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Morphological Differences of Immature Stages between Males and Females in a Korean Wood-Feeding Cockroach (*Cryptocercus kyebangensis*)

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Morphological characteristics of nymphal instars are described for a Korean wood-feeding cockroach, *Cryptocercus kyebangensis*. Eleven instars, including adults, were tentatively identified. Female adults had apicolateral emargination and a truncated apical median prominence in the seventh sternite, and female nymphs of the other instars except for the last had a narrowly rounded apical median prominence in the seventh sternite. In the last instar, the seventh sternite was partially desclerotized and somewhat shriveled at the start of the emarginated area. In contrast, males showed no emargination in adults, and had a rounded broad apical median in this area of all instars. In the ninth sternite, female nymphs had a medium notch on the caudal margin and styli were separated before reaching adulthood, whereas no such notch was observed in male nymphs. The styli remained prominent in the ninth sternite of male adults.

Some characteristics of a wood-feeding cockroach, *Cryptocercus* spp., make them unique among cockroaches. In addition to xylophagy, *Cryptocercus* spp. live as family groups within complex galleries of rotten logs, and in mountainous forests (Cleveland et al., 1934; Seelinger and Seelinger, 1983; Nalepa, 1984; Grandcolas et al., 2001; Park 2002). They live in prolonged pair bonding of a single male and female and provide proctodeal trophallaxis to their young (Cleveland et al., 1934; Seelinger and Seelinger, 1983; Nalepa, 1984; Park et al., 2002).

Such characteristics of *Cryptocercus* spp. life history are uncommon in other cockroaches and have long been studied in relation to well-developed social behavior (Cleveland et al., 1934; Seelinger and Seelinger, 1983; Nalepa, 1984, 1988; Nalepa and Bell, 1997; Nalepa and Mullins, 1992; Park, 2002; Park et al., 2002; Park and Choe, 2003a, b). Different from other wood-feeding cockroaches, only the woodroaches of *Cryptocercus* spp. like primitive termites, include symbiotic protozoa in their gut to digest woody diets and the symbionts between the hosts show close phylogenetic relationship. In addition to the close phylogenetic relationship of the symbionts between *Cryptocercus* and lower termites, some morphological characteristics of *Cryptocercus* are also similar to those of lower termites (McKittrick, 1964, 1965). Thus, *Cryptocercus* has been considered as a key-taxon for understanding relationships and evolution in the Dictyoptera and Isoptera, and the morphology of *Cryptocercus* has been investigated frequently to discern its phylogenetic position (Cleveland et al., 1934; McKittrick, 1964, 1965; Thorne and Carpenter, 1992; Grandcolas, 1994; Park and Choe, 2002).

Although morphological studies are sufficiently reported to date, most of them are for phylogenetic relationship of *Cryptocercus* (McKittrick, 1964). Morphological characteristics needed for field researches, however, were little studied except a North American species, *C. punctulatus* (McKittrick, 1964, 1990; Appel, 1989; Nalepa et al., 1997).

Accurate methods for determining the age and sex of *Cryptocercus* are of considerable value for field and laboratory experiments, allowing a more complete understanding of the many aspects of its ecology and social behavior which may vary accordingly. Although some categories of nymphal age and sexual characteristics related to morphology have been described in *C. punctulatus* (Seelinger and Seelinger 1983, Nalepa, 1990; Appel 1989; Nalepa et al., 1997), they still remain poorly understood in Asian *Cryptocercus* (Park and Choe, 2002).

Woodroaches of the genus *Cryptocercus* include six species. They occur in temperate forest mountains of western North America (*C. clevelandi*), eastern North America (*C. punctulatus*), west China (*C. matilei* and *C. primarius*) and northeast Asia (*C. kyebangensis* and *C. relictus*) (Scudder, 1862; Bey-Bienko, 1950; Nalepa et al., 1997; Nalepa et al., 2001; Grandcolas, 2000; Grandcolas et al., 2001; Park 2002). In an American species, *C. punctulatus*, however, Burnside et al. (1999) have

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separated the species into four different species including *C. punctulatus, including C. wrighti, C. darwini,* and *C. garciai.*

The present study was conducted to provide information which can be easily applied for the determination of *Cryptocercus* instar and sex. It includes descriptions of full nymphal stages and morphological characteristics of immature stages in a Korean wood-feeding cockroach, *C. kyebangensis*, recently described by Grandcolas et al. (2001).

Materials and Methods

Collection

Korean populations of the genus *Cryptocercus* occurred in most of the forested high mountainous regions from the North to the South of South Korea. For the present study, wood-feeding *C. kyebangensis* were collected from Gyebang-san (Mt.), Gangwon Province, Korea. To investigate the morphology of immature instars, collections of *Cryptocercus* were made in July and October, 1997. All the connecting chambers of *C. kyebangensis* in rotten logs were opened with a hammer and wood chisel. Sporadic collections were also made in June, July, October, September, and November, 1997 and April, 1998 to investigate composition of instars in the field. Following Nalepa (1990), nymphs of the first instar were obtained by incubating oothecae until hatching at 25 ± 2°C in glass vials furnished with moist woody fragments.

Measurement and morphological investigation

Body mass and size of the field-caught *Cryptocercus* were measured within 7 d of each collection, and preserved in 70% alcohol. Morphological features were measured using a binocular microscope with a graduated eyepiece (Olympus SZ-STU1). The head capsule width and the number of antennal segments were measured following Nalepa (1990). To determine the head capsule width, the maximal width of the head, including the eyes, was measured. The number of antennal segments was counted in undamaged antennae.

Morphology of the seventh and ninth sternites was examined in nymphs of various instars. The seventh sternite of a male and a female were examined using a scanning electron microscope (SEM). For the scanning electron microscopy, the seventh sternites were separated from the samples preserved in 70% alcohol and dried for 3 d. Samples were mounted on stubs, coated with gold at 6 mA for 3 min using an ion coater (Eiko IB-3), and examined with SEM (Akashi SR 50A) operating at an accelerating voltage of 15 kV.

Abbreviations used in the description of morphology were modified from McKittrick (1964) as follows: ca. = caudal median, ce. = cercus, c.l.m. = caudal-lateral margin, s.g.p. = subgenital plate, st. = stylus, SIIV = seventh sternite, SIIIV = eight sternite, SIX = ninth sternite, pp.= paraproct.

Statistics

One-way ANOVA was conducted at 5% level to test whether each category of instar differs significantly in head capsule width, fresh weight, and body size of individuals, respectively (Table 1).

Results and Discussion

Developmental stages

Frequency distribution of head capsule width was grouped into 11 instar categories in field-caught *C. kyebangensis* (n = 482) (Fig. 1). Multiple comparison test showed the individuals among each instars including adults significantly differed at 0.5% level in head capsule width, fresh weight, and body size (Tukey test; $P < 0.05$). Thus, 11 stages including adults were tentatively identified.

The head capsule widths of adults did not differ significantly between the sexes ($t = 1.07$, df=39, P = ns). Head capsule widths of *C. kyebangensis* adults were compared with those reported previously in *C. punctulatus* (Seelinger and Seelinger, 1983; Nalepa, 1984; Nalepa et al., 1997) and *C. clevelandi* (Nalepa et al., 1997). They were similar in size to those reported for *C. punctulatus* (Seelinger and Seelinger, 1983; Nalepa, 1984; Nalepa et al., 1997), but smaller than that described for *C. clevelandi* (Nalepa et al., 1997). It is known that a larger body size is associated with longer postembryonic development in several cockroach species. Greater adult weight was, for example, a result of their longer duration

Table 1. Instar categories based on the frequency distribution of head capsule widths of the field-caught *Cryptocercus kyebanpensis*

Instar	Sample size (*n*)	Head capsule width (mm)		Fresh weight (mg)	Body size (mm)
		Mean ± SD	Range		
1st	43	0.87 ± 0.05	0.75-0.95	2.7 ± 1.7	2.9 ± 0.5
2nd	61	1.14 ± 0.05	1.00-1.20	5.4 ± 1.6	4.4 ± 0.7
3rd	51	1.38 ± 0.05	1.25-1.45	10.6 ± 2.3	6.8 ± 0.6
4th	65	1.65 ± 0.04	1.55-1.73	16.4 ± 3.9	7.9 ± 0.8
5th	43	1.92 ± 0.06	1.80-2.05	30.8 ± 5.8	10.1 ± 1.1
6th	22	2.26 ± 0.06	2.13-2.35	44.2 ± 9.2	11.5 ± 0.9
7th	50	2.64 ± 0.07	2.50-2.75	76.3 ± 14.3	13.4 ± 1.2
8th	40	2.96 ± 0.10	2.80-3.10	108.4 ± 21.7	14.7 ± 1.1
9th	22	3.34 ± 0.11	3.20-3.55	196.9 ± 38.9	17.7 ± 1.0
10th	26	3.90 ± 0.16	3.65-4.25	337.8 ± 57.8	21.3 ± 1.3
Adult	59	4.37 ± 0.15	4.05-4.73	443.0 ± 70.3	23.1 ± 1.8

Multiple comparisons indicated that instar categories differed significantly in head capsule width, fresh weight, and body size (Tukey test, $P < 0.05$), respectively.

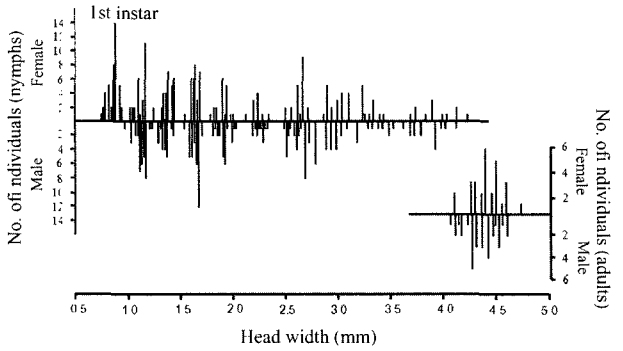

Fig. 1. The frequency distribution of head capsule width of field-caught *Cryptocercus kyebangensis*. Nymphs of the first instar were not sexed.

of larval development in *Blatta orientalis* (Landowski, 1938), *Periplaneta americana* (Wharton et al., 1967), and *Diploptera punctata* (Woodhead and Paulson, 1983). In *Diploptera punctata*, especially, both a higher rate of growth and longer duration of larval development contribute to the larger adult size of females than males (Woodhead and Paulson, 1983). Since *C. kyebangensis* has a smaller head width size than *C. clevelandi*, the former will probably have a shorter developmental period than the one (6-7 yr) described in the latter (Nalepa et al., 1997). The similar head capsule width between *C. kyebangensis* and *C. punctulatus* suggests that the developmental span of *C. kyebangensis* may be similar to that (4-5 yr) known in *C. punctulatus* (Nalepa et al., 1997).

Morphological differences between sexes during nymphal development

Individuals used for morphological descriptions were assigned to an instar, based on categories of wild-caught *C. kyebangensis* (Table 1). The morphology of the seventh and ninth sternites was investigated in males and females of all developmental stages. In nymphs of the first instars, the seventh and eight sternites are crescent-shaped (Fig. 2A). The extended seventh sternite concealed most of the eighth sternite, except for both marginal sections of the sternite at the second instar (Fig. 2B). The eighth sternite was concealed completely under the seventh sternite of the third instar (Fig. 2C). Morphological difference between sexes of the third instar was obsereved in the seventh sternite. The caudal-lateral margin (c.l.m.) of the sternite was shrunk slightly in the female and the apical margin was narrowly rounded. In contrast, male nymphs have a wider rounded apical margin. This shape of the seventh sternite was maintained to the stage prior to the last instar. In nymphs of the last instar (Fig. 2D), the apical margin of males was broadly rounded, whereas that of females was partially desclerotized and a little shriveled at the start part of the

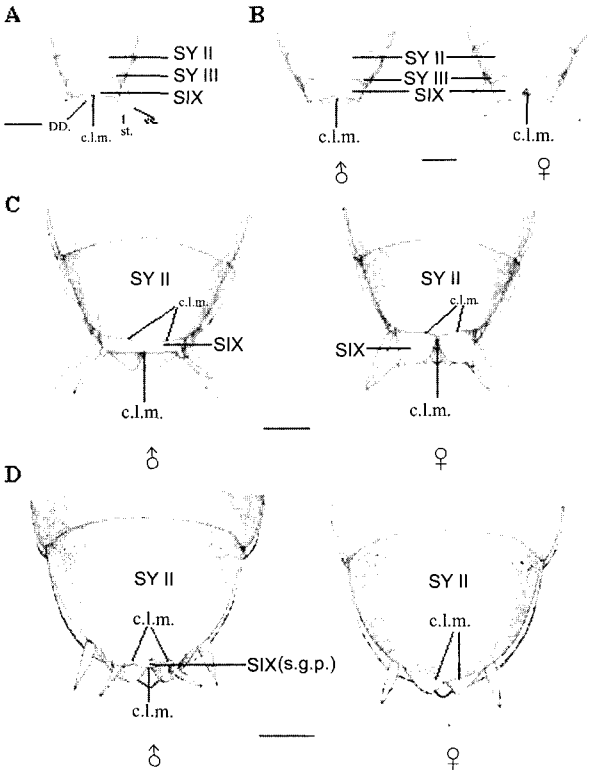

Fig. 2. Morphological differences between sexes of *Cryptocerus kyebangensis* nymphs. ca; caudal median, ce; cercus, c.l.m.; caudal-lateral margin, s.g.p.; subgenital plate, st.; stylus, SIIV;seventh sternite, SIIIV; eight sternite, SIX; ninth sternite, pp; paraproct. Statistics. A, A postero-ventral view of the abdomen of the first instar. Head capsule width: 0.88 mm. Nymphs of the first instar were not sexed. B, A postero-ventral view of the abdomen of second instars. Head capsule width: 1.12 mm (♂), 1.11 mm (♀). C, A postero-ventral view of the abdomen of third instars. Head capsule width: 1.35 mm (♂), 1.32 mm (♀). D, A postero-ventral view of the abdomen of last instars. Head capsule width: 3.90 mm (♂), 4.10 mm (♀). Scale bars = 2 mm(A-C) and 1 mm (D).

emarginated area.

The median caudal margin of the ninth sternite was almost straight or very slightly concave in neonates (n=153 from 6 oothecae). Although the latter could be assumed to be a morphological characteristic of females, morphological differences between the sexes were very indistinct at this stage (Fig. 2A). However, morphological differences in the ninth sternite occurred in an obvious manner between the sexes from the second instar (Fig. 2B). In females, the caudal median margin of the sternite is deeply concave in all nymphal stages. The styli were separated from the ninth sternite prior to at least the last instar (Fig. 2D). Evidence that the styli were separated existed in the ninth sternum of females of the eighth or ninth instar. In contrast, there was no division of the caudal margin of the ninth sternite and styli were also not separated during all stages of nymphal development in males (Fig. 2B-D).

Morphological differences between sexes in adults

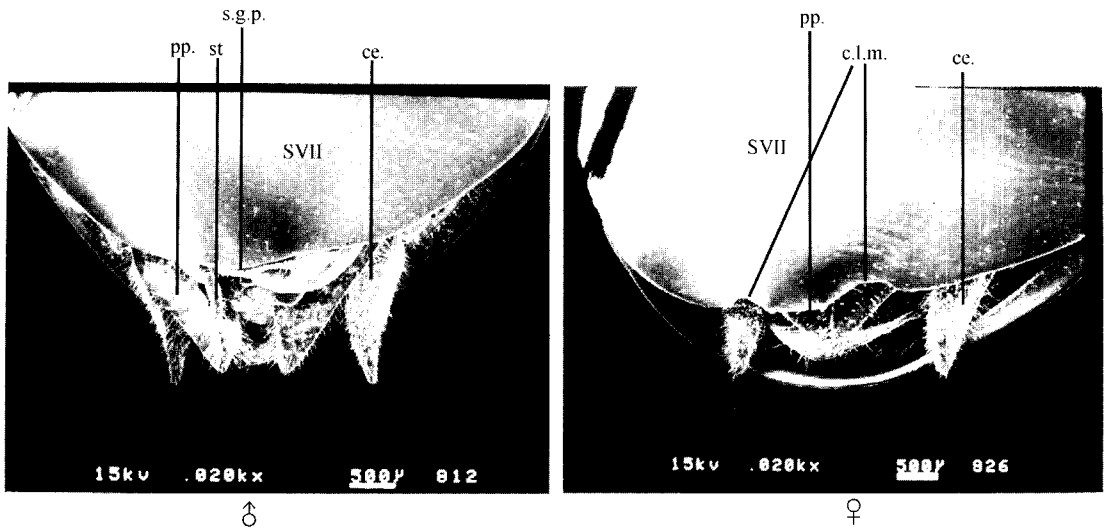

Fig. 3. A postero-ventral view of the abdomen in adults. Head capsule width; 4.35 mm (♂), 4.5 mm (♀).

Morphological characteristics of the seventh sternite of adult females have ever been firstly described for some Asian *Cryptocercus* spp. including *C. kyebangensis* by Grandcolas et al. (2001). For comparative description of the morphological characteristics of the seventh sternite between sexes, we redescribed the sternite of females as well as description of male sternite using scanning electron microscopy (Fig. 3).

In female adults, apicolateral emargination formed on the seventh sternite from the parts which were partially desclerotized and a little shriveled at the marginal areas (Fig. 2D), resulting in a subtruncate apical median prominence in the sternite (Fig. 3). In male adults, however, the apicolateral emargination was not observed in the apical margin of the seventh sternite and the apical margin remained broadly rounded.

The ninth sternite differentiated into the male subgenital plate and the styli were not separated from the sternite (Fig. 3). In the female adults, styli were separated from the ninth sternite, and the sternite differentiated into a pair of third valves of the female reproductive organ.

Conclusions

The morphological features exhibited in the seventh and the ninth sternites can be used as diagnostic characteristics for determining the sexes of *C. kyebangensis*. Since the morphological differences of both sternites were observed obviously in nymphs as well as adults, they were characters which were applied easily for the rapid sex determination during field studies. This study is the first description of immature stages and sexual dimorphism at each stage in an Asian *Cryptocercus*, *C. kyebangensis*, and it will provide comparative information for the Asian *Cryptocercus* spp.

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