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Morphological changes of different castes of the subterranean termites (*Odontotermes proformosanus*) in fungus combs of termitophiles

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A B S T R A C T

Termitidae is the largest family of termites. The workers of the subfamily Macrotermitinae have the extra-ordinary phenomena of making fungus combs inside the termitophiles. Considering the consistency of fungus-bed in the termitophiles, an attempt has been made to observe the morphological changes of nymphs while they stayed within the fungus comb. Biological attributes indicated that *Odontotermes proformosanus* are polymorphic in nature and have exopterygote post-metamorphic development. During the period of study, eggs and nymphs were collected in different seasons from the fungus combs of termite mounds. Eggs of *O. proformosanus* were found profusely in fungus comb in the months of March, May, September and October of 2006 to 2008 Average lengths and standard deviation (mean±SD) of the eggs were (0.66 ± 0.09), width (0.04 ± 0.07) mm and egg shape ratio is 0.53. Eggs were mostly of broad type. Distinct quiescent period was also observed. Morphological changes completed within 1st to 5th stages. In the 1st stage, total length ranged from 0.8-1.5 mm. Terminal form of workers and soldiers were attained at the 5th stage (4.1- 4.5 mm in body length). During the period of observation, it was found that total length of the nymph, head length and head capsule width are significantly correlated at 0.01 level in their five different development stages.

***Keywords*:** Fungus comb, *Odontotermes proformosanus,* morphological changes, nymphs, termitophiles.

Introduction

Termites are found in a wide range of terrestrial environments and are distributed throughout the tropical, subtropical and temperate regions of the world (Krishna & Weesner 1970, Wood & Pearce 1991). All are eusocial and the colony composed of distinct castes, which include workers, soldiers, nymphs and larvae (Krishna 1989). 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, incapable of feeding (Howard & Haverty 1980). The largest family Termitidae of termites is divided into subfamilies Serritermitinae, Amitermitinae, Termitinae, Macrotermitinae and the Nasutitermitinae. 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 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 extra-ordinary phenomenon of making fungus combs inside the termitophiles (Hickin 1971). Several suggestions have been made concerning the role of fungus gardens in termite colonies. They have been considered to be a source of food and vitamins (Grassé 1937) and also temperature (Luscher 1951) and humidity regulating devices. It is generally agreed that the fungus attacks lignin-cellulose complexes making cellulose accessible to termite digestion (Lee & Wood 1971). However, the mechanism by which fungus growing termites utilize cellulose

has been a matter of speculation (Noirot & Noirot-Timothéé 1969, Abo-Khatwa 1978). The nests of Macrotermitinae are unique in that all excreta are used to construct fungus combs and the workers further utilize the fungus combs as food and nursery chambers (Wood & Sands 1978). A large number of workers, soldiers and young nymphs live in fungus combs. Sometimes cluster of eggs were noted within the fungus comb. The study of the post-embryonic development of termites is unusually difficult. Direct observation of the numbers of instars and of the course of caste differentiation is impossible in natural colonies and the plasticity shown in the development of the castes from nymphs of different stages renders it difficult to be certain as to how far some of the developmental processes revealed in artificial colonies are a regular feature of natural development (Imms 1957). Grassé (1949) has given a useful interpretative summary of available information and the diversity of modes of development. So, considering the consistency of fungus-bed in the termitophiles, an attempt has been made in the present study to observe the morphological changes of nymphs while they remain within the fungus comb.

Materials and Methods

Odontotermes proformosanus of Termitidae family was selected as the study material for the morphological changes of different castes. Fungus combs were collected randomly by digging different termite mounds at a depth of 25-35 cm from the ground throughout the study period from Savar, a suburban area of Dhaka city in Bangladesh. Nymphs and eggs were separated from the fungus comb with a camel hairbrush and kept in petridishes. Collected nymphs and eggs were observed and under microscope with ocular-micrometer magnification (18×4x). Egg size and shape were measured and classified according to Roonwal (1975). To observe the morphological differentiation of different caste, total length of the nymph, head length and head capsule width were measured, following the measurement index of Roonwal and Chhotani (1989). According to them, ratio of head length, head width and total body length of the nymph were presented as "head length: head width: total body length", where calculations were showed considering total body length of nymph equals to one. To find out the morphological change of nymphs, the relationship of total length, head length and head capsule width of nymph the collected data were analyzed statistically and the mean, standard deviation, Duncan's Multiple Range Test (DMART) and other calculations were evaluated considering 1% level of significance by a computer packace programme called SPSS (Statistical Package for Social Science) of version 14, now frequently being used to analyze all sort of data and this version was developed by SPSS Inc. in 2006.

Result and Discussion

A queen and a king were collected at the time of digging from a termite mound (Fig.1 a,b). The queen collected was 7.5 cm long and 2.0 cm wide. Length of the king was 1.5 cm. Colour of the egg was milky white. Various number of eggs found in fungus combs in different months were collected from different termite mounds. It was noticed that the number of eggs were huge in March, May, September and October, medium in August and few in the months of April, July and December of 2006 to 2008. Eggs were not arranged in any orderly manner and

were attached loosely to the fungus combs (Fig.1c). The eggs of *O. proformosanus* were mostly of broad type (Ratio was more than 0.45) and a few were of medium type (Ratio was 0.40 - 044). Total length of the eggs varied from 0.5-0.8 mm, with mean and standard deviation 0.66±0.09 mm. Width of the eggs ranged from 0.2-0.5 mm; mean ± SD, 0.4 ± 0.07 mm (Fig.1d). Different developmental post-embryonic stages within eggs were noted chronologically. The eggs were oval and milky white. Distinct embryo was observed within the egg (Fig.1e). Post-embryonic condition was noted in the centre of brown coloured egg, with blackish border. The content of the egg was black within the eggshell (Fig.1 f, g). Pharate larval stage was noted (Fig.1 h). Prequiescent stage of the larva and their ecdysing stages were visible (Fig.1 i, j, k). Quiescent phase of termite was distinctly visible in *O. proformosanus* (Fig.1l) with flexed head, thorax and immobile limbs noted in the plates. Incubation period could not be determined.

From table 1 it was found that *O. proformosanus* was polymorphic in nature. Lengths of the 1st stage were ranged from 0.8-1.5 mm. The nymphs were milky white in colour. Head and pronotum were not distinct, mandible was not developed, soldier and worker could not be differentiated (Fig.1.m, n). In some of the nymphs within the range of 1.0-1.5 mm, transparent soft mandible without denticle was noticed. A total of 42 nymphs were observed in 1st stage, where they varied total length in 1.14± 0.26 mm, head length in 0.51± 0.16 mm and head capsule width in 0.42 ± 0.12 mm and the ratio of total length and head length was 0.36:0.81:1 (Fig. 2 a,b). In the 2nd nymph stage, 1.6-2.5 mm (Fig.2 c, d), soft mandible structure could be recognized. Short mandible with transparent indistinct denticles emerged in immature workers and elongated transparent mandible without denticle in immature soldiers (Fig.2.d). Among the 30 nymphs studied (Table. 1) mean and standard deviation in total length, head length and capsule width were 2.03 ± 0.27 mm, 0.89 ± 0.27 mm and 0.64 ± 0.10 mm and the ratio of total length and head length 32:0.72:1. In the 3rd nymphal stage, when the total length was 2.6-3.5 mm, pre-soldiers (Fig.2 e, f) and pre-workers (Fig.2 g,h) could easily be recognized. Soft, transparent denticle emerged in the mandibles of immature worker and soldier. Mandible of the immature workers became more or less stout. It was also noticed that in few immature workers and soldiers denticle did not develop. Pronotum was found to be fully developed in both soldiers and workers. Head and abdomen were milky white in colour. Under this group, 30 nymphs were measured (Table 1). Ranges of total length, head length and capsule width of the nymphs were 3.23 ± 0.31, 1.25 ± 0.37 and 0.98 ± 0.21mm respectively, with the ratio of total length and head length 0.36:0.36:1.

In the 4th stage, total length of nymph varied from 3.6 to 4.0 mm. Pronotum and mandibular denticles of the pre-soldier could distinctly be differentiated (Fig.2 j). Mandible turned into light brown and head into yellowish brown. Abdomen and pronotum were found milky white. Total length (3.8-4.0 mm) of some preworkers became yellowish white (Fig.2 i). Twenty one nymphs of this group were measured (Table 1). Mean and standard deviation of nymphs, when total length was 3.80 ± 0.14 mm, head length 1.61± 0.20 mm and capsule width 1.12 ± 0.21mm. The ratio of total length and head length was 0.28: 0.67:1.

The 5th stage measured 4.1 to 4.5 mm. Their abdomen turned brown, the outer edge of the mandibles turned dark brown and became stronger. Nymphs ranged from 4.1-4.5 mm were confirmed as soldiers and workers (Fig. 2.k, l). A total of 22 nymphs of this group were studied (Table 1). Mean and standard deviations of total length, head length and capsule width of nymphs were 4.31± 0.14 mm, 1.76± 0.37 mm and 1.25 ± 1.9 mm likewise. The ratio of total length and head length was 0.34:0.84:1.

Mean of total nymph length (TL), head length (HL), and head capsule width (CW) of the five groups, revealed that total length of nymphs were found increasing uniformly up to 2nd stage and again from 3rd stage to 5th stage but a sharp increase was noticed from 2nd to 3rd stage. However, the head length and head capsule width were found to increase more or less at a

Table 1.
Morphological changes observed in different nymphal stages of *O. proformosanus* collected from fungus combs

No. of nymph	Different stages (mm)	TL (mm) Mean± SD	HL (mm) Mean± SD	CW (mm) Mean± SD	Ratio of TL and HL	Morphological characteristics
42	1st Stage (0.8 –1.5)	1.143±0.26 e	0.514± 0.16 d	0.417± 0.12 d	0.36:0.81:1	Head and pronotum not differentiated. In some nymph's soft mandibular structure developed without denticle. Colour of the nymphs' milky white.
30	2nd Stage (1.6-2.5)	2.030±0.27 d	0.893± 0.27 c	0.643± 0.10 c	0.32:0.72:1	Dentition of the mandible just emerged, transparent in colour. Immature worker recognized. Elongated mandible not fully developed. Immature soldiers presumed.
30	3rd Stage (2.6-3.5)	3.227±0.31 c	1.250±0.37 b	0.977± 0.21 b	0.36:0.36:1	Mandible developed with tender denticle, transparent; preworker and presoldier easily differentiated. Head and abdomen milky white in colour.
21	4th Stage (3.6-4.0)	3.795±0.14 b	1.609±0.20 a	1.121± 0.21 b	0.28:0.67:1	Mandible with denticle and pronotum distinctly differentiated. Mandible colour turned into light brown, head yellowish brown. Abdomen and pronotum milky white.
22	5th Stage (4.1-4.5)	4.305±0.14 a	1.755±0.37 a	1.248± 0.19 a	0.34:0.84:1	Colour of the abdomen turned brown. Mandible became hard and its outer edge brown. Nymphs ranging from 4.5-5.0 mm length were mostly recognized as mature soldier and worker. Abdomen colour turned into yellowish brown.

TL = Total length of the nymph, Hl = Head length of the nymph, CW = Head capsule width
* Means followed by same alphabet do not differ significantly (P=0.01) by DMRT

uniform rate (Fig.3). From table 2, caste development was found to be strongly associated with the change of the total length, head length (r=0.89) and head capsule width (r=0.91). Association of head length with total length (r=0.89) and with head capsule width (r=0.90) was also strongly significant. Relative growth was strongly significant in case of head capsule width and total nymph length (r=0.91) and also with head length (r=0.90). Correlations in the five developmental stages showed to be significant at 0.01 levels (2-tailed).

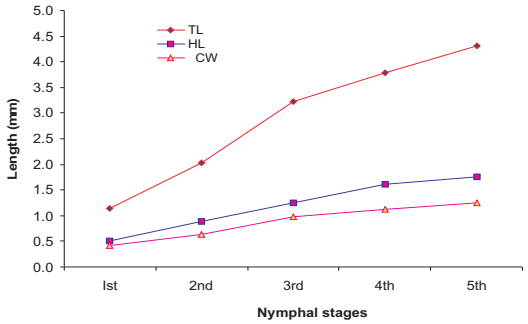

Fig. 3 Correlation of total length, head length and head width of the nymphs of *O. proformosanus*.

In the present study types of eggs found in the fungus combs were mostly broad, while some medium eggs were also noted. This agrees with Roonwall and Chhotani's (1989) finding that advanced Termitidae family consists mostly of the broad type of egg. Hill (1925) mentioned that *Mastotermes* lays cluster of 16-24 eggs cemented together with a gelatinous secretion. In the present investigation cluster of eggs were found to be much more than the number cited by Hill. Moreover, eggs in the fungus combs were found without cementing materials, eggs were arranged in an irregular manner and attached loosely. Juvenile stage observed in the present study was also mentioned by Hopkins (2003). He cited that immature termite like all juvenile insect, must periodically go through the molting process in which they shed their skin i.e. exoskeleton in order to grow. Weyer (1935) and Synder (1913) mentioned about a quiescent period, which is most pronounced in the ecdysis that gave rise to soldiers and primary reproductives. In Macrotermitinae (Termitidae) the insect lies on its sides with head flexed upon the ventral aspect of the thorax and the limbs and other parts remain immobile. Present investigation corresponds with the quiescent period of *O. proformosanus* as shown in the (Fig.1l). Imms (1957) pointed out that caste differences appeared during the course of postembryonic development. The major and minor forms of workers and soldiers may be distinguished in the preceding nymphal stages. In the present investigation it was found in the 1st stage that caste could not be differentiated, but differentiation could be detected from the 2nd stage. Hickin (1971) stated that some soldiers developed from nymphs that are indistinguishable from the nymphs that would produce workers. In addition, the soldier can be produced from a fully developed worker. In the present observation it was found that in the 1st stage appearance of the nymph was like worker, differentiation was found in the 2nd stage. The soldiers are mostly dimorphic (Table 1) which is corresponding to Hickin and Weesner (1960) statement. Transitional form of the nymph was referred by Hickin (1971) as “white soldier”, on account of absence of pigmentation in them and generally observed only between one and two week. The 3rd instar, pigmented individual of *T. tenuirostris* and 4th instar apterous individual of *G. perplexus* and *A. wheeleri* may be validly designated as definite workers (Light & Weesner 1955). It was noticed in the present study that no pigmentation could be observed up to 3rd stage of the nymphs. Pigmented mature worker was

obtained at 4th stage of nymph. This observation agrees with that of Light and Weesner (1955) on *G. perplexus* and *A. wheeleri*.

Hicken (1971) mentioned that in Macrotermitidae there are three stages of nymph. This statement does not agree with the present work, where four stages of nymph were observed (Table. 1). According to Imms (1957) dimorphism occurred in *Macrotermes esterase*, *O.obesus*, *O.redemanni*, *O.horni* and in many species of the genus *Trinervitermes.* The present study revealed that *O. proformosanus* workers and soldiers were dimorphic. The terminal form of workers and soldiers were attained at 5th stage (Table 1 and Fig. 2 k, l).

In the present study it was found that, mandibles were soft and transparent in 2nd and 3rd stage. In the 4th stage the mandibles were hard, having pigmentation. In 2nd and 3rd stage denticles were also soft and transparent (Fig.2c-h). Pigmentation and hardness of denticles were also noticed in 4th stage. Fernando (1960) observed that before the development of a soldier, the larva (all nymphs) not distinguishable from other larvae moults into larval soldier with characteristic head and mandibles but is pigmentless. Fernando's observation is corresponding with the present study. The life cycle of termite is one of incomplete metamorphosis. The eggs hatch into larva. Worker, soldier or alate were reached their terminal form through several instars. A large number of populations of different castes were found in the fungus comb of Macrotermitinae termitophiles. Throughout the study period it was observed that the morphological changes and differentiation were associated with the changes of length, head length and head capsule widths of the nymphs of *O. proformosanus* .

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Table 2.
Relative growth of total length, head length and head capsule width of nymphs of *O. proformosanus.*

		Total nymphal length (mm)	Head length (mm)	Head capsule width (mm)
Total nymphal length (mm)	Pearson correlation	1.000	.893**	.913**
	Sig. (2-tailed)	-	.000	.000
	N	145	145	145
Head length (mm)	Pearson correlation	.893**	1.000	.900**
	Sig. (2-tailed)	.000	-	.000
	N	145	145	145
Head capsule width (mm)	Pearson correlation	.913**	.900	1.000
	Sig. (2-tailed)	.000	.000	-
	N	145	145	145

** Correlation is significant at the 0.01 level (2-tailed).

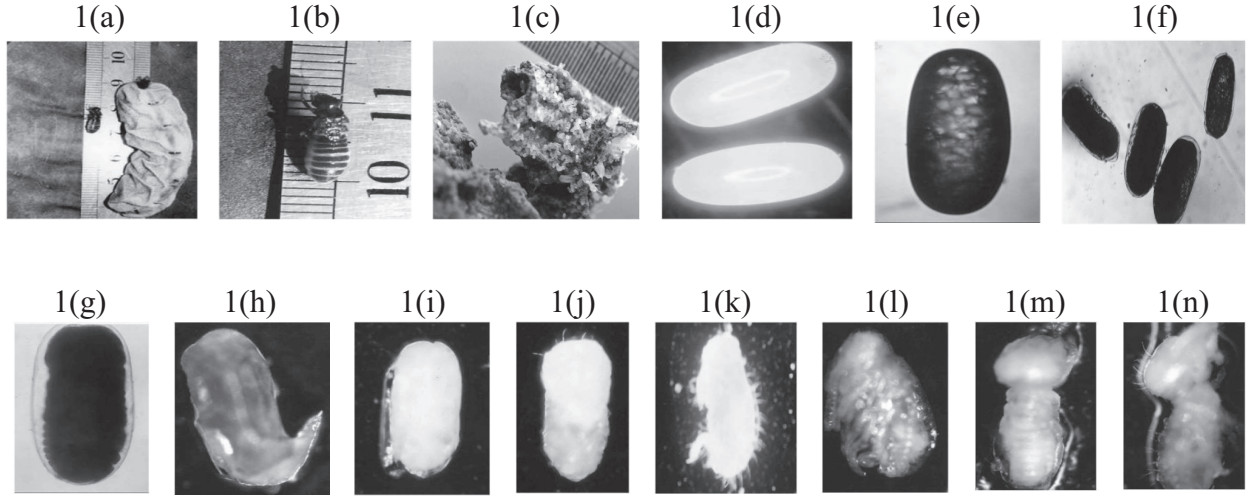

Fig1. Post metamorphosis stages of *Odontotermes proformosanus* (a) king and queen after collection from the nest; (b) king; (c) fungus comb with irregularly arranged eggs; (d) close view of eggs. (e) egg with initial metamorphic stage; (f-g) distinct pharate embryo inside the egg shell; (h) pharate embryonic stage. (i-k) ecdysing stages of the larva; (l) larval quiescent period; (m-n) larval stages of the termite. (Fig. d-n magnification 10x 40x).

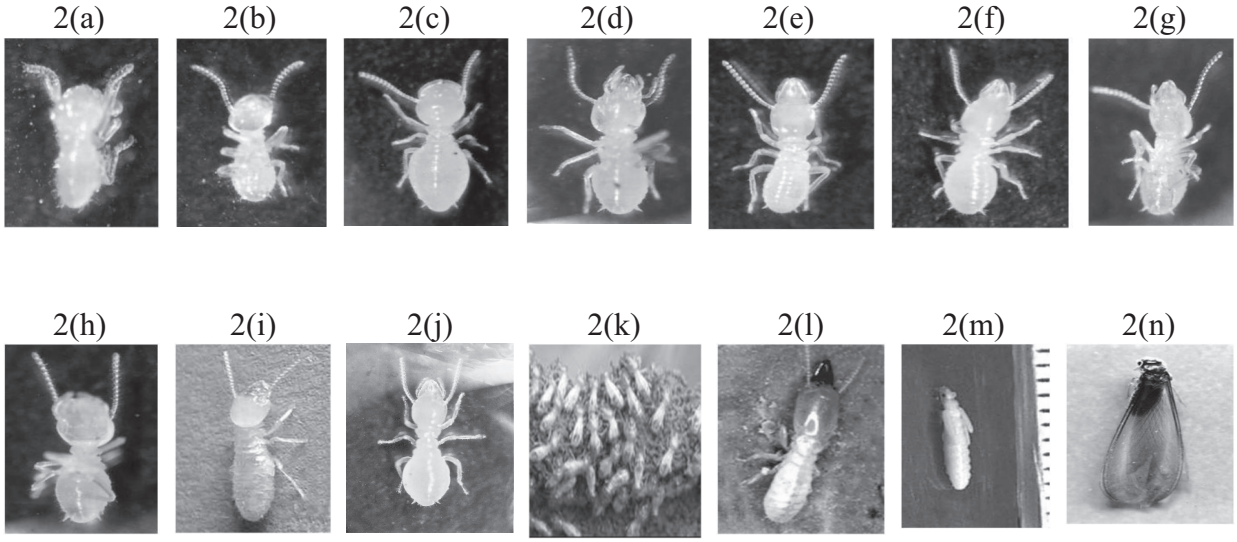

Fig 2. Morphological changes of nymphs of *O. proformosanus* (a-b) 1st stages of the nymph; (c) 2nd stage of the worker ; (d) 2nd stage of the soldier with poorly developed mandibles; (e-f) 3rd stages of dorsal and ventral views of the immature solider with transparent mandibles ; (g-h) ventral and dorsal view of the 3rd stage worker; (i) 4th stage, mature worker (j) 4th stage of the soldier transparent mandible with denticles; (Magnification 10x 25x); (k) 5th stage, fully developed soldier; (l) fully developed workers on the surface of the fungus comb; (n) supplementary queen; (n) collected winged adult.

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