Revista Brasileira de Entomologia
http://dx.doi.org/10.1590/S0085-56262013005000019

Caste polymorphism of apterous line of the Neotropical termite *Nasutitermes corniger* (Motschulsky) (Isoptera, Termitidae)

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ABSTRACT. Caste polymorphism of apterous line of the Neotropical termite *Nasutitermes corniger* (Motschulsky) (Isoptera, Termitidae). Termite societies are structured by individuals that can be grouped into castes and instars. The development of these instars in most species occurs in irregular patterns and sometimes is distinguished subcastes in physical systems that originate polymorphic soldiers and workers. In this study, we characterized the morphological diversity of castes of apterous in *Nasutitermes corniger*. We collected four colonies of *N. corniger*, one every three months between May 2011 and February 2012. Individuals of the nest were separated into groups: larval stages, workers and presoldiers and soldiers. A morphometric analysis was performed on individuals from each group based on head width, metatibia, antenna, and thorax length. The data were submitted to discriminant analysis to confirm different morphological types inside these groups. The apterous line of *N. corniger* is composed of one first larval instar and two second larval instar. The workers caste has two lines of development with four instars in a larger line and three instars in a lower line. Two morphological types were identified in presoldiers and soldiers. The pattern of castes was similar to other species of the genus, in which bifurcation into two lines of workers, one smaller and one larger occurs after the first molt.

KEYWORDS. Arboreal termite; Insecta; morphometry; nasute; Nasutitermitinae.

The caste system in social insects is a central mechanism in the regulation of individuals' interactions. Its evolution in most species occurs in irregular patterns and is sometimes distinguished by systems of physical subcastes that originate polymorphic soldiers and workers, particularly in termites and some ant species (Oster & Wilson 1978; Roisin 2000).

The Isoptera order comprises one of the biggest groups of social insects. Termite societies are structured with individuals that can be grouped into castes and stages of development. The system of castes can vary according with to family, genus and species of termites (Noirot & Pasteels 1987; Roisin 2000). In Termitidae, bifurcation occurs after the first stage of development. An apterous line gives rise to the worker caste after one or two larval instars. Subsequently the worker caste gives rise to soldiers. A nymph line, after five nymphal instars with wing buds, gives rise to alates (Moura *et al.* 2011; Roisin 1992, 1996).

In some species of the genus *Nasutitermes*, it has been observed that after the first molt of the apterous line, a new bifurcation occurs that gives rise to two lines of workers. Vestigial males give rise to smaller workers and vestigial females give rise to larger workers, thus characterizing sexual dimorphism (Noirot 1955; Roisin & Pasteels 1986).

The arboreal termite *Nasutitermes corniger* (Motschulsky, 1855) is widespread in the Americas, occurring from Mexico to northern Argentina (Torales 2002; Constantino 2002). This termite can be found in plains, sandbanks and rainforests (Abreu *et al.* 2002; Vasconcellos *et al.* 2005). In the forests, it is mainly abundant in secondary forests with some degree of disturbance. The high feeding plasticity allows this termite to feed on hard or soft, wet or dry wood of different species (Abreu *et al.* 2002; Reis & Cancello 2007). On the other hand, this species is of great economic importance because it has been reported as urban pest in South America (Bandeira *et al.* 1998; Constantino 2002; Gazal *et al.* 2012).

Nasutitermes corniger has at least two growth phases in the line of workers (McMahan 1970). Polymorphism in the worker caste was described by Thorne (1984), but the number of stages was not determined. In soldier caste two morphological types are usually described, a smaller and a larger one, and presumably they originate from two different lines of workers (Costa-Leonardo 2002).

A major aspect to be understood in insect societies is their social organization. However, no study of the developmental caste pathways has been performed for *N. corniger*, despite its growing importance. The understanding of this phenomenon and the determination of different morphological types within castes can contribute to a better understanding of inter-individual relations and the role of behavioral dynamics of individuals in termite societies. In this work, we characterize the morphological diversity of castes of apterous line of *N. corniger*.

MATERIAL AND METHODS

Termites. Mature nests of *N. corniger*, with presence of alates, were collected every three months, from May 2011 to February 2012, in Campos dos Goytacazes, Rio de Janeiro

(21°45'S, 41°19'W). The nests (n = 4) brought from the field were taken to the laboratory where they were placed in glass boxes and kept in a room maintained at 27 ± 5 °C and RH of 85 ± 10% and 14 hour photoperiod (Gazal *et al.* 2010). After 24 hours, one quarter of the volume of each nest was separated and carefully sectioned. The termites obtained were placed in a plastic tray and immediately classified and separated into the following groups: individuals in the larval stage (immature individuals with no signs of wing buds or soldier morphology), presoldiers (individuals with head morphology similar to that of soldiers, but not sclerotized), soldiers and workers (Roisin 2000).

Individuals of each group were again separated into subgroups of individuals based on differences in body size and degree of pigmentation and size of the head, established by visual assessment. Samples of 100 individuals of each group were placed in tubes containing 10 ml of a solution of formol, alcohol and acetic acid in a ratio of 1:3:1 for 24 hours and then were transferred to 70% alcohol.

Morphometry. The morphological categorization of the different groups of termites established by visual assessment showed three morphological types of larvae, three smaller workers, four larger workers, two presoldiers and two soldiers.

Individuals in each subgroup of the different castes (n = 40) were observed under a stereoscopic microscope and an ocular micrometer was used to measure: (1) maximum width of head capsule, (2) length of the left metatibia, (3) length of the antenna and (4) length of the thorax (Noirot 1955; McMahan & Watson 1975). The number of antenna segments was also counted.

The morphometric variables of the subgroups were subjected to backward stepwise discriminant analysis to confirm the different morphological types inside the group of larvae, workers and presoldiers and soldiers.

RESULTS

The stages identified visually and their respective morphometric data are given in Table I.

Larval instar. Three larval instars were identified. The first instar or larva one (L1) has 12 antennal segments with the segments 3, 4 and 5 very small. After this first instar, division occurs that gives rise to a lower line or small larva 2 (SL2) and a larger line or large larva 2 (LL2), both with 13 antennal segments. The discriminant analysis of this group indicated differences between instar (F = 382.17; P<0.0001) (Fig. 1). Head variable was excluded by the backward stepwise analysis because of its low non significant contribution. The main variable responsible in the first discriminant function was length of the antenna and for the second function were length of thorax and of metatibia.

Workers. Figure 2 shows the increasing degree of sclerotization of the head of the morphological types identified in the worker caste. All worker caste individuals have pigmented intestine, except when undergoing molting. They also have antennae with 14 segments, but LW1 and SW1 have a shorter third antennal segment, which seems to be fused to the fourth segment (Fig. 3 a, e). The second instar of the workers (LW2 and SW2) has those segments more elongated (Fig. 3 b, f). In the other instars (LW3, LW4 and SW3), the dimensions of the third and fourth antennal segments are larger (Fig. 3 c, d, g). The discriminant analysis confirmed the separation of the different worker caste instars initially identified by visual assessment (F = 199.51; P<0.0001) (Fig. 4). However, a small overlapping happens between SW3 and SW4. The four variables remained in the model and explained 99% of the variation (Wilks' Lambda: 0.00149 aprox.). In the first discriminant function, the metatibia length (correlation 71%) was the variable that most contributed to the difference between the groups, while head width was the most important variable in the second function (correlation 85%).

Soldiers. The size of the soldiers showed high variability, but two morphological types were established. Besides the soldiers, one small and one large presoldiers were confirmed (Fig. 5). These individuals are similar in size and form to soldiers, but have no body pigmentation. The discriminant analysis of individuals showed significant differences

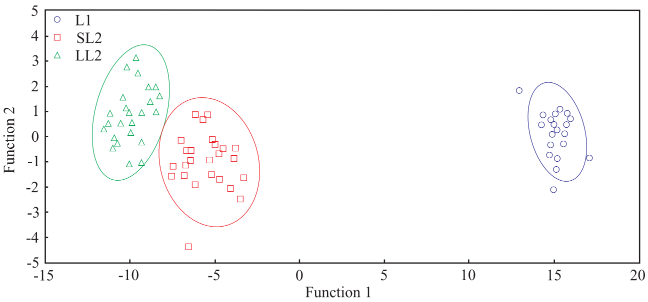

Fig. 1 Discriminant analysis of the distributions of the larval stages of *Nasutitermes corniger*. *L1* first instar larvae; *SL2, LL2* small and large second instar larvae. The ellipses indicate the level of significance (p<0.05).

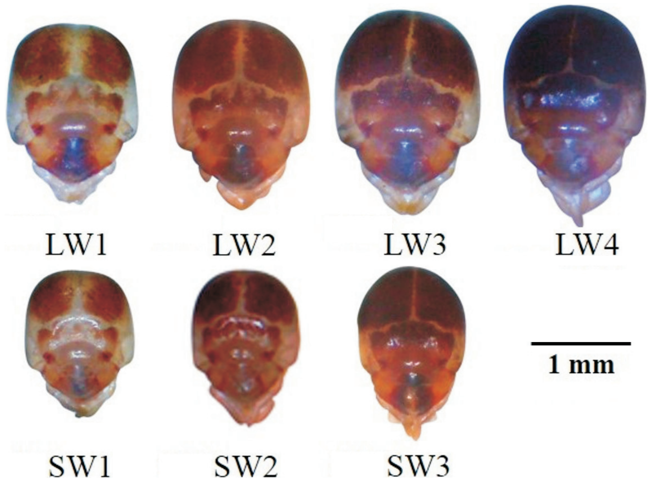

Fig. 2 Level of head sclerotization of the seven morphological types of *Nasutitermes corniger* worker; *SW1 to SW3* instar 1 to 3 of small workers; *LW1 to LW4* instar 1 to 4 of large workers.

Table I. Number of antennal segments and range and mean (in parentheses) of measurements in millimeters of body parts of stages of apterous line in *Nasutitermes corniger*.

Stage	Antennal segments	Head width	Metatibia length	Antenna length	Thorax length
Larva 1 (L1)	12	0.45-0.49 (0.461)	0.25-0.28 (0.270)	0.49-0.58 (0.535)	0.12-0.21 (0.175)
Small larva 2 (SL2)	13	0.66-0.78 (0.715)	0.55-0.61 (0.715)	0.82-0.90 (0.869)	0.10-0.31 (0.281)
Large larva 2 (LL2)	13	0.76-0.90 (0.815)	0.57-0.67 (0.629)	0.91-0.97 (0.956)	0.30-0.39 (0.346)
Small worker 1 (SW1)	14	0.87-0.99 (0.941)	0.85-0.93 (0.888)	0.93-1.02 (0.976)	0.28-0.45 (0.366)
Small worker 2 (SW2)	14	0.90-1.03 (0.995)	0.91-1.02 (0.973)	1.03-1.14 (1.091)	0.37-0.69 (0.511)
Small worker 3 (SW3)	14	0.96-1.12 (1.010)	0.97-1.11 (1.058)	1.14-1.35 (1.199)	0.42-0.64 (0.554)
Large worker 1 (LW1)	14	1.11-1.21 (1.170)	0.94-1.05 (0.996)	1.06-1.17 (1.105)	0.33-0.52 (0.419)
Large worker 2 (LW2)	14	1.20-1.32 (1.254)	1.08-1.17 (1.130)	1.21-1.30 (1.250)	0.42-0.67 (0.544)
Large worker 3 (LW3)	14	1.24-1.42 (1.302)	1.17-1.29 (1.220)	1.29-1.50 (1.380)	0.58-0.79 (0.676)
Large worker 4 (LW4)	14	1.23-1.42 (1.297)	1.20-1.36 (1.307)	1.47-1.62 (1.540)	0.66-0.84 (0.756)
Small presoldier (SPS)	13-14	0.67-0.87 (0.801)	0.87-1.05 (0.946)	1.38-1.48 (1.421)	0.49-0.60 (0.548)
Large presoldier (LPS)	13-14	0.82-0.91 (0.867)	1.05-1.18 (1.140)	1.35-1.65 (1.512)	0.52-0.82 (0.726)
Small soldier (SS)	13	0.88-1.03 (0.954)	1.05-1.23 (1.152)	1.42-1.65 (1.561)	0.37-0.70 (0.609)
Large soldier (LS)	13	0.99-1.18 (1.081)	1.24-1.39 (1.300)	1.59-1.84 (1.729)	0.54-0.82 (0.722)

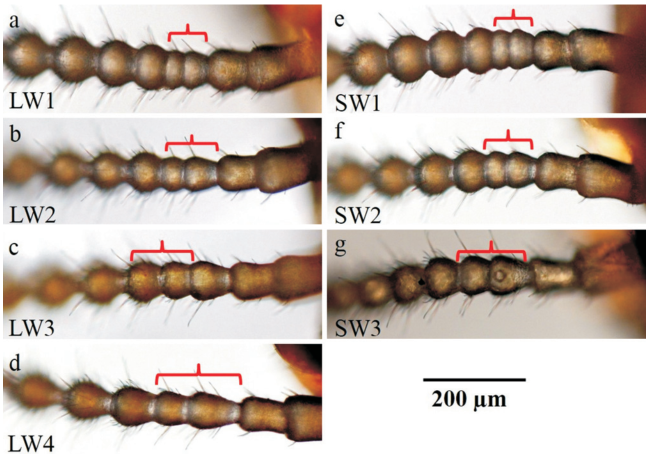

Fig. 3. Bases of antennae of workers of *Nasutitermes corniger*. Third and fourth segments extend in the advanced instars (red braces). *LW1 to LW4* large workers, instar 1 to 4 (a, b, c, d); *SW1 to SW3* small workers, instar 1 to 3 (e, f, g).

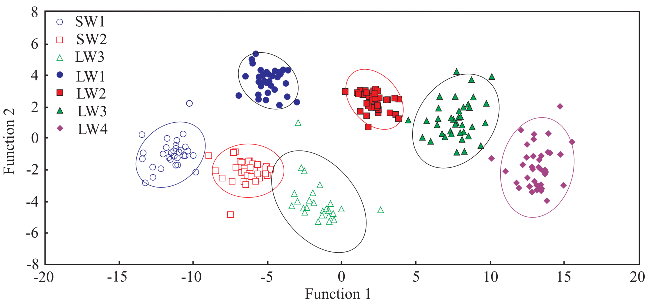

Fig. 4. Discriminant analysis of the distributions of worker caste of *Nasutitermes corniger. SW1 to SW3* instar 1 to 3 of small workers; *LW1 to LW4* instar 1 to 4 of large workers. The ellipses indicate the level of significance (p<0.05).

between the morphological types inside this group and explained more than 99% of the variation (Wilks' Lambda: 0.047 aprox., $F = 69.62$, $P<0.0001$) (Fig. 5). A clear separation can be observed between both types of soldiers and between presoldiers, but there is an overlap between small soldier and large soldier and two presoldiers (Fig. 5). Head width and metatibia length were the variables with the highest contribution to the significance in the first discriminant function and thorax length was in the second function.

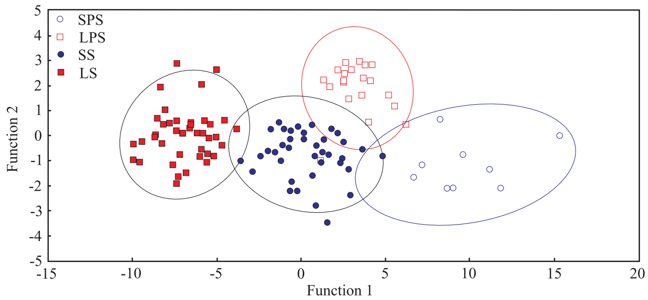

Fig. 5. Discriminant analysis of the distributions of the soldiers and presoldiers *Nasutitermes corniger. SPS, LPS* small and large presoldiers; *SS, LS* small and large soldiers. The ellipses indicate the level of significance (p<0.05).

DISCUSSION

The genus *Nasutitermes* contains the largest number of species in the Isoptera order (Constantino 2012), but although few studies have been conducted to characterize castes of species of this genus, we can see little variability in development pathways of the apterous line. As in other species of the genus, there is bifurcation into two lines after the first molt in *N. corniger* (Noirot 1955; McMahan & Watson 1975; Roisin & Pasteels 1987; Hojo *et al.* 2004). This bifurcation occurs in the second larval instar (SL2 and LL2) in individuals without sclerotization in the head, abdomen or mandible. The morphometric analysis allows inferring that the smaller line of workers reaches the third instar and the larger line reaches the fourth instar. These results are similar to those described in *Nasutitermes arborum* (Noirot 1955) and *N. novarumhebridarum* (Roisin & Pasteels 1987), which have two instars in the smaller line and four in the larger line. Likewise, *Nasutitermes exitiosus* and *Nasutitermes princeps* have two and five instars in the smaller and larger lines, respectively (McMahan & Watson 1975; Roisin & Pasteels 1986).

The different instars were present in all colonies examined in the study, indicating that they are present in nests throughout the year. However, some of these morphological types (*e.g.* SW3 and presoldiers) occurred in a lower frequency. It was not possible to determine what instar of workers gives rise to each presoldier and therefore each type of soldier. Due to the considerable variability in the size of the soldiers, the presoldiers could originate from various instars of workers. According to McMahan & Watson (1975), in *N. exitiosus* presoldiers originate from the first stage of worker (SW1 and LW1), but they also suggested that the second stage (SW2 and LW2) can give rise to these too. In the species *Nasutitermes polygynus, N. princeps, N. novarumhebridarum* (Roisin & Pasteels 1985, 1986, 1987), *N. arborum* and *N. maculiventris* (Noirot 1955), presoldiers originate from the two first instars of the smaller worker line (SW1 and SW2), but in *N. princeps* there is no certainty about the origin of large presoldiers. In *N. corniger* as in *N. exitiosus* (McMahan & Watson 1975), the presoldiers have 13 segments in the antenna, but some individuals can have 14 segments. Since this 14^{th} segment is smaller than the others and since presoldiers originate from workers that have 14 segments, it is likely that individuals with 14 segments are in a transition instar and that the 14^{th} segment is finally lost in these individuals.

Establishing the degree of morphological diversity of individuals in a society is an essential step to determine the degree of complexity of the interactions between individuals that support the social structure. Studies should be undertaken to determine whether the different morphological types identified perform different tasks. The existence of polyethism would allow understanding the causes of the high degree of polymorphism within the castes and explain the possible mechanisms that regulate the formation of distinct stages of development. These aspects are essential to understand the behavioral dynamics of termite societies.

ACKNOWLEDGEMENTS

We thank Hildefonso José de Souza for his help during the field collections and the Rio de Janeiro State Research Foundation (FAPERJ) and Darcy Ribeiro North Fluminense State University for the financial support granted to VLSL.

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Received 9 November 2012; accepted 17 May 2013
Associate Editor: Marcio R. Pie