REPORT OF THE

Two Months Attachment Program on Diagnostics of Leafminers of Agricultural Importance

at
Department of Biological Sciences, Faculty of Science,
Nara Women's University, Nara, Japan

August 01 – September 30, 2016

by

Dr. Hiroki Sato

Organized by:

Nara Women's University
Nara, Japan

In Collaboration with:

bionet aseanet

ASEAN Network on Taxonomy

2016

The Report of the Follow-up Attachment Program
at
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Background

Leafminers are an insect group larvae of which feed on leaf tissues within leaves during a part or the whole of their immature stage. Some species are well-known as pests of vegetables, fruit trees and ornamental plants. Occasionally a leafmining species invades non-native area and becomes a pest there, although it is not a pest its native areas. For example, a gracillariid moth, *Phyllonorycter issikii*, invaded from Eastern Asia to Europe in 1970's and cause serious damage to ornamental lime trees in several countries. It is equally possible that an alien leafminer becomes a pest in South-East Asia. In that case, prompt identification of the leafminer is crucial for application of available control measures. However, there are few researchers on taxonomy and ecology of leafminers in the ASEAN region.

Before the attachment program. a "Training Workshop on Diagnostics of Leafminers of Agriculture Importance" was held at Research Center for Biology, Indonesian Institute of Science, Cibinong, Bogor, from 29 February to 11 March 2016, under Japan-ASEAN Integration Fund through the ASEAN Plant Health Cooperation Network of the ASEANET. I joined this workshop as a resource person to introduce taxonomy and ecology of leafminers and to give instruction on the basics of how to collect leafminers and make genitalia preparations. At the same time, I was invited to organize the "Follow-up Attachment Program" for two months at my laboratory for two selected participants from the workshop.

Objectives

At the "Training Workshop on Diagnostics of Leafminers of Agriculture Importance", all the 20 participants learnt basic knowledge and skills for identification of leafminers. However, their acquisitions are insufficient to identify leafminers, because leafminers are very diverse taxonomically, morphologically and ecologically. Objectives of this "Follow-up Attachment Program" for selected participants are to improve their knowledge and skills for the study of leafminers and to encourage them to contribute towards development of this field in ASEAN region.

Contents of the program

1) Nara Women's University

I gave the two participants, Ms. Ariene GARCIA-CASTILLO and Dr. Yuvarin BOONTOP, lectures and practical courses about the following topics when the two participants were in Nara:

a) Lectures on leafminer taxonomy, morphology and ecology using articles and specialist books
b) Collecting leafminers in natural fields in the vicinity of Nara City
c) Rearing leafminers in the laboratory
d) Making genitalia preparations

In addition, the participants joined a one-day program for learning ancient Nara for foreign students of Nara Women's University. Furthermore, Dr. Y. Nasu took us to the beech forest of Mt. Izumi-Katsuragi, which is the southernmost and isolated beech forest in Japan. We collected leafminers there.

2) Kyoto Prefectural University

We visited Kyoto Prefectural University on 15-16 and 19 August. Dr I. Oshima gave lectures and practical courses about the following topics:

a) Phylogeography on the basis of DNA sequence data.
b) How to use software for aliment of DNA sequences and analysis of population genetic structure and phylogeography
c) How to extracting DNA, amplify COI barcoding region by PCR, purify PCR products and sequence COI barcoding region.
d) How to keep a lepidopteran leafminer, *Acrocercops transecta*, through successive generations in the laboratory.

3) Osaka Prefectural University

We visited Entomological Laboratory of Osaka Prefectural University on 31 August. Drs. N. Hirai and Y. Yoshiyasu showed us around insect specimen stock rooms and gave a short lecture on their taxonomic work on small moths of Thailand.

4) Natural History Museum of Osaka City

We visited Natural History Museum of Osaka City on 1 September. Dr. I. Kanazawa showed us around its insect stock room and explained its activities.

5) Kyushu University

We visited Biodiversity Laboratory and Entomological Laboratory of Kyushu University on 6-8 September. Professors Y. Abe and T. Hirowatari gave lectures and practical courses about the following topics:

a) Two invasive dipteran leafminers, *Liriomyza trifolii* and *L. sativae*, in Japan
b) Taxonomy of Japanese lepidopteran leafminers
c) Observation of genitalia of *Liriomyza trifolii* and *L. sativae*
d) Observation of the larva of *Gronotoma micromorpha* (Hymenoptera, Eucoilidae) attacking dipteran leafminer *Liriomyza trifolii* and *L. sativae*
e) How to keep dipteran leafminers, *Liriomyza trifolii* and *L. sativae*, and their parasitoid, *Gronotoma micromorpha*, through successive generations in the laboratory
f) How to identify the families at basal clades of lepidopteran phylogeny.

In addition, they showed us around its insect stock rooms, which boast the largest number of insect specimens in Japan.

6) Agriculture Research and Developmnet Center of Nara Prefecture

We visited Agriculture Research and Development Center of Nara Prefecture on 12 and 13 September. Mr. I. Takenaka and his colleagues offered us the following observation tours to learn how dipteran leafmininers *Liriomyza trifolii* and *L. sativae* were controlled.

a) Greenhouses in the center
b) Local farmers' greenhouses in which tomatoes and spinach are cultivated
c) A local farmer's field of long onions

7) Hokkaido University

We visited Hokkaido University on 21-23 September. Professor M.T. Kimura took us to the oak forest along Ishikari Coast and introduced us to leafminers attacking *Quercus dentata*. We collected leafminers from oaks in the forest and brought them back to the laboratory to transfer them in rearing boxes. Those leafminers were reared in the Sato's laboratory.

Furthermore, we visited the University Museum. Professor M. Ohara showed us around its insect stock rooms, which boasts the largest number of insect specimens in Japan.

8) Apple Research Institute of Aomori Prefecture

We visited Apple Research Institute of Aomori Prefecture on 26 and 27 September. Mr. Y. Ishiguri gave us lectures on the history and activities of the institute and on an apple leafminer, *Phyllonorycter ringoniella* and its control. He also took us around its apple field and explained how apple trees were protected from pests including apple leafminers.

Outcomes

1) Lectures and field tours enabled the two participants to learn more advanced knowledge about taxonomy and ecology of leafminers.
2) Practical courses enabled them to acquire more advanced techniques for leafminer identification.

3) Visits to insect stock rooms in several museums and universities enhanced their understanding of insect specimen management.
4) The field tours in Agriculture Research and Development Center of Nara Prefecture and Apple Research Institute of Aomori Prefecture improved their knowledge about methods for leafminer control.
5) The short program for learning ancient Nara for foreign students of Nara Women's University and friendship with students of Sato's laboratory deepened participants' knowledge about the history and culture of Japan.

Request

When examining specimens of microlepidopteran moths such as leafminers, we need specific equipment for them, e.g., rearing cases, minute pins, watch dishes, setting boards and cases for storing the boards. These items are expensive and difficult to purchase in participants' countries. In particular, watch dishes and setting boards and cases are very expensive and even impossible to obtain outside Japan because they are handmade by Japanese craftsmen. Hence I was unable to give the participants these items as gifts when they went back their countries. Although I should have requested expenses for those items from JAIF, I hesitated to do it because I was afraid that JAIF rejected the request. I hope that JAIF appropriates sufficient expenses for specific items in the budget.

Acknowledgments

I thank JAIF for its financial support to this program and Dr. S.S. Sastroutomo for all his help. I also thank Prof. T. Hayashii (the dean of Faculty of Science, Nara Women's University), Dr. G. Tanigawa (the director of Agricultural Research and Development Center of Nara Prefecture) and Dr. S. Arai (the director of Apple Research Center of Aomori Prefecture) for their acceptance of the participants as visiting scientists. This program would not have been conducted without helps of Drs. Y. Nasu, I. Ohshima, Y. Yoshiyasu, N. Hirai, Y. Abe, T. Hirowatari, M.T. Kimura, M. Ohara, and Mr. I. Takenaka and Y. Ishiguri. I am appreciative of all their efforts.

SEVERAL PICTURES TAKEN DURING THE ATTACHMENT

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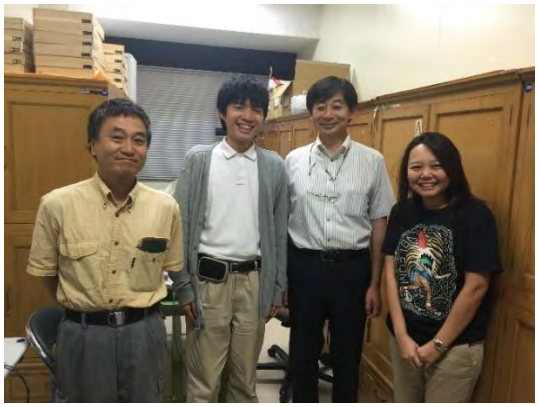
1. Collection of leafminer samples from *Quercus* spp. (Japanese emperor oak) and hybrids at Ishikari Coast, 22 September 2016
2. Dr Hiroaki Sato demonstrating the preparation of genitalia for leafminer identification at Department of Biological Sciences, Faculty of Science, Nara Women's University, 4-5 August 2016



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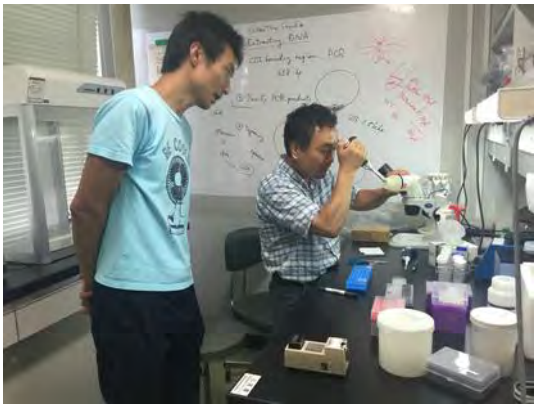

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3. Dr Hiroaki Sato, Mr Taguchi Daisuke , Dr Yoshihisa Abe and Dr Yuvarin Booptop at the Faculty of Social and Cultural Studies, Kyushu University (Ito Campus), 7 September 2016 (after the attachment training).

4. Dr Hiroaki Sato conducting the DNA extraction of leafminer for molecular identification with Dr Issei Ohshima's supervision at the Section of Applied Entomology, Department of Life and Environmental Sciences, Kyoto Prefectural University, 15-16 August 2016.

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5. Dr Hiroaki Sato, Mr Taguchi Daisuke , Dr Yoshihisa Abe and Ms Ariene Garcia-Castillo at the Faculty of Social and Cultural Studies, Kyushu University (Ito Campus), 7 September 2016 (after the attachment training).
6. Participants with Dr Hiroaki Sato, Dr Yoshinori Kunimoto, Dr Isao Takenaka and Dr Takeo Imura in one of the field visits at the Nara Prefecture Agriculture Research and Development Center, 13 September 2016.

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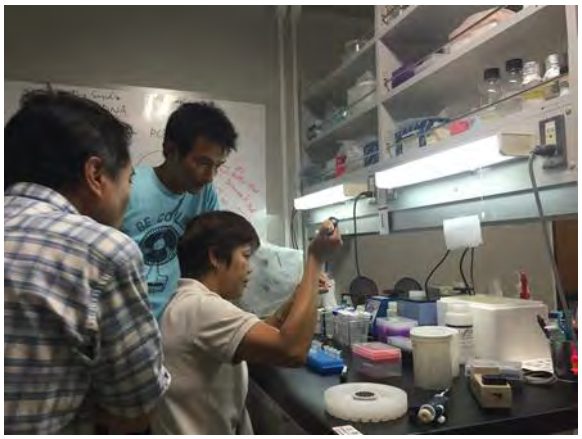
7. Participants with the Staff of Nara Prefecture Agriculture Research and Development Center, 13 September 2016 (after the Welcome Dinner hosted by the Nara Pref. Agr. Staff).
8. Collection of leafminer samples at Ishikari Coast, 22 September 2016.



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9. Hands-on practice of DNA extraction of leafminer for molecular identification at the Section of Applied Entomology, Department of Life and Environmental Sciences, Kyoto Prefectural University, 15-16 August 2016 (On photo: Dr Sato, Dr Ohshima and Ariene).

10. Participants with Dr Hiroaki Sato at the The Kasuga Grand Shrine during the Special Lecture on Nara's History and Excursion to the World Heritage Complex in Nara, 20 August 2016