CASTE DEVELOPMENTAL PATHWAYS OF *Cortaritermes fulviceps* (BLATTODEA, TERMITIDAE)

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ABSTRACT

The study of the developmental pathways in termites contributes to the understanding of caste evolution and resources utilization, as well as other ecological aspects. In this paper, the caste developmental pathways of *Cortaritermes fulviceps* (Silvestri) were analyzed in natural colonies from the Province of Corrientes, Argentina. Five measurements were taken of different body parts of larvae (L), workers (W), pre-soldiers (PS), soldiers (S), nymphs (N) and alates (A) for morphometric analyses. Dissections were also performed to determine the sex of workers and soldiers. The collected data were analyzed using principal component analysis, analysis of variance and Tukey test. In the nymphal line, four nymphal instars and alates were identified. The apterous line included two larval instars. Major workers were females and minor workers, pre-soldiers and soldiers were males. The results suggest that the developmental pathways of *C. fulviceps* is similar to the one of the genus *Nasutitermes* Dudley.

Keywords: Evolution, Nasutitermitinae, polymorphism, sexual dimorphism, termites

PADRÕES DE DESENVOLVIMENTO DAS CASTAS DE *Cortaritermes fulviceps* (BLATTODEA, TERMITIDAE)

RESUMO

O estudo dos padrões de desenvolvimento dos cupins contribui para a compreensão da evolução das castas e utilização dos recursos, bem como de outros aspectos ecológicos. Neste trabalho os padrões de desenvolvimento das castas de *Cortaritermes fulviceps* (Silvestri) foram analisados em colônias naturais na Província de Corrientes, Argentina. Para a análise morfométrica foram tomadas cinco medidas das diferentes partes do corpo de larvas (L), operárias (W), pré-soldados (PS), soldados (S), ninfas (N) e alados (A). Também foram feitas dissecações para a determinação do sexo de operárias e soldados. Os dados foram submetidos à análise de componentes principais, à análise de variância e ao teste de Tukey. Na linha ninfal foram identificados quatro estádios prévios aos alados. Na linha áptera foram diferenciados dois estádios larvais. Operárias grandes (maiores) eram fêmeas e operárias pequenas (menores), pré-soldados e soldados eram machos. Os resultados sugerem que o padrão de desenvolvimento de *C.fulvicep*s é similar àquele do gênero Nasutitermes Dudley.

Palavras-chave: Evolução, Nasutitermitinae, polimorfismo, dimorfismo sexual, cupins

INTRODUCTION

Termites are social insects that have a hemimetabolous development with diploid males, differentiating from Hymenoptera, such as bees, wasps and ants, which are holometabolous with haploid males. The development of termites is characterized by a continuous growth and a series of gradual molts with few morphological changes up to the last molt leading to a winged imago in the nymphal line. However, this general pattern has significant changes in different lineages (ROISIN, 2000).

The most basal families, as Kalotermitidae, show a pattern of linear development, with four or five larval instars, and at least two nymphal instars. Soldiers can be derived from third to seventh instars and pseudoergates originate from fifth to seventh instars. Eventually, neotenic reproductives may arise from the last four instars (MILLER, 1969; ROISIN & PASTEELS, 1991; ROISIN, 2000).

The Rhinotermitidae family, however, shows a varied development pattern. The genus *Prorhinotermes* Silvestri lack workers, and the pseudoergates derived from second to sixth nymphal instars by retrogressive molts. In *Reticulitermes* Holmgren and *Coptotermes* Wasmann the apterous line separates early and workers can have up to nine instars, but pseudoergates can also be derived from fourth to sixth instars nymphs (ROISIN & LENZ, 1999; ROISIN, 2000). In this family, the soldiers generally originate from the last worker instar (ROISIN, 2000).

In Termitidae, on the other hand, an irreversible separation occurs from the first larval instars, where can be differentiate a nymphal line (or "sexual line") leading to alates, and an apterous line (or "neutral line") that leads to workers and soldiers (ROISIN, 2000). The most primitive pattern is found in the genus *Amitermes* Silvestri, in which workers without sexual dimorphism emerge after two larval instars and male and female soldiers from the first worker instars (NOIROT, 1969). *Microcerotermes* Silvestri, however, presents a marked sexual dimorphism in the apterous line. After the first undifferentiated larval instar, the second instar allows to distinguish small larvae (males) and large larvae (females). Males have three stages of minor workers and females have four instars of major workers. Soldiers can be derived from the first instars of major and minor workers (NOIROT, 1969). Roisin (2000), however, shows that the soldiers are always females and derive from the first instar major workers. Reproductive ergatoids derived from the first two stages of major and minor workers and nymphoids from the fourth and fifth nymphal instars (ROISIN, 2000).

The Nasutitermitinae subfamily presents much more marked variations. In *Nasutitermes exitiosus* (Hill) and *Nasutitermes coxipoensis* (Holmgren) a small second larval stage gives rise to minor workers and a large second larval stage gives rise to major workers. Presoldiers and soldiers can arise from the first instar of minor and major workers (MC MAHAN & WATSON, 1975; BUSCHINI & COSTA LEONARDO, 2002). The genus *Velocitermes* Holmgren has a marked polymorphism in the apterous line (ROISIN, 1996). From the egg hatched dimorphic larvae, the major are females and the minor are males. Major larvae originate a second larval stage, which will give rise to two stages of major workers. Minor larvae originate dimorphic second larvae stage, the smaller gives rise to a third larval stage that will rise to presoldiers and soldiers. The biggest molts to a minor worker stage which can origin major soldiers. Instead, *Subulitermes* Holmgren and *Coatitermes* Fontes have a pattern similar to *Amitermes*.

The study of the developmental pathways of different termite genus and species is very useful for understanding the evolution of castes; examine relationships between the observed polymorphism and resources utilization and, from the ecological point of view, for a deeper knowledge of population dynamics (LEGENDRE et al., 2008; ROISIN, 1996). In this paper, the developmental patways of the genus *C. fulviceps* is analyzed for the first time from morphometric data.

MATERIAL AND METHODS

C. fulviceps development was studied by means of the analysis of biometric characters, according to the methods used by ROISIN (1996); BUSCHINI & COSTA LEONARDO (2002).

The biological material was obtained from field colonies located around the city of Corrientes (Argentina). Dissection of nests and separation of termites were performed by a combination of the methodologies proposed by DARLINGTON (1984) and THORNE (1985), which consists of fragmenting the nest into small pieces and separate the population by shaking and flotation in water with the aid of a fine mesh strainer. The samples were obtained and purified by consecutive washes and deposited in jars with fixative (FAA) for 24 hours and then preserved in 80% ethanol.

Measurements were taken in 30 major workers, 30 minor workers, 30 presoldiers, 30 soldiers and 94 larvae. In the nymphal line, 150 individuals were measured, covering all ranges of variation in size. For so, it was necessary to review several colonies of different dates due to seasonality in their production.

The characters measured in all individuals were:

Maximum width of the head (MWH): distance between two parallels marking the outermost edge of the head capsule (including compound eyes, when present), dorsally measured.

Length of the left antenna (LLA): distance between the antennal base and the distal segment.

Median length of pronotum (MLP): Distance between two parallels marking the anterior and posterior edge of pronotum along the longitudinal midline, dorsally measured.

Maximum width of pronotum (MWP): Distance between two parallels marking the lateral margins of pronotum, at its point of maximum separation, dorsally measured.

Length of hind tibia (LHT): Distance between two parallels marking the proximal and distal margins of the tibia, excluding apical spines, with the tibia maintained horizontally.

All measurements were taken using an ocular micrometer attached to an Olympus SZH10 stereomicroscope.

Statistical analysis

The determination of the stages of development was performed by Principal Component Analysis (PCA) with data previously submitted to a logarithmic transformation. The differences between castes and stages of development were determined by analysis of variance and Tukey test, considering the apterous and nymphal line separately.

Sexing

Sexing of workers and soldiers was conducted according to the methodology used by NOIROT (1955). Ten major workers, ten minor workers and ten soldiers were chosen. The specimens were stained with Giemsa for 30-60 min. The abdomen of each caste was divided along the pleura, gastrointestinal tract was carefully removed and the specimens were placed on a microscope slide and coverslip for examination under a binocular microscope with a digital camera.

RESULTS AND DISCUSSION

The PCA performed indicated that the first principal component explained more than 90% of the total variability and was positively correlated with all variables and, thus, gives a general measure of the size of the individuals, while the latter explained 5% of the variability and was positively correlated with the size of pronotum and negatively with the remaining variables (Table 1).

Table 1. Results of principal component analysis.

	Factor 1	Factor 2
Eigenvalue	4.670	0.253
% Variance	93.4	5.1
MWH	0.9599	-0.2389
LLA	0.9802	-0.1267
MLP	0.9060	0.4205
MWP	0.9946	0.0197
LHT	0.9888	-0.0475

Reference: maximum width of the head (MWH); length of the left antenna (LLA); median length of pronotum (MLP); maximum width of pronotum (MWP); length of hind tibia (LHT).

The PCA (Figure 1) identified a first larval stage (L1) in the apterous line, from which a second larval stage with individuals of two sizes: small L2 (SL2) and large L2 (LL2). From that stage, the individuals of the apterous line are grouped on one side: minor workers (WS), presoldiers (PS), soldiers (S) and major workers (WL). On the other side, the members of the nymphal line formed four groups of nymphs (N1 to N4) and the alates (A).

The analysis of variance performed for the apterous line showed differences between groups for all the considered variables (MWH: $F=1749.21$, $p<0.00$; LLA: $F=1506.10$, $p<0.00$; MLP: $F=387.16$, $p<0.00$; MWP: $F=1250.83$, $p<0.00$; LHT. $F=2235.14$, $p<0.00$). The Tukey test (Table 2) showed similarities among minor workers and soldiers in two variables (MWH and MLP) and with presoldiers in MWH, while major workers did not show differences with soldiers in LHT and LLA.

Larval stages can be easily distinguished from workers based on their white color due to the absence of sclerotization. The number of antennal segments increased in each molt, beginning with 11 segments in L1, 12 segments both in SL2 and LL2, 13 segments in PS, S and WS and 13 to 15 segments in WL. Morphometric characteristics of each stage were registered (Table 3).

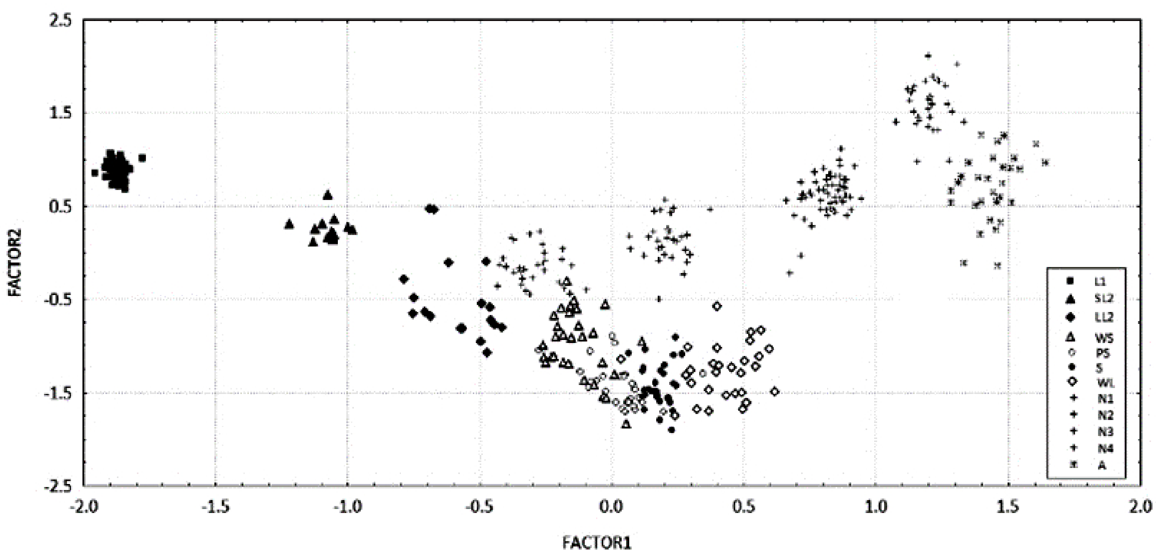

Figure 1. PCA graph of whole caste system in *C. fulviceps*. References: WL: major workers; WS: minor workers; S: soldier; PS: presoldiers; L1: Larva 1; LL2: large larva 2; SL2: small larva 2; N1 to N4: nymph 1 to 4; A: alates

Table 2. Results of Tukey's Test. Same letters mean no significant differences between castes for the variables considered

	MWH	LLA	MLP	MWP	LHT
L1	a	a	A	a	a
SL2	b	b	B	b	b
LL2	c		b,c	c	c
Ps	d	d	C	d,e	d
S	e	e	D	d	d,e
WS	d,e	c	D	e	f
WL	f	e	E	f	e

References: Larva 1 (L1); small larva 2 (SL2); large larva 2 (LL2); presoldiers (PS); soldier (S); minor workers (WS); major workers (WL); maximum width of the head (MWH); length of the left antenna (LLA); median length of pronotum (MLP); maximum width of pronotum (MWP); length of hind tibia (LHT).

Table 3. Mean and standard deviation (n) of the measured variables in individuals of the neutral line

CASTE	MWH	LLA	MLP	MWP	LHT
L1	0.240 (0.009)	0.276 (0.023)	0.053 (0.007)	0.124 (0.016)	0.132 (0.008)
SL2	0.527 (0.073)	0.671 (0.092)	0.128 (0.021)	0.324 (0.027)	0.333 (0.069)
LL2	0.694 (0.105)	0.896 (0.097)	0.143 (0.028)	0.380 (0.056)	0.523 (0.072)
Ps	0.958 (0.062)	1.204 (0.093)	0.158 (0.016)	0.571 (0.043)	0.939 (0.066)
S	1.012 (0.048)	1.343 (0.076)	0.184 (0.020)	0.596 (0.024)	0.968 (0.040)
WS	0.976 (0.079)	0.960 (0.073)	0.176 (0.025)	0.549 (0.039)	0.771 (0.035)
WL	1.225 (0.047)	1.329 (0.104)	0.241 (0.030)	0.684 (0.069)	1.005 (0.079)

References: Larva 1 (L1); small larva 2 (SL2); large larva 2 (LL2); presoldiers (PS); soldier (S); minor workers (WS); major workers (WL); maximum width of the head (MWH); length of the left antenna (LLA); median length of pronotum (MLP); maximum width of pronotum (MWP); length of hind tibia (LHT).

In the apterous line it was determined that the major workers are females (Fig. 2A), whereas minor workers and soldiers are males (Figure 2B, C).

In the sexual line, the analysis of variance showed differences in all the considered variables ($p<0.001$) while Tukey test also showed that the nymphal instars are different from each other and with the alates, in all the measured variables ($p<0.001$). The dimensions of each nymphal instar and alates were registered (Table 4).

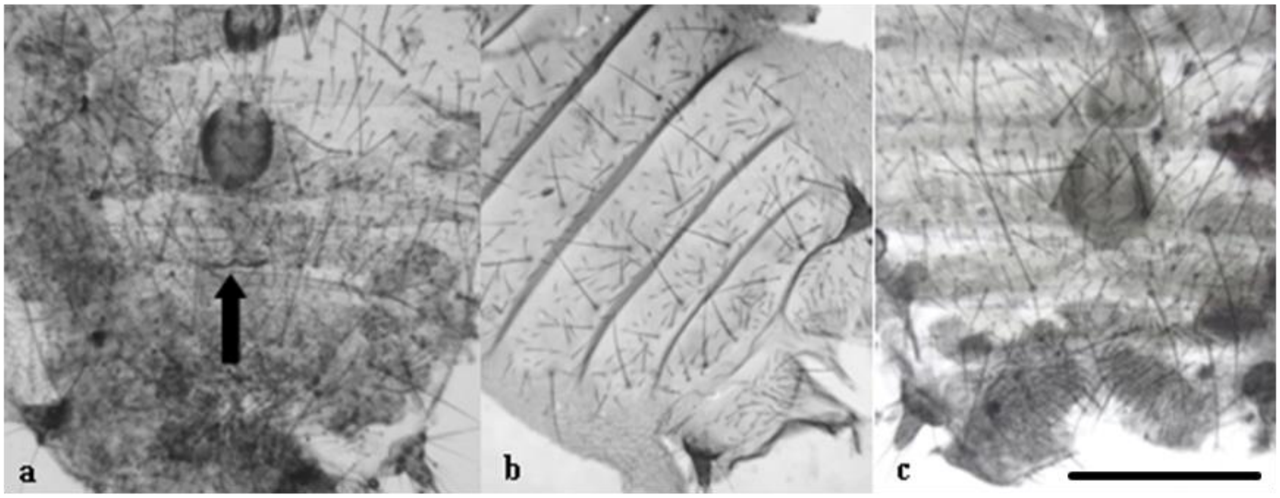

Figure 2. Ventral view of abdomen of major worker (A), minor worker (B) and soldier (C). The arrow indicates the rudiment of spermatheca found only in the eighth sternite of females

Table 4. Mean and standard deviations of the measurements of *C. fulviceps* nymphs and alates

CASTE	MWH	LLA	MLP	MWP	LHT
N 1	0.706 (0.053)	1.035 (0.067)	0.197 (0.018)	0.481 (0.039)	0.734 (0.037)
N 2	0.845 (0.051)	1.241 (0.065)	0.292 (0.022)	0.600 (0.033)	1.038 (0.051)
N 3	1.065 (0.027)	1.508 (0.102)	0.433 (0.028)	0.815 (0.030)	1.302 (0.054)
N 4	1.198 (0.031)	1.631 (0.082)	0.596 (0.029)	0.957 (0.031)	1.391 (0.051)
Alates	1.421 (0.039)	1.841 (0.132)	0.564 (0.044)	1.054 (0.043)	1.535 (0.089)

References: N1 to N4: nymph 1 to 4; A: alates. maximum width of the head (MWH); length of the left antenna (LLA); median length of pronotum (MLP); maximum width of pronotum (MWP); length of hind tibia (LHT).

The morphometric characteristic of the identified stages and the determination of the sex in workers and soldiers, allow us to propose for *C. fulviceps* the development pattern outlined in Figure 3.

According to the characteristics shown by the developmental stages identified in this study for *C. fulviceps*, the nymphal progress leading to the alates responds broadly to the one established by Roisin (2000) for Termitidae. The finding of only four stages in our study could be due, probably, to the absence of the first nymphal stage in the analyzed samples.

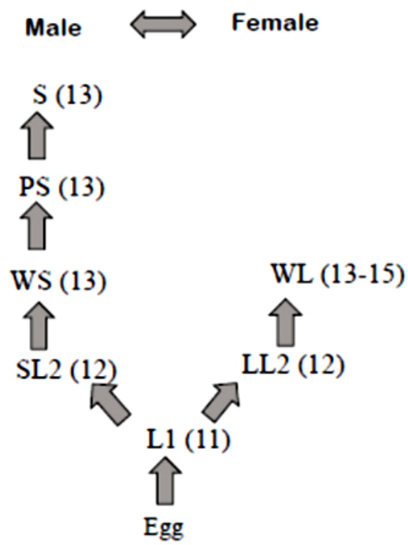

Figure 3. Scheme proposed for the development of the apterous line of *C. fulviceps*. Each arrow indicates a change. The numbers in parenthesis indicate the numbers of antennal segments. Bar indicates 0,5mm

In the apterous line, it could be determined the existence of two larval instars as commonly seen in many Termitidae species with a noticeable dimorphism from the second larval instars (ROISIN, 2000). At this stage, the group of smaller L2 would be male and would give rise to minor workers, while the larger L2 would be female and originate major workers.

In the major workers, the variations registered in the number of antennal segments suggest the existence of at least two stages, without clear morphometrical changes. The characteristics observed in size and numbers of antennal segments of these castes are consistent with the findings for *C. fulviceps* workers (ANNONI et al., 2011) and the developmental pathways matches the ones described for the *Nasutitermes* group (ROISIN 1987, 2000).

In Termitidae, the origin of soldiers varies between species, but always involves a presoldier stage, which is a white individual devoid of sclerotization, but with the morphological characteristics of a soldier. The presoldiers usually develop from workers or from larvae (NOIROT, 1955, 1985). In *Bulbitermes sarawakensis* (Haviland), presoldiers originate from the second larval stage (LOMMEN et al., 2004) while in *Velocitermes heteropterus* (Silvestri) the male presoldiers could be derived both from larvae and minor workers while the major workers are females (HAIFIG et al., 2012). A similar pattern is found in *Constrictotermes cyphergaster* (Silvestri), with the difference that both the workers and soldiers are monomorphic males (MOURA et al., 2011).

In *C. fulviceps*, the origin of the presoldiers from minor workers, as proposed in this paper, is inferred by the presence of the same number of antennal segments in both castes and also because both of them are males.

CONCLUSION

The results obtained in this study show that the developmental pathway of *C. fulviceps* matches the ones of *Hospitalitermes medioflavus* (Holmgren) (MIURA et al., 1998) and the genus *Nasutitermes* (ROISIN & PASTEELS, 1987; BUSCHINI & COSTA LEONARDO, 2002). The similarity between the *Cortaritermes* and *Nasutitermes* developmental patterns, contribute to support the relationship of both genus as proposed by Fontes (1998) that includes *Cortaritermes* in the same clade of *Nasutitermes*.

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REFERENCES

ANNONI, G.; CORONEL, J.M.; LAFFONT, E.R. 2011. Analisis morfométrico de la casta obrera de *Cortaritermes fulviceps* (Isoptera, Termitidae). **Revista de Agricultura**, Piracicaba, 86 (3): 200–206.

BUSCHINI, M.L.T.; COSTA LEONARDO, A.M. 2002. Biometrics studies of caste development in *Nasutitermes coxipoensis* (Isoptera, Termitidae). **Sociobiology**, Bahia, 40 (2): 465–477.

DARLINGTON, J. 1984. A method for sampling the populations of large termite nests. **Annals of Applied Biology**, Wellesbourne, 104: 427–436. doi:10.1111/j.1744-7348.1984.tb03024.x

FONTES, L.R. 1998. Novos aditamentos ao "Catálogo dos isóptera do Novo Mundo", e uma filogenia para os géneros neotropicais de Nasutitermitinae. In: FONTES, L.R., BERTI FILHO, E. (eds.), **Cupins. O desafio do conhecimento**, FEALQ, Piracicaba, SP, Brasil, pp. 309–412.

HAIFIG, I.; COSTA LEONARDO, F.; FERREIRA COSTA, F.; COSTA LEONARDO, A.M. 2012. On the Apterous Line of the Termite *Velocitermes heteropterus* (Isoptera: Termitidae): Developmental Pathways and Cellulose Digestion. **Zoological Science**, Washington, 29 (12): 815–820. doi: 10.2108/zsj.29.815

LEGENDRE, F.; WHITING, M.F.; BORDEREAU, C.; CANCELLO, E.M.; EVANS, T.A.; GRANDCOLAS, P. 2008. The phylogeny of termites (Dictyoptera: Isoptera) base don mitocondrial and nucler markers: Implications for the evolution of the workers and pseudergates caste, and foraging behaviors. **Molecular Phylogenetics and Evolution**, Kidlington, 48: 615–627. doi: 10.1016/j.ympev.2008.04.017

LOMMEN, S.; VAN HUIS, A.; SAJAP, A. 2004. Caste biology and behaviour of *Bulbitermes sarawakensis* (Isoptera, Nasutitermitinae) in Malaysia. **Proceedings of the Netherlands Entomological Society**, The Netherlands, 15: 21–28.

MC MAHAN, E.A.; WATSON, J.A.L. 1975. Non-reproductives castes and their development in *Nasutitermes exitiosus* (Hill) (Isoptera). **Insectes Sociaux**, Switzerland, 22: 183–198. doi: 10.1007/BF02223428

MILLER, M. 1969. Caste differentiation in the lower Termites. In: KRISHNA, K., WEESNER, F.M. (eds), **Biology of Termites,** Vol. I. Academic Press, New York, pp. 283–310.

MIURA, T.; ROISIN, Y.; MATSUMOTO, T. 1998. Developmental Pathways Polyethism of neuter castes in the Processional Nasute Termite *Hospitalitermes medioflavus* (Isoptera: Termitidae). **Zoological Science**, Washington, 15: 843–848. doi: 10.2108/zsj.15.843

MOURA, F.M.S.; VASCONCELLOS, A.; SILVA, N.B.; BANDEIRA, A.G. 2011. Caste development systems of the Neotropical termite *Constrictotermes cyphergaster* (Isoptera, Termitidae). **Insectes Sociaux**, Switzerland, 58: 169–175. doi: 10.1007/s00040-010-0132-z

NOIROT, C. 1955. Recherches sur la polymorphisme des termites' supérieurs (Termitidae). **Annales des Sciences Naurelles, Zoologie et Biologie Animale**, Kidlington, 17: 399–595.

NOIROT, C. 1969. Formation of castes in the higher termites. In: KRISHNA, K., WEESNER, F.M. (eds), **Biology of Termites** Vol. I. Academic Press, New York, pp. 311–350.

NOIROT, C. 1985. Caste system in higher termites. In: WATSON, J.A.L., OKOT-KOTBER, B.M., NOIROT, CH. (eds). **Caste differentiation in social insects**. Pergamon Press, Oxford, pp. 75–86.

ROISIN, Y. 1996. Castes in humivorus and litter-dwelling neotropical nasute termites (Isoptera, Termitidae). **Insectes Sociaux**, Switzerland, 43: 375–389. doi: 10.1007/BF01258410

ROISIN, Y. 2000. Diversity and evolution of caste patterns. In: ABE, T., BIGNELL, D.E., MATSUMOTO, M. (eds.) **Termites: Evolution, Sociality, Symbioses, Ecology**. Academic Publisher, Kluwer, pp. 95–119.

ROISIN, Y.; PASTEELS, J.M. 1987. Caste developmental potentialities in the termite *Nasutitermes novarumhebridarum*. **Entomologia experimentalis et applicata**, United Kingdom, 44: 277–287. doi: 10.1111/j.1570-7458.1987.tb00556.x

ROISIN, Y.; PASTEELS, J.M. 1991. Polymorphism in the giant cocoa termite, *Neotermes papua* (Desneux). **Insectes Sociaux**, Switzerland, 38: 263–272. doi: 10.1007/BF01314912

ROISIN, Y.; LENZ, M. 1999. Caste developmental pathways in colonies of *Coptotermes lacteus* (Froggatt) headed by primary reproductives (Isoptera, Rhinotermitidae). **Insectes Sociaux**, Switzerland, 46: 273–280. doi: 10.1007/s000400050146

THORNE, B.L. 1985. Numerical and Biomass caste proportions in colonies of the termites *Nasutitermes corniger* and *N. ephratae* (Isoptera: Termitidae). **Insectes Sociaux**, Switzerland, 32 (4): 411–426. doi: 10.1007/BF02224018

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