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

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Singapore

Organized by:

Nara Women's University
Nara, Japan

In Collaboration with:

bionet aseanet

ASEAN Network on Taxonomy

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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

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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, 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 *Quercu*s 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 *Phyllonorcyter 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 25^0C 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 trekked to the mountain was characterised by steep ascend 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. Monotrysia (Micropterigoidae) and Ditrysia (Tischeriidae, Opostegidae, Nepticulidae, Gelechidae, 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 recognized 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 Kashihara 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 *Phyllonorcyter 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.

<u>23 September 2016</u>

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, *Phyllonorcyter 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, Neonicotenoids 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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-for the accommodation

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Dr/Prof Yoshitsugu Nasu, and Osaka Plant Protection Office, Osaka, Japan

Dr/Prof Yutaka Yoshiyasu , Dr/Prof Norio Hirai and Dr/Prof Shouhei Ueda, Environmental Entomology and Zoology, Graduate School of Life and Environmental Sciences, Osaka Prefectural University, Osaka, Japan

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

Dr/Prof Yoshihisa Abe and Mr Taguchi Daisuke, Faculty of Social and Cultural Studies Kyushu University (Hakozaki Campus), Fukuoka, Japan

Dr/Prof Toshiya Hirowatari and students of Entomological Laboratory, Faculty of Agriculture, Kyushu University (Ito Campus), Fukuoka, Japan

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Mr Fumiaki Kimura and The Kashihara City Insectarium, Kashihara, Nara Prefecture, Japan

Dr/Prof Masahito T. Kimura, Dr/Prof Tetsuo Kohyama, Mr Matsumura and the Faculty of Environmental Earth Science, Hokkaido University, Sapporo, Hokkaido, Japan

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)

Dr/Prof Hiroaki Sato Training Coordinator Lepidopteran Leafminers Expert Department of Biological Sciences Faculty of Science, Nara Women's University, Nara, Japan	
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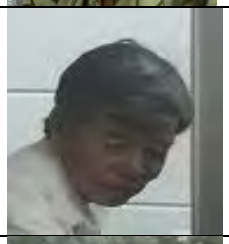
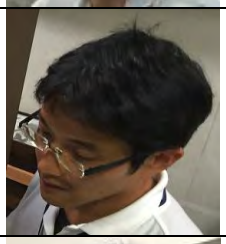
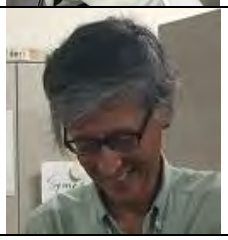
Dr/Prof Yoshitsugu Nasu Microlepidopteran Expert Research Institute of Environment, Agriculture and Fisheries, Osaka Prefecture, Japan	
Dr/Prof Yutaka Yoshiyasu Professor/Lepidopteran expert Environmental Entomology and Zoology, Graduate School of Life and Environmental Sciences, Osaka Prefectural University, Osaka, Japan	
Dr/Prof Norio Hirai Associate Professor Environmental Entomology and Zoology, Graduate School of Life and Environmental Sciences, Osaka Prefectural University, Osaka, Japan	
Dr/Prof Shouhei Ueda Assistant Professor, Environmental Entomology and Zoology, Graduate School of Life and Environmental Sciences, Osaka Prefectural University, Osaka, Japan	
Dr Itaru Kanazawa Entomologist, Osaka Museum of Natural History, Osaka, Japan	

Dr/Prof. Yoshihisa Abe Professor, Entomology Faculty of Social and Cultural Studies Kyushu University (Hakozaki Campus), Fukuoka, Japan	
Mr Taguchi Daisuke PhD Graduate Student, Graduate School of Integrated Sciences for Global Society Kyushu University (Hakozaki Campus), Fukuoka, Japan	
Dr/Prof Toshiya Hirowatari Professor (Phylogenetic Systematics of Insects Mainly on Lepidoptera) Entomological Laboratory Faculty of Agriculture, Kyushu University (Ito Campus), Fukuoka, Japan	
Dr Isao Takenaka Researcher Nara Prefecture Agricultural Research and Development Center, Sakurai, Nara, Japan	
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	
Dr Yoshinori Kunimoto Chief Researcher Nara Prefecture Agricultural Research and Development Center, Sakurai, Nara, Japan	
Mr Fumiaki Kimura Entomologist The Kashihara City Insectarium, Kashihara, Nara Prefecture, Japan	
Dr/Prof Masahito T. Kimura Professor/ Entomologist Faculty of Environmental Earth Science, Hokkaido University Sapporo, Hokkaido, Japan	

Dr/Prof Masahiro Ohara Vice Director, The Hokkaido University Museum and Professor, Systematic Entomology Graduate School of Agriculture, Sapporo, Hokkaido, Japan	
Dr Yoichi Ishiguri Researcher Plant Protection Section, Apple Research Institute, Aomori Prefecture Industrial Technology Research Center, Kuroishi, Aomori, Japan	

Details of Participants (Name, title, profession)

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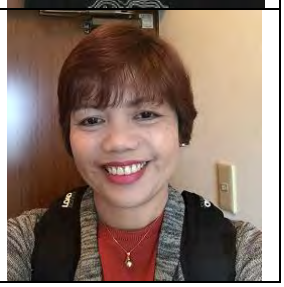
Ms Ariene Garcia Castillo Senior Scientist Agri-Food & Veterinary Authority of Singapore Animal and Plant Health Centre 6 Perahu Road, Singapore 718827	

9. PHOTOS & IMAGES

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

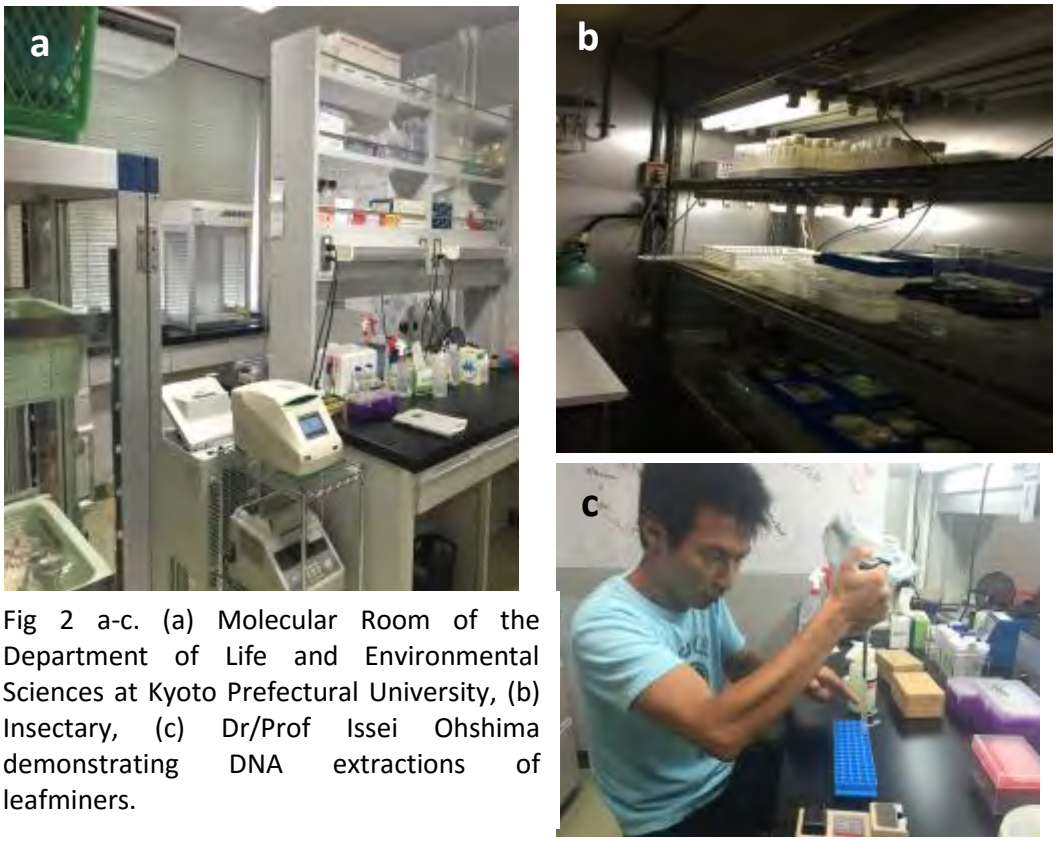


Fig 2 a-c. (a) Molecular Room of the Department of Life and Environmental Sciences at Kyoto Prefectural University, (b) Insectary, (c) Dr/Prof Issei Ohshima demonstrating DNA extractions of leafminers.

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*.

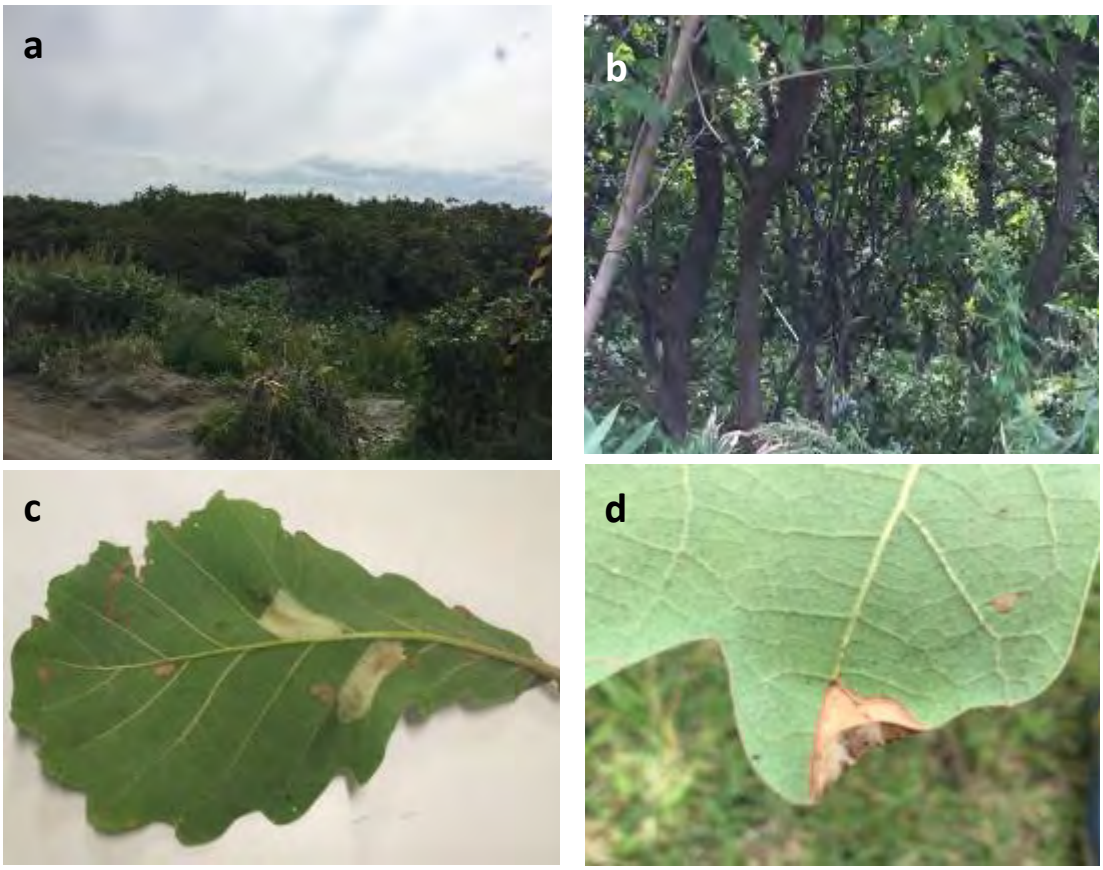


Fig 8 a-d. (a) Ishikari Coast, Hokkaido, Japan, (b) Dr/Prof Hiroaki Sato collecting samples of leafminer from *Quercus* sp. (Oaks), (c) Leafminer damaged caused by the dominant and common leaf-mining species, *Phyllorycter similis* and (d) *Phyllorycter leucocorona* on *Quercus dentata* (Japanese emperor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

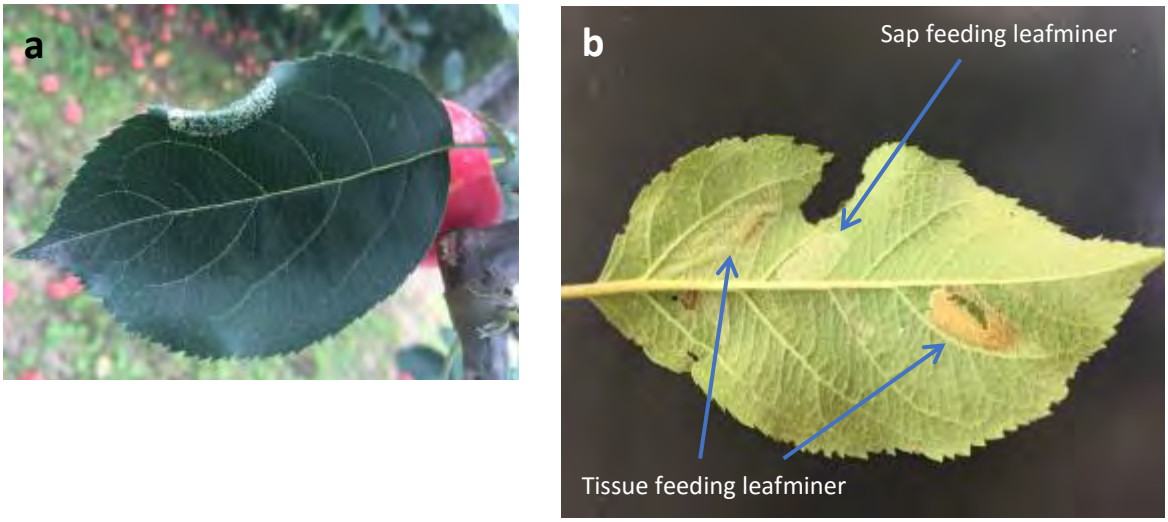


Fig 10 a-b. (a) Leafmining damaged in *Malus* sp. (Apple) (b) Sap and tissue feeding stages of Apple leafminer, *Phyllonorycter ringoniella*.