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

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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 fronto-clypeal 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-

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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 *Asphondylia baca* Monzen, 1937 (Uechi *et al.*, 2004). Thus, five identified 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

Table 1. Specimens used for COI sequence.

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

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

Table 2. Specimens used for 12S sequence.

Gall midge species	Host plant	Collection site (Collector or reference)	Isolation name	Accession No.*
Asphondylia sp.	*Distylium racemosum*	Yona, Kunigami Village, Okinawa Pref.	DistyOki70	AB164444
			DistyOki71	AB164445
		Morinokawa Park, Ginowan City, Okinawa Pref.	DistyOki217	AB164446
Asphondylia yushimai	*Glycine max*	Yoshiki, Chikushino City, Fukuoka Pref.	SoyFuk15	AB164447
			SoyFuk46	AB164448
		Hosoyamada, Kushira Town, Kagoshima Pref. (T. Furukawa)	SoyKg379	AB164449
Asphondylia baca	*Weigela coraeensis*	Inunaki, Wakamiya Town, Fukuoka Pref.	WeiFuk43	AB164450
			WeiFuk56	AB164451
			WeiFukInu62	AB164452
Asphondylia sp.	*Hedera rhombea* (fruit)	Hamaogi, Amatsukominato Town, Chiba Pref.	HedFrChb140	AB164453
		Shikanoshima, Fukuoka City, Fukuoka Pref.	HedFrSk106	AB164454
		Oro-no-shima Is., Fukuoka City, Fukuoka Pref.	HedFrOr109	AB164455
Asphondylia sp.	*Hedera rhombea* (flower bud)	Hamaogi, Amatsukominato Town, Chiba Pref.	HedFlbChb135	AB164456
			HedFlbChb136	AB164457
			HedFlbChb138	AB164458
Outgroup				
Pseudasphondylia matatabi	*Actinidia polygama*	Ino, Hisayama Town, Fukuoka Pref.	PsematAct25	AB164443

* 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_5 meeting with costa a little beyond wing apex; 2 sensory pores present on distal portion of R_1, 1 on basal and 2 or 3 on medial to subdistal portion of R_5. 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 caudo-laterally 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. Fronto-clypeal 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 μm, 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 µm 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 µm; 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 µm, 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 µm 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.

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

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
l/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

* 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		Male		Female
Specimens examined		2		1
		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, and two dead pupae, an adult emerged on Mar. 7, 2001; *ibid.*, Jul. 16, 2001, NU, 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

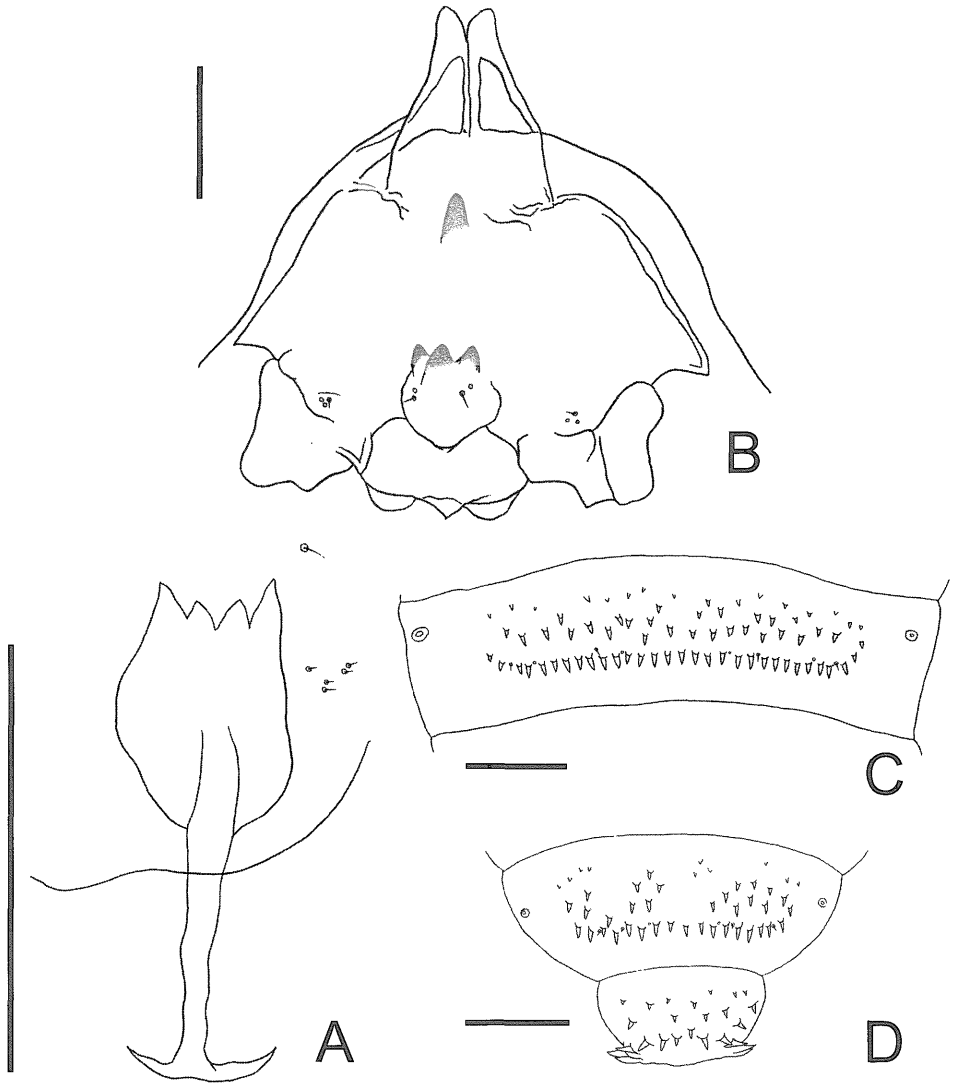

Fig. 1. *Asphondylia itoi* sp. n.: **A**, larval sternal spatula and adjacent papillae. **B**, ventral view of pupal head. **C**, dorsal papillae of larval third abdominal segment in dorsal view. **D**, pupal eighth and terminal abdominal segment in dorsal view. Scale bars = 0.4 mm.

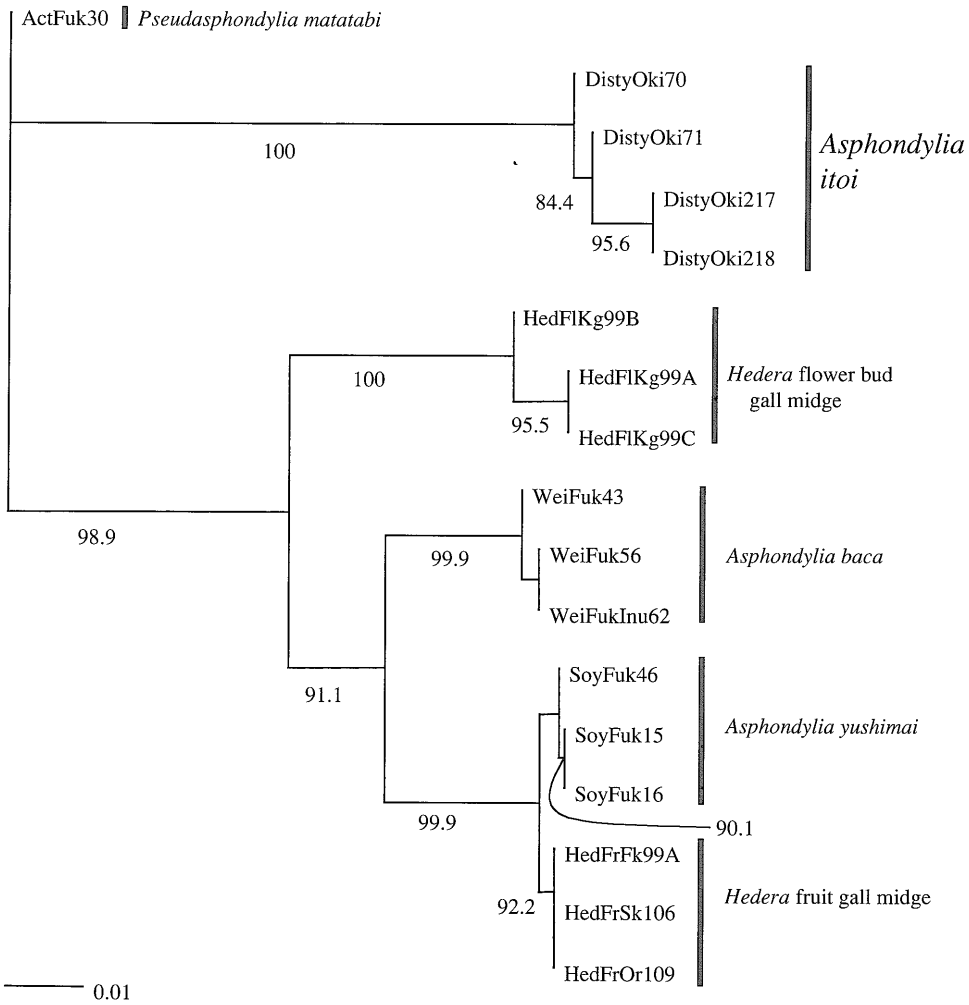

Fig. 2. NJ tree based on 439 bp of the mtDNA COI gene for *Asphondylia itoi* and some Japanese species and segregates. Bootstrap values are indicated for nodes gaining more than 80 % support (1000 replications). *Pseudasphondylia matatabi* was used as an outgroup species. Isolation names correspond to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

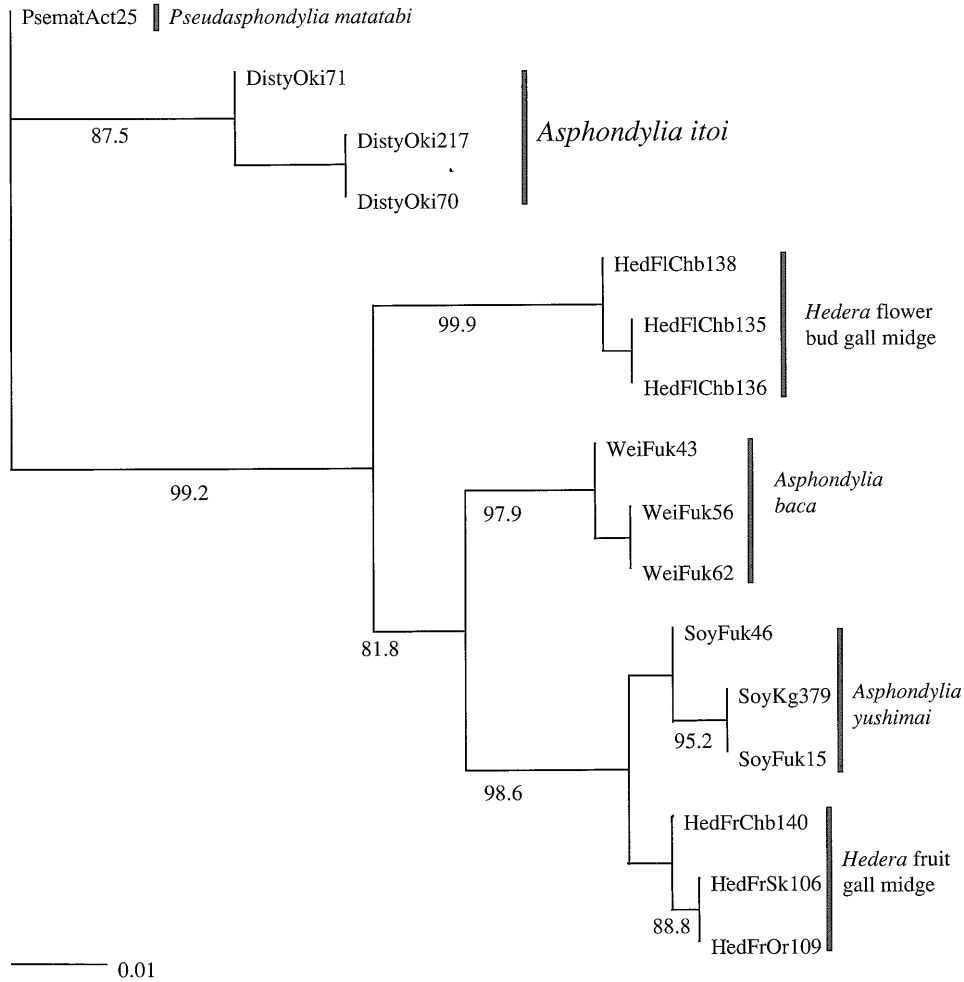

Fig. 3. NJ tree based on partial sequence of 12S region (including gaps) for *Asphondylia itoi* and some Japanese species and segregates. Bootstrap values are indicated for nodes gaining more than 80 % support (1000 replications). *Pseudasphondylia matatabi* was used as an outgroup species. Isolation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

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

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

* 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

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*.

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