

JAIF PROJECT PROGRESS REPORT

PROJECT TITLE:	Taxonomic capacity building to support market access for agricultural trade in the ASEAN region	
PROJECT PROGRESS REPORT:	<input type="checkbox"/> 1st <input type="checkbox"/> 2nd XXX 3rd <input type="checkbox"/> 4th <input type="checkbox"/> 5th	
PROJECT START AND END DATES	From: MAY 2015	To: APRIL 2017
PERIOD COVERED BY THIS REPORT:	From: 02 MAY 2016	To: 31 OCTOBER 2016
IMPLEMENTING AGENCY:	ASEAN Plant Health Cooperation Network (APHCN) - ASEANET	
CONTACT PERSONS:	Names: DR LUM KENG YEANG (Chairperson & Project Manager) & DR SOETIKNO S. SASTROUTOMO (Technical Secretary) Tel: +60-3-8943-2921 Fax: +60-3-8942-6490 E-mail: ky.lum@cabi.org AND s.soetikno@cabi.org	
<p>OVERVIEW: Briefly describe: (i) the objective of the project; (ii) progress in project implementation to date; (iii) any particular issues faced and/or results achieved during this reporting period.</p> <p>(i) Overall objective: The project will develop and strengthen capacities in taxonomic knowledge to identify and manage quarantine risks associated with agricultural commodities and to accurately diagnose pests and diseases among the ASEAN Member States (AMS).</p> <p>Intermediate objective: To increase taxonomic capacity of scientists/officers from AMS in 3 groups of insect pests and diseases, i.e. in plant viruses, aphids and leaf miners of agricultural importance.</p> <p>(ii) Progress till April 2016: Only one activity has been carried out in the period from May to October 2016, i.e. on Activity 1.2. Attachment Program on Diagnostics of Leafminers of Quarantine Importance held in the Department of Biological Sciences, Faculty of Science, Nara Women's University, Nara, Japan from 1 August - 30 September 2016. In addition the website for this project has been updated with the uploading of the 1st & 2nd Progress Reports of the Project, training materials, and reference materials and reports of the attachment program. The project briefs were distributed to the JAIF Leafminers workshop participants and also to participants of the 3 (three) AANZ-FTA workshops, i.e. on DNA extraction and Barcoding in Indonesia and on Diagnostics of Diptera in Thailand.</p> <p>One activity related to Diagnostics of Leafminers (training workshop and attachment program), due to unavailability of the main Japanese resource person, has been dropped and will be replaced by the same on Diagnostics of Weevils of Quarantine Importance. The approval for this change has been granted by JAIF.</p>		

(iii) Results:

1. Report of the Attachment Program in Japan
2. Project Website (<http://aseanet.org/JAIF1.asp>)
3. Tentative Program for the Training Workshop on Diagnostic of Weevils
4. Progress Report to the 18th Meeting of ASEAN EWG-PS

PART A: PROGRESS & RESULTS

A. PROGRESS & ACHIEVEMENTS:

Describe progress in implementation during this reporting period, including key outputs/outcomes, based on the approved project document.

Attachment Program in Nara Women's University, Nara, Japan

The attachment program was held at the Department of Biological Sciences, Faculty of Science, Nara Women's University, Nara, Japan from 1 August - 30 September 2016 with the objective to improve their knowledge and skills for the study of leafminers and to encourage them to contribute towards development of this field in the ASEAN region.

The two plant health/biosecurity officers, one each from Singapore and Thailand, who participated in the attachment program are:

1. Ms Ariene G. Castillo, Scientist, Management Executive 12, Animal and Plant Health Centre, Agri – Food & Veterinary Authority, Singapore
2. DR Yuvarin Boontop, Senior Entomologist, Insect Taxonomy Group, Plant Protection Research Department of Agriculture, Bangkok, Thailand

During the attachment program they were supervised by Assoc. Prof. Hirosaki Sato from the Department of Biological Sciences, Faculty of Science, Nara Women's University, Nara, Japan. The program of the 2 months attachment consisted of:

- a). Series of lectures given by resource persons and invited speakers,
- b). Laboratory work supervised by Dr. Sato and others scientists on the preparation of genitalia slides and molecular study of leafminers
- c) Field studies covering several field collections of leafminers from different locations and habitats
- d) Specimen collection study in different insect museums, i.e. Entomological Laboratory of Osaka Prefecture University, Natural History Museum of Osaka City, etc.
- e) Other activities such as visit and discussion with other scientists working on leafminers from universities and private/national research institutions in Japan.

The full reports prepared by Dr. Sato and the 2 participants are given in the **Attachments 1-3**.

Training Workshop on Diagnostics of Weevils of Quarantine Importance

The proposed training workshop will be organized in the Philippines with the collaboration of the Crop Protection Cluster, UPLB, Los Banos (contact person Ass. Prof. Dr. Sheryl A. Yap) from July 10-22, 2017. The resource person from Japan would be Dr. Hiraku Yoshitake from the Institute of Agro-Environmental Sciences, NARO, Tsukuba.

The tentative program of the training workshop is given in the **Attachment 4**.

Project Brief

Twenty five (25) copies each of the Project Brief has been distributed to participants at the following events:

1. AANZFTA - Workshop on DNA extraction and Barcoding (Bekasi, Indonesia, 23-27 May 2016)
2. AANZFTA – Workshop on Identification of Pest Diptera (flies) of Agricultural Importance (Chiang Mai, Thailand, 6-10 June 2016)

JAIF Project Website – Component 2 of the Project (see Attachment 5)

Objectives of this component include information sharing, information dissemination and mainstreaming and institutionalisation of information through networking. The information gained through networking and exchange of information becomes embedded and enters the common knowledge domain of respective institutions and NPPOs of countries concerned.

Latest project activities updated with announcements of the attachment programs on plant virology held in Tokyo University of Agriculture, Japan and diagnostic of leafminers in Nara Women University, Japan have been uploaded. The meeting of the Expert Working Group on Harmonisation of Phytosanitary Measures in ASEAN was uploaded and announced in this section as well. All relevant outputs from the attachment programs and meeting have been uploaded and made available for downloading. Images from activities have been uploaded into the picture gallery. Sourcing for better images to populate the gallery is on-going.

The expert register is currently being updated to include participants of workshops on plant viruses and leafminers held in Indonesia and the Philippines. Relevant details will be included in the register. The mobile version of the register is being further tested on different devices (subject to availability of devices) to ensure that it is formatted and displayed correctly.

18th Meeting of the ASEAN EWG-PS, Vientiane, Laos, 18-19 July 2016

Dr. Soetikno S.S. participated and presented a Progress Report of the Project to the Meeting. A concept note on the proposed Phase 2 Project was also given and endorsed by the Meeting (See **Attachment 6**).

B. TIMEFRAME AND BUDGETING

Explain whether the project is on-track with regard to: (i) the budget; and (ii) the original timeframe. If either the expenditures and/or timeframe are off-track, please explain and describe the corrective actions being taken.

Project implementation is behind schedule, as a result of the cancellation of the Training Workshop and Attachment Program on Diagnostics of Aphids which would have started in September 2016. The key Japanese resource person on aphids, due to his full commitment with the university, has had to withdraw his involvement as the resource person for this program. However, we managed to organize replacements for these 2 activities, i.e. on Diagnostics of Weevils, which was approved by JAIF for implementation in July 2017.

Budget expenditures are also on track and without any over-spend in all activities that have been carried out so far (**Attachment 7**).

C. OTHER IMPLEMENTATION ISSUES

Describe any significant changes to the project design, context or partners during the reporting period, or any other issues faced, and actions that are being taken in response, if appropriate.

Training Workshop and Attachment Program on Diagnostics of Aphids have been replaced with Diagnostic of Weevils to be implemented in July 2017.

D. OTHER COMMENTS:

Please provide any other relevant information or observations on the project, e.g. on lessons learned, particular challenges or issues that may arise in the next reporting period, changes to the logframe, etc.

None.

Provide a list of key documents (e.g. mission reports, training materials, workshop reports, etc.) produced during this reporting period. Copies of the final versions of these documents should be attached to this report.

1. Report of the Attachment Program on Diagnostics of Leafminers (Attachments 1-3)
2. The Proposed Program of the Training Workshop on Diagnostics of Weevils of Agricultural Importance (Attachment 4)
3. Report of the activities of Component 2 – Project Website (Attachment 5)
4. Progress report to the 18th Meeting of ASEAN EWG-PS (Attachment 6)

E: FINANCIAL OVERVIEW (SEE ATTACHMENT 7)

	JAIF*	In kind / Other**	Total
a) Total project budget (US\$)	892,354.10	-	892,354.10
b) Total amount received to date (US\$)	713,883.28	-	713,883.28
c) Total expenditure during the reporting period*	78,898.34	-	78,898.34
d) Total expenditure to date (US\$)	328,414.84	-	328,414.84
e) Unspent funds a) – d) (US\$)	385,468.44	-	385,468.44



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:



ASEAN Network on Taxonomy

2016

The Report of the Follow-up Attachment Program
at
Department of Biological Sciences, Faculty of Science,
Nara Women's University, Nara, Japan
1 August - 30 September 2016
by
Dr. Hiroaki SATO,
Nara Women's University

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 graccillariid 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 alignment 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, Eucolidae) 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 Development 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 leafminers *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

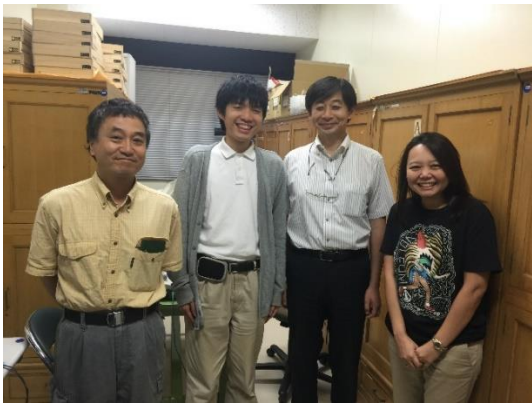


1

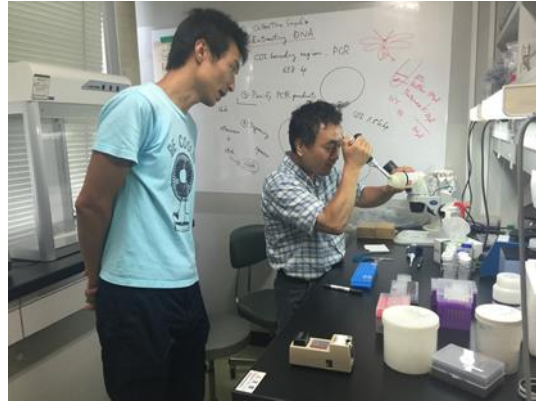


2

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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4

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.



5

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

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.



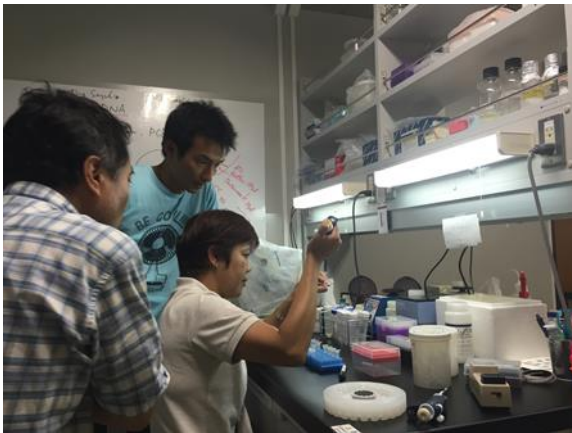
7

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

8. Collection of leafminer samples at Ishikari Coast, 22 September 2016.



9

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).



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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



REPORT OF THE
Two Months Attachment Program on
Diagnostics of Leafminers of Agricultural
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at
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August 01 – September 30, 2016

BY
Ariene Garcia Castillo
Singapore

Organized by:



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2016

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ATTACHMENT PROGRAM

**Japan-ASEAN Integration Fund (JAIF) and ASEAN Plant Health Cooperation Network (APHCN) of ASEANET Training on Leafminers of Agricultural Importance.
(JAIF Funded Project on Taxonomic Capacity Building to Support Market Access for Agricultural Trade in the ASEAN Region)**

Venue:

Nara Women's University, Japan

Duration:

1 August to 30 September, 2016

Participant Name & Position:

Ms Ariene Garcia Castillo, Senior Scientist

Institutional Address and Country:

Agri-Food & Veterinary Authority of Singapore
Animal & Plant Health Centre, 6 Perahu Road
718827 Singapore

1. BACKGROUND INFORMATION

The ASEAN Plant Health Cooperation Network (APHCN) – ASEANET Project “**Taxonomic capacity building to support market access for agricultural trade in the ASEAN region**”, funded by the Japan ASEAN Integration Fund (JAIF) successfully held its second capacity building activity, entitled “Training Workshop on Leafminers of Agricultural Importance”, from 29th February to 11th March 2016 at Museum Zoology, LIPI, Cibinong, Indonesia. Under this ASEAN-endorsed Project, three outstanding participants from the training workshop were selected for a two-month attachment programme based on the endorsement by the resource persons involved in the training workshop, participant’s learning attitude and ability to contribute as a resource person for ASEAN. This two-month attachment was part of the 2nd Phase workshop on Diagnostics Training on Leaf miners of Agricultural Importance organised by the ASEAN Plant Health Cooperation Network (APHCN) of ASEANET to improve the diagnostic capability and the enhancement of development of other diagnostic resources in ASEAN. This is also in line with the APHCN-ASEANET project “Taxonomic capacity building to support market access for agricultural trade in the ASEAN region”. The selected participants (2 from Thailand and Singapore) were attached to the laboratories of Assoc. Prof. Hiroaki Sato at the Nara Women University, Nara, Japan from 1 August to 30 September 2016, while another participant (from Brunei Darussalam) would be attached to Dr. Hari Sutrisno at LIPI, Cibinong, Indonesia for more in-depth technical training. Through this specialised in-depth attachment, the participants would be able to gain more in depth knowledge on taxonomical identification of economically important leaf miners to broaden their diagnostic capability and competency to handle all pest diagnosis. The acquired information would be useful to build up the confidence and skills of the participants thus contributing to capacity development of their Department or their representative country.

2. OBJECTIVES OF THE ATTACHMENT

The objectives of the attachment programme are:

1. To transfer skills and knowledge of Japanese experts on Leaf miners to counterparts in ASEAN countries so as to promote confidence of the participants in the diagnostics of pests and taxonomic identification.
2. To strengthen the diagnosis capacity by providing participants with practical understanding of the concept of leaf miners, identification of important leaf miners and their management practices, and
3. To tap the selected participants that would undergo more intensive technical training towards being a potential ASEAN resource person on identification of

leaf miners to provide them gained expertise to ASEAN member states and the ASEAN Diagnostic Network.

3. PROGRAM OF ATTACHMENT (TIME TABLE)

2016		Programs	
Aug.	1	Mon.	Arrival of Participants at Kansai International Airport
			Move to Nara Women's University (NWU)/Settle Down
	2	Tue	Briefing on Programmes and Activities (AM) Collection of Leafminers in Hills of Nara City (PM)
	3	Wed	Study Taxonomy of Leafminers
	4	Thu	Slide Mount Preparations of Leafminer Genitalia in NWU
	5	Fri	
	6	Sat	
	7	Sun	
	8	Mon.	Slide Mount Preparations of Leafminer Genitalia in NWU
	9	Tue	
	10	Wed	
	11	Thu	Collection of Leafminers in Hills of Yata, Nara
	12	Fri	Slide Mount Preparations of Leafminer Genitalia in NWU
	13	Sat	Slide Mount Preparations of Leafminer Genitalia in NWU (AM)
	14	Sun	
	15	Mon.	Visit to Dr. Issei Ohshima's Laboratory at Kyoto Prefecture University and Learn DNA Sequencing Techniques
	16	Tue	
	17	Wed	Slide Mount Preparations of Leafminer Genitalia in NWU
	18	Thu	
	19	Fri	Visit to Dr. Issei Ohshima's Laboratory, Department of Life and Environmental Sciences at Kyoto Prefecture University and Learn DNA Sequencing Techniques and Rearing Methods
	20	Sat	Special Lecture on History of Nara and Excursion to the World Heritage Complex in Nara
	21	Sun	
	22	Mon.	Review of Articles and Appointment with NWU Account Section
	23	Tue	Slide Mount Preparations of Leafminers in NWU
	24	Wed	
	25	Thu	Collection of Leafminers in Mt. Takamado, Takana, Nara
	26	Fri	Slide Mount Preparations of Leafminers in NWU (AM) Discussion with Dr Lum Keng Yeang and Dr Soetikno S Sastroutomo on Progress of Attachment & 2nd Phase of Project (PM)
	27	Sat	
	28	Sun	
	29	Mon.	Collection of Leafminers in Mt. Izumi-Katsuragi
30	Tue		
31	Wed	Visit to Entomological Laboratory of Osaka Prefecture University	

Sep.	1	Thu	Visit to Natural History Museum of Osaka City
	2	Fri	Learn Taxonomy of Leafminers in NWU
	3	Sat	
	4	Sun	
	5	Mon.	Learn Taxonomy of Leafminers in NWU
	6	Tue	Move to Hakata, Fukuoka Prefecture
	7	Wed	Visit to the Entomological Laboratory, Faculty of Social and Cultural Studies Kyushu University (Hakozaki Campus) and Learn Taxonomy of Leafmining Diptera
	8	Thu	Visit to the Biosystematic Laboratory, Faculty of Agriculture of Kyushu University (Ito Campus), and Learn Taxonomy of Leafmining Lepidoptera
	9	Fri	Move Back to Nara
	10	Sat	
	11	Sun	
	12	Mon.	Visit to the Nara Prefecture Agriculture Research and Development Center, Farms in Sakurai City, and Kashihara City Insectarium in Ohwuda City to Learn about Protection of Crops from Leafminers.
	13	Tue	
	14	Wed	Learn Taxonomy of Leafminers in NWU
	15	Thu	
	16	Fri	
	17	Sat	
	18	Sun	
	19	Mon.	Day off/National Holiday
	20	Tue	Learn Taxonomy of Leafminers in NWU
	21	Wed	Move to Sapporo, Hokkaido Prefecture (AM) Tour of the Hokkaido University (PM)
	22	Thu	Collection of Leafminers in the Forest of Ishikari Coast
	23	Fri	Preparation and Rearing of Leafminers Collected from Ishikari Coast in the Entomology Laboratory of the Faculty of Environmental Earth Science (AM) Visit to the Natural History Museum of Hokkaido University (PM)
	24	Sat	
	25	Sun	Move to Kuroishi, Aomori Prefecture
	26	Mon.	Visit to the Apple Research Institute, Aomori Prefectural Industrial Technology Research Center in Kuroishi, Aomori to Learn About Apple Leafminers
	27	Tue	
	28	Wed	Move back to Nara
	29	Thu	Organization of Data and Specimens, Discussion in NUW and Presentation of Certificate
	30	Fri	Departure of Participants

4. DAILY ACTIVITIES

1-7 August 2016: Arrival in Japan, Familiarisation with the Facilities in NWU and Field Collection

Dr/Prof Hiroaki Sato facilitated the tour of the facilities and conducted the briefing of the attachment programme. In the laboratory, hands-on preparations and slide mounting of male and female genitalia for taxonomic identification of Lepidopteran leafminers as well as proper rearing techniques were demonstrated. Each participant was provided with adult Lepidopteran leafminer specimens for processing, staining and mounting of genitalia for species level identification. The slide mounting of the specimen was performed following the modified protocol of Sato (2016). Review of taxonomic keys and references were also done simultaneously with the laboratory activities.

The collection of leafminer samples from the vicinity of NWU and the hills of Nara City were done for the first week of attachment. In the field, participants were taught to recognise various forms of mines and signs of leafminer damaged from forest trees (e.g. Oaks, Sakura, Japanese lime, etc.) and weeds. The infested samples were brought to the laboratory for rearing into adult and for species identification. The most commonly used moist-paper method of rearing leafminers was employed for the samples collected.

8-14 August 2016: Survey and Collection of Leafminers from Yata Higashiyama Hills of Nara and Preparation of Genitalia in NWU

Survey and collection of leafminers were conducted in Yata Higashiyama hills of Nara (Fig 1). The area surveyed was from the JR Yamato-Koizumi Station to Haginodai Station, an almost 12 km walk from the Yamatokiriyama city area. Sample collection was done by examining plants with symptoms of mining and signs of leafmining larvae or pupae from plants such as *Quercus* sp. (Oak), *Prunus* sp. (Sakura) and weeds. The collected specimens were brought back to the laboratory for rearing. Checking and maintenance of reared leafminers and continuation of slide mounting of genitalia were performed in the laboratory.

15-21 August 2016: Attachment at the Department of Life and Environmental Sciences, Kyoto Prefectural University, Preparation of Genitalia in NWU, Special Lecture on Nara's History and Excursion to the World Heritage Complex in Nara

Visit to Dr/Prof Issei Ohshima at the Department of Life and Environmental Sciences, Kyoto Prefectural University was done on the 15-16 and 19 August. The visit involved the tour into the Molecular laboratory, the Electrophoresis room and the campus Insectary (Fig 2a-b). In the laboratory, Dr Ohshima conducted the briefing and demonstrations of the 2 commonly used methods of DNA extraction of Lepidopteran leafminers (i.e. by using Qiagen DNA extraction kit and by DNA & RNA purification protocol by Y/D [Boom et al., 1990]); the preparation of PCR products for DNA sequencing; and the analysis of data (Fig 2c). The participants including Dr/Prof Sato were given opportunity to perform the two DNA extraction and the preparations of PCR products by using the populations of leafminers collected from Ishikari, Hokkaido and Nara. The non-destructive and destructive method of DNA extractions from the 2 leafminer populations, were also performed. The prepared DNA samples were subjected to gel electrophoresis and same set of samples were sent for external DNA sequencing. Dr/Prof Ohshima showed the different methods of analysing the sequenced data by using online software such as 4Peaks, PAUP, Mesquite, MEGA, BLAST, DNASP and NETWORK. Each software advantages and disadvantages were discussed. The review of the DNA sequenced was also done with the results showing detections of 4 *Phyllonorycter leucocorona* holotypes from the two leafminer populations.

In the Insectary, Dr/Prof Ohshima demonstrated the use of vein-wrapping method in rearing the Lepidopteran leafminer, *Acrocercops transecta* from leaves of *Juglans* spp. (Walnut) or *Rhododendron* spp. (Fig 3a-g). The method employed soaking the cut basal part of the leaf blade with 2% sucrose solution in tissue paper for the long term preservation of infested leaves. Although the vein-wrapping method was laborious than the commonly used moist-filter paper for rearing leafminers, the method could kept the leaf freshness for 6 months at 25°C in the incubator which was essential for the development of the leafminers.

20 August 2016

Dr/Prof Mayuko Kawakami from the Department of International Studies for the History, Sociology and Geography of NWU (DISHSG, NWU) conducted the special lecture on the history of Nara, Japan. The lecture was attended by the participants from the leafminer attachment, 10 foreign and 10 local undergraduate students of the NWU Summer Course. An excursion headed by Dr/Prof Masashi Takada (also from DISHSG, NWU) to the World Heritage complex in Nara followed after the lecture. The tour includes Kasuga Grand Shrine, Todaiji Temple and Nigatsu-do Hall. The Kasuga Grand Shrine is the Shrine of Fujiwara family and its interior is famous for its many bronze lanterns as well as many stone lanterns that lead up to the shrine. The Todaiji temple housed the Great Buddha, which was reported as the world's largest bronze statue of the Buddha. Adjacent to the Todaiji temple is the Nigatsu-do hall, considered as one of the important hall in Todaiji and designated as a national treasure. The

visited sites were registered as UNESCO World Heritage Sites as part of the "Historic Monuments of Ancient Nara".

22-28 August 2016: Collection of Leafminer in Mt. Takamado, Preparations of Genitalia in NWU, Meeting and Discussion with JAIF and APHCN-ASEANET representatives

25 August 2016

Sampling of leafminers was conducted by walking from approximately 10 km from the Nara city to Mt. Takamado, an outskirt of Nara. The trek to the mountain was characterised by steep ascent to bamboo plantations and traverses through hiking trails of oaks, pines and small bamboos (Fig 4a-b). As the condition was so dry, most of the leafminers observed were dead or already emerged from the mines (Fig 4c-d). It was learned that some species of leafminers tend to hibernate during the dry season and emerged only after a year or if the condition is favourable. These leafminers were not collected and left undisturbed. Other leafminers fit for rearing were brought back to the laboratory.

26 August 2016

The preparations of genitalia from specimens collected from *Quercus aliena* were done. In the afternoon, Dr Lum Keng Yeang and Dr Soetikno S Sastroutomo visited the NWU for the discussion on progress of the attachment with the participants and the future plans for the 2nd phase of the JAIF and APHCN-ASEANET project with Dr/Prof Sato and Dr/Prof Hideaki Iwahori. Dr/Prof Iwahori, Plant Nematologist from the Department of Bioresource Sciences, Faculty of Agriculture, Ryukoku University was invited for the possible collaboration on the 2nd phase of the JAIF project.

29 August-4 September 2016: Collection of Leafminer in Mt. Izumi-Katsuragi, Visit to the Osaka Prefectural University and to the Osaka Museum of Natural History

29-30 August 2016

Dr/Prof Yoshitsugu Nasu conducted a guided two days tour for the collection of leafminer in Mount Izumi-Katsuragi. The mountain is 858 metres (2,815 ft) in height located on the Kongo Range between the prefectural boundary of Osaka and Wakayama and is covered by Japanese beech and vast vegetation. To cover the large forest, drive-through sampling and convenience sampling were done for the collection of leafminer from beech trees, weeds and other plants. However, due to heavy rain and approaching typhoon at the time of collection only few specimens were collected and brought back to the laboratory.

31 August 2016

Dr/Prof Yutaka Yoshiyasu, Dr/Prof Norio Hirai and Dr/Prof Shouhei Ueda facilitated the tour in the Environmental Entomology and Zoology, Osaka Prefectural University, by showing some of their activities such as rearing *Ephestiopsis vishnu* (Lepidoptera: Pyralidae), a potential candidate for biological control agent for exotic *Azolla* species (Fig 5a). The larvae of *E. vishnu* caused characteristic leafmining damaged on exotic *Azolla* sp. but it was not harmful to other ornamental water plants and cultivated crops. The rearing methods for invasive and endemic species of butterflies and other animals including fish, salamander and molluscs were also discussed. Dr/Prof Hirai also showed the simulation experiment on different temperature requirements for rearing macrolepidopterans from different prefectures. e.g., Osaka, Fukuoka, etc. Dr/Prof Yoshiyasu conducted the guided tour in the Insect Specimen Rooms and rearing facilities (Fig 5b). The collection includes macro- and microlepidopterans, beetles and other insects in pins and in slides. The techniques on preserving the microlepidopteran specimens without naphthalene were also demonstrated. The discussion on identification of microlepidoterans particularly leafminers was conducted after the tour.

1 September 2016

Dr Itaru Kanazawa gave a tour in the Osaka Museum of Natural History insect collections. The museum contains small exhibit of insects, mostly pinned specimens of butterflies and beetles. These specimens including some leafminers were categorised into Families and were mostly unidentified. The participants were informed that the insect collections were donated by Dr Okamura, a private collector and other insect enthusiasts as well as foreign collectors. It was learned that approximately 4,000 specimens were received monthly from private collectors. Dr Kanazawa also showed their collections of old entomological books and reprints, herbaria and other terrestrial arthropods including spiders. After the tour of the insect collections, the participants were allowed to view the museum's regular exhibits and displays.

6-9 September 2016: Visit to the Entomological Laboratories of Kyushu University (Ito Campus and Hakozaki Campus) to Learn Taxonomy of Leafmining Diptera and Lepidoptera

The participants gave an introductory presentation to Dr/Prof Yoshihisa Abe and to the student of Entomology, Faculty of Social and Cultural Studies, Kyushu University (Ito Campus). This was followed by a presentation entitled "Different displacement directions in two species of exotic Leafminer in different localities" (Abe and Tokumaru, 2007) and "Mode of parasitism of *Gronotoma micromorpha* (Hymenoptera: Figitidae: Eucoilinae)" (Abe, 2001 & Abe, 2006) by Dr/Prof Abe and Mr Taguchi Daisuke, respectively. In Dr/Prof Abe's presentation, he suggested that the

possible causes of displacement of *Liriomyza trifolii* by *Liriomyza sativae* in Japan could be attributed to (1) fecundity, (2) insecticide susceptibility, (3) competition for food sources, (4) reproductive inference, and (5) effects of natural enemies, *Dacnusa sibirica*. Mr Taguchi discussed the mode of parasitism of *G. micromorpha* and its success as the only egg-pupal and larval-parasitoid against *Liriomyza* spp. He explained that the biological features of *G. micromorpha* as being a solitary (feed only on one host), koinobiont (allow host to continue to grow in size after parasitism) and endoparasitoid (develop and feed within host body) made it more successful as a biological control agent against species of *Liriomyza* in Japan.

After the presentation, the lab's microlepidopteran insect collections, rearing facilities and host cultures were observed. Mr Taguchi demonstrated their method of rearing *L. trifolii* and *L. sativae* in common bean (*Phaseolus vulgaris*) and their parasitoids, *G. micromorpha* under laboratory conditions (Fig 6a-c). He also showed the preparation of male genitalia of *L. trifolii* and *L. sativae* by hot-plate boiling in 10% KOH (Fig 6d-e). The participants had hands-on practice on the preparation of genitalia as well as identification of the species. Mr Taguchi also demonstrated the extraction of *G. micromorpha* from parasitized larva and pupa of *L. trifolii*. The different morphological structures and characteristics of egg, larva and pupa of *G. micromorpha* were highlighted and discussed (Fig 6f).

8 September 2016

Dr/Prof Toshiya Hirowatari facilitated the tour of the Entomological Laboratory, the Insect Collection Rooms, Insect Museum and Library at the Faculty of Agriculture, Kyushu University (Hakozaki Campus) on 8 September 2016. The insect collections (pinned and slide mounts) which were distributed in several locations within the campus holds an approximately 4 million specimens with 30,000 holotypes described. Dr/Prof Hirowatari cited that some of these collections were dated back before World War 2. During the time of visit, insect collections were being sorted by the students prior for transfer in the Kyushu University, Ito campus, hence only few specimens were viewed. In the afternoon, participants were taught how to use the illustrated key to the Microlepidoptera authored by Dr/Prof Hirowatari. Under the microscopes, reviewed of the morphological characteristics of the lepidopteran leafminer families, e.g. Monotrypsia (Micropterigoidae) and Ditrypsia (Tischeriidae, Opostegidae, Nepticulidae, Gelechiidae, Heliozeidae, Gracillidae, etc.,) using the illustrated keys was conducted. Briefing of the progress of taxonomy of leafminers in Japan was also provided by Dr/Prof Hirowatari. An e-copy of the illustrated key and article on the recent progress in the taxonomy of Japanese leafminers was given to the participants for future reference.

12-18 September 2016: Visit to the Nara Prefecture Agriculture Research and Development Center, Farms in Sakurai City and Kashihara City Insectarium

Two days visits were conducted at the Nara Prefecture Agricultural Research and Development Center, Sakurai (NPARDC-Sakurai) to learn crop protection practices of leafminers and other pests of economic importance in the region. Before the tour of the centre's facilities, a courtesy call was given to Dr Tanigawa Motokazu, Director of NPARDC. Dr Isao Takenaka led the tour of the facilities and the laboratories such as the plant houses, Entomology Lab., Plant Pathology Lab., and Plant Biology Lab. It was learned that the centre transferred from Ohwuda City to the new centre in Sakurai City, only 2 weeks before the time of the visit, hence most of the plant houses were empty except for a few planted with strawberries (Fig 7a-b). As crop protection measure against soil borne pests, the centre used mixture of soil and wood chips for sowing. It was also observed that plastic films covering the walls of plant houses were raised to allow the naturally occurring parasitoids entrance to the strawberry plots. In the laboratory, the participants were shown specimens of parasitoids such as *Diglyphus isaea*, *Dacnusa sibirica* and *Encarsia formosa* collected from the parasitized larvae or pupa of leafminers, tobacco moth and other pests collected in the Kashihara, Sakurai and Yamatotakada, Nara, Japan. It was learned that the centre was not mass producing these parasitoids and those were naturally occurring in the production areas. In the afternoon, the participants introduced themselves and their company by giving presentation to the staff of NPARDC. The presentation was attended by more than 20 researchers and staff of NPARDC. At the end of the day, the participants were given brochures and articles pertaining to crop protection practices in Japan and other countries.

On 13 September 2016, the participants together with Dr Takenaka and Dr Imura visited some vegetables farms in Kashihara and Sakurai, Nara, Japan. In one onion plantation, plants were observed with *Liriomyza chinensis* reported as the main problem in the production area. The participants were taught how to recognize and identify early leafmining damaged in onions (Fig 7c-d). In another farm, *Liriomyza trifolii* was reported as the main problem in the tomato plant houses. It was informed that control of the pest was done by regular pesticide spraying.

At the NPARDC-Uda-Haiharasanguiji, the visit started with a brief introduction of the participants to Dr Maeda Shigeichi, Director of NPARDC-Uda, followed by the tour of the premises. It was learned that the centre was conducting different studies pertaining to crop productions and integrated pest management. It was observed that pheromone traps against Lepidopteran pests (e.g. *Spodoptera litura*, tobacco moth, leafminers, etc.) were strategically located within the experimental areas. The centre also experimented on the different use of selective and non-selective pesticides against pests of tomatoes. The use of selective pesticides compatible with the naturally occurring parasitoids in the area ensures preservation of the parasitoids even after pesticide applications. It was noted that plastic films covering the plant houses were kept partially open at the sides to allow entry of parasitoids and also for maintaining humidity in the plant houses (Fig 7e). Dr Imura explained that specific pests were noted to attack specific hosts, i.e., thrips in eggplant, *L. sativae* on watermelon, *Spodoptera litura* on spinach, and *L. trifolii* on tomato (Fig 7f). Dr Imura

also reported that *Orius sauteri* and *O. minutus* were the 1st and 2nd predominant species of parasitoids found in Nara, respectively. Although *Orius strigicollis* (dominant in Kyushu) and *O. nagaii* were reported in the locality those were not frequently observed in the production areas. To increase the populations of *Orius* spp., the use of French marigold (*Tagetes* spp.) as trap crop for non-pest thrips and as source of food for *Orius* spp. were employed in the production areas.

The Kashiwara City Insectarium was also visited to learn the rearing methods of macrolepidoterans and other insects in the insectarium. Mr Fumiaki Kimura facilitated the tour of the Exhibit Room, the Insect reference collection room, the rearing facilities and the butterfly garden.

20-24 September 2016: Visit to the Hokkaido University, The Hokkaido University Museum (HoUM) and Collection of Leafminers in Ishikari Coast, Hokkaido

A visit to the Entomology Laboratory at the Faculty of Environmental Earth Science, Hokkaido University was conducted to study the common leafminers in Sapporo and Ishikari. Dr/Prof Masahito T. Kimura and Mr Matsumura showed the Entomology laboratory and the rearing room for the common leafminer species such as *Phyllonorycter leucocorona* and *P. similis* in Hokkaido. A brief tour on the premises of the university was also conducted.

On 22 September 2016, the participant together with Dr/Prof Sato, Mr Matsumura and Dr/Prof Tetsuo Kohyama (Entomologist) conducted the collection of leafminer samples in Ishikari Coast (Fig 8a-b). The coast is designated as protected area consisted of coastal dunes and vegetation, mostly comprising of oaks, bamboos and weeds. The participants were taught to recognise various forms of mines and signs of leafminer damaged caused by the dominant and common leaf-mining species such as *P. similis* and *P. leucocorona* infesting *Quercus dentata* (Japanese emperor oak) and its hybrids (Fig 8c-d). Dr/Prof Sato highlighted the differences in the position of mines on leaves among leafminer species, e.g. mines of *P. similis* were distributed more frequently in the middle section of leaves, whereas those *P. leucocorona* and other species were concentrated in the basal section. More than 300 samples of these infested *Q. dentata* and hybrids were brought back to the laboratory for rearing and diagnosis.

23 September 2016

The participants together with Dr/Prof Sato, Dr/Prof Kimura and Mr Matsumura visited the Hokkaido University Museum (HoUM). Dr/Prof Masahiro Ohara, Vice Director of the HoUM provided the tour by highlighting the collections of micro and macrolepidoptera, specifically the leafminer, *Phyllonorycter similis* attacking *Quercus* spp. Dr/Prof Ohara also reported that the museum holds more than 2.5M collection of specimens and documents. Forty thousand of those were insects of which 10,000 were considered primary types. Currently, those insects were being reviewed for

correct identification and restoration in new boxes. At the end of the day, the participants were allowed to view the museums exhibits and research laboratories in the university.

26-27 September 2016: Visit to the Apple Research Institute, Aomori Prefectural Industrial Technology Research Center in Kuroishi, Aomori

Two-day visits were conducted at the Apple Research Institute (ARI), Aomori Prefectural Industrial Technology Research Center in Kuroishi, Aomori to learn the leafminers attacking apples and their control. The participants first gave an introduction of themselves and their company to Dr Yoichi Ishiguri and this was followed by Dr Ishiguri's presentation of the history of the institute, the major pests of apples, control measures and some projects and researches undertaken by the institute. It was learned that the research on apple started in Aomori Agricultural Experiment Station in 1911 which was renamed in 1931 as Aomori Apple Experiment Station and later as Apple Research Institute (ARI, 2009). Dr Ishiguri cited that ARI was under the Department of Agriculture and Forestry of the Aomori Prefectural Industrial Technology Research Center with 4 sections namely: Cultivation Section, Breeding Section, Plant Protection Section and Kennan Fruit Tree Section. The Plant Protection Section was involved in the development of control measures against diseases and insect pests. It was reported that more than 20 species were major pests of apples which were mostly fruit borers, leafminers, scale insects and mites. Although, *Phyllonorycter ringoniella* (Apple leafminer) and *Lyonetia prunifoliella malinella* (Striped Bent-wing moth) were commonly found in the apple orchard, it was reported that these species were not of a major concern in the apple production as they only feeds on the leaves and they don't damage the fruits. Dr Ishiguri also reported that Pyrethroids, Insect Growth Regulators, Neonicotinoids and Mating pheromone disruptions were currently used to control the major pests. He also stated that there are varieties of naturally occurring parasitic wasps such as *Ageniaspis testaceipes*, *Sympiesis* sp., Ichneumonids and *Apanteles* sp. in the orchard, however the frequent use of pesticides (i.e. 6-10 applications per year) resulted in the death of these natural enemies. Dr Ishiguri also shared his studies on the effect of overwintering and apple bud phenology and the effect of leaf abscission on the populations of *P. ringoniella*.

After the presentation, Dr Ishiguri facilitated the tour and collection of leafminer in the apple orchard inside the institute's premise. It was noted that sticky traps and mating disruption pheromones were placed in few trees to monitor the population of pests and to prevent copulation of pests, respectively (Fig 9a-b). Dr Ishiguri also explained how crop protection practices such as bagging, netting and calendar spraying were done in the production areas. During the walkabout, leaves with leafmining damaged were collected and brought back to the laboratory for diagnosis. It was observed that sap and tissue feeding stages of *P. ringoniella* were predominant in the samples collected (Fig 10 a-b). Few of these samples were parasitized by parasitoid wasps belonging to Family Encyrtidae.

27 September 2016

On the second day of attachment to Apple Research Institute, Dr Ishiguri showed some of the Institute's pest collections. The rearing methods for peach fruit moth (*Carposina sasakii*), Oriental fruit moth (*Grapholita molesta*) and other major pests of apples were also shown. At the end of the day, tour at the Apple Archives Building was conducted. The building formerly houses the Apple Research Institute and now serves as a historical museum about apple cultivars and its history.

28-30 September 2016: Organization of Data and Specimens, Discussion in NUW, Presentation of Certificate and Departure of Participants

Data and specimens were collected at the NWU followed by brief discussion on the outcome of attachment. The attachment was officially closed with the presentation of certificates given by Dr/Prof Sato and departure of the participants.

5. SUMMARY OF THE ATTACHMENT

The Japan-ASEAN Integration Fund (JAIF) and ASEAN Plant Health Cooperation Network (APHCN) of ASEANET Training on Leafminers of Agricultural Importance attachment programme was held from 1 August to 30 September 2016 in Japan. This two-month attachment was part of the 2nd Phase workshop on Diagnostics Training on Leaf miners of Agricultural Importance organised by the APHCN-ASEANET to improve the diagnostic capability and the enhancement of development of other diagnostic resources in ASEAN. The selected participants from Thailand and Singapore attended the attachment under the supervision of Dr/Prof Hiroaki Sato at the Nara Women's University, Nara, Japan for more in-depth technical trainings on leafminers. During the course of the attachment, laboratory and field works, visits to universities and different agencies, lectures and discussions on leafminers as well as other pests were conducted. Under the different experts, the participants were taught (1) the proper collection and handling of leafminer samples for rearing and identification purposes, (2) recognising various forms of mines and signs of some common leafminer damaged from plants, (3) correct rearing methods of leafminers and parasitoids, and (4) proper killing, fixing, setting of Lepidopteran leafmining moths and slide mounting of leafminer genitalia for identification and long-term preservation and storage. The different diagnostic skills using traditional and molecular techniques for common leafminers were also imparted by experts. The participants also gained increase awareness and more insights on how to plan, prepare and evaluate integrated pest management of leafminers under planthouses or field conditions. The acquired knowledge on the different management measures for leafminers, i.e. chemical control, use of different types of traps, (e.g. pheromone traps, sticky traps and correct location of traps), pheromone mating disruptions, use of natural enemies and other

physical controls from different localities gave them broader understanding on the leafminers and its management that could be adopted in farms to monitor pest populations or in preventing pest outbreaks in their countries. The attachment also gave the participants and trainers an opportunity to develop a network that could be used as networking for exchange of information and for tapping diagnostic expert(s) for future identification of leafminer taxa to expedite identification of new or unknown pests.

6. RECOMMENDATION FOR FUTURE ACTIVITIES

- Leaf miners are a group of insect pests often causing serious damage on ornamentals and vegetables throughout the world. Through this JAIF and APHCN–ASEANET Project a more in depth knowledge on taxonomical identification of economically important leaf miners was gained by some members of ASEAN to broaden their diagnostic capability and competency to handle such pest. The correct identification and diagnosis of leafminers are necessary to support pest management decision-making for effective safeguarding of plant health, thus it is recommended that a similar capacity building activity could be offered in the future.
- It is suggested that more hands-on and practical session on molecular diagnostics could be provided for future trainings. As molecular diagnostics coupled with acquired morphological skills and techniques would speed up reliable species identification especially if dealing with unknown or pest of quarantine importance.
- A follow up action by establishing network with the Japanese leafminer experts and with the participants must be done after the attachment. This could be achieved by tapping on each other for pest ID verification. Counter-checking of pre-identified leafminers or other pests could strengthen the diagnostic capability and hence leaning towards being an ASEAN expert on leafminers.
- Conducting a survey of leafminers in ASEAN with samples to be submitted from the region coupled with the expert's verification of identity of the leafminers that could further strengthen the acquired skills is also suggested.
- Most of the Japanese experts were not well versed in English and due to language barrier, sometimes there was limited or no active interaction between experts and participants. Other relevant references (e.g. taxonomic keys, illustrated guides, etc.) were also written in Japanese which posed difficulty in using and understanding. Hence, it is suggested that these issues could be considered in future activities.

- To coincide the timing of the attachment with the pest occurrence (if possible). Due to limited time, the participants were not able to process and conduct preliminary identification of the Lepidopteran leafminers collected by them. The rearing of the leafminers would take more than a month to develop from larvae to adult before it could be processed and identified. Moreover, there were only few samples collected for processing and identification. It was learned that the timing of the attachment did not coincide with the peak of leafminer occurrence. The populations of leafminers were said to be high during May-July and less during the summer season of August-September.
- To have more specimens or for the participants to bring their own specimens for identification. During the training, specimens provided for slide mount preparations of genitalia were very few. The limited number did not allow the participants to practice more and hone their skills on the preparation and identification of female and male genitalia which were the most important features for species level identification of Lepidopteran leafminers.

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Dr Itaru Kanazawa, Osaka Museum of Natural History, Osaka, Japan

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Dr/Prof Masahiro Ohara and the Hokkaido University Museum, Sapporo, Hokkaido, Japan

Dr Yoichi Ishiguri and the Plant Protection Section, Apple Research Institute, Aomori Prefecture Industrial Technology Research Center, Kuroishi, Aomori, Japan

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9. ANNEXES


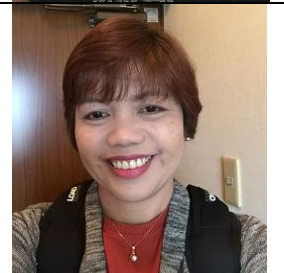
Details of Speakers (Name, title, profession)

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<p>Dr/Prof Yutaka Yoshiyasu Professor/Lepidopteran expert Environmental Entomology and Zoology, Graduate School of Life and Environmental Sciences, Osaka Prefectural University, Osaka, Japan</p>	
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<p>Dr Takeo Imura Chief Researcher, Agriculture Research and Development Center, Division Research and Development Section Basic Technology, Pest Management Unit (Crop Protection Office) Nara Prefecture Agricultural Research and Development Center, Sakurai, Nara, Japan</p>	
<p>Dr Yoshinori Kunimoto Chief Researcher Nara Prefecture Agricultural Research and Development Center, Sakurai, Nara, Japan</p>	
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<p>Dr Yoichi Ishiguri Researcher Plant Protection Section, Apple Research Institute, Aomori Prefecture Industrial Technology Research Center, Kuroishi, Aomori, Japan</p>	

Details of Participants (Name, title, profession)

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9. PHOTOS & IMAGES



Fig 1. Yata Higashiyama Hills, Nara, Japan where leafminer samples were collected.

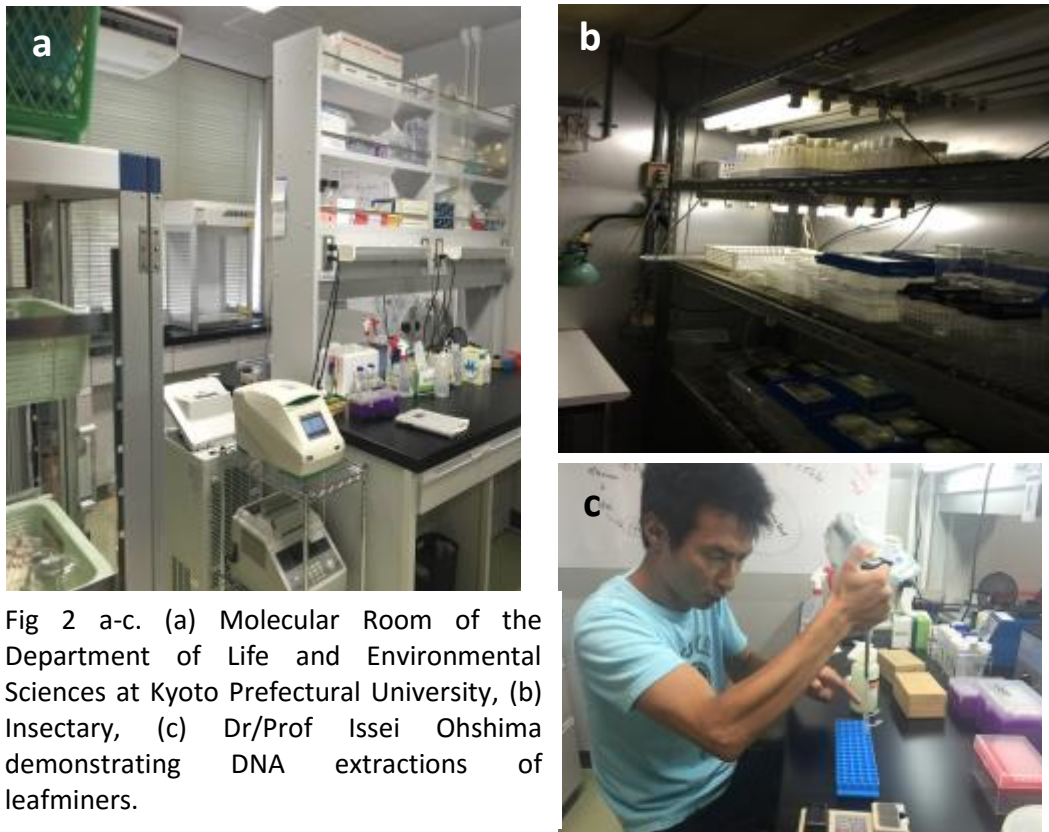


Fig 2 a-c. (a) Molecular Room of the Department of Life and Environmental Sciences at Kyoto Prefectural University, (b) Insectary, (c) Dr/Prof Issei Ohshima demonstrating DNA extractions of leafminers.

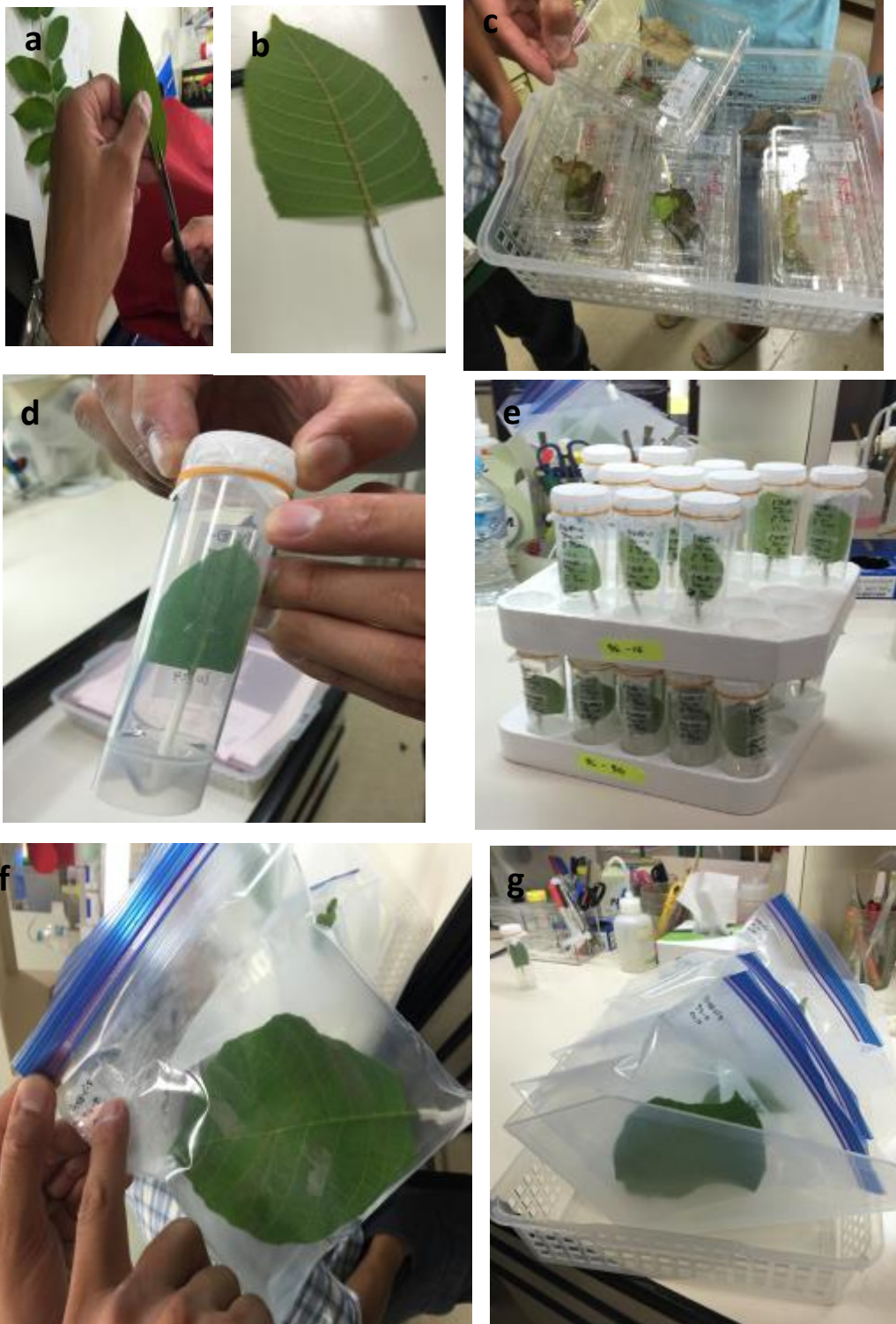


Fig 3 a-g. Vein-wrapping method in rearing the Lepidopteran leafminer, *Acrocercops transecta* from leaves of *Rhododendron* spp. (a) Cutting and removal of leaf blade basal parts, (b) leaf blade wrapped in tissue paper with 5% sucrose, (c) rearing in plastic containers, (d-e) rearing in holed-centrifuge tubes, (f-g) rearing in zip-locked plastics.



Fig 4 a-d. Collection of leafminers in Mt. Takamado, Nara, Japan (a-b) Hiking trails, (c-d) Leafminer damaged on leaves.

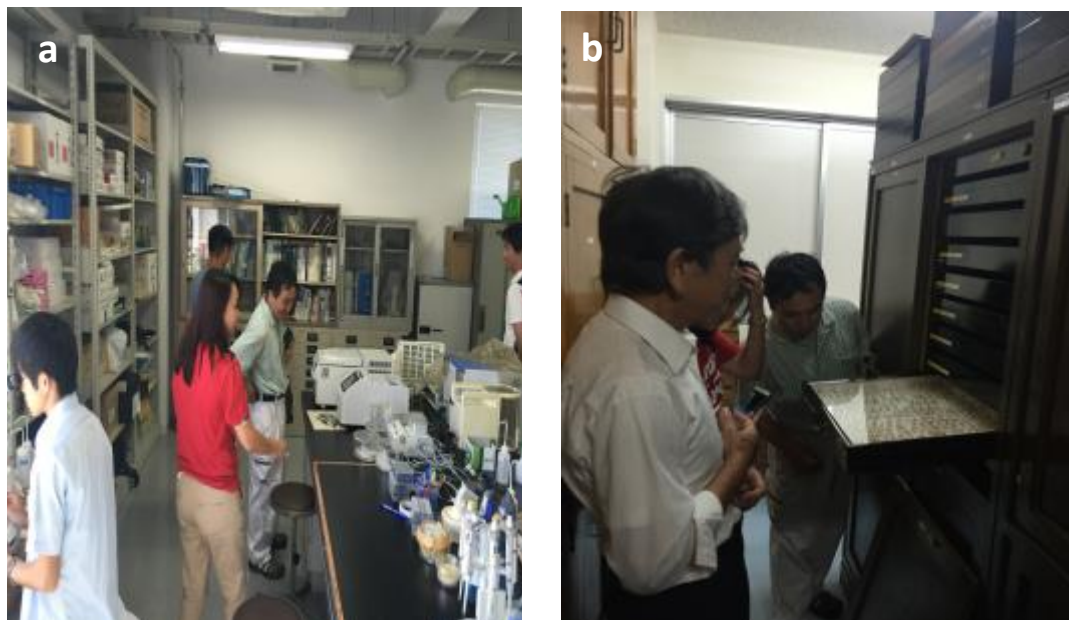


Fig 5 a-b. (a) Laboratory of the Environmental Entomology and Zoology and (b) Insect Specimen Room at the Osaka Prefectural University.



Fig 6 a-f. (a-b) Rearing method of *Liriomyza trifolii* and *Liriomyza sativae* and (c) the parasitoid, *Gronotoma micromorpha* in *Phaseolus vulgaris* (Common bean), (d-e) Preparation of male genitalia of *L. trifolii* and *L. sativae* by hot-plate boiling in 10% KOH, (f) First instar larva of the parasitoid, *Gronotoma micromorpha*.



Fig 7 a-f. (a-b) Planthouses at the Nara Prefecture Agricultural Research and Development Center, Sakurai, Japan, (c) Dr Takeo Imura with participant teaching on symptoms recognition of *Liriomyza chinensis* on onions, (d) Leafmining symptoms of *L. chinensis* in onions, (e) Plastic films covering the plant houses kept partially open to allow entry of parasitoids, (f) Tomato plants infested with *Liriomyza trifolii*.

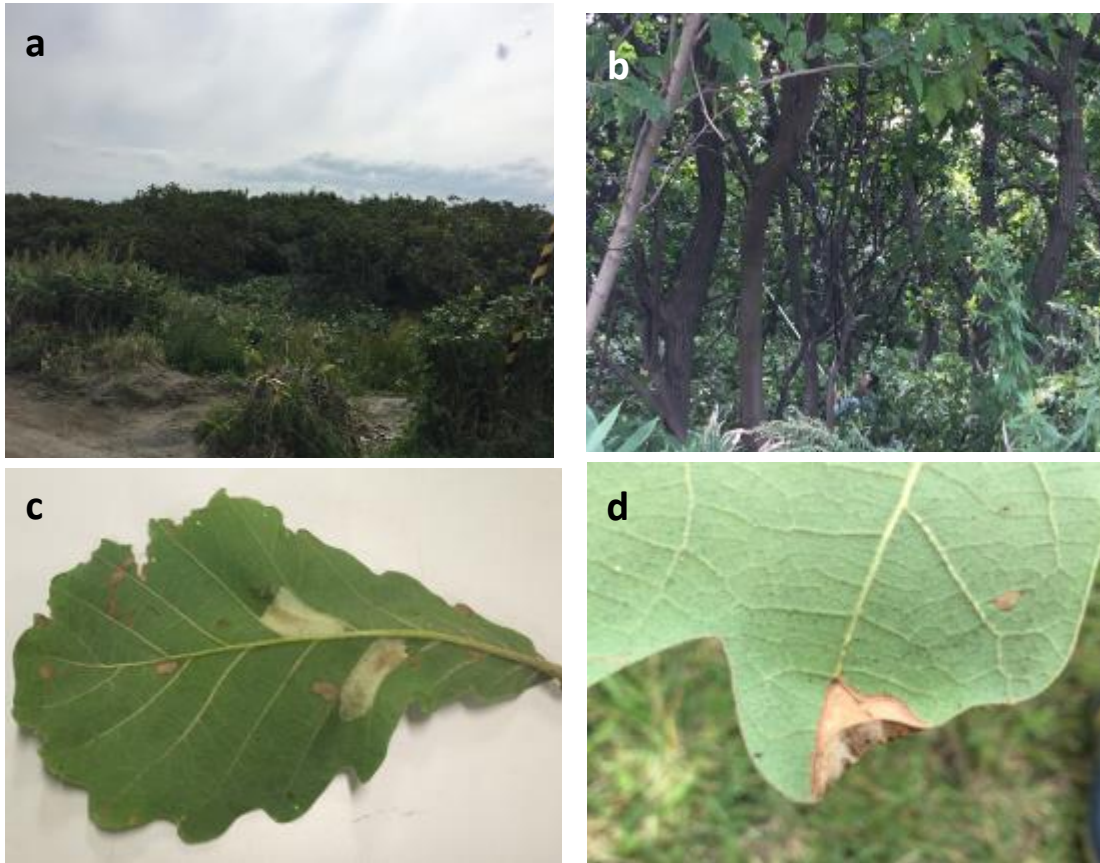


Fig 8 a-d. (a) Ishikari Coast, Hokkaido, Japan, (b) Dr/Prof Hiroaki Sato collecting samples of leafminer from *Quercus* sp. (Oaks), (c) Leafminer damaged caused by the dominant and common leaf-mining species, *Phyllorhynchus similis* and (d) *Phyllorhynchus leucocorona* on *Quercus dentata* (Japanese emperor oak) .

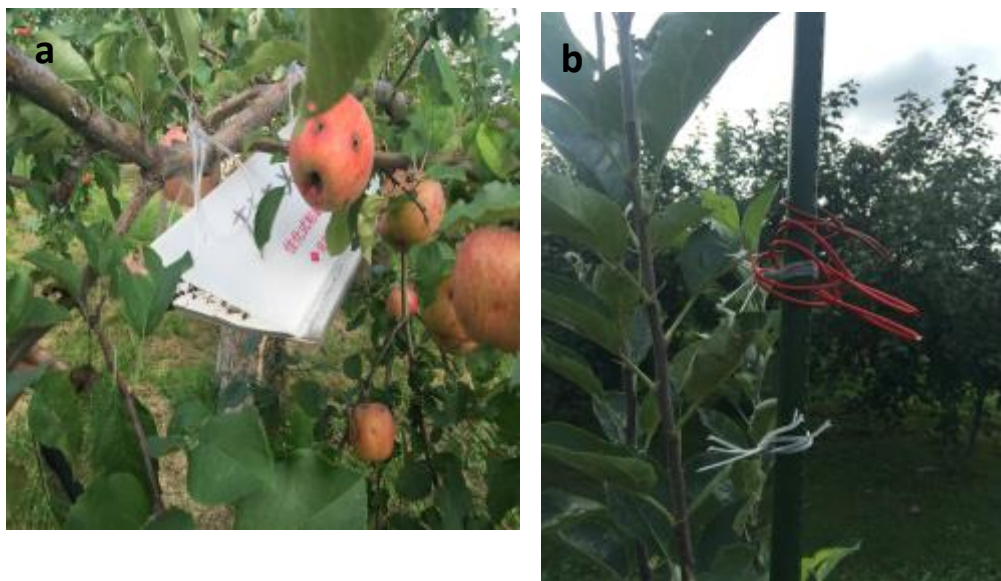


Fig 9 a-b. (a) Sticky trap and (b) mating disruption pheromones used against pests in the Apple Research Institute (ARI), Aomori Prefectural Industrial Technology Research Center in Kuroishi, Aomori, Japan.

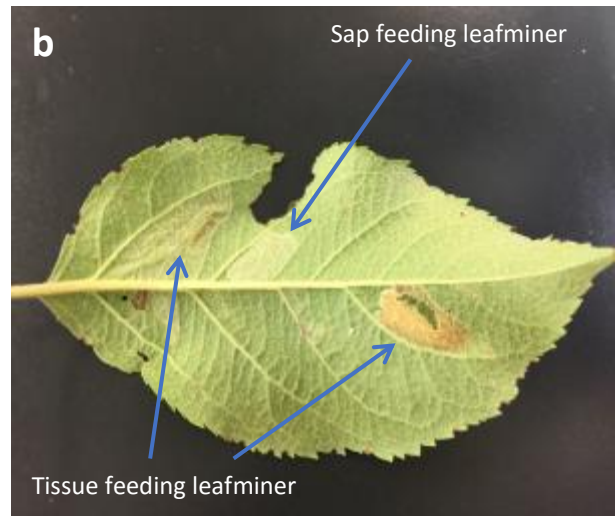


Fig 10 a-b. (a) Leafmining damaged in *Malus* sp. (Apple) (b) Sap and tissue feeding stages of Apple leafminer, *Phyllonorycter ringoniella*.



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 Yuvarin Boontop
Thailand

Organized by:



Nara Women's University
Nara, Japan

In Collaboration with:



ASEAN Network on Taxonomy

2016

Attachment Program: Diagnostics of Leafminers of Agricultural Importance

**(JAIF Funded Project on Taxonomic Capacity Building to Support Market
Access for Agricultural Trade in the ASEAN Region)**

Venue: Nara Women University, Japan

Duration: 1st August to 30th September 2016

Participant Name & Position: Yuvarin Boontop (Entomologist)

**Institutional Address and Country: Department of Agriculture,
Phahonyothin Road, Chattuchak, Ladyao, Bangkok, Thailand**

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1. Background Information

The ASEAN Plant Health Cooperation Network (APHCN)-ASEANET Project “Taxonomic capacity building to support market access for agricultural trade in the ASEAN region”, funded by the Japan ASEAN Integration Fund (JAIF) successfully held its second capacity building activity, a “Training Workshop on Leafminers of Agricultural Importance”, from 29th February to 11th March 2016 at Museum Zoology, LIPI, Cibinong, Indonesia. Under this ASEAN-endorsed Project, three participants from the training workshop were selected for a two-month attachment program at the laboratories of the lead resource person, Professor Hiroaki Sato at the Nara Women University, Nara, Japan, for further training. The attachment focused primarily on leafminers belonging to the order Lepidoptera.

I, **Yuvarin Boontop**, was nominated by the Project Steering Committee, based on the advice of the resource persons of the training workshop, as one of the three successful candidates for this attachment program. The starting date and details relating to the attachment program were finalized by Dr. Sato, the overall supervisor for the attachment program. I was accompanied for the entire program by another participant in the attachment program, Ms Ariene Castillo of the Agri-Food and Veterinary Authority of Singapore.

All expenditures (international travel economy class and DSA) associated with participation in the attachment program was borne by the Project and paid through ASEANET. ASEANET also facilitated processing of travel and other, associated arrangements.

2. Objectives of the Attachment

2.1 The participants in the attachment were to acquire taxonomic skills in identifying the species, genera and families of major pest leafminers, on the basis of external characters and DNA barcoding.

2.2 Participants were also to gain understanding of how seriously leafminers damage horticulture crops and familiarity with appropriate control methods.

3. Program of Attachment (Time Table)

Month	Date	Day	Activities	Notes
Aug.	1	Mon	Arrival at Kansai Airport, Japan	
			Travel to Nara Women's University (NWU)	
	2	Tue	Collect leafminers in hills of Nara City	
	3	Wed	Make preparations of leafminers in NWU	
	4	Thu		
	5	Fri		
	6	Sat		Sato, out of office due to business trip
	7	Sun		
	8	Mon	Day off	
	9	Tue	Day off	
	10	Wed	Collect leafminers in hills of Nara City	
	11	Thu	Make preparations of leafminers in NWU	National Holiday
	12	Fri		
	13	Sat		
	14	Sun		
	15	Mon	Visit to Dr Oshima's lab. of Kyoto Prefecture University to learn DNA sequencing techniques	Under supervision by Dr Ohshima
	16	Tue		
	17	Wed		
	18	Thu	Make preparations of leafminers in NWU	
	19	Fri	Visit to Dr. Oshima's lab. of Kyoto Prefecture University	
	20	Sat		
	21	Sun		
	22	Mon	Collect leafminers in hills of Nara City	
	23	Tue	Make preparations of leafminers in NWU	Dr Sato, out of office due to a special lecture and practice
	24	Wed	Collect leafminers in Mt Takama hill	
	25	Thu	Make preparations of leafminers in NWU	
	26	Fri		

Month	Date	Day	Activities	Notes
	27	Sat		
	28	Sun		
	29	Mon	Collecting leafminers on Mt Izumi-Katsuragi	
	30	Tue		
	31	Wed	Visit Entomological Laboratory of Osaka Prefecture University	
Sep.	1	Thu	Visit to Natural History Museum of Osaka City	
	2	Fri	Make preparations of leafminers in NWU	
	3	Sat		
	4	Sun		
	5	Mon	Learn taxonomy of leafminers in NWU	
	6	Tue	Move to Hakata, Fukuoka Prefecture	
	7	Wed	Visit Entomological Laboratory of Kyushu University, and learn taxonomy of leafmining Diptera and Lepidoptera	Under supervision by Dr Abe and Hirowatari
	8	Thu		
	9	Fri	Travel back to Nara	
	10	Sat		
	11	Sun		
	12	Mon	Visit Agricultural Exp. Station of Nara Prefecture in Ohwada City to learn about protection of crops from leafminers.	Under supervision by Dr Takenaka
	13	Tues		
	14	Wed	Learn taxonomy of leafminers in NWU	
	15	Thu		
	16	Fri		
	17	Sat		
	18	Sun		
	19	Mon	Day off	National Holiday
	20	Tue	Learn taxonomy of leafminers in NWU	
	21	Wed	Travel to Sapporo, Hokkaido Prefecture	
	22	Thu	Collect leafminers in the forest of Ishikari Coast	Under supervision by Dr Kimura
	23	Fri	Visit Natural History Museum of Hokkaido University.	
	24	Sat	Identify the species of leafminers in Hokkaido University	
	25	Sun		
	26	Mon	Visit Apple Research Institute of Aomori Prefecture to learn about apple leafminers	Under supervision by Dr Ishiguri
	27	Tue		
	28	Wed	Return to Nara	
	29	Thu	Organize data and specimens, and final discussions in NUW	
	30	Fri	Return to Thailand	

4. Daily Activities (Laboratory Studies)

A) Laboratory Studies

4.1 Preparation of genitalia slides of leafminers

Professor Sato provided instruction in the preparation of slide mounts of the genitalia of leafminers because the characteristics of genitalia are very important for leafminer identification. Preparation of genitalia slides was conducted at Nara Woman's University.

Materials and methods (Figure 1)

- 1) Leafminer larvae and pupae were obtained from many kinds of trees from the forest. Adults were reared from larvae and pupae collected in the field.
- 2) Genitalia were dissected, stained with 2% fuchsin (in a mixed solution of lactic acid and phenol in the ratio of 3 to 1) for 2 hours at 70°C.
- 3) Genitalia were also stained with chlorazol black (in 70% ethanol)
- 4) Genitalia were mounted in Canada balsam or Euparal. Male genitalia were mounted with widely spread valvae, so that the inner surfaces faced upwards. Female genitalia were left attached to the abdominal segments VII and VIII, and were mounted ventral side up.
- 5) Species were identified from adults emerging from mines and/or by their pupal cremaster.

Laboratory work in Nara also included extensive practice in the identification of leafminers belonging the families Gracillaridae, genera *Phyllonorycter*

4.2 Molecular study

I visited the laboratory of Associate Professor Dr Issei Oshima, Department of Life and Environmental Sciences, Kyoto Prefectural University. His research investigates the

process and mechanism of the evolution of complex traits. His focus is on host shifting events in phytophagous insects, which require evolution of adult female preferences and larval performance. He is interested especially in the genetic basis of ovipositing female preferences and larval performance. His current work involves QTL mapping of the genes responsible for host adaptation using the leaf-mining moth *Acrocercops transecta* (family Gracillariidae) as a model system. I spent three days with Dr Oshima to learn about DNA sequencing techniques (Figure 2 –7). I learnt how to perform DNA extraction, polymerase chain reaction (PCR) amplification, sequencing and purification as detailed below.

Molecular procedure and analysis

- Mitochondrial DNA extraction

The abdomen was removed from each individual for genomic DNA extraction using the ISOLATE II Genomic DNA Kit (Qiagen) following the manufacturer's protocol. A 650 bp fragment of mitochondrial cytochrome c oxidase subunit I (*cox1*) was amplified using the universal invertebrate *cox1* primers:

LCO1490 (forward: GGTCACAAATCATAAAGATATTGG) and

HCO2198 (reverse: TAAACTTCAGGGTGACCAAAAATCA) (Folmer et al., 1994;

Wilson, 2012).

-Polymerase chain reaction (PCR) amplification sequencing and Sanger sequencing

PCR amplification was carried out with 1 µl of template DNA, 2µl of each primer (10 pmol/µl), 2 µl of 10X polymerase buffer, 1.6µl of dNTP, and made up to a final volume of 20µl with distilled deionized water (ACMG).

Amplifications were performed in a thermal cycler with an initial denaturing step at 94°C for 3 minutes followed by 28 cycles at 94°C for 30 seconds, 50°C for 30 seconds, 72°C for 30 seconds, and a final elongation step at 72°C for 5 minutes.

- Gel electrophoresis

One µl of PCR product was separated in 1.5% (w/v) agarose gel using TBE buffer (40 mM Tris acetate, 1 mM EDTA) to confirm the quality of PCR product.

-PCR Purification

Total PCR product was purified using the commercial ISOLATE PCR and Gel Kit following the manufacturer's guidelines. Purified PCR product was amplified in a sequencing reaction containing 1.0 µl of PCR product, 1.0 µl of forward primer (3.2pmol/µl), 0.5 µl of version 3.1 ABI Prism® Big Dye Terminators (Applied Biosystems, California, USA), 3.5 µL of 5x sequencing dilution buffer (400mM Tris pH9, 10mM MgCl₂), adjusted to a total reaction volume of 20µL with dH₂O (double distilled water). Purified PCR were sent to sequencing at the Molecular Genetics Company in Japan.

- Mitochondrial DNA analyses

1) Sequences were aligned by eye and checked for internal stop codons and double peaks (indicative of pseudogenes) using FOURPEAK Sequence Alignment Editor for MAC and BioEdit Sequence Alignment Editor Version 7.2.5 (Hall, 1999) for WINDOW.

2) Analysis of genetic diversity (number of haplotypes) was performed using MEGA version 7.0 (Tamura et al., 2007).

3) Median joining networks among leafminers from Nara and Hokkaido haplotypes were constructed and post-processed under maximum parsimony in Network Version 4.6.1.1 (Bandelt et al., 1999). Median joining networks were considered the most appropriated method in this case over other alternatives (e.g., minimum spanning and maximum parsimony). Minimum spanning networks perform best when sampling of haplotypes across the population is relatively complete; they do not perform well if there

are significant gaps in sampling across the distribution of a species and where some internal node haplotypes are not sampled. On the other, hand, median joining networks incorporate the maximum parsimony criterion and infer internal node haplotypes that may have been missed by incomplete sampling (Cassens et al., 2005).

B) Field Studies

Leafminers were collected from the field and returned to the laboratory where larvae completed development. Adults were reared from those larvae and pupae that were collected in the field. The targeted leafminers depended on availability, but the nominated hosts were species that are typically attacked by Lepidopteran leafminer.

In total, I collected leafminers at five locations in Japan. Specimens were obtained from Nara Hill, Yata Hill, Takamado Hill, Mt Izumi -Katsuragi and Ishikari Coast.

Field collection

1) Nara Hills

Leafminers were collected from the hills of Nara City on the 2 August 2016 (Figures 8). The vegetation of the hills is bamboo predominantly deciduous woodland with evergreen pines. Many species of leafminers were found and taken back to the laboratory.

2) Yata Hill

Leafminers were collected in Yata hills on 22 August 2016 (Figure 9 and 10). The Yata hills are part of Yata Prefectural Natural Park to the northwest of Nara. The vegetation is / similar to Nara Hills. Temples in the park include Ryōsenji, Yatadera, Tōmyōji, and Matsuodera. The Yata hills run from north to south for about 10 km. Many species of leafminers were found and taken back to the laboratory.

3) Takamado Hill

We carried out the leafminer collecting in a red pine (*Pinus densiflora* Siebold & Zuccarini, Family Pinaceae) forest on Takamado Hill near the urban area of Nara on 22 August 2016 (Figure 11). The understory vegetation of the forest was dominated by *E. japonica*. Many species of leafminers were found and taken back to the laboratory.

4) Mt Izumi-Katsuragi

Mount Izumi-Katsuragi is a mountain in the Kongō Range, the border between Osaka and Wakayama Prefectures in Japan. Its peak elevation is 858 metres (Figure 12). The north slope of Mount Izumi-Katsuragi is covered in a wood of Japanese beech. This species is fast growing here because the elevations are high enough to remain fairly cool. This is the southernmost stand of Japanese beech on Honshū. The trees in the area were designated as a nationally protected species in 1923, which has allowed them to continue to thrive under suburban and urban growth. Unfortunately, our visit coincided with a typhoon and we were unable to collect as many leafminers as we had hoped.

5) Ishikari coast, Hokkaido

We also collected leafminers from the Ishikari coast. Many specimens were collected from oak leaves from eight locations along the coast. After collecting we put those mines in boxes and Dr. Sato sent them back to Nara (Figure 13).

C) Specimen Collection Study

1) Visit to Entomological Laboratory of Osaka Prefecture University

We visited the insect collection at the Entomological Laboratory of Osaka Prefecture University (Figure 14). There are many kinds of insects in this collection, including butterflies and moths from Thailand. Professor Dr Yutaka Yoshiyasu, who is an expert on microlepidoptera and also the curator, showed us the insect collection and gave

me valuable reference books for the library associated with the Thai Department of Agriculture insect collection (Microlepidoptera of Thailand: scientific results of the Lepidoptera ecological Expeditions of the University of Osaka Prefecture to Thailand). These books will be very useful because there are many Lepidoptera specimens in the Department's collection that have not yet been identified. I will be able to use these books as a reference for future identification.

2) Natural History Museum of Osaka City

We also visited the Natural History Museum of Osaka city (Figure 15). The collections of recent insect and other terrestrial arthropods, including spiders, comprise an estimated 700,000 prepared specimens. The number of specimens is increasing by more than 10,000 per year due to the cooperation of many people and students. Most of them are dried specimens except for the larvae as specimens with alcohol and minute insects. The collection also includes paratype specimens. The total number of specimens amounted to 649,330 in 2001. Approximately 80% of the specimens were collected from Japan and 20% from other countries.

D) Other activities

1) Visiting Entomological Laboratory of Kyushu University.

We were hosted in Kyushu by Dr Yoshihisa Abe, Professor-Entomology, Faculty of Social and Cultural Studies and his Ph.D. student Mr Taguchi Daisuke, Graduate school of Integrated Sciences for Global Society. Dr Abe is interested in leafminer parasitoids. He gave us a presentation about the different displacement directions in two species of exotics and Mr Taguchi Daisuke also presented about mode of parasitism in *Gronotoma micromorpha* (Hymenoptera; Eucolidae).

In this laboratory, we also learnt about the taxonomy of leafmining Diptera using male genitalia as per the protocol below.

Genitalia preparation: The males were examined for identification.

- The complete abdomen of each individual was removed and placed in 5 ml of 10% potassium hydroxide (KOH) solution
- Boiled at temperature (70 °C); this process softened and cleared the structures for ease of dissection.
- Following softening, abdomens were dissected under water.
- Each aedeagus was removed from the remaining genitalic structures and straightened on a microscope slide.

In addition, we learnt how to rear the dipteran leafminer and its parasites. Parasitic wasps (parasitoids) of the family Eulcoilidae (order Hymenoptera) are important in natural control and, in the absence of insecticides, usually keep this insect at low levels of abundance. Two parasitoid species are known from Kyushu University. Species of Eulcoilidae such as *Gronotoma micromorpha* (Perkins) are generally found to be most important (Figure 16 -17).

Also at Kyushu University, Professor Dr. Toshiya Hirowatari, Entomological Laboratory, Faculty of Agriculture has been working on the taxonomy and phylogenetic systematics of insects, especially on moths and butterflies (Lepidoptera including leafminers). He showed us the Insect exhibition (Figure 18) and taught us how to identify moths to family level and identified specimens from insect collection (Figure 19).

2) Agriculture Research and Development Center, Agricultural Experiment Station of Nara Prefecture in Ohwuda City.

In this research station, we learnt about the protection of crops from leafminers from researchers (Mr Takeo Imura, Chief Researcher and Mr Yoshinori Kunimoto). They showed us the damage from leafminers and the tomato plantations using pesticide and non-insecticide (Figure 20). We also had a chance to visit the insect collection in which

mostly contains leafminers (Diptera and Lepidoptera) from tomato (Figure 21). We surveyed leafminer in onion plantation and found the larvae of the dipteran leafminer (*Liriomyza achiensis*) (Figure 22). We also visited another Experiment station (a branch of Agriculture Research and Development Center, Agricultural Experiment Station of Nara Prefecture) and another Experiment station and farmer's plantations in that area (Figures 23, 24).

3) Kashihara City Museum of Insect, National Museum of Nature and Science.

We had the opportunity to visit the insect museum in Kashihara city. Mr. Fumiaki Kimura showed us the butterfly greenhouse and how to mass rear insects for exhibition (Figure 25). The Kashihara City Insectarium is especially interesting because the museum room is exhibited as a history of life and as insects' physical characteristics. In the Ecological Exhibit Room there is a device with allows you to observe insects with a miniature camera from an insect's perspective (Figure 26).

4) Aomori Prefectural Industrial Technology Research Center

The Apple Research Institute of the Aomori Prefectural Industrial Technology Research Center is located in Kuroishi city and is the only apple experimental research institution in Japan. The Apple Research Institute was established in 1931 and carries out experimental research on apples and other fruit trees. In this institute under supervised Dr Ishiguri, we surveyed the apple orchard and collected mines to be taken back to the laboratory. Four species of leafminer and their parasitoids have been found. We also visited the Apples Archives Building which serves as a historical museum displaying information about apple cultivars and history.

5) Summary of the Attachment

The attachment program on diagnostics of leafminer of agriculture importance took place from 1 August to 30 September 2016. Two participants from Thailand and Singapore were nominated. Because I have been working in the Insect Taxonomy group which is also responsible for the principle insect museum of Thailand, the program in Japan has been very useful for my day-to-day work and long-term responsibilities. I have learnt many things from this attachment program both with regard to the general management and display of reference collections and the identification of leafmining pests.

I have not only learnt how to prepare and identify leafminers (both Lepidopteran and Dipteran leafminers) but also gained knowledge about collecting and mass rearing of these pests. The taxonomic training encompassed family, genus and species level identification and was greatly aided by having extensive authoritatively identified specimens, up-to-date literature and the guidance of experienced taxonomists. I am grateful that the attachment has enable me to establish good working relationships with several specialists with whom I can consult in the future. The practical nature of the work, in which specimens were collected from the field or reared, prepared and then examined, was especially welcome.

Moreover, we have visited much Insect collection throughout Japan such as Osaka Natural museum, the Insect museum in Nara and the Hokkaido museum. That has been a good opportunity for me because I have gained more ideas and knowledge on how to improve my Insect museum in Thailand, both as a reference collection and as a teaching resource.

The most important thing is I have had a chance to learn how some of the most important agricultural products of Japan (vegetable and apple) are grown and how pest problems are managed. Many of these practices are widely applicable. I have a unique

opportunity to learn numerous and diverse techniques from many experienced Professors and specialists. Everything that I have learnt from Japan can be applied to Thai agricultural product, either in principle or practice.

6) Recommendation for Future Activities

Based on my experience of this attachment program, I would like to recommend that for future activities:

- The duration of two months is not sufficient for optimal learning. For challenging and diverse groups such as leafminers at least two months is required to learn how to identify genera and species. This is in addition to the time required to become confident in the specialist techniques necessary for collecting, rearing and preparation of specimens.

7) Acknowledgements

I wish to express my sincere appreciation and profound gratitude to Professor Dr Hiroaki Sato, Nara Women University for his invaluable guidance, constructive comments and kind encouragement throughout my study. As my primary advisor, he helped me with my every need. I extend my sincere appreciation and heartfelt gratitude also to the other lecturers, Associate Professor Dr Issei Oshima, Osaka Prefecture University, Professor Dr Yoshihisa Abe, Entomology, Faculty of Social and Cultural Studies and Professor DrToshiya Hirowatari, Entomological Laboratory, Faculty of Agriculture, Kyusu University, Mr Takeo Imura, Mr Yoshinori Kunimoto and his team from Agriculture Research and Development Center, Agricultural Experiment Station of Nara Prefecture in Ohwuda City, Kashihara City Museum of Insect, National Museum of Nature and Science in Kashihara city for their hospitality, crucial advice and suggestions during the study period.

I am profoundly indebted to Dr Lum Keng Yeang and Dr Soetikno S. Sastroutomo, APHCN-ASEANET for their encouragement, valuable comments and suggestions.

Finally, I would like to express my gratitude to the Japan ASEAN Integration Fund (JAIF). This attachment program would not have been possible without the support of JAIF that have provided financial to keep this project running.

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Bandelt, H. J., Forster, P. & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, 16(1), 37-48.

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9) Images/Photos



Figure 1 Genitalia slide preparation. Dissected parts were passed through a series of graded alcohols in the small, ceramic pots shown in this photograph prior to mounting.

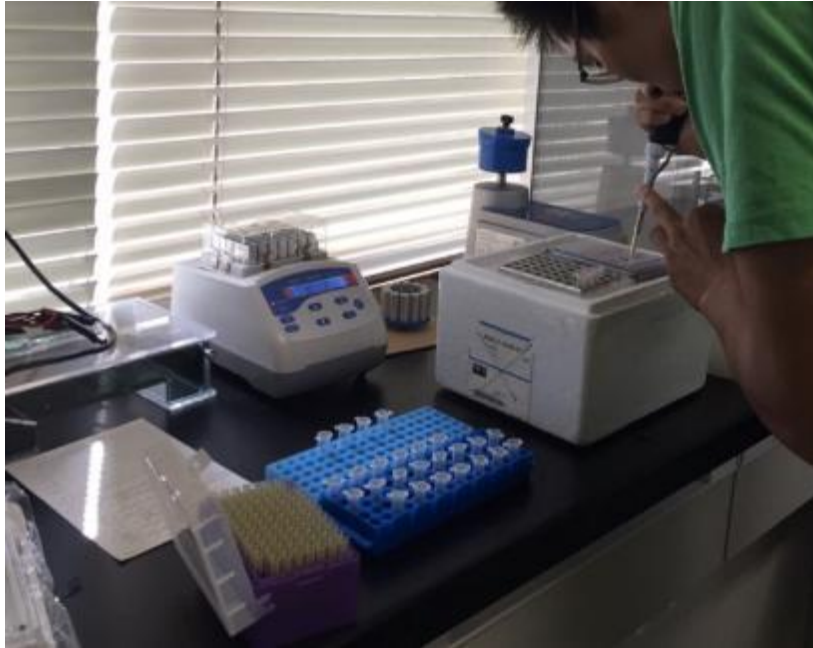


Figure 2 Demonstration how to prepare the PCR reaction by Dr Issei Oshima.

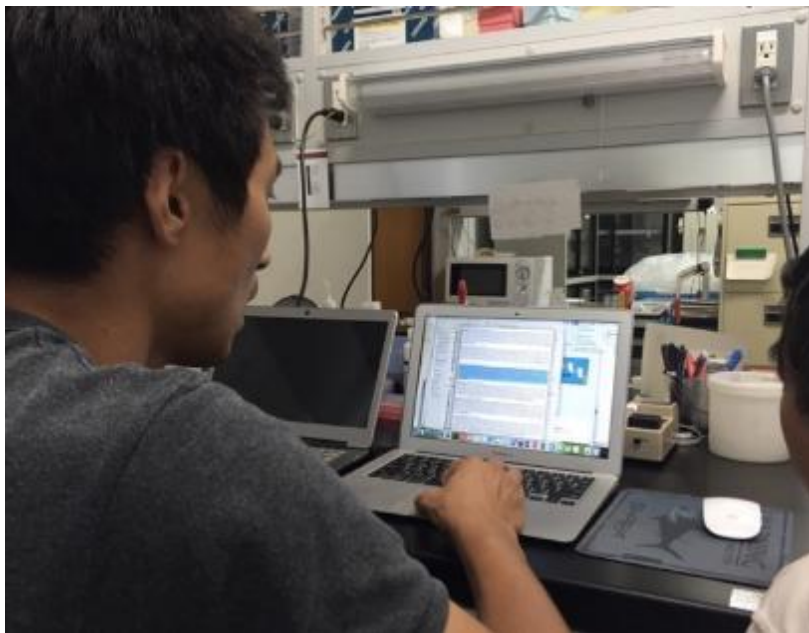


Figure 3 Demonstration of how to obtain information from leafminers sequence by Dr Issei Oshima.

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>AG-01_COIH-PREMI Length=650
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AAAGCTATATCAGGGGCTCCAAGTATAAGAGGAACAAGTCAATTACCAAATCCCCAA
TTATAATTGGTATCACTATAAAAAAATTATAATAATGCATGAGCTGTAAACAATTG
ATTATAAATTTGGTCATCTCCAATTAAGATCCTGGGTTTCTAATTCAGCTCGAATT
ATAATCTTAAAGAAGATCCAATTATTCTGCTCAAATCCAAAATAAAATAAAGAG
TTCCAATNNNNN
```

Figure 4 Sequence of leafminer (fasta file).



Figure 5 Sequence of leafminer (MEGA file).

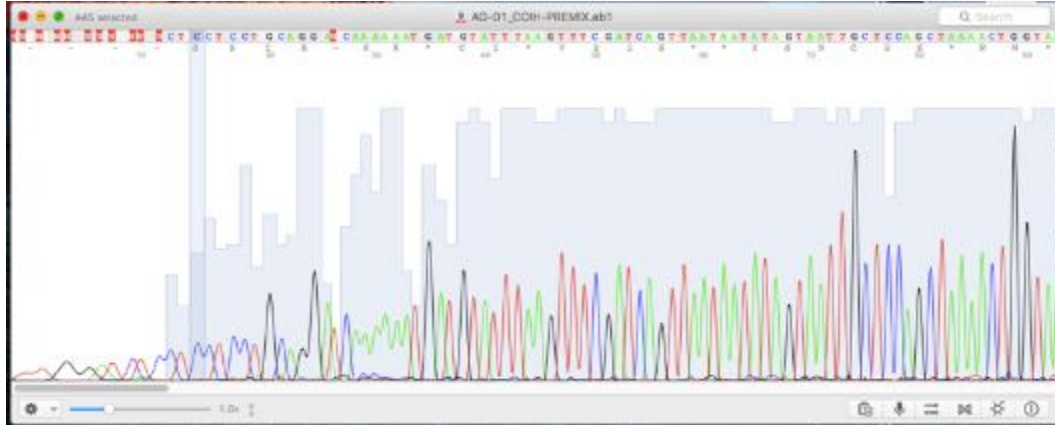


Figure 6 Sequence of leafminer from 4peak program.

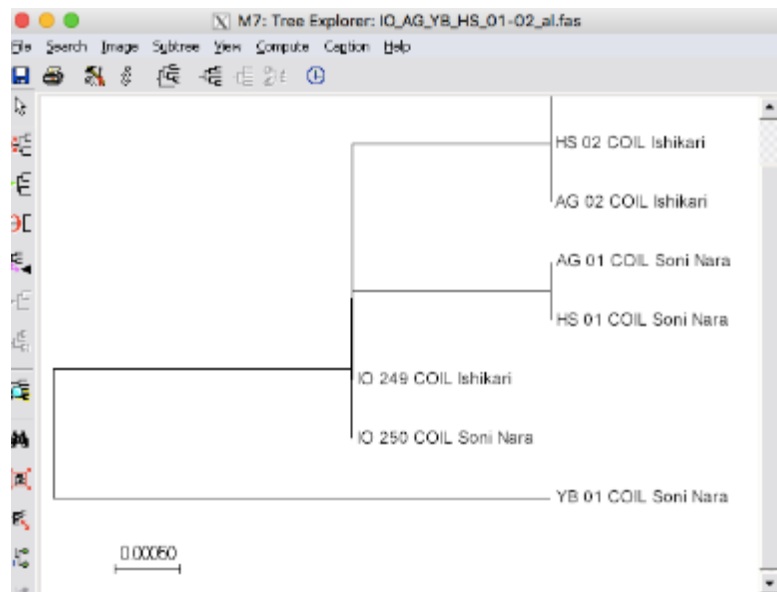


Figure 7 The evolutionary history of the sampled leafminers was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length is shown. The tree is shown the evolutionary distances used to refer the phylogenetics.



Figure 8 Collecting location at Nara Hill.



Figure 9 Collecting location at Yata Hill.



Figure 10 Leafmines at Yata Hill.

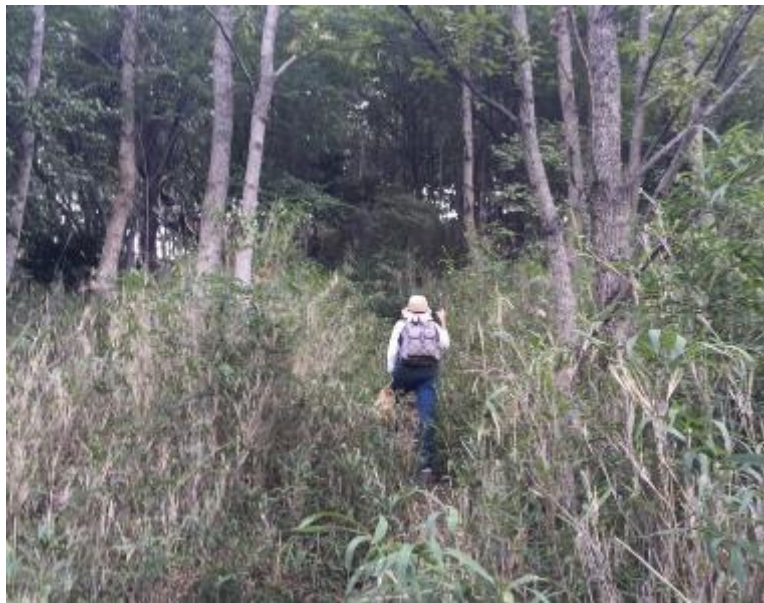


Figure 11 Collecting location at Mt. Takamado.



Figure 12 The collecting location at Mt Izumi-Katsuragi.



Figure 13 Field collection from oak trees along the Ishikari coast and transferring leafminer collections to boxes for dispatch to Nara.



Figure 14 The Insect collection in Osaka Prefecture University.



Figure 15 The Insect collection in Osaka Natural History Museum.

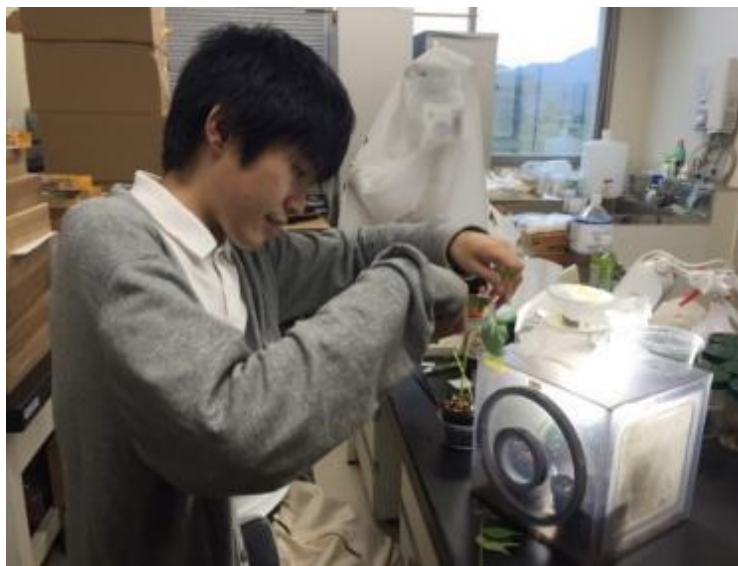


Figure 16 Mass rearing leafminers in laboratory in Kyushu University.

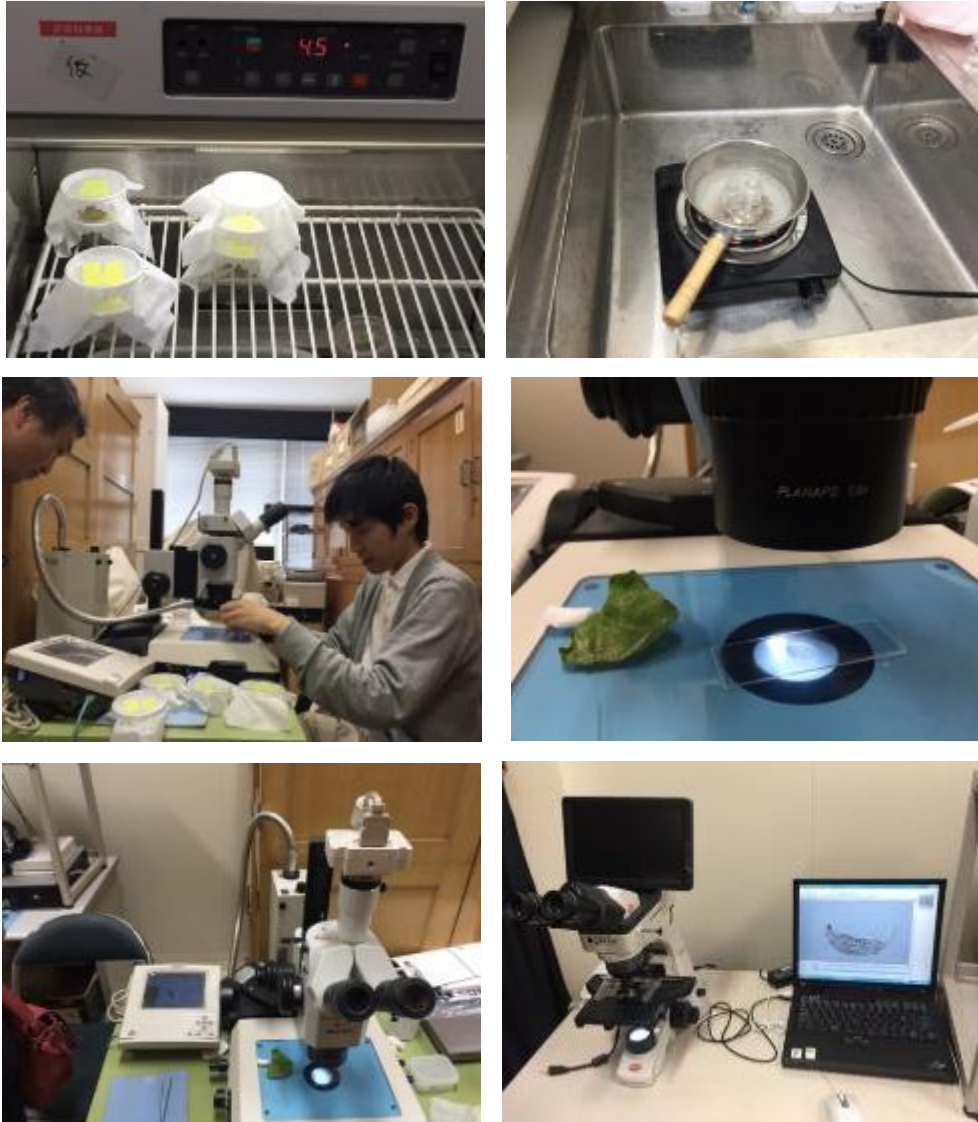


Figure 17 Genitalia study and morphological study of leafminer parasite, Kyushu University.



Figure 18 The Insect exhibition in Kyushu University.



Figure 19 The Insect collection in Kyushu University.



Figure 20 Tomato plantations in Agriculture research and Development Center, Agricultural Exp. Station of Nara Prefecture.



Figure 21 Insect collection in Agriculture research and Development Center, Agricultural Exp. Station of Nara Prefecture.



Figure 22 Onion plantations in Agriculture research and Development Center, Agricultural Exp. Station of Nara Prefecture and leaf mines caused by *Liriomyza achiensis*.



Figure 23 Tomato plantations in Agriculture research and Development Center, Agricultural Exp. Station of Nara Prefecture.



Figure 24 Tomato plantations in the local area.



Figure 25 Insectarium at Kashihara city



Figure 26 The insect exhibition at Kashihara city.



Figure 27 Hokkaido University and Hokkaido university museum.



Figure 28 Leafminer larva and insect pest of apple at Apple Research Institute of the Aomori Prefectural Industrial Technology Research Center, Kuroishi city.



Training Workshop on Diagnostics of Weevils of Quarantine Importance

July 10-22, 2017

College of Agriculture
University of the Philippines Los Baños

Funded by:

Japan – ASEAN Integration Fund (JAIF)



Japan-ASEAN Cooperation

Through

ASEAN Plant Health Cooperation Network of the ASEANET
(APHCN-ASEANET)



TRAINING WORKSHOP ON DIAGNOSTICS OF WEEVILS OF QUARANTINE IMPORTANCE

July 10 - 22, 2017

Crop Protection Cluster
Wing A, Biological Sciences Building
College of Agriculture
University of the Philippines Los Banos

I. Project Title: Training Workshop on Diagnostics of Weevils of Quarantine Importance

II. Project Duration: Two weeks, from July 10 - 22, 2017

III. Training Outline

A. Background

Weevils are small to medium insects that belongs to the Order Coleoptera. They are usually dark-colored, brownish to black. Many are considered as pests of crops or stored products. Most of the small weevils can be present in imported agricultural and stored products. Weevils belonging to genus *Sitophilus*, *Callosobruchus*, *Anthonomus*, *Acanthoscelides*, *Merhynchites*, and *Cosmopolites* are the most serious pests of storage products. The damage that they cause is very destructive and expensive.

On the other hand, there are many other species of weevils that attack agricultural crops, for example, *Sternochetus frigidus*, these weevil is responsible in the down-fall of mango export industry in Palawan Island. Currently, there are observation of weevils feeding or attacking crops in some farm areas especially those areas that where converted from forest or shrubland into agricultural lands. These weevils are not previously seen or recorded feeding on agricultural crops. Hence, quick and accurate identification is vital in quarantine processes and converted farm areas. The proper identification could be able to limit or eliminate entry of these destructive pest to the importing countries as well as proper management or control strategies can be administered.

In this workshop, we aim to teach the participants how to identify weevils with economic importance, and the importance of taxonomy and ecology in managing or controlling pests such as weevils. Also, participants will be able to learn techniques in identification based on morphology and DNA sequences.

B. Course description

This workshop is coordinated by Crop Protection Cluster, College of Agriculture, University of the Philippines Los Banos through the ASEAN Plant Health Cooperation Network (APHCN) of ASEANET project on "Taxonomy capacity building to support market access for agricultural trade in the ASEAN region." This project is funded by the Japan-ASEAN Integration Fund (JAIF) that will be implemented for 2017 covering several activities related to training and attachment programs.

This workshop aims to provide the participants basic knowledge, understanding, and practical skills on the importance of taxonomy, biology, and ecology of weevils in the context of pest management. The training course will cover the following topics: basic taxonomy, biology, ecology, identification and collection techniques, and basic DNA barcoding techniques of weevils with quarantine importance. Likewise, participants will also be taught of proper pest management strategies of these weevils.

C. General Objectives

Lecture: At the end of the training, the participants should be able to analyze the importance of controlling or managing weevils, their biology, and ecology.

Laboratory: At the end of the training, the participants should be able to demonstrate basic skills in identifying the species, genera and families of weevils based on morphology and habits, DNA barcoding, collecting, and preservation and labelling of specimens.

D. Specific Objectives

Lecture:

- 1) To describe the taxonomy, biology, and ecology of weevils with quarantine importance.
- 2) To recognize the importance of weevils in quarantine.
- 3) To discuss that weevils can invade one region to another
- 4) To design appropriate pest management strategies in controlling weevils

Laboratory:

- 1) To describe signs of weevil infestation.
- 2) To identify weevil species based on morphology and DNA barcoding.
- 3) To demonstrate basic techniques in identification, collection in the field, preservation of specimens, and DNA sequencing.

E. Training Methodology

The training will be conducted at the Crop Protection Cluster, UPLB. This would consist of lectures on the proper identification of weevils of quarantine importance and how to manage them. There will also be laboratory or hands-on exercises where participants will be exposed to the actual specimens. Likewise, visit(s) to facilities such as warehouses or agricultural field experiment stations will be conducted so that participants will have an experience on how to collect weevils.

F. Training Contents

a. Course Outline

SESSION 1. OPENING AND INTRODUCTION

- Registration
- Welcome/Opening Program
- Introduction and overview of the training course
- Group photograph

SESSION 2. CLASSIFICATION, BIOLOGY AND ECOLOGY OF WEEVILS

- Lecture: Classification, biology and ecology of weevils
- Lecture: Economic importance of weevils
- Lecture: Taxonomy of Weevils

SESSION 3. HABITUS IDENTIFICATION OF WEEVILS

- Lecture: Morphology and terminology
- Practice: Identification of specimens using keys

SESSION 4. DISSECTION OF GENITALIA

- Practice: Demonstration
- Practice: Hands-on work

SESSION 5: PRESERVATION TECHNIQUES

- Lecture: Preservation techniques
- Practice: Demonstration
- Practice: Hands-on work

SESSION 6. FIELD COLLECTION IN STORAGE FACILITIES AND AGRICULTURAL AREAS

- Practice: Field observation and sampling
- Practice: Preliminary identification of specimens

SESSION 7. WEEVILS AS STORAGE PESTS AND THEIR CONTROL

- Lecture: Common Storage pests
- Lecture: Control strategies and management of storage pests
- Practice: Identification of storage pests

SESSION 8. WEEVILS IN AGRICULTURAL CROPS

- Lecture: Important weevils attacking economically important crops
- Lecture: Control strategies and management of weevils

SESSION 9. DNA SEQUENCING

- Lecture: Proper DNA techniques and application
- Lecture: Extraction of DNA
- Lecture: Detection of DNA
- Lecture: Sequencing and identification of DNA
- Practice: Demonstration of DNA Techniques
- Practice: Extraction of DNA
- Practice: Detection of DNA
- Practice: Sequencing and identification of DNA

SESSION 10. QUARANTINE POLICIES

- Lecture: Quarantine policies and protocols

SESSION 11. RESOURCES OF WEEVILS ON THE WEB

- Browsing of web sites concerning with weevils

SESSION 12. EVALUATION AND CLOSING PROGRAM

- Post-test evaluation
- Closing Program

G. TENTATIVE PROGRAM OF THE TRAINING WORKSHOP

Date/Time/Venue	Topic/Activity	Resource Person(s)/Facilitator
Sunday, 09 July 2016		
	Arrival of Participants	Dr. Sheryl A. Yap and Ms. Priscilla M. Barcial, CPC
Week 1: Monday, 10 July 2016		
Venue: CPC Auditorium	SESSION 1. OPENING AND INTRODUCTION	
08:45 - 09:00	Registration	Ms. Priscilla M. Barcial, CPC
09:00 - 09:30	Welcome Address	Dr. Enrico P. Supangco, Dean, College of Agriculture Dr. Lum Keng Yeang, Chairperson ASEANET-APHCN
	Opening Remark	Dr. Teresita U. Dalisay, Director, CPC
09:30 - 09:40	Workshop Context and overview	Dr. Sheryl A. Yap, CPC
09:40 - 10:00	Introduction of Participants, trainers and training team	Mr. Ace Kevin S. Amarga, CPC
10:00 - 10:15	Group photograph	Mr. JP Aquino, OPR
10:15 - 10:45	Tea/coffee break	
Venue: CPC Auditorium	SESSION 2. CLASSIFICATION, BIOLOGY AND ECOLOGY OF WEEVILS	
10:45 - 12:00	Lecture: Biology and ecology of weevils	Dr. Hiraku Yoshitake, Institute for Agro-Environmental Sciences, NARO
12:00 - 13:00	Lunch break	
13:00 - 14:30	Lecture: Biology and ecology of weevils	
14:30 - 15:00	Lecture: Economic importance of weevils	
15:00 - 15:15	Tea/coffee break	
15:15 - 16:00	Country reports	Ms. Priscilla M. Barcial, CPC
16:00 - 17:00	Country reports (continued)	Ms. Priscilla M. Barcial, CPC
Venue: Kamayan sa Palaisdaan	DINNER RECEPTION	All participants Resource persons Training team

Week 1: Tuesday, 11 July 2016

Venue: Lab room	SESSION 3. HABITUS IDENTIFICATION OF WEEVILS	
09:00 - 10:30	Lecture: Morphology and Taxonomy	Dr. Hiraku Yoshitake
10:30 -10:45	Tea/Coffee break	
10:45 - 12:00	Practice: Identification of specimens using keys	Dr. Hiraku Yoshitake, Dr. Sheryl A. Yap
12:00 - 13:00	Lunch break	
13:00 - 14:45	Practice: Identification of specimens using keys	Dr. Hiraku Yoshitake, Dr. Sheryl A. Yap
14: 45 - 15:00	Tea/coffee break	
15:00 - 17:00	Practice: Identification of specimens using keys	Dr. Hiraku Yoshitake, Dr. Sheryl A. Yap

Week 1: Wednesday, 12 July 2016

Venue: Lab room	SESSION 4. DISSECTION OF GENITALIA	
09:00 - 10:30	Practice: Demonstration	Dr. Hiraku Yoshitake
10:30 -10:45	Tea/Coffee break	
10:45 - 12:00	Practice: Hands-on work	Dr. Hiraku Yoshitake, Dr. Sheryl A. Yap
12:00 - 13:00	Lunch break	
13:00 - 14:30	SESSION 5: PRESERVATION TECHNIQUES	
	Lecture: Preservation techniques	Dr. Sheryl A. Yap
14: 30 - 14:45	Tea/coffee break	
14:45 - 15:45	Practice: Demonstration	Dr. Sheryl A. Yap
15:45 - 17:00	Practice: Hands-on work	Dr. Hiraku Yoshitake, Dr. Sheryl A. Yap

Week 1: Thursday, 13 July 2016

Venue: Storage facilities (Industry: Raw materials)	SESSION 6. FIELD COLLECTION IN STORAGE FACILITIES AND AGRICULTURAL AREAS	
07:00 - 07:30	Preparation for field trip (Venue: Hotel)	Training team All participants All resource persons
07:30 - 09:30	Travel from hotel to storage facilities	
09:30 - 10:30	Arrival and briefing of the facility	
10:30 -11:30	Observation and sampling	
11:30 - 13:30	Lunch	

13:30 -15:00	Observation and sampling
15:00	Return to Hotel
17:00	Arrival at the hotel

Week 1: Friday, 14 July 2016

Venue: Storage facilities (Agricultural products)	SESSION 6. FIELD COLLECTION IN STORAGE FACILITIES AND AGRICULTURAL AREAS (continued)
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07:00 - 07:30	Preparation for field trip (Venue: Hotel)	Training team All participants All resource persons
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07:30 - 09:30	Travel from hotel to storage facilities
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09:30 - 10:30	Arrival and briefing of the facility
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10:30 -11:30	Observation and sampling
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11:30 - 13:30	Lunch/Prayer for Moslems
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13:30AM -15:00	Observation and sampling
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15:00	Return to Hotel
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17:00	Arrival at the hotel
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Week 1: Saturday, 15 July 2016

Venue: Farm areas	SESSION 6. FIELD COLLECTION IN STORAGE FACILITIES AND AGRICULTURAL AREAS (continued)
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07:00 - 07:30	Preparation for field trip (Venue: Hotel)	Training team All participants All resource persons
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07:30 - 09:30	Travel from hotel to the farm
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09:30 - 10:30	Arrival
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10:30 -11:30	Observation and sampling
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11:30 - 13:30	Lunch
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13:30AM -15:00	Observation and sampling
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15:00	Return to Hotel
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17:00	Arrival at the hotel
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Week 1: Sunday, 16 July 2016

REST DAY

Week 2: Monday, 17 July 2016

Venue: Lab room

SESSION 7. WEEVILS AS STORAGE PESTS AND THEIR CONTROL

09:00 - 10:30	Lecture: Common Storage pests	Dr. Pio A. Javier
10:30 - 10:45	Tea/Coffee break	
10:45 - 12:00	Lecture: Control strategies and management of storage pests	
12:00 - 13:00	Lunch break	
13:00 - 14:30	Identification of storage pests	Dr. Pio A. Javier, Dr. Sheryl A. Yap, Dr. Hiraku Yoshitake
14:30 - 15:00	Tea/Coffee break	
15:00 - 17:00	Identification of storage pests	Dr. Pio A. Javier, Dr. Sheryl A. Yap, Dr. Hiraku Yoshitake

Week 2: Tuesday, 18 July 2016

Venue: Lab room

SESSION 8. WEEVILS IN AGRICULTURAL CROPS

09:00 - 10:30	Lecture: Weevils in agricultural crops	Dr. Celia dR. Medina
10:30 - 10:45	Tea/Coffee break	
10:45 - 12:00	Lecture: Control strategies and management of storage pests	
12:00 - 13:00	Lunch break	
13:00 - 14:30	Identification of weevils in agricultural crops	Dr. Celia dR. Medina, Dr. Sheryl A. Yap, Dr. Hiraku Yoshitake
14:30 - 15:00	Tea/Coffee break	
15:00 - 17:00	Identification of weevils in agricultural crops	Dr. Celia dR. Medina, Dr. Sheryl A. Yap, Dr. Hiraku Yoshitake

Week 2: Wednesday, 19 July 2016**SESSION 9. DNA SEQUENCING**

9:00 - 10:30	Lecture: DNA techniques and their application	Dr. Barbara L. Caoili
10:30 - 10:45	Tea/Coffee break	
10:45 - 12:00	Lecture: Extraction of DNA	
12:00 - 13:00	Lunch break	
13:00 - 14:30	Lecture: Detection of DNA	Dr. Barbara L. Caoili
14:30 - 15:00	Tea/Coffee break	

15:00 - 17:00	Lecture: Sequencing and identification of DNA	Dr. Barbara L. Caoili
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Week 2: Thursday, 20 July 2016

SESSION 9. DNA SEQUENCING (continued)

09:00 - 10:30	Practice: Demonstration of DNA Techniques	Dr. Barbara L. Caoili/Insect Path lab team
10:30 - 10:45	Tea/Coffee break	
10:45 - 12:00	Practice: Extraction of DNA	Dr. Barbara L. Caoili/Insect Path lab team
12:00 - 13:00	Lunch break	
13:00 - 14:30	Practice: Detection of DNA	Dr. Barbara L. Caoili/Insect Path lab team
14:30 - 15:00	Tea/Coffee break	
15:00 - 17:00	Practice Sequencing and identification of DNA	Dr. Barbara L. Caoili/Insect Path lab team

Week 2: Friday, 21 July 2016

SESSION 10. QUARANTINE POLICIES

9:00 - 10:30	Lecture: Quarantine policies and protocols	Quarantine officer
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10:30 - 10:45	Tea/coffee break	
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SESSION 11. RESOURCES OF WEEVILS ON THE WEB

10:45 - 12:00	Browsing of web sites concerning with weevils	Dr. Hiraku Yoshitake, Dr. Sheryl A. Yap
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12:00 - 13:00	Lunch break	
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SESSION 12. EVALUATION AND CLOSING PROGRAM

13:00 - 16:00	Post-test evaluation	Dr. Sheryl A. Yap
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14:00 - 14:30	Tea/coffee break	
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14:30 - 16:30	Closing remarks rela	Dr. Hiraku Yoshitake
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	Information related to Attachment Program	ASEAN representative
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	Official closing	Dr. Teresita U. Dalisay
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	Presentation of certificates	Dr. Hiraku Yoshitake
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		Dr. Sheryl A. Yap
--	--	-------------------

		Dr. Teresita U. Dalisay
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	Response from participants	Participant
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Week 2: Saturday, 22 July, 2016

DEPARTURE

H. RESOURCE PERSONS

1. Dr. YOSHITAKE Hiraku, Research Scientist for Insect Systematics, Institute for Agro-Environmental Sciences, NARO, Kannondai 3-1-3, Tsukuba, Ibaraki 305-8604, Japan
E-mail: zoumushi@affrc.go.jp, Tel: 029-838-8348; Fax: 029-838-8354
2. Dr. Pio A. Javier, Research Professor, Crop Protection Cluster, College of Agriculture, University of the Philippines Los Baños, Los Baños, Laguna, Philippines 4031; E-mail: pio.javier@yahoo.com; Tel: +6349-536-1315
3. Dr. Celia dR. Medina, Associate Professor, Crop Protection Cluster, College of Agriculture, University of the Philippines Los Baños, Los Baños, Laguna, Philippines 4031; E-mail: cdrmedina@yahoo.com; Tel: +6349-536-1315
4. Dr. Barbara L. Caoili, Professor, Crop Protection Cluster, College of Agriculture, University of the Philippines Los Baños, Los Baños, Laguna, Philippines 4031; E-mail: blcaoili@up.edu.ph; Tel: +6349-536-1315
5. Dr. Sheryl A. Yap, Assistant Professor, Crop Protection Cluster, College of Agriculture, University of the Philippines Los Baños, Los Baños, Laguna, Philippines 4031; E-mail: sayap3@up.edu.ph; Tel: +6349-536-2617

I. ASEANET-APHCN TRAINING COORDINATION

1. Dr. Lum Keng Yeang, Chairperson, ASEANET-APHCN, c/o CABI SEA, P.O. Box 210, UPM Post, 43400 Serdang, Selangor, Malaysia, E-mail: ky.lum@cabi.org; Telp.: +60-3-89432921.
2. Dr. Soetikno S. Sastroutomo, Technical Secretary, ASEANET-APHCN, c/o CABI SEA, P.O. Box 210, UPM Post, 43400 Serdang, Selangor, Malaysia, E-mail: s.soetikno@cabi.org; Telp.: +60-3-89432921.

3RD PROGRESS REPORT FOR JAIF - NETWORKING AND INSTITUTIONALISATION

Objectives of this component include information sharing, information dissemination and mainstreaming and institutionalisation of information through networking. The information gained through networking and exchange of information becomes embedded and enters the common knowledge domain of respective institutions and NPPOs of countries concerned.

Mainstreaming and institutionalisation of information is achieved through the various activities in the project; training and capacity building workshops, attachment programs with experts, engagement through the project website, online tools and services and other project activities.

The project website provides a platform to host tools and services e.g. expert register databases, online diagnostic tools, pests and diseases information etc.

Latest project activities

The announcements of the latest attachment programs on plant virology held in Tokyo University of Agriculture, Japan and diagnostic of leafminers in Nara Women University, Japan have been uploaded. The section hosts all the very latest updates, images, documents and outputs related to the aforesaid activities.

The meeting of the Expert Working Group on Harmonisation of Phytosanitary Measures in ASEAN was uploaded and announced in this section as well. The meeting saw the reporting of progress made till April 2016 and discussions on the proposal for a possible Phase 2 of the project. The relevant outputs from the meeting have been uploaded and made available for downloading.

The section features a summary, followed by images and a longer description of the activity. The document section is at the bottom of the project activity news body and displays all outputs related to that activity (reports, presentations, publications, etc). The title and format of the document can be seen and downloaded (previously, the document links can only be downloaded after logging but is now available to all).

Attachment Program in Plant Virology at Tokyo University of Agriculture, Japan

Date: 26th Oct - 25th Dec 2015

The attachment program was held at the Laboratory of Tropical Plant Protection, Tokyo University of Agriculture (Tokyo NODAI), Japan from October 26 - December 25, 2015 with the objective of providing a basic understanding of the concepts of plant viruses and practical measures, a diagnosis of infected plants, and to learn the latest technology and management of diseases involving viral diseases



Search

Search site

Activities

18th Meeting of the Experts Working Group on Harmonisation of Phytosanitary Measures in ASEAN

Attachment Program in Diagnostic of Leaf-miners at Nara Women University, Japan

Attachment Program in Plant Virology at Tokyo University of Agriculture, Japan

News

A new species of pest fruit fly (Diptera: Tephritidae: Dacinae) from Sri Lanka and Africa
 Biodiversity and Human Well-being: A Synthesis Report for the Convention on Biological Diversity

Fig. 1 – Section on project activities with summary, images and description

During the attachment program they were supervised by Prof. Keiko Natsuaki from Laboratory of Tropical Plant Protection, Tokyo University of Agriculture, Japan. The selection of participants were based on the individual performance and evaluation results as well as observation and evaluation by all resource persons during the "Training Workshop on the Diagnostic of Plant Viruses" that was organised at UPLB, Philippines from 17-28 August 2015. The program of the 2 months attachment were divided into 4 categories, i.e.:

- Series of lectures given by resource persons and invited speakers
- Individual experiments and laboratory works supervised by Prof. Natsuaki and Dr. M. Pinili
- Participation in the International Congress of International Society for South East Asian Agricultural Sciences (ISSAS) organized by Tokyo NODAI from 7-9 November 2015, and
- Participation in the seminar and defence of PhD students of Tokyo NODAI

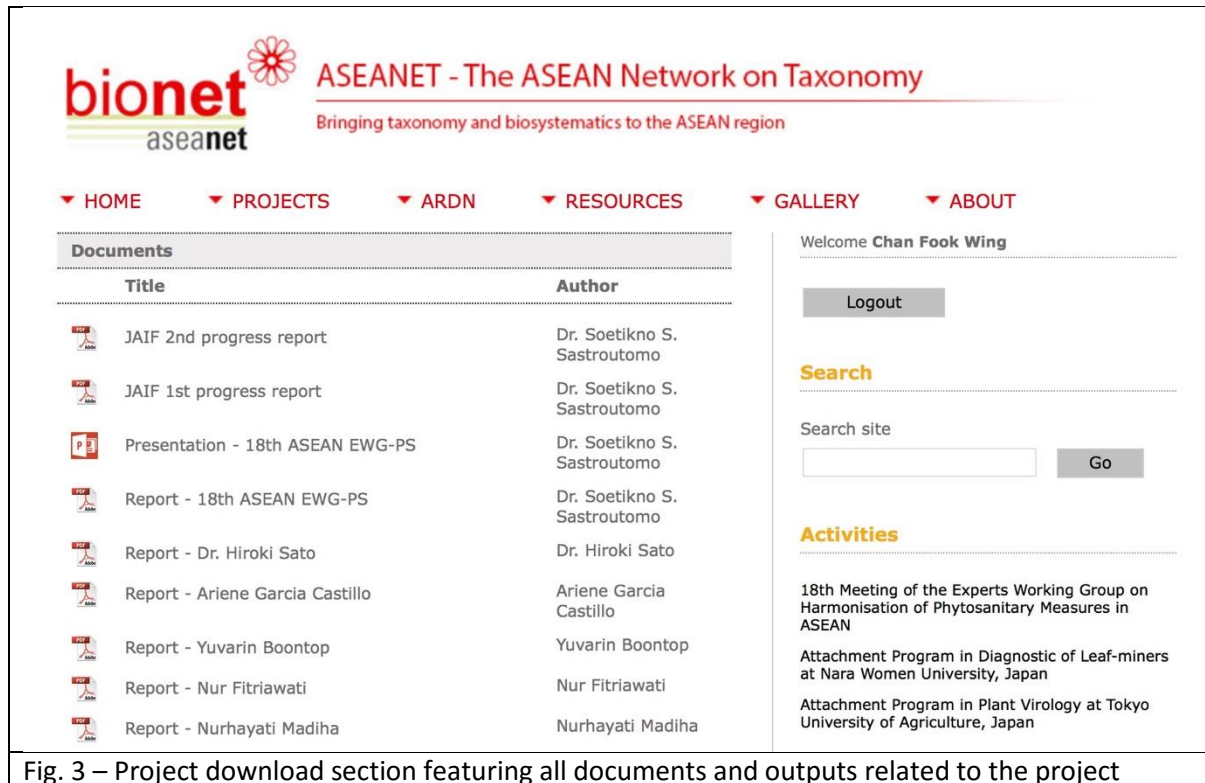
Documents



Fig. 2 – Documents related to project activities now available to all

Project download section

The project download section contains documents and other outputs related to activities carried out during the project. The documents come in different formats depending on the output produced during the project activity. All formats are catered for and different icons are used for the different format to provide visual differentiation when viewing documents.












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Documents

Title	Author
 JAIF 2nd progress report	Dr. Soetikno S. Sastroutomo
 JAIF 1st progress report	Dr. Soetikno S. Sastroutomo
 Presentation - 18th ASEAN EWG-PS	Dr. Soetikno S. Sastroutomo
 Report - 18th ASEAN EWG-PS	Dr. Soetikno S. Sastroutomo
 Report - Dr. Hiroki Sato	Dr. Hiroki Sato
 Report - Ariene Garcia Castillo	Ariene Garcia Castillo
 Report - Yuvarin Boontop	Yuvarin Boontop
 Report - Nur Fitriawati	Nur Fitriawati
 Report - Nurhayati Madiha	Nurhayati Madiha

Welcome **Chan Fook Wing**

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Activities

18th Meeting of the Experts Working Group on Harmonisation of Phytosanitary Measures in ASEAN

Attachment Program in Diagnostic of Leaf-miners at Nara Women University, Japan

Attachment Program in Plant Virology at Tokyo University of Agriculture, Japan

Fig. 3 – Project download section featuring all documents and outputs related to the project

Image Gallery

The gallery now holds updated images from the latest attachment program workshops on plant viruses and leafminers held in universities in Japan. A better selection of images for both workshops is being sought for uploading.

Attachment Program in Diagnostic of Leaf-miners at Nara Women University, Japan, 1st Aug - 30th Sep 2016, Department of Biological Sciences, Faculty of Science, Nara Women's University, Nara, Japan



Attachment Program in Plant Virology at Tokyo University of Agriculture, Japan, 26th Oct - 25th Dec 2015, Laboratory of Tropical Plant Protection, Tokyo University of Agriculture (Tokyo NODAI), Japan



Fig. 4 – Image gallery

Search site

Go

Activities

18th Meeting of the Experts Working Group on Harmonisation of Phytosanitary Measures in ASEAN

Attachment Program in Diagnostic of Leaf-miners at Nara Women University, Japan

Attachment Program in Plant Virology at Tokyo University of Agriculture, Japan

News

A new species of pest fruit fly (Diptera: Tephritidae: Dacinae) from Sri Lanka and Africa

Biodiversity and Human Well-being: A Synthesis Report for the Convention on Biological Diversity

Expert Register

The expert register has already been populated with data from the previous database of contacts comprising participants and resource persons from capacity building workshops on taxonomy carried out over a number of years in the past. The register is currently being updated to include participants of workshops on plant viruses and leafminers held in Indonesia and the Philippines.

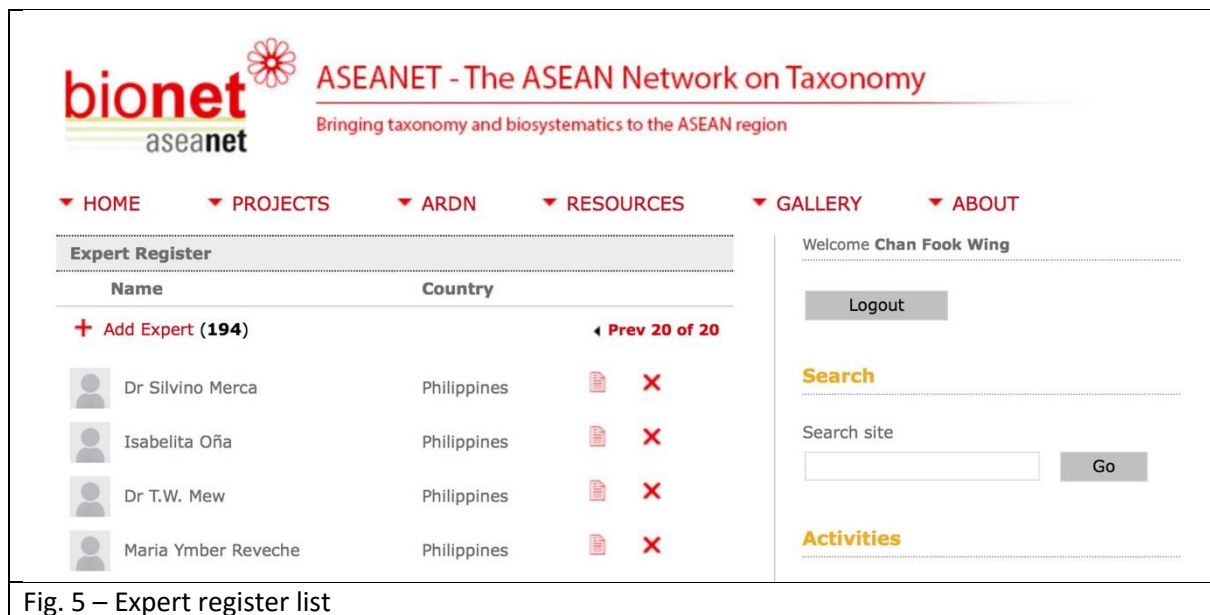


Fig. 5 – Expert register list

Mobile version of the expert register

The mobile version of the register has been developed and is compatible on major mobile platforms, allowing for quick access and reference to the register from smartphones and mobile devices. Further testing on different devices is on-going, subject to availability of devices.

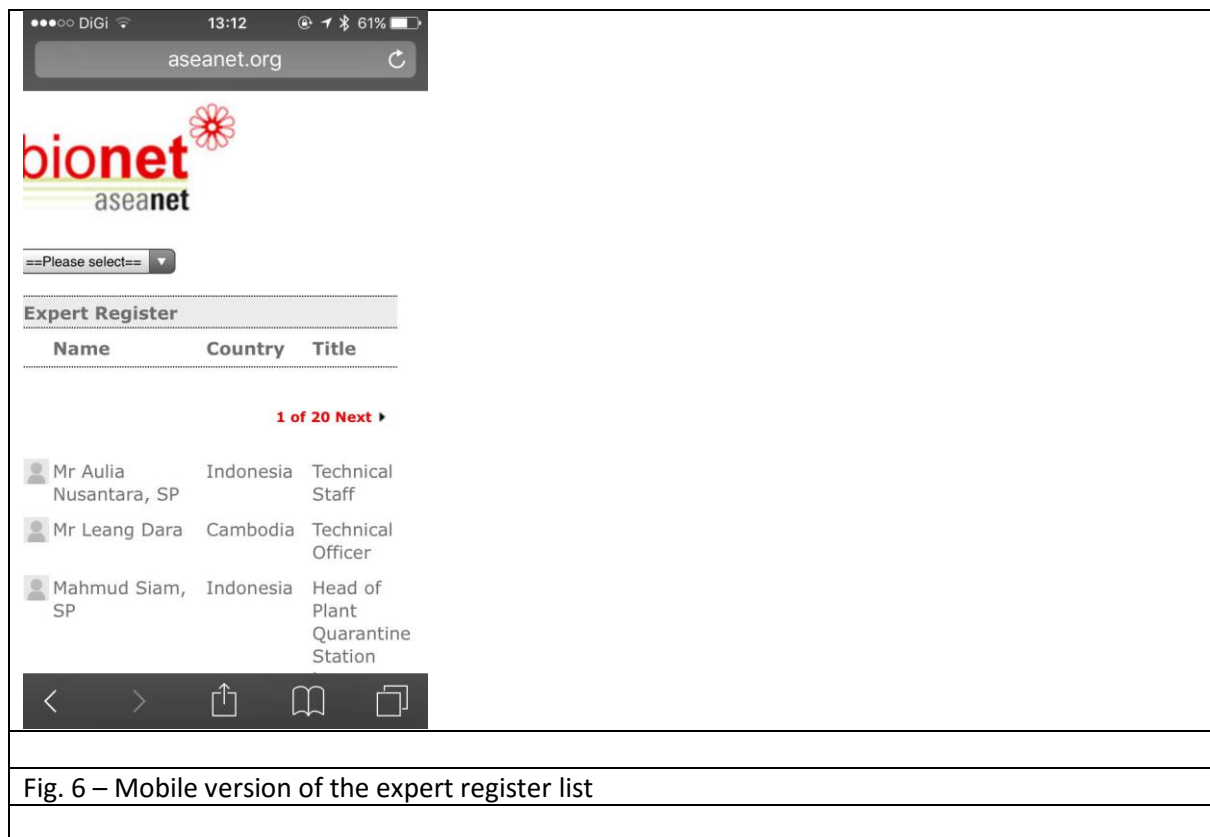



Fig. 6 – Mobile version of the expert register list

DiGi 13:13 aseanet.org 60%

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==Please select==

Expert Register

 **Hoang Kim Thoa**
Technical Officer
☎ 84 4 8513746
☎ 84 4 8513746
✉ thanhtram1992@yahoo.com;
thoa_hoangkim75@yahoo.co

ADDRESS
149 Ho Duc Di Street, Dong Da District,
Hanoi
Vietnam

DEPARTMENT/INSTITUTE
Plant Quarantine Diagnostic Center

EXPERTISE CROPS PATHOGENS

Fig. 7 – Expert details (mobile version)



**JAIF FUNDED PROJECT ON “TAXONOMIC CAPACITY BUILDING TO SUPPORT
MARKET ACCESS FOR AGRICULTURAL TRADE IN THE ASEAN REGION”**

REPORT TO:

**ASEAN EXPERTS WORKING GROUP FOR THE HARMONISATION OF
PHYTOSANITARY MEASURES (EWGHPM)**

&

ASEAN SECTORAL WORKING GROUP ON CROPS (ASWGC)

Vientiane, Laos, 18 – 19 July 2016

The project on “Taxonomic capacity building to support market access for agricultural trade in the ASEAN region” has been approved for funding by the Japan-ASEAN Integration Fund (JAIF) on 15th April 2015 for 2 (two) years starting in 1st May 2015.

This project will develop and strengthen capacities in taxonomic knowledge to identify and manage quarantine risks associated with agricultural commodities and to accurately diagnose pests and diseases among the ASEAN Member States (AMS). Key activities of the project would be a small part of the major activities of the ASEAN Regional Diagnostic Network (ARDN) Strategic Plan, an output of the 2009 Planning Meeting of ARDN held in Vientiane, Lao PDR, organized by ASEANET in collaboration with and supported by NZAid-Plant Health and AusAID SPS Capacity Building programs. The concept of an ARDN has been endorsed repeatedly by the Experts Working Group on the Harmonization of Phytosanitary Measures and the ASEAN Sectoral Working Group on Crops (first in Bali, Indonesia in 2005, then in Langkawi, Malaysia in 2007, and in Nay Pyi Daw, Myanmar in 2008). These meetings recommended pilot activities, in particular the development of a list of regional resources (expertise and laboratories) and taxonomic capacity building on several major invasive pest & diseases.

The three (3) major components undertaken in this project in line with the ARDN Strategic Plan are:

- Project Activity 1 - Training and Capacity Building
- Project Activity 2 - Networking and Institutionalization
- Project Activity 3 - Management and Coordination

The full project document has been given as ANNEX 10 of the 17th EWG-PS Meeting Report held in Jogjakarta, Indonesia in May 2015.

PROGRESS OF THE PROJECT

Five major activities have been carried out during the period May 2015 to June 2016, i.e.:

- Activity 1.1. Training Workshop on Diagnostics of Plant Viruses, and Training Workshop on Diagnostics of Leafminers of Agricultural Importance.
- Activity 1.2. Attachment Program on Plant Viruses
- Activity 2.2. Development of project website
- Activity 2.3. Development of promotional materials
- Activity 3.1. Project Inception Meeting

1. Training Workshop on Diagnostics of Plant Viruses

This “Training Workshop on the Diagnostics of Plant Viruses” was implemented at the Institute of Plant Breeding (IPB) – Crop Science Cluster, College of Agriculture, University of the Philippines – Los Baños from 17-28 August 2015 and was participated by 19 (nineteen) plant pathologists from Brunei Darussalam, Cambodia, Indonesia, Lao PDR, Malaysia, Myanmar, Philippines, Thailand and Vietnam. The majority of them are attached to Plant Quarantine and Plant Protection Centres of their respective countries under the Department of Agriculture and one from Bogor Agricultural University. The ultimate goal of the training workshop was to develop capacity building among plant virologists across the ASEAN region in addressing virus diseases in each country that may pose potential threats (emerging or invasive) in the exchange/movement of crops or planting materials. The training workshop caters to the need to equip our plant virologists who are working in universities, research institutions and plant quarantine and plant protection offices with basic knowledge on disease identification, detection and characterization using available tools (symptomatology, transmission, serology and molecular assay). The main resource persons of the workshop were from Tokyo University of Agriculture (Prof. K. Natsuaki), Japan; Bogor Agricultural University (Prof. Sri Hendrastuti Hidayat), Indonesia and from IPB-UPLB (Dr. Marita Pinili and her team).

2. Training Workshop on Diagnostics of Leafminers of Agricultural Importance

This “Training Workshop on the Diagnostics of Leafminers of Agricultural Importance” was coordinated by the Museum Zoologicum Bogoriense, Centre for Research Development in Biology, LIPI, Cibinong, Bogor, Indonesia from 29 February to 11 March 2016 and was participated by 20 (twenty) entomologists from Brunei Darussalam, Cambodia, Indonesia, Lao PDR, Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam.

The ultimate goal of the training workshop was to develop capacity among entomologists across the ASEAN region in addressing leafminer pests in each country that may pose potential threats (emerging or invasive) to their economic crops intended for export or as planting materials. The training workshop provided the participants with basic knowledge on the ecology and taxonomy of leafminers of agricultural importance with focus on improvement of skills in the identification of leaf miners and their parasitoids using morphological characters and molecular techniques. The main resource persons of the workshop were from Nara Women University, Nara, Japan (Prof. Hiroaki Sato); School of Applied Systems Biology, Centre for Agri-Bioscience, La Trobe University, VIC 3083, Australia (Assoc. Prof. M. Malipatil), and from the Museum Zoologicum Bogoriense Bogor (Dr. Hari Sutrisno, Prof. Rosichon Ubaidillah and Dr. Awit Suwito and his team).

3. Attachment Program on Plant Viruses

The attachment program was held at the Laboratory of Tropical Plant Protection, Tokyo University of Agriculture (Tokyo NODAI), Japan from October 26 – December 25, 2015 with the objective to strengthen understanding of the concepts and practice with plant viruses, diagnosis of infected plants, and to learn the latest technologies associated with the management of diseases attributed to plant viruses.

The attachment were participated by 3 (three) plant health/biosecurity officers, one each from Indonesia, Malaysia and Vietnam, i.e.

1. Ms. Nur Fitriawati, BBUSKP, Plant Quarantine and Biosafety Centre, Indonesian Agriculture Quarantine Agency (IAQA), Jakarta, Indonesia
2. Ms. Norhayati binti Madiha, Agriculture Officer Plant Biosecurity Division Department of Agriculture Jalan Gallagher, 50632 Kuala Lumpur Malaysia

3. Mr. Tran Van Chien, Post Entry Quarantine Centre I, Plant Protection Department, Ministry of Agriculture & Rural Development, Hanoi, Vietnam

During the attachment program they were supervised by Prof. Keiko Natsuaki from Laboratory of Tropical Plant Protection, Tokyo University of Agriculture, Japan. The selection of participants was based on the individual performance and evaluation results as well as observation and evaluation by all resource persons during the “Training Workshop on the Diagnostic of Plant Viruses” that was organised at UPLB, Philippines from 17-28 August 2015. The program of the 2 months attachment comprised four components, i.e.:

- a) Series of lectures given by resource persons and invited speakers,
- b) Individual experiments and laboratory work supervised by Prof. Natsuaki and Dr. M. Pinili,
- c) Participation in the International Congress of International Society for South East Asian Agricultural Sciences (ISSAS) organized by Tokyo NODAI from 7-9 November 2015, and
- d) Participation in the seminar and defence of PhD students of Tokyo NODAI.

4. Development of project website

The website for the project has been developed and is now up and running at the URL address: <http://www.aseanet.org/JAIF1.asp>. The website serves three main purposes: repository for all activities, updates, news and reports for the project; communication, publicity and awareness where the project is publicized to stakeholders and where project partners communicate and interact; knowledge exchange where the website serve as a platform for knowledge exchange and resources for taxonomy.

The website has sections on project activities, news, gallery showcasing images from workshop and project activities, resources where project documents are uploaded, expert register and content management system. Project documents are behind a password protected section and logins will be provided to project partners for download.

The expert register comprises information and contact details of individual experts and diagnostic laboratories that provide identification and diagnostic services and facilities for plant pests and diseases. The register is populated with data from the previous database of contacts comprising participants and resource persons from capacity building workshops on taxonomy carried out over a number of years. The experts and diagnostic laboratories are mainly located in the region i.e. Southeast Asia

The mobile version of the register has been developed and is compatible on major mobile platforms, allowing for quick access and reference to the register from smartphones and mobile devices that users have on them.

5. Development of promotional materials

One thousand copies of the Project Brief flyer were printed and until June 2016 has been distributed to participants at the following events:

- a. Project Inception Meeting (in Malaysia)
- b. 29th Session of the Asia Pacific Plant Protection Commission (in Bali, Indonesia)
- c. JAIF Training Workshop on Diagnostics of Plant Viruses (UPLB, Philippines)
- d. AANZFTA - Training Workshop on Whiteflies (MARDI, Malaysia)
- e. AANZFTA – Training Workshop on Diagnostics of Plant Diseases (Chiang Mai University, Thailand)
- f. JAIF Training Workshop on Diagnostics of Leafminers of Agricultural Importance (LIPI, Cibinong, Indonesia, March 2016)
- g. AANZFTA - Workshop on Diagnostics of Immature stages of Pest Lepidoptera (Bekasi, Indonesia, November 2015)
- h. AANZFTA – ASEAN Diagnosticians Forum (Putrajaya, Malaysia, December 2015)

- i. AANZFTA – Weed Identification and Risk Assessment Workshop (Vientiane, Laos, February 2016)

6. Project Inception Meeting

The meeting was held on 27th July 2015 at the Avillion Admiral Cove Hotel, Port Dickson, Malaysia with the objectives: a) To establish a Steering Committee for the Project b) To finalize details for identified project activities b) To discuss and finalize the budget for each activity c) To discuss and confirm the selection criteria for training workshop participants The meeting was attended by 11 participants composed of NPPO representatives of 6 (six) ASEAN member states, i.e. Malaysia (2), Indonesia (1), Philippines (2), Singapore (1), Thailand (1) and Vietnam (1) and from APHCN-ASEANET (3).

The project steering committee members are the following:

- a. Ms. Faridah Aini binti Muhammad, Director, Plant Biosecurity Division
Department of Agriculture, Ministry of Agriculture & Agro-based Industry, Jln. Sultan Salahuddin, 50632 Kuala Lumpur, Malaysia. E-mail: faridah@doa.gov.my
- b. Ms. Benavidez, Paz Il Joson, Assistant Secretary for Regulations, Department of Agriculture and OIC Director, Bureau of Plant Industry, Elliptical Road, Diliman, Quezon City, 692 San Andres St., Malate, Manila, Philippines. E-mail: asec.regulations2014@gmail.com
- c. Dr. Antario Dikin, Director, Centre for Plant Quarantine and Biosafety, Indonesian Agricultural Quarantine Agency, Ministry of Agriculture, Jalan Harsono RM. No. 3, Building E, 5th Floor, Ragunan-Jakarta 12550, Indonesia. E-mail: antario_dikin@yahoo.com
- d. Ms. Yap Mei Lai, Jenny, Programme Chief (Plant Health) and Director, Plant Health Laboratory Dept., Agri-Food & Veterinary Authority, Animal & Plant Health Centre, 6 Perahu Road, 718827 Singapore. E-mail: YAP_Mei_Lai@ava.gov.sg
- e. Dr. Pornpimon Athipunyakom, Senior Plant Pathologist, Plant Pathology Research Group, Plant Protection Research & Development Office, Department of Agriculture, Chatuchak, Bangkok 10900, Thailand.
E-mail: pathipunyakom@gmail.com
- f. Dr. Duong Minh Tu, Director, Plant Quarantine Diagnostic Centre, Plant Protection Department, Ministry of Agriculture and Rural Development (MARD), 149, Ho Duc Di Street, Dong Da District, Ha Noi, Viet Nam. E-mail: thanhtam1992@yahoo.com

PROPOSED PROJECT ACTIVITIES UNTIL DECEMBER 2016

1. Attachment Program on Leafminers of Agricultural Importance and its Natural Enemies

The attachment on leafminers taxonomy has been planned for 1st August to 30th September at Nara Women University, Japan under the supervision of Assoc. Prof. Hiroaki Sato, Department of Biological Sciences, Faculty of Science. Two participants from the training workshop held in LIPI, Indonesia have already been selected by the resource persons and approved by the Project Steering Committee to attend the attachment program in Japan, i.e.:

- a) Dr. Yuvarin Boontop, Senior Entomologist, Insect Taxonomy Group, Plant Protection Research, Department of Agriculture, 50 Phaholyothin Road, Bangkok 10900, Thailand. E-mail: yuvarin_b@yahoo.com
- b) Ms. Ariene Garcia Castillo, Scientist, Agri-Food & Veterinary Authority of Singapore, Animal and Plant Health Centre, 6 Perahu Road, Singapore 718827. E-mail: Ariene_Garcia_Castillo@ava.gov.sg

The attachment on leafminer natural enemies will be held from 3rd October to 30th November 2016 at Muzeum Zoologicum Bogoriense, LIPI, Cibinong, Indonesia under the supervision of Prof. Rosichon Ubaidillah. A participant from Brunei has been selected, pending their government approval, to attend this attachment program.

2. Training Workshop on Diagnostics of Aphids of Agricultural Importance

This training workshop would be organized in Thailand, pending confirmation from the Japanese resource person, Prof. Shin-ichi AKIMOTO, Laboratory of Systematic Entomology, Graduate School of Agriculture, Hokkaido University.

PROPOSED 2nd PHASE OF JAIF PROJECT

The current project has been scheduled to be completed by April 2017. During the Project Inception Meeting held in July 2015, several potential activities have been proposed and approved by the Project Steering Committee (PSC) for further consideration and approval by the ASEAN NPPOs. The approved PSC training activities together with proposals offered by resource persons during the training workshops and attachment programs have been circulated by e-mail to NPPOs of the 10 ASEAN countries with the request that the activities be ranked based on their respective country priorities. The result of this exercise is given in ANNEX 1.

Ten activities have been selected for possible funding consideration by JAIF in the Phase 2 of the Project. The full proposal of the 2nd Phase Project would be prepared after the approval is sought and after consultation with the relevant authority in Japan.

The meeting is requested to endorse the list of possible activities for approval by the SOM Meeting.