Refinement of Methods for Sexing Instars and Caste Members in *Neotermes koshunensis* (Isoptera, Kalotermitidae)

by

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ABSTRACT

Exact sexing of individuals is important to an understanding of termite societies. We found that the female-specific sexual character of the elongated seventh sternite, known in other termites, is useful for distinguishing the sex of individuals of the dry-wood termite *Neotermes koshunensis* (Shiraki) from the fifth instar onward. The method described is highly efficient at sexing instars, nymphs, soldiers and alates.

Keywords: Dry-wood termites, Kalotermitidae, *Neotermes koshunensis*, morphology, sexual dimorphism.

INTRODUCTION

Insect societies among the Hymenoptera and Isoptera (termites) divide labor between reproductive and non-reproductive castes, whereas in hymenopteran societies males almost never play any social role. In termite societies males and females are assumed to share the same social roles (Krishna 1969, Holldöbler & Wilson 1990, Roisin 2000). However, a growing body of empirical evidence suggests that this premise in termites does not always hold true, and caste-specific sex biases have been reported (Roisin 2000). For a better understanding of the roles of the sexes in termite social systems, we need more empirical data on various termite taxa. With this aim, the sexing of individual termites is indispensable. However, the small morphological differences between male and female termites often make morphological sexing difficult.

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Alates (imagos) of many termites show a female-specific morphological character, viz., a widely extended seventh sternite that covers most or all of the eighth and ninth sternites (Weesner 1969). In some species, immature females also show similar sexual characteristics that can be used for sexing (Thompson & Synder 1920, Mensa-Bonsu 1976, Myles & Chang 1984, Roisin 1992). Zimet and Stuart (1982) described the sexual dimorphism in immature stages of *Reticulitermes flavipes* (Rhinotermitidae), providing currently the most useful information for sexing immature stages of termites. However, they did not identify the stage at which this character becomes practical for sexing. So far no study has examined the validity of this sexing method in every developmental stage of immatures. We did so by observation of the sternite structure in all immature stages of *Neotermes koshunensis* (Kalotermitidae), followed by confirmation of sex by gonadal dissection.

MATERIALS AND METHODS

N. koshunensis is distributed from Taiwan to Okinawa (Ikehara 1966). A colony of *N. koshunensis* collected on the main island of Okinawa in September 2009 was reared in the laboratory (in the dark at ambient temperature) using a plastic box with pine woods as food for about 2 months before the study. A preliminary examination showed that alates of this species show sexual dimorphism in the sternites (Y. Miyaguni, unpublished data). For every instar, nymphal stage and alates, we used the morphology of the sternites to classify individuals into putative females and putative males under a stereomicroscope (×40 magnification at maximum). We then dissected 30 putative females and 30 putative males of each instar and caste to determine the true sex through inspection of the gonads (Thompson and Synder 1920). Instars and castes were defined according to Katoh *et al.* (2007). The sternite structures were recorded on a digital camera attached to the stereomicroscope (Olympus E-System E-330; Olympus Corporation, Tokyo, Japan).

RESULTS

The sexual dimorphism of nymphs was very clear, especially in pre-alates, which could be sexed under a magnifying glass (×10). Sixth instar females showed considerable individual variation in the degree of sternite development, ranging from only a slight extension to near complete coverage of the eighth sternite (Fig. 1). The sexual difference in soldiers was similar to that of sixth instars. It was less conspicuous in fifth instars (Figs. 1, 2). Despite this variation, dissections revealed that our sexing of individuals using this morphological indicator was 100% accurate in those developmental stages and castes (Table 1). Some fourth instar females did not show a sexual character and were misclassified as males, although we correctly determined the sex of other females (Table 1). There was a significant difference in the proportion of misidentified individuals between fifth and fourth instars (Fisher's exact test, $P < 0.001$). All third instars were classified as males by our criterion, as this instar showed no sexual difference in the sternite configuration (Table 1). In first and second instars, the gonads were indistinguishable between

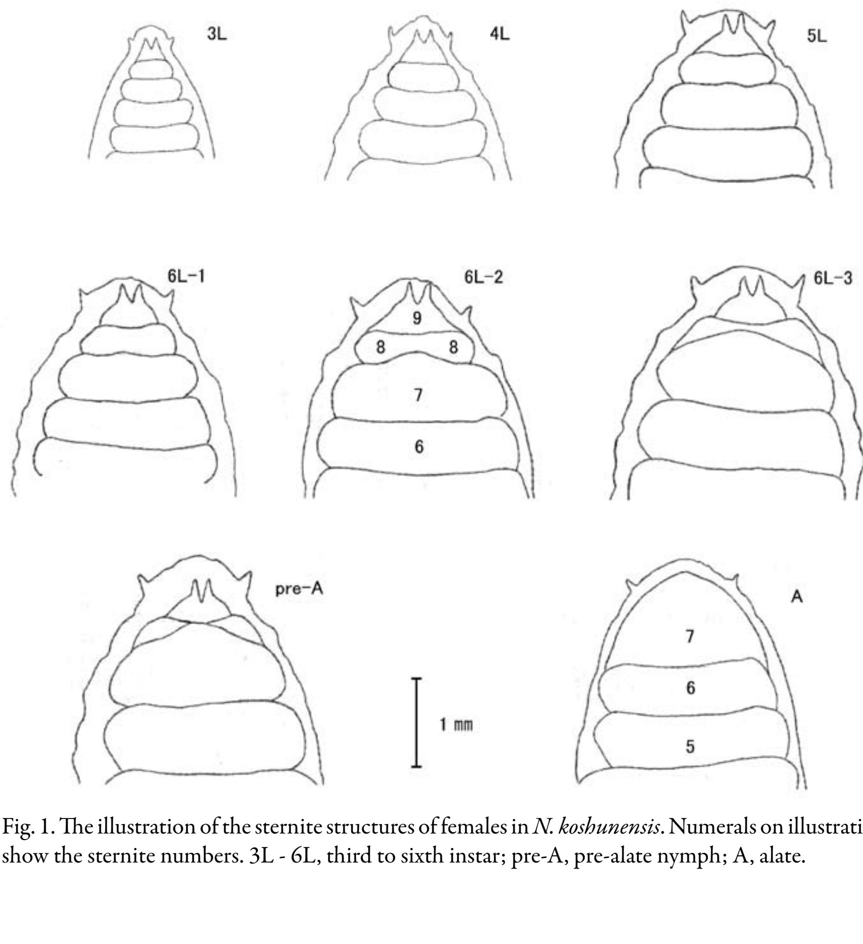

Fig. 1. The illustration of the sternite structures of females in *N. koshunensis*. Numerals on illustration show the sternite numbers. 3L - 6L, third to sixth instar; pre-A, pre-alate nymph; A, alate.

Table 1. The result of sexing based on sexual dimorphism.

Instars & castes	n	True sex		Proportion misidentified
		Male	Female	
Putative male				
3rd instar	30	20	10	0.3
4th instar	30	18	12	0.4
5th instar	30	30	0	0.0
6th instar	30	30	0	0.0
Early nymph	30	30	0	0.0
Pre-alate nymph	30	30	0	0.0
Alate	30	30	0	0.0
Soldier	30	30	0	0.0
Putative female				
3rd instar	-	-	-	-
4th instar	30	0	30	0.0
5th instar	30	0	30	0.0
6th instar	30	0	30	0.0
Early nymph	30	0	30	0.0
Pre-alate nymph	30	0	30	0.0
Alate	30	0	30	0.0
Soldier	30	0	30	0.0

testes and ovaries, and therefore it was not possible to determine the sex. Thus, sexing using the seventh sternite was highly accurate in all stages from the fifth instar onward.

DISCUSSION

The sexual characteristics of *N. koshunensis* females begin to develop from the fourth instar, but the sexual difference in the sternites from the fifth instar on becomes conspicuous enough to be informative as a morphological indicator of sex (Table 1, Figs. 1, 2). In termite species that have a linear caste developmental pathway, the timing or stage at which the wing buds begin to develop differs among individuals (Roisin 2000, Katoh *et al* 2007). The variation that we found in the seventh sternite morphology in the fourth to sixth instar females of *N. koshunensis* may reflect a similar individual difference in development. Although the generality of our finding in *N. koshunensis* is still to be confirmed in other colonies, our study indicates that sternite morphology can be used for sexing some younger stages.

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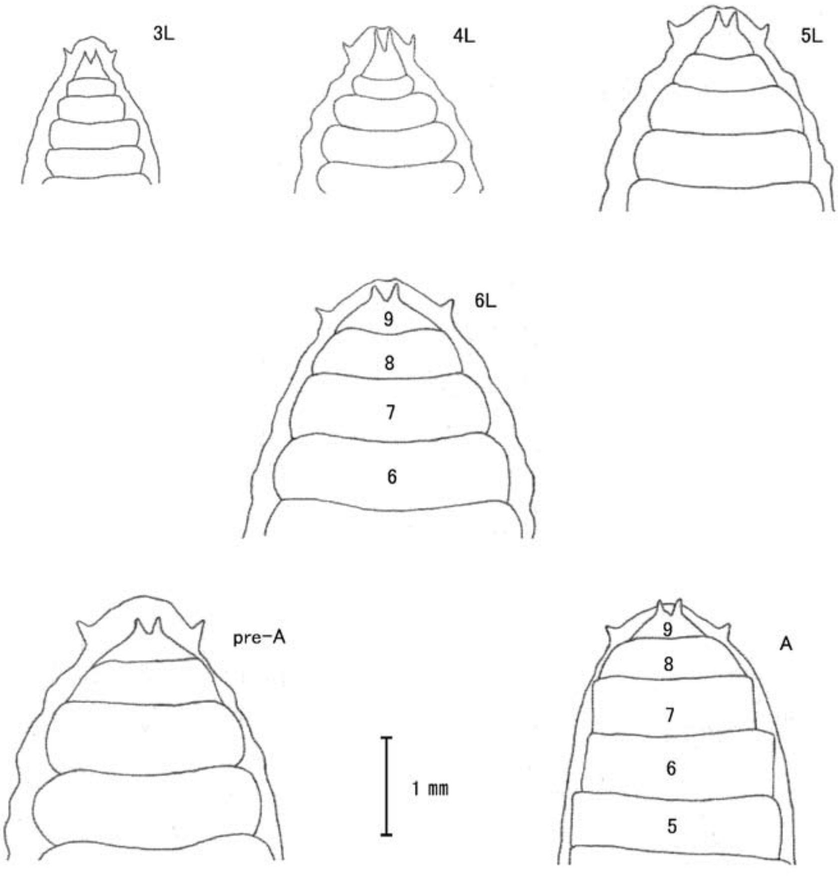

Fig. 2. The illustration of the sternite structures of males in *N. koshunensis*. Numerals on illustration show the sternite numbers. 3L - 6L, third to sixth instar; pre-A, pre-alate nymph; A, alate.

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