'not recorded in the respective locality.

Measurements of the soldier body parts follow those in Thapa (1981) and Tho (1992). The following 12 morphometric characters were measured (in millimetres); (1) Head width at point of constriction (HWC); (2) Maximum head width (HW); (3) Length of rostrum (LR); (4) Head length measured to base of mandible (HLM); (5) Head height (HH); (6) Head length with rostrum (LHR); (7) Head length without rostrum (LHxR); (8) Head width to base of mandibles (WHM); (9) Width of postmentum (WPt); (10) Length of postmentum (LPt); (11) Width of pronotum (WPr); and (12) Length of pronotum (LPr).

C. Statistical Analysis

The measured data were analysed by calculating the minimum value, maximum value, average, standard deviation, coefficient of variance and analysis of variance (ANOVA). Tukey-Kramer and cluster analysis were constructed by using squared Euclidean distance. The data obtained were presented using the unweighted pair group method average (UPGMA) cluster analysis using Minitab version 15.1.3, while ANOVA was conducted using SPSS Version 24.0.

III. RESULTS

A. Diagnostic Feature Examination

A morphological comparison was made among the *Nasutitermes* species used in this study based on the diagnostic features such as head capsule and shape of rostrum (Figure 1), pronotum (Figure 2) and postmentum (Figure 3).

Head capsule: Based on Figure 1, *N. longinasus* have square-shaped of the head with a flat dorsal profile and possess the largest size. Unlike *N. havilandi* and *N. matangensis*, which have a round shape, it is rather difficult to distinguish these two species based on the head capsule. On the other hand, *N. fuscipennis* and *N. matangensiformis* were remarked to own pear-shaped and tear-dropped shape of head capsule (Figure 1D & 1F). However, *N. matangensiformis* was usually smaller in size to *N. fuscipennis* with a darker colour at the rostrum part. In this study, it can be observed that *N. regularis* is distinct from other species in term of their head capsule shape, which it exhibits a rectangle shape with light brown colour and dorsal profile is slightly concave (Figure 1E).

The shape of rostrum: *N. longinasus* and *N. regularis* significantly have a long elongated cylindrical shape of rostrum but there was slight disparate between both species (Figure 1A & 1E). *N. regularis* have a weakly sharp tip with thicker rostrum at the base compare to *N. longinasus* which has a slightly flat tip with cylindrical shape from the middle to the top. It was observed that the shape of the rostrum of *N. longinasus* is distinctly different from other species. Both *N. havilandi* and *N. matangensis* have cone shape nasus with a sharp tip and thicken at the base. Other than that, *N. matangensiformis* and *N. fuscipennis* also share the same rostrum configuration which has cone shape with ogive shape of the tip. Similar to *N. havilandi*, *N. matangensiformis* have longer rostrum compared to *N. fuscipennis*.

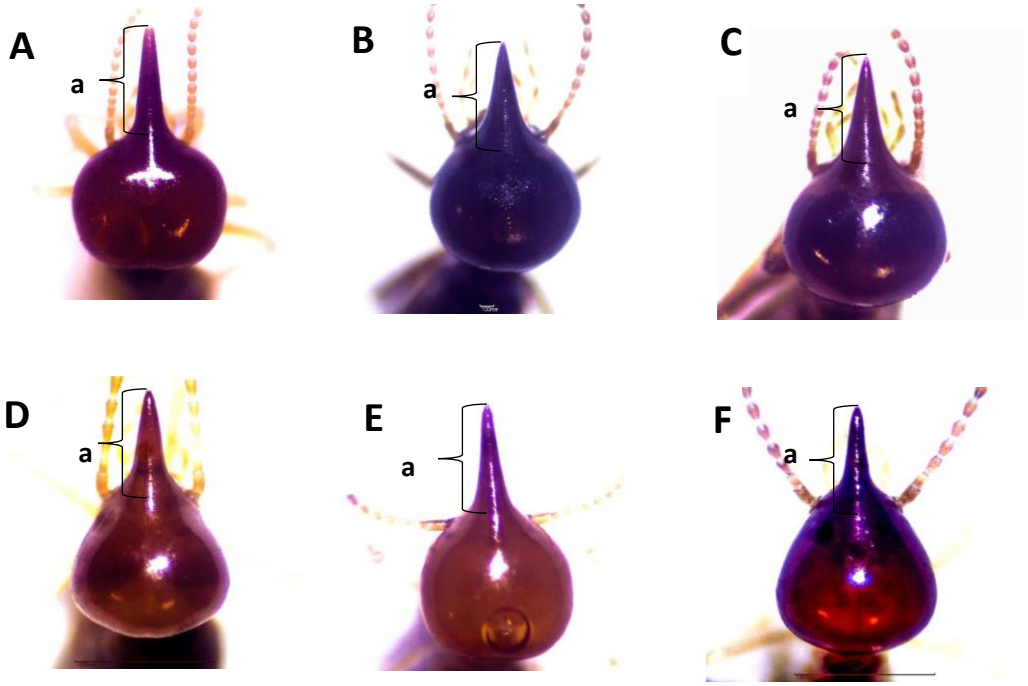

Figure 1. Dorsal view of soldier head and the shape of rostrum (a) of *Nasutitermes* species under various magnification. **A.** *N. longinasus* (30x); **B.** *N. matangensis* (40x); **C.** *N. havilandi* (40x); **D.** *N. fuscipennis* (40x); **E.** *N. regularis* (40x); **F.** *N. matangensiformis* (50x).

Pronotum: Based on Figure 2, all four species have saddle-shaped pronotum, namely *N. longinasus*, *N. havilandi*, *N. fuscipennis* and *N. regularis* with slight differences on their anterior and posterior alignment. *N. havilandi* exhibit anterior margin shallowly emarginated and posterior narrowly rounded, which were the most distinct compared to other species analysed in this study. *N. matangensis* exhibit hemisphere-shaped of pronotum, which anterior nearly flattened and more rounded posterior, differ to *N. matangensiformis,* which exhibit oval-shaped with posterior and anterior part is weakly concave.

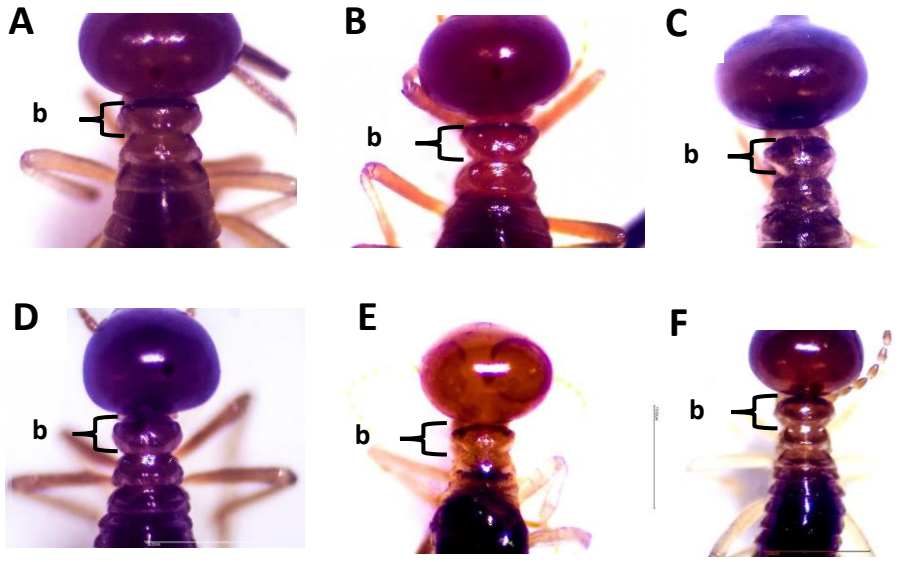

Figure 2. Dorsal view of pronotum (b) of *Nasutitermes* species under 50x magnification.
A. *N. longinasus*; **B.** *N. matangensis*; **C.** *N. havilandi*; **D.** *N. fuscipennis*; **E.** *N. regularis*; **F.** *N. matangensiformis*.

Postmentum: As shown in Figure 3, all species used in this study shared a similar shape of postmentum but the width is varied (Table 1). Therefore, postmentum was not a suitable characteristic to determine the species level of genus *Nasutitermes* since it is overlapping among species. Nevertheless, *N. longinasus* used in this study appeared to be the largest while *N. matangensiformis* was the smallest among the four species.

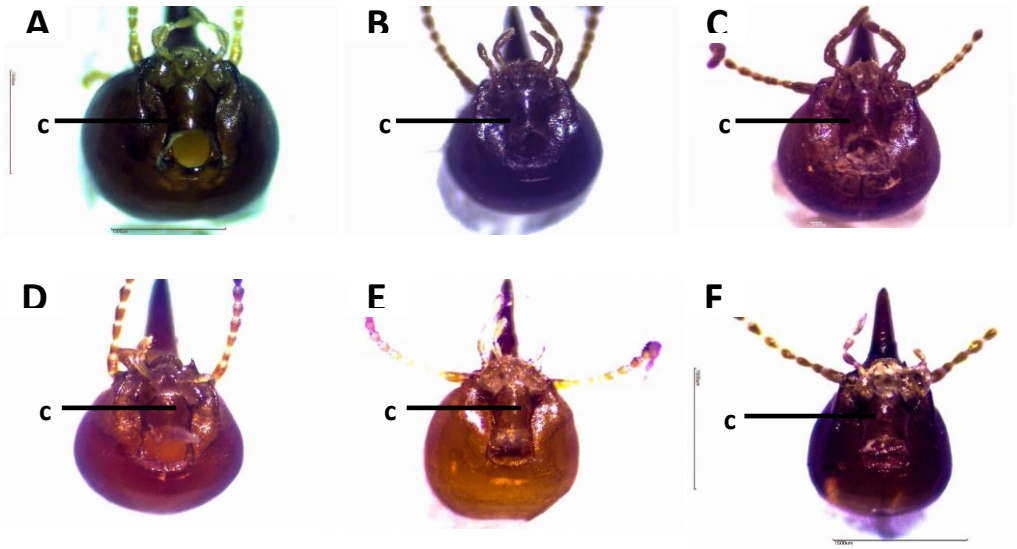

Figure 3. Ventral view of postmentum (c) of *Nasutitermes* species under 50x magnification.
A. *N. longinasus*; **B.** *N. matangensis*; **C.** *N. havilandi*; **D.** *N. fuscipennis*; **E.** *N. regularis*; **F.** *N. matangensiformis*.

B. Morphometric variation

An overview of variation of all characteristics measured in the study was established (Table 2) with ANOVA F-statistics indicating the presence of a significant difference between at least two populations. For 12 morphometric characteristics measured, statistical differences between populations were indicated. Tukey-Kramer testing of individual characteristics provided groupings that indicate significant pairwise population differences (Table 2).

C. Cluster Analysis

Cluster analysis using the unweighted pair group method average (UPGMA) resulted in a dendrogram tree as shown in Figure 4. Based on this study, a total of five species (except *N. longinasus*) were clustered under clade 2, with 31.86% distance value from clade 1. In addition, almost all individuals of *N. longinasus* soldiers clustered together and lies under clade 1a. This shows that there are only slight differences between all six species examined and they might share some of the morphological characteristics.

IV. DISCUSSION

A. Morphological Variation

N. longinasus is the largest species among *Nasutitermes* species, followed by *N. havilandi, N. fuscipennis*, *N. matangensis, N. matangensiformis* and *N. regularis*. However, *N. longinasus* is distinguishable from other species by having square-shaped of the head with the larger size. Even though *N. longinasus* was significantly different from the other *Nasutitermes* species, it shared a small degree of similarity with *N. regularis* as revealed from this study. Comparably, *N. regularis* exhibit rectangle shape of the head and dorsal profile slightly concave. Following the comparison between six *Nasutitermes* species used in this study, it is suggested that *N. regularis* and *N. longinasus* were closely resembled to each other than to the remaining four species; *N. havilandi, N. matangensis, N. matangensiformis* and *N. fuscipennis* based on external morphological characteristics.

Furthermore, while other species were compared to each other, it was difficult to distinguish between *N. havilandi* and *N. matangensis,* also between *N. matangensiformis* and *N. fuscipennis* in terms of head capsule, yet distinguishable with the shape of the rostrum. As clearly shown in Figure 1, both species shared the same configuration in terms of the shape of rostrum, which *N. regularis* and *N. longinasus* have long elongated cylindrical rostrum but there was slight disparate towards the end of nasute. Thapa (1981) stated that both *N. regularis* and *N. longinasus* possess elongated shape of the rostrum. Undoubtedly, other characters including head capsule and pronotum are relevant to be used as keys to delineate *Nasutitermes* termites until the species level, as proven from this study.

According to Thapa (1981) and Ahmad (1965), *N. matangensiformis* and *N. fuscipennis* are similar to each other, where *N. fuscipennis* distinct from *N. matangensiformis* by having pear-shaped head cone shape of rostrum with ogive shape of tips and larger size. This is consistent with the morphometric finding from this study (Figure 1). However, *N. matangensiformis* is remarkable by having a tear-dropped shape of the head as shown in Figure 1, which cannot be easily differentiate using naked eyes or under microscope magnification and almost oval-shaped with posterior and anterior part weakly concave pronotum shape. As stated by Thapa (1981), head without rostrum is astonishing sub-circular and nearly linear at dorsal profile. This species can be easily differentiated from the other *Nasutitermes* species based on size, yet it is still hard to delineate with *N. fuscipennis* if only depending on morphological characters. Nevertheless, the only characteristic besides pronotum that differs between both species *N. fuscipennis* and *N. matangensiformis* are head configuration, which pear-shaped and tear-dropped shape respectively.

The head capsule of *N. matangensis* without rostrum is perfectly rounded as in *N. havilandi* (Thapa 1981; Syaukani and Thompson 2011). From this study, both species were observed to be slightly different to each other, depending on several morphological characters but almost similar among all six species. In terms of the shape of rostrum, both species have the same configuration of rostrum which is cone-shaped nasus with a sharp tip and thickened at the base. The only difference is the length of the nasus, in which *N. havilandi* have longer nasus than *N. matangensis*.

Therefore, the shape of the rostrum is unreliable for species identification. Other than that, both species can be distinguished using pronotum shape as shown in Figure 2, in which *N. havilandi* possessed hairy saddle-shaped pronotum with anterior margin shallowly emarginated and posterior narrowly rounded, while in *N. matangensis* the pronotum is almost hemisphere-shaped, which anterior margin nearly flattened while posterior is rounded. Therefore, this diagnostic feature (Figure 2) is a reliable character to distinguish between *N. havilandi* and *N. matangensis*.

B. Morphometric Analysis of Nasutitermes Based on UPGMA

Presented distance value unravel that *N. longinasus* is significantly different from the other five *Nasutitermes* species, supported with $p<0.05$. Other remarkable characteristics of *N. longinasus* which distinguished it from other species, includes a flattened dorsal profile of the head, an elongated cylindrical shape of rostrum with a slightly flat tip and a larger size of saddle shape pronotum.

In addition, the cluster of *N. havilandi* and *N. fuscipennis* under clade 1a showed that the localities of termites might have a direct effect towards morphological characters especially size. The colony of *N. havilandi* that lies under clade 1a was collected from Rayu trail, Kubah National Park, whereas *N. fuscipennis* were from Borneo Highland. Both sampling site in this study can be considered as highland area, which consists mountainous land at high altitude. Chown and Klok (2003) stated that altitude directly influences the morphological size of insects; the higher latitude which correlated with altitude, the insect may be larger. This is consistent with the result obtained from this study, shown in Figure 4. Nevertheless, two individuals originated from different colony found clustered on the same clade 1b, which both soldiers from *N. havilandi* may have a wider range of morphological size that influence their clade position as shown in the result.

From this study, the cluster analysis showed that *N. havilandi* and *N. fuscipennis* located at both clade 1a and clade 2 indicated that both species almost of the same size. Furthermore, the grouping of species by cluster analysis used as shown in the dendrogram is still reliable for species identification, although it does not fully resolve in the present study. Therefore, extensive sampling needs to be done with a greater number of individuals and colonies to be included in further analysis in order to clarify the relationship between each clade. In addition, the morphological character of a species might differ or similar due to ecological conditions or habitat distribution and historical factors, such as continental drift, orogeny, and climatic variations (Boulogne *et al.* 2017).

Table 2. Descriptive statistics of studied *Nasutitermes* species.

Species	***N. havilandi* (n=25)**			***N. fuscipennis* (n=29)**			***N. longinasus* (n=32)**			***N. matangensis* (n=10)**			***N. matangensiformis* (n=6)**			***N. regularis* (n=5)**		
Character	**Mean±SD**	**Min**	**Max**	**Mean±SD**	**Min**	**Max**	**Mean±SD**	**Min**	**Max**	**Mean±SD**	**Min**	**Max**	**Mean±SD**	**Min**	**Max**	**Mean±SD**	**Min**	**Max**
HWC	0.52±0.15^{a,b}	0.26	0.72	0.41±0.18^{b,c}	0.25	0.62	0.62±0.06^{a}	0.49	0.75	0.44±0.16^{b}	0.29	0.73	0.20±0.01^{d}	0.19	0.21	0.29±0.01^{c,d}	0.27	0.30
HW	1.09±0.09^{b,c}	0.87	1.23	1.12±0.09^{b}	1.03	1.26	1.46±0.12^{a}	1.21	1.65	1.10±0.03^{b,c}	1.08	1.19	0.74±0.03^{d}	0.72	0.79	0.99±0.04^{c}	0.93	1.02
LR	0.72±0.15^{b}	0.40	0.91	0.70±0.12^{b}	0.54	0.13	1.17±0.05^{a}	1.06	1.27	0.74±0.06^{b}	0.65	0.86	0.51±0.01^{c}	0.50	0.52	0.67±0.04^{b}	0.63	0.71
HLM	1.16 ±0.06^{b}	0.95	1.23	1.19±0.16^{b}	0.80	1.47	1.37±0.12^{a}	1.24	1.90	1.09±0.04^{b,c}	1.03	1.16	0.96±0.02^{c}	0.92	0.99	1.17±0.04^{b}	1.06	1.16
HH	0.78±0.05^{b,c}	0.68	0.88	0.83±0.06^{b}	0.63	0.94	0.99±0.18^{a}	0.81	1.44	0.80±0.05^{b,c}	0.71	1.08	0.54±0.03^{d}	0.49	0.59	0.71±0.01^{b}	0.70	0.72
WHM	0.51±0.10^{a}	0.40	0.85	0.43±0.07^{a,b}	0.25	0.55	0.48±0.04^{a}	0.41	0.55	0.44±0.05^{a,b}	0.39	0.53	0.29±0.01^{c}	0.28	0.31	0.39±0.02^{b}	0.37	0.43
LHR	1.70±0.08^{b}	1.51	1.84	1.72±0.18^{b}	1.39	2.05	2.40±0.10^{a}	2.15	2.55	1.73±0.06^{b}	1.62	1.80	1.37±0.05^{c}	1.30	1.42	1.71±0.03^{b}	1.66	1.76
LHxR	0.79±0.09^{b}	0.68	1.04	0.88±0.10^{b}	0.62	1.61	1.09±0.16^{a}	0.81	1.67	0.82±0.10^{b}	0.73	1.06	0.62±0.02^{c}	0.59	0.63	1.04±0.04^{b}	0.99	1.06
WPt	0.32±0.03^{b}	0.25	0.36	0.31±0.04^{b}	0.25	0.39	0.39±0.04^{a}	0.32	0.58	0.29±0.02^{b,c}	0.26	0.32	0.26±0.02^{c}	0.22	0.27	0.30±0.05^{b,c}	0.26	0.38
LPt	0.33±0.03^{b}	0.28	0.39	0.31±0.04^{b}	0.23	0.37	0.43±0.04^{a}	0.33	0.55	0.30±0.04^{b}	0.23	0.34	0.25±0.01^{c}	0.24	0.26	0.32±0.03^{b}	0.29	0.36
WPr	0.53±0.03^{b,c}	0.49	0.58	0.58±0.08^{a,b}	0.49	0.73	0.61±0.04^{a}	0.46	0.61	0.51±0.04^{c,d}	0.44	0.55	0.43±0.01^{e}	0.42	0.43	0.45±0.02^{d,e}	0.43	0.48
LPr	0.24±0.03^{a}	0.20	0.31	0.26±0.03^{a}	0.21	0.33	0.24±0.03^{a}	0.17	0.30	0.25±0.02^{a}	0.22	0.27	0.23±0.09^{a}	0.17	0.41	0.19±0.02^{b}	0.16	0.20
Rostrum-Head Index	1.23±0.38^{b,c}	0.76	1.94	1.28±0.25^{a,b}	0.75	1.88	0.92±0.21^{c}	0.07	1.47	1.12±0.15^{b,c}	0.87	1.41	1.21±0.02^{b,c}	1.17	1.24	1.56±0.10^{a}	1.42	1.67
Head Index	2.28±0.66^{c}	1.68	3.73	2.91±0.66^{b,c}	1.92	4.10	2.38±0.30^{c}	1.63	2.81	2.78±0.79^{b,c}	1.62	3.71	3.69±0.16^{a}	3.39	3.83	3.46±0.12^{a,b}	3.33	3.65
Pronotum Index	2.21±0.24^{b,c}	1.67	2.62	2.23±0.22^{b,c}	1.78	2.90	2.58±0.27^{a}	1.92	3.20	2.07±0.08^{c}	1.90	2.21	2.02±0.58^{c}	1.04	2.54	2.43±0.18^{a,b}	2.18	2.60
Postmentum Index	0.99±0.10^{a}	0.76	1.17	1.01±0.13^{a}	0.86	1.45	0.94±0.08^{a}	0.81	1.15	0.99±0.16^{a}	0.79	1.29	1.02±0.10^{a}	0.84	1.09	0.92±0.07^{a}	0.87	1.04

Note: Mean, within rows, followed by the different letter are significantly different ($p < 0.05$; Tukey's HSD).

HWC = Head width at point of constriction; HW = Maximum head width; LR = Length of rostrum; HLM = Head length measured to base of mandible; HH = Head height; WHM = Head width to base of mandibles; LHR = Head length with rostrum; LHxR = Head length without rostrum; WPt = Width of postmentum; LPt = Length of postmentum; WPr = Width of pronotum; LPr = Length of pronotum.

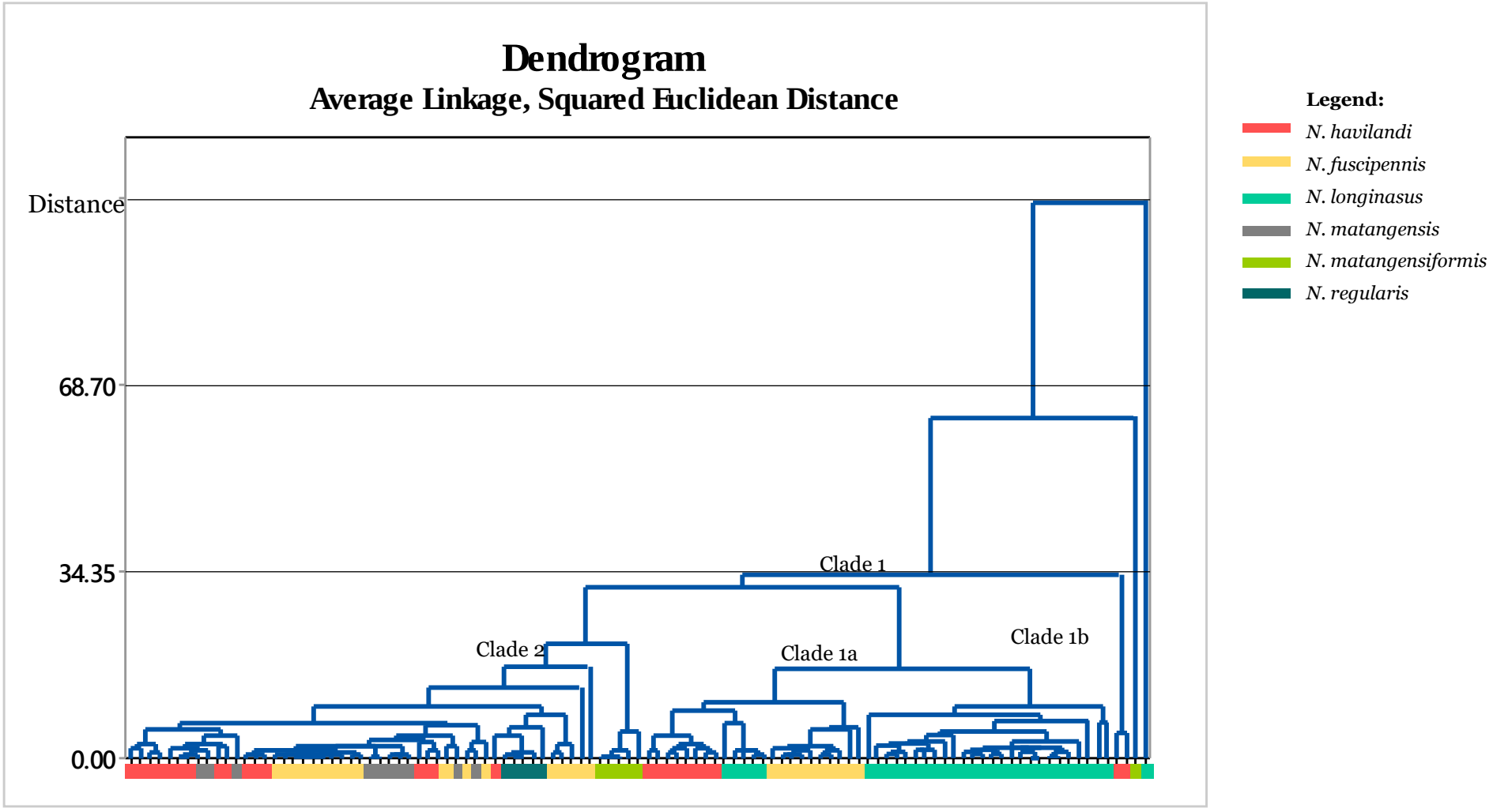

Figure 4. Dendrogram of the morphological relationship of *Nasutitermes* species analysed using 12 morphometric measurements and UPGMA algorithm on standardised variables based on average linkage and squared Euclidean distances.

V. CONCLUSION

In summary, species delineation using morphological characters are rather complicated among *Nasutitermes* species understudied, taking into account most of them fall under the same cluster, which suggested that they may have similar configuration based on phenotypic appearance. This suggested they might be high in homogeneity. However, they still exhibited some heterogeneity that contributes to morphology adaptability and makes them slightly different from other species within the *Nasutitermes* genus. Hence, a phylogenetic study using molecular should be further used for reviewing and to compare the molecular genetics finding with this morphometric result in order to provide comprehensive revision on this particular *Nasutitermes*.

VI. ACKNOWLEDGEMENT

Malaysia Ministry of Higher Education funded this project under Research Acculturation Grant Scheme (RAGS/1180/2014-03). The authors wish to thank Forest Department Sarawak for Research Permit No. NPW.907.4.4 (Jld.14)-63 and Park Permit No. WL32/2017 and Sarawak Forestry Corporation for access to facilities and study sites. The authors would also like to thank the Faculty of Resource Science and Technology, UNIMAS for the administrative supports.

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