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Original article

First discovery of winter-emerging leaf-miner: *Phyllonorycter styracis* (Kumata, 1963) (Lepidoptera, Gracillariidae) from Korea with DNA barcode

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ABSTRACT

In this study, *Phyllonorycter styracis* Kumata, which emerges in winter season, is reported for the first time from Korea. Adult and genitalic characters of the moth are briefly redescribed with available information, including its distributional range and host plant. In addition, DNA barcoding for correct identification of the species is presented.

Introduction

The family Gracillariidae constitutes the major group of leaf miners; so far, 1,880 species have been described from this family worldwide (De Prins and De Prins 2012). Among the genera in Gracillariidae, *Phyllonorycter* is one of the largest groups comprising 401 described species (De Prins and De Prins 2005, 2012). They are generally small and shiny moths showing the characteristic behavior in sitting posture, that is, the body lying parallel to the surface when lowering the head (Davis and Robinson 1999).

In Korea, the first record of the genus *Phyllonorycter* was *Phyllonorycter ringoniella*, listed in "List of Forest Insect Pests in Korea" (Ko 1969) under the name *Lithocolletis ringoniella*. Later, Shin et al (1983) reported 10 species for the genus: *Phyllonorycter nipponicella*, *Phyllonorycter acutissimae*, *Phyllonorycter kamijoi*, *Phyllonorycter aino*, *Phyllonorycter issikii*, *Phyllonorycter koreana*, *Phyllonorycter pastorella*, *Phyllonorycter melacoronis*, *P. ringoniella*, and *Phyllonorycter ulmi*. In addition, Kumata et al (1983) listed 11 species of *Phyllonorycter* adding one species to the aforementioned list: *P. similis*. Park and Han (1986) reported three new species: *Phyllonorycter leucocorona*, *Phyllonorycter orientalis*, and *Phyllonorycter pygmaea*. Thus, 14 species have been known from Korea at present. In addition, one species of Phyllocnistidae, which is closely related to the family Gracillariidae, has been known from Korea (The Entomological Society of Korea and Korean Society of Applied Entomology 1994; Byun et al 2009; Paek et al 2010; Park et al 2012). Recently, *Phyllocnistis citrella* Stainton, belonging to Phyllocnistidae, has become a notorious pest affecting citrus trees in the southern regions of Korea (Lee et al 2015; Kim et al 2015).

In this study, we found a species of *Phyllonorycter*, *P. styracis* Kumata, which was first collected in October 2014 from Mt. Palgong, which is located in the middle-eastern part of Korea, and then re-emerged in January 2015. This species is known to be endemic to Japan until now, but has been reported for the first time from Korea in this study. Adult and genitalic characteristics are briefly redescribed, and available information including the distributional ranges and host plants are also discussed. In addition, DNA barcode for the correct identification of the species was extracted and sequenced in this study.

Materials and methods

All materials examined in this study were collected from Mt. Palgong, Korea, and now preserved at the Systematic Entomology

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Figure 1. Adult and pupa of *Phyllonorycter styracis* (Kumata, 1963). A, adult; B, pupa after emerging.

Laboratory, Hannam University, Daejeon, Korea. Adults of both sexes were dissected for examination of genitalic structures and samples were fixed on glass slides with Euparal mountant in accordance with the procedure recommended by Holloway et al (1987). The images for the species were taken using a digital camera attached on the microscope (Leica M205 C; Leica Microsystems, Wetzlar, Hesse, Germany).

Cytochrome c oxidase subunit I gene was extracted for DNA barcoding according to the protocols of Canadian Center for DNA Barcoding (Biodiversity Institute of Ontario, University of Guelph, Guelph, ON, Canada) and the DNA was amplified using the primer LepF1 (attcaaccaatcataaagatattgg)/LepR1 (taaacttctggatgtc caaaaaatca; Hebert et al 2004). Polymerase chain reaction conditions for amplification were as follows: at 94°C for 5 minutes, 5 cycles of 94°C at 30 seconds/45°C at 30 seconds/72°C at 1 minute, 40 cycles of 94°C at 30 seconds/51°C at 30 seconds/72°C at 1 minute, and 72°C at 7 minutes. All sequences were assembled with CodonCode aligner version 2.0.6 (CodonCode Co., Centerville, MA, USA) after the amplification.

Systematic accounts

Order Lepidoptera Linnaeus, 1758
Family Gracillariidae Stainton, 1854
Subfamily Lithocolletinae Stainton, 1854

Genus *Phyllonorycter* Hübner, 1822
Type species: *Phalaena rajella* Linnaeus, 1758
= *Phyllonorycter* Hübner, 1806
= *Lithocolletis* Hübner, 1825
= *Eucestis* Hübner, 1825
= *Eucesta* Hübner, 1826
= *Hirsuta* Bruand, 1851
= *Lithocolletes* Dyar, 1903
= *Phyllonorycter* Lord Walsingham (de Grey), 1914
= *Hirsuta* Fletcher, 1929

***Phyllonorycter styracis* Kumata, 1963** 때죽나무가는나방 (신칭)

Lithocolletis styracis Kumata, 1963: 56–58. TL: Hikosan, Kyushu, Japan. Holotype: Entomological Laboratory, Kyusyu University.

Adult (Figure 1A). Wingspan 7–8 mm. Length of head and thorax 1.5–1.7 mm. Head smooth with shiny white scales on frons; apex of the head with tufts of rough scales, mixed with white and yellowish brown; ocelli absent and compound eye blackish; antennae ciliate and covered with pale brown scales on the edge of each segment; scape with long scales; haustellum elongate; labial palps straight with white scales. Thorax covered with goldish yellow scales and two whitish vertical streaks. Forewing slender; ground color yellowish brown with white stripes; basal dash white and three pairs of white stripe repeated across up and down with

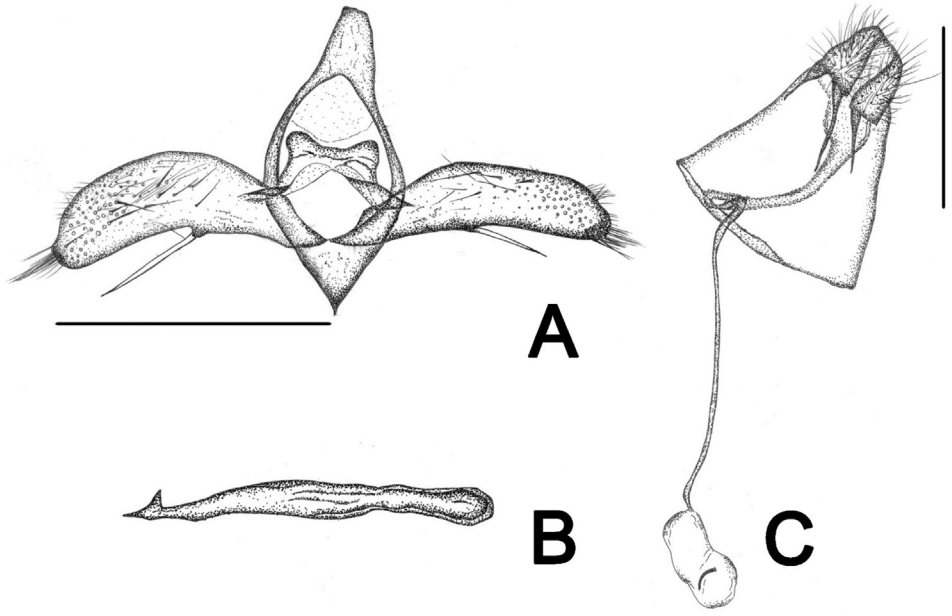

Figure 2. Male and female genitalia. A, male genitalia; B, aedeagus; C, female genitalia. <scale bars: 0.5 mm>

Figure 3. Habitats and leaf mines of *Phyllonorycter styracis*. A, the habitats of the species in Mt. Palgong, Korea, November 2015; B, *P. styracis* mine on the host plants.

blackish spot; whitish "u" on the apex of forewing; blackish brown scales scattered in the outer margin with long pale brownish cilia. Hindwing lanceolate and more narrow than forewing; ground color pale gray with long silver hairs in the outer margin; a vivid brown spot in the proximal region of hindwing.

Male genitalia (Figures 2A and 2B). Uncus absent; tegument narrow and membranous; tuba analis extremely thin membrane; vinculum as long as the upper side of genitalia and Y shaped; saccus absent; valva moderate and asymmetrical; left valva large, 1.4 times than right, with setae on two-third of valva to terminal margin; a pair of pectinifer in one-third of valva. Aedeagus narrow and more slender toward apex; cornuti absent and hook on the apex.

Female genitalia (Figure 2C). Palpillae anales asymmetrical, moderate, and small with a dozen of long hairs. Apophyses posteriores short but as long as apophyses anteriores. Ostium bursae relatively elongate, gradually narrowed downward. Ductus bursae fairly slender, membranous, and as long as three times of corpus bursae. Corpus bursae membranous and ovate with sclerotized projections of signum laterally.

Material examined. 2♂3♀, Mt. Palgong, Daegu, South Korea, 24 × 2014 (leg. BK Byun)-coll. SEL/HNU-5189, 5190, 5205; 1♀, Mt. Palgong, Daegu, South Korea, 12 ii 2016 (leg. BK Byun)-coll. SEL/HNU.

Distribution. Korea (South, new record), Japan (Kyushu, Honshu).

Host plants (Figure 3B). Styracaceae: *Styrax japonicus* Sieb. & Zucc. (Kumata 1963). Ptychonomous mine on the upper side of leaves (De Prins and De Prins 2005).

Biology (Figures 1B and 3A). Adults emerged in winter (in January) after the larval stage in fall. Before the emergence, pupae escape from the leaves of host plants. In Japan, this species overwinters in the pupal stage, and emerges in the spring of next year (Hirowatari 2013).

DNA barcode. Cytochrome c oxidase subunit I gene was extracted and sequenced. The sequence of *P. styracis* (Kumata) in Korea is as follows (655 bp)/Genbank accession no.: KY170862:

AGATATTGAACTCTTATTTATTTTGGAATTGAGCAGGAATAATTGATC
TTCTCTAAGAATTATAATTCGAGCTGAATTAGGAAATCCAGGATCTTTAA
TCGGAGACGATCAAATTTATAATACAATTGTAACAGCTCATGCATTTATC
ATAATTTTTTTCATAGTAATACCTATTATAATTGGAGGATTTGGAAATTGA
CTTGTTCCTCTAATACTCGGAGCTCCAGACATAGCTTTCCCCCGACTTAAT
AACATAAGATTTTGATTATTACCTCCCTCATTATTATTATTAGTTTCAAGA
AGAATTGTAGAAAATGGAGCAGGTACTGGTTGAACTGTTTACCCTCCTTT
ATCTTCTAATATTGCTCACGCTGGTAGATCTGTAGATTTAGCTATTTTTTC
CCTTCACCTTGCAGGAATTTCCTCAATCTTAGGGGCTATTAATTTTATTAC
AACTATTATTAATATACGAACTAATGGTATAAAATTTGATAATATACCTCT
ATTTGTATGAGCTGTAGGTATTACAGCCTTACTTTTATTATTATCATTACC
TGTATTAGCAGGAGCAATTACTATATTACTAACAGACCGAAATTTAAATA
CATCCTTTTTTGATCCTGCTGGAGGAGGAGATCCAATTTTATATCAACAC

Discussion

P. styracis (Kumata) was described from Japan as an endemic species. In this study, we found the species for the first time from the middle-eastern part of Korea. The host plant, *S. japonicus* Sieb. & Zucc., is distributed mainly in the southern regions of Korea. The collecting sites were located near the valley mixed with *Quercus* tree community and the host plants found in the understory vegetation of the *Quercus* trees. The larvae mine the leaves of the host plants forming an oval mine between the veins of leaves. The life cycle of the species was not well studied, but it is interesting to have an emergence season in winter. In this study, we collected the pupae from the fallen leaves of host plants in October 2014 and adults emerged in January 2015. In Japan, the species emerges in October, November, and early spring. However, there is a need to investigate their correct flying season and the reason for having the cold season for their emergence.

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