A New *Dasineura* Species (Diptera: Cecidomyiidae) Associated with Symplocos cochinchinensis (Loureiro) (Symplocaceae) in Japan

Ayman K. ELSAYED^{1,2)}, Koreyoshi OGATA³⁾, Koichi KABURAGI⁴⁾,

Junichi YUKAWA⁵⁾, and Makoto TOKUDA^{1,6)}

¹⁾ The United Graduate School of Agricultural Sciences, Kagoshima University,

Kagoshima, 890-0065 Japan. Email: ayman.khamis77@gmail.com

²⁾ Department of Applied Entomology, Faculty of Agriculture, Alexandria University, Egypt

³⁾ Nishino-omote, Nishino-omote City, Kagoshima, 891-3101 Japan.

⁴⁾ Noma, Nakatane Town, Kagoshima, 891-3604 Japan

⁵⁾ Entomological Laboratory, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan. Email: jzs02305@nifty.ne.jp
⁶⁾ Laboratory of Systems Ecology, Faculty of Agriculture, Saga University, Honjo 1, Saga 840-8502, Japan. Email: tokudam@cc.saga-u.ac.jp
Corresponding address: The United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima, 890-0065 Japan

Abstract Recently, a gall midge induces leaf bud galls on *Symplocos cochinchinensis* (Loureiro) (Symplocaceae) was found on Tanegashima Island, Kagoshima, Japan. Based on morphological observation, the gall midge (Diptera: Cecidomyiidae) was clarified to be an undescribed species of the genus *Dasineura* Rondani (Lasiopteridi, Dasineurini). The species is distinguishable from other congeners by its unique undivided female 8th tergite, which is shorter than the 7th, in contrast with other *Dasineura* species possess longitudinally divided 8th tergite longer than the 7th. The new species, *Dasineura symplocos* Elsayed and Tokuda n. sp., is the first example of *Dasineura* species associated with Symplocaceae.

Introduction

The angiosperm plant family Symplocaceae consists of about 320 woody evergreen species distributed in mountainous areas in tropical America and tropical and subtropical regions of eastern Eurasia and Malesia, and a few north temperate and Australian outliers (Fritsch et al., 2015). Up to present, only three gall midge species have been known to use Symplocaceae as their host plants, i.e. Rhopalomyia ilexifoliae Shinji, 1944 [the generic status of this species should be reconsidered (Yukawa, 2014)], Asphondylia bursaria Felt, 1927, and Contarinia pulcherrima Kieffer, 1909, which are associated with Symplocos chinensis, S. fasciculata and S. theiformis, respectively (Gagné & Jaschhof, 2014). In addition, some cecidomyiid galls induced on Symplocaceae were reported but the gall inducers were still undescribed; e.g. flower galls on Symplocos brandisii in east India (Docters van Leeuwen-Reijnvaan & Docters van Leeuwen, 1926) and leaf and stem galls on Symplocos uniflora in Brazil (Maia & Silva, 2013).



Fig. 1. Terminal bud galls induced by *Dasineura symplocos* n. sp. on *Symplocos cochinchinensis*.

During the course of our taxonomic and faunistic studies of gall midges in Japan, we found an undescribed species of gall midge that induces axillary bud galls (Fig. 1) on *Symplocos cochinchinensis* (Loureiro) (Symplocaceae). Then we noted that the morphological features of this gall midge were consistent with the tribe Dasineurini (Cecidomyiidae: Cecideomyiinae: Lasiopteridi). By comparing the morphological characters with thoses of Dasineurini genera, we concluded that the gall midge belongs to the genus *Dasineura* Rondani, 1840 but is distinguishable from known species by unique female postabdominal tergites. This study aims to describe the gall midge as a new species of *Dasineura*. This is the first example of a *Dasineura* species inducing galls on plants belonging to Symplocaceae.

Materials and methods

1. Collecting of gall midges

Leaf bud galls on *S. cochinchinensis* (Fig. 1) were collected from Mount Kawayasu, Kunigami, Nishinoomote, Tanegashima Island, Kagoshima Prefecture, Japan and were kept in plastic bags until the emergence of adults. Some galls were dissected to obtain mature larvae and pupae of the gall midge. Most of the specimens collected were preserved in 75% ethanol for morphological examinations while some were kept in 99.5% ethanol for molecular phylogenetic studies or observation by a scanning electron microscope.

2. Morphological examination

Some of the ethanol-preserved specimens were mounted on slides in Canada balsam using the technique outlined in Gagné (1989). Morphological features were examined under the light microscope Nikon H550L and drawings were made with the aid of a drawing tube. Some pupae were observed with a scanning electronic microscope (Hitachi S-3400N) after mounting them in various positions on a copper stub by



Figs. 2–8. *Dasineura symplocos* n. sp. 2, Male head; 3, ventral view of female 5th flagellomere; 4, ventral view of male 5th flagellomere; 5, 5th tarsomere; 6, female 7th abdominal tergite; 7, female 8th abdominal tergite; 8, ovipositor. Scale bars = $0.05 \mu m$.

using double face adhesive tape.

Morphological terminology follows McAlpine (1981) and Gagné *et al.* (2014) for adults Gagné (1989) for larvae, and Gagné (1994) for pupae. The holotype and paratypes are deposited in the collection of the Entomological Laboratory, Faculty of Agriculture, Kyushu University, Japan.

3. DNA extraction, sequencing and alignment

The total DNA was extracted from the whole body of third instars using the DNeasy Blood and Tissue Kit (Qiagen, Tokyo, Japan) following the manufacturer's instructions. A region of the mitochondrial cytochrome oxydase subunit I (COI) gene was amplified by polymerase chain reaction (PCR) using the following combinations of primers: LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AA AAT CA-3') (Folmer *et al.* 1994). The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and the sequencing reaction was performed using the BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, Foster City, CA, USA) and was electrophoresed on an ABI 3100 sequencer (Applied Biosystems).



Figs. 9–11. *Dasineura symplocos* n. sp. 9, Male sixth through eighth abdominal segments; 10, male terminalia; 11, larval sternal spatula and associated papillae. Scale bars = $0.05 \mu m$.

The alignment of nucleotide COI sequences were carried out using the software MEGA (ver. 6.0) (Tamura *et al.*, 2013) and were deposited in the DNA Data Bank of Japan (DDBJ), European Molecular Biology Laboratory (EMBL), and GenBank nucleotide sequence databases as the registration numbers shown in the results.

Results

The genus Dasineura Rondani, 1840

Dasineura can be distinguished from other Lasiopteridi genera by the following combination of characters (Gagné

et al., 2014): antennal flagellomeres variable even within a species, not restricted to 12; male flagellomeres with basal node and distinct apical neck; female flagellomeres with almost no neck beyond the node; C wing vein broken after its juncture R5; tarsal claws robust, curved and toothed; empodia about as long as the tarsal claws while the pulvilli about 1/3 the length of the claws; the gonocoxite mediobasal lobe closely juxtaposed to the side of the aedeagus; the ovipositor elongate-protrusible and its cerci fused to form a single lobe. Larvae with bilobed spatula and basic cecidomyiine complement of papillae. Although *Dasineura* is also characterized by the female eighth tergite that is longitudinally divided into two sclerites and longer than the 7th (Gagné &



Jaschhof, 2014), this feature is not applicable for the new species described in this paper.

Dasineura symplocos Elsayed and Tokuda, sp. nov.

Description. Adult: body length: 2.8-3.2 mm in female (n = 9) when ovipositor not protruded and 2.1-2.5 mm in male (n = 10).

Head (Fig. 2): Compound eyes with circular eye facets, eye bridge 3–4 facets long, gap between eyes on vertex 0.75–3.0 times as wide as facet. Antenna 2+17–18 in male (n = 10) and 2+18–19 in female (n = 10); scape conical, pedicel rounded; flagellomeres with two connected rings of circumfila in both sexes, with short neck in female (Fig. 3) and with long naked neck in male (Fig. 4), neck length about as half as node. Front with 48–60 setae (n = 9). Labellum hemispherical, with several strong short setae. Palpus 4-segmented; the first segment 30 μ m (19–44 μ m, n = 21), the second about 46 μ m (37–56 μ m, n = 21), the third about 47 (36–62 μ m, n = 21), fourth about 42 μ m (23–60 μ m, n = 21).

Thorax: Wing (Fig. 12) length 2.09–2.74 mm in female (n = 6), 2.14–2.56 mm in male (n = 5); R1 joining C before mid-length of wing and R5 almost before wing apex. M3+4 connected with Cu, forming a fork. Anepimeral setae 15–18 in male (n = 5) and 16–18 in female (n = 6). Dorsolateral setae 43–60 in male (n = 4) and 41–53 in female (n = 4). Tarsal claws curved, toothed on all legs. Empodia as long as tarsal claw (Fig. 5).

Female abdomen: 1st to 7th tergites rectangular; 1st to 6th with two posterior rows of setae, 7th with 3 rows (Fig. 6), 8th trapezoid (Fig. 7) shorter than 7th tergite (1 : 1.75), weakly sclerotized except for 2 darker, parallel, longitudinal, mesal areas, and with a few scattered posterior setae. 2nd to 6th sternites rectangular with group of band of setae at midlength and 1–2 posterior rows of setae; 7th sternite quadrate with similar setae distribution to other sternites but posterior setae more than 3 rows; 2nd to 7th sternites with 2 anterior pairs of trichoid sensilla; 8th sternite bare, difficult to distinguish the margin from the surrounding tissues, with only one anterior pair of trichoid sensilla. Ovipositor (Fig. 8): protrudable, about 4.5 times as long as the 7th tergite; cerci evenly

microtrichose, setose, with few apical blunt setae; hypoproct

Wing. Scale bar = $200 \,\mu m$.

Fig. 12. Dasineura symplocos n. sp.

Male abdomen: 1st through 7th tergites as in female, 8th very weakly sclerotized and mostly indistinguishable from surrounding tissue, with anterior pair of trichoid sensilla and 3–4 posterior setae (Fig. 9). 2nd through 7th sternites as in female, 8th sternite with only one anterior pair of trichoid sensilla and posterior scattered setae. Terminalia (Fig. 10): Gonocoxite massive, evenly microtrichose, setose, about two times as long as gonostylus. Mediobasal lobe subdivided, the dorsal part short, hemispherical, microtrichose, the ventral part slightly shorter than aedeagus, mostly microtrichose, apically with some short setae on clear papillae. Cerci ovoid, with some scattered setae especially near edges. Hypoproct shorter than cerci, deeply notched. Aedeagus cylindrical, with truncate tip.

narrow, microtrichose, with distal pair of setae.

Full-grown larva: Cylindrical, pointed apically, blunt posteriorly, body length 2.2-2.6 mm (n = 5). Second antennal segment short; cervical papillae without setae. Spiracles present dorsally on the 1st thoracic segment and 1st through 8th abdominal segments; six dorsal papillae present on all thoracic and 1st through 7th abdominal segments, each with seta; 8th abdominal segment with two dorsal papillae, each with seta. Sternal spatula anteriorly bidentate with V-shaped emargination (Fig. 11); three inner and two outer lateral papillae present on all thoracic segments, two of each with seta; two and four sternal papillae without setae on all thoracic segments, and from 1st to 8th abdominal segments, respectively. Ventral papillae with seta on all thoracic segments and from 1st to 8th abdominal segments. Terminal segment with eight terminal papillae, each with seta; anus located ventrally; 2 pairs of anal papillae visible, without setae.

Pupa: Body length 2.65–3.06 mm (n = 5). Antennal horn rather developed (Figs. 13 and 14); apical papillae each with long seta; a pair of lower facial papillae each with seta; lateral facial papillae invisible. Prothoracic spiracle (Fig. 15) 68–85 μ m long (n = 7), slightly curved, connected with trachea almost to rounded tip. Spiracles present on 2nd to 6th abdominal segments. Abdominal segments covered with spinules, dorsal spinules slightly wider than ventral ones; three



Figs. 13–15. Scanning electron micrograph of *Dasineura symplocos* n. sp. 13, Ventral view of pupal head; 14, Lateral view of pupal head and thorax; 15, pupal prothoracic spiracle.

rounded areas without spinules present on both lateral sides of 1st to 7th tergites. 1st to 8th tergite each with anterior pair of trichoid sensilla. Four pairs of dorsal papillae present on 1st to 6th abdominal segments, the two outermost pairs and the second inner pairs each with seta; three pairs of dorsal papillae present on the 7th abdominal segment, the two innermost pairs and outer pairs each with seta; two dorsal papillae on the 8th segment, each with seta.

Holotype: 1 d (on slide; slide no. Cecid. ET0101; Type no. 3464, kept in the Entomological Laboratory, Kyushu University, Fukuoka, Japan); Kawayasuyama, Tanegsashima Island, Kagoshima Prefecture, Japan; collected on 2.ii.2006, emerged on 28.ii.2006; KK leg., reared by MT from a leaf bud gall of *S. cochinchinensis*.

Paratypes: All paratypes were collected by KK from Kawayasuyama, Kagoshima Prefecture, Japan and reared by MT from leaf bud galls of *S. cochinchinensis*. $3\overset{\circ}{\supset}$ [on slides; slide nos. Cecid. ET0102-ET0104], $2\overset{\circ}{\subsetneq}$ [slide nos. Cecid. ET0105-ET0106] and 4 pupae [on slides; slide nos. Cecid. ET0107-ET0108]: collected on 2.ii.2006 & adults emerged on 28.ii.2006; $1\overset{\circ}{\subsetneq}$ [on slide; slide no. Cecid. ET0109]: collected on 11.ii.2006 & emerged on 5.iii.2006; 2 full-grown larvae [on slide; slide no. Cecid. ET0110]: collected on 11.ii.2006 & galls dissected on 23.ii.2006.

DDBJ accession numbers: LC120314 -LC120316.

Distribution. At present, this gall midge is only known from Tanegashima Island, Japan.

Etymology. The species name is derived from the host plant genus name *Symplocos*.

Biology. This species is considered to be univoltine, adults emerging in February and March and probably oviposit onto host leaf buds. Galls are 7.5-10.8 mm in diameter (n = 10) and 6.7-9.9 mm in height (n = 10) (Fig. 1). Galls are multichambered and contain about 20 larvae. Pupation takes place inside the galls in spring.

Remarks. The new species is clearly distinct from the described *Dasineura* species and can be separated from them as follows: *Dasineura* have almost no neck beyond the female flagellomere nodes (Gagné *et al.*, 2014) and the 7th abdominal tergite is usually shorter than the 8th and is divided into two longitudinal sclerites (Gagné & Jaschhof, 2014). In contrast, females of the new species have distinct short female flagellomere necks and longer 7th tergite than 8th that is undivided. In *Dasineura, D. erodiicola* and *D. marginemtorquens* also possess undivided female 8th tergite, but is distinguishable from the new species by its shape: rectangular in these two species (Sylvén & Tastás-Duque, 1993) while trapezoidal in *D. symplocos*. In addition, the length of ovipositor is clearly much shorter in *D. erodiicola* and *D. marginemtorquens* (Sylvén & Tastás-Duque, 1993)

than in *D. symplocos*. Therefore, *D. symplocos* is considered as a species new to science.

Discussion

Dasineura is a large cosmopolitan genus in which at least 476 species have been described to date from various host plant groups (Gagné & Jaschhof, 2014). In the genus, *D. symplocos* is the first described species associated with Symplocaceae, though some undescribed species identified as *Dasineura* were recorded from Symplocaceae; e.g. a *Dasineura* sp. associated with leaf and stem galls on *Symplocos uniflora* in Brazil (Maia & Silva, 2013). Further investigations are needed to clarify the diversity of *Dasineura* on Symplocaceae.

So far, the female eighth tergite that is longitudinally divided into two sclerites and longer than the 7th has been regarded as a common morphological feature of Dasineura (Gagné & Jaschhof, 2014). Although D. symplocos does not have these characters, we concluded to treat it as a member of Dasineura in a wide sense. The characters of female post-abdominal tergites in Dasineura are plesiomorphic because some Dasineura species, e.g. D. erodiicola and D. marginemtorquens, have undivided female 8th tergite (Sylvén & Tastás-Duque, 1993). Such a case is not only known in Dasineura within Dasineurini but also in Gephyraulus (Sylvén & Solinas, 1987), indicating that we cannot rely only on the characters of female post-abdominal tergites in creating a new genus separately from Dasineura in Dasineurini. Because Dasineura is a large, polyphyletic and more or less catchall genus, when several more congeners associated with Symplocaceae are found to have some synapomorphies, they, including D. symplocos, may be treated as an independent genus from Dasineura in future.

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