Immature Stages and Reproductive Characteristics of the Lizard Tick, *Ixodes asanumai* (Acarina: Ixodidae)

Fumio HAYASHI and Masami HASEGAWA

*Department of Biology, Faculty of Science, Tokyo Metropolitan University,
Setagaya-ku, Tokyo 158, Japan*

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We reared all stages of *Ixodes asanumai* (Acarina: Ixodidae), a tick parasitic on the lizard *Eumeces okadae* in Miyake-jima, Izu Islands and described the morphological characteristics of its larva and nymph revealed by scanning electron microscope observation. In addition, body size, weight, and life cycle parameters were measured under the laboratory condition. The number of eggs was positively correlated with the body weight of engorged female. The number of eggs produced per mg body weight and the percentage of total egg weight to the body weight were calculated to be 11.1 and 49.4%, respectively.

INTRODUCTION

KITAOKA (1973) described *Ixodes asanumai* (Acarina: Ixodidae) as a new species based on the characteristics of adult specimens collected mainly by the flagging method in Sukomo-banare, Amami Islands. He also described that a semi-engorged female was collected from the lizard, *Takydromus tachydromoides*¹ in Miyake-jima, Izu Islands. However, there is no exact record on the larvae and nymphs of this species.

On the ticks parasitic on lizards in Japan, FUJITA and TAKADA (1978) reported that the larvae and nymphs of *I. nipponensis* were found on the lizard *T. tachydromoides*, but no adult was found on it. According to KITAOKA and SAITO (1967), *I. nipponensis* has a wide host range including cattle, horse, dog, badger, wild hare, weasels, and man in the case of adult stage, and small rodents and birds in immature stages. According to these literatures, it is considered that *I. asanumai* is unique among the ticks belonging to the genus *Ixodes*, because it is parasitic on the lizard *Eumeces okadae* in adult stage. Therefore, it is interesting to investigate the host-parasite relationship between this tick and *E. okadae*.

In this study, we reared all stages of *I. asanumai* on the lizard *E. okadae* in the laboratory and examined some reproductive characteristics of this species.

MATERIALS AND METHODS

Three engorged females dropped from the lizards, *E. okadae* which were collected from Miyake-jima, Izu Islands in May 1981, oviposited and the obtained eggs were

¹ Since it is known that *Takydromus tachydromoides* does not distribute in Miyake-jima, KITAOKA probably described erroneously *Eumeces okadae* as the former.

reared up to adult stage by using *E. okadae* as a host. An another engorged female dropped in May 1980 and 13 females in April 1982 oviposited and the larvae hatched from the obtained eggs. They were also collected from Miyake-jima.

An additional datum on oviposition was obtained from 11 females dropped from *E. okadae* which were collected from Aoga-shima, Izu Islands in May 1982.

Body length, body width, total length of each developmental stage were measured on living ticks by an ocular micrometer. Body length means the length from tips of scapulae to posterior body margin and total length from the tip of capitulum to posterior body margin. Setaceous length of larvae and nymphs was measured on the specimens mounted on slides. These measurements were made on at least ten specimens.

Detail observations on the morphology were conducted by scanning electron microscope (JEOL, JSM-25SII). Ticks killed by 70% ethanol, were dried at 50°C for 3 hr after keeping in room temperature for 1 hr. Then these specimens were coated with Au. An unfed female collected on *E. okadae* of Miyake-jima in May 1981, was also added to SEM observation.

During feeding experiments of this ticks, the host lizards were freely allowed to bask in the daytime.

RESULTS AND DISCUSSION

Species identification of experimental materials

Figures 3–16 show the adult of *I. asanumai*. As mentioned by KITAOKA (1973), this species was distinguished from other species of *Ixodes* by the following characteristics, (1) narrower clavate palpi (Figs. 5 and 11), (2) stout retrograde horn-like auriculae of the female (Fig. 13), (3) hypostome with 4/4 denticular formula of the female (Fig. 14), and (4) longer setae beside the HALLER's organ of the male (Fig. 9). KITAOKA also pointed out its pale yellowish brown body-color, but in this study, both living and preserved ticks in alcohol were not yellowish. This difference might be attributed to the geographical color variation between Amami and Izu Islands.

Fig. 1. Larva of *Ixodes asanumai*. A: dorsal view, B: ventral view.

Fig. 2. Nymph of *Ixodes asanumai*. A: dorsal view, B: ventral view.

Figs. 3–9. Male of *Ixodes asanumai* by SEM. 3: dorsal view, 4: ventral view, 5: capitulum (dorsal view), 6: coxae, 7: capitulum (ventral view), 8: hypostome, 9: HALLER's organ.

Description on immature stages

Larva (Figs. 1 and 17–24): Body was subcircular, widest near midlength. The length of four pairs of central dorsal setae (abbreviated as Cd and the unit was shown in μm with ranges in parentheses) was as follows; $\text{Cd}_1=32$ (29–33), $\text{Cd}_2=33$ (26–35), $\text{Cd}_3=40$ (37–42), and $\text{Cd}_4=41$ (37–45). Of ten examined materials, six had four pairs of Cd, but three lacked Cd_4 , one lacked both Cd_3 and Cd_4 . The length of seven

Figs. 10–16. Female of *Ixodes asanumai* by SEM. 10: dorsal view, 11: capitulum (dorsal view), 12: capitulum (ventral view), 13: auricula, 14: hypostome, 15: coxae, 16: HALLER's organ.

Figs. 17–24. Larva of *Ixodes asanumai* by SEM. 17: dorsal view, 18: ventral view, 19: capitulum (ventral view), 20: scutal and marginal dorsal setae, 21: capitulum (dorsal view), 22: coxae, 23: HALLER's organ, 24: ventral seta.

Figs. 25–32. Nymph of *Ixodes asanumai* by SEM. 25: dorsal view, 26: ventral view, 27: capitulum (ventral view), 28: hypostome, 29: capitulum (dorsal view), 30: coxae, 31: HALLER's organ, 32: dorsal seta.

pairs of marginal dorsal setae (Md in μm) was $\text{Md}_1=35$ (33–36), $\text{Md}_2=39$ (35–42), $\text{Md}_3=48$ (45–53), $\text{Md}_4=50$ (45–53), $\text{Md}_5=45$ (45–45), $\text{Md}_6=41$ (39–42), and $\text{Md}_7=41$ (37–45). The length of a paired supplementary dorsal setae (Sd in μm) was $\text{Sd}_1=40$ (36–44). Scutum was widest near its midlength. Five pairs of scutal setae (Sc) were rather short, 9 (8–10) μm . Ventral setae were 13 pairs (13–43 μm). Anal setae were one pair and the length was 19 (17–20) μm . As shown in Fig. 22, coxa-I had an external spur and an internal spur, coxa-II and -III had an external spur. HALLER's organ was shown in Fig. 23. Auriculae were triangular spurs (Fig. 19). Hypostome had a rounded apex and nine or ten denticles in file-1, seven or eight in file-2, and four or five in file-3.

Nymph (Figs. 2 and 25–32): Body was oval. Total number of dorsal setae was 38 to 49 with the length of 7 to 56 μm . Scutal setae were 10 to 16 with the length of 12 (10–15) μm . Ventral setae were 44 to 54 with the length of 12 to 26 μm . The length of three pairs of anal setae was 26 (21–29) μm . As shown in Fig. 30, coxa-I had an external spur and an internal spur, coxa-II, -III, and -IV had an external spur. HALLER's organ was shown in Fig. 31. Capitulum had distinct cornua which extended backward (Fig. 29). Auriculae were large and pointed sharply towards the back (Fig. 27). Hypostome had 3/3 denticular formula as shown in Fig. 28.

Distinction from other species

Immature stages of *I. asanumai* closely resembled those of *I. nipponensis* described by KITAOKA and SAITO (1967), but could be distinguished by the following characteristics,

(1) shorter scutal setae, (2) more pointed auriculae, and (3) smaller body size. The length of scutal setae in larvae and nymphs was 9 (8–10) and 12 (10–15) μm in *I. asanumai*, respectively, as compared with 17 (13–23) and 12–28 μm in *I. nipponensis*. In *I. asanumai*, body length and width were 0.55 and 0.44 mm in larvae, and 1.00 and 0.69 mm in nymphs, while in *I. nipponensis*, they were 0.57 and 0.48 mm, and 1.15 and 0.82 mm, respectively. In addition, the nymph is easily distinguished from *I. nipponensis* because only the latter possesses a very sharply pointed hypostome.

Life cycle in the laboratory

Egg: In the case of specimens obtained from Miyake-jima, a minor axis of eggs after about 20 days from laying at $20\pm 1^\circ\text{C}$ was 0.408 ± 0.019 mm (mean $\pm$ S.D.) and a major axis 0.529 ± 0.017 mm. Fresh egg weight was 0.044 ± 0.003 mg. These values were quite similar to those observed in Aoga-shima, that is 0.404 ± 0.011 mm, 0.519 ± 0.017 mm, 0.046 ± 0.003 mg, respectively. Egg period was 33 to 34 days at $23\text{--}27^\circ\text{C}$ and 41 to 49 days at $20\pm 1^\circ\text{C}$.

Larva: Body length, body width, total length, and the fresh weight of unfed larvae were 0.55 ± 0.01 mm, 0.44 ± 0.00 mm, 0.70 ± 0.01 mm, and 0.022 ± 0.003 mg, respectively. The average feeding period on *E. okadae* was 6 days with a range of 5–7 days at $25\text{--}28^\circ\text{C}$, and 5 (4–7) days at $28\text{--}31^\circ\text{C}$. Body length, body width, total length, and the fresh weight of engorged larvae were 1.09 ± 0.04 mm, 0.81 ± 0.02 mm, 1.24 ± 0.01 mm, and 0.30 ± 0.01 mg, respectively. Postparasitic period was 16 to 18 days at $26\text{--}30^\circ\text{C}$ and 20 to 22 days at $25\pm 1^\circ\text{C}$.

Nymph: Body length, body width, total length, and the fresh weight of unfed nymphs were 1.00 ± 0.01 mm, 0.69 ± 0.01 mm, 1.30 ± 0.02 mm, and 0.13 ± 0.00 mg, respectively. The average feeding period on *E. okadae* was 6 (4–7) days at $28\text{--}31^\circ\text{C}$. Body length, body width, total length, and the fresh weight of engorged nymphs were 2.03 ± 0.12 mm, 1.55 ± 0.04 mm, 2.35 ± 0.09 mm, and 2.30 ± 0.26 mg, respectively. Postparasitic period to molt into males was 28 to 32 days at $25\pm 1^\circ\text{C}$ and 45 to 46 days at $20\pm 1^\circ\text{C}$. That into females was 29 to 33 and 48 to 51 days, respectively.

Male: Body length, body width, total length, and the fresh weight of unfed males were 1.84 ± 0.03 mm, 1.32 ± 0.03 mm, 2.16 ± 0.09 mm, and 0.98 ± 0.13 mg, respectively. Males did not feed.

Female: Body length, body width, total length, and the fresh weight of unfed females were 2.03 ± 0.06 mm, 1.42 ± 0.07 mm, 2.69 ± 0.11 mm, and 1.03 ± 0.15 mg, respectively. Feeding period on *E. okadae* was about 10 days (maximum, 14 days) at $23\text{--}27^\circ\text{C}$. Body length, body width, and the fresh weight of engorged females were very different among the individuals as shown in Table 1. The average preoviposition period was 7 (6–8) days at $23\text{--}27^\circ\text{C}$ and 9 (7–10) days at $20\pm 1^\circ\text{C}$. Oviposition period was 16 to 20 days at $23\text{--}27^\circ\text{C}$ and 20 to 27 days at $20\pm 1^\circ\text{C}$.

Figure 33 summarizes the growth pattern of *I. asanumai* from the relation between the body length and its weight. In this graph, two distinct phases, that is unfed and engorged ones, are easily separated. The equations calculated for unfed phase and engorged one were

$$W=0.134 X^{3.036} \quad n=4, r=0.998$$

and

$$W=0.225 X^{3.253} \quad n=15, r=0.997,$$

respectively. In both phases, the body weight (W) was expressed by about 3 power

Table 1. Oviposition of individual engorged female

Island	Body length (mm)	Body width (mm)	Body weight (X mg fresh wt.)	Total weight of eggs (Y mg fresh wt.)	$\frac{Y}{X} \times 100$ (%)	No. of eggs	No. of eggs per mg body weight
Miyake-jima	4.45	3.15	20.08	3.06	15.2	70	3.5
	4.95	4.25	47.81	22.97	48.0	547	11.4
	5.20	4.30	47.89	27.38	57.2	685	14.3
	5.00	4.50	53.48	29.53	55.2	671	12.5
	6.05	4.70	73.52	26.85	36.5	624	8.5
	6.00	4.48	74.54	26.39	35.4	602	8.1
	6.25	4.95	82.63	37.08	44.9	882	10.7
	6.30	5.10	85.02	47.76	56.2	955	11.2
	6.20	5.00	86.79	52.06	60.0	1001	11.5
	6.30	5.10	92.66	58.63	63.3	1303	14.1
	6.30	5.30	96.99	58.96	60.8	1371	14.1
	6.68	5.45	105.89	(42.75)	(40.4)	(972)	(9.2)
	6.60	5.40	107.58	64.99	60.4	1413	13.1
			Mean $\pm$ S.D.		49.4 $\pm$ 14.3	11.1 $\pm$ 3.1	
Aoga-shima	4.89	3.54	28.23	14.59	51.7	331	11.7
	4.43	3.45	28.56	11.47	40.2	280	9.8
	4.76	3.75	32.67	14.62	44.8	327	10.0
	4.85	4.02	38.60	11.59	30.0	247	6.4
	5.51	4.25	51.28	18.67	36.4	413	8.1
	5.84	4.40	58.07	27.58	47.5	648	11.2
	5.83	4.42	59.00	30.91	52.4	631	10.7
	5.81	4.50	60.07	30.20	50.3	739	12.3
	5.75	4.52	62.04	29.91	48.2	610	9.8
	6.00	4.42	62.32	33.38	53.6	762	12.2
	6.60	4.92	86.81	46.10	53.1	922	10.6
			Mean $\pm$ S.D.		46.2 $\pm$ 7.7	10.3 $\pm$ 1.8	

Parentheses indicate an abnormal death in the middle of oviposition and this female is excluded in calculating the mean value.

of the body length (X).

The larva acquires a great increase in weight, 13.6 times, when once attached on an appropriate host. In the case of nymphs, its body weight increases 17.7 times by engorging. In the females, the increase in body weight is conspicuous and comes up to 75.0 times on the average (maximum, 104.4 times). Thus, the tick must experience such a great change in its body weight three times in order to accomplish the life cycle.

Since whether or not the ticks are able to attach on the host lizards is quite indefinite, it is very difficult to predict the actual life cycle of this tick under field conditions.

Total weight and number of eggs

Total weight and number of eggs produced by respective females at $20 \pm 1^\circ\text{C}$ are shown in Table 1. The relationship between the number of eggs (Y) and the body weight (X) of engorged females was expressed by the following equation,

Fig. 33. Relationship between the body length and the weight of *I. asanumai* in Miyake-jima. A: unfed ($\circ$), $\log W = 3.036 \log X - 0.872$, $n = 4$, $r = 0.998$. B: engorged ($\bullet$), $\log W = 3.253 \log X - 0.648$, $n = 15$, $r = 0.997$. E: ($\times$), L, N, M, and F indicate egg, larva, nymph, male, and female, respectively.

Fig. 34. Relationship between the body weight of engorged female and the number of eggs produced. Each solid and open circles indicate an individual female collected from Miyake-jima and Aoga-shima, respectively. $Y = 13.8 X - 162.5$, $n = 23$, $r = 0.936$. A point in parenthesis indicates an abnormal death in the middle of oviposition and this female is excluded in calculation.

$$Y = 13.8 X - 162.5$$

$$n = 23, r = 0.936$$

as shown in Fig. 34. The similar relationship has been reported on *I. signatus* by ASANUMA and FUKUDA (1957), and other ixodid ticks except for *Ixodes* (see, DRUMMOND et al., 1971). The percentage of total egg weight to the body weight of engorged females was 49.4% in the case of specimens obtained from Miyake-jima and 46.2% in Aoga-shima. This difference was not significant ($t = 0.66$, $0.5 < p < 0.6$). The number of eggs produced per mg body weight of engorged females was calculated to be 11.1 in the case of Miyake-jima and 10.3 in Aoga-shima. This difference was not also significant ($t = 0.75$, $0.4 < p < 0.5$). The number of eggs per mg body weight of other *Ixodes* females was 5.6 ± 0.8 (mean $\pm$ S.D.) in *I. signatus* infesting a black tailed gull (ASANUMA and FUKUDA, 1957), and 14.3 ± 2.2 in *I. trianguliceps* infesting small mammals (RANDOLPH, 1975). This interspecific difference must have a relation to the percentage of total egg weight to the body weight and the weight of a single egg. The latter is probably connected closely to the size of larva at hatching. However, the available information on such a point is quite insufficient.

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