

Larva and Host of the Okinawan Sawfly *Macrophya liukiuana* (Hymenoptera, Tenthredinidae) Determined by DNA Barcodes

Akihiko Shinohara¹, Tatsuya Ide¹ and Masaaki Kimura²

¹Department of Zoology, National Museum of Nature and Science,
4–1–1 Amakubo, Tsukuba, Ibaraki 305–0005, Japan

E-mail: shinohar@kahaku.go.jp/ide@kahaku.go.jp

²GA-SHOW Ltd., Tomari 1-chome 35–5, Naha, Okinawa 900–0012, Japan

E-mail: ga-show.kimura@nifty.ne.jp

(Received 14 May 2020; accepted 24 June 2020)

Abstract Newly discovered sawfly larvae feeding on *Sambucus chinensis* var. *formosana* in Ogimi-son, northern Okinawa-jima Island, have been identified as *Macrophya liukiuana* Takeuchi, 1926, by molecular methods using mitochondrial COI gene sequences (DNA barcodes). This is the first record of the host plant and immature stages of *M. liukiuana*. The larvae are briefly described and illustrated. Bionomics of sawflies was totally unknown in the Ryukyu Islands.

Key words: *Macrophya liukiuana*, larva, new host record, *Sambucus chinensis* var. *formosana*, DNA barcode, Okinawa-jima Island, Ryukyus.

Introduction

The sawfly genus *Macrophya* Dahlbom, 1835, contains 29 species in Japan (Shinohara, 2019, 2020). Inomata (1989) listed host plants for 16 Japanese species, but no additional host records have been published in the last three decades. In the Ryukyu Islands, only two species of *Macrophya* have been recorded (Shinohara, 2015), *Macrophya liukiuana* Takeuchi, 1926, endemic to Okinawa-jima Island and *Macrophya minutifossa* Wei and Nie, in Wei *et al.* (2003), known from vast areas in central, southern and eastern China and Taiwan (Li and Wei, 2013), besides Okinawa-jima Island. Host plants and immature stages are unknown for the two Okinawan species.

In April 2019, Shinohara and Kimura found several sawfly larvae feeding on the leaves of a perennial plant, *Sambucus chinensis* var. *formosana* [Adoxaceae], in Oshikawa, Ogimi-son, northern Okinawa-jima Island. This is the first

finding of any kind of sawfly larvae in the Ryukyus. However, we were not able to identify the species only from the larvae. Kimura made an attempt to obtain the adults for identification by rearing the larvae but failed. In March, 2020, Shinohara and Kimura were able to collect a series of adult specimens of *M. liukiuana* at the same site and they also found numerous larvae feeding on *Sambucus chinensis* var. *formosana* nearby, which may suggest that the larvae possibly belonged to *M. liukiuana*. We have tested this hypothesis by comparing the mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene sequences (DNA barcodes) of the larvae with those of the adults of *M. liukiuana* and finally confirmed the specific identity of the larvae. DNA barcoding has been found useful for identification of the larvae in Symphyta (e.g., Shinohara *et al.*, 2017, 2018; Liston *et al.*, 2020). Here we give the result of the molecular analysis and notes on the larva. This is the first record of the host plant of *M. liukiuana* and the first report on the bionomics of sawflies from the Ryukyu Islands.

Materials and methods

Specimens used in this work are as follows: 2 ♀ 12 ♂, 4 larvae, Oshikawa (ca. 160 m, N26°40'21" E128°8'28"), Ogimi-son, Okinawa-jima Island, Japan, 15. III. 2020, A. & N. Shinohara & M. Kimura, all deposited in the National Museum of Nature and Science, Tsukuba.

Protocols of DNA barcoding, including the DNA extraction, the polymerase chain reaction, sequencing and analyzing, follow Ide and Abe (2019). DNA was extracted from legs in adults or from a part of thoracic muscle in larvae.

Rearing was made by Kimura in a room in Naha city. The temperature was controlled at about 25°C in the room. The larvae were reared in a large plastic container and each matured larva was individually released in a plastic container (200 ml) with soil. For the morphological terminology, we follow Viitasaari (2002). Photographs were taken by Kimura with a digital camera, Olympus Stylus TG-4 Tough. The digital images were processed and arranged with Adobe Photoshop Elements® 9 and 15 software.

Results

Molecular analysis

The sequenced barcode regions (658 bp) of the two adults of *M. liukiwana* and four larval samples were completely identical or different only in one base pair. The result very strongly supported the hypothesis that the larvae belong to *M. liukiwana*. All these sequences have been deposited in the GenBank database under accession numbers MT396212–MT396217.

Host plant

Adoxaceae: *Sambucus chinensis* Lindl. var. *formosana* (Nakai) H. Hara. (new record).

Field observations and rearing records

On April 12, 2019, heavy damage of the leaves of *Sambucus chinensis* var. *formosana* by sawfly larvae was found in Oshikawa, Ogimi-son, northern Okinawa-jima Island. The larvae were soli-

tary external feeders, irregularly eating margins of leaves. Some of the host plants were almost skeletonized (Fig. 1A) by the infestation of the larvae. Kimura collected nine larvae and reared them. The nine larvae matured and went into the soil on April 13 to 18, two larvae on 13, 14 and 17 and one larva on 15, 16 and 18. No adults emerged by April 2020. On April 18, 2020, Kimura examined inside of the soil kept in nine plastic containers and found only one living prepupa in an earthen cell (Fig. 1I–K). Other eight larvae did not survive.

Larva

Middle instar (Fig. 1B–C): Head pale orange, with round spot at top of vertex near posterior margin and large spot including ocellarum and stemmatum black. Trunk translucent opaque whitish, slightly grayish in dorsal half and slightly yellowish in ventral half; large black spot on supraspiracular line in anterior part of each thoracic and abdominal segment; small black spot on subspiracular lobe of meso- and metathoraces and each abdominal segment; large very obscure blackish spot on each surpedal lobe. *Late instar* (Fig. 1D–F): Length ca. 28 mm. Head pale orange, with round spot at top of vertex near posterior margin and large spot including ocellarum and stemmatum black. Trunk dark brownish gray to black in dorsal half above supraspiracular line and slightly yellowish opaque white in ventral half; subdorsal region in abdomen with broad obscure line throughout; large black spot on supraspiracular line in anterior part of each thoracic and abdominal segment; black spot on subspiracular lobe of meso- and metathoraces and each abdominal segment; small blackish spot on surpedal lobe of each abdominal segment. *Mature larva* (Fig. 1G–H): Head pale orange, with ocellarum and stemmatum black. Trunk entirely pale emerald green.

Earthen cell (Fig. 1I–J)

Without fiber and rather easily broken with fingers; inside wall rather smooth.



Fig. 1. *Macrophya liukuana*, damaged leaves of *Sambucus chinensis* var. *formosana* (A), larvae (B–H, K) and earthen cell (I, J), all photographed by M. Kimura.—A, Skeletonized leaves and a larva on a leaf (arrowed), Ogimi-son, April 12, 2019; B, C, middle instar; D–F, late instar; G, last larval molt; H, mature larva; I, J, broken pieces showing inside; K, prepupa found in earthen cell, April 18, 2020.

Discussion

Identification of the adults and larvae

The adult of *M. liukiuana* can be identified by the characters given in Shinohara's key (2020). It belongs to the group of species having entirely black antennae, subhyaline wings without a dark band below the stigma, and the metepimeral appendage without a setiferous basin. The large peculiar creamy white marks on the mesoscutellum and abdominal tergum 1 in both sexes and the creamy white harpe of the male genitalia are quite distinctive.

The larvae of Japanese *Macrophya* are little known. Host plants are known for 16 species in Japan (see below), but larvae have been described or illustrated for only five species (Okutani, 1959; Inomata, 1989). The middle instar larva of *M. liukiuana* (Fig. 1B–C) may resemble the larvae of *M. carbonaria* (Okutani, 1959) and *M. imitator* (Inomata, 1989), but the head capsule has a dorsal black round spot at the posterior margin in *M. liukiuana* (Fig. 1B) whereas such black spots are absent in the latter two species (Okutani, 1959; Inomata, 1989). The late instar larva of *M. liukiuana* may be separated from the known larvae of Japanese *Macrophya* by the dark brownish gray to black dorsal half of the trunk in addition to the black spot on the dorsum of the head (Fig. 1D–F).

Host plant

Host plant records of Japanese *Macrophya* were summarized by Inomata (1989) who listed host plants for 16 species. The host plants include families Adoxaceae, Oleaceae, Rosaceae, Chloranthaceae, Orobanchaceae, Asteraceae and Lamiaceae. Of the 16 species, *M. apicalis* Smith, 1874, *M. infumata* Rohwer, 1925, and *M. carbonaria* Smith, 1874, feed on *Sambucus racemosa* L. subsp. *sieboldiana* (Miq.) H. Hara [Adoxaceae]. *Macrophya liukiuana* is the fourth Japanese species of the genus associated with *Sambucus*.

Of the four Japanese species feeding on *Sambucus*, *M. apicalis* and *M. infumata* are closely

related to each other, having white-tipped antennae and a setiferous basin on the metepimeral appendage (Takeuchi, 1937), whereas *M. carbonaria* and *M. liukiuana*, having entirely black antennae and no setiferous basin on the metepimeral appendage, belong to a group of species very different from the former two species. Gibson (1980) included *M. apicalis* and *M. infumata* in his *trisyllaba* group and *M. carbonaria* in his *epinota* group, both species groups having close host association with *Sambucus* (see also Chevin, 1975). Gibson (1980) examined *M. liukiuana* but did not include it in any species groups. *Macrophya liukiuana* agrees with Gibson's (1980) diagnosis of the *epinota* group in such important characters as the shape of the penis valve and metepimeral appendage, but does not agree with it in the white-marked abdominal sterna of the male and the entirely black postocellar area of the female. The affinity of *M. liukiuana* in the genus is still little known.

Life cycle

So far as we know, sawflies of the genus *Macrophya* are univoltine (Okutani, 1959), with an exceptional case where the larva overwintered twice in the soil (Inomata, 1989; Shinohara, 2015). In univoltine sawflies, the adults usually emerge in spring, the larvae come out shortly, feed on the host leaves for a few weeks, go into the soil on maturity, and enter prolonged diapause usually until next spring, when the pupation takes place in the soil; after a short period of pupal stage, the adults emerge. *Macrophya liukiuana* is expected to follow this pattern of life cycle. The published collection records of *M. liukiuana* are few (Takeuchi, 1926, 1937; Shinohara, 2015) and all known specimens were collected in March (from March 1 to 26). We found the larvae on April 12, 2019, and collected the adults and larvae on March 15, 2020, at the same locality. The occurrence of both the adults and larvae in mid-March suggests fairly a long period of adult emergence, undoubtedly beginning in February. The egg and larval periods are still unknown. *Macrophya liukiuana* is probably a

univoltine species with adult emergence in February to March and feeding larvae in March to April.

Acknowledgements

We thank D. R. Smith (U. S. Department of Agriculture, Washington, D.C.) for his careful review of the manuscript.

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