Appl. Entomol. Zool. 34 (1): 189-194 (1999)

Morphology and biology of a phytophagous ladybird beetle, *Epilachna pusillanima* (Coleoptera: Coccinellidae) newly recorded on Ishigaki Island, the Ryukyus

Susumu Nakano and Haruo Katakura[1]

Division of Life Science, Faculty of Commercial Sciences, Hiroshima Shudo University, Asaminami, Hiroshima 731-3195, Japan

[1] Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo, Hokkaido 060-0810, Japan

(Received 28 September 1998; Accepted 25 November 1998)

Abstract

The occurrence of *Epilachna pusillanima* was confirmed on Ishigaki Island, the Ryukyus, southernmost Japan, in June, 1997. This is the first record of the species from Japan. *E. pusillanima* infested two cucurbitaceous weeds, *Trichosanthes bracteata* and *Trichosanthes* sp. 1, but was not observed on cucurbitaceous crops, though it is known as a notorious pest of cucurbits in Southeast Asia. *E. pusillanima* is externally similar to another cucurbit feeder, *E. boisduvali*, which also occurs on Ishigaki Island, in that both have 12 spots on the elytra and similar body size. However, the two species are easily distinguished by 1) the structure of the genitalia of both sexes, 2) the color and maculation of prepupae and pupae, and 3) diet. Results of a mate choice experiment suggest that *E. pusillanima* and *E. boisduvali* are reproductively isolated by ethological isolation and/or gametic isolation.

Key words: *Epilachna pusillanima, Epilachna boisduvali, Trichosanthes* spp., Ryukyus

INTRODUCTION

Phytophagous ladybird beetles of the subfamily Epilachninae are most diversified in tropical and subtropical regions of the world (Dieke, 1947; Li and Cook, 1961; Liu, 1965; Gordon, 1975; Pang and Mao, 1979; Schaefer, 1983; Katakura et al., 1988), and only a scarce number are distributed in cooler regions (Sasaji, 1971; Richards, 1983; Li, 1993). Up to now, only nine species have been known in Japan (Sasaji, 1971; Kurosawa et al., 1985; Katakura, 1988; Fujiyama et al., 1998). In 1997, Nakano collected another species of epilachnine beetles on Ishigaki Island, the Ryukyus, and the species was identified by Katakura as *Epilachna pusillanima* Mulsant, 1850 (for the application of this species name to the present species, see Booth and Pope, 1989; Shirai and Katakura, 1999), which is known to be a serious pest of cucurbitaceous crops in Southeast Asia (Katakura et al., 1988, 1992; Nakano and Abbas, 1994; Shirai and Katakura, 1999).

In the present paper we will describe the morphological features, host plant utilization, food preference and larval duration of the Ishigaki population of *E. pusillanima*. The results of a mate choice experiment between *E. pusillanima* and *E. boisduvali* Mulsant, 1850, another cucurbit-feeding epilachnine known to occur in the Ryukyus, are also given.

MORPHOLOGICAL FEATURES

The morphology of *E. pusillanima* was described in detail by Li and Cook (1961), Dieke (1947 as *E. dentulata parvinotata* Dieke, 1947; see Li and Cook, 1961), Kapur (1963, 1967 as *E. dodecastigma* Wiedemann, 1823) and Katakura et al. (1988, as *E. dodecastigma*). All the Ishigaki specimens that we examined possessed 12 spots on the elytra, although *E. pusillanima* in Southeast Asia has 12, 14 or more spots (Katakura et al., 1988; Shirai and Katakura, 1999).

On Ishigaki Island, *E. pusillanima* might be confused with sympatric *E. boisduvali*, because both species possess 12 spots on the elytra (Fig. 1) and feed on cucurbits. As shown in Fig. 2, however, the two species have quite different structures of the genitalia of both sexes. The

two species are also different in 1) body proportion (*E. pusillanima* appears more circular when viewed from above; Fig. 1), 2) adult body color (ground color slightly more reddish in *E. pusillanima*; ventral side consistently brownish in *E. pusillanima*, whereas the ventral side of thoracic segments and the first and last visible abdominal segments darker than the rest in *E. boisduvali*), 3) the color of scoli (i.e., long and branching processes of body wall) of final

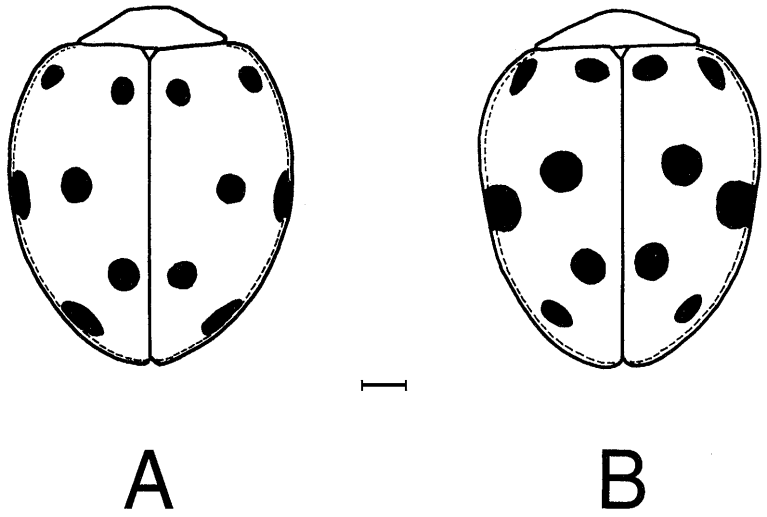

Fig. 1. *Epilachna pusillanima* (A) and *E. boisduvali* (B). Bar scales, 1 mm.

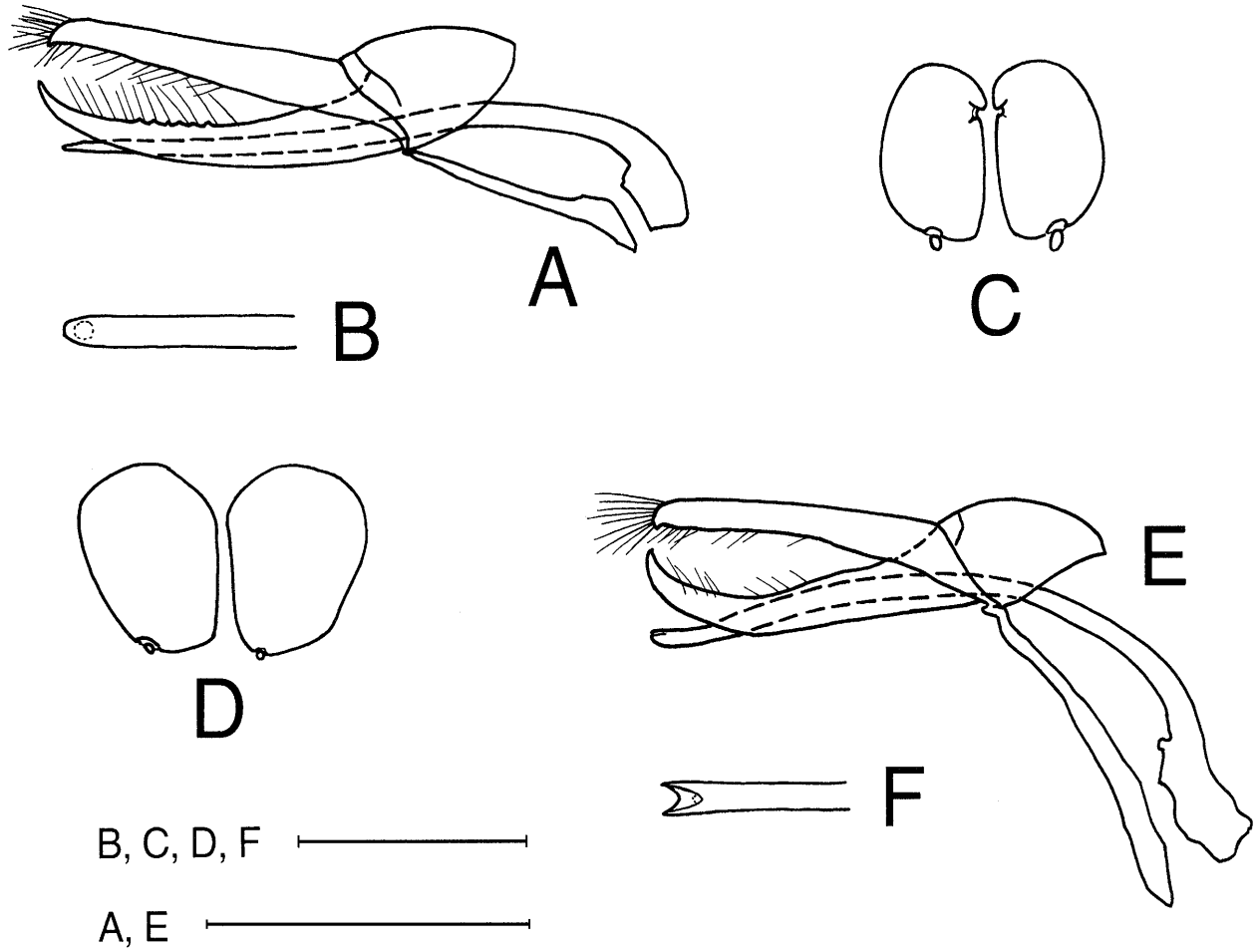

Fig. 2. Male and female genitalia of *Epilachna pusillanima* (A–C) and *E. boisduvali* (D–F). A and E, male genitalia, B and F, tip of sipho from above, C and D, female genitalia (coxites). Bar scales, 1 mm.

(fourth) instar larvae (black from the base to the top in *E. pusillanima*, white at the basal half and black at the proximal half in *E. boisduvali*), and 4) the color and maculation of prepupae and pupae (whitish yellow with many dark parts in *E. pusillanima*, yellowish green with only four small black spots on dorsum in *E. boisduvali*). Furthermore, *E. pusillanima* is distinctly different from *E. boisduvali* in that it does not accept *Diplocyclos palmatus*, one of the host plants of *E. boisduvali* (see below).

BIOLOGY

Materials and methods

Food plant utilization in the field was studied on Ishigaki Island in late June and late October, 1997 by checking seven species of cucurbitaceous plants consisting of three crops and four wild weeds: squash (*Cucurbita moschata*), wax gourd (*Benincasa cerifera*), loofah (*Luffa cylindrica*), *Diplocyclos palmatus, Trichosanthes bracteata, Trichosanthes ishigakiensis*, and *Trichosanthes* sp. 1.

In addition, the following three experiments were made at the Hiroshima Shudo University laboratory. All laboratory experiments, maintenance of adults, and rearing of larvae were carried out under a constant temperature and day length (22.5°C, 16L8D).

1) Acceptability of various plants under laboratory conditions

A preliminary food acceptability test was carried out using only one adult (female) of *E. pusillanima* in late October, 1997. Eight cucurbit species were used: *Cucurbita moschata, Diplocyclos palmatus, Trichosanthes bracteata, Trichosanthes ishigakiensis*, and *Trichosanthes* sp. 1 collected on Ishigaki Island, and *Trichosanthes kirilowii, Sicyos angulatus*, and *Melothria japonica* collected in Hiroshima. A piece of leaf of one plant species was given for one day and the plant species was changed every day. The leaf area consumed was checked every day and rated as follows: − no feeding, + consumed area up to 100 mm^2, ++ consumed area up to 400 mm^2, +++ consumed area more than 400 mm^2. The detailed method of this test is described in Nakano and Abbas (1994).

2) Duration required from oviposition to adult eclosion

Second instar larvae of *E. pusillanima* were collected on *Trichosanthes bracteata* in late June, 1997. They were reared in the laboratory on fresh picked leaves of an alternative food plant, *Sicyos angulatus*, because no original host plants are available in Hiroshima, where the experiments were performed. Newly emerged adults were fed leaves of *Sicyos angulatus* and/or *Cucurbita moschata* until maturation. *Sicyos angulatus* and *Cucurbita moschata* are known as the most favored food plants for several species of cucurbit-feeding *Epilachna* in Japan and Indonesia (Nakano, unpubl.). Eggs deposited by these adults were isolated and hatched larvae were reared on fresh leaves of *Sicyos angulatus* in a transparent plastic case (10 × 12 × 4.4 cm). The dates of hatching and larval molting were recorded daily to determine the time from oviposition to the adult eclosion.

3) Mate choice experiment

On Ishigaki Island, *E. pusillanima* is sympatric with another species of cucurbit-feeding *Epilachna, E. boisduvali*. Since *E. pusillanima* and *E. boisduvali* may meet on the same host

Table 1. Food plant utilization of *E. pusillanima* in the field and its food acceptance under the laboratory condition. The intensity of food acceptance is shown by the leaf area consumed by one adult per one day.

Plant species	In the field[a]	In the laboratory[b]
Crops		
Cucurbita moschata	−	++
Benincasa cerifera	−	
Luffa cylindrica	−	
Weeds		
Diplocyclos palmatus	−	−
Trichosanthes bracteata	+	++
T. ishigakiensis	−	+++
T. sp. 1	+	+
T. kirilowii		+++
Sicyos angulatus		+++
Melothria japonica		+++

[a] −: not utilized, +: utilized.
[b] −: no feeding, +: consumed area up to 100 mm^2, ++: consumed area up to 400 mm^2, +++: consumed area more than 400 mm^2.

Table 2. Duration of successive immature stages (in days) of *E. pusillanima* reared under 22.5°C, 16L8D. Numerals in parentheses indicate the numbers of individuals examined.

Egg	L1	L2	L3	L4	Prepupa	Pupa	Total (range)
6.3	6.0	3.9	4.3	5.9	2.9	5.7	35.0 (33–36)
(39)	(35)	(35)	(35)	(31)	(28)	(27)	

plant, we examined the degree of reproductive isolation by a mate choice experiment. Third instar larvae of *E. pusillanima* (abbreviated as Ep) and second instar larvae of *E. boisduvali* (Eb) were collected on *Trichosanthes bracteata* and *Diplocyclos palmatus* respectively on Ishigaki Island in late October, 1997. They were brought to the laboratory and reared separately on *Sicyos angulatus*. They were sexed immediately after emergence, and females and males were kept separately. About two weeks after emergence, one virgin female from each of the two species and one unmated male from one of the two species were put together and kept in a trio in a plastic case (10×12×4.4 cm). After keeping five trios each of (Ep♀+Eb♀)×Ep♂ and (Ep♀+Eb♀)×Eb♂ combinations for ten days, all females were dissected and the number of retained spermatozoa was estimated by counting a fraction of sperm fluid using a haemocytometer (cf. Katakura, 1986).

Results and notes

1) Food plant utilization in the field and acceptability of various cucurbits under laboratory conditions

E. pusillanima was collected on only two species of *Trichosanthes*, *T. bracteata* and *T.* sp. 1 (Table 1). Moreover, its occurrence was confirmed at only the following two sites though the two *Trichosanthes* weeds were distributed all over the island: Site A at the foot of Mt. Banna, where one plant each of *Trichosanthes bracteata* and *Trichosanthes* sp. 1 were intertwined with one another; Site B near Kannonzaki where only one plant of *Trichosanthes bracteata* grew.

In the food acceptability test *E. pusillanima* accepted *Cucurbita moschata*, four *Trichosanthes* species, *Sicyos angulatus* and *Melothria japonica*, but did not accept *Diplocyclos palmatus*, one of the host plants of *E. boisduvali* (Table 1).

All stages of *E. pusillanima* were found on *Trichosanthes bracteata* at sites A and B, whereas only one individual each of the second and fourth instar larvae were found on *Trichosanthes* sp. 1 at site A. Since the *Trichosanthes* sp. 1 plant was intertwined with *Trichosanthes bracteata* that was heavily damaged by *E. pusillanima*, the larvae may have moved from *Trichosanthes bracteata* to *Trichosanthes* sp. 1. Thus, *Trichosanthes* sp. 1 may be a subsidiary food plant for *E. pusillanima*. The fact that this plant was not preferred by a single adult under laboratory conditions supports this view (Table 1), although food acceptability tests using more individuals will be necessary.

2) Duration required from oviposition to adult eclosion and sex ratio of newly emerged adults

Table 2 shows the duration of successive immature stages of *E. pusillanima*. The total duration required for the completion of embryonic and larval growth was 35.0 days. Newly emerged adults (15 males, 12 females) showed no significant deviation from the expected 1 : 1

Table 3. Results of mate choice experiment between *E. pusillanima* (Ep) and *E. boisduvali* (Eb). Estimated numbers of the spermatozoa preserved in conspecific and heterospecific females were counted 10 days after the start of experiment.

Combination	Estimated number of spermatozoa ($\times 10^4$)[a] preserved in	
	Conspecific female	Heterospecific female
(Ep♀+Eb♀)×Ep♂	29.6±9.9 (20.1–44.8)	0
(Ep♀+Eb♀)×Eb♂	0	44.6±10.2 (35.6–61.3)

[a] Average±SD (range).

sex ratio ($p=0.351$, binomial test). The larval periods of *E. pusillanima* were similar to those of *E. boisduvali* (32–34 days; Nakano unpubl.).

3) Mate choice experiment

The result of the mate choice experiment was straightforward (Table 3). In either combination, conspecific females possessed hundreds of thousands of spermatozoa, whereas no stored sperm was found in heterospecific females. The results indicated that reproductive isolation between *E. pusillanima* and *E. boisduvali* is complete, owing to ethological isolation and/or gametic isolation. Even if they meet on the same *Trichosanthes* plant, therefore, the production of hybrids as demonstrated for certain species of Japanese *Epilachna* (Nakano, unpubl.; Takita and Katakura, unpubl.; also see Katakura, 1997) will not occur at all.

REMARKS

It is not yet certain whether *E. pusillanima* is native to the Ryukyus or it was introduced recently either by human activity or by their own effort. *E. pusillanima* is widespread in Southeast Asia, including Indonesia, Indochina, India, China, Philippines, and Taiwan (sometimes under the name of *E. dodecastigma* or other names; Dieke, 1947; Katakura et al., 1988), where it is known as a serious pest of cucurbitaceous crops such as squash (*Cucurbita moschata*), wax gourd (*Benincasa cerifera*), cucumber (*Cucumis sativus*), loofah (*Luffa acutangula*), watermelon (*Citrullus lanatus*), and chayote (*Sechium edule*) (Katakura et al., 1988, 1992; Nakano and Abbas, 1994; Shirai and Katakura, 1999).

It is therefore important to gather information on the biology of this species in the Ryukyus from the economical viewpoint. At present, the presence of *E. pusillanima* on Ishigaki Island seems very restrictive. It is virtually confined to only two species of wild cucurbitaceous weed, *Trichosanthes bracteata* and *T.* sp. 1 (Table 1). However, attention must be paid to whether *E. pusillanima* stays stenophagous or extends its food repertoire. The result of the food acceptability test (Table 1) suggests that *E. pusillanima* may become a serious pest of crops such as *Cucurbita moschata* in Japan as well.

ACKNOWLEDGEMENTS

This study was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (No. 09440258).

REFERENCES

Booth, R. G. and R. D. Pope (1989) A review of the type material of Coccinellidae (Coleoptera) described by F. W. Hope and by E. Mulsant in the Hope Entomological Collections, Oxford. *Ent. Scand.* 23: 343–370.

Dieke, G. H. (1947) Ladybeetles of the genus *Epilachna* (sens. lat.) in Asia, Europe, and Australia. *Smithsonian Miscellaneous Collections* 106: 1–183.

Fujiyama, N., H. Katakura and Y. Shirai (1998) Report of the Mexican bean beetle, *Epilachna varivestis* (Coleoptera: Coccinellidae) in Japan. *Appl. Entomol. Zool.* 33: 327–331.

Gordon, R. D. (1975) A revision of the Epilachninae of the Western Hemisphere (Coleoptera: Coccinellidae). *Technical Bull. USDA* 1493: 1–409.

Kapur, A. P. (1963) The Coccinellidae of the third Mount Everest Expedition, 1924 (Coleoptera). *Bull. Br. Mus. Nat. Hist. (Ent.)* 14:1–48.

Kapur, A. P. (1967) The Coccinellidae (Coleoptera) of the Andamans. *Proc. Nat. Inst. Sci. India* 32B: 148–189.

Katakura, H. (1986) Evidence for the incapacitation of heterospecific sperm in the female genital tract in a pair of closely related ladybirds (Insecta, Coleoptera, Coccinellidae). *Zool. Sci.* 3: 115–121.

Katakura, H. (1988) *Insects in Japan.* Vol. 10. *Epilachna vigintioctomaculata Complex.* Bun-ichi Sôgo Press, Tokyo. 159 pp. (in Japanese).

Katakura, H. (1997) Species of *Epilachna* ladybird beetles. *Zool. Sci.* 14: 869–881.

Katakura, H., I. Abbas, K. Nakamura and H. Sasaji (1988) Records of epilachnine crop pests (Coleoptera, Coccinellidae) in Sumatera Barat, Sumatra, Indonesia. *Kontyû* 56: 281–297.

Katakura, H., S. Nakano, S. Kahono, I. Abbas, W. A. Noerdjito and K. Nakamura (1992) Epilachnine ladybird beetles (Coleoptera, Coccinellidae) of West Sumatra and West Java. In *Evolutionary Biology and Population Dynamics of Herbivorous Ladybeetles in Indonesia* (K. Nakamura and H. Katakura eds.). Sapporo, pp. 2–21.

Kurosawa, Y., S. Hisamatsu and H. Sasaji (1985) *The Coleoptera of Japan in Color.* Volume 3. Hoikusha, Osaka. 501 pp. (in Japanese).

Li, C. S. (1993) Review of the Australian Epilachninae (Coleoptera: Coccinellidae). *J. Aust. Ent. Soc.* 32: 209–224.

Li, C. S. and E. F. Cook (1961) The Epilachninae of Taiwan (Col.: Coccinellidae). *Pacific Insects* 3: 31–91.

Liu, C. L. (1965) *Economic Insects of China, 5 Coleoptera-Coccinellidae.* Science Publisher, Peking. 101 pp. (in Chinese).

Nakano, S. and I. Abbas (1994) Reproductive isolation in four phytophagous ladybeetles (*Epilachna*, Coccinellidae, Coleoptera) in West Sumatra. *Tropics* 3: 121–129.

Pang, X. F. and J. L. Mao (1979) *Economic Insects of China, 14, Coleoptera, Coccinellidae (II).* Science Publisher, Peking. 170 pp. (in Chinese).

Richards, A. M. (1983) The *Epilachna vigintioctopunctata* complex (Coleoptera: Coccinellidae). *Int. J. Entomol.* 25: 11–41.

Sasaji, H. (1971) *Fauna Japonica Coccinellidae (Insecta: Coleop-*

tera). Academic Press, Tokyo. 340 pp.
Schaefer, P. W. (1983) Natural enemies and host plants of species in the Epilachninae (Coleoptera:Coccinellidae)—a world list. *Agric. Exp. Stn., Univ. Delaware, Bull.* 445: 1–42.
Shirai, Y. and H. Katakura (1999) Host plants of the phytophagous ladybird beetle, *Epilachna vigintioctopunctata* (Coleoptera: Coccinellidae), in Southeast Asia and Japan. *Appl. Entomol. Zool.* 34: 75–83.