Description of Asphondylia itoi sp.n.(Diptera : Cecidomyiidae) Inducing Fruit Galls on Distylium racemosum (Hamameelidaceae) in Japan

Uechi, Nami

Yukawa, Junichi

https://doi.org/10.5109/2680

出版情報:ESAKIA. 44, pp.27-43, 2004-03-31. Entomological Laboratory, Faculty of Agriculture, Kyushu University バージョン: 権利関係:

# Description of Asphondylia itoi sp. n. (Diptera: Cecidomyiidae) Inducing Fruit Galls on Distylium racemosum (Hamamelidaceae) in Japan\*

## Nami UECHI

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. A new gall midge that is responsible for fruit galls on *Distylium racemosum* (Hamamelidaceae) in Japan is described as *Asphondylia itoi* sp. n. (Diptera: Cecidomyiidae). This species is distinguishable from the other *Asphondylia* species and segregates by relatively small numbers of frontoclypeal and mesepimeral setae in adult and by morphological features of pupal head characterized by smooth antennal horns, a simple upper frontal horn, and three lobes of lower frontal horn that are arranged almost linearly. In addition to the morphological features, the DNA sequence data and biological information indicate that *A. itoi* is a distinct, univoltine, and monophagous species. This gall midge is now known only from Okinawa and Fukuoka Prefectures, Japan.

Key words: Asphondylia itoi, Cecidomyiidae, fruit gall, Distylium racemosum, new species.

## Introduction

The genus Asphondylia H. Loew, 1850 (Diptera: Cecidomyiidae) contains 271 spe-

<sup>\*</sup> Contribution from the Entomological Laboratory, Faculty of Agriculture, Kyushu University, Fukuoka (Ser. 5, No. 108).

## N. UECHI & J. YUKAWA

cies in the world (Gagné, 2004). In Japan, five identified species and 14 unidentified segregates of *Asphondylia* were enumerated in Yukawa & Masuda (1996) and then a new segregate, the *Callicarpa* fruit gall midge, was added to them (Uechi *et al.*, 2002). Thereafter, two of the 15 segregates, the soybean pod gall midge and the *Prunus* fruit gall midge, were combined and described as a new species under the name *Asphondylia yushimai* Yukawa et Uechi, 2003, which was confirmed by DNA analysis to exhibit host alternation between fabaceous plants and *Prunus zippeliana* Miquel (Rosaceae) (Yukawa *et al.*, 2003). At the same time, the *Hedera* flower bud gall midge was divided, based on DNA sequence data, into two segregates, the *Hedera* (= ivy) flower bud gall midge and the *Hedera* (= ivy) fruit gall midge (Yukawa *et al.*, 2003). The *Weigela* leaf bud gall midge, which had been misidentified by Shinji (1938) as a North American species, *Asphondylia diervillae* Felt, 1907, was identified as one of the Japanese species are now recognized to exist in Japan and 14 segregates have been still left unidentified until today.

Besides the morphological similarity among most of these segregates, the lack of information on their annual life cycle has postponed species identification of the segregates, as in the case for the identification of *A. yushimai* (Yukawa *et al.*, 2003). In particular, the existence of polyphagous and host-alternating *Asphondylia* species requires the confirmation of host range and life cycle for respective segregates before species identification.

The *Distylium* fruit gall midge is one of the aforementioned 14 segregates. In 1981, Prof. Emeritus Y. Itô (Nagoya University, Japan) and Mr. S. Yamauchi (Ryukyu Sankei Co. Ltd., Japan) found some midge galls induced on the fruit of *Distylium racemosum* Siebold et Zuccarini (Hamamelidaceae) on Okinawa Island, Okinawa Prefecture, Japan and forwarded the galls to one of us, JY, for species identification. The gall midge was identified as a species of *Asphondylia*, but left unnamed due to the insufficient number of specimens for description and a lack of biological information (Yukawa, 1983). In 2000 and 2001, this gall midge was collected again from Okinawa Island, and found in 2001, for the first time, on Ishigaki Island, Okinawa Prefecture and in Sasaguri Town, Fukuoka Prefecture, Japan (Uechi *et al.*, 2002). In 2002, further specimens and biological information were obtained from Okinawa and Fukuoka Prefectures.

DNA analysis and morphological studies with these specimens, together with biological information, revealed that the *Distylium* fruit gall midge is a distinct species. In this paper, we describe this gall midge as a new species of *Asphondylia* and refer to its univoltine life history, monophagous habit, distribution range, and genetic relationship to the other congeners in Japan.

## Materials and Methods

#### Collection and preservation of specimens

Fruit galls induced on *D. racemosum* were collected from various localities in Okinawa and Fukuoka Prefectures. Some of the collected galls were dissected under a binocular microscope to obtain larval and pupal specimens. Remaining galls were maintained in plastic containers (10 cm in diameter, 6 cm in depth) to rear adult midges. Full-grown larvae, pupae, pupal cases, and emerged adults were put into 70-75 % ethanol for morphological observation or 99.5 % acetone for DNA analysis.

## Morphological comparison

The larvae, pupae, pupal cases, and adults of this gall midge that had been stored in 70-75 % ethanol were mounted on slides in Canada balsam for microscopic study, based on the techniques outlined both in Yukawa (1971) and in Gagné (1989).

Fronto-clypeal and mesepimeral setae were counted for the slide-mounted adult specimens and compared with those of several Japanese *Asphondylia* species and segregates. Drawings were made with the aid of a drawing tube.

Adult morphological terminology follows usage in McAlpine (1981), except that the terminology of thoracic setae follows usage in Yukawa & Ohsaki (1988). Morphological terminology of immature stages generally follows usage in Möhn (1955, 1961; originally written in German) that were translated into English in Yukawa (1971), and the terminology of pupae follows that in Gagné (1994).

# DNA extraction, amplification, and sequencing

Four individuals of the gall midge from D. racemosum were used for DNA analysis (Tables 1, 2). For every individual, total DNA was extracted from the whole body with the Dneasy tissue kit (Qiagen, Japan), following the manufacturer's instructions. A region of the cytochrome oxidase subunit I (COI) gene of mtDNA was amplified, purified, sequenced, and electrophoresized following the methods described by Yukawa et al. (2003). DNA of each specimen was amplified using the following primers: forward: 5'-GGATCACCTGATATAGCATTCCC-3' (COIS) and reverse; 5'-CCCGGTAAAATTAAAATATAAACTTC-3' (COIA). These primers have been effectively used for many gall midges to determine intra- and inter-specific relations (e.g., Shirota et al., 1999; Yukawa et al., 2003; Uechi et al., 2003; Uechi et al., 2004). The amplified COI gene fragment of mtDNA was 439 bp long. This region corresponded to the bases 1752-2190 of the genome of Drosophila yakuba Burla (Diptera: Drosophilidae) (Clary & Wolstenholme, 1985; Shirota et al., 1999).

In addition, a region of the mitochondrial small subunit ribosomal DNA (12S) was

|                    |                |   | To let's success | Accession |
|--------------------|----------------|---|------------------|-----------|
| Gall midge species | Host plant     | Collection site (Collector or reference)      | Isolation name   | No.*      |
| Asphondylia sp.    | Distylium      | Yona, Kunigami Village, Okinawa Pref.         | DistyOki70       | AB162344  |
|                    | racemosum      |   | DistyOki71       | AB162345  |
|                    |                | Morinokawa Park, Ginowan City, Okinawa Pref.  | DistyOki217      | AB162346  |
|                    |                |   | DistyOki218      | AB162347  |
| Asphondylia        | Glycine max    | Yoshiki, Chikushino City, Fukuoka Pref.       | SoyFuk15         | AB085786  |
| yushimai           |                | (Yukawa et al., 2003)                         | SoyFuk46         | AB085787  |
|                    |                |   | SoyFuk16         | AB085868  |
| Asphondylia baca   | Weigela        | Inunaki, Wakamiya Town, Fukuoka Pref.         | WeiFuk43         | AB086426  |
|                    | coraeensis     | (Yukawa et al., 2003)                         | WeiFuk56         | AB086427  |
|                    |                |   | WeiFukInu62      | AB086428  |
| Asphondylia sp.    | Hedera rhombea | Yakuoji, Koga City, Fukuoka Pref.             | HedFrFk99A       | AB085878  |
|                    | (fruit)        | Shikanoshima, Fukuoka City, Fukuoka Pref.     | HedFrSk106       | AB085881  |
|                    |                | Oro-no-shima Is., Fukuoka City, Fukuoka Pref. | HedFrOr109       | AB085884  |
|                    |                | (Yukawa et al., 2003)                         |                  |           |
| Asphondylia sp.    | Hedera rhombea | Kurino Town, Aira, Kagoshima Pref.            | HedFlKg99A       | AB085874  |
|                    | (flower bud)   | (Yukawa et al., 2003)                         | HedFlKg99B       | AB085875  |
|                    |                |   | HedFlKg99C       | AB085877  |
| Outgroup           |                |   |                  |           |
| Pseudasphondylia   | Actinidia      | Ino, Hisayama Town, Fukuoka Pref.             | ActFuk30         | AB085873  |
| matatabi           | polygama       | (Yukawa et al., 2003)                         |                  |           |

Table 1. Specimens used for COI sequence.

\* Nucleotide sequence data used in this study are available from DDBJ, EMBL, and GenBank.

# Table 2. Specimens used for 12S sequence.

| Cull with second   | Host plant                                       | Collection site (Collector or other set)      | Isolation name | Accession |
|--------------------|--|---|----------------|-----------|
| Gall midge species |  | Collection site (Collector or reference)      |                | No.*      |
| Asphondylia sp.    | ia sp. Distylium Yona, Kunigami Village, Okinawa |   | DistyOki70     | AB164444  |
|                    | racemosum  |   | DistyOki71     | AB164445  |
|                    |  | Morinokawa Park, Ginowan City, Okinawa Pref.  | DistyOki217    | AB164446  |
| Asphondylia        | Glycine max                                      | Yoshiki, Chikushino City, Fukuoka Pref.       | SoyFuk15       | AB164447  |
| yushimai           |  |   | SoyFuk46       | AB164448  |
|                    |  | Hosoyamada, Kushira Town, Kagoshima Pref.     | SoyKg379       | AB164449  |
|                    |  | (T. Furukawa)                                 |                |           |
| Asphondylia baca   | Weigela  | Inunaki, Wakamiya Town, Fukuoka Pref.         | WeiFuk43       | AB164450  |
|                    | coraeensis                                       |   | WeiFuk56       | AB164451  |
|                    |  |   | WeiFukInu62    | AB164452  |
| Asphondylia sp.    | Hedera rhombea                                   | Hamaogi, Amatsukominato Town, Chiba Pref.     | HedFrChb140    | AB164453  |
|                    | (fruit)  | Shikanoshima, Fukuoka City, Fukuoka Pref.     | HedFrSk106     | AB164454  |
|                    |  | Oro-no-shima Is., Fukuoka City, Fukuoka Pref. | HedFrOr109     | AB164455  |
| Asphondylia sp.    | Hedera rhombea                                   | Hamaogi, Amatsukominato Town, Chiba Pref.     | HedFlbChb135   | AB164456  |
|                    | (flower bud)                                     |   | HedFlbChb136   | AB164457  |
|                    |  |   | HedFibChb138   | AB164458  |
| Outgroup           |  |   |                |           |
| Pseudasphondylia   | Actinidia  | Ino, Hisayama Town, Fukuoka Pref.             | PsematAct25    | AB164443  |
| matatabi           | polygama   |   |                |           |
|                    |  |   |                |           |

\* Nucleotide sequence data used in this study are available from DDBJ, EMBL, and GenBank.

amplified by PCR following the methods described in Kambhampati & Smith (1995). Purification, sequencing, and electrophoresis of the PCR products followed the methods described by Yukawa *et al.* (2003). This region was effectively used for the analysis of intra- and inter-generic variations in gall midges of the tribe Lasiopterini (Diptera: Cecidomyiidae) (N. Dorchin, 2002, personal communication). The primers used for the amplification of 12S region were as follows: forward; 5'-TACTATGTTACGACTTAT-3' (SR-J-14199) and reverse; 5'-AAACTAGGATTAGATACCC-3' (SR-N-14594) (Kambhampati & Smith, 1995). Length of the mitochondrial 12S rRNA gene fragment varied among species from 374 to 390 bp. This region corresponded to the bases 14200-14593 of the *D. yakuba* genome (Clary & Wolstenholme, 1985).

## Molecular phylogenetic analysis

The DNA sequence data were edited using DNASIS (Hitachi Software Engineering Co.). DNA sequences of COI region were easily aligned with the naked eye and those of 12S region were aligned using the CLUSTAL X program (Thompson *et al.*, 1997). Evolutionary distances were computed by Kimura's two-parameter distances (Kimura, 1980). Phylogenetic analysis was conducted by the neighbor-joining (NJ) method (Saitou & Nei, 1987) using the software package PHYLIP ver. 3.573c (Felsenstein, 1993).

The resulting trees were evaluated by the bootstrap test (Efron, 1982; Felsenstein, 1985) based on 1,000 replications for the NJ tree. As an outgroup species in the analysis, *Pseudasphondylia matatabi* (Yuasa et Kumazawa, 1938) (Diptera: Cecidomyiidae) that is responsible for fruit galls on *Actinidia polygama* Siebold et Zuccarini (Actinidiaceae) was used. In the analysis, the DNA sequence data of the Japanese *Asphondylia* species and segregates were also included for the comparison (Tables 1, 2).

## Distributional and ecological information

Distributional information on this gall midge was obtained from the previous (Yukawa, 1983) and current collecting data of the midge galls in 2000, 2001, and 2002. Ecological information was gathered from dissecting data of the midge galls. Each collecting and dissecting data consists of locality, collecting date, collector, developmental stages of the gall midge, and some other biological information. In collector's name, N. Uechi is abbreviated as NU.

#### Asphondylia itoi Uechi et Yukawa sp. n.

(Japanese name: Isunoki Hario Tamabae) (English name: The *Distylium* fruit gall midge) Generic synopsis of Asphondylia: See Gagné (1989) and Gagné & Waring (1990).

## Male.

Eye bridge 7 facets long medially. Palpus consisting of 2 segments; first palpal segment 2.0 to 2.6 times as long as wide; second 1.4 to 2.2 times as long as first. Basal enlargement of third flagellomere 3.6 to 5.1 times as long as wide, basal enlargement of fifth flagellomere 3.6 to 4.7 times as long as wide. Wing length 2.3 to 2.9 mm, 2.6 to 3.0 times as long as wide; R<sub>5</sub> meeting with costa a little beyond wing apex; 2 sensory pores present on distal portion of R<sub>1</sub>, 1 on basal and 2 or 3 on medial to subdistal portion of R<sub>5</sub>. Fore legs with femur slightly longer than tibia and slightly longer than second tarsomere, fourth tarsomere 1.8 to 2.0 times as long as fifth; middle leg with femur nearly as long as or slightly shorter than tibia and distinctly longer than second tarsomere, fourth tarsomere 1.7 times as long as fifth; hind leg with femur nearly as long as tibia and distinctly longer than second tarsomere, fourth 2.0 to 2.2 times as long as fifth; empodia nearly as long as claws in all legs. Genitalia showing the typical shape for Asphondylia; cerci divided into 2 lobes; tegmen rather deeply emarginated dorsally, rather shallowly emarginated ventrally; gonostylus subglobular, apically with a sclerotized and bidentate tooth; aedeagus laterally sclerotized, distally tapering, basally with a rather weakly sclerotized plate-like structure, which is developed into a pair of small lobes caudolaterally and connected laterally to inner portion of gonocoxite. See Tables 3, 4 for fronto-clypeal and mesepimeral setal counts and measurements of wing, palpus, and flagellomeres.

# Female.

First palpal segment 1.7 times as long as wide; second 2.5 times as long as first. Basal enlargement of fifth flagellomere 4.0 times as long as wide. Wing length 2.8 mm, about 3.4 times as long as wide. Fourth tarsomere of fore leg about 2.9 times as long as fifth; those of middle and hind legs 1.4 and 1.5 times as long as fifth, respectively. Ovipositor showing the typical shape for *Asphondylia*; needle part of ovipositor 0.98 mm, 2.1 times as long as the length of seventh sternite. Otherwise practically as in male. Frontoclypeal and mesepimeral setal counts and measurements of wing, palpus, flagellomeres, seventh sternite, and ovipositor are given in Tables 3, 4.

Full-grown larva (Fig. 1A, see also Fig. 2B in Yukawa, 1983).

Second antennal segment short, conical, about  $10 \,\mu\text{m}$ , 1.6 times as long as maximum width; 2 ventral and 2 lateral cervical papillae each with a seta. Number and position of spiracles normal; inner 4 of 6 dorsal papillae each with a seta on all abdominal segments except eighth; 3 pleural papillae present on each side, each with a seta; (most outer papillae are included in pleural papillae in some cases when they are close to stigmatal protuberance: see Möhn, 1955 and Yukawa, 1971); 2 dorsal papillae of eighth

abdominal segment each with a seta; 2 of 6 terminal papillae somewhat cone-shaped, the remaining 4 each with a short seta. Sternal spatula strongly sclerotized, about 220  $\mu$ m in length, 2.7 times as long as maximum width, distally with 4 lobes, which are usually pointed apically; outer lobes longer than inner lobes; width between tips of 2 outer lobes 53 about  $\mu$ m; sternal and inner pleural papillae each with a seta on all thoracic segments; 3 inner and 2 outer lateral papillae each with a seta on all thoracic segments; 2 anterior ventral papillae and 2 posterior ventral papillae each with a seta; 2 ventral papillae of eighth abdominal segment each with a seta; anal papillae without setae.

## Pupa (Fig. 1B; see also 1C in Yukawa, 1983).

Antennal horn long, 300 to 310  $\mu$ m, acutely pointed, inner margin of antennal horn not distinctly denticulate; upper frontal horn simple, strongly sclerotized, pointed apically; lower frontal horn consisting of 3 pointed lobes, of which outer 2 are slightly longer and situated slightly more anteriorly than central lobe; usually a pair of lower facial papillae each with a short seta; 1 of 3 lateral facial papillae each with a seta; apical papillae with setae, which are 60 to 65  $\mu$ m long. Arrangement of spines on dorsal surface of third, eighth, and terminal segments as in Figs. 1C & 1D. Four of 8 dorsal papillae each with a seta (Fig. 1C).

## DNA analysis.

The sequence data of both COI and 12S regions for *A. itoi* did not coincide with those of the other *Asphondylia* species and segregates in Japan, and monophyly of the clade including only *A. itoi* was supported by a 100 % bootstrap value in the sequence data of COI and 12S region, respectively (Figs. 2, 3).

## Host plant.

Distylium racemosum Siebold et Zuccarini (Hamamelidaceae).

# Gall.

Subglobular or ellipsoidal swelling of fruit, normally with 1 to 3 spine-like apical protuberances, which are about 1.8 mm in length; galled fruit is significantly smaller than normal ones; surface pale greenish brown; normally 2, sometimes 1, rarely 3 larval chambers per gall; each chamber containing one midge larva or pupa.

*Etymology*: The specific name, *itoi*, honors Prof. Emeritus Yoshiaki Itô (Nagoya University) who first collected this gall midge from Okinawa Prefecture, Japan.

*Specimens examined*: Holotype, male (on slide, Type No. 3189, Cecid. No. E4801, a gall was collected from Mt. Katsuudake, Kunigami Village, Okinawa Island, Okinawa Prefecture, Japan in November 22, 1981, and an adult emerged on November 28, 1981). Paratypes, 1 male, 1 female, 1 larva, 4 pupae (on slides, Cecid. Nos. E4802-4808, see Table 5 for collecting data). These specimens are kept in the collection of the Entomological Laboratory, Faculty of Agriculture, Kyushu University, Fukuoka, Japan.

## N. UECHI & J. YUKAWA

| Sex                     |      |        | Male        | Female |
|-------------------------|------|--------|-------------|--------|
| Specimens examined      |      |        | 2           | 1      |
|                         |      | ' Mean | Range       |        |
| Fronto-clypeal setae    |      | 18.5   | 17 - 20     | 12     |
| Mesepimeral setae       |      | 21.5   | 21 - 22     | 15     |
| Wing length             |      | 2606   | 2325 - 2886 | 2756   |
| Wing width              |      | 947    | 775 - 1118  | 819    |
| 1/w                     |      | 2.8    | 2.6 - 3.0   | 3.4    |
| Palpus 1                |      | 67.5   | 60 - 75     | 42.5   |
| Palpus 2                |      | 107.5  | 100 - 115   | 107.5  |
| Flagellomere 3*         | ds   | 8.8    | 7.5 - 10.0  | 5.0    |
|                         | be   | 187.5  | 160 - 215   | 130    |
|                         | W    | 43.8   | 42.5 - 45.0 | 32.5   |
|                         | be/w | 4.3    | 3.6 - 5.1   | 4.0    |
| Flagellomere 5*         | ds   | 8.8    | 7.5 - 10.0  | 5.0    |
|                         | be   | 176.5  | 153 - 200   | 130    |
|                         | W    | 42.8   | 42.5 - 43.0 | 32.5   |
|                         | be/w | 4.1    | 3.6 - 4.7   | 4.0    |
| 7th sternite            |      | -      | -           | 455    |
| Ovipositor              |      | -      | -           | 975    |
| Ovipositor/7th sternite |      | -      | -           | 2.1    |

Table 3. Asphondylia itoi sp. n.: fronto-clypeal and mesepimeral setal counts and measurements of wing, palpus, flagellomeres, seventh sternite, and ovipositor in  $\mu$ m.

\* ds; distal stem, be; basal enlargement, w; width.

*Distribution: Asphondylia itoi* is now known to occur only in Fukuoka Prefecture and on Okinawa Island and Ishigaki Island, Okinawa Prefecture, Japan (Yukawa, 1983; Yukawa & Masuda, 1996; Uechi *et al.*, 2002; the current data), although *D. racemosum* is widely distributed in Japan (from central Honshu to Okinawa Prefecture), Korea (Jeju Island), Taiwan, and China (central and southern parts) (Satake *et al.*, 1989). Therefore, *A. itoi* possibly occurs not only in areas between the northernmost (Fukuoka) and the southernmost (Okinawa) prefectures in the Kyushu-Okinawa District, Japan, but also more widely within the distribution range of *D. racemosum*.

*Collecting and dissecting data*: The previous and current collecting and dissecting data of *Distylium* fruit galls from Fukuoka and Okinawa Prefectures are as follows: **[Fukuoka]** Hakozaki Shrine, Fukuoka City, Sep. 3, 2002, NU & M. Tokuda, 1st instars;

| Sex                |             |      | Female      |      |  |
|--------------------|-------------|------|-------------|------|--|
| Specimens examined |             |      | 2           |      |  |
|                    |             | Mean | Range       |      |  |
| Fore leg           | Femur       | 1136 | 1050 - 1222 | 988  |  |
|                    | Tibia       | 1138 | 975 - 1300  | 1092 |  |
|                    | Tarsomere 1 | 125  | 120 - 130   | 117  |  |
|                    | Tarsomere 2 | 911  | 820 - 1001  | 910  |  |
|                    | Tarsomere 3 | 523  | 460 - 585   | 403  |  |
|                    | Tarsomere 4 | 257  | 234 - 280   | 260  |  |
|                    | Tarsomere 5 | 158  | 120 - 195   | 91   |  |
| Mid leg            | Femur       | 970  | 900 - 1040  | 780  |  |
|                    | Tibia       | 998  | 825 - 1170  | 884  |  |
|                    | Tarsomere 1 | 120  | 110 - 130   | 117  |  |
|                    | Tarsomere 2 | -    | 550         | 689  |  |
|                    | Tarsomere 3 | -    | 325         | -    |  |
|                    | Tarsomere 4 | -    | 200         | 169  |  |
|                    | Tarsomere 5 | -    | 120         | 117  |  |
| Hind leg           | Femur       | 1233 | 1100 - 1365 | 1014 |  |
|                    | Tibia       | 1156 | 1050 - 1261 | 1053 |  |
|                    | Tarsomere 1 | 127  | 110 - 143   | 117  |  |
|                    | Tarsomere 2 | 770  | 630 - 910   | 559  |  |
|                    | Tarsomere 3 | 465  | 410 - 520   | 312  |  |
|                    | Tarsomere 4 | 302  | 240 - 364   | 195  |  |
|                    | Tarsomere 5 | 146  | 110 - 182   | 130  |  |

Kyushu Univ. Forest, Sasaguri Town, May 18, 2001, NU, 1st instars; *ibid.*, Sep. 18, 2000, NU, 1st instars; *ibid.*, May 26, 2001, NU, 1st instar; *ibid.*, Apr. 10, 2001, NU, 1st instar; *ibid.*, Apr. 26, 2001, NU, old galls; *ibid.*, Aug. 19, 2001, NU, 1st instars; **[Okinawa]** Mt. Katsuudake, Nago city, Nov. 22, 1981, Y. Itô & S. Yamauchi, old galls, pupae, 2nd & 3rd instars, an adult emerged on Nov. 28, 1981; Yona, Kunigami Village, Mar. 24, 2000, NU, 1st instars; Hijigawa River, Kunigami Village, Feb. 24, 2001, J. Yukawa, 2nd & 3rd instars, and old galls; Mt. Bannadake, Ishigaki City, Feb. 26, 2001, NU, 1st instars; Morinokawa Park, Ginowan City, Mar. 4, 2001, NU, old galls with pupal cases and 1st instars; *ibid.*, Oct. 15, 2001, NU, 1st instars; *ibid.*, Feb. 23, 2002, NU, an adult emerged on Mar. 7, 2002; *ibid.*, Feb. 27, 2002, NU, six dead 1st instars



**Fig. 1.** *Asphondylia itoi* sp. n.: **A**, larval sternal spatula and adjacent papillae. **B**, ventral view of pupal head. **C**, dorsal papillae of larval third abdominal segment in dorsal view. **D**, pupal eighth and terminal abdominal segment in dorsal view. Scale bars = 0.4 mm.



ActFuk30 *Pseudasphondylia matatabi* 

**Fig. 2.** NJ tree based on 439 bp of the mtDNA COI gene for *Asphondylia itoi* and some Japanese species and segregates. Bootstrap values are indicated for nodes gaining more than 80 % support (1000 replications). *Pseudasphondylia matatabi* was used as an outgroup species. Isolation names correspond to the respective accession numbers registered in DNA database.



PsematAct25 *Pseudasphondylia matatabi* 

**Fig. 3.** NJ tree based on partial sequence of 12S region (including gaps) for *Asphondylia itoi* and some Japanese species and segregates. Bootstrap values are indicated for nodes gaining more than 80 % support (1000 replications). *Pseudasphondylia matatabi* was used as an outgroup species. Isolation names correspond to the respective accession numbers registered in DNA database.

|        | Locality                          | Date of coll. | Leg.* | Date of em.*   | Cecid. No. |
|--------|-----------------------------------|---------------|-------|----------------|------------|
| Male   | Mt. Katsuudake, Kunigami Village, | Nov. 22, 1981 | I & Y | Nov. 28, 1981  | E4801**    |
|        | Okinawa Pref., Japan              |               |       | (reared by JY) |            |
| Male   | Morinokawa Park, Ginowan City,    | Feb. 23, 2002 | NU    | Mar. 2, 2003   | E4805      |
|        | Okinawa Pref., Japan              |               |       | (reared by NU) |            |
| Female | Morinokawa Park, Ginowan City,    | Feb. 23, 2002 | NU    | Mar. 2, 2003   | E4806      |
|        | Okinawa Pref., Japan              |               |       | (reared by NU) |            |
| Pupa   | Mt. Katsuudake, Kunigami Village, | Nov. 22, 1981 | I & Y | _              | E4803,     |
|        | Okinawa Pref., Japan              |               |       |                | E4804      |
| Pupa   | Morinokawa Park, Ginowan City,    | Feb. 23, 2002 | NU    | _              | E4807,     |
|        | Okinawa Pref., Japan              |               |       |                | E4808      |
| Larva  | Mt. Katsuudake, Kunigami Village, | Nov. 22, 1981 | I & Y | _              | E4802      |
|        | Okinawa Pref., Japan              |               |       |                |            |

Table 5. Asphondylia itoi sp. n.: list of slide-mounted specimens examined.

\* I & Y: Y. Itô & S. Yamauchi, JY: J.Yukawa, NU: N. Uechi. \*\* Holotype.

and 12 old galls, other galls contained chalcid pupae; *ibid.*, Oct. 29, 2002, NU, 1st instars, other galls contained chalcid eggs or larvae; Urasoe-dai-kouen Park, Urasoe City, Oct. 28, 2002, NU, 1st instars, other galls contained chalcid eggs.

Biological notes. Based on the collecting and dissecting data, the life history of A. *itoi* is summarized as follows: A. *itoi* is fundamentally univoltine, utilizing the fruit of D. racemosum as an annual host. In Fukuoka, the adult emerges in April, synchronizing with the flowering season of D. racemosum from April to May (Satake et al., 1989). On Okinawa Island, many old galls with pupal cases were found and many flowers were observed opening on March 4, 2001, indicating that the gall midge emerges in early March, the flowering season, in Okinawa Prefecture. The female lays its eggs into the young fruit of D. racemosum in March. The first instar passes through the summer, autumn, and winter in the galled fruit and develops into the second and third instar in the following February. Then, it pupates in the gall on the host tree from late February to March.

Some individuals emerged from the fruit galls in November (Yukawa, 1983). They may not be able to lay their eggs into the D. racemosum fruit because it contains seeds inside and the surface of the fruit is hard. Their future development or contribution to the following generation is unknown at present. A eurytomid species (Hymenoptera: Eurytomidae) has been reared from the galls, but has not been identified.

*Remarks. Asphondylia itoi* is characterized and distinguishable from the other species and segregates of *Asphondylia* by the combination of adult and pupal morphological features as follows: in adult, fronto-clypeal and thoracic setae relatively small in number (Table 3); in pupa, inner margin of antennal horn not denticulate, upper frontal horn

#### N. UECHI & J. YUKAWA

simple, lower frontal horn consisting of three pointed lobes, of which outer two are slightly longer and situated only slightly more anteriorly than central lobe (Fig. 1B), spiracular tubercles absent on first abdominal segment.

In most Japanese Asphondylia species and segregates, outer two of three lobes of pupal lower frontal horn are situated distinctly more anteriorly than central lobe (Yukawa, 1971; Yukawa & Miyamoto, 1979; Uechi *et al.*, 2004), while the three lobes are arranged almost linearly in *A. itoi* (Fig. 1B) as in many Holarctic species of *Asphondylia* (e.g., Hawkins *et al.*, 1986; Gagné & Orphanides, 1992; Yukawa *et al.*, 2003).

The smooth inner margin of pupal antennal horn can be seen in the following species other than *A. itoi*: *Asphondylia anthocercidis* Kolesik, 1997 that induces fruit galls on *Anthocercis littorea* La Billardière (Solanaceae) in Australia (Kolesik, 1997); *Asphondylia glomeratae* Gagné, 2001 that induces leaf galls on *Mikania glomerata* Sprengel (Asteraceae) in southeastern Brazil, and *Asphondylia moehni* Skuhravá, 1989 that induces stem galls on *M. glomerata* in southeastern Brazil (Gagné *et al.*, 2001). However, *A. itoi* is distinguishable from *A. anthocercidis* by having almost linearly arranged three lobes of pupal lower frontal horn and the smaller number of eye facets, and from *A. glomeratae* and *A. moehni* by having three lobes of pupal lower frontal horn that are subequal in size and length.

In addition, the DNA sequence data indicate that *A. itoi* is different from the other Japanese congeners, *A. yushimai*, *A. baca*, and the *Hedera* flower bud gall midge (Figs. 2, 3).

Biological information also supports the independence of A. *itoi*, because it is univoltine and can complete its annual life cycle on a single plant species, D. *racemosum*.

#### Acknowledgements

We wish to express our gratitude to Prof. Emeritus Y. Itô (Nagoya University), Mr. S. Yamauchi (Ryukyu Sankei Co. Ltd.), Dr. M. Tokuda (National Agricultural Research Center for Kyushu Okinawa Region), and Mr. T. Furukawa (Kagoshima Agricultural Experiment Station, Osumi Branch) for their kindness in offering the materials. We thank Dr. O. Tadauchi, Dr. S. Kamitani, and Mr. D. Yamaguchi (Entomological Laboratory, Kyushu University) for their help in various ways.

#### References

Clary, D. O. & D. R. Wolstenholme, 1985. The mitochondrial DNA molecule of

*Drosophila yakuba*: nucleotide sequence, gene organization, and genetic code. J. Mol. Evol., **22**: 252-271.

- Efron, B., 1982. *The Jackknife, the Bootstrap, and Other Resampling Plans*. 92 pp., Society for Industrial and Applied Mathematics, Philadelphia.
- Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**: 783-791.
- Felsenstein, J., 1993. *PHYLIP (Phylogeny Inference Package) Version 3.5c.* University of Washington, Seattle.
- Gagné, R. J., 1989. *The Plant-feeding Gall Midges of North America*. 355 pp., Cornell University Press, Ithaca, New York.
- Gagné, R. J., 1994. *The Gall Midges of the Neotropical Region*. 576 pp., Cornell University Press, Ithaca, New York.
- Gagné, R. J., 2004. A catalog of the Cecidomyiidae (Diptera) of the world. *Mem. Entomol. Soc. Wash.*, **25**: 1-408.
- Gagné, R. J. & G. M. Orphanides, 1992. The pupa and larva of *Asphondylia gennadii* (Diptera: Cecidomyiidae) and taxonomic implications. *Bull. ent. Res.*, **82**: 313-316.
- Gagné, R. J., R. A. M. Oda & R. E. Monteiro, 2001. The gall midges (Diptera: Cecidomyiidae) of *Mikania glomerata* (Asteraceae) in Southeastern Brazil. *Proc. Entomol. Soc. Wash.*, **103**: 110-134.
- Gagné, R. J. & G. M. Waring, 1990. The Asphondylia (Cecidomyiidae: Diptera) of creosote bush (Larrea tridentata) in North America. Proc. Entomol. Soc. Wash., 92: 649-671.
- Hawkins, B. A., R. D. Goeden & R. J. Gagné, 1986. Ecology and taxonomy of the Asphondylia spp. (Diptera: Cecidomyiidae) forming galls on Atriplex spp. (Chenopodiaceae) in southern California. Entomography, 4: 55-107.
- Kambhampati, S. & P. T. Smith, 1995. PCR primers for amplification of four insect mitochondrial gene fragments. *Insect Mol. Biol.*, 4: 233-236.
- Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide-sequences. J. Mol. Evol., 16: 111-120.
- Kolesik, P., R. Whittemore & H. M. Stace, 1997. Asphondylia anthocercidis, a new species of Cecidomyiidae (Diptera) inducing fruit galls on Anthocercis littorea (Solanaceae) in Western Australia. Trans. R. Soc. S. Aust., 119: 171-176.
- McAlpine, J. F., 1981. Morphology and terminology-adults, pp. 9-63, In McAlpine, J. F.,
  B. V. Peterson, G. E. Shewell, H. J. Teskey, J. R. Vockeroth & D. M. Wood (eds.), *Manual of Nearctic Diptera Volume 1*. Canadian Government Publishing Centre, Hull.
- Möhn, E., 1955. Beiträge zur Systematik der Larven der Itonididae (= Cecidomyiidae,

Diptera). 1. Porricondylinae und Itonidinae Mitteleuropas. *Zoologica*, **105**: 1-247, 30 pls.

- Möhn, E., 1961. Gallmücken (Diptera, Itonididae) aus El Salvador. 4. Zur Phylogenie der Asphondyliidi der neotropischen und holarktischen Region. Sencken. Biol., 42: 131-330.
- Saitou, N. & M. Nei, 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, 4: 406-425.
- Satake, Y., H. Hara, S. Watari & T. Tominari, 1989. *Wild Flowers of Japan. Woody Plants II.* 303 pp., Heibonsha, Tokyo. (In Japanese.)
- Shinji, O., 1938. 4 unrecorded species of the Cecidomyiidae from Japan. *Insect World*, 42: 270-274. (In Japanese.)
- Shirota, Y., K. Iituka, J. Asano, J. Abe & J. Yukawa, 1999. Intraspecific variations of mitochondrial cytochrome oxidase I sequence in an aphidophagous species, *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae). *Entomol. Sci.*, 2: 209-215.
- Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin & D. G. Higgins, 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res.*, **25**: 4875-4882.
- Uechi, N., M. Tokuda & J. Yukawa, 2002. Distribution of *Asphondylia* gall midges (Diptera: Cecidomyiidae) in Japan. *Esakia*, (42): 1-10.
- Uechi, N., M. Tokuda, J. Yukawa, F. Kawamura, K. K. Teramoto & K. M. Harris, 2003. Confirmation by DNA analysis that *Contarinia maculipennis* (Diptera: Cecidomyiidae) is a polyphagous pest of orchids and other unrelated cultivated plants. *Bull. ent. Res.*, 93: 545-551.
- Uechi, N., J. Yukawa & D. Yamaguchi, 2004. Host alternation by gall midges of the genus *Asphondylia* (Diptera: Cecidomyiidae), pp. 53-66. In Evenhuis, N. L. & K.Y. Kaneshiro (eds.), *Contributions to the Systematics and Evolution of Diptera*. D. Elmo Hardy Mem. Vol. Bishop Mus. Bull. Entomol., 12.
- Yukawa, J., 1971. A revision of the Japanese gall midges (Diptera: Cecidomyiidae). Mem. Fac. Agr. Kagoshima Univ., 8: 1-203.
- Yukawa, J., 1983. A new midge gall of *Asphondylia* species (Diptera, Cecidomyiidae) from Okinawa. *Mem. Kagoshima Univ. Res. Center S. Pac.*, **3**: 139-145.
- Yukawa, J. & H. Masuda, 1996. Insect and Mite Galls of Japan in Colors. 826 pp., Zenkoku Nôson Kyôiku Kyôkai, Tokyo. (In Japanese with English explanations for color plates.)
- Yukawa, J. & K. Miyamoto, 1979. Redescription of Asphondylia sphaera Monzen (Diptera, Cecidomyiidae), with notes on its bionomics. Mem. Fac. Agr. Kagoshima Univ., 15: 99-106.
- Yukawa, J. & N. Ohsaki, 1988. Separation of the aucuba fruit midge, Asphondylia

*aucubae* sp. nov. from the ampelopsis fruit midge, *Asphondylia baca* Monzen (Diptera: Cecidomyiidae). *Kontyû*, **56**: 365-376.

Yukawa, J., N. Uechi, M. Horikiri & M. Tuda, 2003. Description of the soybean pod gall midge, *Asphondylia yushimai* sp. n. (Diptera: Cecidomyiidae), a major pest of soybean and findings of host alternation. *Bull. ent. Res.*, 93: 73-86.

.

.