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Findings of New Cecidomyiid Galls Induced by *Asphondylia* Segregates (Diptera: Cecidomyiidae) in Japan

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Abstract. After 2006, eight sorts of gall induced by eight segregates of *Asphondylia* (Diptera: Cecidomyiidae) were newly found on eight plant species belonging to six families in Japan. As a result, six described species and 18 seregates of Japanese *Asphondylia* are now hosted by a total of 32 plant genera belonging to 25 families. Newly recorded host plants include three alien species. These findings imply the second, third and fourth examples of host range expansion to alien plants by Japanese gall midges unless they are alien *Asphondylia*. In general, gall-inducing cecidomyiids are mono- or oligophagous and hardly expand their host range to newly encountered plants. However, *Asphondylia* species may be able to expand their host range to alien plants more easily than other gall-inducing cecidomyiids, because some *Asphondylia* species are polyphagous, multivoltine and sometimes exhibit host alternation. Further information on their morphological features, ecological traits, distribution records and DNA sequencing data will enable species identification and clarify their life history patterns and host ranges.

Key words: *Asphondylia*, Cecidomyiidae, flower gall, fruit gall, host alternation, host range, polyphagous.

Introduction

The genus *Asphondylia* (Diptera: Cecidomyiidae) includes 289 species in the world (Gagné, 2010). Species of *Asphondylia* exhibit diverse patterns in life history (univoltine or multivoltine) and host plant utilization (monophagous, oligophagous, or polyphagous) (e.g. Uechi & Yukawa, 2006b). In particular, some polyphagous species exhibit host alternation across different plant families (e.g. Yukawa *et al*., 2003; Uechi *et al*., 2004).

In Japan, five identified species and 14 segregates of *Asphondylia* were known to occur on 25 plant genera of 17 families (Yukawa & Masuda, 1996). 'Segregate' has been defined as a gall midge that may be a new species or identical to one of the described species but has been left unidentified because of morphological similarity and biological dissimilarity to congeners (e.g. Yukawa *et al*., 2003; Uechi & Yukawa, 2004; Uechi *et al*., 2004). Each segregate induces galls on one or a few congeneric plant species and adults appear in a particular season. Therefore, most segregates cannot complete their annual life cycle on restricted plant species. DNA analysis has effectively identified segregates to the species level and sometimes demonstrated that the existence of host plant alternation by combining two or more segregates or described species bearing the same DNA sequencing (e.g. Yukawa *et al*., 2003; Uechi *et al*. 2004). Thus, host plant alternation enables the annual lifecycle to be completed.

In Uechi *et al*. (2002b), the distribution records of the Japanese *Asphondylia* gall midges were renewed based on

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collecting data after Yukawa & Masuda (1996). Thereafter, eight segregates and one misidentified species of *Asphondylia* gall midges were identified and five of them were described as new species or combined with described species (Yukawa *et al*., 2003; Uechi & Yukawa, 2004; Uechi *et al*., 2004: Uechi *et al*., 2005; Uechi & Yukawa, 2006a; Uechi & Yukawa, 2006b). Identification of these segregates was based mainly on molecular data because Japanese *Asphondylia* species and segregates are morphologically quite similar to one another. As a result, the number of identified *Asphondylia* species increased from five to six and that of segregates decreased from 13 to 10 by 2006.

In this paper, we describe, together with photographs, galls that were newly found in Japan after 2006. They are all induced by *Asphondylia* segregates, of which biological information is also provided. These findings will contribute to future identification of the segregates and detection of further combinations of host plant alternation.

Materials and Methods

Our colleagues listed in the Acknowledgements and we collected cecidomyiid galls from various wild and cultivated plants in Japan during the period from 2007 to 2011. We identified host plants relying upon Satake *et al*. (1989) and Katanotda (1999). Galls and gall–bearing plants collected were brought back to the laboratory and some galls were dissected under a binocular microscope to identify gall–inducer and to obtain larval and pupal specimens. At the same time, the developmental stage of the gall midges was recorded. Some galls were kept in plastic bags to obtain adults and pupal exuviae. Gall midge specimens were preserved in 75% ethanol for morphological observation and in 99% ethanol or acetone for future DNA analysis.

Results

After 2006, eight sorts of gall induced by *Asphondylia* segregates were newly found in Japan. They are briefly described below together with photographs (Figs. 1–10) and biological information on the segregates. A new Japanese name was provided for each gall sort. Galls are arranged according to the taxonomical order of host plants (Yonekura & Kajita, 2003).

(1) Leaf bud gall on *Schoepfia jasminodora* Sieb. et Zucc. (Olacaceae)

Japanese name: Boroboronoki-me-fukure-fushi.

Galled buds look similar to normal ones but they are slightly swollen (Fig. 1). Gall size is 3.9-8.3 mm in height, 3.0-4.3 mm in maximum width, and 2.7-4.7 mm in minimum width.

Collecting data are summarized in Table 1.

Biological information: Collecting data (Table 1) shows that this gall midge is multivoltine, adults emerging in May, June and September. Annual life cycle can be completed only on *S. jasminodora* because host buds are available throughout the year.

(2) Fruit gall on *Phytolacca americana* L. (Phytolaccaceae)

Japanese name: Youshuyamagobou-mi-fukure-fushi.

Galled fruit is similar to normal mature fruit in size and shape but slightly malformed and partly tinged with pale green to red-purple, which contrasts to greenish young fruit.

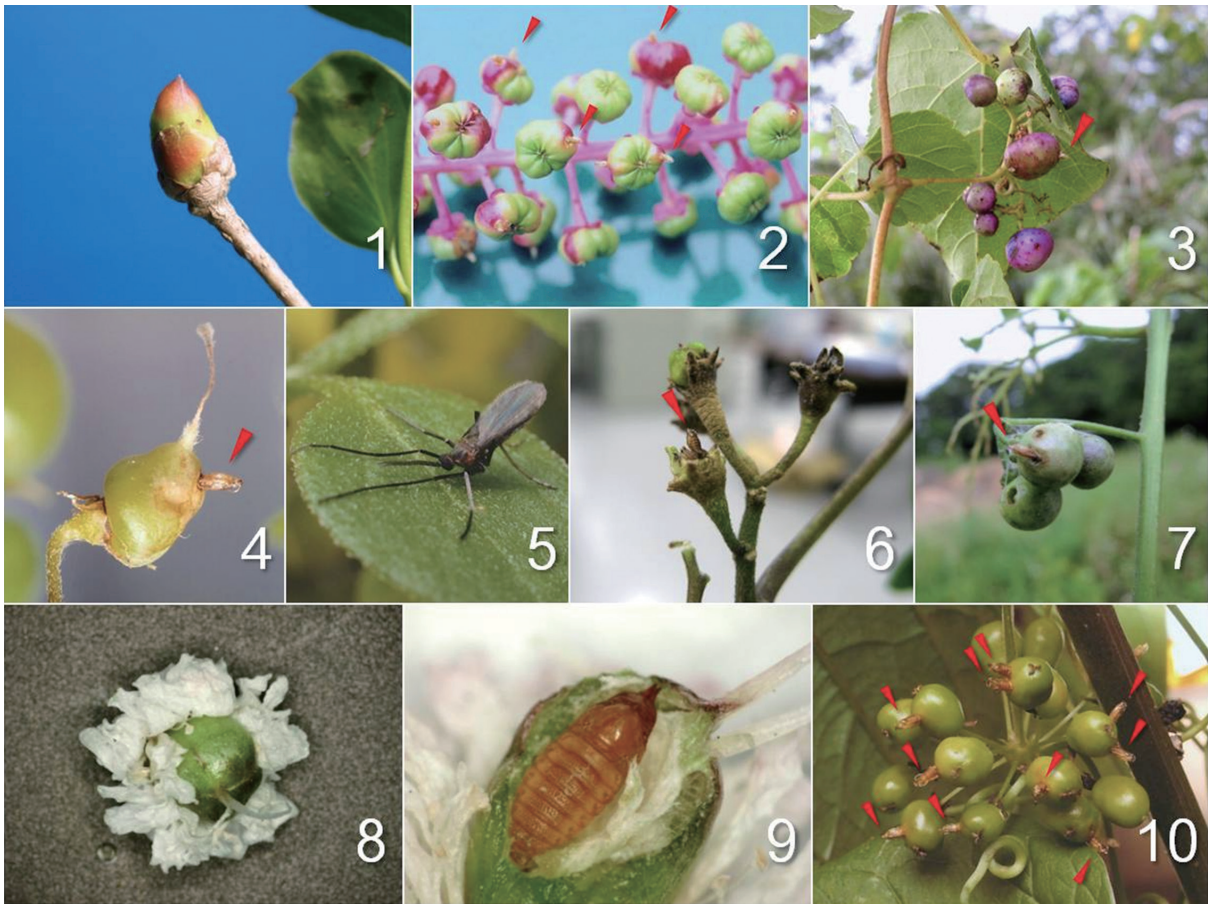
Collecting data: [Tomioka City, Gunma Pref.] Galls were collected on 16 July 2005 by H. Tsuruta. Adults were obtained by S. Usuba on 24 July 2005; [Kan-ra Town, Gunma Pref.] Galls were collected by S. Usuba and I. Kogure on 23 July 2005, and adults emerged on 10 August 2005; [Fukuyama City, Hiroshima Pref.] Galls were found by R. Kobashi on 25 July 2005; [Kiryu City, Gunma Pref.] Galls were collected by I. Kogure on 10 September 2006; [Other information] S. Usuba found galls with emergence holes at Shibuya and Ueno, Tokyo, and Utsunomiya City, Tochigi Prefecture, but could not obtain adults.

Biological information: *Phytolacca americana* is an alien plant, originating from North America (Shimizu et al., 2001). We do not know at the moment whether this gall midge came to Japan together with its host plant or it has expanded its host range to the alien plant from Japanese host plants. At least, this gall midge needs to use alternative host plants for overwintering because no fruit of *P. americana* is availabe in the winter. Okamoto *et al*. (unpublished data) susupect that *Hedera rhombea* (Miq.) Sieb. ex Bean (Araliaceae) is one of the possible overwintering hosts but the data have not yet been published (Usuba, 2007).

(3) Fruit gall on *Ampelopsis brevipedunculata* (Maxim.) Trautv. var. *glabrifolia* Honda (Vitaceae)

Japanese name: Minami-nobudou-mi-fukure-fushi.

Galled fruit is similar to normal fruit but somewhat larger in size and irregular in shape (Fig. 3). This gall is quite similar in shape to that induced by *Asphondylia baca* Monzen on the same host plant in Japan (Hokkaido, Honshu, Shikoku and Kyushu, other than the Nansei Islands) and the Korean Peninsula (see Fig. C-386 in Yukawa & Masuda, 1996). However, we confirmed by a



Figs. 1-10. Galls and gall midges treated in this paper (arrows point toward pupal exuvia). **1.** Leaf bud gall on *Schoepfia jasminodora*; **2.** Fruit galls on *Phytolacca americana* with pupal exuviae; **3.** Fruit galls on *Ampelopsis brevipadunculata* with a pupal exuvia and an exit hole; **4.** Fruit gall on *Salpichroa origanifolia* with a pupal exuvia; **5.** Adult emerged from a fruit gall on *S. origanifolia*; **6.** Fruit galls on *Solanum biflorum* and a pupal exuvia; **7.** Fruit galls on *Solanum photeinocarpum* with a pupal exuvia and exit holes; **8.** Flower gall on *Gypsophila paniculata*; **9.** Pupa in a flower gall on *G. paniculata*; **10.** Fruit galls on *Smilax riparia* var. *ussuriensis* with pupal exuviae.

Table. 1. Collecting data of the leaf bud galls on *Schoepfia jasminodora*.

Collecting site	Collecting date and collector(s)*	Date of adult emergence	Additional information
Mt. Koura, Kurume City, Fukuoka Pref.	13 June 2010 (NG)	20 June 2010	—
	6 Sep 2010 (NG)	—	Chalcid wasps emerged on 13 Sep 2010.
	24 April 2011 (NG)	30 April, 8 & 13 May 2011	Adults were obtained by NG & KZM. Parasitoid wasps emerged on 13 May 2011.
	14 June 2011 (NG)	16 June 2011	Galls were immature.
	11 Sep 2010 (NG)	—	Braconid wasps emerged on 12, 13 Sep 2010.
Yoshii Town, Ukiha City, Fukuoka Pref.	30 Apr 2011 (NG)	—	—
	3 Sep 2011 (NG)	—	Pupal exuviae were obtained. On 4 Sep 2011, braconid and eulophid wasps emerged.
Mt. Kiyomizu, Miyama City, Fukuoka Pref.	17 May 2011 (NG)	—	On 29 May 2011, galls were dissected by KZM & WGK, and pupae were obtained.
Tsuko, Ogori City, Fukuoka Pref.	2 Sep 2011 (KYM)	—	Pupal exuviae were obtained.

*Collector's names are abbreviated as follows: NG: Naohisa Gyoutoku, KYM: Kiyoko Matsunaga, KZM: Kazunori Matsuo, WGK; W-G. Kim.

preliminary DNA analysis that these gall-inducing *Asphondylia* species are different from each other (Uechi & Yukawa, 2006).

Collecting data: [Mt. Banna, Ishigaki Is., Okinawa Pref.] Galls were collected by N. Uechi and M. Tokuda on 2 March 2002 (Uechi *et al*., 2002b). Further galls were collected by N. Uechi on 25 May 2004. In this season, adults had emerged from the galls in many cases and some galls contained 1st, 2nd and 3rd instars, and pupae. [Hoshino, Ishigaki Is., Okinawa Pref.] Galls were collected on 26 May 2004. Similarly, adults had emerged from the galls in many cases and some galls contained 1st and 3rd instars, and larvae of a braconid wasp.

Biological information: *Asphondylia baca* alternates summer and winter host plants between *A. brevipedunculata* and *Weigela* spp. (Caprifoliaceae) (Uechi *et al*., 2004) because fruit of vitaceous plants is not availabe during the winter. On Ishigaki Island, however, fruit of *A. brevipedunculata* is available during the winter, which enable this gall midge to repeat generations on a single host plant throughout the year.

(4) Fruit gall on *Salpichroa origanifolia* (Lam.) Baill. (Solanaceae)

Japanese name: Hakobehoozuki-mi-midori-fushi.

Galled fruit is similar to normal fruit in size and shape but slightly malformed.

Collecting data: [Inari, Fujisawa City, Kanagawa Pref.] Fruit galls (some with pupal exuviae) were collected by Y. Oguri on 20 July 2009. Pupae and adults were obtained from the galls by S. Usuba on 25 July 2009.

Biological information: not available.

(5) Fruit gall on *Solanum biflorum* Lour. (Solanaceae)

Japanese name: Mejirohoozuki-mi-midori-fushi.

Galled fruit is smaller than normal fruit in size and malformed in shape.

Collecting data: [Oshikawa, Ogimi Village, Okinawa Pref.] Fruit galls were collected on 6 March 2008 by N. Uechi, Y. Sadoyama and K. Nishida. Adults emerged from the galls by 19 March 2008. [Gushiken, Motobu Town, Okinawa Pref.] Fruit galls were collected on 15 March 2008 by N. Uechi, Y. Sadoyama. Adults emerged from the galls by 29 March 2008.

Biological information: not available.

(6) Fruit gall on *Solanum photeinocarpum* Nakam. et Odash. (Solanaceae)

Japanese name: Teriminoinuhoozuki-mi-midori-fushi.

Galled fruit is similar to young or mature normal fruit in size and shape but almost greenish in color.

Collecting data: [Maesato, Itoman, Okinawa Pref.] Fruit galls and pupal exuviae were collected by N. Uechi on 15 May 2007. Adults emerged on 25 and 27 May 2007. [Yonaguni Is., Okinawa Pref.] Galled fruit was collected by T. Matsuyama *et al*. on 22 June 2005. Adults emergerd on 12-19 July 2005.

Biological information: not available.

(7) Flower gall on *Gypsophila paniculata* L. (Caryophyllaceae)

Japanese name: Kasumisou-hana-fukure-fushi.

Ovary of the flower is enlarged. Galled flower becomes obvious when the flower blooms. Gall size is about 5 mm in diameter.

Collecting data: [Yanohara, Showa Village (open field), Fukushima Pref. and Fukushima Agricultural Technology Centre (open field), Hiwada Town, Koriyama, Fukushima Pref.] Galled flower buds were collected by T. Mitamura on 12 October 2007. From these galls, 3rd instars and pupae were obtained. Adults emerged on 29 October 2007 from the galls collected from Showa Village.

Biological information: not available. This gall midge is a pest of flowers of *G. paniculata* cultivated in open fields.

(8) Fruit galls on *Smilax riparia* A. DC. ssp. *ussuriensis* (Regel) Kitag. (Smilacaceae)

Japanese name: Shiode-mi-midori-fushi.

Galled fruit is oval- or spindle-shaped, sometimes stigma remains at the tip of galled fruit. Color is green or partly yellowish green. Gall size is similar to normal fruit and 4.5 to 6.3 mm in diameter.

Collecting data: [Ishikawa, Fujisawa City, Kanagawa Pref.] Fruit galls were first found by Y. Oguri on 8 August 2009, then collected by him on the next occasion, 19 July 2010. The galls were reared by S. Usuba and adults emerged on 30 July 2010.

Remarks

By adding newly found segregates, their galls and host plants, six described species and 18 segregates of Japanese *Asphondylia* are now hosted by a total of 32 plant genera belonging to 24 families (Table 2). Among the host plants, *Ampelopsis brevipedunculata* and *Hedera rhombea* are utilized by at least two *Asphondylia* gall midges, respectively. These are only examples of a single plant species that is used by more than one *Asphondylia* species in Japan. In contrast, more than several species are associated with particular plant species in the U.S.A. For example, seven species induce different sorts of gall on

Atriplex canescens (Pursh) Nutt. (Chenopodiaceae) (Hawkins *et al*., 1986) and 15 species on *Larrea tridentata* (DC.) Coville (Zygophyllaceae) (Gagné & Waring, 1990). They are considered to have diversified on a particular host plant (Gagné, 2010). There are no such examples in Japan.

Generally, gall inducers, especially Cecidomyiidae, are associated with their host plants tightly and have difficulty using newly encountered alien species. Yukawa & Uechi (1999) demonstrated that most Japanese gall inducers have not expanded their host range to alien plants that have naturalized in Japan since the 1850s when the Japanese Government opened the country to foreign intercourse after a long-term isolation for more than 250 years. Actually no gall midges were recorded from alien plants in Yukawa & Uechi (1999). Thereafter, Nohara *et al*. (2007) found that *Rhopalomyia yomogicola* (Matsumura) had expanded its host range to an alien *Artemisia* sp. (Asteraceae). This was the only example of host range expansion by Japanese gall midges until today. In this paper, we record three alien plant species, *Phytolacca americana*, *Salpichroa origanifolia* and *Gypsophila paniculata*, as hosts of *Asphondylia* gall midges. Therefore, this paper affords the second, third and fourth examples of host range expansion to alien plants by Japanese gall midges unless they are alien *Asphondylia*. As far as we observed morphological features of their pupal facial spines, they exhibited an arrangement of facial spines that is typical among Japanese *Asphondylia* species (e.g. Figs. 4 & 5 in Yukawa *et al*., 2003; Fig. 2 in

Table. 2. A list of the Japanese *Asphondylia* species, segregates, their host plants and galled organs (modified and updated from Uechi *et al*., 2005). Species are arranged in alphabetical order of their species name, and segregates are arranged in taxonomic order of their host plant family.

Gall midge	Host plant family and species	Galled organ[a]
Asphondylia aucubae	[Cornaceae] *Aucuba japonica*	FR
**A. baca*	[Vitaceae] *Ampelopsis brevipedunculata, Cayratia japonica*	FR
	[Caprifoliaceae] *Weigela hortensis, W. coraeensis, W. japonica, W. middendorffiana*	LB
A morivorella	[Moraceae] *Morus alba, M. australis, M. bombycis*	LB
**A. sphaera*	[Oleaceae] *Ligustrum obtusifolium, L. japonicum, L. lucidum, L. ovalifolium*	FR
	[Anacaridiaceae] *Rhus succedanea, R. sylvestris*	FLB or FR
**A. yushimai*	[Fabaceae] *Glycine max, G. soja, Lespedeza bicolor, Desmodium racemosum, D. podocarpum, Indigofera pseudo-tinctoria, Sophora flavescens, Vicia amoena*	P
	[Caesalpiniaceae] *Chamaecrista nomame*	
	[Rosaceae] *Prunus zippeliana*	FR
	[Oleaceae] *Osmanthus heterophyllus*	FR
A. itoi	[Hamamelidaceae] *Distylium racemosum*	FR
***Asphondylia* sp.	[Phytolaccaceae] *Phytolacca americana*	FR
***Asphondylia* sp.	[Caryophyllaceae] *Gypsophila paniculata*	FL
Asphondylia sp.	[Ericaceae] *Pieris japonica*	FLB
Asphondylia sp.	[Myrsinaceae] *Ardisia japonica*	FLB
Asphondylia sp.	[Cornaceae] *Helwingia japonica*	FR
***Asphondylia* sp.	[Olacaceae] *Schoepfia jasminodora*	LB
Asphondylia sp.	[Celastraceae] *Celastrus orbiculatus*	FR
Asphondylia sp.	[Euphorbiaceae] *Sapium japonicum*	LB
***Asphondylia* sp.	[Vitaceae] *Ampelopsis brevipadunculata* var. *grablifolia*	FR
Asphondylia sp.	[Araliaceae] *Hedera rhombea*	FR
Asphondylia sp.	[Araliaceae] *Hedera rhombea*	FLB
***Asphondylia* sp.	[Solanaceae] *Salpichroa origanifolia*	FR
***Asphondylia* sp.	[Solanaceae] *Solanum biflorum*	FR
***Asphondylia* sp.	[Solanaceae] *Solanum photeinocarpum*	FR
Asphondylia sp.	[Verbenaceae] *Callicarpa japonica*	FR
Asphondylia sp.	[Rubiaceae] *Paederia foetida*	FLB
Asphondylia sp.	[Zingiberaceae] *Alpinia intermedia*	FR
***Asphondylia* sp.	[Smilacaceae] *Smilax riparia* var. *ussuriensis*	FR

*: Species exhibiting host plant alternation; **: newly recorded segregates in this paper.
a: FL: Flower, FLB: flower bud, FR: fruit, LB: leaf bud, P: pod.

Uechi *et al*., 2004). In the case of *Asphondylia* species, they can expand their host range to alien plants more easily than other gall-inducing cecidomyiids because some *Asphondylia* species are polyphagous (Orphanides, 1975; Gagné & Wuensche, 1986), multivoltine, and sometimes exhibit host alternation (e.g. Yukawa *et al*., 2003; Uechi *et al*., 2004).

In conrast, some alien gall midges arrived in Japan more recently together with their host plants. They are *Procontarinia mangicola* (Shi) on mango, *Mangifera indica* L. (Anacardiaceae) (Uechi *et al*., 2002a), *Contarinia maculipennis* Felt on orchid flower buds and other host plants (Tokuda *et al*., 2002; Uechi *et al*., 2011), and *Obolodiplosis robiniae* (Haldemann) on *Robinia pseud-acacia* L. (Fabaceae) (Kodoi *et al*., 2003). However, there is no interception record of alien *Asphondylia* species at Japanese sea- or airports in recent years (Iwaizumi *et al*., 2007).

The *Asphondylia* gall midges recorded in this paper have not yet been identified to the species level. Further information on their morphological features, ecological traits, distribution records and DNA sequencing data will enable species identification and clarify their life history patterns and host ranges.

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