PUBS-SATREPS The 3rd WORKSHOP

15-16 July 2023 @ Tsukuba International Congress Center















●Venue

Conference Room: 1F 102, Tsukuba International Congress Center (EPOCHAL Tsukuba) Address: 2-20-3 Takezoko, Tsukuba, Ibaraki, 305-0032, Japan つくば国際会議場 (エポカルつくば) 住所: 茨城県つくば市竹園 2-20-3 https://www.epochal.or.jp/en/

•Access to Tsukuba center (to TX Tsukuba station/Bus terminal)

From Tokyo Area: Tsukuba Express (TX) Akihabara Station – (Rapid/about 45 min.) – Tsukuba Station

From Narita International airport: Kanto Tetsudo bus Narita Airport Terminal– (about 60 min.) – Tsukuba Station Terminal



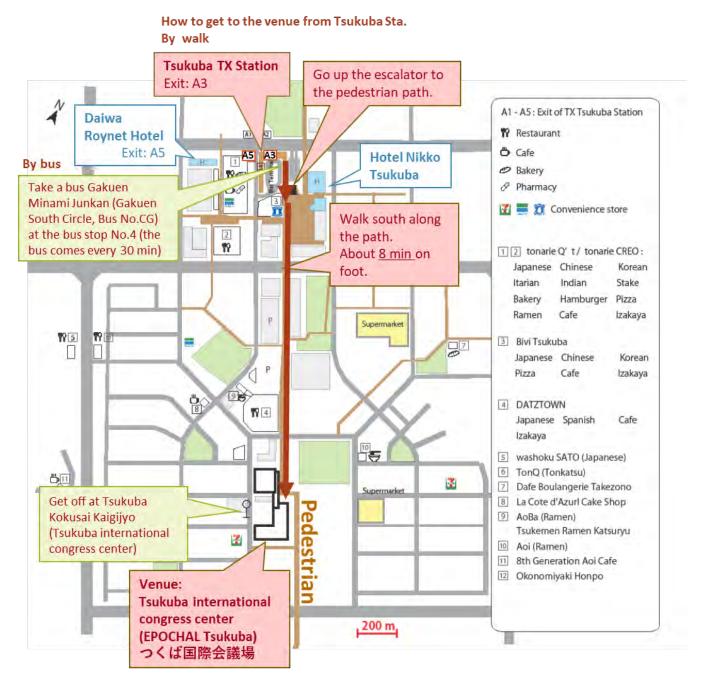
Access to Tsukuba International Congress Center (EPOCHAL Tsukuba) from Tsukuba center

-By walk

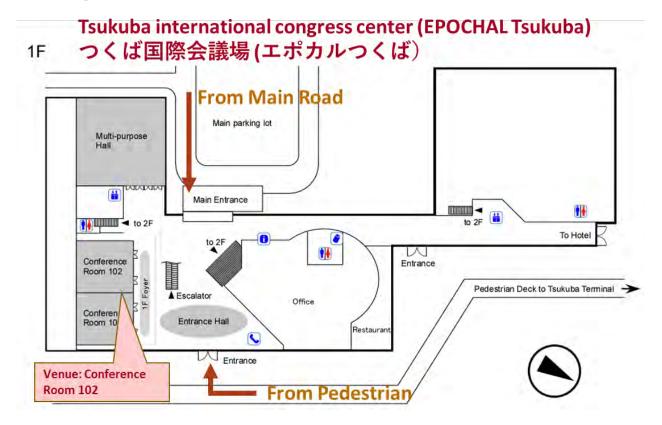
From the bus terminal, go up the escalator to the pedestrian path. Walk south approximately 800m along the path. About 8 min on foot.

-By bus

Take a bus, Gakuen Minami Junkan(Gakuen South Circle, Bus No.CG) from the Bus Terminal at No. 4. The bus comes every 30 min. Get off at Tsukuba Kokusai Kaigijyo (Tsukuba international congress center). It will take about 2~3 min, 170 yen. (adult/one way).



●Venue Map



2023 PUBS-SATREPS workshop program

*All times in Japan time (UTC +9).

Day 1: 15 July 2023

14:00-14:30 **Opening remarks & Plenary** Takao Itioka Professor, Graduate School of Human and Environmental Studies, Kyoto University

14:30-17:10 Flash Talk

PLANT

- 01 Kaya Shimizu
- 02 Yayoi Takeuchi
- 03 Akira Ito
- 04 Jacqualine Henry
- 05 Tatsuhiro Ohkubo
- 06 Seiya Okuno
- 07 Iku Asano

FUNGI/MICROORGANISMS

- 08 Satoshi Yamashita
- 09 Hirotoshi Sato
- 10 Yuho Ando
- 11 Shirley Anak Chip
- 12 Holed Juboi
- 13 Mohd Farith Kota

BREAK (10 MINUTES)

INSECT

14 Hasumi Kawagoe

- 15 Taisuke Kanao
- 16 Takeshi Yamasaki
- 17 Yoko Takematsu
- 18 Paulus Melang

VERTEBRATE

- 19 Kanto Nishikawa
- 20 Azroie Daniel
- 21 Yosuke Kojima
- 22 Manabu Onuma

SOCIAL SCIENCE

- 23 Shinya Numata
- 24 Susumu Takahashi
- 25 Asami Shikida
- 26 Masatomo Ogawa
- 27 Yuma Akaho
- 28 Ayumi Onuma
- 29 Kriskkumar Karunanidhi

17:10-17:15 **Closing remarks for the first day of the workshop**

18:30-20:30 **Dinner**

Day 2: 16 July 2023

09:30-12:00 Progress from Master and PhD candidate students

Species diversity of phyllosphere fungi on leaves of Macaranga species

Khairunnisa Othman

Research officer, Forest Department Sarawak / Master student, Graduate School of Global Environmental Studies, Kyoto University

Biogeography of ectomycorrhizal (ECM) fungi inhabiting the lowland forests

Ajuwin Lain

Research Officer, Sarawak Biodiversity Centre / Master Student Graduate School of Global Environmental Studies, Kyoto University

Molecular Analysis of Ganoderma and Amauroderma Species in the Tropical Rainforest of Sarawak, Borneo

Jamilah Hassan

Research Officer, Sarawak Biodiversity Centre / Master Student, Graduate School of Global Environmental Studies, Kyoto University

BREAK (10 MINUTES)

Progress Update on Temporal Trends in Populations of Phytophagous Hemipteran Insect Species in a Bornean Tropical Rainforest

Clement Het Kaliang

Conservation Officer, Sarawak Forestry Corporation / Doctoral Student, Graduate School of Global Environmental Studies (GSGES), Kyoto University

Bornean Clouded Leopard Project - a Progress Report

Voon Mufeng

Wildlife Officer, Sarawak Forestry Corporation / Doctoral Student, Graduate School of Urban Environmental Sciences, Tokyo Metropolitan University

- 12:00-14:00 Lunch Break
- 14:00-15:30 **Progress from Postdocs**

Progress and future plans of botanical research in PUBS: biogeography and inventory work

Natsuki Komada Postdoctoral researcher, Graduate School of Global Environmental Studies, Kyoto University

Progress of researches on biodiversity science under PUBS

Takafumi Mizuno Researcher, Graduate School of Global Environmental Studies, Kyoto University

Arrangement and database development of entomological collection in Forest Department Sarawak

Koichi Arimoto Postdoctoral researcher, Graduate School of Global Environmental Studies, Kyoto University

- 15:30-16:30 Free time to discuss with each other
- 16:30-16:40 Closing remarks

Species diversity of phyllosphere fungi on leaves of Macaranga species

Khairunnisa Othman^{1,2}*, Hirotoshi Sato³, Takafumi Mizuno³, Usun Shimizu-Kaya⁴, Satoshi Yamashita⁵, Hasumi Kawagoe³, Kanto Nishikawa^{2,3}, Jamilah Hassan^{2,6}, Ajuwin Lain^{2,6}, Takao Itioka^{2,3}

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Abstract: The accumulated leaf surface globally has approximately twice the land surface area, harboring tremendous microbial species diversity with many beneficial effects on plants and environments. However, the phyllosphere microorganisms, including fungi on leaves, have not been studied in depth, especially in tropical rainforest areas, so far. Various factors, such as biogeography and host plant species, have been reported to influence the species compositions of the phyllosphere fungi community. The aim of this study is to investigate the effect of location and host plant species on the species composition of phyllosphere fungi community in Southeast Asian tropical rainforests. The study focused on the phyllosphere of five Macaranga species at the two sites, Santubong National Park and Lambir Hills National Park, and targeted internal transcribed spacer (ITS) region by sequencing the amplicons with Illumina MiSeq. The high-throughput sequencing of the fungal ITS1 region identified 1,214 OTUs with 54,991 reads. The majority of phyllosphere fungi on Macaranga sp. belong to the phylum Ascomycota with 85.6% of all sequences, followed by Basidiomycota with 12.6%, and unclassified with 1.8%. The results of NMDS and Permutational Multivariate Analysis Variance (PERMANOVA) based on Raup-Crick index also showed that both location and plant species had significant effects on the species composition of the phyllosphere fungi community (p < 0.001 for location and p < 0.0001 for plant species). These results suggest that the phyllosphere fungi communities exhibit variability in relation to locality and host plant species. Some plausible factors, such as myrmecophytism (plant-ant attendance), leaf characteristics, and environmental conditions of microhabitats, that may be associated with the significant effects will be discussed as well as what remains to be done for elucidating the factors that affect the differences in the future.

Keywords: Borneo, fungi diversity, phyllosphere microorganisms, tropical forests, Macaranga

Biogeography of Ectomycorrhizal (ECM) Fungi Inhabiting the Lowland Forests in Sarawak

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Abstract: Ectomycorrhizal (ECM) fungi involved in obligatory symbiosis with several major tree families are invaluable to most forest ecosystems. Most studies on the ecology and biodiversity of ECM fungi have focused on temperate forests in the Northern Hemisphere. Thus, we need to extend our current knowledge about the biodiversity of ECM fungi in tropical rainforests to better understand the global biodiversity of ECM fungi. To improve our understanding of species diversity and endemism of ECM fungi inhabiting tropical rainforests in Sarawak, Malaysia, we aim to compare the biogeography of ECM fungi and soil saprotrophic (SAP) fungi inhabiting tropical rainforests in Sarawak. In this study, 288 root samples in total were collected in the Lambir Hills and Santubong National Parks. The internal transcribed spacer1 (ITS1) region of ribosomal RNA, the fungal barcode region, was sequenced using the Illumina MiSeq. Then, 103 ECM and 106 SAP operational taxonomic units (OTUs at the 97% sequence similarity level) were obtained. Using the Basic Local Alignment Search Tool (BLAST), highly similar sequences ($\geq 97\%$ sequence similarity and \geq 70% query coverage) with these queries were searched. The number of ECM and SAP OTUs that were shared between this study and each country was counted. Using two-step binomial model, the probability that each OTU obtained in this study is present in each country was estimated, together with the deposition rates of ITS sequences in each country. ECM fungal OTUs were shown to be highly present only in Southeast Asian countries. Furthermore, results indicated the presence of Dipterocarpaceae and annual precipitation strongly increased the probability of occurrence of ECM OTUs, whereas temperature showed significant negative influences on the probability of ECM occurrences. Unlike ECM, SAP fungi were distributed not only in Southeast Asia but also in distant areas, including Africa and South America. Moreover, the presence of Lithocarpus tree strongly increased the probability of SAP OTUs occurrences whereas the distribution of the Dipterocarpaceae tree negatively influences the occurrences. These findings highlight the endemism of the ECM fungi in tropical rainforests in Southeast Asia, presumably attributed to the strong host specificity of ECM fungi.

Keywords: Ectomycorrhizal, Fungi, Tropical Forest, Sarawak

Molecular Analysis of *Ganoderma* and *Amauroderma* Species of the Tropical Rainforest of Sarawak, Borneo

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Abstract: Ganoderma australe (Polyporaceae) is one of the dominant fungal species in the tropical rainforest of Borneo Island. A previous study conducted in Lambir Hills National Park, Sarawak, Borneo, revealed that this species contained at least six genetic types. The genetic types of G. australe observed in the two areas are expected to differ from each other due to its wide distribution in Borneo and the different environmental conditions between northeastern and western Sarawak. Amauroderma subrugosum (Polyporaceae) is phylogenetically closely related to Ganoderma and widely distributed in Borneo. As seen in G. australe, this species also exhibits highly variable fruiting bodies. Therefore, it is suggested that A. subrugosum contains multiple genetic types in this species. In this study, we aimed to record the genetic types of both G. australe and A. subrugosum with their morphological and ecological traits. G. australe sensu lato, A. subrugosum sensu lato, and some Ganoderma species were studied in three national parks in Sarawak: Santubong, Kubah (western area), and Lambir Hill (northeastern area). The decay stage and diameter of fallen logs from which fungal fruiting bodies appeared were recorded. The size of the pores and length of the tube of fruiting bodies were measured and recorded in the laboratory. Molecular experiments were conducted to obtain phylogenetic information. Ganoderma is the most common polypore fungus collected, followed by Amauroderma and others. In the phylogenetic tree analysis, which contains several Ganoderma species and A. subrugosum, A. subrugosum was grouped into a single genetic type. As for Ganoderma, it has been divided and classified into seven types. A phylogenetic tree that focuses on the phylogenetic relationship within G. australe reveals that G. australe was classified into four genetic types: type 1, type 2, type 3, and type 5. All genetic types of G. australe fruiting bodies frequently appear in the intermediate stages (stage 3) of decayed wood from small and medium-sized (diameter) fallen trees or logs. In this study, no novel genetic types of G. australe were observed, and several genetic types were not observed in A. subrugosum. All genetic types of G. australe were found in the northeast (Lambir Hills) and west (Kubah, Santubong) areas of Sarawak, suggesting that these genetic types are widely distributed across Sarawak. Further study is needed to reveal the mechanisms that drive speciation and maintain genetic diversity in G. australe and the mechanisms that prevent speciation in A. subrugosum.

Keywords: Ganoderma, Amauroderma, Sarawak, Borneo

Progress Update on Temporal Trends in Populations of Phytophagous Hemipteran Insect Species in a Bornean Tropical Rainforest

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Abstract: Insect populations show various patterns in their temporal fluctuation in response to various environmental factors, such as climatic features and patterns, and ecological factors, such as phenology of vegetation, abundance, and biomass of other organisms that interact with the insect population, life history as adaptive responses to the factors. In particular, climatic conditions often significantly influence seasonal and temporal patterns in temporal population fluctuation in insects. Therefore, seasonal patterns in the climatic conditions are known to be tightly associated with the seasonality in fluctuation patterns of insect populations inhabiting habitats in subtropical, dry, temperate, and subarctic zones, where the climate conditions show clear seasonal patterns. However, seasonality in temporal population patterns has not been fully understood in tropical rainforest areas, where the seasonal patterns in climatic conditions are rather weak. So far, studies on the seasonality in temporal population patterns have been limited only for some insect taxa in the Neotropics and Chrysomelidae, Scarabaeidae, Passalidae, and the giant honeybee in the Southeast Asian tropics. In this study, we investigate the seasonality in the temporal patterns of population fluctuation for phytophagous insects and several cicada species, based on specimens that were collected by monthly light-trapping for almost seven years from 1993 to 1999 in a tropical rainforest at Lambir Hills National Parks. At current progress, we selected the following ten cicada (Hemiptera: Cicadidae) species with >100 individuals as our target species: Purana sp., Nelcyndana tener, Orientopsaltria phaeophila, O. maculosa, Dundubia vaginata, D. rufivena, Platylomia spinosa, Pomponia spp., Abroma maculicollis, and Purana guttulavis. Most of the species shows non-seasonal temporal patterns, neither responding to the general flowering events. Of the 10 species, only Dundubia vaginata showed a clear seasonal pattern, while the other cicada species did not show seasonal patterns and most of them constantly occur irrespective of calendar month almost throughout the study period. These results suggest that most cicada species don't have clear seasonal life cycles in the tropical rainforest areas in the Southeast Asian tropics, which is likely to be quite different from the seasonal pattern shown in some hemipteran insects in the Neotropics.

Keywords: Bornean, Cicada, life history, population, seasonal pattern, temporal

Bornean Clouded Leopard Project - A Progress Report

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Abstract: The largest predator on the Borneo Island is the Bornean Clouded Leopard (*Neofelis diardi borneensis*), a subspecies described in 2011, along with the Batu-Sumatran subspecies (*Neofelis diardi diardi*). The *N. diardi borneensis* is native to Borneo and is distinctly different from its mainland cousin *Neofelis nebulosa*. Unlike *N. nebulosa*, the Bornean Clouded Leopard is still understudied and although researchers have started to look into the spatial ecology and genetics aspects of the species, there are still a lot of research gap that needs to be bridged urgently, with the subspecies currently listed as Vulnerable in the IUCN Red List. This paper outlined the progress of the on-going research done at Pulong Tau National Park, Bario, Sarawak. The field work was conducted from 16 April to 21 May 2023 with a total of 49 camera traps installed. Set up of the camera traps follow the Spatially Explicit Capture-Recapture (SECR) design, where each camera trap station has two camera traps on the left and right side of the trail. The camera traps will be left to run for 90 nights before shifting to different locations to expand the trapping area. With this project, we hope to subsequently obtain sufficient empirical data that can be used to advocate for better management decision for the protected areas and its surrounding forests.

Keywords: Bornean Clouded Leopard, progress

Progress and future plans of botanical research in PUBS: biogeography and inventory work

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Abstract: The presentation focuses on the progress and outcomes of floristic field surveys conducted as part of the Survey on Biodiversity at Protected Areas in Sarawak (SBPS) under the Project on Development of Management Systems for Multiple Utilization of Biodiversity in the Tropical Rainforests at the Protected Areas in Sarawak (PUBS). The surveys were conducted at Lambir, Niah, Santubong, and Similajau National Parks between August 2022 and May 2023, to accumulate biogeographic information on the plants found in the tropical rainforests of Sarawak region. The presentation outlines the number of collected specimens and future plants, as well as noteworthy characteristics of certain plant species.

In addition to the explanation of biogeography and inventory work, I introduce the studies on the diversity and ecology of epiphytes as introduction of my research works so far. Vascular epiphytes (hereafter epiphytes) grow non-parasitically on other plants. I have sought to elucidate the composition and host tree utilization patterns of epiphytes in a lowland tropical rain forest in Lambir Hills National Park in Borneo (hereafter Lambir). I compared the number of species and the component percentage of each epiphyte taxon, among 11 sites from the Southeast Asian tropics, Afrotropics, and Neotropics. The results suggested that the epiphyte flora of Lambir is characterized by, for instance, the highest diversity of Apocynaceae and Moraceae. As ecological studies, I examine the relationships between epiphyte species richness and the abundance and diameter at breast height (DBH) of host trees. The results indicate that number of epiphyte species and number of epiphyte individuals both increase exponentially with DBH. The greatest increase was observed in trees with DBH > 40 cm. In addition, approximately half of all epiphyte species recorded in this study were observed exclusively on trees with DBH > 60 cm.

Keywords: canopy, ecology, epiphytes, plant diversity, taxonomy

Progress of researches on biodiversity science under PUBS

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Abstract: Biodiversity studies are one of main subjects and a foundation of the other subjects in PUBS (the project on development of management systems for multiple utilization of biodiversity in the tropical rainforests at the protected areas in Sarawak). To promote greater understanding of entire biodiversity in Sarawak we have conducted field work in several national parks and also are conducting studies using specimens collected before this project. In these studies, we plan to obtain DNA data from a massive number of samples with next generation sequencing (NGS) technology that have been installed in Sarawak. In this presentation I will show progress of two ongoing studies.

The topic of the first study is mutualistic relationship between *Crematogaster* ants and myrmecophytic *Macaranga* where the plants provide ants with foods and nest site, in return, the ants protect their host plants from herbivory. Although the *Crematogaster-Macaranga* system have been well studies, host range of the ants and variation of the host range among sites are still unclear mainly due to difficulty in morphological species identification of the *Crematogaster* ants and lack of sample collection from multiple sites. I conducted species identification of *Crematogaster* ants inhabiting several *Macaranga* species from multiple national parks based on DNA data obtained by NGS and will show the results.

The topic of the second study is population dynamics of Carabid beetles. Population dynamics of insects in aseasonal tropical rainforest in Sarawak have been studied using leaf beetles, passalid beetles and scarab beetles collected by monthly light-trapping for over six years in Lambir Hills National Park. However, population dynamics of predatory insects such as *Carabidae* have never been investigated. I have been sorting Carabid beetles collected by the same light traps since I joined this project. In this presentation I will show the pattern of *Carabidae*'s population dynamics, effect of mass flowering and severe drought on the population dynamics, and response of *Carabidae*'s population dynamics to population dynamics of other insects studied in previous researches.

Keywords: Crematogaster - Macaranga mutualism, NGS, population dynamics, Carabidae

Arrangement and database development of entomological collection in Forest Department Sarawak

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Abstract: Insects account for more than half of the species described in the animal kingdom and a significant proportion of the biodiversity on Earth. Borneo has one of the oldest rainforests in the world, containing a concentrated number of insect-diverse and endemic species. Recent entomological collection in Borneo has been built in Forest Department Sarawak, Kuching, with well over 100,000 pinned specimens and numerous unmounted specimens in alcohol, many of which were collected during regular surveys using light traps and Malaise traps in Lambir Hills National Park, Miri since the 1990s. The collection is useful for understanding not only the Borneo entomo-fauna but seasonal and annual population dynamics of insects, however, it is inconvenient to use because specimen arrangement is not complete. As the first task, I picked out elaterid beetles from the unorganized pinned specimen and prepared 20 new specimen boxes for sorting to species level. I was able to find some undescribed species, rare species, for which only a few specimens exist in the world, and many specimens of the common species without continuous distributional records among about 10,000 specimens. Consequently, the importance of the collection was reemphasized for understanding insect species diversity in Borneo. In order to further enhance the usefulness of the collection, the unorganized specimens need to be sorted into families and some to the generic and species level. In this presentation, valuable species found in my short-term research will be introduced. In addition, future plans for specimen maintenance and the construction of a database of entomological collection will be presented.

Keywords: Insects, Lambir Hills National Park, new species, species diversity

