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Sexual differentiation and developmental stage identification of the Indian Cockroach, *Pycnoscelus indicus* (Blattodea: Blaberidae)

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Abstract. We found that sexual differentiation of all the nymphal stages of *Pycnoscelus indicus* (Fabricius) was possible by observing the developmental features of their posterior abdominal segments. Using this observation, the sex of even the 1st stage instar nymph could be determined. The female of the 1st to 6th stage instar nymph possess a V-shaped notch at the middle of the posterior edge of the 9th sternite. This notch is not seen in the male nymph. In the female 7th stage (final stage) instar nymph, the styli were not apparent and, the 8th and 9th sternites became degenerated and were covered over by the profoundly developed 7th sternite. In contrast, all stages of the male nymph showed the presence of styli. Thus, it is possible to differentiate the sex of all the stages, from 1st to 7th, of the nymph of *P. indicus* taxonomically. Moreover, it is also possible to identify the various specimens as to which stage the nymphal instar belong to, by counting the number of cercal segments from the ventral view.

INTRODUCTION

It is known that age differentiation of cockroach nymphs has been conducted based on the length and breadth of the head, thorax and abdomen, the number of segments on the antenna as well as on the number of sensory organs (Funaki, 1958; Wigglesworth, 1964; Sugimoto, 1967; Makiya, 1969; Mackay, 1978; Takeda, 1993; Gullan, 2000).

Sexual differentiation of the cockroach nymph for all the instar stages of *Blatta orientalis* Linnaeus (Qadri, 1938), *Supella longipalpa* (Fabricius) (Hafez & Afifi, 1956) and *Periplaneta fuliginosa* (Serville) (Saito & Hayashi, 1973) has been reported. Furthermore, sexual differentiation of *Blattella germanica* (Linne), *Periplaneta americana* (Linne) and *Periplaneta japonica* Karny were also reported to be possible, despite the observation of only the 1st stage instar nymph.

For the identification of the various developmental stages of *B. germanica*, it was reported that it can be done by counting the number of segments in the antennae, which were observed to increase correspondingly with age (Ishii, 1971). However, the antennae of the nymph are usually broken and thus this organ is not reliable for age determination. Since the number of cercal segments were observed to be different among the 1st to 5th stage instar male nymph, this can be used to identify their developmental stages. However, this criterion cannot be used to determine the age of the 5th and 6th stage female nymph because of overlapping number of segments in those age groups (Hasegawa, 1977; Saito, 1986). Thus, for determining the developmental stage of

the cockroach nymph, a combination of taxonomical features such as the number of segments on the antennae as well as on the cerca, coupled with the characteristic morphology of the sternite is required.

We report herein the criteria for the sexual differentiation of all the different nymphal stages of the Indian cockroach or Burrowing cockroach, *Pycnoscelus indicus*, based on our laboratory-reared specimens.

MATERIALS AND METHODS

Pycnoscelus indicus was originally collected in Ishigaki island, Taketomi-cho, Yaeyama district, Okinawa prefecture, Japan. The cockroaches were reared in the laboratory and had been passaged for more than 10 generations. The cockroaches used in our experiments were reared in a plastic container of diameter 90mm and a height of 50 mm. The beddings were made up of 10mm thick hydrated insect-rearing mat (Fujicon Co., Japan) and holes were made in the container lid for aeration. The cockroaches were fed slices of carrot cut to 10mm thickness. The whole container was placed in an incubator (Sanyo Co., Japan), with temperature set at 26-28°C, humidity at 50-70% and left in natural light condition. The rearing-mat and the carrot were replaced at appropriate time to prevent the growth of fungus.

The hatched nymphs were immediately segregated into those that have a V-shaped notch at the ventral abdominal caudal region and those that do not possess such structure. They were then reared separately in two groups. Immediately after hatching and every 4 days, the nymphs were anaesthesized with carbon dioxide gas, placed in a small transparent plastic bag and their ventral abdominal caudal region examined under a dissection microscope.

RESULTS

Morphology of the ventral abdominal caudal region of the male and female nymphs at different stages

1st stage nymph: Female has a notch at the 9th sternite posterior edge (A type; Fig. 2-A). No notch was seen in male (A type; Fig. 1-A). Besides the aforementioned features, no other differences were noted between the male and the female.

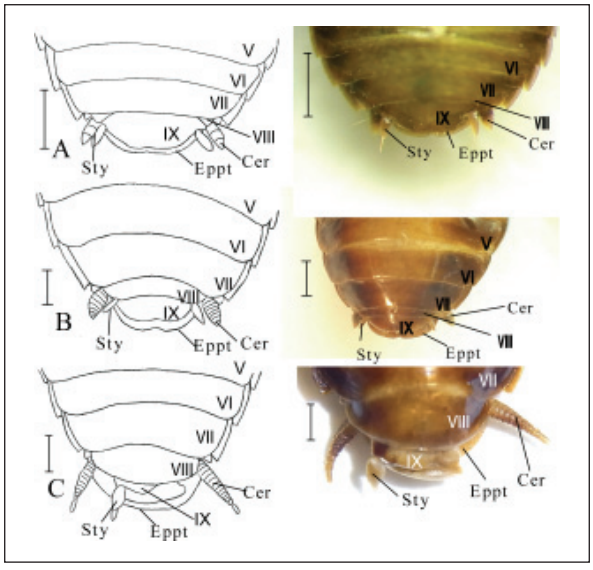

Figure 1. Development of the terminal abdominal sternite of male nymphs of *Pycnoscelus indicus* (Ventral view).
A: First instar nymph. B: Penultimate instar nymphs. C: Adult.
Cer: Cercus. Sty: Stylus. Eppt: Epiproct.
Scale bars. 0.5 mm for A. 1.0 mm for B-C.

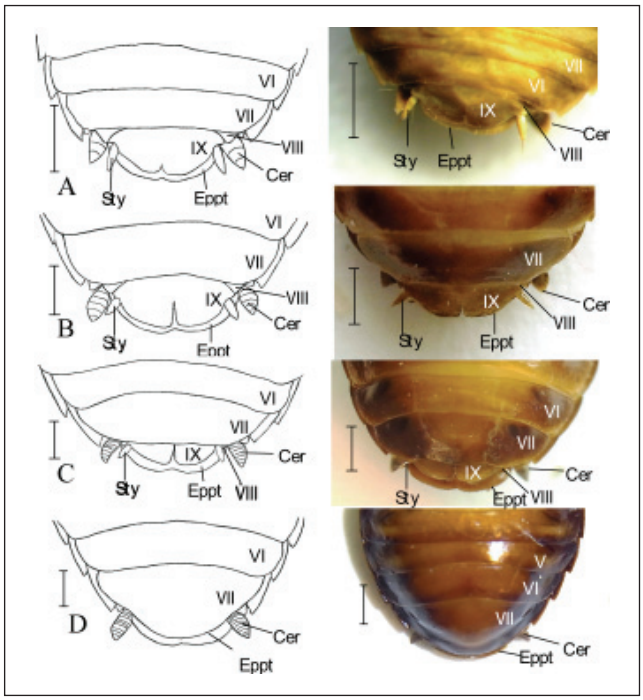

Figure 2. Development of the terminal abdominal sternite of female nymphs of *Pycnoscelus indicus* (Ventral view).
A: First instar nymph. B: Early age instar nymphs. C: Middle instar nymphs. D: 7th instar nymphs and adult. Cer: Cercus. Sty: Stylus. Eppt: Epiproct.
Scale bars. 0.5 mm for A; 1.0 mm for B-D.

2nd stage nymph: Just like in the 1st stage nymph, female has a notch at the 9th sternite posterior edge (A type; Fig. 2-A). No notch was seen in male (A type; Fig. 1-A).

3rd stage nymph: The notch in the female has become deeper and more conspicuous (B type; Fig. 2-B). Male was morphologically similar to 1st and 2nd stage (A type; Fig. 1-A).

4th stage nymph: The female nymph morphology was essentially the same as the 3rd stage (B type; Fig. 2-B). The 8th sternite of the male nymph was completely visible (B type; Fig. 1-B).

5th stage nymph: The notch in the female nymph, which could be seen on only the 9th sternite during the 4th stage, has become deeper and more conspicuous, reaching into the 8th sternite, as though splitting the sternal plate into two halves. (B, C type; Fig. 2-B, C). The male nymph did not show any changes with that of the 4th stage (B type; Fig. 1-B).

6th stage nymph: Both the female and the male nymph did not show any changes with that of the 5th stage (Female: B, C type; Fig. 2-B, C; Male: B type; Fig. 1-B).

7th stage nymph: The 8th and 9th sternite were being covered over by the 7th sternite and were no longer visible, and the styli had also disappeared in the female nymph (D type; Fig. 2-B). The male nymph show almost the same morphology as the 6th stage (B type; Fig. 1-B).

Adult: The 8th and 9th sternite, as well as the styli were no longer visible (D type; Fig. 2-B). The pair of styli, which was still apparent in the 7th stage male nymph, changed to become only one, with only the left stylus remaining on the male adult cockroach (C type; Fig. 1-C).

Cercal segments in all the stages of both female and male nymphs, and adults

The number of cercal segments from the dorsal view in male nymph are as follows: 1st stage, 3; 2nd & 3rd stage, 4; 4th stage, 5; 5th stage, 6; 6th stage, 7; and 7th stage (final stage)

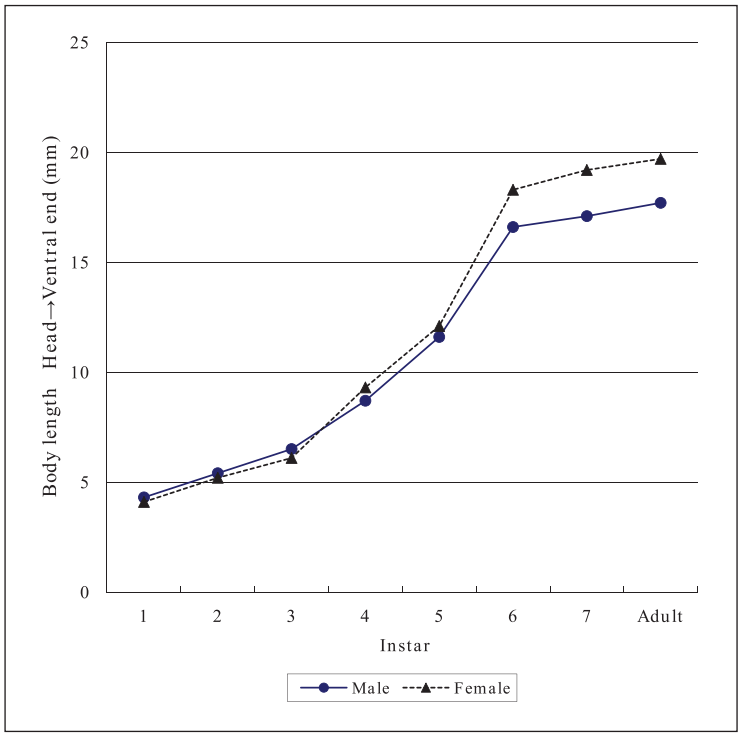

Figure 3. Body length in relation to each age term and sex of *Pycnoscelus indicus*.

8. The adult male has 11 segments on its cerca when viewed dorsally. There was no change in the number of cercal segments from 2nd to 3rd stage male nymph, remaining at 4.

On the contrary, the number of cercal segments from the ventral view in male nymph are as follows: 1st stage, 3; 2nd stage, 4; 3rd stage, 5; 4th stage, 6; 5th stage, 7; 6th stage, 8; and 7th stage (final stage) 9. The adult male has 12 segments on its cerca when viewed ventrally. Thus, there is a variation in the number of cercal segments among the different developmental stages (Table 1).

The number of cercal segments from the dorsal view for all the stages of the female, right up to the 7th stage nymph, were the same as that of the male nymph. The adult female has 9 segments on its cerca when viewed dorsally. Furthermore, the number of cercal segments of all the stages of the female, right up to the 7th stage nymph, when viewed ventrally were the same as that of the male nymph. The adult female has 10 cercal segments when viewed ventrally (Table 2).

Body length and type of sternites in all stages of both female and male nymphs, and adults

The sternite of male nymph of 1st to 3rd stages showed A type, 4th and 5th stage showing A, B type, 6th and 7th stage showing B type, while that of the adult showed the C type.

The sternite of female nymph of 1st and 2nd stages showed A type, 3rd and 4th stages showing B type, 5th stage showing B, C type, 6th stage showing C, D type, 7th stage showing D type, while that of the female adult also showed the D type (Table 3).

DISCUSSION

In recent years, the Indian cockroaches has been reported to invade into the dwellings of humans (Harada & Tsuji, 1995; Tawatsin *et al.*, 2001; Sriwichai *et al.*, 2002; Harunari *et al.*, 2009; Yamauchi & Kato, 2009; Komatsu *et al.*, 2013). From a public health view of point, this warrants a further detailed study of their developmental stages and morphology.

Table 1. The number of cercal segments in male *Pycnoscelus indicus*

Instar		No. of insects	Number of cercal segments											
			1	2	3	4	5	6	7	8	9	10	11	12
Dorsal view	1	15			15									
	2	15				15								
	3	15				15								
	4	15					15							
	5	15						15						
	6	15							15					
	7	15								15				
	Adult	15											15	
Ventral view	1	15			15									
	2	15				15								
	3	15					15							
	4	15						15						
	5	15							15					
	6	15								15				
	7	15									15			
	Adult	15												15

Definition of instar age.
First instar : 3.5-5.5 mm (4.3±0.623), Second instar: 4.5-6.5 mm (5.4±0.767), Third instar: 5.5-7.5 mm (6.5±0.598), Fourth instar: 7.5-13.0 mm (8.7±1.613), Fifth instar: 7.0-16.5 mm (11.6±2.941), Sixth instar: 15.0-19.0 mm (16.6±1.271), Seventh instar: 16.0-18.0 mm (17.1±0.617), Adult: 16.5-19.0 mm (17.5±0.862).

Table 2. The number of cercal segments in female *Pycnoscelus indicus*

Instar		No, of insects	Number of cercal segments											
			1	2	3	4	5	6	7	8	9	10	11	12
Dorsal view	1	15			15									
	2	15				15								
	3	15				15								
	4	15					15							
	5	15						15						
	6	15							14	1				
	7	15								15				
	Adult	15									13	2		
Ventral view	1	15			15									
	2	15				15								
	3	15					15							
	4	15						15						
	5	15							15					
	6	15								15				
	7	15									15			
	Adult	15										13	2	

Definition of instar age.
First instar : 3.5-4.5 mm (4.1±0.530), Second instar: 4.5-6.0 mm (5.2±0.649), Third instar: 5.5-7.0 mm (6.1±0.471), Fourth instar: 7.5-15.0 mm (9.3±1.971), Fifth instar: 9.0-16.0 mm (12.1±2.271), Sixth instar: 16.5-20.5 mm (18.3±1.318), Seventh instar: 17.0-22.0 mm (19.2±1.646), Adult: 17.5-21.5 mm (19.7±0.902).

Table 3. The relation of the instar age, body length and sex to the type of sternite forms in the growth of *Pycnoscelus indicus*

Instar	Male				Female			
	Type of sternites*	No. of insects	Length (mm)	Average± S.D. (mm)	Type of sternites**	No. of insects	Langth (mm)	Average± S.D. (mm)
1	A	15	3.5-5.5	4.3±0.623	A	15	3.5-4.5	4.1±0.530
2	A	15	4.5-6.5	5.4±0.767	A	15	4.5-6.0	5.2±0.649
3	A	15	5.5-7.5	6.5±0.598	B	15	5.5-7.0	6.1±0.471
4	A-B	15	7.5-13.0	8.7±1.613	B	15	7.5-15.0	9.3±1.971
5	A-B	15	7.0-16.5	11.6±2.941	B-C	15	9.0-16.0	12.1±2.271
6	B	15	15.0-19.0	16.6±1.271	C-D	15	16.5-20.5	18.3±1.318
7	B	15	16.0-18.0	17.1±0.617	D	15	17.0-22.0	19.2±1.646
Adult	C	15	16.5-19.0	17.7±0.862	D	15	17.5-21.5	19.7±0.902

*: With reference to Fig. 1.
**: With reference to Fig. 2.

In our study on *P. indicus*, it was observed that among the 1st to 6th stage instar nymph, only the female has a notch at the central posterior edge of the 9th sternite. This notch is not seen in the male nymph. In the female after the 7th stage instar nymph, the 8th and 9th sternites had degenerated but these sternites were still conspicuous in the male. Thus, male and female adult sexual differentiation could be performed based on the number of sternite. This observation conform to that of *B. orientalis*, *S. longipalpa* and *P. fuliginosa* (Qadri, 1938; Hafez & Afifi, 1956; Saito & Hayashi, 1973)

In the 1st to last stage male nymph of *P. fuliginosa*, the 7th, 8th and 9th sternite were wholly conspicuous, but in the 1st to 3rd stage male nymph of *P. indicus*, the 8th sternite was covered over by the 7th sternite, with only a small portion of its tip could be seen.

On the contrary, in the 1st stage female nymph of *P. fuliginosa*, the 7th, 8th and 9th sternite were wholly conspicuous, but in the 1st stage female nymph of *P. indicus*, the 8th sternite was covered over by the 7th sternite, with only a small portion of its tip could be seen. In the mid-stage till the last stage female nymph of *P. fuliginosa*, the 9th sternite posterior periphery was covered over by the 7th sternite, and could not be observed from the outside. Similarly, the 8th sternite was also covered over by the 7th sternite and thus could not be seen.

In the mid stage (3rd and 4th stage) female nymph of *P. indicus*, no difference was noted between that of the 1st stage nymph. However, in the 6th stage female nymph, the 9th sternite central posterior edge notch became narrower and deeper, reaching to near the base of the 7th sternite, as though cutting through 8th sternite into the left and right halves. Furthermore, in the 7th stage female nymph, the 8th and 9th sternites were covered over by the 7th sternite, resulting in the hinderance of the observation (Saito & Hayashi. 1973). Moreover, Asahina (1991) reported that male adult of *P. indicus*, can be differentiated from the female by identifying the former as having 7 abdominal segments and the latter with 9 segments. Thus, our results supported and provided the reason for that report.

It has been reported that there were 3 cercal segments, as viewed from the dorsal and ventral aspects, in the 1st stage male and female nymph of both species of *B. germanica* and *P. indicus* (Hasegawa, 1977; Saito 1986). However, in the 2nd stage nymph of *B. germanica*, there were 6 cercal segments, while in that of *P. indicus*, there were only 4.

In the 3rd stage nymph of *B. germanica*, 7 cercal segments could be seen both dorsally and ventrally, while in that of *P. indicus*, 4 cercal segments could be seen dorsally but 5 could from the ventral view.

From the 4th to the final stage nymph of both species of *B. germanica* and *P. indicus*, the cercal segment increased by one for each of the stages (Hasegawa, 1977; Saito 1986). For *B. germanica*, since the dorsal view of the cercal segment number was found to be constant, it could be used for determining the stage of the nymph. However, this criterion on the cercal segment cannot be applied for the ventral view because of the greater variation in the number of the cercal segments (Hasegawa, 1977; Saito 1986). Nevertheless, the number of cercal segments from the nymph to the adult in *P. indicus*, were constant and can be used for determining the developmental stages. This criterion cannot be used for the female nymph of *B. germanica* because the number of cercal segments of the 5th and 6th stage nymph overlap with each other.

No difference in the body length of 1st to 5th stage male and female nymphs of *P. indicus*, were observed (t test; $p>0.05$. However, from 6th stage nymph onwards, the female nymph became larger than the male nymph ($p<0.05$). This indicates that this parameter can be considered for use in sexual differentiation from the 6th stage nymph onwards. Such observation has not been reported for *P. fuliginosa* and *B. germanica*. Thus, our study has demonstrated that it is possible to differentiate the sex of the Indian cockroach, *P. indicus*, at different developmental stages.

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