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Generic Position of Two Unidentified Japanese Gall Midges (Diptera: Cecidomyiidae) on *Artemisia* Species (Asteraceae)*

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Abstract. Among 19 Japanese gall midge species on *Artemisia*, 3 species that were known only from galls and larvae have been left unnamed. The morphological study proved that the 2 of them belonged to the genus *Rhopalomyia* of the tribe Rhopalomyiini. The larvae of both species are characterized by the reduction of terminal papillae from eight to six, the absence of sternal spatula, and reduction in the number of lateral papillae. They were likely to be new species, but were not described because their adult specimens were not available. Unfortunately, remaining 1 species could not be studied due to the lack of specimens. As a result, the number of Japanese *Rhopalomyia* species on *Artemisia* totaled up to 14. This number is remarkably large for the species associated with a single plant genus.

Key words: *Rhopalomyia*, gall midge, Rhopalomyiini, larval morphology, sternal spatula, identification.

Introduction

At least 19 species of gall midges (Diptera: Cecidomyiidae) that produce galls on the genus *Artemisia* (Asteraceae) have been known to occur in Japan (Yukawa & Masuda, 1996; Table 1). They consist of 12 species of the genus *Rhopalomyia*, 2 of *Lasioptera*, 1 of *Dicrodiplosis*, 1 of *Silvestrina*, and 3 unidentified species. The last 3 species (US 1, US 2, and US 3, hereafter, respectively) have been left unnamed, because

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Table 1. Japanese cecidomyiids that produce galls on *Artemisia* species.

Gall midge[1]	Galled part[2]	Distribution[3]	Host plant[4]					
			A. capillaris	*A. feddei*	*A. japonica*	*A. montana*	*A. princeps*	*A. stolonifera*
Dicrodiplosis? minuta Shinji, 1939	stem	unknown					+	
Silvestrina? artemisiae Shinji, 1939	stem	unknown					+	
Lasioptera artemisifoliae Shinji, 1939	leaf	Hk, Hn, Ky			+		+	
Lasioptera sp.	stem	unknown				+	+	
R. abdominalis Shinji, 1938	t-bud	Hk				+		
R. artemisiae (Bouché, 1834)	f-bud	Hn	+	+	+			
R. caterva Monzen, 1937	a-bud	Hn, Ky	+	+	+			
R. cinerarius Monzen, 1937	leaf	MJ, S, Y, Sw, Ru				+	+	
R. giraldii Kieffer & Trotter, 1900	stem	Hn, Sh, Ky, S, T, Ch, Kr, Ru		+		+	+	
R. japonica Monzen, 1937	leaf	Hn			+		+	
R. iwatensis Shinji, 1938	t-bud	Hn, Ky, T			+		+	
R. longitubifix (Shinji, 1939)	a-bud	Hn					+	
R. struma Monzen, 1937	stem	MJ, S, Y, Kr, Ru				+	+	
R. shinjii Gagné, 1975	a-bud	MJ				+	+	
R. yomogicola (Matsumura, 1931)	leaf	MJ, S, T, Y, Sw, Kr			+	+	+	
Rhopalomyia sp.	leaf	MJ, Sw					+	
Unidentified species 1	a-bud	Ky					+	
Unidentified species 2	leaf	Hn, Ky						+
Unidentified species 3	leaf	Hk				+		

1) Generic positions of gall midges with a question mark are doubtful.

2) a-bud: axillary bud, f-bud: flower bud, t-bud: tarminal bud.

3) Hk: Hokkaido, Hn: Honshu, Sh: Shikoku, Ky: Kyushu, S: Sado, T: Tanegashima, Y: Yakushima, Sw: The Southwest Islands, MJ: the mainland of Japan including Hokkaido, Honshu, Shikoku, and Kyushu, Ch: China, Kr: Korea, Ru: The Russia Far East.

4) Host range of respective species is indicated with the mark of plus.

they are known only from larvae and galls, and adult specimens have never been obtained. US 1 is responsible for a globular gall on the axillary bud of *Artemisia princeps* Pampan (Fig. 1), US 2 for a relatively short tube-like gall on the lower leaf surface of *Artemisia stolonifera* (Maxim.) Komorov (Fig. 2), and US 3 for a tiny subconical gall on the leaves and stems of *Artemisia montana* Pampan.

A diversified gall midge complex on a single host genus like *Rhopalomyia* species

on *Artemisia* has been known for *Asphondylia* on *Larrea* (Zygophyllaceae) (Gagné, 1989), *Semudobia* on *Betula* (Betulaceae) (Roskam, 1977), *Rabdophaga* on *Salix* (Salicaceae) (Nijveldt & Yukawa, 1982; Yukawa & Masuda, 1996), etc. Such a gall midge complex is suitable for the study of phylogenetic relationships among species constituting the complex and the mechanism of speciation and coexistence of congeners. Prior to these studies, the establishment of taxonomic positions for unidentified species is fundamentally necessary.

This paper intends to determine the generic position of US 1 and US 2 based chiefly on larval morphology, although they were not identified at the species level due to the lack of their adult specimens. Unfortunately, US 3 could not be studied here because its galls and larval specimens were not available.

Materials and Methods

Collection and preservation of specimens

Galls of US 1 were collected from various parts of Fukuoka, Miyagi, and Oita Prefectures, Japan during the period from March 2000 to November 2002. Galls of US 2 were collected on November 10 from Hiraodai, Kitakyushu City, Fukuoka Prefecture. Some of these galls were dissected under a binocular microscope and unparasitized full-grown midge larvae were picked out of the galls and kept in 70-75% ethanol for morphological studies and in 99.5% acetone for future DNA analysis.

Morphological study and classification of Cecidomyiidae

The full-grown larvae of US 1 and US 2 that had been stored in 70-75% ethanol were mounted on slides in Canada balsam for microscopic study, based on the techniques outlined in Gagné (1989). In particular, the sternal spatula, its adjacent papillae, and anal segment were examined by bright-field and phase-contrast microscopy using a Nikon ECLIPSE E400 microscope. These morphological features were compared with those of other genera of the supertribe Lasiopteridi in the light of larval diagnostic characters described by Möhn (1955) and Gagné (1989).

The slide-mounted specimens examined in this study are kept in the collection of Entomological Laboratory, Faculty of Agriculture, Kyushu University.

In the classification of Cecidomyiidae, we followed supertribal and tribal groupings that are going to be proposed by R. J. Gagné (in press, A Catalog of the Cecidomyiidae (Diptera) of the World. *Proc. Entomol. Soc. Wash.*).

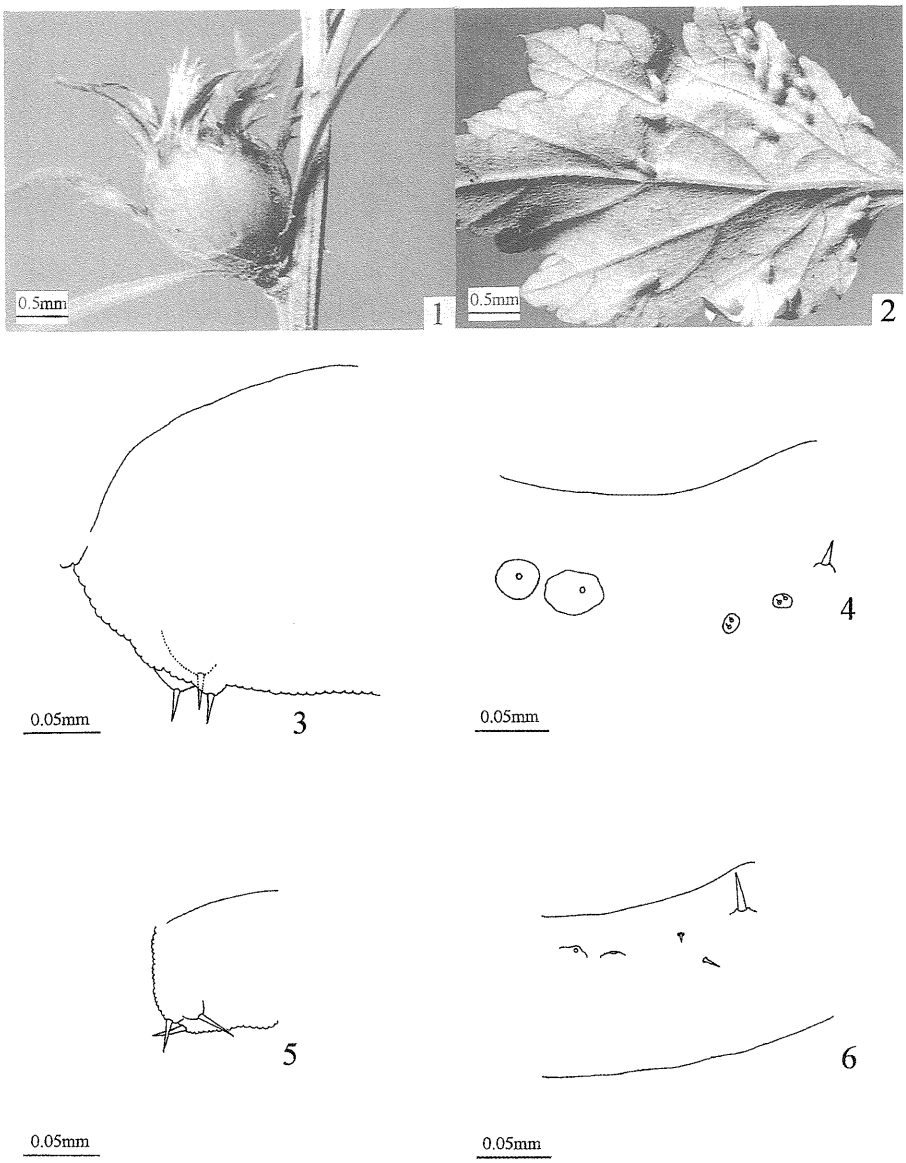

Fig.1. An axillary bud gall produced by US 1 on *Artemisia princeps*; **Fig.2.** Leaf galls produced by US 2 on *Artemisia stolonifera*; **Fig.3-4.** US 1; 3: Papillae on the larval prothoracic segment (ventral view); 4: Terminal papillae on the ninth larval abdominal segment (dorsal view); **Fig.5-6.** US 2; 5: Papillae on the larval prothoracic segment (ventral view); 6: Terminal papillae on the ninth larval abdominal segment (dorsal view).

Results

US 1.

Mature larva. Six dorsal papillae present on all thoracic and first to seventh abdominal segments, each with 6.2 to 7.5μm long seta; two dorsal papillae of eighth abdominal segment each with 6.2 to 7.5μm long seta; two pleural papillae on all thoracic and abdominal segments each with 27.5 to 28.8μm long seta; six terminal papillae present, each with 23.7 to 25.0μm long seta (Fig. 3); sternal spatula absent (Fig. 4); lateral papillae reduced to two groups of two setose papillae per side; inner pleural papillae each with 17.5 to 18.8μm long seta; sternal papillae without a seta on all thoracic segments; four anterior ventral papillae present on first to seventh abdominal segments, each without seta; two posterior ventral papillae present on first to seventh abdominal segments, each with 11.2 to 12.5μm long seta; four ventral papillae of eighth abdominal segment each with a minute seta; four anal papillae each without seta.

Specimens examined: 2 mature larvae (on slides), galls collected from Inoseto, Oita Prefecture on August 31, 2001, M. Nohara & J. Yukawa leg.

US 2.

Mature larva. Six dorsal papillae present on all thoracic and first to seventh abdominal segments, each with 18.7 to 21.3μm long seta; two dorsal papillae of eighth abdominal segment each with 22.5 to 25.0μm long seta; two pleural papillae on all thoracic and abdominal segments each with 23.7 to 25.0μm long seta; six terminal papillae present, each with 16.2 to 18.8μm long seta (Fig. 5); sternal spatula absent (Fig. 6); lateral papillae reduced to two papillae per side, each with 6.2 to 10.0μm long seta; inner pleural papillae each with 22.5 to 25.0μm long seta; sternal papillae without a seta on all thoracic segments; two anterior ventral papillae present on first to seventh abdominal segments, each without seta; two posterior ventral papillae present on first to seventh abdominal segments, each with 20.0 to 22.5μm long seta; two ventral papillae of eighth abdominal segment each with 22.5 to 25.0μm long seta; four anal papillae each without seta.

Specimens examined: 6 mature larvae (on slides), galls collected from Hiraodai, Fukuoka Prefecture on November 10, 2002, M. Nohara & J. Yukawa leg.

Discussion

The comparison of US 1 and US 2 with other genera in the supertribe Lasiopteridi revealed that these species belonged to the genus *Rhopalomyia* of the tribe

Rhopalomyiini (R. J. Gagné, in press), because they showed some of typical characteristics of the genus *Rhopalomyia* as follows: reduction of terminal papillae from eight to six, absence of sternal spatula, reduction in the number of lateral papillae (Möhn, 1955; Gagné, 1989). The genus *Rhopalomyia* comprises at least 253 species in the world and most species are responsible for complex galls on Asteraceae and chiefly on the tribe Anthemideae (R. J. Gagné, in press). Therefore, beside the morphological features, the host plant association with *Artemisia* is another important evidence supporting that these species are members of *Rhopalomyia*.

At least 18 previously described genera have been synonymized with the genus *Rhopalomyia* (R. J. Gagné, in press). This means that *Rhopalomyia* has a wide range of superficial adult characters and adaptive larval and pupal characters.

The larva of US 1 (*Rhopalomyia* sp. 1, hereafter) is similar to that of *Rhopalomyia artemisiae* (Bouché), which was previously placed in the genus *Bouchéella*, one of the 18 genera that had been synonymized with *Rhopalomyia*. However, *Rhopalomyia* sp. 1 differs from *R. artemisiae* by having both inner and outer lateral papillae. In addition, *Rhopalomyia* sp. 1 produces globular swellings on the axillary buds of *A. princeps*, while *R. artemisiae* makes terminal galls on *Artemisia japonica* Thunberg, *Artemisia feddei* Léveille et Vaniot in Japan (Yukawa, 1971), and other *Artemisia* species in Europe (Möhn, 1955).

The larva of US 2 (*Rhopalomyia* sp. 2, hereafter) is similar to that of *Rhopalomyia foliorum* (Loew, H.), which was previously placed in the genus *Diathronomyia*. However, *Rhopalomyia* sp. 2 is distinguished from *R. foliorum* by the shape and position of gall: *Rhopalomyia* sp. 2 produces relatively short tube-like galls on the lower leaf surface of *A. stolonifera*, while *R. foliorum* makes oval and yellowish or reddish green leaf vein galls on the upper leaf surface of *Artemisia vulgaris* L. (Plate 22 in Rübsaamen & Hedicke, 1926).

Both *Rhopalomyia* sp. 1 and *Rhopalomyia* sp. 2 are likely to be new species, but they are not described here because their adult specimens were not available at the moment as mentioned earlier.

As a result of this study, the number of Japanese *Rhopalomyia* species on *Artemisia* totaled up to 14 (Table 1), in which 11 were nominal species. The total number, 14, is remarkably numerous for a gall midge complex that are associated with a single plant genus.

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