

New Host and Distribution Records for *Schizomyia castanopsisae* (Diptera: Cecidomyiidae) from Fukuoka, Northern Kyushu, Japan

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Abstract Inflorescence galls on *Castanopsis sieboldii* (Makino) Hatus and *Castanopsis cuspidata* (Thunb.) Schottky (Fagaceae) were found in Fukuoka Prefecture, northern Kyushu, Japan. Based on morphological and molecular studies, the gall inducer was identified as *Schizomyia castanopsisae* Elsayed & Tokuda (Diptera: Cecidomyiidae). Partial sequences (472 bp) of mitochondrial DNA cytochrome oxidase subunit I (COI) region were identical among individuals collected from *C. sieboldii* and *C. cuspidata* in Fukuoka and an individual from *C. sieboldii* Kagoshima, southern Kyushu. Genetic divergence between individuals collected from Kyushu and those from Hachijojima Island (the Izu Islands, Tokyo) was 0.68%. This is the first report of *S. castanopsisae* from northern Kyushu and *C. cuspidata* is a new host plant record for this gall midge.

Introduction

Schizomyia castanopsisae Elsayed et Tokuda, 2018 (Diptera: Cecidomyiidae) induces inflorescence galls on *Castanopsis sieboldii* (Makino) Hatus (Fagaceae) (Yukawa and Masuda, 1996; Tokuda *et al.*, 2013; Elsayed *et al.* 2018a). Since the first finding of its galls on Okinawa Island in 1980 (Yamauchi *et al.*, 1982), there have been no records of the galls for approximately three decades. However, in recent years, the galls were reported from Miyazaki and Kagoshima Prefectures, southern Kyushu (Nagai, 2010; Tokuda and Kawauchi, 2013a), and from the Izu Islands, Tokyo, Japan (Tokuda *et al.*, 2012, 2013, 2015; Tokuda & Kawauchi, 2013b). In 2018, one of us KM found peculiar swellings on inflorescences of *C. sieboldii* and *Castanopsis cuspidata* (Thunb.) Schottky (Fagaceae) in Chikushino, Fukuoka, northern Kyushu (Fig. 1). The shape was very similar to galls induced by *S. castanopsisae*, and cecidomyiid larvae were found in the swellings.



Fig. 1. Inflorescence galls (arrows) induced by *Schizomyia castanopsisae* on *Castanopsis cuspidata* in Chikushino City, Fukuoka, Japan.

To identify the gall inducer, we examined in this study the larval morphology of the gall midge found in Fukuoka and compared its DNA sequences with those of *S. castanopsisae* reported by Elsayed *et al.* (2018a).

Materials and Methods

1. Collecting and dissecting of galls

Inflorescence galls on *C. sieboldii* and *C. cuspidata* were collected from Haruda (N 33° 27'; E 130° 33'), Chikushino City, Fukuoka Prefecture on 19 November 2018. After measuring the size, they were dissected under a stereoscopic microscope to obtain larvae. Larvae were preserved either in 75% or 99.5% ethanol for morphological and molecular studies, respectively. Specimens used in this study were deposited in the Laboratory of Systems Ecology, Faculty of Agriculture, Saga University.

2. Morphological examination and molecular analyses

For morphological examination, some larval specimens were mounted on slides following the method shown in Elsayed *et al.* (2018b). Total DNA was extracted from larval specimens and a partial sequence of the mitochondrial cytochrome oxidase subunit I (COI) region was sequenced following the methods shown in Elsayed *et al.* (2017). The primer sets used in the analysis are: J-1718 (5'-GGA GGA TTT GGA AAT TGA TTA GTT CC-3') (Simon *et al.* 1994) and COIA (5'-CCC GGT AAA ATT AAA ATA TAA ACT TC-3') (Funk *et al.*, 1995). In addition to specimens collected from Fukuoka, a larval specimen collected from Kagoshima Prefecture on 1 November 2012 and preserved in 99.5% ethanol (Tokuda and Kawauchi, 2013a) was used for the molecular analysis. The sequence data were registered in the DNA Data Bank of Japan (DDBJ), the European Molecular Biology Laboratory (EMBL) and GenBank under the accession numbers: LC480919-LC480921.

Results and Discussion

Galls collected from *C. sieboldii* and *C. cuspidata* in Haruda, Chikushino City were similar in size (*C. sieboldii*: n

= 13, 10.0 ± 5.0 mm in diameter, 7.8 ± 2.4 mm in length; *C. cuspidata*: n = 4; 9.6 ± 6.0 mm in diameter; 6.1 ± 1.0 mm in length) (Fig. 1). In galls collected from *C. sieboldii*, four out of eight cecidomyiid larvae (third instars) were parasitized by unidentified ectoparasitoid larvae. All five cecidomyiid larvae found in galls on *C. cuspidata* were parasitized by ectoparasitoid larvae (so we isolated parasitized cecidomyiid larvae for molecular and morphological examinations), and two pupae of the ectoparasitoid were found in other larval chambers.

Larval morphology of gall midges collected from *C. sieboldii* and *C. cuspidata* in Fukuoka was fundamentally the same to that of *S. castanopsisae* shown in Elsayed *et al.* (2018). The partial sequence of mitochondrial COI region (472bp) of gall midges collected from *C. sieboldii* and *C. cuspidata* in Fukuoka as well as an individual collected from *C. sieboldii* in Kagoshima (Tokuda and Kawauchi, 2013a) were identical. Then the sequence divergence between individuals collected from Kyushu (Fukuoka and Kagoshima) and those from Hachijojima Island, the Izu Islands, Tokyo (the type locality of *S. castanopsisae*; Elsayed *et al.*, 2018a) was 0.68%. Based on these results, we identify the gall midge collected from *C. sieboldii* and *C. cuspidata* in Fukuoka as *S. castanopsisae*. This is the first report of *S. castanopsisae* in northern Kyushu, and *C. cuspidata* is a new host record for this gall midge.

On the Izu Islands, densities of galls induced by *S. castanopsisae* are very high in recent years especially in southern islands such as Miyakejima, Hachijojima, and Aogashima Islands (Tokuda, 2014; Naito and Tokuda, 2018; unpublished data). In contrast, the density was reported to be very low in Kagoshima Prefecture (Tokuda and Kawauchi, 2013a). In addition, galls induced by *S. castanopsisae* have never been reported from the Southwest Islands of Japan after the first finding of this gall from Okinawa Island in 1980 (e.g. Yukawa and Masuda, 1996), including Tanegashima Island (Yukawa *et al.* 2013) and the Tokara Islands (Tokuda, 2017, 2018). So the population density of *S. castanopsisae* has been low in Kyushu and the Southwest Islands of Japan.

Although we have never obtained any parasitoids associated with *S. castanopsisae* on the Izu Islands (Elsayed *et al.* 2018a; unpublished data), we found an unidentified ectoparasitoid in galls collected from Fukuoka in this study. Ectoparasitoid larvae were also found in gall collected from Miyazaki Prefecture, southern Kyushu (J. Yukawa and K. Matsuo, personal communications). These facts imply that parasitism by the ectoparasitoid is possibly one of the important factors regulating *S. castanopsisae* populations and the lack of parasitoids may contribute more or less to the recent outbreaks of *S. castanopsisae* on the Izu Islands.

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