

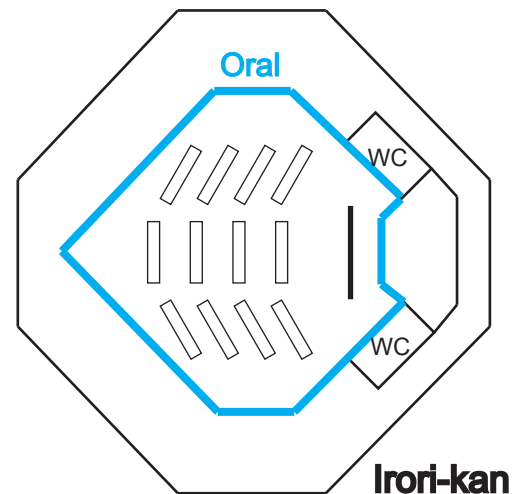
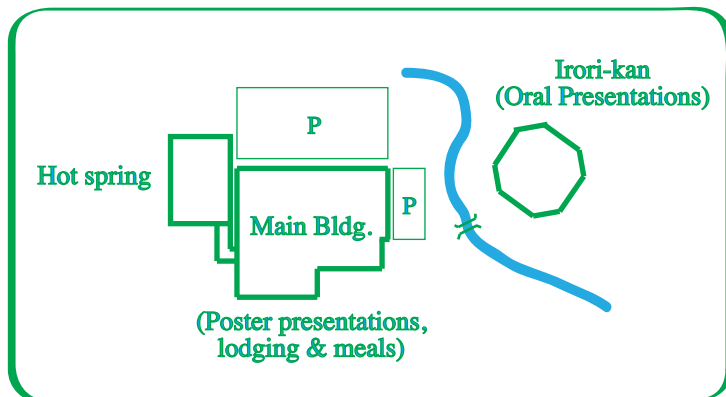
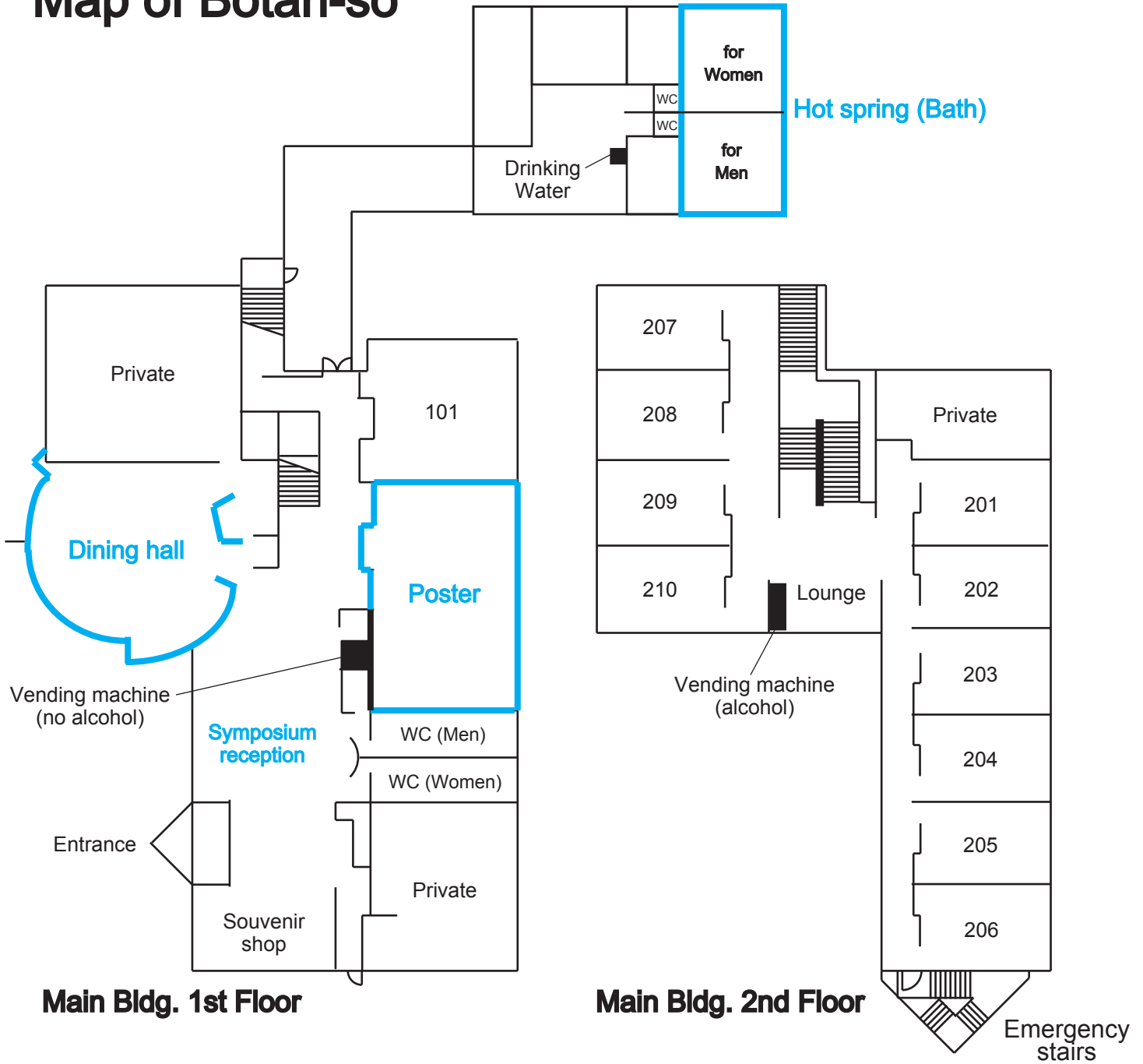
# **International Symposium on Gracillariidae**



**September 26-28, 2014  
Botan-sō Lodge  
Kozagawa, Wakayama, Japan**



# Map of Botan-so



# Program at a Glance

Sept 26 (Fri)	Sept 27 (Sat)	Sept 28 (Sun)	Sept 29 (Mon)
	7:00-9:00 <i>Breakfast</i>	7:00- <i>Breakfast</i>	7:00-9:00 <i>Breakfast</i>
	9:00- Oral Session 1	8:00-        Field Excursion	-10:00 <i>Departure</i>
	10:30- <i>Coffee Break</i>		
	10:45- Oral Session 2		
	12:10- <i>Lunch</i>		
	13:00- Oral Session 3		
	14:30- <i>Coffee Break</i>		
	14:45- Oral Session 4		
15:00- <i>Registration</i>	16:30- <i>Coffee Break</i>		-15:30
	16:45- <i>Keynote Lecture 2</i>		
18:00- <i>Welcome Dinner</i>	18:00- <i>Dinner</i>	18:00- <i>Fairwell Dinner</i>	
19:30- <i>Keynote Lecture 1</i>	19:30- <i>Poster Session</i>	20:30- <i>Training Course (rearing leaf miners)</i>	

**We thank our sponsors for their generous support:**

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 The Motoo Kimura Trust Foundation for the Promotion of Evolutionary Biology  
 The Kyoto University Foundation  
 Center for Ecological Research, Kyoto University

# Program

## **Friday, September 26 (Day 1)**

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15 : 00~ *Registration*

18 : 00~19 : 30 *Welcome Dinner*

19 : 30~20 : 30 **Keynote Lecture 1**

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Tosio Kumata

## **Saturday, September 27 (Day 2)**

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7 : 00~ *Breakfast*

9 : 00~10 : 30 **Talk Session 1**

T-01 **Recent progress in the taxonomy of Japanese leafminers**  
Toshiya Hirowatari

T-02 **Korean species of *Caloptilia* (Lepidoptera, Gracillariidae)**  
Young Min Shin, Bong-Woo Lee, Da-Som Kim and  
Bong-Kyu Byun

T-03 **Comparison of Holarctic lepidopterous leafminer faunas**  
Erik J. van Nieukerken

T-04 **A revision of the Japanese species of the subfamily Phyllocnistinae,  
with description of the pupal morphology**  
Shigeki Kobayashi and Toshiya Hirowatari

10 : 30~10 : 45 *Coffee Break*

10 : 45~12 : 10 **Talk Session 2**

T-05 **Phylogeography and genetics of invasion of the lime leaf miner**



***Phyllonorycter issikii***

Natalia Kirichenko, Paolo Triberti, Sylvie Augustin, Alain Roques,  
and Carlos Lopez-Vaamonde

**T-06 Obligate pollination mutualism between *Epicephala* moths and  
Phyllanthaceae plants and the diversification of *Epicephala***

Atsushi Kawakita

**T-07 Coping with a senescing environment: when insects reveal to plants  
the secret of eternal youth**

David Giron, Mélanie Body, Jérôme Casas, Franck Dedeine,  
Florence Gützwiler, Elisabeth Huguet, Wilfried Kaiser,  
Gaëlle Glevarec, Arnaud Langue and Carlos Lopez-Vaamonde

**T-08 Chemical ecology of *Epicephala* moths: from behavior to  
electrophysiology**

Tomoko Okamoto

12 : 10~13 : 00 *Lunch*

13 : 00~14 : 30 **Talk Session 3**

**T-09 Winter microclimate and the overwintering success of invasive  
*Cameraria ohridella***

Tea Ammunét, Karin Eklund, Christer Björkman

**T-10 Can top-down effects determine the within-leaf distribution in a  
gracillariid moth?**

Yoshiko Ayabe and Naoki Hijii

**T-11 Species level phylogenies provide insight on the role of ecological  
and allopatric speciation in the evolution of species-rich clades of  
leaf-mining moths**

Camiel Doorenweerd

**T-12 Patterns of host utilisation by herbivore assemblages of the genus  
*Caloptilia* (Lepidoptera: Gracillariidae) on congeneric maple tree  
(*Acer*) species**

Ryosuke Nakadai, Masashi Murakami and Atsushi Kawakita

14 : 30~14 : 45 *Coffee Break*

14 : 45~16 : 30 **Talk Session 4**

**T-13 Preliminary molecular phylogeny of the endangered Hawaiian leaf mining moth genus *Philodoria* (Lepidoptera: Gracillariidae)**

Chris A. Johns

**T-14 Co-radiation of *Epicephala* and their host plants (Phyllanthaceae: *Phyllanthus* s. l.: *Glochidion*) in Southeastern Polynesia**

David Hembry, Tomoko Okamoto, Issei Ohshima, Bruce Baldwin, Rosemary Gillespie and Atsushi Kawakita

**T-15 Genetics and genomics of adaptation to a novel host plant**

Issei Ohshima

**T-16 DNA barcoding reveals that the reverse latitudinal gradient of Gracillariidae is an artifact of tropical under-sampling**

Carlos Lopez-Vaamonde, Akito Kawahara, Rodolphe Rougerie, Issei Ohshima, Atsushi Kawakita, Olivier Bouteleux, Jurate De Prins, David C. Lees

**T-17 Utilizing a next-gen anchored hybrid enrichment probe set of 800 genes to resolve deep-level relationships within Gracillariidae**

Akito Kawahara

16 : 30~16 : 45 *Coffee Break*

16 : 45~17 : 45 **Keynote Lecture 2**

**Linking of the information sets based on a global, comprehensive, verified digital and web-based collection of Gracillariidae with special emphasis on the tropics**

Jurate De Prins and Willy De Prins

18 : 00~19 : 30 *Dinner*

19 : 30~21 : 30 **Poster Session**

### **Sunday, September 28 (Day 3)**

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7 : 00~ *Breakfast*

8 : 00~15 : 30 **Field Excursion**

Wakayama Experimental Forest, Hirai  
(Hokkaido University)

17 : 30~19 : 30 *Farewell Dinner*

20 : 30~ **Training Course “Rearing Leaf Miners”**

### **Monday, September 29 (Day 4)**

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7 : 00~ *Breakfast*

~10 : 00 *Departure*





# Keynote Lectures



P-01

19:30-20:30, Friday, September 26 (Day 1)

### Classification of the family Gracillariidae: a morphological approach

Tosio Kumata (The Hokkaido University Museum)

As described in my papers, Gracillariidae may be classified into four subfamilies as follows:

- I. Subfamily Gracillariinae
  1. *Parornix* group (Subfamily Ornichinae)
  2. *Gracillaria* group
  3. *Parectopa* group (Subfamily Ornixolinae)
  4. *Acrocercops* group
- II. Subfamily Oecophyllembiinae
- III. Subfamily Lithocolletinae
- IV. Subfamily Phyllocnistiinae

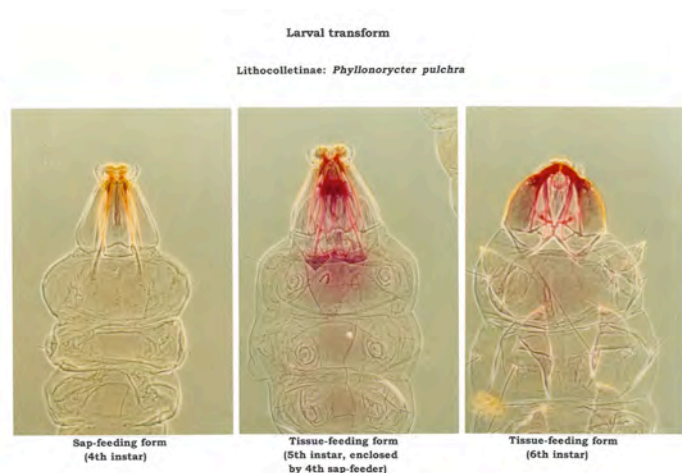
In this scheme the genera *Marmara* and *Dendrorycter* are included in the *Acrocercops* group of the subfamily Gracillariinae, but their true placement is doubtful. Some authors have argued that they are situated in a separate group in the subfamily Gracillariinae. In order to delimit these major groups, I have used adult and larval characters. In this lecture, I will introduce and explain these characters.

In addition to the broad-scale classification based on morphological characters of adults and larvae, I wish to mention the grains occurring on the cocoons of some species belonging to the *Acrocercops* and *Parectopa* groups. As I said many times, the first instar larvae of Gracillariidae are sap feeders, a very unusual form of lepidopteran larvae. So I had expected that “normal”

tissue-feeding-form larval embryos develop into sap feeding larvae inside the eggs. But Ohshima and I found that tissue feeding forms (e.g. “normal” forms) do not develop inside eggs, but rather, during embryonic development, larvae develop initially as sap feeding forms. Thus the family Gracillariidae is unique in Lepidoptera. Finally I wish to talk about the sensory pores found on the ends of wing veins.

These sensory pores may be

important in determining the venation for very narrow wings such as those of Gracillariidae.



**P-02**

**16:45-17:45, Saturday, September 27 (Day 2)**

**Linking of the information sets based on a global, comprehensive, verified digital and web-based collection of Gracillariidae with special emphasis on the tropics**

Jurate De Prins (Natural History Museum, London)

Willy De Prins (Natural History Museum, London)

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Recent advances in digital technology have completely changed the way a museum curator (of all disciplines) needs to work. Curation of collections has always been concerned with access to specimens and the information they hold. Digitisation is the most important advance that collections have seen and the curator has had to adapt accordingly. At the Natural History Museum, London we are committed to digitising 20 million specimens in the next five years and making the images and data associated with the specimens freely accessible via the museum's data portal which will become live late 2014. The Gracillariidae collection at the Natural History Museum is currently being recurated with all collections being amalgamated into one series by species. Every species represented and all type specimens will be digitised and made fully available online. These data will enable taxonomists, molecular biologists, bioinformatics specialists, ecologists, agricultural entomologists and other interested parties to work together to further the collaborative study of the Gracillariidae.

The global taxonomic dataset of Gracillariidae [www.gracillariidae.net](http://www.gracillariidae.net) presents detailed information on every 2663 Gracillariid species-group name (1935 valid species) described up to the present day and has been compiled based on 4716 publications. This information set includes: 1) the verified spelling of the genus-group and species-group names; 2) the verified authorship and date; 3) the original combination; 4) the original reference; 5) information on type specimens and their depository; 6) type locality; 7) referenced larval host plants; 8) referenced parasitoids and 9) referenced detailed distribution by country / province or state.

In the recent post-genomic era with its molecular methods rapidly developing, the voucher specimens of Gracillariidae need to be kept as intact as possible. We linked the taxonomic dataset with a preliminary DNA barcode reference library (De Prins *et al.* 2013). The molecular reference library, based on authoritatively identified voucher specimens and freely accessible online ([dx.doi.org/10.5883/DS-GRAC3](https://dx.doi.org/10.5883/DS-GRAC3)), is intended for matching the obtained sequences from many different environmental samples taken in many tropical places to detect rapidly invasive species, to record new distribution data and to monitor the occurrence and/or spread of pest species. Despite the efforts that have already been made to document the diversity of Gracillariidae moths in the tropics and to verify their taxonomic status (De Prins & Kawahara 2012), we are well aware of probably hundreds of undescribed new species of Gracillariids from tropical areas. There are many tropical Gracillariid species present at the Natural History Museum, London that are awaiting description. To accelerate the study and discovery of tropical Gracillariidae by the broadest community of Lepidopterists, we suggest



the creation of a collaborative consortium of key stakeholders and to keep the Global Gracillariidae dataset [www.gracillariidae.net](http://www.gracillariidae.net) up to date. Advances in digital imaging, the presence of a complete web-based taxonomic informative dataset and tools already available in the bioinformatics of web-based platforms, allow the Global Gracillariidae dataset to be the ultimate web based resource for this family.





# Oral Presentations





T-01

9:00-9:20, Saturday, September 27 (Day 2)

### Recent progress in the taxonomy of Japanese leafminers

Toshiya Hirowatari\* (Faculty of Agriculture, Kyushu University, Japan)  
Shigeki Kobayashi (Graduate School of Life and Environmental Sciences, Osaka Prefecture  
University, Japan)  
E-mail address: hirowat\_t@agr.kyushu-u.ac.jp

After the publication of “Moths of Japan” (MJ) in 1982, many microlepidopteran species including leafminers have been recorded and/or described from Japan. In 2013, “The Standard of Moths in Japan III & IV”(SMJ), which include most of the recent updated data, were published. The latter books (SMJ) indicate that the species numbers of Japanese leafminers increased dramatically during these 30 years as follows: Eriocraniidae: 11 (4), Nepticulidae: 67 (2), Heliozelidae: 22 (8), Tischeriidae: 7(4), Bucculatricidae: 23 (2) Gracillariidae: 216 (135), Lyonetiidae : 20 (15), Elachistidae: 37 (4), Coleophoridae 64 (22). [the numbers in parentheses indicate number of species of each family treated in MJ]  
Here we summarize recent progress in the taxomony of Japanese leafminers especially focusing on the families Eriocraniidae (Fig.1), Bucculatricidae (Fig.2) and Lyonetiidae (Figs.3-4).



Fig. 1. Mine of *Eriocrania carpinella*.



Fig. 2. Mine and cocoonet of *Bucculatrix* spp.



Fig. 3. Adult of *Lyonetia* sp.



Fig. 4. Cocoon of *Lyonetia yasudai*.

**T-02**

**9:20-9:45, Saturday, September 27 (Day 2)**

**Korean species of *Caloptilia* (Lepidoptera, Gracillariidae)**

**Young Min Shin<sup>\*1</sup>, Bong-Woo Lee<sup>1</sup>, Da-Som Kim<sup>2</sup>, and Bong-Kyu Byun<sup>2</sup>**

<sup>1</sup> Division of Forest Biodiversity, Korea National Arboretum

<sup>2</sup> Department of Biological Science & Biotechnology, Hannam University

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This study was conducted to review the genus *Caloptilia* in Korea with taxonomic arrangement. Genus *Caloptilia* Hübner, 1825 is one of the largest group in the family Gracillariidae with 314 described species in the world. Totally 15 species have been recorded from Korea to date. In the present study, four species of *Caloptilia* are newly reported from Korea: *C. fidella* (Reutti), *C. hidakensis* Kumata, *C. illicii* Kumata, and *C. pulverea* Kumata.

In this study, 19 species were recognized from Korea, with four newly recorded species. All the known species are enumerated with illustrations of adults, genitalic structures, and all available information including host plant, life history and distributional ranges.

**Key words:** *Caloptilia*, Lepidoptera, Gracillariidae, new record, Korea

**T-03**

**9:45-10:10, Saturday, September 27 (Day 2)**

### **Comparison of Holarctic lepidopterous leafminer faunas**

Erik J. van Nieukerken (Naturalis Biodiversity Center, The Netherlands)

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The leafmining lepidopterous fauna of the Holarctic region shows many similar patterns between the various subregions, particularly in the deciduous forest zones. Hostplant use and general faunal composition resemble each other, so that e.g. oaks (Fagaceae) are very important hosts for most families in Europe, East Asia and North America, whereas herbaceous hosts are overall rare. There are also striking differences, with radiations in Heliozelidae, Tischeriidae, *Bucculatrix* and *Cameraria* in North America, nepticulids, particularly *Trifurcula*, and *Leucoptera* in Europe, and e.g. Incurvariidae and *Lyonetia* in East Asia. Gracillariidae are most diverse in North America, with 427 known and unnamed species in total, but expected to be more abundant in East Asia, where particularly the Chinese fauna needs focused sampling.

Differences in hostplant patterns can especially be seen in drier habitats: North America harbours a very rich Asteraceae feeding fauna, whereas especially southern Europe has many specialists on brooms (Fabaceae: Genisteae), Cistaceae and shrubby Lamiaceae. In Asia tropical faunas intermix with the Holarctic ones, resulting in many hosts of tropical origin. For understanding the causes of these patterns, we need not only detailed phylogenetic studies, but also provide the hidden information on the leafminers. As yet only for Europe a website figuring the complete fauna exists (with keys), but also that does not include information on known but unnamed species. DNA barcoding can assist in better understanding the patterns and BOLD does contain information on unnamed species. I plead for providing more information on hosts, biology and mine shapes of species for which full descriptions are still lacking, or adults still unknown and not to keep such knowledge hidden until a formal description can be made. Such information could be provided on platforms such as scratchpads or similar, that allow individual users to post data. Also the inclusion in mine keys should be promoted.

T-04

10:10-10:30, Saturday, September 27 (Day 2)

**A revision of the Japanese species of the subfamily Phyllocnistinae,  
with description of the pupal morphology**

Shigeki Kobayashi\* (Graduate School of Life & Environmental Sciences, Osaka Prefecture  
University, Japan)

Toshiya Hirowatari (Faculty of Agriculture, Kyushu University, Japan)

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For more than a century, the placement of the largely cosmopolitan genus *Phyllocnistis* has vacillated between several families, and recently it has been treated as a member of the subfamily Phyllocnistinae. Recent authors treated Oecophyllembiinae as a synonym of Phyllocnistinae, in which they recognized seven genera. Because the position of this sister-group remains unclear, we follow Kumata (1998) in placing six genera in Oecophyllembiinae, and *Phyllocnistis* as a sole member of the Phyllocnistinae.

In Japan, we recorded two new *Phyllocnistis* species and three new Oecophyllembiinae species, and described the pupae of the genera *Corythocestis*, *Guttigera* and *Eumetriochroa* for the first time (Kobayashi & Hirowatari, 2011; Kobayashi *et al.*, 2011ab, 2013). We found three diagnostic differences in pupal morphology from other genera of Oecophyllembiinae and *Phyllocnistis*.

As a result of our field work in Japan, a total of 38 species of the genus *Phyllocnistis* were recorded from more than 80 different plant species of 26 plant families. We recognized at least 25 new species with life histories (Fig. 1). Pupae of 28 species are described for the first time. We compare pupal characters among the genus *Phyllocnistis* and six genera of Oecophyllembiinae. For example, all species of *Phyllocnistis* have tergal spines with a pair of setae and hooks, while all species of genera of Oecophyllembiinae have tergal spines with only a pair of setae (Fig. 2). Three pupal characters, the cocoon cutter, tergal spines and cremaster, may provide a useful diagnosis between species of Oecophyllembiinae genera and *Phyllocnistis*.



Fig. 1. Mine of *Phyllocnistis* species.

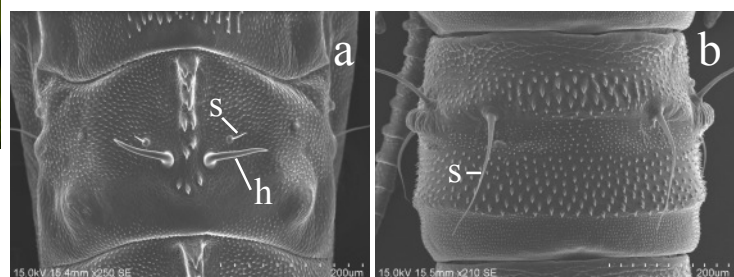


Fig. 2. Pupal spines of abdominal tergum.

a. *Phyllocnistis toparcha*.

b. *Corythocestis yaeyamensis*. s: seta; h: hook.

T-05

10:45-11:10, Saturday, September 27 (Day 2)

**Phylogeography and genetics of invasion of the lime leaf miner *Phyllonorycter issikii***

Natalia Kirichenko\* (V.N. Sukachev Institute of Forest SB RAS, Krasnoyarsk, Russia; INRA - Institut National de la Recherche Agronomique, UR 0633 Zoologie Forestière, Orléans, France)

Paolo Triberti (Museo Civico di Storia Naturale, Verona, Italy)

Sylvie Augustin (INRA, UR 0633 Zoologie Forestière, Orléans, France)

Alain Roques (INRA, UR 0633 Zoologie Forestière, Orléans, France)

Carlos Lopez-Vaamonde (INRA, UR 0633 Zoologie Forestière, Orléans, France)

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The lime leaf miner *Phyllonorycter issikii* Kumata (Lepidoptera: Gracillariidae) (fig. 1) is a moth originating from Eastern Asia. It was described in 1963 by T. Kumata from the island Hokkaido, Japan (Kumata, 1963). Few years later, the species was also reported from Eastern China, Korea and the Russian Far East. Since the mid-1980s, the moth has invaded Western Russia, nearly the whole Eastern Europe and reached some countries in Western Europe. This tiny insect provides a great example of a fast range expansion from East to West throughout Eurasia.

The insect develops on leaves of several lime species *Tilia* spp. (Malvales: Malvaceae). It represents an ornamental pest causing aesthetical damage to the plants in urban parks and gardens. In Western Russia, it has a negative effect on the reproductive and productive characteristics of lime forests which play an important role in honey production (fig. 2).

Here we use molecular sequenced data from three genes: mitochondrial COI and two nuclear genes, 28S and Histone3 to reconstruct the phylogeography of the species. Our preliminary results show a loss of genetic diversity from the East to the West following the range spread. We also found a deep genetic split, clearly individualizing one clade from the Russian Far East, which suggests the possible existence of a new sibling species of *P. issikii*.

This study is supported by LE STUDIUM\* (France).



Fig. 1. An emerged adult of *P. issikii*.

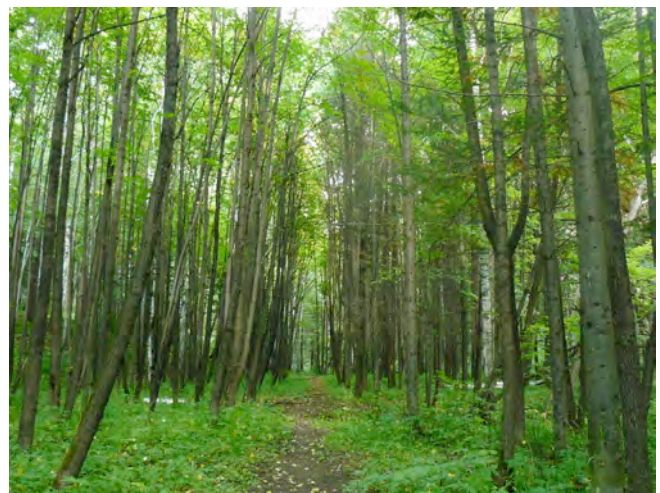


Fig. 2. *Tilia* plantation in Russia.



T-06

11:10-11:25, Saturday, September 27 (Day 2)

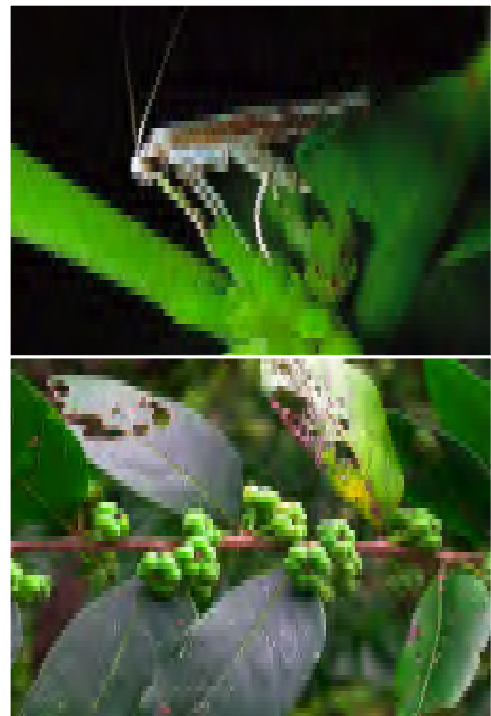
**Obligate pollination mutualism between *Epicephala* moths and Phyllanthaceae plants and the diversification of *Epicephala***

Atsushi Kawakita (Center for Ecological Research, Kyoto University, Japan)

E-mail address: kawakita@ecology.kyoto-u.ac.jp

Leaf-mining habit is a hallmark of Gracillariidae and a likely key innovation that has led to their evolutionary success. However, the *Parectopa*-group of the subfamily Gracillariinae contains considerably large number of species that do not produce typical leaf mines. Examples include flower-bud feeders in *Stomphastis* and *Chileoptilia*, fungal gall feeder in *Polysoma*, aphid gall feeder, leaf droppers in *Cuphodes*, and seed feeders in *Conopomorpha* and *Epicephala*. Although the ultimate and proximate causes of the diversity of larval ecology in *Parectopa*-group remain unknown, their adaptation to previously unused food source likely opened new opportunity for further evolutionary diversification of Gracillariidae.

Among the most remarkable radiation of non-leaf-mining gracillariid are the *Epicephala* seed feeders associated with Phyllanthaceae hosts. *Epicephala* females lay eggs in the host female flower, and, prior to oviposition, actively pollinate host flowers using the proboscises to secure food for their seed-feeding larvae. The Phyllanthaceae plants, in turn, depend entirely on *Epicephala* for pollination, resulting in an obligate mutualism between plants and moths. Host specificity of *Epicephala* moths is usually higher than those of other gracillariid genera feeding as miners on Phyllanthaceae leaves, a pattern likely driven by tight co-adaptation between plant floral odor and moth odor preference. Based on the number of presently known *Epicephala* species and global diversity of host Phyllanthaceae plants, *Epicephala* likely comprise 400–500 species found throughout the world tropics. Comparison of plant and moth phylogenies suggest that host association is generally conserved at plant genus or subgenus level, although host shifts are common, and major jumps between distantly related clades of Phyllanthaceae have occurred at least four times. Recent findings suggest that, although *Epicephala* diversified mainly as pollinating seed feeders of Phyllanthaceae, further diversification in life history has occurred. One such example involves a group of species that induce galls on Phyllanthaceae flowers, and an analysis suggests that the galling habit is likely an adaptation to escape attack from a specialized braconid parasitoid.



(Upper) An *Epicephala* female actively pollinating host flower. (Lower) Larvae are seed feeders of Phyllanthaceae plants.

T-07

11:25-11:50, Saturday, September 27 (Day 2)

**Coping with a senescing environment:  
when insects reveal to plants the secret of eternal youth**

David Giron\* (Institut de Recherche sur la Biologie de l’Insecte, CNRS – Université  
François-Rabelais de Tours, France)

Mélanie Body, Jérôme Casas, Franck Dedeine, Florence Gützwiller, Elisabeth Huguet,  
Wilfried Kaiser (Institut de Recherche sur la Biologie de l’Insecte, CNRS – Université  
François-Rabelais de Tours, France)

Gaëlle Glevarec, Arnaud Lanoue (Biomolécules et Biotechnologies Végétales – Université  
François-Rabelais de Tours, France)

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Nutritional requirements of animals are multidimensional and change qualitatively and quantitatively over time. In parallel, the nutritional environment is frequently highly variable both in space and time and food sources are composed of a wide array of nutrient molecules in various ratios and concentrations combined with non-nutritive molecules. Finally, plants are known to rapidly respond to herbivore attacks by reconfiguring their primary and secondary metabolism in order to reduce herbivore food acquisition. While endophagous insects by their feeding habit secure their nutrition and shelter, they also have to adopt specific feeding strategies allowing them to meet their energetic requirements, to face variations in food composition and nutritional needs, and to counteract plant defensive mechanisms.

Our results on the *Malus domestica/Phyllonorycter blancardella* plant-leaf mining system show the ability of leaf-miner insects to manipulate their host plant physiology and to create an “optimal” nutritional micro-environment through cytokinin production by their endosymbiotic partners. This symbiotic association impacts not only nutrient acquisition but also the feeding behavior of leaf miner insects. A close investigation at the interspecific level also shows a strong correlation between the presence of the endosymbiotic bacteria *Wolbachia* and the induction of green-islands (figure 1.) suggesting that insect symbionts most probably played a major role in the evolution and/or radiation of the endophagous feeding mode. Emerging data also strongly suggest converging strategies between gall-inducer and leaf-miner arthropods.



Fig. 1. Apple leaf infected with the phytophagous leaf-mining moth *Phyllonorycter blancardella*. This insect relies on bacterial endosymbionts to manipulate the physiology of its host-plant resulting in a 'green-island' (insect feeding area) where plant tissues remain green and photosynthetically active while the remaining leaf tissues undergo leaf senescence.



T-08

11:50-12:10, Saturday, September 27 (Day 2)

### Chemical ecology of *Epicephala* moths: from behavior to electrophysiology

Tomoko Okamoto\* (Forestry and Forest Products Research Institute, Japan)

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The reproductively highly specialized clades of the tribe *Phyllanthae* (Phyllanthaceae) which are pollinated by species-specific, seed-parasitic *Epicephala* moths (Gracillariidae). Female adults of *Epicephala* moth pollinate actively – they purposefully collect pollen on male flowers and deliberately pollinate female flowers to secure food for their seed-feeding larvae (Fig. 1).

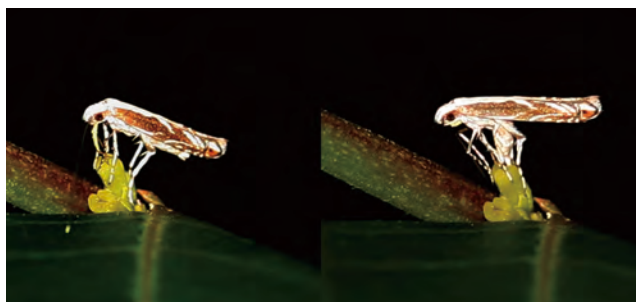
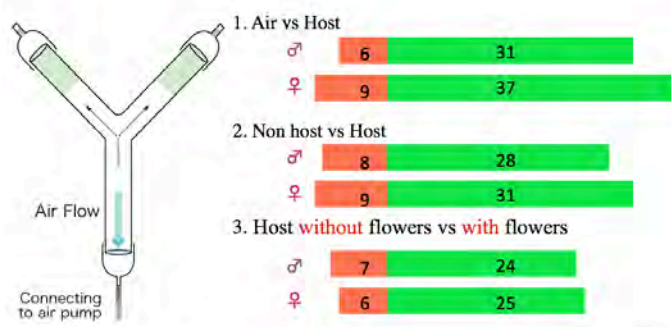


Fig. 1. Pollination behavior of female *Epicephala* moth.

Nocturnally active female *Epicephala* moths use the olfactory signals, especially floral scent of host plant species (Fig. 2). Chemical profiles of floral scent in *Epicephala* pollinated species can be distinguished by relative chemical composition (Okamoto et al. 2007).



Binomial test  $P < 0.01$

Fig. 2. Y-tube assay system. Pollinating *Epicephala* moths of *G. laceolatum* were attracted by host flower scent.

*Epicephala* pollination system differs in behavior of pollinator insects with generalized pollination system in which pollination is usually a byproduct that arises as the insect visitor moves between flowers to gain constant floral rewards. In *Epicephala*-pollinated plants, the major qualitative differences in scent between male and female flowers were observed, and the mated female *Epicephala* moths preferred the scent of male flowers over that of female flowers. These suggest that male floral scent elicits pollen-collecting behavior (Okamoto et al. 2013).

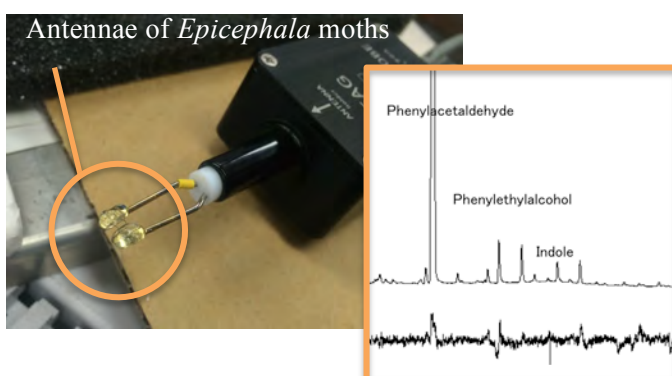


Fig. 3. The GC-EAD system and antennal response to floral scent volatiles in *Epicephala* moth.

Finally, I have focused on the application of electrophysiology to determine the key volatile compounds that mediate the encounters of the species-specific partners in *Epicephala*-Phyllanthaceae mutualism (Fig. 3).

T-09

13:00-13:25, Saturday, September 27 (Day 2)

### Winter microclimate and the overwintering success of invasive *Cameraria ohridella*

Tea Ammunét\* (Department of Ecology, Swedish University of Agricultural Sciences, Sweden)

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Invasive species encounter many obstacles that need to be overcome before establishing. At northern latitudes, winter conditions act as a strong limiting factor particularly for insect species. Yet species, such as the horse chestnut leaf miner (*Cameraria ohridella*, Lepidoptera: Gracillariidae), has been able to invade most of Europe, from the south (Macedonia, 41°N) to the north (Sweden, 59°51'N) in mere 30 years. The invasion has resulted in high densities of the leaf miner turning the leaves of its host tree (the horse chestnut, *Aesculus hippocastanum*) brown already in the middle of the summer. In order to see if the species continues its success at the northern latitudes particularly in light of the predicted climatic changes, conditions affecting the winter survival of the horse chestnut leaf miner were studied at its northern limits in Uppsala, Sweden. In more detail, the effects of snow cover and microclimatic conditions were investigated in an experimental field study. Snow cover was manipulated by adding or removing snow from on top of leaf piles containing overwintering leaf miner pupae inside the leaves (Figure 1.). The total number of hatched adults (Figure 2.) and the rate of hatching in the following spring were recorded. In the light of predicted increases in winter precipitation at northern latitudes, the results indicate better suiting climatic conditions for the invasive horse chestnut leaf miner in the future. In particular, snow cover in interaction with temperature reduced the survival of the overwintering leaf-miner pupae. In general, the results highlight the importance of the often-neglected microclimatic effects on overwintering invasive insects.



Figure 1: Overwintering *Cameraria ohridella* pupae (some of the dark spots) inside the mines in a horse chestnut leaf.



Figure 2: Successfully hatched *Cameraria ohridella* adults

T-10

13:25-13:45, Saturday, September 27 (Day 2)

**Can top-down effects determine the within-leaf distribution in a Gracillariid moth?**

Yoshiko Ayabe\* (Graduate School of Bioagricultural Sciences, Nagoya University, Japan)

Naoki Hijii (Graduate School of Bioagricultural Sciences, Nagoya University, Japan)

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Leafminers make visibly conspicuous feeding tracks called mines and spend the larval stage (and also pupal stage in some species) inside their own mine. Conspicuousness of mines and restricted mobility during the mining stages can expose them to a high risk of parasitism. In lepidopteran leafminers, the species which use the lower leaf surface as a mining site have developed widely over the phylogenetic tree, compared to the species which use the upper surface. For this reason, we hypothesized that leafminers making mines on the lower leaf surface are less recognized by parasitoids and thus are more advantageous in the avoidance of parasitism than those on the upper surface. We therefore examined the relationship between parasitism and the within-leaf distribution of the Gracillariid leafminer *Phyllocnistis* sp., which can use both the upper and lower leaf surface of the Japanese privet *Ligustrum japonicum*, and prefers the lower to the upper leaf surface (Fig. 1). We performed a non-destructive investigation by following the fate of each individual leafminer in the field population over three years (2010-2012). We also performed a destructive investigation by collecting mines and identifying their fates under a microscope to improve the accuracy of evaluation in the destructive investigation. Non-destructive investigation provided different results among years. In one year, larvae of the upper-surface mines were more frequently parasitized than those of the lower-surface mines, while the opposite result was obtained in another year. Thus, the susceptibility of leafminer to parasitism depended on their position within a leaf, but has no consistency. The result of the destructive investigation suggested that upper-surface mines were more susceptible to parasitism than lower-surface mines, and supported our hypothesis. We discussed the extent to which upper-surface mines are susceptible to parasitism and the possibility that the prevailing use of the lower leaf surface in this species is attributed to an interaction with parasitoids.



Fig. 1. Upper-surface mines of *Phyllocnistis* sp.

### Species level phylogenies provide insight on the role of ecological and allopatric speciation in the evolution of species rich clades of leaf-mining moths

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Two species rich gracillariid genera are found globally in temperate regions, and primarily feed on woody plants: *Phyllonorycter* and *Cameraria*. Within the more primitive family Nepticulidae, there are also two species rich leafmining genera with a preference for similar hosts and climatic conditions: *Ectoedemia* and *Stigmella* (Fig. 1). Ideas on the role of sympatric speciation, through adaptation to different ecological environments (i.e. ecological speciation) versus allopatric speciation in animals have been shifting for over a century. Using a molecular phylogeny we estimated how many ecological speciation events may be recognized within *Ectoedemia* s.s., by comparing host ranges between sister species, and within sympatric species complexes. We then used fossils to calibrate the phylogeny, which places the origin of the vast majority of all extant species in the Miocene (Fig. 2). We hypothesize that climatic conditions in the Miocene caused diversification primarily through ecological speciation in allopatry.



Fig. 1: Leafmines of *Stigmella* (upper left), *Ectoedemia* (upper right), *Phyllonorycter* (lower left) and *Cameraria* (lower right)

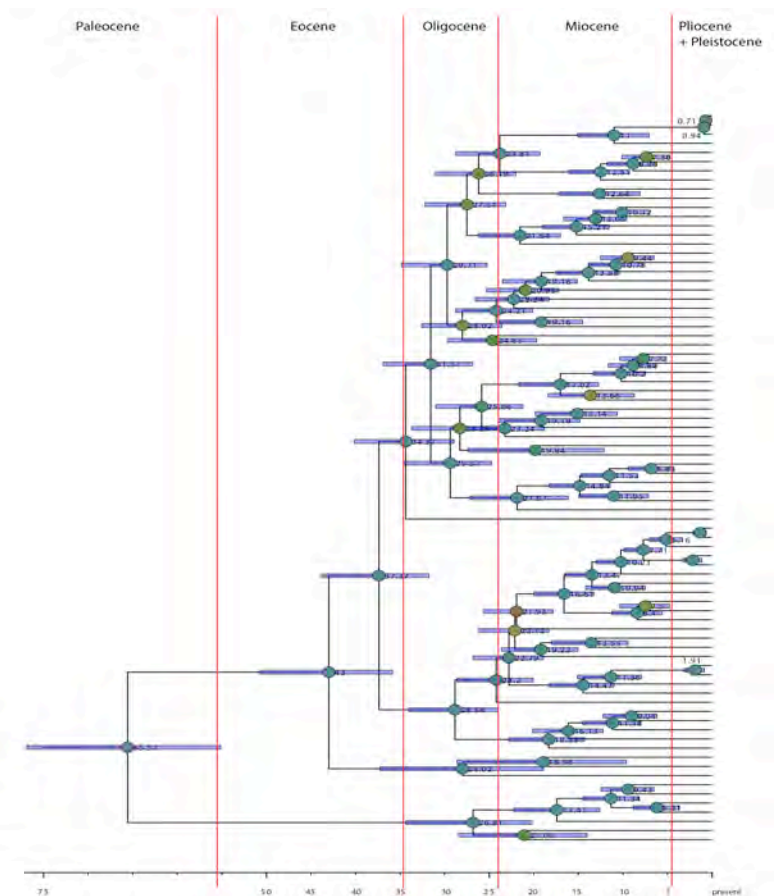


Fig. 2: Relaxed clock molecular phylogeny of the subgenus *Ectoedemia* (Nepticulidae)



T-12

14:10-14:30, Saturday, September 27 (Day 2)

**Patterns of host utilisation by herbivore assemblages of the genus *Caloptilia* (Lepidoptera: Gracillariidae) on congeneric maple tree (*Acer*) species**

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Masashi Murakami (Faculty of Science, Chiba University, Japan)

Atsushi Kawakita (Center for Ecological Research, Kyoto University, Japan)

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We investigated the pattern of host utilisation by congeneric *Caloptilia* caterpillars on 14 different species of *Acer* (maple) in temperate mixed forests of central Japan. A multi-filtering model of host plant utilisation was proposed to address how phylogenetically related herbivore assemblages are constructed on phylogenetically related host plant species. We examined two hypotheses. First, we asked whether a negative relationship exists between the phylogenetic distance of plants from the most suitable host species and the abundance of herbivorous insects on the host. Second, we investigated whether the assemblage dissimilarity of herbivorous insects among host plant species increases with increasing distance of plant phylogeny and traits. We used Mantel and partial Mantel tests to measure the relationship between assemblage dissimilarity of *Caloptilia* species and the distance of plant phylogeny and leaf traits. Both hypotheses were confirmed, clearly suggesting that the utilisation and suitability of hosts for *Caloptilia* caterpillars was strongly influenced by phylogenetic relatedness and leaf trait similarity among *Acer* species. This implies that phylogenetic distance is an integrated measure of phenotypic and ecological attributes of congeneric *Acer* species that can be used to explain specialisation and constraints of host utilisation of congeneric herbivore species even on a short evolutionary time scale.



Fig. 1. Host utilization by *Caloptilia* species on *Acer* species.

T-13

14:45-15:10, Saturday, September 27 (Day 2)

**Preliminary molecular phylogeny of the endangered Hawaiian leaf mining moth genus  
*Philodoria* (Lepidoptera: Gracillariidae)**

Chris A. Johns\* (University of Florida, USA)  
johns.chris.a@ufl.edu

The leaf mining moth genus *Philodoria* (Lepidoptera: Gracillariidae) includes 30 poorly studied species that are endemic to the Hawaiian Islands. Most species are monophagous, feeding internally within the leaf of a single endemic plant species, but the genus as a whole is known to feed on as many as 12 different plant families. Approximately 75% of the group's host plants are threatened or endangered, making these moths particularly vulnerable. A comprehensive systematic treatment has not been conducted in over thirty years, and the systematics, phylogenetics, and conservation status of many of these moths remains largely unknown. Here, we present preliminary data from recent systematic and phylogenetic work on *Philodoria*, and discuss future plans to study the biogeography of the group. In addition, we outline our efforts to raise awareness for *Philodoria* conservation in both conservation and public audiences alike.



Fig.1: *Philodoria auromagnifica*



Fig.2: *Philodoria splendida*

**Co-radiation of *Epicephala* and their host plants (Phyllanthaceae: *Phyllanthus* s. l.: *Glochidion*) in Southeastern Polynesia**

David Hembry\* (Kyoto University, Japan)

Tomoko Okamoto (Forestry and Forest Products Research Institute, Japan)

Issei Ohshima (Kyoto Prefectural University, Japan)

Bruce Baldwin, Rosemary Gillespie (University of California, Berkeley, USA)

Atsushi Kawakita (Kyoto University, Japan)

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Oceanic islands have long been used as laboratories for evolutionary processes. Here I present research on the endemic co-radiations of *Epicephala* (Gracillariinae) and their host plants (Phyllanthaceae: *Phyllanthus* s. l.: *Glochidion*) in Southeastern Polynesia, a region of young, volcanic archipelagos in the central south Pacific. Despite being mutually dependent, *Epicephala* and *Glochidion* have successfully colonized Southeastern Polynesia multiple times independently and non-congruently; these multiple colonizations also reveal rapid host-shifts associated with colonization of new islands. Patterns of species-specificity are also reduced relative to those seen in continental regions. Ongoing work seeks to examine the transcriptomics and population genomics of *Epicephala* associated with different species of *Glochidion* on the islands of Tahiti and Mo'orea, as well as inter- and intraspecific variation in floral odor. I will also briefly introduce what is known about the Gracillariidae fauna of Southeastern Polynesia.

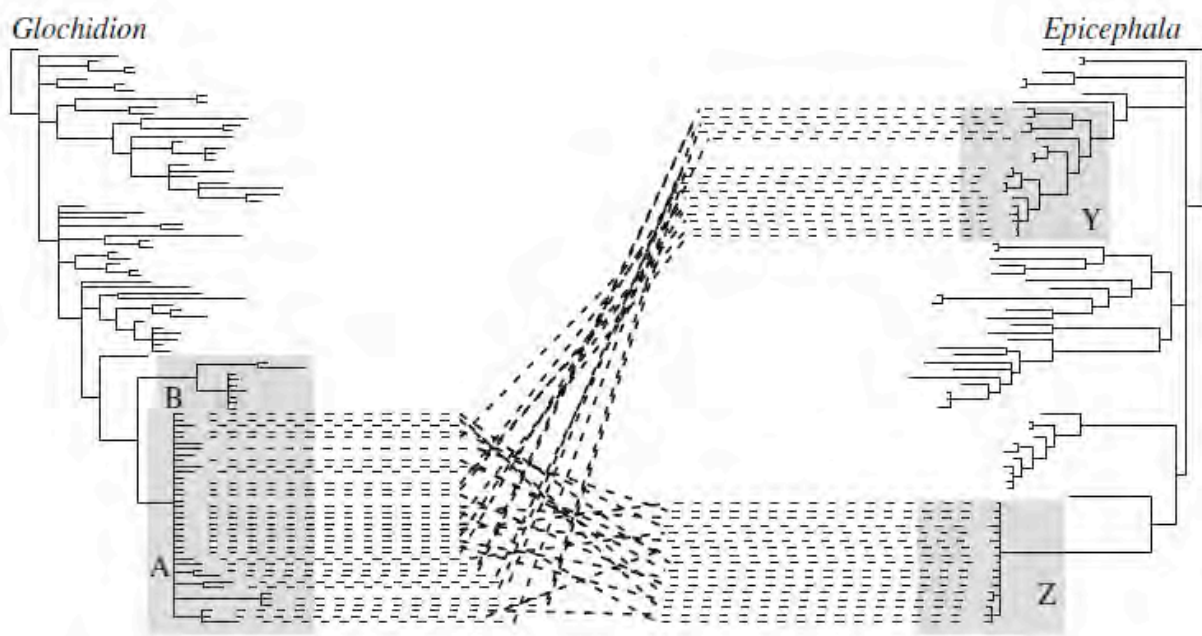


Figure: Global phylogenies of *Glochidion* and their *Epicephala* reveal that both are non-congruently polyphyletic in Southeastern Polynesia (grey boxes): two clades of *Epicephala* (Y and Z), representing separate colonizations, are associated with a single clade of *Glochidion* (A). Hembry et al. (2013)

T-15

15:25-15:40, Saturday, September 27 (Day 2)

### Genetics and genomics of adaptation to a novel host plant

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Kyoto Prefectural University, Japan)  
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Adaptation to a novel environment often requires evolution in multiple traits in a derived population. However, we know little about the genetic backgrounds underlying the evolution of multiple traits. In plant feeding insects, a precise combination between larval assimilability against plant secondary compounds (performance) and preference of ovipositing adult females for particular host plants is crucial. Here I show that the localization of loci for host-plant adaptation to a restricted genomic region in the leaf-mining moth *Acrocercops transecta*. *A. transecta* consists of two host races, the ancestral Juglandaceae race and the derived *Lyonia* race, which are associated with either Juglandaceae plants or *Lyonia ovalifolia* (Ericaceae). Crossing between the two host races and subsequent linkage analysis and QTL mapping using a backcross generation reveal that only ca 180 kb genomic region of a single autosome is responsible for the larval performance. I will report the progress of genomics approaches (whole genome sequencing, RNA-seq and Fosmid library screening) to reveal the genetic mechanisms of host shifting in *A. transecta*.



Top: An adult of a leaf-mining moth, *Acrocercops transecta*, resting on a one-cent coin.

Bottom left: Leaf mines on a walnut tree *Juglans mandshurica* formed by larvae of the Juglandaceae-associated race of *A. transecta*. Bottom right: A leaf mine on *Lyonia ovalifolia* (Ericaceae) formed by a larva of the *Lyonia*-associated race of *A. transecta*. There are no morphological differences between the two host races, and the two host races mate readily under laboratory conditions

without detectable intrinsic incompatibilities.



T-16

15:40-16:05, Saturday, September 27 (Day 2)

**DNA barcoding reveals that the reverse latitudinal gradient of Gracillariidae is an artifact of tropical under-sampling**

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Rodolphe Rougerie (INRA, UR633 Zoologie Forestière, F-45075 Orléans, France)  
Issei Ohshima (Department of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto, Japan)

Atsushi Kawakita (Center for Ecological Research, Kyoto University, Kyoto, Japan)

Olivier Bouteleux (INRA, UR633 Zoologie Forestière, F-45075 Orléans, France)

Jurate De Prins (Entomology Section, Royal Museum for Central Africa, Leuvensesteenweg 13, B-3080 Tervuren, Belgium)

David C. Lees (Department of Zoology, Cambridge University, CB2 3EJ, UK)

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Higher taxa often show increasing species richness towards tropical low latitudes, a pattern known as the latitudinal biodiversity gradient (LBG). A rare reverse LBG (with greater richness towards temperate high latitudes) is exhibited by Gracillariidae moths, in which most described species, occur in northern temperate areas. We carried out the first assessment of gracillariid species diversity in two Neotropical regions to test whether the relatively low tropical species diversity of this family is genuine or caused by insufficient sampling and a strong taxonomic impediment. Field surveys in six French Guianan (Figure 1) and one Ecuadorian sites produced 516 gracillariid specimens that were DNA barcoded to facilitate identification and to match larvae inside leaf-mines with adults. We obtained 485 barcodes (372 adults and 104 larvae). Species delineation from sequence data was approximated using Automatic-Barcode-Gap-Discovery and Refined-Single-Linkage-Analysis through the Barcode Index Number system, and the proportion of described/undescribed species was estimated after comparison with types. The total number of candidate species ranged from 142 (ABGD) to 151 (BINs). For Nouragues we obtained 108 BINs. 64 BINs (59.3%) are represented by singletons. 33% of specimens were identified down to genus level. We were able to assign 17 species names to 38 specimens. Strikingly, at least 85% of the species collected as adults were found to be undescribed. Nearly all barcodes were novel to BOLD. The results from both our molecular and morphological analyses indicate that most of the gracillariid fauna in the studied region is unknown and undescribed. The estimated lower bound of species richness of Gracillariidae for Nouragues ranged from 240 species (Chao1) to 260 species (ACE). Our results show that DNA barcoding allows researchers to overcome the taxonomic impediment and carry out rapid biodiversity assessments in poorly documented regions. Finally, we show that the current reverse LBG seen in this group is an artifact of insufficient sampling and the Linnean shortfall.



Fig. 1 Our major study site at Nouragues research station (French Guiana) with the Inselberg on the right. The reserve of 100 000 Ha contains pristine lowland tropical rainforest, over 1700 angiosperm species and c. 200 tree species per hectare.

**T-17**

**16:05-16:30, Saturday, September 27 (Day 2)**

**Utilizing a next-gen anchored hybrid enrichment probe set of 800 genes to resolve deep-level relationships within Gracillariidae**

Akito Y. Kawahara\*, David Plotkin, Christopher A. Johns, Jesse W. Breinholt (Florida Museum of Natural History, University of Florida)  
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Gracillariid leaf mining moths constitute a diverse family with nearly 2000 described species. Recently, a molecular phylogeny of Gracillariidae was constructed based on 57 taxa and up to 21 protein-coding genes (Kawahara et al. 2011, BMC Evol. Biol). The study revealed that 21 genes cannot adequately provide strong branch support for deep-level relationships among major gracillariid groups. Here we constructed a preliminary next-gen dataset of gracillariid leaf miners using approximately 781 genes, targeted using a newly-developed anchored hybrid enrichment probe set designed from transcriptomes and reference genomes. The talk will include an initial gracillariid phylogeny and a new lepidopteran phylogeny based on this approach. Discussion will focus mainly on capture efficiency of the probe set, and how it can greatly enhance phylogenetic resolution in Gracillariidae and broadly across Lepidoptera.

# Poster Presentations



**P-01**

**19:30-21:30, Saturday, September 27 (Day 2)**

**Two new leafminer species of *Phyllocnistis* Zeller (Phyllocnistinae) from South America**

Rosângela Brito\* (Department of Zoology, Federal University of Paraná State, Brazil)

Gislene L. Gonçalves (Department of Zoology, Federal University of Rio Grande do Sul State, Brazil)

Olaf H.H. Mielke (Department of Zoology, Federal University of Paraná State, Brazil)

Gilson R. P. Moreira (Department of Zoology, Federal University of Rio Grande do Sul State, Brazil)

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*Phyllocnistis* Zeller is a highly diverse (ca. 120 species) genus that exploits a wide range of host plants worldwide. It is poorly-known in the Neotropical region, from where a small fraction of lineages have been described (= 17) so far. Aiming to unravel such a cryptic biodiversity of microlepidopterans, we have been performing a long-term field study in the Centro de Pesquisas e Conservação da Natureza (CPCN Pró-Mata/PUCRS; 29°28'36''S, 50°10'01''W) within the Atlantic Rain Forest domain in southern Brazil. Herein we describe and illustrate two new species of *Phyllocnistis* based on tegumentary morphology (larva, pupa and adults) and provide a preliminary characterization of their life history. Also, a phylogenetic analysis based on DNA sequences is presented. The larvae of *Phyllocnistis* sp. 1 are leafminers of *Begonia fruticosa* (Klotzsch) A.DC. (Begoniaceae). The sap-feeding instars have yellowish color, switching to whitish when they molt to the spinning instar. Pupae present a set of dorsal spines in a "V" shape arrangement, from abdominal segments A2 to A7. The forewings present dark-gray color interspersed with striking bright orange, sinuous longitudinal fascia. Moreover, the transverse fascia is partially interrupted by the posterior margin of the longitudinal fascia, and thus markedly differing from all other Neotropical species. The larvae of *Phyllocnistis* sp. 2 are leafminers of *Drimys angustifolia* Miers (Winteraceae). Sap-feeding and spinning instars in this case present yellowish color. The pupa is characterized by having dorsally on the abdominal segments two pairs of strong spines, except on A6 and A7 where only a single pair of spines is found. Adults present wings with light-gray color. The longitudinal fascia show similar color to the whole wings, which have margins in dark tones, and thus differing from the color pattern found on *P. drimiphaga* Kawahara, Nishida & Davis. Bayesian phylogenetic reconstruction using 640 base pairs of cytochrome oxidase subunit I mitochondrial DNA sequences from *Phyllocnistis* sp.1 and *Phyllocnistis* sp.2 strongly supported both as monophyletic and quite distinct lineages, divergent ca. 16% from each other. Also, a phylogeny tree, which included others *Phyllocnistis* species, indicated that both fall within the genus. Genetic divergence at the interspecific level ranged from 14 to 19%.

P-02

19:30-21:30, Saturday, September 27 (Day 2)

**Pollen dispersal distance of *Epicephala* moths associated with self-compatible *Glochidion acuminatum***

Ko Mochizuki\* (Center for Ecological Research, Kyoto University, Japan)  
Atsushi Kawakita (Center for Ecological Research, Kyoto University, Japan)  
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The monoecious tree genus *Glochidion* (Phyllanthaceae, Phyllanthaeae) consists of >300 species widely occurring in the tropical to subtropical parts of Asia, Australia, and the Pacific. All *Glochidion* species studied to date are pollinated exclusively by species-specific, seed-parasitic moths of the genus *Epicephala*. Female *Epicephala* moths actively collect pollen from the anthers of *Glochidion* male flowers (Fig. 1) and subsequently deposit pollen on the stigmas of female flowers (Fig. 2) to ensure that fruits are produced for their seed-feeding larvae. The female moths then oviposit into ovules, and the hatched larvae consume a subset of the seeds in each fruit, leaving the rest intact.

Because their larvae feed on seeds, moth fitness is strongly affected by fruit set, i.e., the proportion of flowers that mature as fruits. As with most plants in general, *Glochidion acuminatum* has lower fruit set after self-pollination than after cross-pollination. Therefore, whether the female moths deposit self- or cross-pollen to the stigmas must have crucial effect to their fitness.

In *G. acuminatum*, male and female flowers are born close to each other (Fig. 3), which makes it is easy for moths to pollinate neighboring flowers (self pollination) that likely results in low fruit set and hence low larval survival rate. Alternatively, moths may gain high larval survival rate by transporting pollen to another tree after collecting pollen (cross pollination). However, the latter exerts a large cost to female moths because of the time and energy required to fly between trees and the risk of predation during flight.

In this study, we investigate the fruit set, outcrossing rate and pollen dispersal distance in *G. acuminatum* using pollination experiment and paternity analysis of pollen that female moths use for pollination (arrow in Fig. 2), in order to explore whether the cross-pollination behavior evolves when cross pollination results in higher fruit set than self pollination. We will present results in progress.



Fig.1 Female *Epicephala* moth collecting pollen from the anthers of male flower of *G.acuminatum*.



Fig.2 Female *Epicephala* moth depositing pollen to the stigma of female flower of *G.acuminatum*. The Yellow arrow indicates pollen which is used for paternity analysis.



Fig.3 Flowering branch of *G.acuminatum*. The blue and pink arrow indicates male and female flower, respectively.

P-03

19:30-21:30, Saturday, September 27 (Day 2)

### Study on oviposition stimulant for the leaf-mining moth, *Acrocercops transecta*

Tomoko Katte\* (Graduate school of Agriculture, Kyoto University, Japan)

Ritsuo Nishida (Graduate school of Agriculture, Kyoto University, Japan)

Issei Ohshima (Department of Life and Environmental Sciences, Kyoto Prefectural University, Japan)

Hajime Ono (Graduate school of Agriculture, Kyoto University, Japan)

The leaf-mining moth, *Acrocercops transecta*, is associated with several Juglandaceae trees and *Lyonia ovalifolia* (Ericaceae), and it is known that the *Lyonia*-associated population was derived from the Juglandaceae-associated population. The Juglandaceae-associated females oviposit on *Juglans*, *Pterocarya* and *Platycarya*, while the *Lyonia*-associated females oviposit only on *L. ovalifolia*. To elucidate the mechanisms underlying the differences in oviposition preference between the two host-associated populations, we first investigated oviposition stimulant (s) for the *Lyonia*-associated population. When the *Lyonia*-associated female was given a cover glass thinly coated with an ethyl acetate extract from leaves of *L. ovalifolia*, the female showed oviposition behavior (Fig. 1). The oviposition stimulant activity, therefore, was evaluated by the number of eggs or trials of oviposition. The crude extract was fractionated according to the procedure shown in Fig. 2. The extract was separated to four fractions by a silica gel column. Of them, a fraction eluted with acetone showed oviposition activity. The fraction was separated by a reverse phase ODS column to six fractions. The less polar fractions including Fr. 4, 5 and 6 showed the oviposition activity. Identification of an active fraction is now in progress.

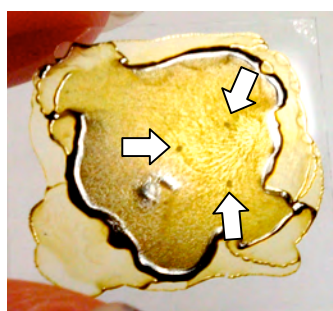


Fig. 1 Oviposition stimulant bioassay. A cover glass was coated with each fraction. The arrows indicate eggs laid on the extract.

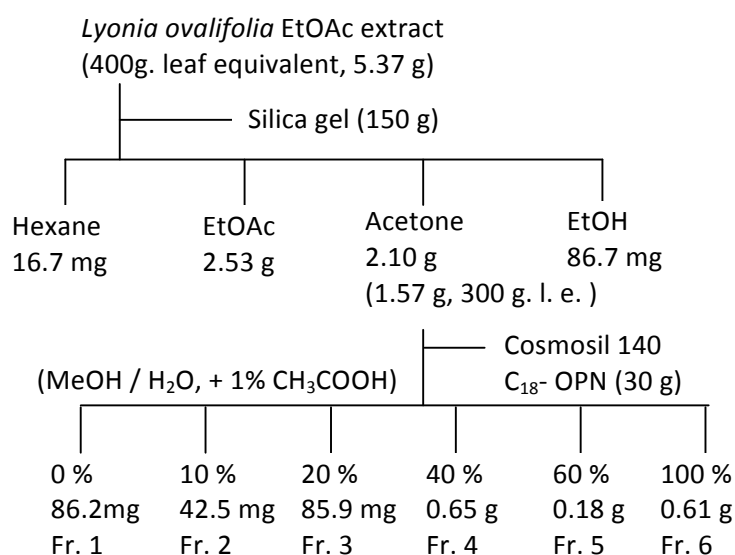


Fig. 2 Separation procedure of the EtOAc extract.



**P-04**

**19:30-21:30, Saturday, September 27 (Day 2)**

**A new genus and species of leaf mining Oecophyllembiinae associated with *Drimys* (Winteraceae) in the Atlantic Rain Forest, Brazil**

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Louise T. Pereira (Department of Zoology, Federal University of Rio Grande do Sul State, Brazil)

Denis S. Silva (Department of Zoology, Federal University of Rio Grande do Sul State, Brazil)

Gislene L. Gonçalves (Department of Zoology, Federal University of Rio Grande do Sul State, Brazil).

Héctor A. Vargas (Agriculture College, Tarapacá University, Chile)

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The larva, pupa and adults of a remarkable new genus and species of Oecophyllembiinae are described and illustrated under light and scanning electron microscopy. The larva is a leaf miner of *Drimys angustifolia* Miers (Winteraceae) in the Atlantic Rain Forest. The study was carried out with a population existing in the Centro de Pesquisas e Conservação da Natureza (CPCN Pró-Mata / PUCRS; 29°28'36''S, 50°10'01''W), 900 m, São Francisco de Paula municipality, Rio Grande do Sul state, Brazil. Like other oecophyllembines, larvae have the thoracic spiracles opening on the mesothorax. Pupae and adults share several characteristics with *Eumetriochroa* Kumata. The new genus differs from the latter one, however, by having forewings without CuA. Also, the sternum eight of males is unique, bearing a pair of short, finger-like processes on posterior margin. Male genitalia has a short, rounded tegumen, with a pair of dorso-posteriorly located, rounded areas that are densely covered by stout setae. Valves show a patch of spine-like setae on distal half; without plumose setae. Unlike the other oecophyllembine pupae, the dorsal, subdorsal and lateral setae on both thorax and abdomen are longer than the width of each segment; especially those of abdominal segments 8-9 are double in size, threadlike, and distally club-shaped. In addition, the first sap-feeding instar builds a slender, serpentine-like, sub epidermal mine that turns into a blotch-type in the later instars. Before molting, the last sap-feeding instar moves deeper into the parenchyma, building centrally on the leaf lamina, a second, smaller, under-layered blotch-mine, where pupation occurs. Prior pupation, the spinning instar builds a tied silky cocoon, folding the central portion of the leaf margins upwards, at the end the leaf looking as been strangling. Morphology and life-history of the new taxon are discussed in comparison to remaining oecophyllembiines and other gracillariids.

P-05

19:30-21:30, Saturday, September 27 (Day 2)

**Seed feeding by *Epicephala* moth selects for elongation of remarkable stalk in the fruit of its host plant**

Saori Furukawa\* (Center for Ecological Research, Kyoto University, Japan)

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Phytophagous insects have brought about various defense mechanisms to their host plants by their feeding damage. Plant defense mechanisms can be largely classified into two categories. One is chemical defense in which the plant produces toxins or digestion inhibitors that are stored in the plant body. The other is physical defense in which the plant modifies the physical properties of the plant body in order to obstruct the feeding damage by phytophagous insects. Although most physical defenses take the form of trichome on plant surface or transformation of plant body into hard tissues, physical defense by other means is little known.

An *Epicephala* moth (Fig.1) and its host plant, *Breynia vitis-idaea*, are engaged in a one-to-one, obligate pollination mutualism, because the *Epicephala* moth and *Breynia vitis-idaea* benefit each other by exchanging seed and pollination service. The female moth is the only pollinator for *Breynia vitis-idaea*. A larva hatched from the egg deposited in female flower eats 2 or 3 of the 6 seeds in a fruit to complete larval development.

Some individuals of *Breynia vitis-idaea* elongate gynophores, or stalk-like structure in a fruit, to varying lengths (Fig.2). We tested whether the elongated gynophores in *Breynia vitis-idaea* was selected to reduce the impact of seed feeding by *Epicephala* moths. We found that longer gynophores increase the mortality of eggs and first instar larvae in the fruit. Female moths laid eggs independently of gynophore length, suggesting that moth cannot distinguish plant defense level at oviposition. Therefore, elongation of gynophores in *Breynia vitis-idaea* is a defense trait selected for to reduce feeding damage by *Epicephala* moths.



Fig. 1. *Epicephala* moth on the leaf of *Breynia vitis-idaea*.

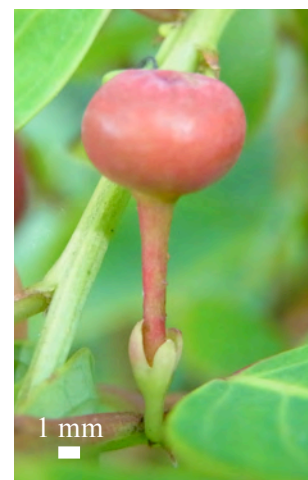


Fig. 2. *Breynia vitis-idaea*

fruits.

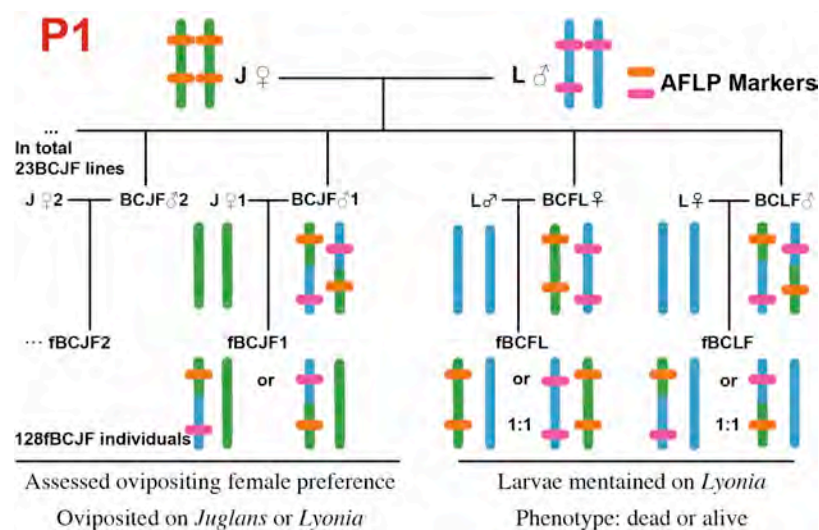
P-06

19:30-21:30, Saturday, September 27 (Day 2)

### A mechanism of maintaining linkage of two host-shift related loci in leaf-mining moth

Masayuki Kobayashi\* & Issei Ohshima (Department of Life and Environmental Sciences, Kyoto Prefectural University, Japan)  
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Approximately 25% of the number of described species are phytophagous insects, and this mega diversity has been ascribed to the evolutionary consequences of association with host plants. Shifting to a novel host plant, host shifting, is considered as the first step of speciation in phytophagous insects, thus the genetic background of host adaptation is ongoing scrutiny. Shifting to a novel host plant requires the evolution both in larval performance (assimilability to secondary compounds in host plants) and in ovipositing adult female preference. Therefore, the evolutionary association between the two traits is crucial for the successive host shifting. However, previous studies have revealed that in general the two traits have an independent genetic basis, indicating that genetic mechanisms creating the preferable allele combination could play a key role in the evolution of novel host associations. Theoretical studies have suggested that loci under epistasis or gene interaction tend to be physically linked. To test this hypothesis, we used the leaf-mining moth *Acrocercops transecta* Meyrick (Lepidoptera: Gracillariidae), which consists of Juglandaceae- and *Lyonia*-associated host races. We assess the oviposition preference and larval performance in the backcross generations between the two host races (Fig). First, we establish a linkage map using hybrids backcrossed to the *Lyonia* race and detect a linkage group responsible for larval performance using AFLP markers. 13 markers are included in the key linkage group and we further assess whether female oviposition preference is also governed by the linkage group using hybrids backcrossed to the Juglandaceae race. Here, we report the progress of our attempt to test the classic theory by using a newly established gracillariid model system, *A. transecta*.



P-07

19:30-21:30, Saturday, September 27 (Day 2)

**Factors regulating host ranges of parasitoid wasps: a case study from host races of *Acrocercops transecta* and its parasitoid wasp *Aneurobracon philippinensis***

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In phytophagous insects, majority of their enemies are parasitoids, and the structure of parasitoid community is influenced by the host plants the former consume. Several host-plant characters have been proposed as factors determining parasitoid communities, but we know little about the extent to which factors regulate host ranges of parasitoids. The leaf-mining moth *Acrocercops transecta* (Gracillariidae) consists of two host races, the Juglandaceae race and the *Lyonia* race. The two host races show distinct host plant association, and their parasitoid communities are also drastically differentiated. We assessed the phenological dynamics of the parasitoid communities using sympatric populations of the two host races for three years and revealed that one braconid wasp *Aneurobracon philippinensis* exclusively attacked the Juglandaceae race. *Lyonia ovalifolia* has a peculiar secondary chemical compound, lyoniatoxin, and we assume that this toxin hinders *An. philippinensis* from parasitization, resulting in the evolution of avoidance of *L. ovalifolia* by ovipositing *An. philippinensis* females. In order to test the hypothesis, we conducted experiments on oviposition preference of *An. philippinensis* females against mines on *J. regia* or *L. ovalifolia*. We also assessed viability of *An. philippinensis* larvae that were laid in *Ac. transecta* larvae as eggs. Oviposition experiments revealed that females do oviposit on mines on *Lyonia* in the laboratory condition, and larvae survived on *Lyonia* as well as on *Juglans*. These results suggest that other factors such as interspecific competition among parasitoids could play a key role in the regulation of parasitoid communities of the two host races.



Figure 1: An adult female of *An. philippinensis* is tracking mines to find larvae of *Ac. transecta* for oviposition.

Figure 2: Pupae of parasitoid (left, *A. philippinensis*) and *A. transecta* (right).

P-08

19:30-21:30, Saturday, September 27 (Day 2)

**Tracking origins of the highly invasive horse-chestnut leafminer using herbaria and minibarcodes**

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Determining the origin of alien invasive species is crucial to developing invasive species management strategies (Roques et al. 2011). However, the origin of many alien species remains uncertain because of the lack of historical data. For instance, the moth *Cameraria ohridella* (Gracillariidae) was described in 1986, as a genus new to Europe and had managed to invade almost all Europe since 1989. Its larvae are leaf miners on the white flowering horse-chestnut (*Aesculus hippocastanum*), causing significant damage to their summer foliage. The fact that the appearance of *C. ohridella* in much of Western Europe has been so recent and dramatic, without earlier detection by entomologists, has made its origin a subject of debate (Lees et al 2011a). Originally thought to be a relict species in the Balkans, a more recent hypothesis is that the moth is an example of a sudden host plant shift to horse-chestnut, probably from maple or sycamore (*Acer spp.*), maybe combined with long distance translocation. Examination of horse-chestnut samples in seven historic herbarium collections revealed that almost half of 71 sheets had leaf mines with larvae/pupae inside. This material came from natural populations in Albania and Greece and dated from 1981 back to 1879.

We extracted DNA from 54 archival larvae and used five COI minibarcode primer pairs developed specifically for *C. ohridella*. We successfully amplified DNA minibarcode fragments from 10 larvae extracted from herbarium specimens from 1936 to 1981. These archival sequences confirm an identity and Balkan origin for *C. ohridella* and the herbarium data set its history back by over a century. The herbaria reveal three previously unknown mitochondrial haplotypes. We also detected local outbreaks back to 1961 and dynamic frequency changes, which may be associated with road development (Lees et al. 2011). In particular, comparison with a temporal series of herbarium samples (1936, 1974 and 1981) with a modern sample from Karitsa in E. Greece suggests the frequency of the invasive haplotype A has been increasing rapidly even within the Balkans. This case history demonstrates that herbaria are greatly underutilised in studies of invasive species origins, herbivore biodiversity and insect-plant interactions.



## REFERENCES

Lees D, Lopez-Vaamonde C, Augustin,S (2011a). Taxon page for *Cameraria ohridella* Deschka & Dimic 1986. In: EOLspecies, <http://eolspecies.lifedesks.org/pages/8675>.

Lees D, Lack HW, Rougerie R, Hernandez A, Raus T, Avtzis ND, Augustin S, Lopez-Vaamonde C (2011b) Tracking origins of invasive herbivores using herbaria and archival DNA: the case of the horse-chestnut leafminer. *Frontiers in Ecology and the Environment*, 9: 322-328

Roques, A., Kenis, M., Lees, D., Lopez-Vaamonde, C., Rabitsch, W., Rasplus, J.-Y. and Roy, D., (eds). (2010). *Alien Terrestrial Arthropods of Europe*, volumes 1 and 2 [BioRisk 4 (Special Issue)]. 1028 pp. Pensoft Publishers, Sofia.



Fig. 1 Pressed L4-stage larva of *C. ohridella* extracted from archival leaf mine



# User's Guide to Botan-sō





## User's Guide to Botan-sō

Welcome to Botan-sō, a Japanese-style lodge and hot spring along the Koza River in Wakayama Prefecture, Japan. Some aspects of Botan-sō are different from conventional hotels in Japan and other countries, so we've put together this user's guide to help make your stay here more enjoyable.

### General

The address of Botan-sō is:

〒649-4106 和歌山県東牟婁郡古座川町月野瀬 881-1

881-1, Tsukinose, Kozagawa-cho, Higashimuro-gun, Wakayama, Japan 649-4106

The building does not lock, even at night, so you can leave and enter anytime (for instance, while blacklighting).

The front desk can be reached from within Japan by dialing 0735-72-0376 and from outside Japan by dialing +81-735-72-0376. Front desk staff do not speak English; please ask a Japanese participant or David Hembry for assistance translating.

If you need to place an outgoing call in case of emergency, please speak with one of the organizers.

Free wireless internet is available. (ID: [botan-guest](#); password: kozagawa)

### Your room

Bedrooms are located upstairs. At traditional inns or lodges in Japan, it is common for multiple people of the same sex to share a room. Instead of sleeping in beds, people sleep in individual futons spread out on the floor. The futon consists of two parts, a *shikibuton* which is analogous to a mattress, and a *kakebuton* which is like a thick blanket or comforter. You sleep on top of the *shikibuton* and under the *kakebuton*. There is no need to make the futon in the evening or to put it away in the morning—the staff will do so for you.

The lodge will provide you a towel which you can use in the bath (?) and a bathrobe-like garment called a *yukata*. The *yukata* is customarily put on after bathing, and worn while sleeping (like pyjamas). The *yukata* may be worn outside the room as well.

## Restrooms

Bedrooms have individual restrooms with Western-style toilets. Below, we provide a translation of some of the controls on the keypad on the side of the toilet seat.



止: stop

おしり: normal function

ビデ: bidet

乾燥: drying

水量: amount of water

ムーブ: moving the position of nozzle

Each room has its own, limited supply of hot water, so please don't use too much hot water or you and the people in your room will run out.

## Bath and hot spring

Individual rooms do not have private baths or showers. Instead, participants use the baths (*o-furo*) located on the first floor, which are also hot springs (*onsen*). These can be recognized by the colored hanging cloths (called *noren*) which hang over the doorway and bear the hiragana character *yu* (ゆ), meaning "hot water". Japanese customarily bathe in the evening before going to sleep. [The baths at Botan-sō are open in the morning from 6:00–8:30 and in the afternoon and night from 13:00–23:00.](#)

There are separate baths for men and women; the women's bath (L) has a red noren and the men's bath (R) has a blue one.



Public Japanese baths are communal, meaning multiple people of the same sex use the bath at the same time. The bath itself is a large, hot pool of water that multiple people can sit in simultaneously. Differently from Western baths, people wash themselves clean before getting in the bath itself; this way, many people can use the same bath water over the course of an evening. Japanese baths are therefore not simply utilitarian; they are venues for socializing with friends and co-workers, and chemicals in hot spring water are believed to have healthful properties. (These aspects of Japanese baths may be familiar if you have seen the film *Spirited Away*.)

Here's how to use the bath:

In the first room, take off all your clothes and put them in one of the baskets.

Go through the doors into the bathroom. Before you get in the bath, sit at one of the stools facing a shower head and, using a towel (provided in the room) and liquid soap, thoroughly scrub yourself. Wash all the soap off. Make sure you are as clean as possible before getting in the bath.

Go sit in the bath. It may be hotter than you expect, but it's not dangerous. People usually do not spend more than about five minutes in the bath.

The bath is not just used by people staying at Botan-sō, but also by other people from the community who come to the baths to socialize with friends. Don't be surprised, therefore, if you meet or see people who don't look like symposium participants, don't speak English, or if they somehow do, don't know what a gracillariid is.

## **Food and drink**

Meals are provided in the restaurant located on the first floor.

At any time of day, bottled and canned beverages are available from the vending machines located on the [first and second](#) floor. (Alcoholic beverages are also dispensable from a machine on the second floor and do not require ID.)

Please see next page for an English translation and explanation of the menu for the opening night *kaiseki* dinner. *Kaiseki* is a highly refined style of Japanese cuisine associated with the historic capital, Kyoto, and distinguished by its emphasis on many small dishes with subtle flavors.

## List of Attendees

Talk No.	Name	Affiliation	Room No.
T-09	AMMUNÉT, Tea	Swedish Univ of Agricultural Sciences	204
P-07	ARIYOSHI, Keiko	Kyoto Prefectural Univ, Japan	202
T-10	AYABE, Yoshiko	Nagoya Univ, Japan	202
	BYUN, Bong-Kyu	Hannam Univ, Korea	203
Keynote 2	DE PRINS, Jurate	Natural History Museum, London	207
	DE PRINS, Willy	Natural History Museum, London	207
T-11	DOORENWEERD, Camiel	Naturalis Biodiversity Center, Netherlands	201
	FUNAMOTO, Daichi	Univ of Tsukuba, Japan	101
P-05	FURUKAWA, Saori	Kyoto Univ, Japan	204
T-07	GIRON, David	CNRS, France	205
T-14	HEMBRY, David	Kyoto Univ, Japan	101
T-01	HIROWATARI, Toshiya	Kyushu Univ, Japan	208
T-13	JOHNS, Chris	Univ of Florida, USA	201
P-03	KATTE, Tomoko	Kyoto Univ, Japan	209
T-17	KAWAHARA, Akito	Univ of Florida, USA	210
T-06	KAWAKITA, Atsushi	Kyoto Univ, Japan	101
	KIM, Da-Som	Hannam Univ, Korea	202
T-05	KIRICHENKO, Natalia	INRA, France	202
P-06	KOBAYASHI, Masayuki	Kyoto Prefectural Univ, Japan	101
T-04	KOBAYASHI, Shigeki	Osaka Prefectural Univ, Japan	210
Keynote 1	KUMATA, Tosio	Hokkaido Univ, Japan	208
	LOPEZ GONÇALVES, Gislene	Federal Univ of Rio Grande do Sul, Brazil	206
T-16, P-08	LOPEZ-VAAMONDE, Carlos	INRA, France	203
P-02	MOCHIZUKI, Ko	Kyoto Univ, Japan	101
T-12	NAKADAI, Ryosuke	Kyoto Univ, Japan	101
T-03	NIEUKERKEN, Erik	Naturalis Biodiversity Center, Netherlands	208
T-15	OHSHIMA, Issei	Kyoto Prefectural Univ, Japan	101
T-08	OKAMOTO, Tomoko	Forestry & Forest Prod Res Inst, Japan	209
	ONO, Hajime	Kyoto Univ, Japan	210
	OSADA, Yohei	Kyushu Univ, Japan	101
P-04	PIRES MOREIRA, Gilson Rudinei	Federal Univ of Rio Grande do Sul, Brazil	206
P-01	BRITO, Rosângela	Federal Univ of Paraná, Brazil	209
	SATOH, Hiroaki	Nara Women's Univ, Japan	203
T-02	SHIN, Young-Min	Korea National Arboretum	204





## Organizers and contacts

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**URL: [http://eureka.kpu.ac.jp/~issei/International\\_Symposium\\_on\\_Gracillariidae/Welcome.html](http://eureka.kpu.ac.jp/~issei/International_Symposium_on_Gracillariidae/Welcome.html)**