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Tokuda, Makoto

Yukawa, Junichi

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**Morphological Features of the Mature Larva and Pupa of
Pseudasphondylia rokuharensis Monzen
(Diptera: Cecidomyiidae)***

Makoto TOKUDA

Entomological Laboratory, Graduate School of Bioresource and Bioenvironmental Sciences,
Kyushu University, Fukuoka, 812-8581 Japan

and

Junichi YUKAWA

Entomological Laboratory, Faculty of Agriculture,
Kyushu University, Fukuoka, 812-8581 Japan

Abstract. The mature larva of *Pseudasphondylia rokuharensis* is described, for the first time, and the pupa is redescribed based on specimens reared from fruit galls produced on *Viburnum dilatatum* (Caprifoliaceae). Immature stages of *P. rokuharensis* were compared with those of Japanese and exotic congeners. *Pseudasphondylia rokuharensis* appeared to be characterized by having spiracular rudiments on the larval metathorax, a larval sternal spatula with four pointed distal lobes, and relatively long stigmatal tubercles on the second to fourth pupal abdominal segments. These morphological features of immature stages indicated the close relation of *P. rokuharensis* to *P. matatabi* that is responsible for fruit galls on *Actinidia polygama* (Actinidiaceae).

Key words: Asphondyliini, Cecidomyiidae, gall midge, morphological features, *Pseudasphondylia rokuharensis*, *Viburnum dilatatum*.

Introduction

The genus *Pseudasphondylia* consists of five species, of which two are exotic species.

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Pseudasphondylia rauwolfiae Coutin, 1980 is responsible for flower galls on *Rauwolfia schumanniana* (Schl.) Boiteau (Apocynaceae) in New Caledonia (Coutin, 1980) and *Pseudasphondylia diospyri* Mo et Xu, 1999 produces stem galls on *Diospyros kaki* L. (Ebenaceae) in China (Mo & Xu, 1999). Remaining three are Japanese species (Yukawa, 1971; Yukawa, 1974; Yukawa & Masuda, 1996). *Pseudasphondylia rokuharensis* Monzen, 1955 produces fruit galls on *Viburnum dilatatum* Thunb. (Caprifoliaceae) (Fig. 1) (Monzen, 1929; Monzen, 1955), *Pseudasphondylia matatabi* (Yuasa et Kumazawa, 1938) is responsible for fruit galls on *Actinidia polygama* (Sieb. et Zucc.) Maxim. (Actinidiaceae) (Yuasa & Kumazawa, 1938), and *Pseudasphondylia neolitsea* Yukawa, 1974 forms leaf galls on *Neolitsea sericea* (Blume) Koidz. (Lauraceae) (Yukawa, 1974).

Pseudasphondylia rokuharensis is the type species of the genus *Pseudasphondylia* Monzen, 1955 and was originally described based on male and female specimens alone (Monzen, 1955). Later Yukawa (1971) added the description of pupa using the Monzen's specimens that had been kept under relatively poor conditions. However, the mature larva of this species was not described previously. In order to analyze phylogenetic relationship among genera of the tribe Asphondyliini, more detailed examinations of larval and pupal morphology are fundamentally necessary, because immature stages are frequently described and used for phylogenetic studies of galling cecidomyiids (e.g., Möhn, 1961; Roskam, 1979; Roskam & Zandee, 1992).

Recently we had an opportunity to collect galls of *P. rokuharensis* from Miyagi Prefecture, Northern Honshu, Japan and to rear them in the laboratory to obtain mature larvae and pupae. In this paper we describe, for the first time, the mature larva of *P. rokuharensis* and redescribe its pupal characters, particularly those that were not fully described in Yukawa (1971) due to the poor conditions of the Monzen's specimens.

Materials and Methods

Fruit galls on *Viburnum dilatatum* were collected on September 17, 2001 in Akiu-ohtaki, Akiu Town, Miyagi Prefecture and on September 18, 2001 in Nuruyu, Hanayama Village, Miyagi Prefecture. We immediately dissected some of these galls in the field and found that all larvae were in the first stadium. Then we tried to rear remaining galls in the laboratory to obtain mature larvae, because the first instar of this gall midge is known to mature rapidly in October (Yukawa & Masuda, 1996). The galls were kept in an incubator for three weeks under the conditions of L11:D13 at 15°C (following the natural day length and mean air temperature of mid-October in Miyagi Prefecture). After three-week incubation, the larvae matured in the galls.

In addition, we obtained mature larvae by dissecting some of galls that had been collected by Mr. Katsuo Goukon (Tohoku Gakuin University) on November 16, 2001 in

Miyatoko, Taiwa Town, Miyagi Prefecture. Because many galls were sent to us from him, we reared remaining galls (about 100 in number) under artificial conditions to obtain pupae earlier than usual season in early summer. The mature larvae were incubated for about four months under the conditions of L10:D14 at 5°C (following the natural day length and mean air temperature of mid-December in Miyagi Prefecture). On February 16, 2002, they were transferred into an incubator with the rearing conditions of L13:D11 at 15°C (following the natural day length and mean air temperature of mid-April in Miyagi Prefecture). During the incubation, the galls were kept in a plastic bag (40 x 25 cm in size) to maintain humidity. The galls were monitored everyday from February 16 to March 26 to examine the emergence of adults. To observe the development of the gall midge, 10 out of the 100 galls were randomly dissected on February 17, 22, 27, March 4, 9, and 14, 2002, respectively, until the first emergence of adult. We collected, for morphological study, pupal skins that had been left on the galls or in the plastic bag.

The mature larvae and pupae were preserved in 75% ethanol for morphological studies and in 99.5% acetone for observation by a scanning electron microscope (SEM) and for future DNA analysis. Some of the ethanol-preserved specimens were mounted on slides for microscopic study in Canada balsam using the techniques outlined in Gagné (1989). Drawings were made with the aid of a camera lucida. Some important structures of pupa were examined with a SEM (HITACHI-3000N). All specimens examined in this study are kept in the collection of Entomological Laboratory, Faculty of Agriculture, Kyushu University.

Pseudasphondylia rokuharensis Monzen

Pseudasphondylia rokuharensis Monzen, 1955; Yukawa, 1971.

Mature larva: Second antennal segment short, conical, about 15 µm in length, 1.5 times as long as basal width; cervical papillae without seta. Number and position of spiracles somewhat different from other congeners, a pair of spiracular rudiments present on metathoracic segment; 6 dorsal papillae present, 4 (2 outer and 2 inner) of them with a seta; eighth abdominal segment with 4 dorsal papillae, each with a seta; 3 pleural papillae present on each side of prothorax (if 1 of them is not inner pleural papilla), without seta; 2 pleural papillae present on each side of other thoracic and first to eighth abdominal segments, each without seta on mesothorax, each with a seta on metathorax and abdominal segments; terminal papillae 2 in number, each with a minute seta.

Sternal spatula (Fig. 2) 0.35 to 0.50 mm in length, distally with 4 pointed lobes, 2 inner lobes little longer than the 2 outer; 3 inner lateral papillae present on all thoracic segments, 2 of them with a seta; 1 outer lateral papilla without seta on pro- and mesothorax, with a

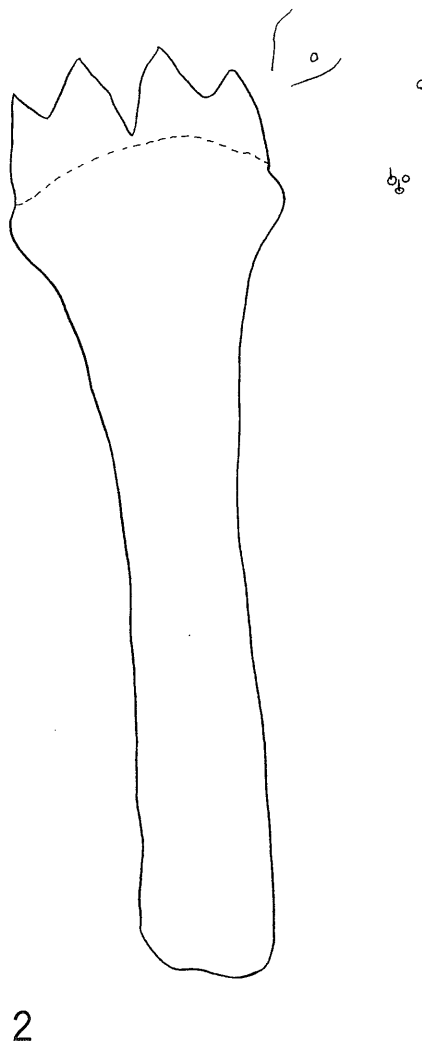
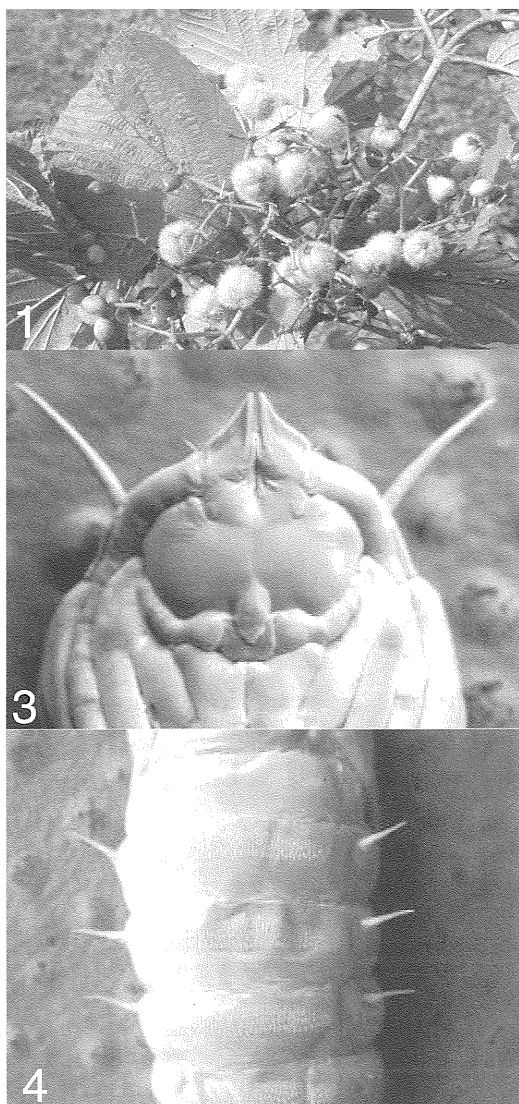


Fig. 1. Fruit galls caused by *Pseudasphondylia rokuharensis* on *Viburnum dilatatum*. The galls are larger than normal fruit and covered with whitish fine hairs.

Fig. 2. *Pseudasphondylia rokuharensis*; sternal spatula of mature larva and adjacent papillae.

Fig. 3. *Pseudasphondylia rokuharensis*; pupal head and frontal area.

Fig. 4. *Pseudasphondylia rokuharensis*; first to fourth abdominal segments of pupa (dorsal view), showing relatively long stigmatal tubercles on the second to fourth segments.

seta on metathorax; sternal papillae without seta on pro- and mesothorax, with a seta on metathorax; inner pleural papillae absent; 2 anterior and 2 posterior ventral papillae each

with a seta; 2 ventral papillae visible on eighth abdominal segment, each with a seta; 4 anal papillae without seta. Each abdominal segment, except terminal one, with many transverse rows of minute spines antero-ventrally, and with many small triangular spines dorsally.

Pupa: Apical spine (Fig. 3) long, 0.20 to 0.25 mm, acutely pointed with finely denticulate outer margin; apical papilla situated on a small protuberance and with a seta, which is about 60 to 80 μm ; upper and lower frontal horns absent; usually 2 pairs of lower facial papillae present, a pair of them with a minute seta; three pairs of lateral facial papillae present, a pair of them with a minute seta; prothoracic horn long, about 0.45 mm; stigmatal tubercles present on second to seventh abdominal segments, sometimes a very short or inconspicuous tubercles present on first abdominal segment; stigmatal tubercles on second to fourth abdominal segment unusually long, 0.20 to 0.25 mm (Fig. 4); first abdominal segment densely with minute spines; second to eighth abdominal segments with several transverse rows of rather long spines on anterior half of dorsal surface and densely with minute spines on ventral surface and on posterior half of dorsal surface. Four dorsal papillae present on first abdominal segment, each with a minute seta; first abdominal segment with a pair of additional papillae, each papilla present at a position posterior to outer dorsal papilla, with a relatively long (20 to 25 μm) seta; 6 dorsal papillae present on second to seventh abdominal segments, 4 (2 outer and 2 inner) of them with a minute seta; usually 2 pleural papillae present on each side, each with a minute seta.

Specimens examined: 4 pupae (on slides), galls collected from Rokuhara, Tono City, Iwate Pref., Honshu, Oct. 24, 1949, K. Monzen leg., emerged on May 5, 1950, reared by K. Monzen; many larvae (in ethanol), galls collected from Akiu-ohtaki, Akiu Town, Miyagi Pref., Honshu, Sep. 17, 2001, M. Tokuda & J. Yukawa leg., dissected by M. Tokuda on Oct. 16, 2001; 4 mature larvae (on slides), many others (in ethanol), galls collected from Nuruyu, Hanayama Village, Miyagi Pref., Honshu, Sep. 18, 2001, M. Tokuda & J. Yukawa leg., dissected by M. Tokuda on Oct. 14, 2001; 6 mature larvae (on slides), many others (in ethanol), galls collected from Miyatoko, Taiwa Town, Miyagi Pref., Honshu, Nov. 16, 2001, K. Goukon leg., dissected by M. Tokuda on Nov. 18, 2001; 3 pupae (in acetone), galls collected from Miyatoko, Taiwa Town, Miyagi Pref., Honshu, Nov. 16, 2001, K. Goukon leg., dissected by M. Tokuda on Feb. 27, 2002; 12 pupae (on slides), many others (in ethanol), galls collected from Miyatoko, Taiwa Town, Miyagi Pref., Honshu, Nov. 16, 2001, K. Goukon leg., emerged from Mar. 14 to 23, 2002 reared by M. Tokuda.

Remarks

The most unique larval morphological feature of *P. rokuharensis* is the presence of spiracular rudiments on the metathoracic segment. This feature has never been observed

in other congeners. The shape of sternal spatula of *P. rokuharensis* is similar to that of *P. rauwolfiae*. However, the sternal spatula is quite different in shape from that of two other congeners in Japan, *P. matatabi* and *P. neolitseae*. In other larval morphological features than the stigma and sternal spatula, *P. rokuharensis* is similar to *P. matatabi*, but differs from *P. neolitseae* whose larval papillae are reduced in number.

The pupa of *P. rokuharensis* has unusually long stigmatal tubercles on the second to fourth abdominal segments (Fig. 4). One of the congeners, *P. matatabi*, has also relatively long stigmatal tubercles (0.17 to 0.19 mm) on the second to fifth abdominal segments (Yukawa, 1971). The prothoracic horn of *P. rokuharensis* and *P. matatabi* is two times longer than that of *P. neolitseae*. Such long stigmatal tubercles may function when the pupae submerge in fluid decaying matter of the fruit galls, because the galls frequently drop to the ground before adult emergence (Sulaiman & Yukawa, 1992; Yukawa & Masuda, 1996). The size and shape of apical spine were quite similar among three Japanese species. Pupal papillae of *P. matatabi* and *P. neolitseae* were reduced in number (Yukawa, 1971; Yukawa, 1974) comparing with *P. rokuharensis*. We could not compare the pupa of *P. rokuharensis* with that of the Chinese and New Caledonian congeners due to the lack of description in Coutin (1980) and Mo & Xu (1999).

Because adults of *Pseudasphondylia* species are morphologically quite similar to one another, morphological phylogenetic analysis requires the comparison of immature stages among species. However, we cannot analyze the phylogenetic relationship among the *Pseudasphondylia* species owing to the lack of information on the immature stages of the exotic species. At the moment, we consider that the morphological features of immature stages indicate the close relation of *P. rokuharensis* to *P. matatabi*.

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