

*Proceedings Of*  
**SEAMEO BIOTROP  
THIRD INTERNATIONAL CONFERENCE  
ON TROPICAL BIOLOGY**

**“Conservation, Enhancement and Sustainable Use  
of Indigenous Tropical Flora and Fauna”**



**Edited by**  
Jesus C. Fernandez  
Cahyo Wibowo

Bogor, 20-21 September 2018  
SEAMEO BIOTROP Convention Hall



Southeast Asian Regional Centre for Tropical Biology

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OF  
SEAMEO BIOTROP  
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## PREFACE

We are pleased to publish the proceedings of our Third International Conference on Tropical Biology held on 20-21 September, 2018 in Bogor, West Java Province, Indonesia. The conference theme, “Conservation, Enhancement, and Sustainable Use of Indigenous Tropical Flora and Fauna”, was in response to the urgent need in ensuring a sustainable use of indigenous tropical flora and fauna, as well as the conservation of species facing extinction due to rapid habitat loss caused by rampant deforestation for agricultural purposes and massive infrastructure development, unregulated collection and trafficking of indigenous plants and animals. We believe that there are several past experiences of sustainable use and conservation schemes by governments and non-governmental organizations that have been successful. Lessons learned from these experiences are critical to generate and formulate practical and sustainable conservation strategies for indigenous tropical flora and fauna, determine and prioritize research needs based on current policies and research results, and strengthen participation and contribution of stakeholders in eliminating current problems and at the same time, enhancing the conservation and sustainable use of the region's biodiversity and natural resources.

We were fortunate to convene 227 scientists and practitioners from eight countries during the conference to share useful lessons, address challenges, and generate commitments to strengthen policy decisions and work collaboratively towards conservation and sustainable use of indigenous tropical flora and fauna, especially in the Southeast Asia region.

This volume of our conference proceedings contains the full papers and abstracts of the keynote addresses, panel discussion, and parallel session oral and poster presentations. The keynote addresses attempt to illustrate the gains and challenges, the diversity and resiliency as well as the approaches, technologies and innovations in conservation, enhancement and sustainable use of indigenous tropical flora and fauna. The panel discussions focus on the policies and other legal frameworks as well as the future directions in conservation, enhancement and sustainable use of indigenous tropical flora and fauna. The parallel session papers provide actual experiences on the four conference subthemes, namely: (1) Diversity and Resiliency of Indigenous Tropical Flora and Fauna and Their Ecosystem; (2) Approaches, Technologies and Innovations in Conservation, Enhancement and Sustainable Use of Indigenous Tropical Flora and Fauna; (3) Socio-economic, Cultural and Ethical Aspects in Conservation, Enhancement and Sustainable Use of Indigenous Tropical Flora and Fauna; and (4) Policies and Other Legal Frameworks in Conservation and Sustainable Use of Indigenous Tropical Flora and Fauna. As much as we would have wanted full papers included in this publication, we respect the presenters' decision to just submit the abstracts of their presentations. We thank all of them for their contributions in making this publication possible. We hope that the papers and abstracts, much more the synthesis and recommendations as well as future agenda generated from the conference, could spark new and continuing efforts to pursue conservation, enhancement, and sustainable use of indigenous tropical flora and fauna in the region.

Our deepest appreciation goes to Southeast Asian Ministers of Education Organization (SEAMEO), Ministry of Environment and Forestry of the Republic of Indonesia, National Committee for Indonesian Germ Plasm, Ministry of Agriculture of the Republic of Indonesia, Lembaga Ilmu Pengetahuan Indonesia (Indonesian Institute of Science/LIPI), Institut Pertanian Bogor (IPB University), Universiti Putra Malaysia, National University of Singapore, Forest Stewardship Council (FSC) Indonesia, Central Luzon State University Philippines, Burung Indonesia, Pampanga State Agricultural University Philippines, Cagayan State University

Philippines, PT Sinarmas Tbk. Indonesia, Bank Mandiri, Bank Mandiri Syariah and PT Garuda Food for supporting us to hold this conference. We highly value the time and effort of the Scientific Committee members for reviewing all the submitted abstracts and helping us finalize the list of paper and poster presenters. We recognize the valuable contributions of SEAMEO BIOTROP staff members for ensuring the smooth implementation of the conference and in packaging this publication.

We look forward to our Fourth International Conference on Tropical Biology in 2020.

Conference Coordinator and Proceedings Editors

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## 1. Welcome Remarks

Dr Irdika Mansur  
SEAMEO BIOTROP Director

- Dr Kirsfianti Linda Ginoga, Director of Forest Research Centre, Forest Research Development and Innovation Agency, Ministry of Environment and Forestry of the Republic of Indonesia,
- Governing Board Members, Deputy Directors and Staff of Southeast Asian Ministers of Education Organization (SEAMEO) for Tropical Biology (BIOTROP),
- Dr Maria Ulfah, Chairperson of the 3<sup>rd</sup> International Conference on Tropical Biology,
- Our Colleagues from Indonesian universities, research institutes, schools and private companies,
- Representatives of local governments from all over Indonesia,
- Distinguished speakers and participants,
- Ladies and Gentlemen,

Assalamu'alaikum warahmatullaahi wabarakatuh,

I am pleased to welcome you all to Bogor City and to SEAMEO BIOTROP for our 3<sup>rd</sup> International Conference on Tropical Biology starting today until 21 September 2018 which we are now conducting in our Convention Hall. We expect this conference to be a venue for sharing knowledge, perspectives, and experiences among the participants and speakers on the theme “Conservation, Enhancement, and Sustainable Use of Indigenous Tropical Flora and Fauna.” As SEAMEO BIOTROP Director, I am honored for our Centre to host this conference.

First, allow me to briefly introduce our Centre to all of you. SEAMEO BIOTROP is one of 24 specialist centres of the Southeast Asian Ministers of Education Organization (SEAMEO). Our Centre was established on 6 February 1968 and is mandated to conduct research, capacity building, and information exchange toward addressing biology-related problems in Southeast Asia. Since 2012 up to now, SEAMEO BIOTROP's vision is to be “A leading Centre in enriching and promoting the real values of tropical biology in Southeast Asia”. Our mission is to provide scientific knowledge and build capacities of institutions and communities in conserving and managing tropical biology sustainably for the well-being of communities and the environment of Southeast Asia. For the next five years, we will be focusing our activities on three program thrusts, namely: (1) Restoration of Degraded Landscapes/Ecosystems, (2) Sustainable Management of Intensively Used Landscapes/Ecosystems, and (3) Conservation and Sustainable Use of Unique Ecosystems/Landscapes of High Biodiversity. We believe that organizing an international conference on tropical biology (ICTB) is one of the ways through which we can realize our vision and mission and address our program thrusts.

Ladies and Gentlemen,

In recent years, we have witnessed an increasing concern on ensuring a sustainable use of indigenous tropical flora and fauna, as well as the conservation of species facing extinction due to rapid habitat loss caused by rampant deforestation for agricultural purposes and massive infrastructure development, unregulated collection and trafficking of indigenous plants and animals. This scenario led us to focus on “Conservation, Enhancement and Sustainable Use of Indigenous Tropical Flora and Fauna” as the theme of this year's conference.

I am very pleased to see delegates from various countries in and outside the Southeast Asian region as well as representatives from many Indonesian institutions. I believe that with the various expertise of the participants and speakers present here, we would have interesting and enthusiastic discussions during our conference. I sincerely hope that this conference will be able to generate consensus among participants to formulate practical and sustainable ways, based on current policies and research results, to strengthen participation and contribution of stakeholders in eliminating current problems and, at the same time, enhancing the conservation and sustainable use of the region's biodiversity and natural resources.

Ladies and Gentlemen,

I would like to express my gratitude to the Southeast Asian Ministers of Education Organization (SEAMEO) and partner-institutions for supporting us to hold this conference. Let me take this opportunity to acknowledge them here, namely: the Ministry of Environment and Forestry of the Republic of Indonesia, National Committee for Indonesian Germ Plasm, Ministry of Agriculture of the Republic of Indonesia, Indonesian Institute of Science, Institut Pertanian Bogor, Universiti Putra Malaysia, National University of Singapore, Forest Stewardship Council (FSC) Indonesia, Central Luzon State University Philippines, Burung Indonesia, Pampanga State Agricultural University Philippines, Cagayan State University Philippines, PT Sinarmas Tbk Indonesia, Bank Mandiri, Bank Mandiri Syariah and PT Garuda Food. I would also like to express my heartfelt appreciation to all the members of our Conference organizing and scientific committees for their hard work and dedication in making sure that all things are in place and running well. And to all of our speakers and participants, thank you so much for your presence and interest to be a part of this important conference, because without you this event could not be realized.

I wish everyone a productive conference and I hope that you will find your stay in SEAMEO BIOTROP a pleasurable one. Once again, I extend our warm welcome to all of you.

Thank you very much.

Wassalamu'alaikum warahmatullaahi wabarakaatuh.

## 2. Opening Remarks

Dr Kirsfianti Linda Ginoga

Director of Forest Research Centre, Forest Research Development and Innovation  
Agency, Ministry of Environment and Forestry of the Republic of Indonesia

The honorable

Director of SEAMEO BIOTROP

Prof Mohamed Zakaria Hussin, Faculty of Forestry, Universiti Putra Malaysia, Malaysia

Prof Ani Mardiasuti, Faculty of Forestry, Institut Pertanian Bogor, Indonesia

Dr Chew Fook Tim, National University of Singapore, Singapore

Prof Enny Sudarmonowati, Indonesian Institute of Science, Indonesia

Dr Urdujah Alvarado-Tejada, Cagayan State University, Philippines

Distinguished Participants, Ladies and Gentlemen,

It is a great honor for me to represent our institution, the Ministry of Environment and Forestry, and give a keynote speech and officially open this 3<sup>rd</sup> International Conference on Tropical Biology. I would like to congratulate SEAMEO BIOTROP for the excellent arrangements of this meeting and for your warm welcome. I hope that all participants will benefit from this event by exchanging knowledge and experiences during the various sessions of the Conference. I also would like to recognize the great efforts of the Conference secretariat in gathering experts from various fields of tropical biology to shed light on the theme and subthemes.

Ladies and Gentlemen,

Today, I would like to share two interrelated topics, namely: the development of the Paris Agreement at the end of last year and its relationship to biodiversity in Southeast Asia. The Paris Agreement has brought 178 countries to agree in keeping global temperatures below two degrees, with best efforts at 1.5 degree Celsius. To achieve this objective, it is essential to limit greenhouse gas emissions and maximize the potential to reabsorb them from the atmosphere.

There are at least four key elements of the Paris Agreement, comprising the following:

1. Keeping global temperatures "well below" 2 °C above pre-industrial times and "try to limit" them even lower to 1.5 °C
2. Limiting the amount of greenhouse gases emitted by human activities to the same levels that trees, soil and oceans can absorb naturally, beginning at some point between years 2050 and 2100
3. Reviewing each country's contribution to reduce emissions every five years to be able to improve their efforts to achieve the target
4. Mandating rich countries to help poorer nations by providing "climate finance" to adapt to climate change and switch to renewable energy.

This agreement reflects global commitment to cope with the problems of climate change where the ASEAN Member States (AMS) may need to contribute to the effort. Today, let me first start with the role of AMS in the development of the Paris Agreement, then I will talk about the role of Land Use, Land-Use Change, and Forestry (LULUCF) in addressing climate change in AMS, and conclude my speech with an invitation to collaborate intensively amongst AMS to cope with climate change in forestry sector.

Ladies and Gentlemen,

Some of you may still remember AMS'effort to propose forestry sector as a crucial part in solving global climate change. The AMS'proposal succeeded when forestry issues were explicitly mentioned in article 5 in the Paris Agreement, especially in the second paragraph where forest sector can play an important role to reduce emissions by reducing deforestation and forest degradation (REDD+).

The next step for AMS is to continue their contribution in good faith on developing mechanisms, modalities, procedures and guidelines to be collectively called as Paris Agreement Work Program (PAWP), taking into account the principles of CBDR-RC. This PAWP needs to be finalized and adopted in COP-24 Katowice, Poland.

Regarding to REDD+ implementation, AMS also need to maintain agenda under COP related to coordination of support for implementation of REDD+. This agenda is very important as a platform for communication among REDD+ countries and countries/institutions providing support including climate finance.

Based on these processes and our experience as a group, we can do something great if we work together in influencing international policies. I strongly believe that we can achieve big targets if we work together as a group.

Ladies and Gentlemen,

According to the Intergovernmental Panel on Climate Change (IPCC), Land Use, Land Use Change and Forestry (LULUCF) significantly contributes to the global GHG emissions. In the past 20 years, emissions from LULUCF have reached 1.65 Gt Carbon per year or about 17% of the global emissions. Of this figure, about 75% have been contributed by developing countries, especially AMS that have large areas of tropical forests.

Based on documents on Nationally Determined Contributions (NDC), AMS has a significant potential contribution, both individually and collectively, in reducing 5.48% of the global GHG emission. Most of AMS contributions for reducing global GHG emissions come from forestry and agriculture as the main sectors.

These commonalities in NDCs of AMS can be a strong basis for establishing partnerships as well as for strengthening the implementation of our NDCs taking positive lessons of similar initiatives under different ASEAN colleagues. Therefore, ASEAN as a regional group should take advantage of these commonalities, which allow us to work in partnership, as well as in helping each other to achieve the commitment individually and collectively, to demonstrate ASEAN contribution to the global goal.

Ladies and Gentlemen,

It is obvious to most of AMS, that REDD+ implementation is important in fulfilling NDC targets under the Paris Agreement regime. However, there are various stages among AMS in implementing REDD+. To optimize in achieving the NDC targets, AMS need to work together, enhance coordination mechanisms, and develop effective institutional arrangements.

I propose that we should keep our coordination, share information, and exchange technologies to address LULUCF in climate change mitigation and adaptation and REDD+ implementation. For

example, Indonesia has put a 17.2% contribution of its Forestry sector to achieve 29% of its NDC targets with its own resources. This is an ambitious target taken from forestry contribution to achieve Indonesian NDC through four main strategies including:

1. Maintaining deforestation rate by maximum of 0.45 million ha per year until 2020 and 0.35 million ha per year from 2021 until 2030;
2. Improving implementation of sustainable forest management principles, both in natural forests (degradation reduction) and in plantations;
3. Rehabilitation of 12 million ha of degraded land by 2030 or 800,000 ha/year with survival rates of 90%; and
4. Restoration of 2 million ha of peat land by 2030 with a success rate of 90%

Based on our need to gain maximum benefit when addressing climate change, our ability to work together and keeping our coordination, I highly expect that this meeting could help us in defining the forestry contribution on the NDC implementation.

Ladies and Gentlemen,

Policies on climate change are also linked to biodiversity. We understand that the biodiversity in Southeast Asia has unique and distinctive properties. A slight disturbance to the biodiversity can disrupt the natural cycles that have occurred over millions of years through the process of evolution. I believe that we are also aware that Southeast Asia's population is steadily increasing and meeting their daily needs also increases biodiversity use. Unregulated and continuous use of biodiversity would result to its depletion in due time which could culminate in decreased ability of a country to produce quality products, increased poverty, loss of access to genetic resources and encourage an increase in invasive alien species in Southeast Asia. Therefore, I encourage the management and utilization of biodiversity to be done wisely and fairly by applying the principles of sustainable use.

Indonesia's experience shows that in managing biodiversity, it is necessary to establish a conservation area that involves community participation to safeguard and preserve forests. In 1993, Indonesia developed a Biodiversity Action Plan (BAPI) and was revised in 2003 to become Indonesia Biodiversity Strategies and Action Plan (IBSAP) 2003–2020. Then in 2015, the document was improved to become IBSAB 2003-2015 by incorporating global and national issues such as Biodiversity Action Plan 2020, Aichi Target, Access and Benefit Sharing (ABS), Economics of Biodiversity and Climate Change. One form of biodiversity utilization in Indonesia is environmental/ecosystems services, biotechnology and social benefits. In relation to natural resources, the utilization of forest microbes in Indonesia forests has not received proper attention. The high benefits of tropical forest microbes can disappear if the tropical forests in Indonesia decline in function.

Ladies and Gentleman,

The main consequences of climate change that we are facing now include a decrease in natural resources and the loss of many genetic resources in tropical flora and fauna. These need our commitment to restore and strengthen our capacity in conservation and sustainable use technology to restore our remaining natural resources. Some examples are as follows:

1. The use of tropical forest microbes as an agent to trigger forest growth and restore natural forest resources. FOERDIA also has developed mycorrhiza inoculant to mass produce forest plant seeds.



2. Bioinduction agent can be used to help the formation of agarwood. FOERDIA has done long-term research of microbes and has found the key fungus to produce agarwood by inoculation.
3. Bioremediation agent also can be used to neutralize pollution. Microbes (bacteria and fungus) can be utilized to accelerate the process of decomposition of targeted pollutants.
4. Alternative energy resources such as biogas (CH<sub>4</sub>).
5. Development of bioplastic resources to support the green economic design and resolve societal problems on plastic wastes.

From all I mentioned earlier, I believe that we have an opportunity to create positive growth of tropical biodiversity and use them sustainably. In order to stand to our commitments, I would like to invite all institutions which are engaged in the protection of natural resources of flora and fauna to collaborate with each other. I have a strong commitment to support international cooperation because it is a small step to gain benefit from tropical flora and fauna utilization.

Ladies and Gentlemen,

Once again, allow me to sincerely appreciate your participation and valuable contribution in all the discussion sessions during this conference. I hope you will have a good time here in Bogor. I hope that what we will achieve in this conference could be used as a tool to improve our environmental quality. I do sincerely hope that this conference will provide us with useful insights to create better policies for ASEAN countries.

I have now the honor to officially open this conference.

Thank you very much for your kind attention and I wish you all a successful conference.

### 3. Keynote Address 1

## GAINS AND CHALLENGES OF CONSERVATION, ENHANCEMENT AND SUSTAINABLE USE OF INDIGENOUS TROPICAL FLORA AND FAUNA

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### ABSTRACT

Tropical forests host the vast majority of indigenous biodiversity in our planet. The indigenous flora and fauna have long been recognized as important and valuable natural resources that provide many vital ecosystem services directly and indirectly for humans. These benefits vary in forms such as the provision of carbon sinks to combat climate change, improvement of forest productivity, and livelihood improvement through income generation from non-timber forest products. Unfortunately, many indigenous tropical forest flora and fauna species have gone extinct, and more are threatened due to unsustainable exploitation of their benefits. In general, only about 5% of indigenous vegetation is spared to survive in a highly fragmented state. Several factors have been identified to pose serious threats to the conservation of indigenous tropical species. They are climate change, introduction of invasive exotic species, lack of information regarding wood characteristics, limited knowledge on indigenous species pool, expansion of the global market on timber export to include indigenous species, tropical forest disease, consumptive value of indigenous species, discovery of new medicines, human-animal conflicts and other anthropogenic activities which include irrational setting up of plantation areas, forest deforestation and degradation particularly due to urbanization, industrialization and intensive agriculture. Therefore, these call for a holistic approach in the conservation and sustainable use of indigenous tropical flora and fauna. In recent times, enhancement approach had assisted the recovery of indigenous tree species through human interventions, such as fencing, to improve rangelands' productivity, control livestock/wild animal grazing, weed control, and fire protection. The success of enrichment planting is pivotal on planted seedling performance, which varies with complex environmental factors. Thus, the development and adoption of plans and strategies for resolving these critical issues are highly imperative.

**Keywords:** Anthropogenic activities, conservation, enhancement approach, indigenous species, unsustainable exploitation

#### 4. Keynote Address 2

### DIVERSITY AND RESILIENCY OF INDIGENOUS TROPICAL FLORA AND FAUNA AND THEIR ECOSYSTEMS: INDONESIA CASE

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#### ABSTRACT

Flora and fauna in most areas of the world have been experiencing a mass species loss, including those in the tropics. Ecosystems as the habitat of flora and fauna also have decreased and/or degraded, mostly as consequences and impact of human-dominated activities, leading to new geologic time called Anthropocene epoch. The objective of this paper is to provide a general overview on the current situation of the species diversity and ecosystem resilience in Indonesia. Although Indonesia is known to have an extremely rich biodiversity (720 species of mammals; 1,771 birds; 723 reptiles; 385 amphibians; 1,900 butterflies; 28,000 flowering plants) and the number of species of some taxa has been increasing, it does not mean that the condition of flora and fauna is getting better. Discovery of new species is mostly due to a speciation in remote areas, implying that the list of the endangered species is getting longer and prone to extinction. Species relaxation (the gradual loss of species through time after a habitat is fragmented) has been reported in many areas for many taxa, including big mammals (i.e. Sumatran elephant, Sumatran rhino, Sumatran tiger), birds (mainly song-birds, hornbills, raptors), herpetofauna, fishes, insects, as well as slow growing trees (ebony, ironwood, some Dipterocarp). The resiliency (the capacity to respond to a disturbance by resisting damage and recovering quickly) of some ecosystems (e.g. mangroves, peatland, heath forest, coral reef) has been known to be low, although long-term study is still lacking.

The loss of a certain ecosystem can lead to a chain-reaction effect to species diversity. Conservation efforts are urgently needed, especially for the critically endangered species and ecosystem that show a low resilience. Otherwise, Indonesia will lose many species in the future, while some low resilience ecosystem may fail to recover.

**Keywords:** Anthropocene, biodiversity, community relaxation, extinction, species relaxation

## 5. Keynote Address 3

# APPROACHES, TECHNOLOGIES AND INNOVATIONS IN CONSERVATION, ENHANCEMENT AND SUSTAINABLE USE OF INDIGENOUS TROPICAL FLORA AND FAUNA

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### ABSTRACT

The use of molecular tools for identification and differentiation of indigenous tropical species offers a greater promise in the field of conservation as well as enhancement and sustainable use of these flora and fauna. Information on genetic diversity is usually the first step to ascertain genetically subtypes as well as the presence of deteriorated populations, so that better management and conservation plans can be established. Molecular markers are also versatile tools for the identification of populations with genetic crisis, by comparing genetic diversities which in turn helps to resolve taxonomic uncertainties and to establish management units within species. The genetic marker analysis also provides sensitive and useful tools for traceability and prevention of illegal logging, for instance. Additionally, marker associated traits as well as the use of new –omics tools could be implemented to enhance the propagation of selected flora or fauna for sustainable use.

## 6. Panel Discussions

### 6.1. Policies and Other Legal Frameworks in Conservation and Sustainable Use of Indigenous Tropical Flora and Fauna

# POLICIES AND OTHER LEGAL FRAMEWORKS IN CONSERVATION AND SUSTAINABLE USE OF INDIGENOUS TROPICAL FLORA AND FAUNA

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## ABSTRACT

One of underlying causes of the degradation of ecosystems and habitats which lead to biodiversity loss is weak law enforcement which is reflected on the overall legal framework of a country. There are relevant regulations including Acts, Government Regulation, and Presidential Decrees related to biodiversity and its conservation, but the implementation has not been in line with each other in several aspects. The reduction of biodiversity in terms of number of species and genetic quality has been detected in several areas in Indonesia although more surveys and exploration are needed to regularly monitor and widen the scope of areas. Status and distribution of fauna, flora and microorganism in Indonesia as compared to world's diversity as well as the utilization of bioresources including breeding experiments and other research on indigenous flora and fauna are crucial for future conservation strategy and implementation. The role of LIPI as a National Scientific Authority on biodiversity in Indonesia is important in updating the status of biodiversity, determining export's quota and providing recommendation related to research and commercial trade to meet CITES's mechanisms and regulation. Halting and reducing the number of cases related to international trade, such as illegal trade need collaborative efforts. One of the efforts is the improvement of database and information exchange on biodiversity such as the one developed by LIPI i.e. Indonesian Biodiversity Information Facility (InaBIF). Multisectoral and multidisciplinary approach and strengthening collaboration as well as increasing synergy among key players are a must for the conservation and sustainable use of tropical bioresources.

**Keywords:** Biodiversity, CITES, conservation, InaBIF, legal framework, scientific authority

## 6.2. The Future Directions in Conservation, Enhancement and Sustainable Use of Indigenous Tropical Flora and Fauna

### CONSERVATION AND SUSTAINABLE USE OF GENETIC RESOURCES FOR AGRICULTURE

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#### ABSTRACT

The richness of Indonesia's agroecosystem leads to the diversity of the adapted Agricultural Genetic Resources (AGR). Management of AGR faces several problems. There is an increasing demand for genetic resources to feed the growing population, but the collection is threatened by several factors. An integrated genetic resources management is needed to ensure conservation and sustainable utilization of the genetic resources. An efficient management should consider an effective collaboration among the genetic holders and the users. The National Commission of Genetic Resources (NCGR) assists the conservation and utilization of AGR through in situ and ex situ approaches as well as legal policy for the property right protection. In situ conservation is being implemented throughout the 34 provinces in Indonesia by the Local Commission of Genetic Resources and Assessment Institute for Agricultural Technology (AIAT) with the assistance of Indonesia Agency for Agricultural Research and Development (IAARD). On the other hand, ex situ conservation is being conducted in gene banks in research institutes under IAARD. Ex situ management of genetic resources consists of a series of activities to ensure the genetic integrity and quality of the collections. There are several principles to be adopted to implement an efficient ex situ conservation. The utilization of AGR can be done directly and indirectly. The indirect utilization of AGR through crop improvement can produce improved varieties that are widely used in agricultural productions in Indonesia. The legal policies are adopted as a genetic-resources-base property right. The protections are conducted through the implementation of Geographical Indication, Farmer Right, Plant Variety Protection, and Access and Benefit Sharing.

**Keywords:** Agricultural genetic resources, conservation and utilization, ex situ conservation, in situ conservation

# **STRATEGY IN UTILIZING TIMBER OF NATURAL FOREST PRODUCTION BASED ON SPECIES: A NEW APPROACH TOWARD SUSTAINABLE USE AND CONSERVATION OF FOREST BIODIVERSITY**

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## **ABSTRACT**

In the last two decades, the licenced areas for selective logging in natural production forests, used to be known as HPH and now called as IUPHHK, have decreased significantly from 60 million ha in 1993 to only 20 million ha in 2013. Production system that is still based on volume to produce mass/commodity products, causes a high exploitation rate. It also indicates that sustainable forest management is not applied which cause the decline of forest area and quality.

Forestry businesses should shift to management system and product strategy that are in accordance with the nature of the forest. The benefits of natural forests are not only for timber, but also for Non-Timber Forest Products (NTFP) and Ecosystem Services (ES) and therefore, they should be managed in balance to gain those benefits equally. Timber from natural forests that requires decades or even hundred years to grow and mature, should be no longer harvested to make mass products/commodities such as plywood and/or sawn timber and its derivative products. Natural forest should produce material for high end and high value product which is more specific and exclusive, e.g. furniture or interior. Therefore, the timber production system should only be based on individual species rather than species grouping such as meranti groups, mixed tropical hardwood and luxury wood which occur today. The importance to have knowledge of wood characteristics and properties of individual species is a must to utilize timber for high value products.

Management of natural production forest should acknowledge all benefits of the forest i.e. timber, non-timber products and ecosystem services that should be managed in balance to gain optimum benefits. Extracting small volume timber with high value product could maintain economic viability of business and be environmentally appropriate to maintain or even enhance ecosystem services such as water supply, carbon storage and biodiversity, as well as social benefit of forest for the local community. Additionally, the effort will build better relationship with local community through assisting local community to have better management of utilizing NTFP.

For mass/commodity products, main source of timber should be from plantations. The latest research in wood science and technology will help to overcome quality and constraints of timber from plantations which are usually lower than natural forest timber. Science and technology will also help to increase forest productivity in fulfilling higher demand of wood consumption. The development of plantation forest could be incorporated with social forestry program initiated by the Ministry of Environment and Forestry (MoEF), through allocating million of hectares of state forest to be managed by local communities. It could be a potential source of timber for various purposes i.e., construction wood, fiber wood, and bioenergy. Therefore, grand design or strategic planning of social forestry program must be developed from beginning to ensure its sustainable management and benefit through link and match with demands for timber.

## CONSERVATION OF INDONESIA'S ENDEMIC SPECIES: CHALLENGES AND OPPORTUNITIES

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### ABSTRACT

Conservation of endemic species in Indonesia is being challenged by habitat loss and illegal trade of the species. The role of communities in conserving the species is critical as some of the species are used for consumption and source of livelihood. Community based approach combined with regulatory improvement at local level is tested in two habitats of Moluccan megapode *Eulipoa wallacei* (VU). The species is facing extinction as a result of overharvesting of the eggs and loss of the nesting ground from coastal area development and abrasion. Certain populations exist only in three habitats: Galela and Kao in North Halmahera and Haruku Island in Maluku. To address this threat, two community-based programs were initiated since 2016. In Haruku, local communities are engaged in the conservation of the species by incorporating the value of the species into *sasi*, a local wisdom that hinders extraction of natural resources within a certain period of time to balance the need of the communities and the availability of the natural resources. While in Galela, to maintain the wild population of the bird, egg harvesters are engaged in agreement to leave some of the eggs in their nests to hatch and return to the wild as new birds. Hatchery facilities are also built in the two sites to increase the success rate of the hatching eggs. Results indicated that there has been enhancement of the species' breeding season from typically 5 months to become 11 months in Galela, resulting in even better yield for the harvesters. In Haruku, the community facility was able to hatch 130 chicks, of which 108 survived and were released to the wild. Results from the case studies demonstrated that local participation is key for successful species conservation. The governance of natural resources that accommodates local communities is proven to be paramount in delivering local solution to sustainability. Nevertheless, regulatory support from the government is still required to ensure long term conservation outcomes. A combination of these approaches will likely be still relevant in the future.

**Keywords:** Bird, conservation, endemic



# **COMMUNITY EMPOWERMENT THROUGH SCIENCE AND TECHNOLOGY, EDUCATION, ENVIRONMENT PROTECTION AND HEALTH (CESTEEPH): A COLLABORATIVE COMMUNITY-BASED PROGRAM FOR THE CONSERVATION, RESTORATION AND SUSTAINABLE UTILIZATION OF MANGROVE ECOSYSTEMS IN GONZAGA, CAGAYAN**

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## **ABSTRACT**

With crucial and urgent need to promote biodiversity conservation, the Cagayan State University through its President Dr. Urdujah A. Tejada, and Commission on Higher Education Commissioner Dr. Lilian A. De Las Llagas, established a partnership with SEAMEO Regional Center for Tropical Biology (BIOTROP), Department of Science and Technology and Local Government Units in the Philippines. The cooperation focuses on joint implementation of research, development, and extension (RDE) activities on tropical biology with priority concerns on the conservation and sustainable use of mangrove ecosystem.

The 4-year program aims for a comprehensive ecological development of poor and climate change vulnerable communities in three coastal villages of Gonzaga, Cagayan, Philippines and building their resiliency with the protection, restoration, conservation and sustainable utilization of mangrove ecosystem through research, development and extension, bioengineering and hydrology, livelihood, ecotourism, education, health and nutrition.

The program was launched in April 20, 2018 attended by more than 400 individuals from the target community consisting of fisher folks, farmers, municipal and village officials, housewives, youth, and academe.

The program has so far implemented the following activities:

1. A benchmarking visit and basic training on mangrove conservation for the program management team was conducted in Puerto Galera Mangrove Conservation and Ecotourism Area, Puerto, Galera, Mindoro.
2. A series of consultation and participatory planning was conducted, involving members of communities, people's organizations, local government units, local and national agencies, and other stakeholders.
3. A benchmark survey titled “Socio-Economic Component, Coastal Resource Use and Community Participation of Coastal Communities/Fishing Households in Gonzaga, Cagayan” was conducted.

Other surveys conducted in the area were “Bio-Physical and Chemical Assessment of Mangrove” and “Knowledge, Attitude and Practices of Barangays Caroan, San Jose, And Tapel, Gonzaga, Cagayan on Mangrove Benefits, Conservation and Rehabilitation.” All of these studies were aimed to analyze socio-economic, demographic and environmental status of the area and identify appropriate interventions for the development of communities.

To date, a total of 50,000 mangrove propagules and other coastal trees have been planted to rehabilitate the degraded ecosystem. In some areas, bioengineering with the use of coconut nets, indigenous trees, mangrove propagules and bamboos was applied for site restoration.

On the livelihood development, several projects are on-going which include: (1) Aquasilviculture of Mudcrab (*Scylla serrata*): An Environmentally Friendly Mariculture System towards Food Security of Mangrove Communities (2) Farmers Business School on Pineapple Production, Processing and Marketing (3) Farmers' Field School on Rice and Vegetables.

The program continues to receive support and commitment from various individuals and institutions. More developmental projects and other interventions are lined up for implementation in the future which are geared towards producing ecologically restored mangrove sites and building resilient communities.



## 7. Paper Presentation Sessions

### 7.1. Session 1: Diversity and Resiliency of Indigenous Tropical Flora and Fauna and Their Ecosystem

#### **PRESERVATION OF NATIVE TREES USED FOR BOGOR LOCAL CUISINE TO SUPPORT URBAN LANDSCAPE IDENTITY**

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#### **ABSTRACT**

Local cuisine is a reflection of local knowledge in managing local natural resources including plants and animals. In Bogor area, several local cuisines utilize some part of plants especially from native tree. However, the increasing urbanization and , land conversion have decreased the amount of open space and resulted in the decrease of native tree species and even extinction of species. The aims of this research were to identify the native tree species used for Bogor local cuisine and to analyze the spatial distribution of native tree species. This study also proposed strategy for native tree preservation. The study was conducted in Bogor City area from February-May 2018. Data of native tree species used for local cuisine were obtained through direct interview and literature study. Direct observation was held to learn the native tree distribution in Bogor City. The results showed that six native trees were used for Bogor local cuisine. Of the six native trees, only four trees were found in Bogor City area. The four native trees grow in several areas such as agricultural farm, fields, yard, green corridor, office and school parks. The results of this study can be used as the basis for determining the green open space potential and advanced strategy for native tree preservation in Bogor City.

**Keywords:** Local cuisine, native tree, spatial distribution, sustainability, urban landscape identity

#### **INTRODUCTION**

Native plants are those which have evolved naturally under the local environmental conditions (Miller *et al.* 1996). In natural landscaping such plants can be utilized for sustainable landscaping because they are adapted to local environmental conditions (Kermath 2007; Phondani *et al.* 2015). Long term sustainable landscaping using native plant resources requires management to take into account not only ornamental purposes, but also ecological and environmental values (e.g. soil, water, biodiversity) as well as socio-economic issues (Muhtaman *et al.* 2000). It has long been recognized as important and valuable natural resources and provide many vital ecosystem services which directly and indirectly benefit humans, including agricultural production, cultural benefits, and support services (Yapp *et al.* 2010).

Native plant for sustainable landscaping will be promoted in a time-critical manner to ensure that current and future development plans support the country's goal of sustainable development and enhancing the quality of the environment (Phondani *et al.* 2016). Vegetation having similar structure and form of native, natural or semi-natural vegetation requires the least

resource inputs (Kristensen *et al.* 2008). Urban biodiversity conservation theory and practice generally assumes that only native plants effectively support native animal biodiversity (DEFRA 2008).

Several local cuisines utilize some parts of plants especially from native tree in Bogor area. According to UNESCO (2006), local cuisine included in an intangible heritage associated with practices, representations, expressions, knowledge and skills transmitted from generation to other generation, becomes an identity and sustainability for community. However, the potential native plants are under threat due to global warming, the impact of climate change, overgrazing, other anthropogenic pressures and developmental activities such as urbanization and construction of mega-structures (Yasseen 2011; Phondani 2016). The present study promotes the use of native plant species for sustainable landscape which have good survival rate, high adaptation potential, low maintenance requirements, little pruning demand and disease resistance, salinity tolerance, nitrogen fixation ability and multiple value uses.

The aims of this research were to identify the utilization of native tree species for Bogor cuisine and to analyze spatial distribution of native tree species. This study also proposed to make strategy for native tree's preservation.

## MATERIALS AND METHODS

The study was conducted in Bogor City area in West Java, Indonesia from February-May 2018. Study area administratively covers 6 subdistricts i.e., West Bogor, South Bogor, Middle Bogor, East Bogor, North Bogor, and Tanah Sareal subdistricts (Fig. 1). Geographically, Bogor City is located between 106°48' E and 6°26' S, with a total area of 118.50 km. Bogor is located at an altitude of 190 to 330 meter above sea level. The air is relatively cool with monthly average air temperature of 26 °C and air humidity of about 70%. The lowest average temperature in Bogor is 21.8 °C.

This study used descriptive qualitative method by providing an explanation of native tree preservation and spatial analysis. Primary data collection was performed by direct semi-structured interview and direct field observation. Spatial distribution data was collected from native tree point data using Garmin GPS. Secondary data were collected from literature study, while relevant data were obtained through government agencies and results of previous studies. Data analysis was conducted using spatial and descriptive analyses to explain native tree conservation strategies in Bogor City to support the preservation of urban landscape identity.

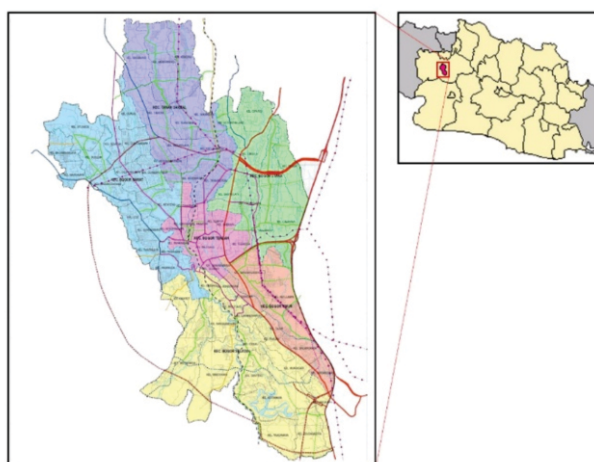


Figure 1 Research Location

## RESULTS AND DISCUSSION

### Utilization of Native Tree's Part for Bogor Local Cuisine

Based on interview with culinary expert, there are 14 types of Bogor's cuisine. But only 5 local cuisines utilize native tree part. There are 6 native trees species which usually be utilized in the 5 local cuisines (Table 1). Almost all of the local cuisines utilize the fruit part of native trees. Nutmeg tree (*Myristica fragrans*) is the most widely used plant for Bogor's cuisine. The use of nutmeg is influenced by the local knowledge of Bogor community that nutmeg is highly used for Bogor's cuisine and history during the colonization by the Dutch which sought spices in Indonesia (Deryanti *et al.* 2014).

Table 1 Utilization of native tree species for local cuisine

No	Local Cuisine	Species	Tree Part Utilization
1	Pickled fruit	<i>Baccaurea racemosa</i>	Fruit
		<i>Myristica fragrans</i>	Fruit
		<i>Mangifera caesia</i>	Fruit
2	Candied fruit	<i>Baccaurea racemosa</i>	Fruit
		<i>Myristica fragrans</i>	Fruit
		<i>Mangifera caesia</i>	Fruit
3	Nutmeg ice	<i>Myristica fragrans</i>	Fruit
4	“Rujak”	<i>Mangifera caesia</i>	Fruit
		<i>Syzygium polycephalum</i>	Fruit
		<i>Bonea macrophylla</i>	Fruit
5	“Lobi-lobi” ice	<i>Flacourtia inermis</i>	Fruit

### Spatial Distribution of Native Trees

In total, 118 native trees were recorded, consisted of 6 tree species. The highest percentage found was 60.71% for nutmeg tree (*Myristica fragrans*), while the lowest percentage was 16.7% for *Syzygium polycephalum* and *Flacourtia inermis*, respectively. These trees can be found in most areas in Bogor, except East Bogor Subdistrict (Table 2).

Native tree species are distributed unevenly in almost all subdistricts in Bogor City (Fig. 2), with the West and Central subdistricts as dominant areas. The highest percentage of tree distribution in Bogor was 53.21% (green corridor) and the lowest was 2% in city park (Fig. 3).

Table 2 Native tree species and distribution in Bogor

Species	Percentage (%)	Distribution					
		NB	WB	SB	EB	TS	MDB
<i>Bonea macrophylla</i> Griff.	12.71			√			
<i>Mangifera caesia</i> Jack.	5.93			√			
<i>Flacourtia inermis</i>	1.69	√				√	
<i>Baccaurea racemosa</i>	17.80	√	√	√			
<i>Myristica fragrans</i> Hoult.	60.17	√	√	√		√	√
<i>Syzygium polycephalum</i>	1.69			√			

Notes: NB: North Bogor, WB: West Bogor, SB: South Bogor, EB: East Bogor, TS: Tanah Sareal, MDB: Middle Bogor

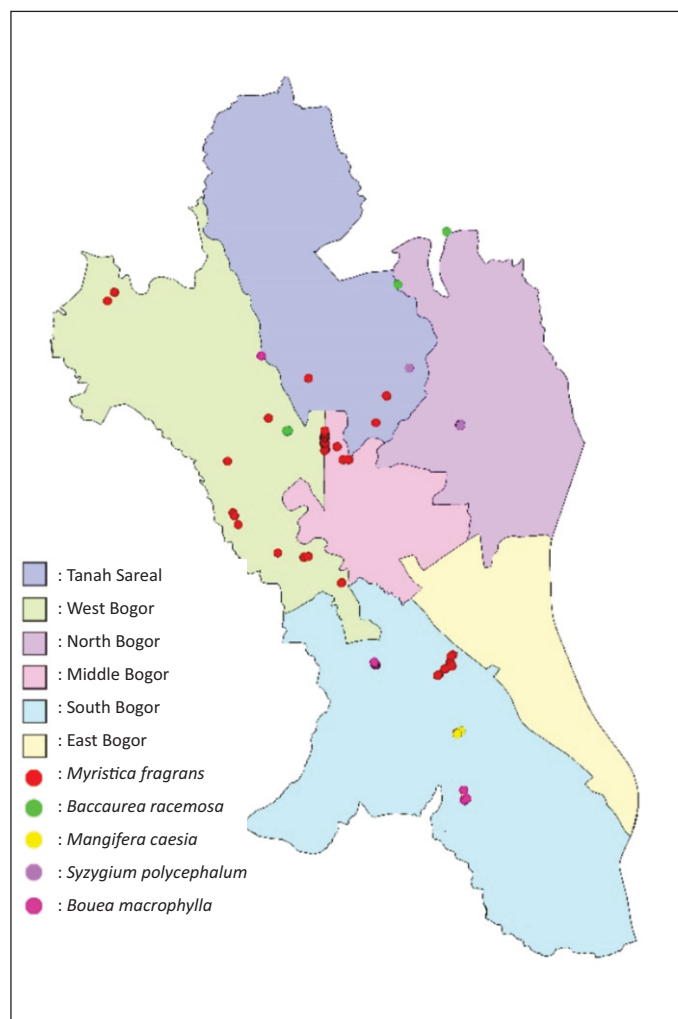


Figure 2 Spatial distribution of native trees in Bogor subdistricts

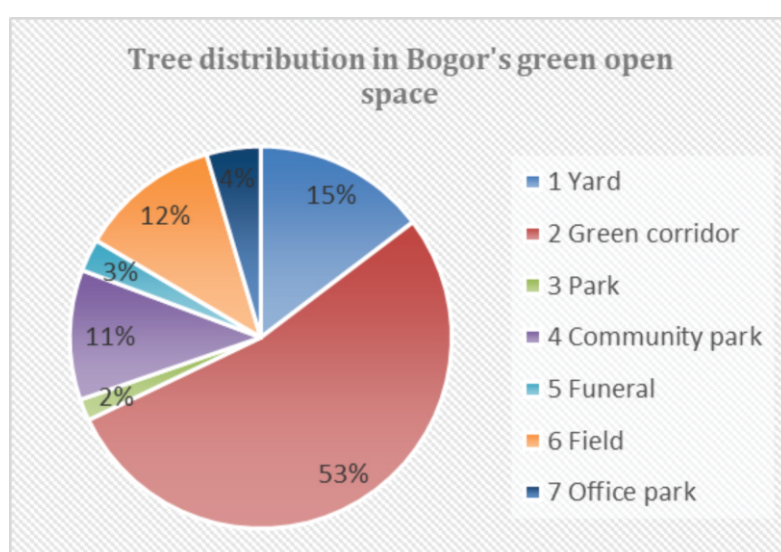


Figure 3 Percentage of tree distribution in Bogor's green open space

### Tree Preservation Priorities

Preservation of native trees in Bogor should be based on conservation status of trees. According to World Conservation Monitoring Centre (1998), four tree species i.e. *Bonea macrophylla*, *Flacourtia inermis*, *Baccaurea racemosa*, and *Syzygium polycephalum* are included in Not Evaluated category. However, on direct observation in Bogor area, the four tree species have the lowest percentage. Based on the observation result, it is important to first preserve and plant the four tree species in several green open spaces in Bogor. Nutmeg (*Myristica fragrans*) is included in the category of data deficient because information is insufficient to estimate the risk of extinction based on distribution and population status. *Mangifera caesia* is included in the least concern category, namely IUCN category given to species that have been evaluated but not included in any conservation categories.

Table 3 Preservation Priorities Based on Conservation Status

Species	NE	DD	LC	NT	VU	EN	CR	EW	EX
<i>Bonea macrophylla</i> Griff.									
<i>Mangifera caesia</i> Jack.	√		√						
<i>Flacourtia inermis</i>									
<i>Baccaurea racemosa</i>	√								
<i>Myristica fragrans</i> Houutt.		√							
<i>Syzygium polycephalum</i>	√								

Notes: Source: World Conservation Monitoring Centre (1998)

NE= Not Evaluated; DD= Data Deficient; LC= Least Concern; NT= Near Threatened; VU= Vulnerable; EN= Endangered; CR= Critically Endangered; EW= Extinct in the Wild; EX= Extinct

### Recommendation of Native Tree Preservation in Bogor City

Based on observation results, several recommendations are offered based on number of tree species and spatial distribution. Fruit of nutmeg tree (*Myristica fragrans*) is most frequently used in Bogor's cuisine, proven by its highest percentage of existence in the sampling areas (60.71%). However, nutmeg trees can be easily found. Therefore, nutmeg tree can be considered for preservation. Conservation can also be done by planting several subdistricts with tree species having the highest percentage of existence. The Government of Bogor City can also develop thematic villages with the aim of increasing the potential of local community wisdom, improving the economy, and supporting the sustainability of nutmeg tree utilization in Bogor communities. Additionally, it is necessary to develop thematic fruit villages based on native tree distribution.

Preservation strategy should also be considered based on tree existence in study area. Planting native tree in private green open spaces, such as home yards, can support local food availability as well as improve the aesthetic quality of the yard and increase ecological quality of the environment. Yard empowerment based on local wisdom, culture and ecological knowledge should elevate the yard status to functional land, which means the yard becomes productive for fulfilling subsistence food in economical scale (Arifin 2013). Utilization of the yard is very strategic in the context of conserving agricultural biodiversity. Nassauer (1993) has found that yards incorporating native plants can be attractive; to homeowners as oppose to conventional yards that do not include native plants.

Preservation of native tree is closely related with landscape sustainability. Landscape sustainability refers to the local or regional scale. Turner *et al.* (2013) defined landscape sustainability as the capacity of socio-ecological systems to provide a desired set of landscape sustainability for current and future generations in the face of human land use and a fluctuating



environment. In some analyses, the ecological branch of sustainability is also regarded as ecological integrity (Miller 2005; Kandziora *et al.* 2013). Conservation can also be done by planting potential subdistricts with native tree having the highest percentage of existence, such as nutmeg.

## CONCLUSION

A total of 118 native trees were found in 5 subdistricts in Bogor City. There were 6 native tree species found. The highest percentage of tree distribution in Bogor was 53.21% in green corridor and the lowest was 2% in city park. Strategy for preservation must be considered based on tree growth requirement and potential open space for preservation area.

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## SOIL PROPERTIES AND TREE COMPOSITION IN 27-YEAR-OLD *Acacia mangium* Willd. PLANTATION ON ABANDONED MINING AREA AT PHANGNGA FORESTRY RESEARCH STATION

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### ABSTRACT

In this research, we studied the important role of *Acacia mangium* plantation in rehabilitating an ex-mining area. Soil properties and tree composition were investigated in a 27-year-old *Acacia mangium* plantation, grown on sandy soil (S27) and on clayey soil (C27). The study was also conducted in a mixed plantation (MP) grown on clayey soil. Soil properties and tree composition in these three plantations were then compared with soil properties and tree composition in an abandoned mining area (AB), a secondary forest (SF), and a primary forest (PF) at the Phangnga Forestry Research Station. Three permanent plots of 40x40 m size were established and soil samples were randomly collected from depths of 0-10, 10-20, 20-30 and 30-50 cm, in S27, C27, MP, AB, SF and PF. The physical and chemical properties of soil were analyzed and the importance value index (IVI), Shannon-Wiener index, and similarity index were evaluated. The results showed that the bulk density in S27, C27, and MP was lower than that in AB and was similar to that in SF and PF, especially on the top soil. Total nitrogen, available phosphorus, organic matter, exchangeable potassium, and magnesium in S27, C27, and MP were higher than those in AB, which were lower than SF and PF. These results indicated that soil development in S27, C27, and MP was slower than that in SF. A lower bulk density and higher soil nutrients were positively affected by *A. mangium* plantation. The Shannon-Wiener index obtained for S27, C27, and MP (1.43, 2.51 and 2.77, respectively) was found to be lower than that for SF (3.89). Similarity index of tree species found in S27, C27, MP, and PF was low, ranging from 5.83-8.00, which indicated that the development of forest community was slow compared to SF. Enrichment planting with poorly dispersed shade tolerant trees could be taken up to increase forest diversity and improving forest structure in this area and other degraded lands.

**Keywords:** *Acacia mangium*, mining restoration, soil properties, tree composition

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## GROWTH PERFORMANCE OF DIPTEROCARPACEAE SPECIES PLANTED ON ABANDONED MINING AREA IN THE PHANGNGA FORESTRY RESEARCH STATION

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### ABSTRACT

Dipterocarp trees have large canopy and hold a significant importance in the ecology and economics of tropical forests. Six Dipterocarp species, located in an abandoned mining area, namely *Dipterocarpus alatus* Roxb. (DA), *D. gracilis* Bl. (DG), *Hopea odorata* Roxb. (HO), *Shorea gratissima* Dyer (SG), *S. roxburghii* G. Don (SR), and *Parashorea stellata* Kurz (PS), were investigated. These species were planted in a 6-year-old *Acacia mangium* and in open plots, located at the Phangnga Forestry Research Station. Survival rate, diameter at root collar ( $D_0$ ), and total height (H) of the Dipterocarp seedlings were calculated and measured. Soil samples in the open and *A. mangium* plots were collected and the physical and chemical properties were analyzed. The survival rate,  $D_0$ , H, and relative growth rate (RGR) of  $D_0$  and H were analyzed using One Way Analysis of Variance (ANOVA). The results indicated that survival rate of all Dipterocarp tree species planted in the *A. mangium* plot was higher than that in the open plot. The survival rate of 1 to 3-year-old DA, HO, SG, SR, and PS was relatively high in the *A. mangium* plot and exceeded 75%, except that of DG.  $D_0$  of SR, DA, HO, and PS was high in the *A. mangium* plot. However, the  $D_0$  and H of SR, DA, and HO in the open plot were high and similar to that in the *A. mangium* plot.  $RGR_{D_0}$  and  $RGR_H$  of SR, DA, and HO were high in both *A. mangium* and open plots. The results demonstrated that the Dipterocarp seedlings should be planted with *A. mangium* for improving the survival rate and growth of Dipterocarp trees to rehabilitate ex-mining area. SR, DA, and HO can be planted in open areas with poor soil conditions and extreme environment conditions. Human interventions such as thinning should also be practiced for improving the growth of Dipterocarp seedlings.

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## PHENETIC AND PHYLOGENETIC STUDIES IN *Usnea* spp. BASED ON MORPHOLOGICAL AND MOLECULAR CHARACTERS

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### ABSTRACT

Lichens of the genus *Usnea* are used as a traditional herbal remedy. This genus has thallus which shape is very similar among species, so it is very difficult to distinguish among species in this genus. Based on morphological characters, identification of species in Indonesia is still limited. Morphological characters can only be used to identify up to family and genus levels. Molecular characters based on Internal Transcribed Spacer (ITS) rDNA sequences which have conserved region (5.8S) and varied region (ITS1 and ITS2) are needed to strengthen and support the identification process and phylogenetic analysis. The objective of this research was to identify and make phylogenetic analysis of *Usnea* species from the forest of Gunung Lawu (LW), Center Java and Turgo (T), Yogyakarta. Identification had been conducted based on morphological, anatomical, microchemistry, and microcrystals characters. ITS rDNA sequence was analyzed by Bioedit software. Sequence cutting based on chromatogram was analyzed using DNA Baser, BLAST to determine the level of homology. Sequences alignment samples were analyzed using Clustal-X. Nucleotide similarity with Phytid and clustering (phylogeny tree reconstruction) were constructed with Maximum Likelihood (ML). Model of evolution Kimura-2 parameter was developed with the bootstrap 1000 on MEGA-5.05. Based on morphological characters there were 7 species found from 16 of the morphotype i.e. *U. pectinata* (LW3), *U. rubrotincta* (LW4), *U. himalayana* (LW1, LW2, LW5, LW11), *U. fragilesceus* (LW6, LW9), *U. nidifica* (LW8, LW10), *U. baileyi* (LW7, T1, T3), and *U. bismolliuscula* (T2, T4, T5). Based on ITS rDNA only 6 species were identified because *U. pectinata* and *U. baileyi* were identified as the same species, i.e. *U. baileyi*. Dendrogram based on morphological characters showed that *U. pectinata* was closely related to *U. bismolliuscula*; *U. rubrotincta* was closely related to *U. fragilesceus*; *U. himalayana* was closely related to *U. nidifica*. It was also found that *U. baileyi* was distantly related to *Usnea*. Topology of the phylogenetic based on ITS rDNA sequences showed that the clade of subgenus *Usnea*, *U. himalayana* was closely related to *U. nidifica*; *U. bismolliuscula* was more closely related to *U. rubrotincta* than to *U. fragilesceus*, and the clade of subgenus *Eumitria*, *U. pectinata* was closely related to *U. baileyi*.

**Keywords:** ITS rDNA, lichen, morphological, phylogenetics, *Usnea*

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## DIVERSITY AND VERTICAL DISTRIBUTION OF VASCULAR EPIPHYTES IN MANGROVE ISLAND OF PENINSULAR MALAYSIA

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### ABSTRACT

Many studies have been conducted to explain the diversity and abundance of epiphytic plants in major ecosystems worldwide. However, study on the abundance of epiphytic plants in mangrove is rather scanty. The aim of this research was to study the diversity and distribution of vascular epiphytes in a mangrove forest in Peninsular Malaysia. The sampling took place in a 0.1 hectare area of Pulau Telaga Tujuh, a mangrove island in Terengganu, Malaysia. Trees with vascular epiphyte were divided into three strata i.e. basal, trunk and canopy. Vascular epiphytes were identified and the number of individuals were recorded from each stratum. In total, 8 species of vascular epiphytes from 6 genera and 4 families were recorded. Pulau Telaga Tujuh mangrove forest showed a relatively low diversity of vascular epiphytes ( $H'=1.43$ ). The dominance of *Hydnopytum formicarum* contributes to a significant effect on the diversity of vascular epiphyte in this forest. Meanwhile, the highest abundance of epiphytes was recorded on trunk region of the host trees, which can be explained by the adaptation for water level in mangrove ecosystem. In conclusion, Pulau Telaga Tujuh had a high density of vascular epiphytes but had less diversity compared to some ecosystems.

**Keywords:** Epiphyte ecology, mangroves, Setiu Wetlands, spatial distribution

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## LIMITED SEED DISPERSAL MAY SHAPE GENETIC STRUCTURE OF *Hydnophytum formicarum* JACK. POPULATIONS IN MANGROVE ECOSYSTEM

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### ABSTRACT

*Hydnophytum formicarum* is an epiphytic plant that is highly distributed throughout Southeast Asia from Myanmar to Fiji. The distribution of this plant is declining due to forest fragmentation as well as habitat changes. Using Random Amplified Polymorphic DNA (RAPD) marker, we assessed genetic variation and genetic differentiation among three populations to identify the potential conservation management of this species in mangrove ecosystem in Malaysia. A total of 10 highly reproducible primers were used in population analysis, which have resulted in a total of 221 discernible fragments. The genetic variation of this species is high, where 98% of the fragments were found to be polymorphic. AMOVA showed significant genetic differentiation among populations ( $\Phi_{PT} = 0.554$ ,  $p \leq 0.001$ ) with pairwise genetic distances between the populations ranged from 0.495-0.589. PCoA clustering analysis separated the populations according to their geographical locations. The high genetic variation within population and high genetic differentiation between populations and clear separation in cluster analysis might indicate a restricted seed dispersal of this species.

**Keywords:** AMOVA, epiphyte, myrmecophyte, RAPD, Setiu Wetlands

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## QUANTITATIVE STRUCTURE OF ENDANGERED SPECIES *Cotylelobium melanoxylon* IN BONA LUMBAN FOREST, CENTRAL TAPANULI, NORTH SUMATRA

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### ABSTRACT

At present time, *Cotylelobium melanoxylon* is hard to find in the wild because its bark has been intensively harvested for making traditional alcoholic drink. The bark is sold by kilogram in traditional market in North Sumatra and Riau, which activity threatens the existence of this species in their habitat. A study was conducted to determine the quantitative structure of the species from seedling to tree stages at Bona Lumbang Forest, Central Tapanuli. Purposive sampling technique was implemented by making line transect to forest areas determined by local people as having naturally grown *C. melanoxylon*. There were 4 lines transects implemented. Each line transect was consisted of five plots. For each growth stage there were 20 plots. Thus, total number of plots for all stages were 80 plots. Results shown for the tree, pole, sapling and seedlings stages indicated that Important Value Index (IVI) for *C. melanoxylon* was 66.33, 17.65, 11.82, and 12.90; Diversity Index (H) was 2.9, 1.90, 2.88, 2.53 and 12.90; Index of Evenness (E) was 0.844, 0.534, 0.85, and 0.935; and Index of Richness (R) was 5.71, 7.13, 5.37, and 3.67; respectively. These results showed that *C. melanoxylon* still grows naturally at Bona Lumbang Forest comprising of all stages from tree to seedling stages. However, based on different values of IVI for tree stage compared to its younger stages, there might be a problem in its natural regeneration. Regeneration status of *C. melanoxylon* was classified as fair because the existing mature trees remaining are no longer productive and healthy due to excessive harvesting for its bark.

**Keywords:** Endangered species, quantitative, rare, structure

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## MACROFUNGAL SPECIES LISTING IN CALABARZON REGION PROTECTED AREAS FOR FOREST SUSTAINABILITY AND CONSERVATION

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### ABSTRACT

Collection, identification and listing of macrofungal species were conducted in five protected areas in CALABARZON Region. Species listing on macrofungi was done to account the existing macroscopic fungal species in Mt. Palaypalay-Mataas na Gulod Protected Landscape, Mt. Banahaw-San Cristobal Protected Landscape, Mt. Maculot-Taal Volcano Protected Landscape, Hinulugang Taktak Protected Landscape and Quezon Protected Landscape for forest conservation and management. The documentation and collections were done during wet and dry seasons. Transect, quadrat and opportunistic sampling methods were used. The study resulted in the identification of 254 species belonging to 97 genera, 44 families, and 5 classes. Further studies for these landscapes are anticipated because they are currently experiencing some degrees of anthropogenic disturbances such as minor forest products gathering, slash-and-burn farming, and quarrying. Fungal diversity research efforts need to be encouraged to evaluate the effects of these human disruptions on the ecology of these landscapes.

**Keywords:** CALABARZON, conservation, macrofungal species, protected landscape

### INTRODUCTION

Fungi are a significant component of the terrestrial ecosystem which aid nutrient cycling (Angelini *et al.* 2015; Klemm *et al.* 2005; Cooke & Rayner 1984). They are eukaryotic that have a cell wall which is mainly composed of polysaccharide chitin and other substances such as lipids and proteins (Chang & Miles 1992). Macroscopic fungi or macrofungi are described as having spore-bearing structures visible to the naked eye which can be mushrooms, brackets, puffballs, false-truffles, cup fungi, etc. (Mueller *et al.* 2007). Aside from ecological importance, macrofungal species indeed have economical and medicinal significance, for they are good sources of food and nutraceutical products (Reyes *et al.* 2009).

The CALABARZON Region in the Philippines is composed of the provinces of Cavite Laguna, Rizal, and Quezon. It has a total land area of 1,636,303 hectares and 74,378 ha of it are classified as Protected Areas (PA). The PA is mandated to maintain the existing ecosystem by preserving plants, animals and other organisms for conservation and sustainable management (CALABARZON Regional Physical Framework 2008).

These Protected Areas are known for its high diversity of flora and fauna (Arsenio *et al.* 2011; Lagat 2009; Salibay & Luyon 2008; Alejandro & Madulid 1999) and minimal studies on the macrofungal species (Tadiosa 2013), and therefore, researchers led in the documentation and collection of macrofungi, and they came up with species listing for forest conservation and management.



## MATERIALS AND METHODS

Field collections were performed for each site namely, Cavite at Mts. Palaypalay–Mataas na Gulod Protected Landscape (MPMGPL); Laguna, Mt. Banahaw–San Cristobal Protected Landscape (MBSCPL); Batangas, Mt. Maculot–Taal Volcano Protected Landscape (MMTVPL); Rizal, Hinulugang Taktak Protected Landscape in Rizal (HTPL); and Quezon Protected Landscape (QPL) using five 1,000 meters transect lines (TL). Transect lines served as the guide during the collection, and quadrat and opportunistic sampling were employed in searching for the sample taxa in the field. Macrofungi observed along and both sides of the TL were also photographed in their habitat.

The five sites were visited twice on wet or rainy and in the dry seasons. Fungal host, color, shape, size of the fruiting bodies and Global Positioning System (GPS) data were recorded. Macroscopic fungi inhabiting the soil and ground litter were dug using trowel while the bolo and knife were used for those attached to tree branches or barks (Lodge *et al.* 2004).

Morphological characterization of the fruiting bodies was done. On the other hand, spore prints were taken from fleshy samples and tissue sectioning for woody samples. Spore analysis covers spore color, shape, and size (Cui & Zhao 2012). Proper identification and classification were initially made using the published works of De Leon *et al.* (2015), dela Cruz (2013), Kuo, (2011), Tadosa, (2011) and Quimio (2001). The verification of the initial identification was done at the Philippine National Herbarium of the Botany Division, National Museum of Natural History.

## RESULTS AND DISCUSSION

The documentation of macrofungal species resulted in the identification of 254 species, represented by 22 Ascomycota and 232 Basidiomycota, under five classes, 44 families, and 97 genera. Moreover, the collections of samples at all sites were summarized, the MPMGPL has the most number of individual samples documented with 95 species under 69 genera and 38 families, while MMTVPL has 92 species, belonging to 57 genera and 33 families. On the other hand, MBSCPL having the least land coverage (3.20 ha) has 62 species, 52 genera, and 30 families, which is the least number of species documented in the area. The collection resulted in the identification of 16 species under 12 genera and 11 families. Lastly, the macrofungi documented in QPL has a total of 69 species, under 39 genera and 23 families.

The dominance of Family Polyporaceae is viable because the tree species in the mountain are diverse. Thus, more wood-rotting macrofungi can be seen in the forested area compared to other habitats. A study in the tree and macrofungal species revealed that the presence of tree, is positively correlated with fungal diversity (Lodge *et al.* 2004). Thus, most of the macrofungi were documented on rotten trunks or branches of dead trees while others on soil and forest leaf litters.

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## THE IMPORTANCE OF LANDSCAPE CONNECTIVITY FOR BIRD CONSERVATION IN URBAN AREA

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### ABSTRACT

Birds are a common form of wildlife found in urban areas that use green spaces as their habitats. In urbanized areas, the green spaces tend to be small and scattered, resulting from land development. It is quite obvious that the probability of habitat isolation is increased. Using the Probability of Connectivity (PC) Index and Sørensen Similarity Index, this study analyzed the connectivity of bird habitat patches in Bogor City and its surroundings. The results showed that there were four core habitat patches, seven stepping stone patches, and three isolated habitat patches in Bogor City and its surroundings. All of core habitat patches had low bird community similarity with most of other habitat patches. Conversely, all of stepping stones and isolated habitat patches had high bird community similarity with most of other habitat patches. From these results, it can be concluded that stepping stones had important roles for creating and maintaining the connectivity for bird habitat in urban areas.

**Keywords:** Landscape management, species richness, urban landscape, urban wildlife

# COMMUNITY STRUCTURE OF AQUATIC INSECTS IN FOUR FRESHWATER LAKES IN BOGOR, WEST JAVA

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## ABSTRACT

Aquatic insects play an important role in aquatic ecosystems. However, not much information available on aquatic insects inhabiting lakes in Indonesia. A study was conducted to determine and compare aquatic insect diversity in four freshwater lakes in Bogor. Nine sites were established at each lake. Distance between sites was set at 100 m. Insect samples were collected from each site in the littoral zone using *D-net*. Sampling was carried out in March, April, and May 2017. A total of 6,868 individuals representing 82 species, 28 families, and 7 orders were recorded. The highest number of insects collected were from Situ Babakan (2,396 individuals) and the least number was from Situ Gede (526 individuals). The Shannon-Wiener diversity index was 3.11, 2.93, 2.83 and 1.51, respectively for Situ Gede, Situ Burung, Situ Tonjong, and Situ Babakan. The most dominant species found, especially in Situ Babakan, were *Microneecta ludibunda* of water bug and *Chironomus* sp. of midges. Ordination analysis using NMDS showed two aquatic insects assemblages based on species composition, that separated Situ Gede from three other lakes. Furthermore, ANOSIM revealed that aquatic insect species composition differed significantly among the lakes ( $R = 0.448; p < 0.001$ ).

**Keywords:** Aquatic insects, biodiversity, lakes

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## 7.2. Session 2: Approaches, Technologies and Innovations in Conservation, Enhancement and Sustainable Use of Indigenous Tropical Flora and Fauna

### **BALI STARLING (*Leucopsar rothschildi*) NATURAL HABITAT IN BALI BARAT NATIONAL PARK INDONESIA**

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#### **ABSTRACT**

Tropical savannas and dry forests in Indonesia are important types of ecosystems which provide habitat to support various endemic wildlife. Several of these endemic species are now seriously threatened and accordingly have high conservation status according to IUCN, including the Bali starling (*Leucopsar rothschildi*) which is mostly now restricted to Bali Barat National Park. Given the high extinction risk facing such species, conservation programs are likely to require multidisciplinary approaches that address both the biological attributes of the species itself, as well as their habitat requirements. Regrettably, for many species, their habitat ecology remains inadequately understood. The objectives of this paper are to: 1) characterize the habitat of the Bali starling in terms of structure and floristic composition; and 2) document evidence of vegetation cover changes in Bali Barat National Park. Analysis of remote sensing imagery as well as field sampling for vegetation attributes was conducted to address these objectives. Normalized Difference Vegetation Index (NDVI) was calculated from Landsat imageries using red and near infrared bands. Tree cover percentage data was downloaded from Vegetation Continuous Fields (VCF) product from University of Maryland's website. Results showed that forest and savanna are the dominant land cover types in Bali Barat National Park, but their distribution is somewhat dynamic with changes in vegetation cover and greenness found across the years, in which increasing cover of woody plants is the general trend. In Bali Barat National Park, the Bali starling is mostly found at or near distinct vegetation boundaries, such as the border between savanna-forest; savanna-cropland; savanna-shrubland; settlement-cropland; and forest-shrubland. Although Cekik area had plant species that has been known to be able to provide shelter and food for Bali Starling (so was Brumbun), the bird has not been observed to be present in the area since the 1990s. These results further confirm the importance of examining habitat patterns of endemic bird within a landscape that are influenced by multiple factors that interact in space and time. Addressing data shortage in habitat patterns within endemic species distribution is important for conservation managers in developing conservation management strategies. Evaluating the remaining habitat of the species is important for conservation of Bali starling and useful for the reintroduction and release program to their natural habitat.

**Keywords:** Bali starling, habitat suitability, savanna

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## SITE INDEX OF SIAMESE ROSEWOOD (*Dalbergia cochinchinensis* Pierre) IN PLANTATIONS OF THAILAND

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### ABSTRACT

Decreased productivity of Siamese Rosewood (*Dalbergia cochinchinensis* Pierre) is a problem. Currently, the site index is a used measure to predict forest productivity before promoting the appropriate site for Siamese rosewood plantation. The objective of this research was to predict the site index for Siamese rosewood in Thailand. The 78 temporary plots from 26 sites located in 16 provinces in Thailand were selected for constructing site index on reference age at 30 years. The results showed that the mean age of Siamese Rosewood was 28 (range 13-55) years, the mean of DBH was 20.03 (range 7.99-33.80) cm and the mean of dominant height was 20.79 (range 7.32-24.82) m. Site index equation was estimated as:  $SI = e(\ln H_{do} - 12.476(A^{-1} - A_b^{-1}))$   $R^2=0.406$ ;  $p<0.0001$  and shown for 14, 18, 22, 26 and 30 m on site class I, II, III, IV and V, respectively. In the natural forest, Siamese rosewood was distributed in dry evergreen forest and mixed deciduous forest. In this case, the study showed several sites of the in situ conservation area. The ex situ conservation area was represented by good and very good sites. Thus, both areas can be planted to promote productivity of Siamese Rosewood.

**Keywords:** Siamese Rosewood, Site Index

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## DEVELOPMENT OF WOODCERAMICS FROM TROPICAL FLORA

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### ABSTRACT

Woodceramics are porous carbon or hybrid materials, derived from woody material impregnated with a thermosetting resin and carbonized in vacuum at high temperature. During the carbonization of the raw materials, the woody material changes into soft and amorphous carbon, while the impregnated resin transforms into hard glassy carbon. Woodceramics find a variety of uses in many appliances such as heaters, gas filters, absorbents, humidity, and temperature sensors. The aims of this study were to determine the suitable conditions for producing woodceramics from various materials and recommending the potential applications. The Specific Surface Area (SSA), pore size, pore volume, pore diameter, and adsorption isotherm of woodceramics made from Bamboo, Pine, Eucalyptus, Rubberwood, and Oil palm shell particleboards were obtained by the Brunauer-Emmett-Teller (BET) method. The average pore size was classified as being mesoporous (2-50 nm). The SSA and adsorption isotherms of woodceramics made from Eucalyptus can be used as an activated charcoal. From the study, the volume electrical resistivity of woodceramics depends on the maximum carbonization temperature. The woodceramics obtained during this study have a volume electrical resistivity close to that of a semiconductor. The electromagnetic shielding effectiveness of woodceramics, carbonized at maximum temperature of 800 °C and 1,000 °C was approximately 20-60 dB within a frequency range of 800-2,200 MHz. The electromagnetic shielding effectiveness of woodceramics was higher than that woven from boron carbon fiber, carbon boron, and stainless steel fiber filled thermoplastics. Moreover, the woodceramics production was lower than the commercial materials. This study also found that the volume electrical resistivity of woodceramics depended on the maximum carbonization temperature, which decreased with increasing maximum carbonization temperature.

**Keywords:** Adsorption isotherms, electromagnetic shielding, specific surface area, volume electrical resistivity, woodceramics

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## SITE INDICATOR SPECIES FOR PREDICTING THE PRODUCTIVITY OF TEAK PLANTATIONS IN PHRAE PROVINCE, THAILAND

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### ABSTRACT

Site quality is important for tree planting. In terms of forest plantation, site quality assessment may serve a range of management purposes for optimizing the estimation of the productivity. The objective of this study was to evaluate the site quality for teak plantation using plant indicator species. The study sites were located in 3 teak plantations in Northern Thailand belonging to the Forest Industry Organization (FIO). Twenty-four sample plots were chosen to cover all growth classes within the ages of 6-39 years. The site index of teak was established using anamorphic technique relating dominant height and age at a base age of 30 years; divided into 3 site index classes as 24 (good), 21 (moderate), and 18 (poor), site quality types, respectively. The plant indicator species was classified using the Indicator Species Analysis (ISA) and Two Way Indicator Species Analysis (TWINSPAN).

The understory was classified into 110 species and 38 families. The results of ISA indicated that the significant indicator species under the good site class were *Streblus ilicifolius*, *Lagerstroemia floribunda*, and *Dalbergia cana*; while *Mimosa pudica* fell under the moderate site class, and *Dalbergia nigrescens* under the poor site class. The results from TWINSPAN indicated that the understory community could be grouped according to the area characteristics. In the lowlands, Mae Sa-Roy (MS) and Wang Chin (WC) plantations were found. These two understory species are similar to each other. Meanwhile, Khun Mae Kham Mee (KM) plantation was found in the highlands. Suitable classes to be used as plant indicators could not be determined for the low and moderate site classes. On the other hand, species indicators for the good site class in KM and WC were *Dalbergia cultrate* and *Croton stellatopilosus*, respectively. To our knowledge, this is the first study reporting specific indicators of teak suitable sites in Thailand. To prove this result, the study should be conducted in other nearby areas, especially in the natural forest with teak. However, indicators resulted from this study can be a guideline for farmers who are interested in planting teak.

**Keywords:** Productivity, site indicator species, site quality, teak plantation, Thailand

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## GENETIC VARIANCE OF TEAK MISTLETOE (*Dendrophthoe pentandra* (L.) MIQ. INFERRED BY RAPD MARKER

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### ABSTRACT

Padangan teak clonal seed orchard (CSO), in East Jawa Province, Indonesia, has management purposes including controlling mistletoe to keep up the growth of teak trees which produce quality seeds. The aim of this study was to analyze the level of teak mistletoe (*Dendrophthoe pentandra*) genetic variance using RAPD marker. Leaf samples were taken by sampling from each tree of five teak hosts. The samples were collected from three parts of the crown (upper, middle, below). The sampled teak trees were located in sub OMP units (Observation Measure Plots), which were randomly selected within every OMP unit. There were four OMP units within OSP (Observation Sample Plots) units (n=3, 50 x 50 meters in size) having several different infestation levels (light, moderate, heavy), referring to modified EForTS/CRC990. Results of genetic variance study showed that genetic variance of AT, AB, AA upper crown types (He 0.1806-0.2551) was greater than that of T middle crown type (He 0.2271) and BW below crown type (He 0.1135). Also, the largest genetic distance occurred between AB and BW types (0.310), while the smallest genetic distance occurred between AA and AT types (0.0375). AB and BW types were originated from different parts of crown, i.e. AB was from upper crown and BW was from below crown. AA and AT types were originated from the same upper crown part. For Perhutani (State owned forestry enterprise), practical implication of this study is that mistletoe must be wholly eradicated by completely pruning mistletoe from the crown part.

**Keywords:** *Dendrophthoe pentandra*, genetic variance, mistletoe, RAPD, teak

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## EFFECTS OF FIRE ON STRUCTURES AND COMPOSITIONS OF VEGETATION COMMUNITY IN PINE FORESTS, NAM NAO NATIONAL PARK

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### ABSTRACT

Anthropogenic forest fires have been widely reported in many forests of Thailand, including pine forests. Although pine forests are fire dependent, too frequent burning could negatively affect vegetation community and nutrient cycle leading to ecosystem degradation. This study aimed to examine effects of forest fire to structures and compositions of vegetation community in a degraded pine forest (PF) and a mixed pine-oak forest (O-PF) of Phu Kum Khao, Nam Nao National Park, Phetchabun Province, Thailand. Structures and compositions of the vegetation community, and effect of fire were studied using three 50x50 m plots in each of the forest type. The results demonstrated that fire significantly caused higher tree mortality rate in the degraded pine forest than in the mixed pine-oak forest. Additionally, it was found that the seedling ratio of vegetation in the mixed pine-oak forest was more than that in the degraded pine forest. Furthermore, there were no significant effects of burning to sapling and seedling structures in terms of density, diameter and height. This insignificant difference could be due to high variation of fire behavior in each plot. The study also found that sapling and seedling diameters were slightly different. As effects of forest fire could be a potential parameter causing forest degradation in the study area, it is encouraged that further studies on effects of fire frequency on vegetation structures and compositions should be conducted in order to provide sufficient measure for efficient forest fire management to prevent further ecosystem degradation and succession of savanna ecosystem.

**Keywords:** Burning, degraded pine forest, Nam Nao National Park, pine-mixed oak forest

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## ASSESSMENT OF HOUSEHOLD ORGANIC SOLID WASTE DECOMPOSITION USING BLACK SOLDIER FLY (BSF) LARVAE

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### ABSTRACT

Solid waste is normally concerned as something with no economic value. This is due to the low benefit that can be gained from organic solid waste management where organic fraction is the majority of solid waste. In response to this condition, it is necessary to make an effort to increase the economic value of the domestic waste. One of the solutions of this situation is the utilization of Black Soldier Fly (BSF) larvae as the decomposer of the organic waste. This study aimed to analyse the use of BSF larvae as decomposer for household organic waste. The experiment used household scale composting bin constructed from red brick that was covered by mortar plaster and was finished with cement layer. Waste decomposition process using Black Soldier Fly can reduce organic waste, up to 50 percent until reaching stable condition. The main output of this process is a blackish stable product similar to compost that can be used as plant nutrition source and plant growing media. Every month, this waste decomposition process can produce 14 kg fine compost, which is equal to IDR 21,000, based on compost market price in Bogor. This study also found that BSF larvae is a potential agent to decompose domestic organic solid waste.

**Keywords:** Black soldier fly, compost, decomposition, economic value, solid waste

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## SOIL PROPERTIES OF EXOTIC TREE PLANTATIONS AT SAITHONG SILVICULTURAL RESEARCH STATION, PRACHUAP KHIRI KHAN PROVINCE

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### ABSTRACT

Exotic trees can grow very fast in new areas and are sometimes faster than the native trees. This happens because exotic trees have often been grown more in other places. Some exotic trees may be tougher and live through harsher conditions. For example, they may be drought resistant. This means that they can survive in a long period of time without rainfall. Exotic trees can be beneficial when they are introduced into a new area. For example, they may cast out pests and diseases that damage other trees in that area. This means that they may grow very fast to start with. The objective of this study was to compare soil physical and chemical properties of different tree plantations, namely *Eucalyptus urophylla*, *Acacia crassicaarpa* and *A. aulacocarpa*. The study was conducted in the Saithong Silvicultural Research Station. Three soil samples were collected from 0-10, 10-30, 30-50 cm soil depths, using a completely randomized design in *E. urophylla*, *A. crassicaarpa* and *A. aulacocarpa* stands aged 27 years, planted in 2 x 2 m spacing. The results showed that soil texture in these stands was sandy loam soil. Soil moisture percentage was the highest in the *E. urophylla* plantation (12.85%). Phosphorus, magnesium and potassium contents were the greatest in *E. urophylla* plantation (18.2, 6.98 and 6.98 mg kg<sup>-1</sup>, respectively). Organic matter content was the greatest in the *A. crassicaarpa* plantation (7.59%). Nitrogen and calcium contents were the greatest in the *A. aulacocarpa* plantation (0.04% and 36.17 mg kg<sup>-1</sup>, respectively) In conclusion, there were differences in soil bulk density and porosity caused by structure, tillage, cropping practices, soil depth and compaction. Characterization of soil pore system is important for understanding soil physical and parent material composition, which has direct impact on soil chemistry and fertility. Parent materials rich in soluble ions (calcium, magnesium, potassium and sodium) are desirable, as these chemicals are easily dissolved in water and thus, available for plants. *E. urophylla*, *A. crassicaarpa* and *A. aulacocarpa* might enhance aboveground stand production on poor nutrient soils in warm and humid tropical climates with low water limitations and could be planted to restore the soil and used in the form of forest plantations or agroforestry.

**Keywords:** Exotic tree plantation, Saithong Silvicultural Research Station, soil properties

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### 7.3. Session 3: Socio-economic, Cultural and Ethical Aspects in Conservation, Enhancement and Sustainable Use of Indigenous Tropical Flora and Fauna

## MOBILIZING CITIZEN TO DOCUMENT HERPETOFAUNA DIVERSITY IN INDONESIA

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### ABSTRACT

Involving public to collect data on species through citizen science is a new tool that has not been fully utilized in Indonesia. A citizen science project called GO ARK (*Gerakan Observasi Amfibi Reptil Kita* or Movement to Observe Our Amphibian and Reptile) were launched in Bogor as part of *Amfibi Reptil Kita* (ARK) program that started in 2016. The aim of this program is to gather distributional data of amphibians and reptiles in Indonesia using citizen science approach, which will be used for the development of Indonesian Amphibian and Reptile Atlas. GO ARK were carried out as a rapid field survey effort or Herpblitz, that was conducted in 21-27 April 2017. We developed four phases of activity i.e. development phase, preparation phase, live phase and analysing and reporting phase. Data from the resulted herpblitz were analyzed to see the distribution of participants based on province, number of species found and any special findings. Ninety-six people from 34 groups participated in GO ARK, resulting in 1159 observations. The number of participants conducting herpblitz in Java and Bali was 79% from the total participants with 82% of total observations. A total of 153 species were reported (67 species of amphibians and 86 species of reptiles) with 67 records of unknown species which consisted of 11 amphibian species and 13 reptile species. There were several challenges in this programs, 1) identification of species, 2) knowledge in using iNaturalist application to report finding, 3) low participation from outside Java, and 4) continuity of citizen science after GO ARK. We recommend that continuous training will be held, especially in outside Java to increase knowledge in species identification and using the applications.

**Keywords:** Citizen science, herpetofauna, Indonesia, monitoring biodiversity

### INTRODUCTION

Conserving biodiversity usually depends on the amount of information that can be gathered by scientists during research, especially on the distribution of the species and the number of species occurring in any given habitats. Current practices in biodiversity conservation generally rely on professional scientists or conservationists with additional involvements of

university students under the supervision of professional scientists. This approach has been carried out in many countries, including countries with high biodiversity. Unfortunately, a meta-analysis has shown that conservation research is low in areas with high diversity, including Indonesia (Wilson *et al.* 2016). The limited number of professionals working as conservationist or scientist in Indonesia can be shown by the number of conservation programs in Indonesia that mostly focus on selected charismatic species (i.e. orang-utans, elephants, tigers and rhinos in selected areas), while neglecting other small and less charismatic species such as amphibians and reptiles with high risk population decline due to human consumption, habitat change, pollutions and many other factors.

In the last decade, a volunteer based scientific program has been adopted in the effort to increase public participation in science or known as citizen science (CS). The largest utilization of this methods are in research on biology, conservation and ecology, where CS is used to collect and classify data (Kullenberg & Kasperowski 2016). In this scheme, general public participated in the collection of scientific data on certain taxonomic groups, geographic locations and time-frame, usually under the supervision of professional scientist. The volunteer-based scientific monitoring scheme has been used around the world to overcome insufficient monitoring of diverse taxa (Aceves-Bueno *et al.* 2015, Theobald *et al.* 2015).

Citizen science program is a new tool that has not been fully utilized in Indonesia. Although volunteers-based program i.e. clean up project and planting trees has been done for many years in Indonesia (Lailia 2014; Hambali & Suardi 2017; Rubiantoro & Haryanto 2013). However, there are few reports on the use of volunteer to collect data on species. The only available information regarding citizen science effort for monitoring of wildlife is the Indonesian bird atlas (Taufiqurrahman *et al.* 2016).

In 2017, a citizen science project called GO ARK (*Gerakan Observasi Amfibi Reptil Kita* or Movement to Observe Our Amphibian and Reptile) were launched in Bogor by Faculty of Forestry of Institut Pertanian Bogor (IPB) and Indonesian Herpetological Society. The citizen science program is part of bigger program called *Amfibi Reptil Kita* (ARK) that started in 2016 in Bogor. The aim of this program is to gather distributional data of amphibian and reptile in Indonesia using citizen science approach, which will be used for the development of Indonesian Amphibian and Reptile Atlas. Java and Bali were selected as the initial focus, due to the importance of both islands for Indonesia in terms of politics, socio-economics and wildlife conservation. This paper reports the development of the citizen science programs, result of the first citizen science monitoring and the challenges to keep the program alive.

## MATERIALS AND METHODS

Planning to mobilize public participation in citizen science program were carried out since 2016. To conduct the GO ARK Herpblitz we developed four phases of activity which is enclosed in the *Amfibi Reptil Kita* (ARK) mission. The activities included development phase, preparation phase, live phase and analysing and reporting phase (Fig. 1). The development of this project is similar to the approach followed by many scientists, according to Bonney *et al.* (2009). During the development phase we identified target participants, data storage and processing and develops protocols. Afterwards, during preparation phase we conducted training to selected targets and developed promotional modules. Trained students were then mobilized during the live phase to lead observation in their community during the GO ARK Herpblitz. Data were sent through the iNaturalist application and analyzed by our team. Fig. 1 shows the schematic development of GO ARK.

1	<b>Development Phase</b>
	- Identifying Target Participants
	- Selecting Data Storage and Processing
	- Developing Protocol
2	<b>Preparation Phase</b>
	- Selecting Trainee
	- Training and Festivals in Three Locations: Bogor, Yogyakarta and Bali
	- Developing Promotional Material
3	<b>Live Phase</b>
	- Accepting Data Through GO ARK/Herpblitz
4	<b>Analyse and Reporting Phase</b>
	- Complete Data Analysis and Interpretation
	- Report results
	- Evaluate lesson learned

Figure 1 Schematic development of GO ARK citizen science program

To recruit our lead volunteers, we mobilized the network of Herpetological Society of Indonesia or *Perbimpunan Herpetologi Indonesia*, mostly through mailing list and social media. The society was established in 2007 (Eprilurahman 2008) and around 400 people were listed in the society's network of mailing list, FB group and WhatApps group which included professional researchers working in research institutions and universities, university students, conservation practitioners, captive breeders and herp enthusiast. Flyers regarding the trainings were developed and distributed through this network. Three 4-days trainings were organized in Bogor (West Java), Ubud (Bali) and Yogyakarta. Each training was organized in partnership with local university i.e. Faculty of Forestry, Institut Pertanian Bogor for Bogor session; Faculty of Biology, Universitas Gadjah Mada for Yogyakarta session and Faculty of Basic Science and Mathematics, Universitas Udayana for Ubud session. The trainings consisted of a one-day class lecture at University and a three-days field course at selected natural areas. We selected at least 20 participants for each training, about 2/3 come from Java and Bali. Although we specifically focusing on Java and Bali, there were lots of request from other participants residing outside the islands to be involved in the program which prompted us to widen the geographic distribution for the trainees. In addition of training, we also conducted a one day festivals (*Festival Amfibi Reptil Kita*) that included talks shows, photograph exhibitions and amphibian and reptile exhibitions. The Festival was held a day after the end of training and open to public.

The citizen science program uses bioblitz approach or a rapid field survey effort by volunteers who document as many species as possible in a defined location during a defined period (Parker *et al.* 2018). As we focus on amphibian and reptile, the bioblitz is called as Herpblitz. A week long herpblitz was organized in 21-27 April 2017 after all three trainings were completed. Information regarding the herpblitz were sent through social media, mailing list and individual email to our former training participants.

To enable volunteers reporting the finding of amphibian and reptile in case, data from the herpblitz were collected using mobile application through iNaturalist (<https://www.inaturalist.org>). This application act as a crowd-sourced database for biodiversity and readily available for Android or Apple hardware (mobile phone, computer or tablet) for free. Participants need to be registered in iNaturalist at the start of the herpblitz and form an organized group consisting of 4-8 participants. Groups can request assistance for herpblitz expenses in their selected locations (no more than Rp 1,000,000 or around USD 67) and are encouraged to avoid overlap of survey area with other groups. As an incentive, after the final tally, one group and one participant with the highest point (given based on the number of species found) were given a prize.

Based on percentage of usable data reported in the mobile apps, we analyzed the distribution of citizen scientist based on province, number of record, number of species and conservation status based on IUCN Red list. We overlaid data points from GO ARK records to see the contribution of this effort in increasing the locality data of herpetofauna in Java and Bali.

## RESULTS AND DISCUSSION

In total, 209 participants from 34 groups registered in GO ARK. However, four groups did not conduct any reporting in iNaturalist (n = 22 participants) and only 96 participants put their findings in iNaturalist, resulting in 1,159 observations. The number of participants conducting herpblitz in Java and Bali was 79% from the total participants with 82% of total observations (Table 1, Fig. 2). A total of 153 species were reported (67 species of amphibians and 86 species of reptiles) with 67 records of unknown species (11 amphibian species and 13 reptile species).

Table 1 Number of participants and groups involved in GO ARK 2017 (21-27 April 2017) that reported their findings in Amfibi Reptil Kita project at iNaturalist

Province	N participants	N groups	N amphibian reports	N amphibian species	N unknown amphibian species	N reptile reports	N reptile species	N unknown reptile species
Banten	1	1	6	4	1	5	5	0
West Java	31	14	213	27	3	142	33	7
Central Java	11	5	112	15	0	131	24	3
Yogyakarta	15	4	60	19	0	37	18	5
East Java	17	7	131	21	2	109	30	2
Bali*	4	1	6	5	0	4	3	0
West Sumatra	2	1	20	14	3	1	1	0
North Sumatra	7	2	49	23	5	41	21	1
West Kalimantan	8	1	23	22	1	14	12	2
East Kalimantan	2	2	19	14	1	9	7	2
Southeast Sulawesi*	2	1	5	5	0	7	6	1
Total	100	39	644	67	11	500	86	13

Notes: \*One group that reported the findings in Bali and Sulawesi were in fact originated from Java in which their members reported findings in other islands. Four participants reported their findings in more than one province

Almost all amphibian species reported were Least Concern (LC) in the IUCN Red list status (85%, IUCN 2018); two species had not been evaluated against criteria of IUCN Red List (*Microhyla orientalis* and *Polydectes iskandari*), five species were in Near Threatened (NT) status (*Lepolalax gracilis*, *Limnonectes malesianus*, *L. paramacrodon*, *Microhyla petrigena* and *Nyctixalus pictus*), two species were Vulnerable (*Huia masonii* and *Limnonectes macrodon*) and one species was Critically



Endangered (*Leptophryne cruentata*). Most of the high number of reported amphibian were species that widely distributed in Indonesia and had Least Concern (LC) status in IUCN Redlist, except for *Limnonectes macrodon* (Vulnerable/VU) (Table 2). Two of the highest number of reported amphibians were *Chalcorana chalconota* and *Duttaphrynus melanostictus*, which were not only distributed widely in Indonesia, but also known as species having ability to thrive in human habitat (Church 1960; Inger *et al.* 2009; Moore *et al.* 2015). The result also showed new distributional record of at least two species of amphibians, *Microhyla orientalis* (reported in East Java, only known from Bali) and *Polypedates macrotis* (reported in West Java). The distribution of *M. orientalis* is previously known only from Bali (Matsui *et al.* 2013) whereas *P. macrotis* is previously known in Sumatra and Borneo (Brown *et al.* 2010; Diesmos *et al.* 2004). There is a need to conduct a thorough survey to verify this new distribution.

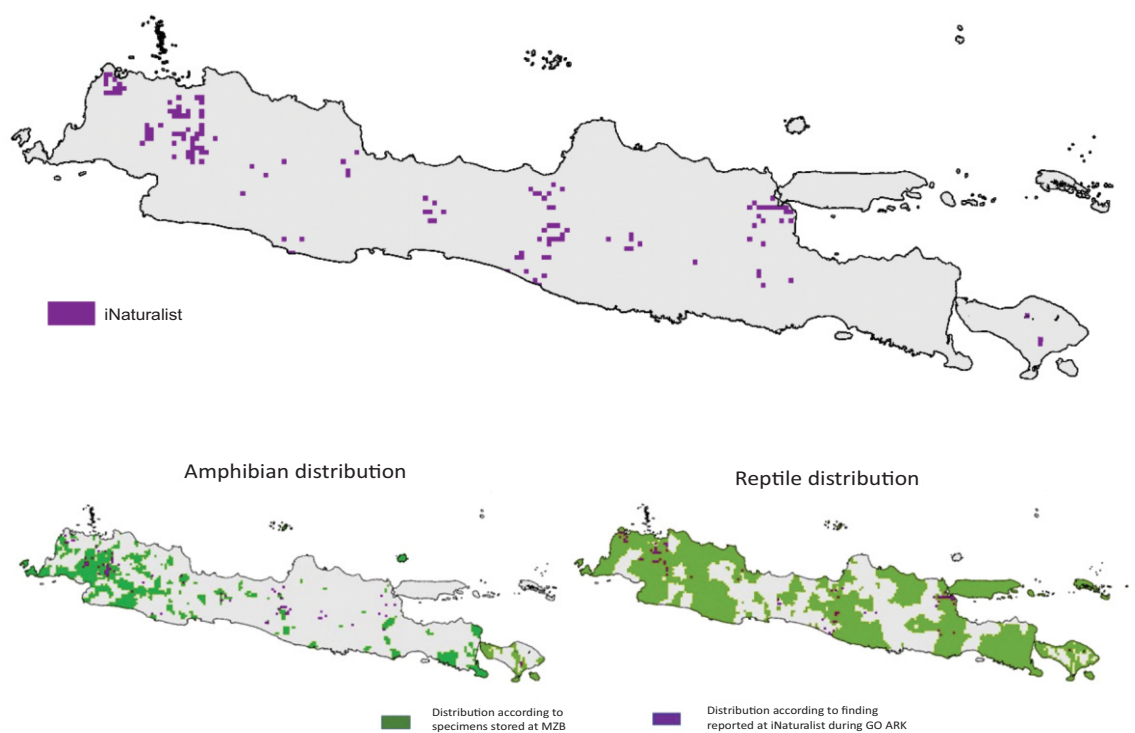


Figure 2 Distribution of GO ARK herpbliitz records in Java and Bali. Dots represent data available in the 5 x 5 km grids

Almost half of the reptile species (46.5%) were considered as the Least Concern, and more than half (52.3%) had not been evaluated against IUCN red list criteria. Only one species was considered in threatened status at IUCN Red list, which was the Olive Ridley *Lepidochehys olivacea*. Most of the frequently reported reptile were widely distributed in Indonesia with Least Concern (LC) status in IUCN Redlist, or had not yet evaluated (Table 3). Four of the highest number of reported reptiles were *Cyrtodactylus marmoratus*, *Bronchocela jubata*, *Eutropis multifasciata* and *Hemidactylus frenatus*. The marbled bow-fingered gecko (*C. marmoratus* species complex) are distributed widely in Southeast Asia (Mecke *et al.* 2016), as well as the other three species. Both *E. multifasciata* and *H. frenatus* are species that are able to thrive in human habitat all over Indonesia (see Gillespie *et al.* 2005 for Sulawesi and Janiawati *et al.* 2016 for Bali). The occurrence and widely distribution of the Oriental Garden Lizards (*Calotes versicolor*) in Java Island is officially reported here. There is no official report from Java before in any paper, including in Reptile Database

(<http://reptile-database.reptarium.cz/>) a database that is used widely by herpetologist (Uetz & Stylianou 2018). This is also an opportunity to prove whether the distribution of this species in Java is natural or introduced.

Table 2 The most common amphibian species recorded during GO ARK 2017 (21-27 April 2017) in AmfibiReptil Kita project at iNaturalist, with the total record and IUCN Red List status

Species	IUCN RL	a	b	c	d	e	f	g	h	i	j	k	N record
<i>Chalcorana chalconota</i>	LC	+	+	+	+	+	+		+	+			58
<i>Duttaphrynus melanostictus</i>	LC		+	+	+	+		+	+		+		56
<i>Polypedates leucomystax</i>	LC		+	+	+	+	+		+		+		47
<i>Limnonectes kublii</i>	LC		+	+	+			+	+	+			37
<i>Phrynoidis asper</i>	LC		+	+	+	+			+		+		35
<i>Fejervarya limnocharis</i>	LC	+	+	+	+	+	+		+				32
<i>Huia masonii</i>	VU		+	+	+	+							32
<i>Odorrana bosii</i>	LC		+	+	+	+			+				31
<i>Leptobrachium basseltii</i>	LC		+	+	+	+							27
<i>Microhyla achatina</i>	LC		+	+	+	+							25
<i>Megophrys montana</i>	LC		+	+		+							20

Notes: Distribution note: a = Banten, b: West Java, c: Central Java, d = Yogyakarta, e = East Java, f = Bali, g = West Sumatra, h = North Sumatra, i = West Kalimantan, j = East Kalimantan, k = Southeast Sulawesi

Conflicts between human and reptile are mostly caused by venomous snakes, especially where human activities overlap with the habitat of these species i.e. in plantation where agricultural activities occurred (Rifai *et al.* 2017). Other potential conflicts are between salt water crocodile and reticulated python. There is an indication that conflict between humans and salt water crocodile (*Crocodylus porosus*) become more often in Sumatera and Java (Ardiantiono *et al.* 2015). The occurrence of *C. porosus* in East Java in Brantas River is one of the example that wild population of this species still exists nearby the high density of human settlements. Further monitoring in Brantas River (Surabaya) is needed to ensure the reduction of potential conflict. Although there is no referred paper on human and python conflict in Indonesia, scattered reports in newspaper or other media showed cases of strangulation and death of local people caused by reticulated python i.e. in Sulawesi during 2017 and 2018.

There were new records of reptile reported from GO ARK. However, further information on voucher specimen was needed to ensure the validity of species identification i.e. for *Pareas margaritophorus* and *Cryptoblepharus cursor*. The distribution of *Pareas margaritophorus* was previously reported in China, and then Thailand to West Malaysia (Stuart *et al.* 2012). However, during GO ARK 2017, a report of this species came from North Sumatra. Similarly, the distribution of *C. cursor* was formerly reported from Bali, Lombok and Sulawesi (Das 2015; Koch 2011). The record from southern Java extended its distribution.

In the case of alien species, *Lamprolepis smaragdina* was reported existed outside its natural geographical range. This skink is native to Sulawesi, Moluccas, Lesser Sunda and Papua. However, report from GO ARK showed that a population of this species was established in West Java. This skink is becoming popular in pet industry for its beautiful bright green coloration. There is a possibility that the occurrence of this species in West Java comes from unintentional release of captive *L. smaragdina* and potentially becoming a competitor with the native species such as *Dasia olivacea* which occupies similar niche.

Table 3 The most common reptile species recorded during GO ARK 2017 (21-27 April 2017) in *AmfibiReptil Kita* project at iNaturalist, with the total record and IUCN Red List status

Species	IUCN													N record
	Red List	a	b	c	d	e	f	g	h	i	j	k		
<i>Cyrtodactylus marmoratus</i>	NA		+	+	+	+			+					45
<i>Bronchocelea jubata</i>	LC	+	+	+	+	+	+							38
<i>Eutropis multifasciata</i>	NA		+	+	+	+			+		+			37
<i>Hemidactylus frenatus</i>	LC		+	+	+	+			+					24
<i>Pareas carinatus</i>	LC		+	+	+	+			+		+			20
<i>Abaetulla prasina</i>	LC	+	+	+	+	+								18
<i>Gekko gekko</i>	NA		+		+	+							+	17
<i>Dendrelaphis pictus</i>	NA	+	+	+	+	+			+					15
<i>Gonocephalus kublii</i>	NA		+		+	+								15
<i>Calotes versicolor</i>	NA	+	+			+			+					15
<i>Takydromus sexlineatus</i>	LC		+	+	+	+								14
<i>Lygosoma bowringii</i>	NA				+	+								10

Notes: Distribution note: a = Banten, b: West Java, c: Central Java, d = Yogyakarta, e = East Java, f = Bali, g = West Sumatra, h = North Sumatra, i = West Kalimantan, j = East Kalimantan, k = Southeast Sulawesi

Table 4 Reptile species recorded during GO ARK 2017 (21-27 April 2017) in *AmfibiReptil Kita* project at iNaturalist, which have a potential risk to human

Species	IUCN													N record
	Red List	a	b	c	d	e	f	g	h	i	j	k		
<i>Bungarus candidus</i> *	LC		+		+									2
<i>Calliophis intestinalis</i> *	LC					+								4
<i>Caloselasma rhodostoma</i> *	LC			+										1
<i>Naja sumatrana</i> *	LC								+					1
<i>Trimeresurus albolabris</i> *	NA		+			+								4
<i>Trimeresurus macrops</i> *	NA								+					1
<i>Trimeresurus puniceus</i> *	LC			+		+								2
<i>Tropidolaemus subannulatus</i>	LC										+			1
<i>Malayopython reticulatus</i> *	NA											+		1
<i>Crocodylus porosus</i> *	LC					+								1

Notes: Distribution note: a = Banten, b: West Java, c: Central Java, d = Yogyakarta, e = East Java, f = Bali, g = West Sumatra, h = North Sumatra, i = West Kalimantan, j = East Kalimantan, k = Southeast Sulawesi. \* denotes venomous snake

Data points from GO ARK for amphibian and reptile in Java and Bali enabled us to add current locality record to the distribution maps that was produced based on MZB specimens (Kusrini *et al.* 2018, in preparation). Although most of the reported species was considered widely distributed, however, data from GO ARK was able to increase information on species distribution in finer scale especially in areas that had no previous report. However, the finding of new records needed to be verified through peer-review process of scientific publications.

In this project, data compiled by citizen science will be used for the development of atlas of amphibian and reptile in Indonesia. Thus, species identification is an essential requirement and will be crucial for the credibility of the program which is important in any citizen science project (Freitag *et al.* 2016). Participants must have basic understanding of species, which include understanding the morphological character needed to identify species, differentiate between similarly looking species, and how to search species. This basic expertise is sometimes taught at

university level for zoology students, however participants that have no prior knowledge might be able to learn by themselves using available guidebooks. Results have shown that several participants are unable to report findings to species level. Trainings have been conducted prior to GO ARK, however, the number of participants exceed participants of training and not all trainees are able to lead groups. There are more unknown reptile's records than amphibians, which might be caused by lack of training and also due to lack of identification books. To date, there are not many identification books on amphibians and reptiles in Indonesia. Books on amphibians of Java and Bali are slightly numerous than reptiles, although it is not easily accessed (Iskandar 1998; Kurniati 2003; Kusriani 2013; McKay 2006; Rusli 2016; Somaweera 2018). The situation is similar for other islands, and in some cases like in Kalimantan and Sumatra, books from the neighboring countries i.e. Malaysia and Sumatera were used (Cox *et al.* 1998; Mistar 2003; Inger 2005; Das 2015).

Based on the result where only half of the total participants reported their findings with iNaturalist app, it can be shown that we have underestimated participants' knowledge in using iNaturalist app. Although Indonesia is one of the country with high number of media social user (Ambardi *et al.* 2014), but there is a need to increase digital literacy in the country (Kurnia & Astuti 2017). Follow up interview to several groups showed that reasons of this low participations are either by limited understanding on how to use iNaturalist application or incorrect assumption that report should only be sent by the leader of the group, not by all members. Results also showed that participation outside Java is low, including Bali that was included in the trainings. This result is to be expected as Java has higher number of students' community interested in herpetology compared to other islands. However, the fact that several trainees came from outside Java and Bali indicate that there is a growing number of students interested in herpetology that should be facilitated by the Indonesian Herpetological Society. We believe that we need to continue trainings and educate students either through face-to-face meeting or through other means.

Finally, as the aim of the program is to develop an atlas, there is a need to maintain the continuation of citizen science after GO ARK. Sustainability of such program will be achieved if all stakeholders are fully participated. GO ARK acts as incentive to keep the public engaged and motivated to report amphibians and reptiles, however, during the week of the herpblitz we only recorded eight people outside the GO ARK participants that reported amphibian and reptile using iNaturalist. Therefore, the citizen science should be designed to increase participations of public to report the finding of amphibians and reptiles at any time, not only during the herpblitz.

## CONCLUSION

A rapid inventory data collection for reptiles and amphibians may work well with citizen science approach despite current lack of trainings. In our experience with GO ARK, we found a relatively high interest of general public to be involved in this program which resulted in an increased information on distribution of herpetofauna species in Indonesia. However, there is a need to continue capacity building across Indonesia to increase participations.

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## PLANTS USED FOR TRADITIONAL POSTPARTUM CARE BY SAMA-BAJAU PEOPLE IN KOTA BELUD, SABAH, MALAYSIA

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### ABSTRACT

This study aimed to present local traditional plants used by Sama-Bajau women during postpartum care. From the face to face interview with local people, seven species were identified to have been used traditionally to care for women during their confinement period. The seven species are *Aloe vera*, *Bambusa* sp., *Cosmos caudatus*, *Curcuma longa*, *Curcuma zanthorrhiza*, *Momordica charantia*, *Zingiber zerumbet*. The part of plants that have been utilized in preparations including leaves, young shoot, tuber, and fruits. The preparations were not just consumed orally, but also used as bathing mixture for both mother and new born infants. The traditional knowledge of postpartum care seldom scientifically documented, only passed through oral communication or by observing practice through generations.

**Keyword:** Kota Belud, postpartum, Sama-Bajau

### INTRODUCTION

Sabah state of Malaysia is described as having tropical climate and 4200 mm annual rainfall that advocate her establishment of biodiversity. The forest cover in Sabah encompassed approximate area of 37,600 km<sup>2</sup>, which is considered high according to Southeast Asia region's standard (Reynolds *et al.* 2011). Sabah is also home for 40 indigenous ethnics and sub-ethnic groups, and each group has their own identity, lifestyle, and traditional culture. There are five main ethnics, Kadazandusun, Bajau, Murut, Chinese, Brunei Malay (Lailawati *et al.* 2014). Except for Chinese, the other four main indigenous ethnics are also called as *Bumiputra* Sabah. The indigenous people speaks more than 50 languages and 80 dialects (Muhammed & Muthu 2015).

The Bajau community is the second largest native ethnics after Kadazandusun group, the Bajau make 13% of total native population in Sabah (Muhammed & Muthu 2015). Bajau or its variants (e.g. Badjao, Bajo, Bajao) is a generic word used to group several closely related subethnics. Their diaspora covers the Sulu Archipelago and Borneo shorelines and islands, which today is known as modern territory of Malaysia, Philippines, and Indonesia (Miller 2011; Halina 2013). In the state of Sabah, Malaysia, Sama-Bajau and Aa'a Sama, are the two large subgroups of Bajau (Saat 2003). The demarcation of culture, religion, and language between two subgroups is rather in harmony, and the common understanding is that Bajau is a muslim (Saat 2003). However, there are some more animistic rituals still being practiced (Halina 2013). Sometimes, Aa'a Sama would identify themselves as Aa'a Dilaut due to their customary nomadic maritime-coastline lifestyle, especially in Semporna District, east coast of Sabah (Ismail & Ahmad 2015). Meanwhile, The Sama-Bajau or the Jomo Sama of Kota Belud, is acknowledged as inland Bajau due to their livelihood that is more related to activity such as paddy agriculture, forest foraging, livestock rearing, and also as skillful horsemen (Miller 2011; Halina 2013; Lailawati *et al.* 2014).

Other than Kota Belud, the Sama-Bajau settlements in west coast of Sabah also include Kudat, Tuaran, Putatan, and Papar (Halina & Saidatul 2008; Halina 2013). In previous study, it was noted that decline of traditional knowledge among groups with higher household income were compared to the lower income group (Muhammed & Muthu 2015). Moreover, traditional knowledge in Sabah is not well documented orthographically, it often remains in the memory of elderly people, and merely passed down to younger generation through informal oral communication and by observing practice (Awang-Kanak *et al.* 2018a; Awang-Kanak *et al.* 2018b). Therefore, this study aimed to present local plants that have been used traditionally by Sama-Bajau women in Kampung Taun Gusi and Kampung Menunggu, during postpartum care.

## MATERIALS AND METHODS

Semi structured interviews were conducted among villagers to collect the information on utilization of natural resources as freshly eaten vegetables and its application as herbal medicine including for postpartum care (Martin 1995). These six informants were chosen by snowball sampling technique. Informants were selected based on (i) their willingness to share their knowledge on freshly eaten vegetable and its utilization as herbal medicine including for postpartum care. (ii) Their ability to describe how the herbal medicine is administrated to women during their confinement period. The demographic detail of informants and data of plant parts that have been used for postpartum care were also recorded.

## RESULTS AND DISCUSSION

A total of six Sama-Bajau informants were interviewed for their traditional knowledge. Three informants were from Kampung Taun Gusi, and another three were from Kampung Menunggu. Informants that came from the same villages are related to each other as family. However, only four from six informants had knowledge on plants used for Sama-Bajau's traditional postpartum care. The ages of informants were between 29 to 67 years old at the time of interviews were conducted. In common, all six are practicing muslims, literate, married, and at minimum with secondary education. The demographic data of informants is as detailed in Table 1. Eight species of plants have been used for women during postpartum care period, which lasted from 40 to 90 days after giving birth. The eight species are *Aloe vera*, *Bambusa* sp., *Benincasa hispida*, *Cosmos caudatus*, *Curcuma longa*, *Curcuma zanthorrhiza*, *Momordica charantia*, *Zingiber zerumbet* (Table 2). The part of plants that have been utilized in preparations including leaves, young shoot, tuber, and fruits. The preparations were not just consumed orally, but also used as bathing mixture for both mother and new born infants. The daily dietary uptake for new mothers includes boiled young shoot of *Bambusa* sp., fresh leaves of *Cosmos caudatus*, fresh cut of *Aloe vera*, and decoction made from tuber of *Curcuma longa*. The utilization of *Curcuma longa* and other species from Zingiberace family e.g. *Zingiber aromaticum*, *Zingiber officinale* during postpartum care has been also traditionally practiced by Malay in state of Johor and Negeri Sembilan. The gingers grounded and mix with hot water, and consumed as drink to improve blood circulation (Jamal *et al.* 2011).

The fruit and leaves of *Momordica charantia* were soaked in lukewarm water and the preparation is used as bathing water for new mothers. Bathing water for women during confinement period is often infused with herbs, and this mixture would help the mothers to refresh and revitalize their health and wellness (Jamal *et al.* 2011; Othman *et al.* 2014). The traditional Malay midwife or confinement lady would help to prepare the bathing ritual that is



known as “mandi serom”. The Malay in Northern of Peninsular Malaysia, commonly used *Cymbopogon nardus*, *Lawsonia inermis*, and *Pandanus amaryllifolius* for traditional postpartum bathing water (Razak *et al.* 2018). In Kampung Menunggu, plants are also used for the new born infant, the leaves of *Centella asiatica* were drenched into the water, and the combination was used as bathing water to treat infant with jaundice. Meanwhile in Kampung Taun Gusi, the remedy for post-natal jaundice is bathing the affected infant with water that pre-soaked with fruit of *Benincasa hispida*. Interviews revealed that, there was no record of animistic element assimilated into traditional postpartum care among Sama-Bajau in both villages. This could be related with the Sama-Bajau people in Kota Belud who are committed Sunni Muslims, therefore, any animistic embedded rituals or customs are declining in practice. According to Informant 5 (INF 5), they already have access to modern healthcare facilities and services, e.g. government hospital, clinics, and government midwife nurse, thus the dependency on traditional medical care is much lesser than what had been practiced by previous generation.

Table 1 Demographic details of informants from Kampung Taun Gusi and Kampung Menunggu, Kota Belud, Sabah

Code	Gender	Age	Marital status	Religion	Source	Education	Occupation
INF 1 <sup>a</sup>	Male	67	Married	Islam	Older generation and experience	Teaching college	Native chief
INF 2 <sup>a</sup>	Male	64	Married	Islam	Older generation	Secondary	Headman
INF 3 <sup>a</sup>	Male	65	Married	Islam	Older generation	Secondary	Food vendor
INF 4 <sup>b</sup>	Female	29	Married	Islam	Older generation	University	Government servant
INF 5 <sup>b</sup>	Female	59	Married	Islam	Older generation	Teaching college	Pensioner
INF 6 <sup>b</sup>	Female	49	Married	Islam	Older generation	Secondary	Housewife

Notes: INF1-INF6= Informants, Address of informant  
<sup>a</sup> = Kampung Taun Gusi, <sup>b</sup> = Kampung Menunggu

Table 2 List of plants used as postpartum care by Sama-Bajau people in Kampung Menunggu and Kampung Taun Gusi, Kota Belud, Sabah

Scientific name	Local name	Locality	Part used	Preparation
<i>Aloe vera</i>	Lidah buaya	TG	Leaves (flesh & bud)	Freshly eaten, mixed with drink or soup.
<i>Bambusa sp.</i>	Rebung buluh	M	Young shoot	Boiled before eaten.
<i>Benincasa hispida</i>	Buah kundur	TG	Fruit	Fruit soaked with water, used as bathing water for jaundice baby.
<i>Cosmos caudatus</i>	Ransa ransa	M	Leaves	Freshly eaten.
<i>Curcuma longa</i>	Kunyit	M	Tuber	Decoction, drink.
<i>Curcuma zanthorrhiza</i>	Temulawak	TG	Young leaves	Freshly eaten, briefly boiled before eaten.
<i>Momordica charantia</i>	Peria	M	Fruit	Fruit soaked with water, used as bathing water for jaundice baby.
<i>Zingiber zerumbet</i>	Lempoyang	TG	Young leaves	Freshly eaten, briefly boiled before eaten.

Notes: TG = Kampung Taun Gusi, M= Kampung Menunggu

## CONCLUSION

In conclusion, this study had recorded traditional knowledge on preparations of eight plant species used for postpartum care by Sama-Bajau people in Kampung Taun Gusi and Kampung Menunggu, Kota Belud, Sabah. Various parts of plants have been prepared differently for oral consumptions, e.g. freshly eaten, boiled before eaten, decoction, drink, and also for external use, e.g. bathing mixture. Sustainable utilization of plant resources would give more value to the plant species, and strengthen the justification for species conservation. Similar study should be carried out with other Bajau subgroups from different geographical areas such as Kudat, Tuaran, Putatan, Papar, Lahad Datu, and Semporna, in order to compile more data on the use of plant for Bajau's traditional postpartum care.

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## ETHNOBOTANY OF TRADITIONAL MEDICINAL PLANTS AT THE FOOTHILLS OF MT. ARAYAT, PAMPANGA, PHILIPPINES

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### ABSTRACT

This study documented from key informants at the foothills of Mt. Arayat, Pampanga, Philippines, a total of 165 species of medicinal plants in 63 families with Family Lamiaceae having the most number of species. Majority of the plants were herbs (75%) and terrestrial (94%). Herbarium specimens of representative species were prepared and properly labeled as to description and uses. There were twenty-six ailment categories reported with “gastro-intestinal problems” as Rank 1, “Asthma” (Rank 2), “kidney problems” (Rank 3), and “dermatological disorders” (Rank 4). The Factor informant consensus (Fic) was highest for dengue (Fic=0.92). The Relative frequency citation (RFC) was highest in *Plectranthus amboinicus* (0.51). The Fidelity Level (FL) value was the highest (100%) for *Muntingia calabura* (for gastro-intestinal), *Senna alata* (for dermatological disorders), *Hibiscus rosa sinensis* (for boils), and *Aloe vera* (for dermatological disorders). A plant locally named as “*Bacali*” (no taxonomical classification as of this report) cited for kidney problems, registered an FL value of 98%. Leaves were the most preferred form of medicinal material, prepared mostly by females, generally through boiling/decoction and most of the time taken orally by the patients. Both male and female administered the medicinal material in various forms and dosages. The knowledge on ethno medicine, handed over by the elders/ancestors to the next generations, was used mostly by those informants aged 60-65 who were generally elementary graduates and had been residing in the area for 20 years or more. The use of medicinal plants by the informants was still a viable option even when there are commercialized medicinal materials available because of their availability, accessibility and effectivity through the years. This study recommends a more comprehensive study using other quantitative indices on Relative Cultural Importance (RCI). Plants in the study with high Fic, RFC and FL values not included in the top ten plants endorsed by the Department of Health and those studied by the National Integrated Research Program on Medicinal Plants (NIRPROMP) can be subjected to bioassay and pharmacological investigations whereas, those with low-value scores requires analyses of their bioactivity.

**Keywords:** Ethnobotany, ethnomedicine, local wisdom, medicinal plants, Mt. Arayat

#### 7.4. Session 4: Policies and Other Legal Frameworks in Conservation and Sustainable Use of Indigenous Tropical Flora and Fauna

### MECHANICAL AND CHEMICAL WEED CONTROL COMPARISON ON TWO AGROECOSYSTEMS AS AN EFFORT TO REDUCE INVASIVE SPECIES ON AGRICULTURAL LAND

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#### ABSTRACT

Invasive species are commonly found in agroecosystem as weeds. They are usually controlled because weeds can reduce agricultural productivity. Mechanical and chemical weed controls are the most common methods used in Indonesia. Lack of information regarding effectiveness of both methods in reducing weeds leads to ineffective weed control. Therefore, this study was conducted to see the effectiveness of each weed control method. The observation was carried out at an organic agroecosystem SO (main crop *Lactuca sativa*) and a conventional agroecosystem KN (main crop *Brassica juncea*). Weeds in SO were controlled mechanically every 1-2 days, while weeds found in KN were controlled by chemical using Fenoxaprop-p-ethyl 0.1 kg/ha on the 10<sup>th</sup> day after planting. Ten plots of 1 x 1 m<sup>2</sup> quadrat were placed randomly on both agroecosystems to record weeds species richness in one cultivation period. Thirty one (31) weeds species (16 families) were found in two agroecosystems, and dominated by Asteraceae. SO agroecosystem was invaded by *Oxalis intermedia* (Importance Value Index, IVI 44%) and *Cyperus rotundus* (IVI 17.2%). KN agroecosystem was dominated by *Portulaca oleracea* (IVI 83.7%) and *Amaranthus blitum* (IVI 18.5%). Weeds diversity in SO was higher (24 species, Shannon-Wiener diversity index H'=2.62) than that in KN (18 species, H'=1.61). Species dynamics in every three days observation showed the impact of weed control. In KN agroecosystem, the number of species decreased by 30% five days after herbicide application, while mechanical method only reduced weeds species by 9.5%. However, both methods were considered not effective in reducing invasive species because there was a total species richness increased by 48% in KN and 30% in SO. It can be assumed that high species number in SO was related to plant's ability to reproduce vegetatively or generatively after the mechanical weed control.

**Keywords:** Agroecosystem invasive species, weed, weed control

Note: Full paper of this abstract is in the process of publication in BIOTROPIA journal

## GENETIC CONSERVATION OF SUMATRAN RHINO BASED ON D-LOOP MITOCHONDRIAL DNA SEQUENCE

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### ABSTRACT

Genetic conservation of Sumatran rhino was studied to understand the determining level of genetic diversity of inter-species individual, to determine the phylogeny of inter-species individuals, and to find out genetic distance of four individuals. Blood samples were taken from 2 female Sumatran rhinos (Rosa and Bina) and 2 male Sumatran rhinos (Torgamba and Andalas) from the SRS (Sumatran Rhino Sanctuary), Way Kambas National Park. Analysis was conducted using the D-loop mtDNA. The primers used to amplify sequence of D-Loop partial were RHDLF and RHDLR. Data were analyzed using the MEGA (Molecular Evolutionary Genetic Analysis) program with African White rhino, African black rhino and Indian rhino from GenBank as the out-groups. The phylogeny was analyzed using Neighbor-Joining (NJ) method with 1000 bootstraps. The PCR product of D-Loop partial was 677 bp-size DNA fragments. The D-loop DNA sequence also showed that the Sumatran rhinos were significantly different from the African rhinos despite their similarity in appearance, in which both species of rhinos had two horns. The Sumatran rhinos apparently had a closer genetic distance with Indian rhinos which had single horn. This suggested that the geographical distribution was more influenced by the phylogeny rather than by the number of horns.

**Keywords:** Conservation, DNA, mitochondrial, Sumatran Rhino

Note: Full paper of this abstract is in the process of publication in BIOTROPIA journal

## 8. Poster Presentation Session

### ODONATA FAUNA OF RIPARIAN HABITATS IN SELECTED AREAS OF LUZON AND MINDORO REGION

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#### ABSTRACT

Odonata considered as potential indicator of environmental disturbances. Despite of great efforts in recording the Odonata fauna of the Philippines in general, the riparian habitats in Bataan/Subic Bay (Luzon) and Mt. Hinunduang/Baroc River Catchment (Mindoro) are still unexplored. Due to continuous forest and freshwater habitats destruction, faunal survey of Odonata species is urgent. The present study surveyed selected riparian habitats in these regions in December 2015 to April 2016. A total of two hundred six species belonging to twenty genera (*Heteronaias*, *Brachydiplax*, *Diplacodes*, *Macrodiplax*, *Neurothemis*, *Orthetrum*, *Pantala*, *Potamarcha*, *Tritthemis*, *Zyxoma*, *Neurobasis*, *Cyrano*, *Rhinocypha*, *Agriocnemis*, *Ischnura*, *Pseudagrion*, *Teinobasis*, *Euphaea*, *Coeliccia*, and *Risocnemis*) were recorded and seven endemic species of the family Platycnemididae, Euphaeidae, and Calopterygidae were documented in both regions. From the collected specimens, one species under the family Platycnemididae is new to science but additional collection and evaluation are needed. The data collected contribute to the understanding of Odonata diversity and distributions in the regions and support future conservation and management strategies.

**Keywords:** Distribution, diversity, faunal survey, Odonata, riparian habitats

#### INTRODUCTION

Order Odonata is an order of carnivorous insects, encompassing the dragonflies (Anisoptera) and the damselflies (Zygoptera) and they are among the most ancient of winged insects, which have existed since the Triassic (Kalkman *et al.* 2008). Dragonflies are generally larger, and perch with their wings held out to the sides, while damselflies have slender bodies, and hold their wings over the body when at rest (Orr *et al.* 2004). They are generally found at or near fresh water although some species roam widely and may be found far from their larval habitats (Orr *et al.* 2004; Corbet 1999). The larvae are predatory and, aquatic, and can be found in various kinds of inland waters (Kalkman *et al.* 2008; Orr *et al.* 2004; Corbet 1999).

The Philippines Odonata and their taxonomy are still insufficiently known (Hämäläinen 2004). As described by Hämäläinen (2004) the country has high percentage of endemism in terms of its Odonata fauna. Luzon, being the largest island in the Philippines, is a home for a wide number of Odonata species, some of which are endemic to the island or in a particular region of the island (Villanueva *et al.* 2012; Gapud 2004; Hämäläinen 2004).

Present literature provides data of new discovered species of Odonata from several islands in the country (Villanueva & Gil 2011; Villanueva *et al.* 2012) and these discoveries denote that there are still more to explore (Villanueva 2011) from virtually unexplored main islands up to smallest islands in the archipelago. However, the number of critical or nearly endangered species is also significantly increasing (International Union for Conservation of Nature 2011). Species that were previously identified are not actually seen and remain elusive at present time (Villanueva 2011). Human activities and increasing number of populations contribute to the deterioration and destruction of habitat, which leads to the increased number of Odonata species that are considered critical or nearly endangered (Hämäläinen 2004).

Odonata became a subject as ecological indicators (Tiple & Koparde 2015; Henning 2008; Smith *et al.* 2007), their occurrence in a particular area could indicate good land water condition (Septianella 2014; Henning 2008) since odonates have both aquatic and terrestrial life stages (Bried 2005) and they are also very sensitive to sudden change in environmental factors such as temperature, oxygen levels, and amount of forest covers (Ramirez 2000). Recent studies also revealed that certain Odonata species demonstrate high association with particular habitats (Gómez-Anaya & Novelo-Gutiérrez 2010; Smith *et al.* 2007).

Despite the effort in understanding Odonata fauna and diversity in the country in general and in Luzon in particular, the Odonata of selected riparian habitats in Bataan/Subic Bay (Luzon) and Mt. Hinunduang/Baroc River Catchment (Mindoro) are still unexplored. Due to continuous forest destruction and other habitat stresses, a faunal survey of Odonata species is urgent. Specifically, the study aims to document the Odonata species found in selected riparian habitats in Bataan/Subic Bay (Luzon) and Mt. Hinunduang/Baroc River Catchment (Mindoro), including different altitudes and land use and identify new or endemic species found in the areas based on their morphological structure. Collected data will contribute to the information about Odonata in the island and will support future conservation and management strategies.

## MATERIALS AND METHODS

### Study Areas

The study was conducted in selected riparian habitats in Bataan (ca. 14°64' N, 120°48' E) / Subic Bay (ca. 14°79' N, 120°23' E) in Luzon and Mt. Hinunduang / Baroc River Catchment, Roxas, Oriental Mindoro (ca. 12°35' N, 121°30' E) (see Fig. 1).

Before the field study was conducted, official consent was obtained from the respective local government units (LGUs), DENR, SENRO, PENRO, and NCIP.

### Sampling Sites

**Site 1 Boton River** (ca. 14°78'67"N 120°29'76"E). This site has an elevation of 110 m asl. and is located within the Subic Bay Metropolitan Area. This sampling site is considered as disturbed primary forest. The Boton Falls is one of the main attractions in Subic Bay wherein travelers from nearby cities visit the area.

**Site 2 Batalan River, Subic Bay** (ca. 14°43'01"N 120°18'41"E). This area has an elevation of 65 m asl. and classified as secondary forest.

**Site 3 Orani River** (ca. 14°44'15" N 120°24'58" E). The elevation of this site is 460 m asl. The area is located at the foot of Mt. Natib in Bataan. The area is characterized by very steep forested slope.

**Site 4 Lower Baroc River** (ca.12°35'51"N 121°28'11"E). This area is located in Roxas, Oriental Mindoro and has an elevation of 27 m asl. The sampling area is considered as disturbed farmland. The area is also near the quarrying site.

**Site 5 Tagaskan River** (ca.12°34'39"N 121°22'24"E), a Hinundugan River Tributary at the upper Baroc River Catchment. The area is located in Brgy. San Vicente, Roxas Oriental Mindoro and classified as extensive farmland with an elevation of approx. 410 m asl.

**Site 6 Taugad Daka** (ca.12°38'05"N 121°19'33"E), a Taugad River Tributary at the upper Baroc River Catchment. The area is located in Brgy. San Vicente, Roxas Oriental Mindoro and classified as disturbed primary forest with an elevation of approx. 530 m asl.

**Site 7 Taugad Diit** (12°36'39"N 121°20'47"E), upper Taugad Diit River, a Taugad River tributary at the upper Baroc River Catchment; this site has an elevation of approx. 525 m asl. and is characterized as a secondary forest.

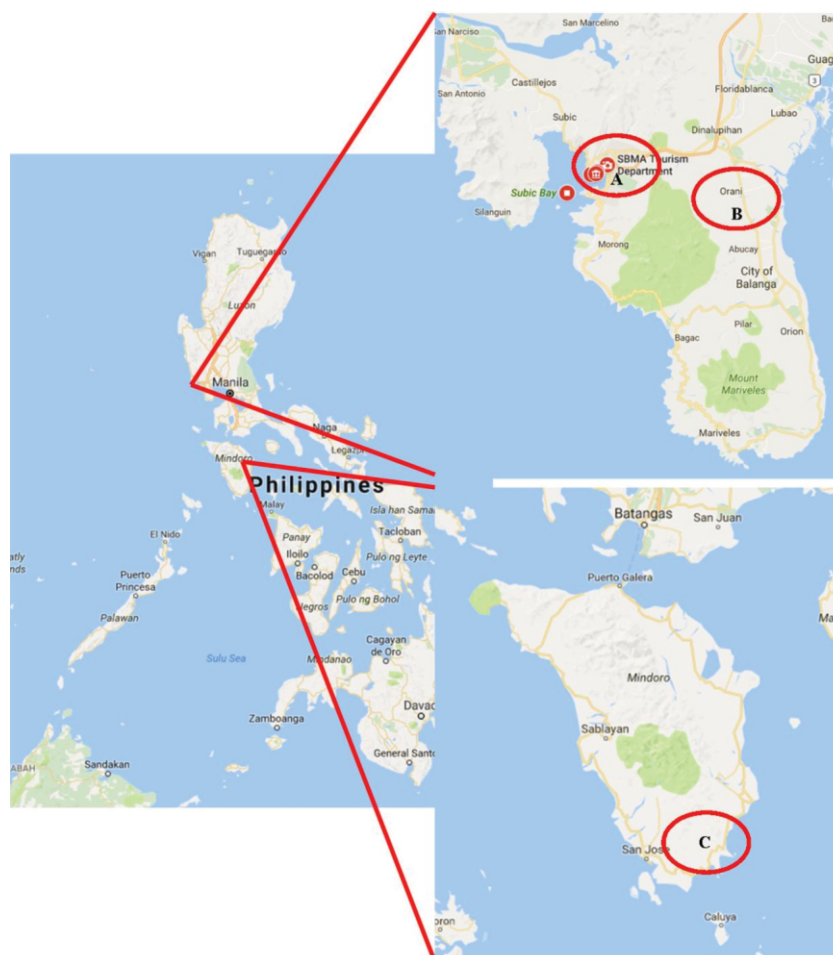


Figure 1 Map of the three sampling sites: (A) SBMA, Zambales; (B) Bataan; and (C) Municipality of Roxas, Oriental Mindoro (Google Maps 2017)





Figure 2 Sampling sites: (A) Boton Falls; (B) Boton River; (C) Batalan River; (D) Orani River; (E-F) Tagaskan River; (G) Taugad Daka; and (H) Taugad Diit

### Collection and Preservation

Sample collection was conducted twice at all sites during the months of December 2015 and April 2016. Opportunistic sampling (Jumawan *et al.* 2012) was employed to all sampling sites. Odonata were captured through hand picking and by a catching net made from silk cloth with a measurement of 25 x 60 cm. A stretch of 10-15 m of the river served as sampling site. Upon arriving at the site, the researcher stayed and caught samples for 60-120 minutes, then proceeded to the next sampling site. Samples were collected from eight o'clock in the morning to five o'clock in the afternoon. Preservation of the specimen was based on the methods used by Mapi-ot, Taotao, Nuñeza, and Villanueva (2013). Each specimen was properly placed in an empty white envelope with their wings folded. Each envelope was properly labeled according to the time, date, and collection locality. Specimens from each site were euthanized using small amount of acetyl acetate. Preservation of the specimen using acetone depended on the respective suborder (24 hours for dragonflies while 12 hours for damselflies). After being soaked in acetone, specimens were air dried and placed in tissue paper and stored in a cool dry place.

## Specimen Identification

Odonata specimens were identified based on their morphological characteristics, such as head, thorax, abdomen, anal appendages, and wing venation. Specimens were examined and measured using OLYMPUS CX21 compound microscope, OLYMPUS SZ40 stereo microscope equipped with digital adapter LW Scientific MiniVid DCM310 and LEICA EZ4 dissecting microscope. Species were identified from family up to species level using published identification keys (van Tol & Gassmann 2007; van Tol 2005; Gassmann & Hämäläinen 2002; Hämäläinen & Müller 1997; Hämäläinen 1991; Needham & Gyger 1939; Needham & Gyger 1937).

## Data Presentation

The taxon names are presented, based on current systematic classification (Dijkstra *et al.* 2014), from order up to the species level. It includes the morphological diagnosis (wings, head, thorax, and abdomen) of representative specimens, along with notes on their distribution and ecology.

The collected specimens were deposited in the Ateneo de Manila University, Biology Department Laboratory for safe keeping and future references. In addition, collections of Dr Reagan Joseph T. Villanueva in Davao City and Dr Victor L. Gapud in University of the Philippines, Los Baños were visited for comparison, confirmation and verification of the collected specimens.

## RESULTS AND DISCUSSION

### Documented Diversity of Odonata Species of Riparian Habitats in Selected Areas of Luzon and Mindoro Region

The study surveyed selected riparian habitats in these regions in December 2015 to April 2016. A total of 206 specimens belonging to twenty-eight species in twenty genera (*Agriocnemis*, *Ischnura*, *Pseudagrion*, *Teinobasis*, *Risicnemis*, *Coeliccia*, *Rhinocypha*, *Cyrano*, *Euphaea*, *Neurobasis*, *Heteronaias*, *Diplacodes*, *Neurothemis*, *Orthetrum*, *Pantala*, *Potamarcha*, *Trithemis*, *Brachydiplax*, *Macrodiplax*, and *Zyxomma*) were recorded and seven endemic species of the family Platycnemididae, Euphaeidae, and Calopterygidae were documented in the study sites (Table 1 and 2).

Majority of Anisoptera were found in the low altitude area. Similar results were also found in the study of Medina, Cabras, and Villanueva (2015). This is due to the fact that dragonflies have a robust body size, which allows fast and efficient flight when warmed-up by higher temperatures in the lowlands (van Tol & Gassmann 2007). In addition, they are more often found in open areas where they can receive direct light from the sun for thermoregulation. Consequently, they disperse and distribute widely (van Tol & Gassmann 2007). In contrast, Zygoptera were more commonly found at high altitudes. Due to smaller body size, thermoregulation seems to be maintained by convection, which allows them to occupy shaded areas such as forest (van Tol & Gassmann 2007). The majority of the species collected in this study were anisopterans, since most of the sampling sites were located in open areas and degraded riparian habitats.

Libellulidae was the most diverse family and with the highest number of species being collected (Fig. 3). This cosmopolitan family is considered to be the largest among other families with 1,012 species identified worldwide and 190 species of this family are oriental and most commonly found in disturbed areas (Mapi-ot *et al.* 2013).

Table 1 Species' abundances in different sampling sites in Luzon

	Luzon Region			Total %	
	Boton River	Batalan River, Subic Bay	Orani River		
Altitude	ca. 110 m asl	ca. 65 m asl	ca. 460 m asl		
Land Cover	Disturbed Primary Forest	Secondary Forest	Steep Forested Slope		
<b>Suborder Zygoptera</b>					
Family Calopterigidae					
<i>Neurobasis l. luzoniensis</i> <sup>b</sup>	1	1	9	11	18.0
Family Chlorocyphidae					
<i>Cyano unicolor</i>	-	-	4	4	6.6
<i>Rhinocypha colorata</i>	13	3	5	21	34.4
Family Euphaeidae					
<i>Euphaea refulgens</i> <sup>b</sup>	6	-	4	10	16.4
Family Platycnemididae					
<i>Risio cnemis pulchra</i> <sup>a</sup>	2	-	2	4	6.6
<i>Risio cnemis serrata</i> <sup>a</sup>	-	-	1	1	1.6
<i>Risio cnemis</i> sp. <sup>a</sup>	1	-	-	1	1.6
Family Coenagrionidae					
<i>Pseudagrion p. pilidorsum</i>	-	-	2	2	3.3
<b>Suborder Anisoptera</b>					
Family Corduliidae					
<i>Heteronias heterodoxa</i>	-	-	2	2	3.3
Family Libellulidae					
<i>Diplacodes trivialis</i>	2	-	-	2	3.3
<i>Pantala flavascence</i>	-	1	-	1	1.6
<i>Trithemis aurora</i>	1	-	-	1	1.6
<i>Zyxomma obtusum</i>	-	-	1	1	1.6
Total Number of Individuals	26	5	30	61	100
Total Number of Species	7	3	9		
Total Number of Endemic Species	4	1	4		

Note: <sup>a</sup>Endemic species in Luzon, <sup>b</sup>Endemic species in Luzon and Mindoro

Table 2 Species' abundances in different sampling sites in Mindoro

	Mindoro Region				Total	%
	Lower Baroc River	Tagasakan	Tauga Daka	Tauga Diit		
Altitude	ca. 27 m asl	ca. 410 m asl	ca. 530 m asl	ca. 525 m asl		
Land Cover	Disturbed Farmland	Extensive Farmland	Disturbed Primary Forest	Secondary Forest		
<b>Suborder Zygoptera</b>						
Family Calopterigidae						
<i>Neurobasis l. luzoniensis</i> <sup>b</sup>	-	4	1	18	23	16.1
Family Chlorocyphidae						
<i>Rhinocypha colorata</i>	-	-	4	14	18	12.6
Family Euphaeidae						
<i>Euphaea refulgens</i> <sup>b</sup>	-	-	-	3	3	2.1
Family Platycnemididae						
<i>Coelicia brachysticta</i> <sup>a</sup>	-	4	-	-	4	2.8
<i>Risio cnemis asabina</i> <sup>b</sup>	-	10	4	15	29	20.3

Table 2 (Continued)

	Mindoro Region				Total	%
	Lower Baroc River	Tagasakan	Tauga Daka	Tauga Diit		
Altitude	ca. 27 m asl	ca. 410 m asl	ca. 530 m asl	ca. 525 m asl		
Land Cover	Disturbed Farmland	Extensive Farmland	Disturbed Primary Forest	Secondary Forest		
<b>Family Coenagrionidae</b>						
<i>Agriocnemis f. femina</i>	9	3	-	-	12	8.4
<i>Ischnura senegalensis</i>	2	-	-	-	2	1.4
<i>Pseudagrion p. pilidorsum</i>	-	8	3	2	13	9.1
<b>Suborder Anisoptera</b>						
<b>Family Libellulidae</b>						
<i>Brachydiplax chalybea</i>	-	2	-	-	2	1.4
<i>Diplacodes trivialis</i>	5	-	-	-	5	3.5
<i>Macrodiplax cora</i>	2	-	-	-	2	1.4
<i>Neurothemis ramburii</i>	-	-	-	1	1	0.7
<i>Neurothemis terminata</i>	-	1	-	2	3	2.1
<i>Orthetrum chrysis</i>	-	1	-	-	1	0.7
<i>Orthetrum pruinosum delia</i>	-	1	-	-	1	0.7
<i>Orthetrum sabina</i>	-	-	-	1	1	0.7
<i>Pantala flavascence</i>	-	-	-	2	2	1.4
<i>Potamarcha coengener</i>	-	-	-	-	-	-
<i>Trithemis aurora</i>	-	1	-	-	1	0.7
<i>Trithemis festiva</i>	-	5	3	4	12	8.4
<i>Trithemis festiva</i>	-	6	1	-	7	4.9
<i>Trithemis pallidinervis</i>	-	1	-	-	1	0.7
Total Number of Individuals	18	47	16	62	143	100
Total Number of Species	4	13	6	10		
Total Number of Endemic Species	0	3	2	3		

Note: <sup>a</sup>Endemic species in Mindoro, <sup>b</sup>Endemic species in Luzon and Mindoro

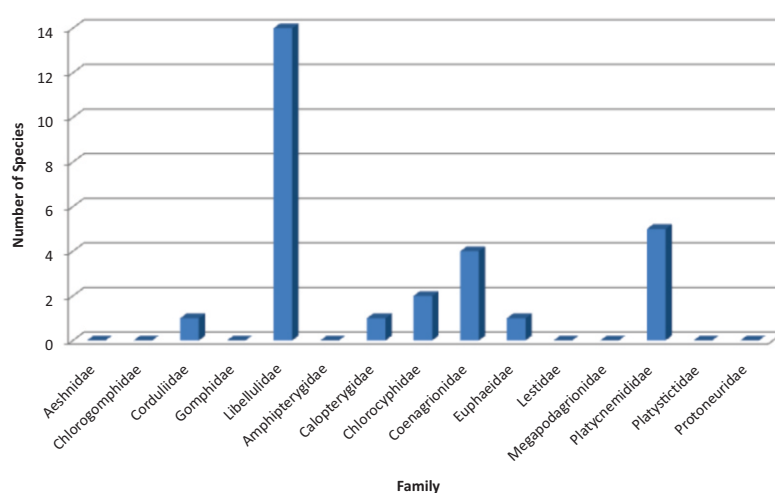


Figure 3 Species records among families occurring in Luzon and Mindoro

Habitats in high altitude host endemic species such as *Risioenemis pulchra* (Hämäläinen 1991) and *R. asabinai* (Kitagawa 1990). Most endemic species of Odonata prefer forested and apparently undisturbed areas. In addition, several species recorded from the islands in focus were not found during the survey, such as *Paracercion luzonicum* (Asahina 1968), *P. malayanum* (Selys 1876), *P. pendulum* (Needham & Gyger 1939), *Sangabasis furcata* (Brauer 1868), *S. bulba* (Villanueva & Dow 2014), *S. janvantoli* (Villanueva & Dow 2014), *R. truconii* (Selys 1981) and *Vestalis mealnia* (Selys 1873). The absence of these previously documented species in the regions might be due to the weather condition during the survey periods. Further surveying on the regions is necessary during more favorable weather conditions to find those species that were historically documented as well as further species that might be new regional records.

From the collected specimens, one species under the family Platynemididae is new to science but additional material and evaluation are needed for a proper scientific description.

## CONCLUSION

Tagaskan River and Taugad Diit at the upper Baroc River Catchment in Mindoro Region had the highest number of species collected consisting mostly widespread orientals. Anisoptera were predominant species found in the two regions because most of the sampling areas were mainly surrounded by open areas and degraded riparian forest. Anisoptera were found in the low altitude area, while Zygoptera were found in high altitude area.

Both regions were diverse areas and species were moderately distributed, although endemism was low. Libellulidae was most diverse family and with the highest number of species being collected, while endemic species such as *Risioenemis pulchra* and *R. asabinai* were found in high altitude due to habitat preferences.

From the collected specimens one species under the family Platynemididae is new to science, but additional collection of the specimen and evaluation are needed.

Previously recorded species from the respective regions were not found during the survey due to unfavorable weather condition affecting the region during the survey and the increased disturbance (e.g. conversion of forest into agricultural land). Further surveys in these sites are necessary to prove whether these taxa are still existent in the areas and to record further rare taxa that were probably missed out. Further surveying on the regions is necessary during more favorable weather conditions. This is especially important to locate the “expected” species and find more material of some interesting species.

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## SEED PRE-COLLECTION ASSESSMENT OF CIBODAS TROPICAL SUBMONTANE FOREST ECOSYSTEM

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### ABSTRACT

Due to integrated approach to conservation and contribution to Target 8 of the Global Strategy for Plant Conservation GSPC, Botanic Gardens of the Indonesian Institute of Sciences (LIPI), particularly Cibodas Botanical Garden (CBG) seed bank, is committed to increase its ex situ plant collection through seed banking. This research aimed to assess population and collect seeds from Cibodas remnant forest and its neighboring area, Mt. Gede Pangrango National Park forest, for seed banking purpose. During September to December 2017, thirty-two number of seeds belong to 15 families were collected, particularly from Rubiaceae (seven species), Arecaceae (five species) and Moraceae (five species). Most of the species have not yet been assessed on IUCN red list except for *Saurania cauliflora* (VU) and *Magnolia blumei* (LC). *Pinanga javana* is currently classified as endangered species by World Conservation Monitoring Unit (WCMC). However, 65% of the population were possible to collect without affecting seed availability in nature, but we also collected a small number of seeds (<500 seeds) and estimated the date to return and collect seeds'. Around half of the seeds were collected from natural dispersal stage to ensure the seeds maturity. Of the collected seeds, 91% are expected to be orthodox seeds and can be stored in Cibodas Seed Bank after being dried, and three species were recalcitrant and cannot be stored in Cibodas Seed Bank i.e. *Calamus ciliaris*, *C. reinwardtii*, *Daemonorops rubra*. Although two species, *Ficus ribes* and *F. variegata*, were already in Millenium Seed Bank (MSB) list, they were stored in Cibodas Seed Bank. Cut-test result showed that 78% seeds collected were all full seeds without infested, empty and immature seeds. The findings of this study proved that seed collecting and banking are needed to save plants under threats in the Cibodas tropical submontane forest ecosystems.

**Keywords:** Cibodas, Millenium Seed Bank (MSB), seed bank, seed collection, seed population assessment

### INTRODUCTION

Tropical forests in Java play a critical role as biodiversity refuge because of land conversion into human settlement. Conservation areas serve as last defense for biodiversity loss. Botanic gardens are one of the main institutions involved in ex situ conservation of wild plant species through its plant collections and seed banking. Seed banking involves collecting seeds from wild plants, drying, and storing them in cool conditions. Seed banks offer solution against threats to plants in situ, including habitat loss and degradation, introduction of alien species, over-exploitation, pollution, disease and climate change (Heywood 2017; O'Donnell & Sharrock 2017).

Due to integrated approach to conservation and contribution to Target 8 of the Global Strategy for Plant Conservation GSPC, Botanic Gardens of the Indonesian Institute of Sciences (LIPI), particularly Cibodas Botanical Garden (CBG) seed bank, is committed to increase its ex situ plant collection through seed banking. To support this ex situ conservation program, LIPI



joined the Millenium Seed Bank (MSB) Partnership in 2016 which focused on seed collecting and banking of Indonesian native plants (Hardwick *et al.* 2017).

This research aimed to assess population and collect seeds from Cibodas remnant forest and neighboring Mt. Gede Pangrango National Park forest for seed banking purpose. The seed collected from this study were stored in Cibodas Seed Bank and duplicated in Bogor Seed Bank for research and restoration purposes, as well as for regular viability testing.

## MATERIALS AND METHODS

### Study Site and Materials

Seeds were collected from Mt. Gede Pangrango National Park and several areas of remnant natural forest in Cibodas Botanical Garden (CBG) from September to December 2017. The study site has annual rainfall of 2,082 mm. Monthly precipitation during study is shown in Figure 1. Climatic data were obtained from Cibodas Botanical Garden Field Station. The seed collection focused on native Indonesian trees and shrubs species was predicted to have orthodox seeds, which are not already present on the Millennium Seed Bank's Data Warehouse Base List. We prioritized species that are endangered, endemic or useful.

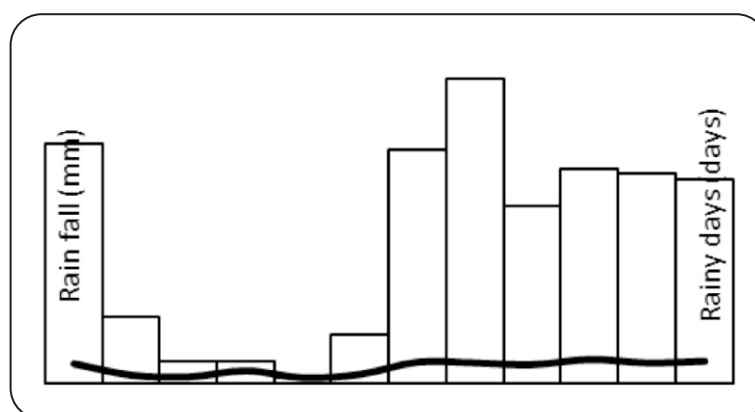


Figure 1 Monthly precipitation in Cibodas

### Pre-collection Assessment

Pre-collection assessment included plant identification, population assessment, and readiness of population for seed collection, seed physical quality check up and seed availability. Cut-test was held to assess seed quality. Ten seeds from several individuals in the population were cut-tested. A record was made on the number of full, empty, infested, and immature seeds. Number of full (viable) seeds for collection were estimated using this formula (Way & Gold 2014):

$$\text{Estimated number of full (viable) seeds for collection} = \text{Maximum number of seed that can be collected} \times \text{Percentage of full seeds}$$

$$\text{Maximum number of seeds that can be collected} = \text{Available population} \times 20\%$$

$$\text{Available population} = \text{Total population} \times \text{Percentage of full seeds}$$

$$\text{Total population} = \text{Estimated number of plants at natural dispersal} \times \text{Average number of fruits or dispersal units per individual plants} \times \text{Average number of seeds per fruits or natural dispersal units}$$

## Seed Collecting

Fully ripe fruits were collected from wild plants. Seeds were collected using different collecting techniques, such as by hand picking, using pole over a tarpaulin, or climbing. Technique used depends on the species and type of dispersal unit. Seeds were harvested using pole (pruner) and secateur at around natural dispersal stage to maximize longevity in long-term storage. Seeds were removed from its fleshy fruits immediately after the field trip to prevent fermented seeds. Collected seeds were stored in cloth bags and immediately transferred to CBG seed bank for drying and processing.

Number of seeds collected was large enough to be held in CBG seed bank. A duplicate of seed collection was stored at Botanic Gardens seed bank. Seeds were also made available for distribution. Ideally, 10,000 healthy seeds were collected around natural dispersal without taking more than 20% of available seeds. All seed collections were accompanied by a fertile (flower or fruit) herbarium specimen, if possible, and field data form, linked to a unique collection number. Herbarium specimen were sent to the herbarium of Research Centre for Biology - LIPI for botanical identification and verification purposes.

## RESULTS AND DISCUSSION

Seed pre-collection assessment was done in 9 locations which covers Mt. Gede Pangrango NP (1,421 to 1,802 m asl) and forest areas inside Cibodas Botanical Garden (1,313 to 1,411 m asl) (Table 1) with 32 seed species found at fruiting period. The floristic composition of the study area is typical submontane tropical rain forest and dominated by *Villebrunea rubescens*, *Ostodes paniculata*, *Strobilantbes hamiltoniana*, *Cyrtandra picta*, *Diplazium pallidum*, and *Calamus reinwardtii* (Mutaqien & Zuhri 2011).

Table 1 Locations of seed pre-collection assessment

Location	Latitude	Longitude	Altitude (m asl)
Mt. Gede Pangrango National Park			
Cibodas	06.74311 S	107.00598 E	1,421
Mandalawangi	06.73105 S	106.99256 E	1,683
Gunung Putri	06.76642 S	107.00295 E	1,802
Cibeureum	06.74837 S	106.99345 E	1,592
Lumut forest	06.74480 S	107.00320 E	1,385
Cibodas Botanical Garden			
Wornojiwo forest	06.74176 S	107.01044 E	1,378
Kompos forest	06.74251 S	107.00648 E	1,356
Jalan akar forest	06.74074 S	107.00563 E	1,411
Cibogo forest	06.74201 S	107.00604 E	1,313

## Seed Pre-collection Assessment

Thirty-two number of seeds belonging to 15 families were found and assessed from forests (Table 2), particularly from Rubiaceae (seven species), Arecaceae (five species) and Moraceae (five species). Most of the species have not yet been assessed on IUCN red list, except for *Saurauia cauliflora* (VU) and *Magnolia blumei* (LC). *Pinanga javana* is currently classified as endangered species by World Conservation Monitoring Unit (WCMC).

A total of 65% of the population were possible to collect without affecting the availability of seeds in nature. However, in this study a small number of seeds (< 500 seeds) were also collected. Date to return and re-collect seeds was also estimated. Multi-year collecting and/or

propagation may be necessary to achieve a good-sized seed collection for such species (Way & Gold 2014). Ideally, 5,000-10,000 healthy seeds were collected around natural dispersal without taking more than 20% of available seeds. The seed collection can cover seeds for several purposes i.e. developing base collection (kept, in case of wild population), developing an effective germination protocol and viability monitoring, implementing viability monitoring over the anticipated 200 year lifespan of the collection, implementing seed collection duplication at another seed bank for safety, implementing seed distribution for users, and developing future propagation and restoration projects.

Population assessment was known by approximating the area of population, number of accessible individual plants, and evidence of damage or disturbance. Population areas were estimated using Google Maps Area Calculator Tools. It was easy to calculate area using a Google maps interface and found the latitude and longitude of a specific point. Population area in this study ranged from 50 to 200,000 m<sup>2</sup>. *Vaccinium korthalsii* and *Schefflera scandens* were presented only in small area with limited number of individuals. Number of plants were estimated after scanning the area and counting the population member. Number of seeds collected were sampled randomly and evenly across the extent of the population to ensure the representative of population (Way & Gold 2014). Damages in study areas were caused by invasion of invasive alien species, especially *Chimonobambusa quadrangularis* and *Strobilanthes laevigatus* in forest adjacent area.

Table 2 Seed pre-collection assessment

No	Species	Population area (m <sup>2</sup> )	Number of plants	Full seeds (%)	Seeds per fruit	Fruits per plant	Estimated number of viable seeds to collect	Collection possible?*
1	<i>Antidesama tetrandum</i>	10,000	51-100	100	1	18,480	40,656	Yes
2	<i>Ardisia fuliginosa</i>	20,000	51-100	100	1	30,360	303,600	Yes
3	<i>Ardisia villosa</i>	40,000	51-100	100	1	32	384	No
4	<i>Boehmeria diversifolia</i>	40,000	11-50	100	50	50	5,000	Yes
5	<i>Calamus ciliaris</i>	10,000	51-100	90	1	246	3,587	No
6	<i>Calamus reinwardtii</i>	10,000	11-50	90	1	197	1,596	No
7	<i>Daemonorops rubra</i>	40,000	11-50	100	1	188	1,880	No
8	<i>Dichroa febrifuga</i>	20,000	1-10	100	50	198	9,900	Yes
9	<i>Ficus ampelas</i>	10,000	11-50	100	46	3,848	221,260	Yes
10	<i>Ficus deltoidea</i>	20,000	11-50	100	4	11	132	No
11	<i>Ficus fistulosa</i>	40,000	11-50	100	696	6,480,000	13,530,240	Yes
12	<i>Ficus ribes</i>	40,000	51-100	100	244	38,016	185,518,080	Yes
13	<i>Ficus variegata</i>	20,000	11-50	100	1,388	1,000	6,940,000	Yes
14	<i>Homalanthus giganteus</i>	40,000	11-50	60	2	19,320	41,731	Yes
15	<i>Lasianthus laevigatus</i>	20,000	11-50	100	1	1,920	7,680	Yes
16	<i>Lasianthus rigidus</i>	20,000	51-100	100	4	160	8,960	Yes
17	<i>Lasianthus</i> sp.	20,000	11-50	70	5	700	10,290	Yes
18	<i>Magnolia blumei</i>	5,000	11-50	100		1,200	2,400	No
19	<i>Medinilla speciosa</i>	40,000	11-50	100	195	8,000	7,800,000	Yes
20	<i>Montanoa grandiflora</i>	40,000	11-50	100	5	100	2,000	No
21	<i>Morinda sarmentosa</i>	10,000	11-50	100	14	2,240	188,160	Yes
22	<i>Mycetia cauliflora</i>	20,000	11-50	100	200	20	9,600	Yes
23	<i>Pinanga coronata</i>	10,000	101-1000	90	1	1,200	48,600	Yes
24	<i>Pinanga javana</i>	40,000	1-10	100	1	6,600	10,560	Yes
25	<i>Psychotria angulata</i>	20,000	11-50	100	2	100	1,600	No
26	<i>Psychotria montana</i>	10,000	51-100	80	2	162	4,147	No
27	<i>Saurania cauliflora</i>	40,000	1-10	100	100	238	47,600	Yes
28	<i>Saurania nudiflora</i>	100,000	1-10	100	758	2,576	3,905,216	Yes
29	<i>Saurania pendula</i>	200,000	51-100	80	240	50	115,200	Yes
30	<i>Schefflera scandens</i>	50	11-50	100	5	65,000	2,600,000	Yes
31	<i>Tetrastigma</i> sp.	10,000	1-10	100	2	160	320	No
32	<i>Vaccinium korthalsii</i>	50	11-50	100	4	100	3,200	No

Note: \* Possibility to collect 5,000-10,000 healthy seeds around natural dispersal area without taking more than 20% of available seeds

Physical quality of seeds were showed by cut-test of 10 randomly selected seeds from the examined seeds. The test indicated that the most frequently occurring seed stages were full seeds, infested seeds, empty seeds, and immature seeds. Generally, a full seed is firm and white inside, not shrivelled or overly dry. Empty seeds contained little or no seed tissue inside and may even have evidence of insect damage or an aborted seed. Twenty five seed species were consisted of 100% full seeds and the others were empty and immature seeds. There were no mouldy and infested seeds in the cut-test result. Mouldy seeds were consisted of dead and necrotic seeds, and often seeds having brown tissue. Infested seeds showed evidence of insect predation. Cut-tests are subjective and although most people will obtain similar results, interpretations may vary greatly (Kolotelo 1997).

Readiness of population for seed collection is shown by the most frequently occurring stages. Seeds must be collected at the optimum stage of development to maximize longevity in long-term storage. Most seeds in the process of natural dispersal are suitable for collection and showed by changes in fruit color, changes in seed coat color, fruits splitting or breaking open, seeds rattling, seeds that are hard and dry, and some seeds already dispersed (Way & Gold 2014). Four species was found in full natural dispersal stages, i.e. *Boehmeria diversifolia*, *Dichroa febrifuga*, *Ficus deltoidea*, *F. fistulosa*, and *Saurauia cauliflora* (Fig. 2). For population which seeds have not yet reached natural dispersal stage, an estimated date was set up to return and collect seeds, such as *F. ribes* and *Pinanga javana* which were still in the immature seeds stage when the seeds collection was conducted.

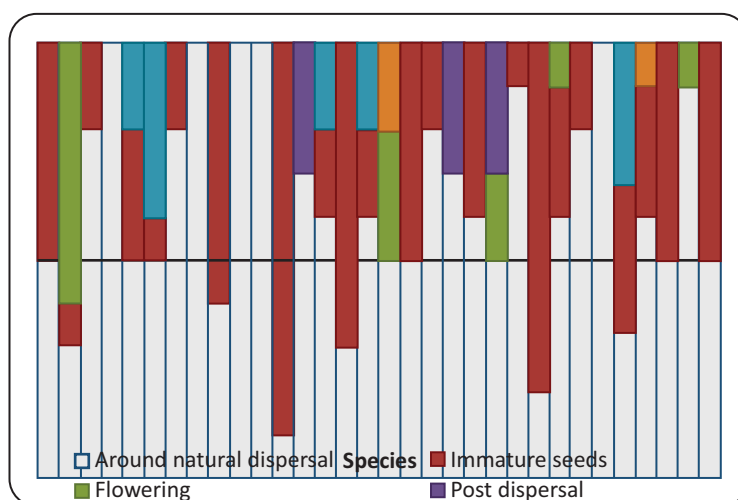


Figure 2 Readiness of population for seed collection; species number refer to Table 1

### Seed Collection

All seed species in the list of seed pre-collection assessment (Table 1) were collected and stored in Cibodas Seed Bank. Even though 11 species were not possible to collect at an ideal number of seeds, it is still collected and estimated date was set to return and re-collect of seeds. Three species were recalcitrant after being checked in Seed Information Database of Kew Royal Botanic Gardens (<http://data.kew.org/sid/>) and cannot be stored in Cibodas Seed Bank i.e. *Calamus ciliaris*, *C. reinwardtii* and *Daemonorops rubra*. Recalcitrant seeds need to be used immediately after collection or they may die. Moreover, recalcitrant seeds are shed at high water content, desiccation sensitive and cannot be stored under conditions conventionally employed for desiccation-tolerant orthodox seeds (Baskin & Baskin 2001; Pammenter & Berjak 2013).

Meanwhile, 91% collected seeds are expected to be orthodox seeds and can be stored in Cibodas Seed Bank after being dried. Orthodox seeds can be dried up to 2-5% without losing viability and tolerate relatively to long storage periods, and hence have inherent primary seed dormancy. Moreover, orthodox seeds are seeds that acquire desiccation tolerance during development, can be dried to low water contents (generally less than 5%), and retain viability in the dry state for predictable periods (Pammenter & Berjak 2000; Baskin & Baskin 2001; Mng'omba 2007).

Most of the collected seeds (37.5%) were from trees consisting of 12 species i.e. *Antidesma tetrandum*, *Ardisia fuliginosa*, *Ficus ampelas*, *F. fistulosa*, *F. ribes*, *F. variegata*, *Homalanthus giganteus*, *Lasianthus* sp., *Magnolia blumei*, *Saurauia cauliflora*, *S. nudiflora* and *S. pendula* (Table 3). It is in accordance with the study which focused on collection of native Indonesian trees and shrubs. Besides, the other life forms occurred in the study area were epiphytic treelet, liana, tree palm, and woody climber.

Table 3 Seed species collected based on life form

Life form	Number of seed species	
Tree	12	37.50%
Shrub	10	31.25%
Herb	0	0%
Epiphytic treelet	4	12.50%
Liana	1	3.12%
Tree palm	2	6.25%
Woody climber	3	9.37%
Total	32	

Five species of figs i.e. *F. ampelas*, *F. deltoidea*, *F. fistulosa*, *F. ribes* and *F. variegata* were collected from forest patch areas of Cibodas Botanical Garden and interior forest of Mt. Gede Pangrango National Park. Seeds of subgenus *Ficus* have a sticky mucilaginous coat i.e. when this material dries, seeds become attached to the surface of tree branches, leaves, soil, rocks and so on (Baskin & Baskin 2001). Fig seeds are orthodox, and large number of seeds were located inside its syconium. Moreover, fig seeds can remain dormant for a long period of time, at least several years, in dry and cool (artificial) conditions (Berg & Corner 2005). The most common seed was *F. variegata*, the edible fig, which reach more than 1,000 minute seeds/fruit.

Three species of *Lasianthus* were present in the field i.e. *L. laevigatus*, *L. rigidus*, and *Lasianthus* sp. *Lasianthus* plants were encountered frequently along line track with number of fruits per plant for *L. laevigatus* i.e around 1,900 fruits. Genus *Lasianthus* has 4-9 locular ovaries and a single, erect, basal ovule in each locule, which normally develops into a drupe with 4-9 pyrenes (Zhu 2015; Backer & van den Brink 1965). *Lasianthus* species are usually present in large numbers in the tropical forests of Asia and may therefore, represent an ecologically important element and also show interesting distribution patterns (Zhu 2002; Zhu *et al.* 2012).

## CONCLUSION

Thirty-two number of seeds belonging to 15 families were collected, particularly Rubiaceae (seven species), Arecaceae (five species) and Moraceae (five species). Most of the species have not yet been assessed on IUCN red list except for *Saurauia cauliflora* (VU) and *Magnolia blumei* (LC). *Pinanga javana* is currently classified as endangered species by World Conservation Monitoring Unit (WCMC). A total of 65% population were possible to collect without affecting the availability of seeds in nature However, a small number of seeds (<500 seeds) were also collected. Date to return and re-collect seeds was also estimated. Around half of seed collection

were collected from natural dispersal stage to ensure the seeds maturity. A total of 91% collected seeds are expected to be orthodox seeds and can be stored in Cibodas Seed Bank. Cut-test result showed 78% seed collected were all full seeds without infested, empty and immature seeds. The findings of this study proved that seed collecting and banking are needed to save plants under threats in the Cibodas tropical submontane forest ecosystems.

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## REVISITING THE TAXONOMIC POSITION OF *Collybia reinakeana* P. Henn. FROM THE PHILIPPINES THROUGH ITS MOLECULAR PROFILE

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### ABSTRACT

*Collybia reinakeana* P. Henn is an indigenous wild edible fungi with a notably aggregate fruiting bodies. Classical identification of this species was argued belonging to other genera. Thus, this study aimed to identify taxonomic position of *C. reinakeana* collected from different research areas such as Agoo, Bataan, Bicos, Binalonan, Dipaculao, San Roque and Urdaneta. The pure culture of the fruiting bodies of each strain was prepared using standard laboratory procedure. The DNA extraction was done following the manufacturer's protocol. Internal Transcribed Spacer (ITS), ITS5F and ITS4R primers were utilized in the amplification of the extracted samples. Basic Local Alignment Search Tool (BLAST) was employed in the species molecular authentication, while Molecular Evolutionary Genetics Analysis (MEGA) version 6.06 software was used to recognize relatedness of the species. The BLAST search of *C. reinakeana* strains revealed that 99% of *C. reinakeana* is homologous to genera *Macrocybe* and *Calocybe*. Based on the BLAST search and Maximum Likelihood tree topology, none of the samples of *C. reinakeana* were matched and grouped on its known genus *Collybia*. Therefore, further and thorough analysis must be conducted to establish the identity of the species *Collybia reinakeana*.

**Keywords:** *Collybia reinakeana*, DNA extraction, internal transcribed spacer, molecular authentication

### INTRODUCTION

*Collybia reinakeana* P. Henn was first documented in Barangay Puncan in the town of Caranglan, Nueva Ecija Province, Philippines. This mushroom is a saprophyte with huge and thick basidiocarp that can weigh up to 5,965 grams, Furthermore, it is economically valuable, being edible and with nutraceutical properties (Reyes *et al.* 1997, 2010).

*C. reinakeana* belongs to the family Tricholomataceae which is described as a taxonomic depository group of pale-spore, gilled mushroom. Nowadays, the emergence of molecular authentication using DNA sequences leads to the construction of other families in Basidiomycetes like the Omphalotaceae, Phasalacriaceae, Marasmiaceae and Mycenaceae and even genus under this group split into other genera (Kuo 2015). For instance, in the study of Pegler *et al.* (1998) seven associated species of genus *Tricholoma* were designated into a new genus *Macrocybe*. Likewise, through the aid of DNA analysis, the species under the genus *Collybia* were transferred to other genera (Kuo 2013).

The complexity of the description in the family Tricholomataceae including the genera *Tricholoma* and *Collybia* led the researchers to conduct molecular authentication to confirm the identity of *C. reinakeana*.

## MATERIALS AND METHODS

### Sample Collections and Preparation

Seven strains of *C. reinakeana* were collected from the towns of Agoos, Binalonan, Dipaculao, San Roque and Urdaneta. The secondary mycelia obtained from the fresh fruiting bodies were rescued conforming the standard protocol of mycelial isolation (Reyes *et al.* 2009; Kalaw *et al.* 2016). The pure cultures were kept in a petri dish in a Potato Dextrose Agar (HiMedia Laboratories Pvt. Ltd., India) medium.

### DNA Extraction, Amplification, Purification and Sequencing

DNA extraction was carried out following the Machery Nagel microbial DNA extraction kit standard protocol. The Internal Transcribed Spacer (ITS) 5 forward and 4 reverse primers were used in the amplification and sequencing of samples. The positions of ITS5F and ITS4R in the nuclear ribosomal DNA are presented in Figure 1. Polymerase Chain Reaction (PCR) amplification was done using the following concentrations (25 $\mu$ L): 16.3 $\mu$ L of molecular bio. H<sub>2</sub>O, 2.5 $\mu$ L of 10 x buffer, 1 $\mu$ L MgCl<sub>2</sub> (25mM), 1.0 $\mu$ L dNTP mix, (10 mM), 1.0 $\mu$ L of each primer (8  $\mu$ M), 0.2 units of Taq polymerase (Accutag) and 1 $\mu$ L Genomic DNA. The reactions were attained using BIO-RAD Thermal Cycler with the following cycling parameters: starting denaturation at 94 °C for 5 minutes, followed by 94 °C for 30 seconds, 50 °C for 45 seconds in annealing of primers, 72 °C for 1 minute in primer extension and the final extension at 72 °C for 10 minutes and completed with 30 cycles. PCR products were subjected to agarose gel electrophoresis (Arenas *et al.* 2017). While the PCR products purification was done following the manufacture's instructions (Machery Nagel microbial purification kit) and sent to MACROGEN, South Korea for DNA sequencing.

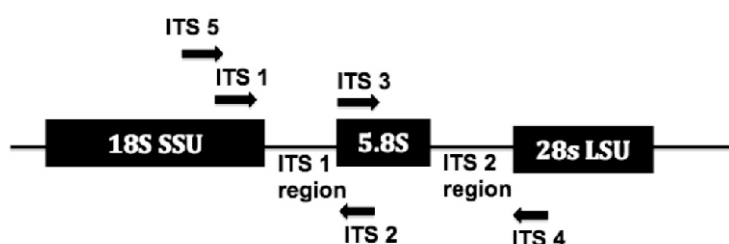


Figure 1 Position of ITS5 and ITS4 primers in the nuclear ribosomal DNA which are utilized in the amplification of ITS region (Arenas *et al.* 2017)

### Data Analysis

The Basic Local Alignment Search Tool (BLAST) was used to analyze the nucleotide sequences for percent identity of each sample. Alignment of sequences was achieved on Muscle in the MEGA 6.0 software. The species resolution was analyzed through the construction of a Maximum Likelihood Phylogenetic tree in Kimura 2- parameter (K2P) distances.



## RESULTS AND DISCUSSION

The nucleotide sequences generated were all subjected to the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) to match the identity of seven strains nucleotide sequences. BLAST homology of seven strains nucleotide sequences displayed 99% homologous to *Tricholoma giganteum* and *Macrocybe gigantea*. The results showed that none of the seven strains of *C. reinakeana* matched with the genus *Collybia*.

The maximum likelihood (ML) phylogenetic topology tree of nucleotide sequences of seven *C. reinakeana* strains revealed that the strain from Bataan, Dipaculao and Urdaneta group with *Macrocybe gigantea*. While the other strains from Agoos, Bicos, Binalonan, and San Roque group with *Calocybe indica*. The molecular authentication of seven strains *C. reinakeana* confirmed that the strains belong to Genus *Macrocybe* and *Calocybe*. The ML phylogenetic topology tree is shown in Figure 2.

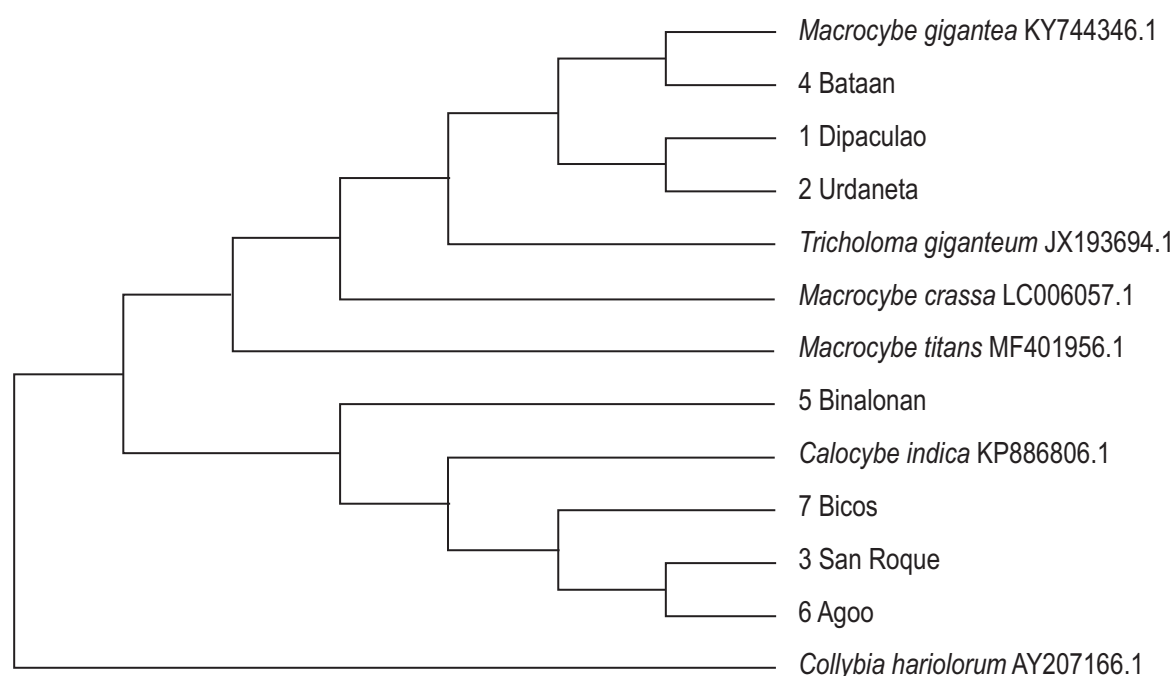


Figure 2 Resolution of species using Maximum Likelihood Phylogenetic tree

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## TYOLOGY OF CAPTIVE BREEDING IN LESSER BIRD-OF- PARADISE (*Paradisaea minor* Shaw, 1809)

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### ABSTRACT

Lesser bird-of-paradise (*Paradisaea minor* Shaw, 1809) is an endemic bird species in Papua. The population of this species experiences annual decrease due to illegal hunting, illegal trading and habitat destruction. Nowadays, lesser bird of paradise ex situ conservation in Indonesia has not succeeded, especially in breeding effort. Several efforts in ex situ conservation have been conducted in Indonesia such as the establishment of zoo and bird parks, however, those establishments lacked breeding success. Typology is needed to determine the key factor that supports successful breeding. This research aimed to identify, analyze, and formulate typology determining components to support successful breeding in a lesser bird of paradise. This research was conducted from January to March 2018 in Taman Mini Indonesia Indah (TMII) Bird Park, Mega Bird and Orchid Farm (MBOF) Bogor, and Al Walbra Wildlife Preservation. Kaiser Mayer Olkin (KMO) test result showed that the value of analysis was 0.682. The significance of Bartlett test of sphericity was 0.001. The value of representation was 79.981%. There are five variables that become the principal factors to determine the typology supporting successful breeding, such as age, diet, facility of cage, nest and keeper ability.

**Keywords:** Breeding, conservation, ex situ, lesser bird-of-paradise, typology

### INTRODUCTION

Lesser bird-of-paradise (*Paradisaea minor* Shaw 1809) is confined in Misool Island, the Bird's Head and Neck, Northwest New Guinea Lowlands, Sepik-Ramu, and northern scarp of Huon east at least to Singarokai (Beehler & Pratt 2016). Lesser bird-of-paradise is a well known, sexually selected, radiation of species, known for their bewildering diversity of courtship behaviors and exotic plumages (Irestedt *et al.* 2009; Stavenga *et al.* 2011). Nowadays, this species has been threatened by illegal hunting, illegal trading and habitat destruction. Even ex situ conservation of lesser bird-of-paradise in Indonesia has not succeeded in captive breeding, especially for successful breeding effort. Nevertheless, the success of breeding in ex situ conservation is rare. Ex-situ conservation is needed to preserve the existence of this species. Several efforts in ex situ conservation have been conducted in Indonesia such as establishing zoo and bird parks (Sulandari & Zein 2012), but those efforts are not successful because only a few numbers were successful in captive breeding.

Typology is needed to determine the key factor that supports successful breeding. This typology has several indicators that must meet a number of scientific and practical criteria, for example they must be quantitative, responsive to changes, representative of daily activity of the bird, practical in terms of data collection, and easily understood. The more these requirements are fulfilled, the more effective an indicator will be (van Strien *et al.* 2009). A typology is as a base information and base management to increase the population of lesser bird-of-paradise and as an effort to support a successful ex situ conservation. The aims of this research were to identify, analyze, and formulate typology determining components to support successful breeding in a lesser bird-of-paradise.

## MATERIALS AND METHODS

This research was conducted from January to March 2018 in Taman Mini Indonesia Indah (TMII) Bird Park, Mega Bird and Orchid Farm (MBOF) Bogor, and Al Walbra Wildlife Preservation. Tools and equipment used were tallysheet, stationery, dry wet thermometer, camera, gauge, computer with SPSS 23 software, and lesser bird of paradise.

## RESULTS AND DISCUSSION

Kaiser Mayer Olkin (KMO) test result showed that the value of analysis was 0.682. The significance of Bartlett test of sphericity was 0.001. The value of representation was 79.981%. The matrix of component from this research showed that there are three components supporting the success of ex situ conservation (Table 1).

Table 1 Matrix of component to determine the success of captive breeding of *Paradisaea minor* based on Kaiser Mayer Olkin (KMO) test

Variable	Component		
	1	2	3
Age	.693	-.098	.145
Adaptation	-.203	.560	-.017
Disturbance	.236	.381	.549
Diet	.596	.401	.259
Size of cage	-.132	.364	.538
Facility of cage	.769	-.217	.439
Construction of cage	-.157	-.671	-.125
Nest	.806	-.048	.016
Health and care	.467	.289	-.748
Availability of specialist	-.387	.069	.497
Keeper ability	.541	-.057	-.235
Fund	-.456	.671	-.435

Results of Principal Component Analysis (PCA) showed that there are three determinant factors to formulate typology to support successful breeding. There are five variables that become the principal factors to determine the typology supporting successful breeding, such as age, diet, facility of cage, nest and keeper ability (Table 1).

Sexual maturity age is the most important variable to determine successful breeding. Sexual maturity age is reached when a bird is ready for mating indicated by the time when a bird forms a couple (a sexual companionship) and shows a mating activity; while copulation is used as an indicator of sexual maturity (Todd & Berry 1980). In some birds-of-paradise, males may take up to seven years to obtain full adult plumage. Maturity age is important because it can influence the performance of 'correct' courtship displays. Several females will choose plumed males who can perform the correct courtship (Laska *et al.* 1992). Male birds-of-paradise have evolved extravagant ornamental traits, with intricate sounds and ritualized sets of dance steps in courtship and movements accompanied by simultaneous elaborate feather movements, all combined in beautiful displays to win the favor of females (Wilts *et al.* 2014).

Diet is a source of energy to support the daily activity of the lesser bird-of-paradise, especially for breeding (Gilliard 1969). Adult birds in captive are offered a varied diet of mixed fruit, greens (chopped endive occasionally mixed with chopped spinach), meat and live food with supplements (Todd & Berry 1980). The diet should be cleaned from parasites. Parasitism of

critical organs such as the kidneys, which have such a limited ability for regeneration, could be a significant cause of morbidity in some species (Unwin *et al.* 2013).

Cage is a habitat for bird in captivity. Breeding success depends on cage size and facility. Cage must be similar with the natural habitat condition, so enrichment in cage is the key factor to support the correct courtship and the successful breeding (Citra *et al.* 2016). Cage size and facility (including plants in the cage) is a determinant factor to support a successful breeding and rearing. Cage size is important factor to support bird's health, so that bird can move actively like in natural habitat (Searle 1979). One example of proper bird cage is the bird cage at TMII Bird Park (Fig. 1).

Type of nest is influenced by genetic factor, nest component, place of nest, experience, and ability of female (Jiang *et al.* 2017). Nest component is the most important facility of the cage to support the success of breeding. The nest was a bowl-shaped structure with an exterior diameter of ca 150 mm and an interior diameter of ca 110 mm. It was made of entwined supple roots, vines and leaves, lined with an extensive mat of thin, wire-like fern stems. A live epiphytic orchid and live fern were part of the structure of the cup (Davis & Beehler 1994).

Bird in captivity depends on the management from an animal keeper (Todd & Berry 1980). Therefore, animal keeper needs a broad knowledge about bioecology of lesser bird-of-paradise, especially about animal welfare, so that the bird in captivity can behave normally like in natural habitat (Hammer *et al.* 2008), including the bird's breeding behavior.



Figure 1 Cage of Lesser Bird-of-Paradise's at TMII Bird Park

## CONCLUSION

PCA results showed that there are five variables that become the principal factors to determine the typology supporting successful breeding, such as age, diet, facility of cage, nest and keeper ability. Kaiser Mayer Olkin (KMO) test result showed that the value of analysis was 0.682. The significance of Bartlett test of sphericity was 0.001.

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## SHIFTING DISTRIBUTION OF LEAFMINER FLIES *Liriomyza* spp IN ALTITUDINAL CORRIDOR AND ITS RELATION TO TEMPERATURE CHANGES

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### ABSTRACT

Leafminer flies has been considered as important pests in some vegetables in Indonesia. This research aimed to assess the change of distribution pattern of leafminer, *Liriomyza* in an altitudinal corridor and its relation to temperature changes, because it can be used as a preliminary information for pest control management for national food security. Survey was done in 2011 in different elevations from lowland to highland (0-2100 m asl). Sampling of imagos was carried out in 100 m elevation interval in the morning (4.5 hours) and afternoon (3 hours) for each location. The most severe attack symptom was found in pea beans. However, most imagos emergence were found in red beans. Comparisons with distribution data in 2007 and 2008 indicated that elevational shifting distribution occurred in *Liriomyza huidobrensis* and *L. sativae* in which *L. sativae* known as “warm species” colonized higher altitude and found in 1600 m asl. Temperature as an indication of the shifting distribution is discussed.

**Keywords:** Altitude, distribution, *Liriomyza* spp., temperature, vegetables

### INTRODUCTION

Leafminer *Liriomyza* spp. are important invasive insects in most of the world. This insect invaded Africa from 1976, Europe in 1973, and Asia in 1990's (Bartlett & Powell 1981; Spencer & Steyskal 1986; Rauf 1995; Abe & Kawahara 2001; He *et al.* 2002). The insects were found and reported for the first time in Indonesia i.e. in Puncak area (Rauf 1995), but now it spread to almost all vegetables crops –(van de Fliert *et al.* 1999).

*Liriomyza* spp are an important pests because they can cause great damage to crops, which is difficult to manage. The damage on leaves is caused by the diet habit of larvae and imago. Parella (1987) stated that the vegetable's damage by *Liriomyza* can cause (1) the leafminer's role as disease vector, (2) breaking of the seedlings, (3) declining of the crops, (4) the shedding of fastened leaves, (5) decreasing of the aesthetic value, and (6) strict vegetables and fruit trading regulation among countries.

Several researches have been done to observe and show many aspects of *Liriomyza*, such as biology (Parrella 1987; Pettitt & Wietlisbach 1994; Supartha 1998; Lanzoni *et al.* 2002; Rifai 2003; Facknath 2005), genetics-taxonomy (Pettitt & Wietlisbach 1992; Scheffer 2000; Scheffer & Lewis 2001; Reitz & Trumble 2002; Shiao 2004; Scheffer & Lewis 2005; Zheng *et al.* 2007), ecology (Shepard *et al.* 1998; Reitz & Trumble 2002; Patel *et al.* 2003; Salvo & Valladares 2004), and the development of management methods (Murphy & LaSalle 1999; Weintraub 2001; Prijono *et al.* 2004; Hidrayani *et al.* 2005). The observation indicated that temperature was the limiting factor on the distribution of the dominant species of this groups i.e. *Liriomyza huidobrensis* and *Liriomyza*

*sativae*. *L. buidobrensis* invades colder area, such as high altitude and subtropics areas, whereas *L. sativae* is commonly found in warmer habitat (Rauf *et al.* 2000; Zhao & Kang 2000; Chen *et al.* 2003; Kang *et al.* 2009). However, so far there is no research showing the shifting distribution of *Liriomyza* spp in relation to temperature change. Therefore, we investigated the shifting distribution of *Liriomyza* spp. on altitudinal corridor in relation to temperature change. This information can be used as a preliminary step to manage national food security.

## MATERIALS AND METHODS

Research was carried out in Dieng mountains which includes six regencies, Banjarnegara, Wonosobo, Pekalongan, Purbalingga, Batang, and Temanggung. The observation of leafminer distribution was done by implementing direct collection and counting the emerged imago from the leaves that were collected. The surveys were done twice on March and July 2011 in three lines (Wonosobo, Batang and Banjarnegara) with altitude interval of 100 m asl. There were 50 sampling points along the three lines from 0-2100 m asl (Tabel 1).

### Direct Collection

Leafminer imagos were collected using vial with diameter of 18 mm and length of 150 mm. The collection was done in the morning (06:30-11:00) and afternoon (15:00-18:00). The periods of time were selected because they were the active time periods for the leafminer. The collected imagos were put in 70% ethanol in separate vials. Imagos were carried to Entomology Laboratory, Zoology Division, Research Center for Biology, LIPI for identification and counting purposes.

### Infected Leaves Collection

At each sampling location, infected leaves collection was done on all vegetables and weeds. We collected 50-100 infected leaves. Vegetables with broad leaf such as cucumber, cabbage, and loofa, were only collected in small amount compared to vegetables with narrow leaf, like potato, red bean and pea. Sampling on infected leaves was done on 24 host plants (Table 1).

The infected leaves were brought to the laboratory. The leaves were put in plastic glass container. Tissue paper was placed at the bottom of the glass container to keep the humidity and to prevent rapid decay of the leaves sample. The sample was kept for 4-6 weeks until all the imagos hatched and died. The imagos were then identified and counted under microscope. Afterwards, the imagos were put in vial submerged in 70% ethanol.

### Analysis

Data from the two surveys were combined because data from the first survey was insufficient to be analyzed independently. Data insufficiency was caused by ants attack on the sample, due to unprepared deposit room.

Distribution data this year were compared with the data from 2008 (Tantowijoyo 2008). Infected preference was analyzed by comparing the amount of imagos that hatched on each leaf with ANOVA 5% and advanced analysis was done with Duncan test.



## RESULTS AND DISCUSSION

Out of 24 host plants, the most infectious leaf was found in pea and the least was on eggplant and chili. In general, *Liriomyza* host in lowland were dominated by long bean and cucumber, whereas potato and pea dominated in highland.

Table 1 Locations, imagos and collected host plant species

No	Location			Altitude (m asl)	Imago	Host plant
	Village	District	Regency			
1	Cipawon	Bukateja	Purbalingga	92	8	Long bean, cucumber
2	Tulis	Bandar	Batang	99	0	Cucumber
3	Adipasri	Rakit	Banjarnegara	179	4	Long bean
4	Karanggedang	Bukateja	Purbalingga	138	4	Long bean, loofa
5	Rakit	Rakit	Banjarnegara	159	4	Weed, eggplant, cucumber
6	Badaminta	Rakit	Banjarnegara	213	0	Weed, eggplant, cucumber
7	Simpur	Bandar	Batang	254	0	Chinese cabbage
8	Cirit	Madukara	Banjarnegara	326	7	Lentils, loofa
9	Dawuhan	Madukara	Banjarnegara	330	4	Spinach, Chinese spinach, long bean
10	Pucanggading	Bandar	Batang	379	0	Cucumber, long bean
11	Selokromo	Selomerto	Wonosobo	491	0	Long bean
12	Tunggara	Sigaluh	Banjarnegara	413	0	Cucumber
13	Maduretno	Kertek	Wonosobo	738	6	Spring onion, bok choy, eggplant
14	Semayu	Kertek	Wonosobo	711	6	Brokoli, cucumber, tomato
15	Kenjer	Kertek	Wonosobo	808	26	Long bean, tomato
16	Kalianget	Wonosobo	Wonosobo	711	31	Mung bean, long bean, eggplant, tomato
17	Sindangsari	Garung	Wonosobo	977	0	Bok choy
18	Karangkobar	Karangkobar	Wonosobo	1080	15	Chilli, tomato
19	Kalijeruk	Garung	Wonosobo	1088	43	Mung bean, pea, cabbage, bok choy
20	Kuripan	Garung	Wonosobo	1188	5	Chinese spinach, weed
21	Madukara	Kertek	Wonosobo	1182	0	Weed, pea, Chinese cabbage
22	Pesantren	Wanayasa	Banjarnegara	1140	3	Mung bean, sweet potato
23	Purwajiwa	Batur	Banjarnegara	1140	3	Potato
24	Balun	Wanayasa	Banjarnegara	1164	13	Mung bean, pea
25	Candiyasan	Kertek	Wonosobo	1261	14	Chinese spinach
26	Gumelar	Garung	Wonosobo	1206	12	Weed, long bean, potato, cabbage, cucumber, tomato
27	Krincing	Kledung	Temanggung	1247	13	Cabbage, Chinese cabbage
28	Seruni	Wanayasa	Banjarnegara	1215	65	Mung bean, weed, tomato
29	Sirukem 1	Kalibening	Banjarnegara	1256	0	Chinese cabbage
30	Sirukem 2	Kalibenning	Banjarnegara	1323	0	Potato
31	Telahap	Kledung	Temanggung	1293	56	Weed, long bean, pea
32	Banyu urip	Kertek	Wonosobo	1340	23	Weed
33	Jatilawang	Wanayasa	Banjarnegara	1358	0	Faba bean
34	Kledung	Kledung	Temanggung	1388	0	Spinach, mung bean, bok choy, weed, potato, cabbage
35	Penusupan	Pajawaran	Banjarnegara	1388	9	Chinese cabbage, tomato
36	Rejosari	Kejajar	Wonosobo	1335	44	Mung bean, weed, cabbage, Chinese cabbage, celery, tomato

Table 1 (Continued)

No	Location			Altitude (m asl)	Imago	Host plant
	Village	District	Regency			
37	Wanaraja	Wanayasa	Banjarnegara	1332	78	Weed, potato
38	Grogol	Pejawaran	Banjarnegara	1407	25	Weed, potato
39	Kejajar	Kejajar	Wonosobo	1454	57	Pea
40	Ratamba	Batur	Banjarnegara	1403	31	Mung bean, pea
41	Gerlang I	Bandar	Batang	1597	23	Weed, faba bean, pea, red bean, potato
42	Tembok	Pejawaran	Banjarnegara	1575	53	Faba bean, pea, red bean, potato
43	Pasurenan	Batur	Banjarnegara	1634	46	Weed, red bean, potato
44	Tieng	Kejajar	Wonosobo	1634	37	Long bean, potato
45	Gerlang 2	Bandar	Batang	1753	0	Faba bean, potato
46	Buntu	Batur	Banjarnegara	1957	2	Faba bean, pea, celery
47	Kradenan	Bandar	Batang	1971	13	Potato
48	Parikesit	Kejajar	Wonosobo	1937	6	Faba bean, potato
49	Dieng	Kejajar	Wonosobo	2078	40	Weed, faba bean, potato, celery
50	Tieng	Kejajar	Wonosobo	1671	0	Potato

Based on host plant species, number of imagos were significantly different ( $F=10.34$ ;  $df=9.292$ ;  $p=0,00$ ). The highest number were obtained from red bean, whereas the lowest were from long bean leaf (Fig. 1).

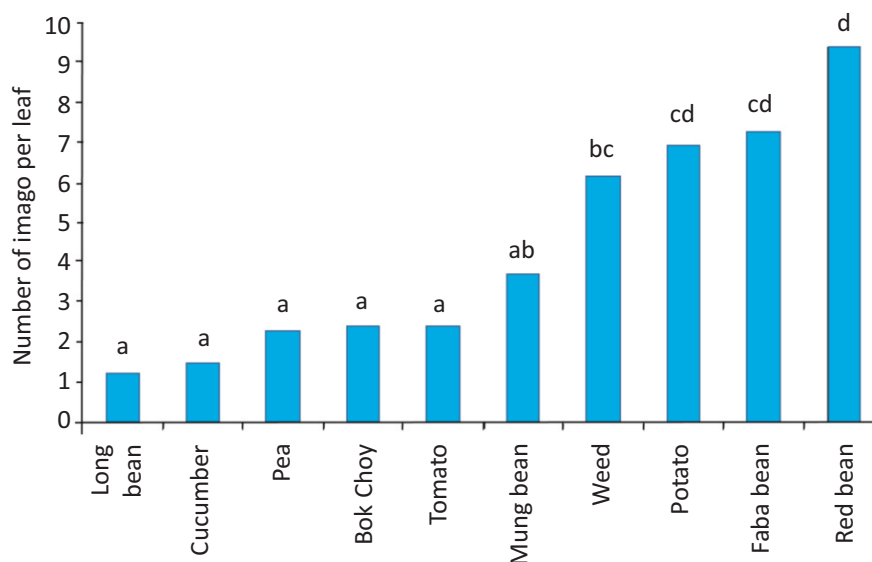


Figure 1 Number of emerged imagos from infected leaves collection on various species of host plants. Different letters above the bar chart indicates significant difference on Duncan test 5%

The difference in imago numbers could be used as indication of leafminer *Liriomyza* preference. This is also an evidence that the smaller leaf as in red bean has higher number of imagos compared to broader leaf as in cucumber (Fig. 1). Leafminer preference was not affected by area, but more by epidermis thickness and trichome presence (Wei *et al.* 2000). Leafminer prefers leaf with thick epidermis, but with less trichome. *Liriomyza* does not like cucumber, which has thick trichomes and thinner epidermis, relatively to nuts.

Comparing distribution data from 2007-2008 and 2011m (Tantowijoyo 2008; Tantowijoyo & Hoffmann 2010), there are shifting distribution of *L. huidobrensis* and *L. sativae* (Fig. 2). *L. sativae* was indicated to invade habitat in higher altitude. In 2007-2008, *L. sativae* only dominated habitat located in altitude lower than 600 m asl. But, in 2011, this species has dominated higher elevation, above 1,000 m asl. The real composition was found in 1,600 m asl, where in the year of 2007-2008, in this habitat, there was only *L. huidobrensis*.

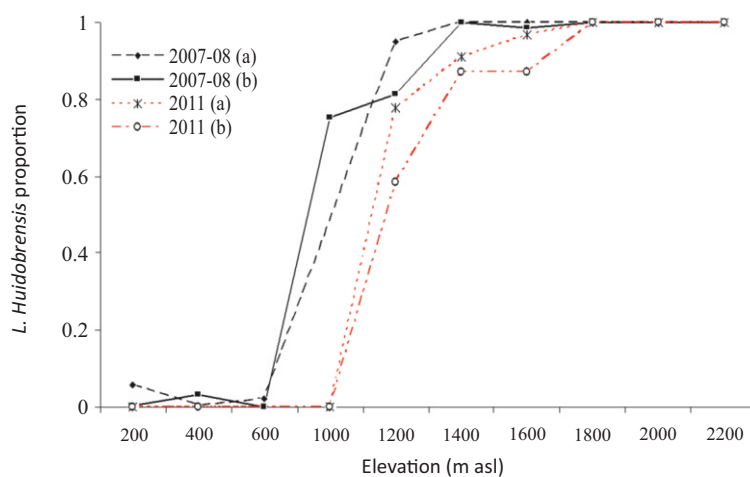


Figure 2 Composition of *L. huidobrensis* and *L. sativae* at different elevations based on 2007-2008 and 2011 distribution data that were obtained from direct collection (a) and infected leaf (b). Number close to 1 indicated that *L. huidobrensis* is dominant to *L. sativae* and vice versa

Shifting distribution of these two leafminer species is suspected to be triggered by temperature rise. However, this inference needs to be further verified by collecting more data. Our findings only showed that *L. sativae* distribution moved to higher elevation, sharing area with *L. huidobrensis*, but infected different host plants. Possibly, there were slightly temperature changes in the area that made *L. sativae* survived in low temperature at higher altitude. This can indicate that the most frequent stresses that confronting insects is mild temperature hardening (Huang *et al.* 2007). Besides, the host plants of *L. sativae* were planted continuously from low to high elevation in 2011. This could be another reason, *L. sativae* can be found in high altitude. *L. sativae* is “warm species” that the origin habitat and distribution apt to be in habitat with warm temperature. Moreover, this species also can be put as “opportunistic survival” insect as Bale’s classification (1996) on the limit of insect cold hardiness. They cannot survive to have a normal metabolic activity on very low temperature (Zhao & Kang 2000). However, in mild cold area, they can overcome the hardiness by an opportunistic behavioral response, such as making an infestation to the same vegetable plants that were planted in higher elevation. Thus, its metabolism made an

adaptation to the surrounding weather. On the contrary, *L. huidobrensis* is “cold species” that apt to invade habitat in colder temperature (Haghani *et al.* 2007; Huang *et al.* 2007; Tantowijoyo 2008; Kang *et al.* 2009; Tantowijoyo & Hoffmann 2010). This species cannot survive in high temperature probably because they have low heat resistance (Huang *et al.* 2007).

## CONCLUSION

*Liriomyza* spp. host plants in lowland were dominated by long bean and cucumber, meanwhile in highland were dominated by potato and pea. The highest number of imago was obtained from red bean, whereas the lowest number of imago was obtained from long bean leaf. Leafminers prefer to infect leaf with thick epidermis and less trichome as in red bean. The distribution pattern of *L. huidobrensis* and *L. sativae* in 2007-2008 compared to 2011 showed shifting distribution. In 2011, *L. sativae* dominated habitat in the higher altitude up to 1,600 m asl. In 2007-2008, it only distributed under 600 m asl. This shifting is probably caused by temperature rise. However, this presumption should be observed more in the future.

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## SPECIES RICHNESS, DIVERSITY AND ABUNDANCE OF DRAGONFLIES AND DAMSELFLIES IN BOGOR

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### ABSTRACT

Dragonflies and damselflies of the order Odonata are part of Indonesia's biodiversity and of a great ecological importance. They are among biological indicators of environmental quality. This study was conducted to examine species richness, diversity, and abundance of Odonata in Bogor. Fieldworks were conducted in lakes, rivers, water falls, and rice fields from January to August 2017. Opportunistic sampling methods using direct observation or sweep nets was employed. The results showed that there were 54 species, representing 10 families. Species diversity and richness were higher in lakes, rivers, and water falls compared to rice fields. Further surveys in early 2018 found 8 additional species, and thus, totaling 62 species of Odonata. The most common species were *Orthetrum sabina*, *Brachytemis contaminata*, *Pantala flavescens* of dragonflies and *Vestalis luctosa* of damselflies. Six species found were endemic to Java: *Paragomphus reinwardtii*, *Rhinocypha fenestrata*, *Rhinocypha heterostigma*, *Drepanosticta gazella*, *Drepanosticta sundana* and *Amphibaeschna ampla*. The presence of endemic species suggests the urgent need to employ conservation measures to safeguard the freshwater ecosystems in Bogor.

**Keywords:** Conservation, damselflies, dragonflies, species diversity, species richness

### INTRODUCTION

Dragonflies and damselflies of the order Odonata are part of Indonesia's biodiversity and of a great ecological importance. They are among biological indicators of environmental quality. Lieftinck (1954) reported there were 156 species of Odonata on the island of Java, consisting of 99 species of dragonflies and 57 species of damselflies, with 28 species were endemic to Java. Survey conducted in Java in the period of 2010-2014 by the Indonesia Dragonfly Society (IDS) collect 88 species, with 7 species being endemic to Java (Setiyono 2014). It seems that in recent decades there has been a decrease in the number of Odonata species in Java as predicted earlier by Orr (2004). The decline in the number of Odonata species during that period was due to the loss of dragonfly habitats mainly due to deforestation (Moore 1997) and land conversion (Kalkman *et al.* 2008). This study was conducted to examine species richness, diversity and abundance of dragonflies and damselflies in Bogor.

### MATERIALS AND METHODS

Surveys were conducted from January to August 2017 in various habitats in Bogor, representing lakes, rivers, waterfalls, and rice fields. Opportunistic sampling methods using direct observation or sweep nets was employed from 08.00 AM to 06.00 PM local time. Identification was done directly in the fields using published photographs and pictorial keys. The diversity of Odonata species in each habitat was measured by the Shannon Weiner index (H') with the help of PAST 3.0 software.

## RESULTS AND DISCUSSION

There were 6,651 individuals of Odonata found during the survey, consisted of 73.2% dragonflies (Anisoptera) and 26.8% damselflies (Zygoptera). The highest number of individuals was found in lakes (3,122 individuals), followed by rivers (1,669 individuals), rice fields (1,334 individuals), and the least was in waterfalls (526 individuals) (Table 1). There were 10 families in this study, with Libellulidae as the most common (72%). According to Kalkman *et al.* (2008) Libellulidae is one of the two largest families of Odonata that dominates freshwater fauna in almost all parts of the world. This is related to its high adaptability and tolerance to various environmental conditions, even to extreme environments such as low dissolved oxygen habitats (Clausnitzer 2003).

Table 1 Species composition and abundance of Odonata in Bogor

Taxa	Waterfalls	Lakes	Rivers	Rice Fields	Total	Relative Abundance (%)
<b>ANISOPTERA</b>						
<b>Aeshnidae</b>						
<i>Anax guttatus</i>		4			4	0.06
<i>Gynacantha</i> sp.			1		1	0.02
<i>Gynacantha subinterrupta</i>		2			2	0.03
<b>Gomphidae</b>						
<i>Ictinogomphus decorates</i>		42	1		43	0.65
<i>Leptogomphus lansbergei</i>	1				1	0.02
<i>Paragomphus reinwardtii</i> *				1	1	0.02
<b>Macromiidae</b>						
<i>Epophthalmia</i> sp.		3			3	0.05
<i>Epophthalmia vittata</i> .		6			6	0.09
<b>Libellulidae</b>						
<i>Acisoma panorpoides</i>		14	1		15	0.23
<i>Agrionoptera insignis</i>		5			5	0.08
<i>Brachydiplax chalybea</i>		181	5		186	2.80
<i>Brachythemis contaminata</i>		1,109	55	6	1,170	17.59
<i>Crocothemis servilia</i>		60	8	64	132	1.98
<i>Diplacodes trivialis</i>	2	2	14	2	20	0.30
<i>Lathrecista asiatica</i>		5			5	0.08
<i>Neurothemis fluctuans</i>	1	3	10	1	15	0.23
<i>Neurothemis ramburii</i>	6	34	21	3	64	0.96
<i>Neurothemis terminata</i>	4	27	30	5	66	0.99
<i>Onychothemis culminicola</i>			1		1	0.02
<i>Orthetrum cbrysis</i>	4	7	16	2	29	0.44
<i>Orthetrum glaucum</i>	6		6		12	0.18
<i>Orthetrum luzonicum</i>		10			10	0.15
<i>Orthetrum pruinosum</i>	13	5	1	3	22	0.33
<i>Orthetrum sabina</i>	105	365	222	800	1,492	22.43
<i>Orthetrum testaceum</i>	1	75	96	66	238	3.58
<i>Orthetrum triangulare</i>	1				1	0.02
<i>Pantala flavescens</i>	11	410	187	202	810	12.18
<i>Potamarcha congener</i>		14	21	6	41	0.62
<i>Rhodothemis rufa</i>	1	25			26	0.39
<i>Rhyothemis phyllis</i>		145			145	2.18
<i>Rhyothemis variegata</i>		2			2	0.03
<i>Tholymis tillarga</i>		30	1		31	0.47
<i>Urothemis signata</i>		12			12	0.18
<i>Zygonyx ida</i>	6				6	0.09
<i>Zyxomma obtusum</i>		236			236	3.55
<i>Zyxomma petiolatum</i>		18			18	0.27

Table 1 (Continued)

Taxa	Waterfalls	Lakes	Rivers	Rice Fields	Total	Relative Abundance (%)
<b>ZYGOPTERA</b>						
Platystictidae						
<i>Drepanosticta gazelle</i> *	1				1	0.02
<i>Drepanosticta sundana</i> *	12				12	0.18
<b>Calopterygidae</b>						
<i>Vestalis luctuosa</i>	189	5	352	9	555	8.34
Chlorocyphidae						
<i>Libellago lineata</i>			184		184	2.77
<i>Rhinocypha fenestrata</i> *	40	6	316	18	380	5.71
<i>Rhinocypha heterostigma</i> *	6		1		7	0.11
<b>Euphaeidae</b>						
<i>Euphaea variegata</i>	59		3	1	63	0.95
<b>Platycnemididae</b>						
<i>Coelliccia membranipes</i>	52	86			138	2.07
<i>Copera marginipes</i>	1	29	87	28	145	2.18
<i>Prodasinевра autumnalis</i>			6		6	0.09
<b>Coenagrionidae</b>						
<i>Agriocnemis femina</i>		94	5	73	172	2.59
<i>Agriocnemis minima</i>		6			6	0.09
<i>Agriocnemis pygmaea</i>		19	1	8	28	0.42
<i>Ceriagrion praetermissum</i>		4			4	0.06
<i>Ischnura senegalensis</i>		20			20	0.30
<i>Pseudagrion microcephalum</i>			1		1	0.02
<i>Pseudagrion pruinsum</i>	4	1	16	36	57	0.86
<i>Pseudagrion rubriceps</i>		1			1	0.02
Total number of individuals	526	3,122	1,669	1,334	6,651	
Total of number species	23	40	30	20	54	
Java endemic species	4	1	2	1	5	
Diversity index (H)	2.04a	2.36b	2.33b	1.48c		

Note: \*Java endemic species

Total number of species found during 2017 survey were 54 species of Odonata. Further survey conducted during in early 2018 revealed eight additional species i.e. *Amphibiaeschna ampla* (Aeshnidae), *Aethriamanta brevipennis* (Libellulidae), *Cratilla lineata* (Libellulidae), *Hydrobasileus croceus* (Libellulidae), *Tetrathemis irregularis* (Libellulidae), *Tramea transmarina* (Libellulidae), *Nososticta insignis* (Platycnemididae), and *Ceriagrion cerinorubellum* (Coenagrionidae). Altogether, there were six Java endemic species found during study i.e. *Paragomphus reinwardtii*, *Rhinocypha fenestrata*, *Rhinocypha heterostigma*, *Drepanosticta gazelle*, *Drepanosticta sundana* and *Amphibiaeschna ampla*.

The Shannon-Wiener index (H') showed that the diversity of Odonata species in lakes and rivers was not significantly different ( $p=0.41$ ), but both were significantly different ( $p=0.001$ ) from those in waterfalls and rice fields. Rice fields have the lowest number of species of Odonata; this might be related to frequent disturbances such as land preparation, harvesting, and pesticide applications.

In conclusion, various freshwater habitats (lakes, rivers, waterfalls, rice fields) in Bogor were inhabited by species richness of dragonflies and damselflies. The presence of six Java endemic species suggests the urgent need to employ conservation measures to safeguard the freshwater habitats in Bogor.



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## FIRST REPORT ON TWO ATYID SHRIMPS OF THE GENUS *Caridina* FROM LOMBOK ISLAND, INDONESIA

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### ABSTRACT

Studies of freshwater shrimps, genus *Caridina* in Lesser Sunda Islands (Lombok, Sumbawa, Flores and Sumba) have been focused in Sumbawa, Flores and Sumba. Freshwater shrimps from Lombok Island are poorly reported. Therefore, the aim of this study is to determine the diversity of freshwater shrimps of the genus *Caridina* in Lombok Island, Indonesia. Determination of sampling sites was performed by purposive sampling method. Samples were collected by hand net. The collected material from throughout the sampling sites showed that there were 2 species of *Caridina* (*C. serratirostris* and *C. typus*) which are new records for Lombok Island. The results included the diagnosis, ecological information, and distribution of the two species of genus *Caridina*.

**Keywords:** *Caridina*, freshwater shrimps, Lombok Island-Indonesia, new records

### INTRODUCTION

Freshwater shrimps in Indonesia comprise three families, i.e. Palaemonidae, Atyidae and Alpheidae (Wowor *et al.* 2004). The most diverse freshwater shrimps belongs to family Atyidae, genus *Caridina*. This genus consists of 290 species (Grave & Fransen 2011). Studies of freshwater shrimps, genus *Caridina* in Lesser Sunda Island (Lombok, Sumbawa, Flores and Sumba) were conducted by some authors (Roux 1928; Holthuis 1978). There were 10 species of freshwater atyid shrimps reported, especially from Sumbawa, Flores and Sumba (Holthuis 1978). However, there was no specimen collected from Lombok Island.

Based on Cai (2003) one species, *Caridina gracilipes* was collected from Lombok Island by V. Kampeen on 26 January 1907 at the vicinity of Ampenan, Lombok Island, Indonesia. The specimen was deposited in Zoologisch Museum Amsterdam, Universiteit van Amsterdam, the Netherlands. Up to the present time, there is no new report on freshwater shrimp from Lombok Island. Therefore, the objective of this research was to study the diversity of freshwater shrimps of Lombok Island, West Nusa Tenggara Province, Indonesia.

### MATERIALS AND METHODS

Sampling was conducted in July, August, and September 2017 in Lombok Island, West Nusa Tenggara Province, Indonesia (Fig. 1). Purposive sampling method was applied in 13 sampling sites. The coordinate and altitude of each sampling location were overlaid to the map of

sampling sites. Morphological identification was conducted in Crustacean Laboratory, Division of Zoology, Research and Development Center for Biology, Indonesian Institute of Sciences (LIPI), Cibinong. The samples were collected using hand nets of 0.3 mm mesh size. All specimens obtained were preserved in 70% alcohol in the field, then fixed in 96% alcohol in the laboratory. All samples were identified using identification key developed by Cai (2003) based on morphological characters i.e. length of rostrum, teeth of rostrum, carapace, first pereiopods, and second pereiopods.

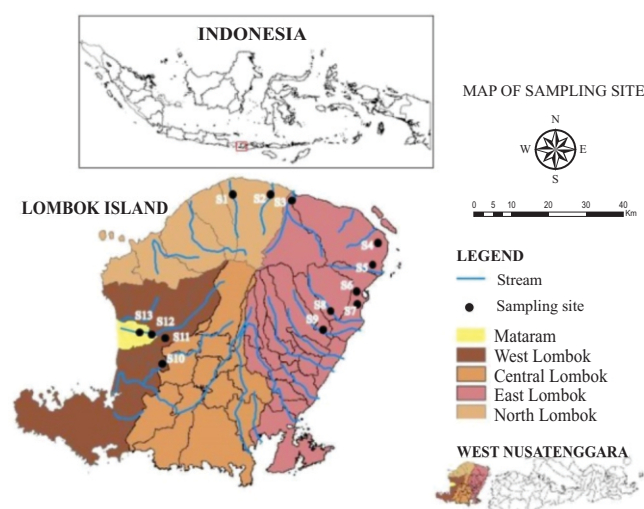


Figure 1 Map of sampling sites

## RESULTS AND DISCUSSION

Freshwater shrimps of Lombok Island were collected for the first time by V. Kampeen in 1907 (Cai 2003). Since then, for more than 100 years there was no report on freshwater atyid shrimps from Lombok Island. According to Cai (2003), only one specimen was collected from Lombok Island. The study did not include description of morphological characters from each species because there were no full grown specimens in his material. Our present study is the second crustacean report from Lombok island completed with its morphological description. This study discovered two new records of atyid shrimp, i.e. *Caridina serratiostris* and *C. typus*.

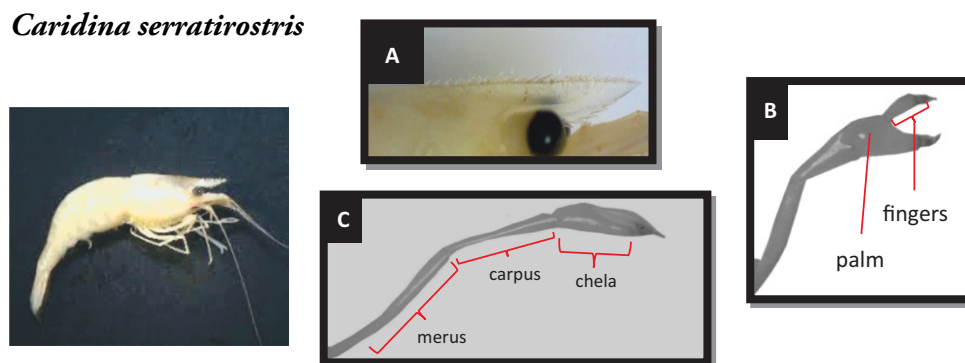


Figure 2 *Caridina serratiostris*. Morphological character of *C. serratiostris*: A. Rostrum; B. First pereiopod; C. Second pereiopod

**Diagnosis:** Rostrum straight, reaching near end of basal segment, or end of second segment of antennular peduncle. Rostrum armed with 15-25 dorsal teeth, with 7-10 postorbital teeth and 4-6 ventral teeth. First pereiopod reaching end basal segment of antennular peduncle, merus as long as carpus, carpus shorter than chela, fingers distinctly longer than palm. Second pereiopod reaching end second segment of antennular peduncle, merus shorter than carpus, fingers as long as palm.

**Remarks:** *Caridina serratirostris* differs from other *C. typus* by having straight rostrum, numerous postorbital teeth and the dorsal teeth are distributed equally along the dorsal margin of the rostrum.

**Habitat and ecological informaton:** *Caridina serratirostris* is found in freshwater and brackishwater.

**Distribution:** Ryukyu, and Philippines (Cai and Shokita 2006). This study reports the presence of *C. serratirostris* in Lombok Island for the first time.

### *Caridina typus*

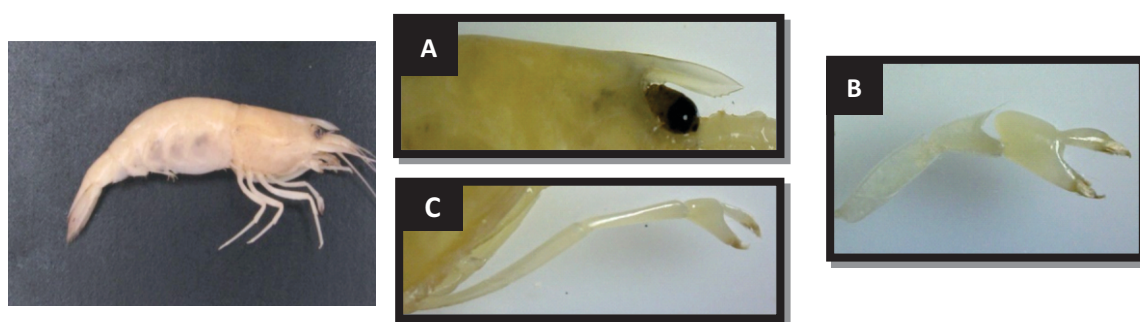


Figure 3 *Caridina typus*. Morphological character of *C. typus*: A. Rostrum; B. First pereiopod; C. Second pereiopod

**Diagnosis:** Rostrum reaching near end of basal segment, or end of second segment of antennular peduncle. Rostrum unarmed dorsally, armed ventrally with 1-4 teeth. First pereiopods short, reaching end basal segment of antennular peduncle, merus slightly longer than carpus, carpus shorter than chela, fingers variable, at shorter than palm, sometimes as long as or even slightly longer. Second pereiopods reaching end second segment of antennular peduncle, merus shorter than carpus, carpus distinctly longer than chela, fingers as long as palm.

**Remarks:** *Caridina typus* differs from other *C. serratirostris* species by not having dorsal rostral tooth.

**Habitat and ecological information:** *Caridina typus* is a common freshwater species, occurs in rivers or streams on islands or coastal areas, prefer to hide under rocks or stones during the day time.

**Distribution:** Indo-West Pacific region (Chace 1997), Malaysia (Ng 1995), Thailand (Naiyanetr 1998), Vietnam (Bouvier 1925), Philippines (Cai and Anker 2004; Cai and Shokita 2006), Taiwan

(Hung *et al.* 1993), Australia (Riek 1953), and Sulawesi-Indonesia (Cai and Ng 2009). This study reports the presence of *C. typus* for the first time in Lombok Island.

Based on the morphological characters, *Caridina serratirostris* differs from *C. typus* by the number of dorsal rostral tooth. *Caridina serratirostris* has numerous postorbital teeth and the dorsal teeth are distributed equally along the dorsal margin of the rostrum, but *C. typus* does not have dorsal rostral tooth.

## CONCLUSION

There are two new records of freshwater shrimps for Lombok Island, i.e. *Caridina serratirostris* and *C. typus*. These make three species of Atyid shrimps known from Lombok Island, i.e. *C. serratirostris*, *C. typus* and *C. gracilipes*.

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## NUCLEOTIDE VARIATION IN CODING REGION OF *DRD1* GENE IN KARAPAN, SONOK, AND BEEF MADURA CATTLE

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### ABSTRACT

*Karapan* (bull racing), *sonok* (cattle beauty contest) and beef madura cattle are different types of livestock based on their maintenance purposes. Environmental and genetic factors may affect the performance of madura cattle where *karapan*, *sonok*, and beef madura cattle have different levels of aggressiveness. This study aimed to analyze the variation of Dopamine receptor D1 gene (*DRD1*) on *karapan*, *sonok*, and beef cattle. Therefore, alteration in the coding sequence (CDS) nucleotide sequences may lead to disruptions in regulating the *DRD1* gene that affects aggressiveness or tameness. Ten blood samples of *karapan*, three saliva samples of *sonok*, and ten blood samples of beef cattle were used in analysis of nucleotide variation on CDS of *DRD1* gene. Samples of the same type was pooled and sequenced together. The sequencing results indicated that nucleotide variant of G357A, T832C, T867C, and A931C were found in *karapan* cattle, C865T and C867T were found in *sonok* cattle, as well as nucleotide variant of A67C was found in beef cattle. This results showed that the nucleotide variation in CDS of *DRD1* gene could be the utilities difference of madura cattle.

**Keywords:** CDS, *DRD1* gene, *karapan*, madura cattle, *sonok*

### INTRODUCTION

Madura cattle is one of the local Indonesian cattle that has been selected naturally and maintained its authenticity in the Madura Island (Setiadi & Diwyanto, 1997). Madura cattle was the result of crossing a banteng (*Bos javanicus*) with a zebu (*B. indicus*) (Nijman *et al.* 2003; Uggla 2008). The interaction between humans and cattle causes high socio-cultural value of madura cattle. This value has led to the formation of cultural practices such as *sonok* (beauty contest) and *karapan* (bull racing). Madura cattle that do not meet these criteria will be used as beef cattle (Kutsiyah 2012).

Dopamine, an abundant neurotransmitter in the central and peripheral nervous system, is involved in a wide variety of behavioral and physiology functions, such as locomotor activity, cognition and endocrine function in mammals (Baskerville & Douglas 2010; Wang *et al.* 2013). Dopamine receptors are members of the G protein-coupled receptors with seven transmembrane domains. In mammals, five dopamine receptors subtypes, *DRD1–DRD5*, have been identified and classified into two major subfamilies: D1-like receptors (*DRD1* and *DRD5*) and D2-like receptors (*DRD2*, *DRD3* and *DRD4*) based on their pharmacological, biochemical and physiological differences (Missale *et al.* 1998). *DRD1* gene in mammals was found to express in the nucleus accumbens, cerebral cortex, hypothalamus, and thalamus (Xu *et al.* 2010). Different segments of coding sequence (CDS) of *DRD1* that influenced different aggressiveness of three

types of madura cattle may contribute to the diversity of *DRD1* expression between *karapan*, *sonok*, and beef madura cattle. Therefore, variation in CDS of *DRD1* gene may be the determination key of *karapan*, *sonok*, and beef madura cattle. This study aimed to analyze nucleotides variation in CDS of *DRD1* gene in three types of madura cattle.

## MATERIALS AND METHODS

The sample used consisted of blood samples of 10 *karapan* cattle and 10 beef cattle, as well as saliva samples from 3 *sonok* cattle. In this study, blood and saliva samples of madura cattle were collected using a non-invasive sampling method, so there was no need for Animal Ethics Statement for this study. DNA extraction was carried out on the blood and saliva samples using Thermo® GeneJET Whole Blood (Thermo® Scientific) DNA extraction kit.

### DRD1 Gene Amplification

Amplification of CDS was performed on each sample using forward primer AF559 (GATGAGGACTCTCAACACGTCTA) and reverse primer AF560 (TCAAGAGAGACGTCAGTGTCATA) with amplicon length of 1298 bp. The amplifying primers and two internal primers, forward primer AF561 (ACCTACACCAGGATCTACAGGAT) with amplicon length of 661 bp and reverse primer AF562 (CTGTAGATCCTGGTGTAGGTGAC) with amplicon length of 656 bp (Fig. 1). PCR reaction used 1x *GoTaq® Green Master Mix* alongside with buffer containing MgCl<sub>2</sub> and dNTPs, *nuclease-free water*, 1-3 µL DNA sample, and 0.5-1 µL for each primer. PCR was performed using ESCO Swift Maxi Thermal Cycler consisted of predenaturation at 95 °C for 2 minutes, then continued with 30 cycles including denaturation at 95 °C for 45 seconds, annealing at 60 °C for 1 minute, and elongation at 72 °C for 1 minute. After completing 30 cycles, it was continued with final elongation at 72 °C for 5 minutes. PCR products were separated on 6% polyacrilamide gel electrophoresis (PAGE) on 200 V for 45 minutes. Silver staining was conducted to visualize DNA bands (Byun *et al.* 2009).

### Nucleotides Sequencing of DRD1 Gene

Samples of the same type of madura cattle was pooled and sequenced at once because the resulting amplicon is assumed to have very low nucleotide variations (Quist & Chapela 2001). Sequencing was carried out on 30 µL of PCR products, each consisting of 3 µL of PCR products from 10 amplicons of blood from *karapan* cattle and 10 amplicons from beef cattle, as well as consisted of 10 µL of PCR products from 3 amplicons of saliva from *sonok* cattle.

### DNA Sequence Analysis

Nucleotide sequencing were edited using BioEdit version 7.1.11 (Hall 1999). The nucleotide sequence of each sample was aligned using Muscle align on MEGA (Molecular Evolutionary Genetics Analysis) version 6 manually (Tamura *et al.* 2011).

## RESULTS AND DISCUSSION

Chromatogram's peak reading showed several numbers of overlapping peaks on *karapan*, *sonok*, and beef madura cattle. This matter was caused by nucleotides variations of pooled samples in sequencing. The overlapping peaks showed that in pooled samples there were heterozygous alleles or genetic variations. *Bubalus bubalis* (XM\_025296394), *Bison bison bison* (XM\_010862102),

*B. indicus* (XM\_019967896), *B. mutus* (XM\_005906159), and *B. taurus* (NC007308.6) were used as reference sequence.

This study found a low nucleotide variation in the CDS of *DRD1* gene in three types of madura cattle, which was 0.47% in *karapan* cattle, 0.28% in *sonok* cattle, and 0.19% in beef cattle with amplicon length of 1,071 bp. Low nucleotide variation in madura cattle was thought to be caused by gene flow that occurred due to hybridization between ancestors of Auroch (*B. primigenius*). During the Neolithic, at least two domestication events occurred i.e. *B. taurus* and *B. indicus*. Other species of wild bovines were also domesticated, namely the buffalo (*Bubalus bubalis*), gaur (*B. gaurus*), and yak (*B. grunniens*) (Lenstra & Bradley 1999). The statement was also supported by the presence of alleles in the madura cattle *DRD1* gene which were identical to *B. indicus*, *B. mutus*, *Bubalus bubalis*, and *Bison bison*, G allele in position 660. In *sonok* cattle, the T allele at 865 and 867 was identical to *B. mutus* (wild yak) (Table 1). Other alleles at positions 67, 357, 360, 832, 897 and 931 were thought to be identical to alleles in *B. javanicus* which was one of the ancestors of madura cattle. Madura cattle was the result of crossing between *B. indicus* (zebu) and *B. javanicus* (banteng); which have been proven based on mitochondrial DNA, satellite fragment length polymorphisms (SFLP), amplified fragment length polymorphisms (AFLP), and microsatellite DNA (Nijman *et al.* 2003).

Nucleotide variations can be found in individuals with the same type of madura cattle or individuals in each type of different cattle utilities. It occurs in the form of nucleotide substitutions which due to transitional mutations and transversion mutations. Transition mutations occurred in A357G, T832C, C865T, and C867T due to changes in purine to other purine or changes in pyrimidine to other pyrimidine and transversion mutations in A67C, G897T, and A931C due to changes in purine to pyrimidine. In *karapan* cattle, A/G allele at positions 357, G/T allele at 897, as well as C allele at positions 832 and 931 can be used as differentiators between *karapan* cattle with *sonok* and beef cattle. Similar phenomena were found in *sonok* cattle with T allele at positions 865 and 867, and beef cattle with C allele at position 67.

In *karapan* cattle, amino acid changed from phenylalanine (TTT) to leucine (CTT) and was caused by nucleotide variations of T832C. Amino acid changed from asparagine (AAT) to histidine (CAT) and was caused by nucleotide variation of A931C. The change in amino acids from phenylalanine to leucine is called a neutral mutation because the amino acid substitution that occurs has similar base on the -R group. Therefore, the protein function does not change. This can be seen from the absence of changes in phenotypes due to mutations, as well as changes in amino acids from asparagine to histidine (Russel 1992). In *karapan* cattle, nucleotide variations of A357G and G897T are called silent mutations because they do not cause changes in amino acids (Nei, 1987). In *sonok* cattle, amino acid change from leucine (CTC) to proline (TTT/TTC) was caused by nucleotide variations of C865T and C867T. In beef cattle, amino acid changes from methionine to leucine was caused by variations nucleotides of A67C. Amino acid changes that occurred in *sonok* and beef cattle were also called a neutral mutation, whereas amino acid changes that occurred due to changes in nucleotide sequences such as A67C, T832C, C865T, C867T, and A931C were called missense mutations (Nei 1987).

## CONCLUSION

This study showed that selection and maintenance systems, as well as special care for *karapan* and *sonok* cattle can distinguish madura cattle genetically based on variation of CDS of *DRD1* gene.



Table 1 Alignment result of CDS of *DRD1* gene from *karapan* (10 pooled samples) *sonok* (3 pooled samples), and beef madura cattle (10 pooled samples) towards *Bubalus bubalis* (XM\_025296394), *Bison bison bison* (XM\_010862102), *B. indicus* (XM\_019967896), *B. mutus* (XM\_005906159), and *B. taurus* (NC007308.6)

Breed	Nucleotide position number in CDS (bp)								
	67	357	360	660	832	865	867	897	931
<i>Bubalus bubalis</i> (XM_025296394)	A	G	T	G	T	C	C	G	A
<i>Bison bison bison</i> (XM_010862102)	A	G	T	G	T	C	C	G	A
<i>Bos indicus</i> (XM_019967896)	A	G	T	G	T	C	C	G	A
<i>Bos mutus</i> (XM_005906159)	A	G	T	G	T	T	T	A	A
<i>Bos taurus</i> (NC007308.6)	A	G	T	A	T	C	C	G	A
Madura cattle ( <i>Karapan</i> )	A	<b>G/A</b>	<b>T/C</b>	G	<b>T/C</b>	C	C	<b>G/T</b>	<b>A/C</b>
Madura cattle ( <i>Sonok</i> )	A	G	<b>T/C</b>	G	T	<b>C/T</b>	<b>C/T</b>	G	A
Madura cattle (beef)	A/C	G	<b>T/C</b>	G	T	C	C	G	A

Table 2 Summary of amino acids translation in CDS of *DRD1* gene in *karapan* (10 pooled samples) *sonok* (3 pooled samples), and beef madura cattle (10 pooled samples) towards *Bubalus bubalis* (XM\_025296394), *Bison bison bison* (XM\_010862102), *B. indicus* (XM\_019967896), *B. mutus* (XM\_005906159), and *B. taurus* (NC007308.6)

Breed	Codon position number in CDS							
	23	119	120	220	278	289	299	311
<i>Bubalus bubalis</i> (XM_025296394)	M	G	N	S	F	L	T	N
<i>Bison bison bison</i> (XM_010862102)	M	G	N	S	F	L	T	N
<i>Bos indicus</i> (XM_019967896)	M	G	N	S	F	L	T	N
<i>Bos mutus</i> (XM_005906159)	M	G	N	S	F	P	T	N
<i>Bos taurus</i> (NC007308.6)	M	G	N	S	F	L	T	N
Madura cattle ( <i>Karapan</i> )	M	G	N	S	<b>F/L</b>	L	T	<b>N/H</b>
Madura cattle ( <i>Sonok</i> )	M	G	N	S	F	<b>L/P</b>	T	N
Madura cattle (beef)	<b>M/L</b>	G	N	S	F	L	T	N

Note: Nucleotide position numbers are counted from start codon. Bold letters show mutation in CDS. M=Methionine (start codon); I=Isoleucine; G=Glycine; N=Asparagine; S=Serine; F=Phenylalanin; L=Leucine; T=Threonine; H=Histidin; P=Proline

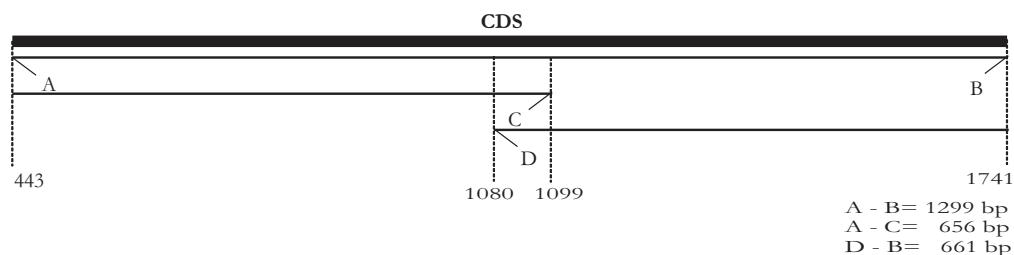


Figure 1 Position of primer a). AF559, b). AF560, c). AF562, and d). AF561 on CDS of *DRD1* gene

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## *Paphiopedilum* spp. (ORCHIDACEAE) COLLECTION AT CIBODAS BOTANIC GARDEN

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### ABSTRACT

*Paphiopedilum* is one of the important genera within Orchidaceae, with showy flower which make them an interesting object for horticulturists. In conservation point of view, most *Paphiopedilums* are already on the IUCN red list, ranked as vulnerable to endangered species. Therefore, in the CITES all of *Paphiopedilum* were placed in Appendix I. Cibodas Botanic Garden (CBG) as an ex situ plant conservation conserved *Paphiopedilum* spp. among all plant collection in the garden. Until now, CBG has collected 12 species of *Paphiopedilum*, of which eight are native to Indonesia (*P. bulenianum*, *P. glaucophyllum*, *P. javanicum*, *P. lowii*, *P. tonsum*, *P. victoria-reginae*, *P. wilhelminae*, *Paphiopedilum* sp), and the rest are from other countries (*P. armeniacum*, *P. concolor*, *P. hirsutissimum*, *P. villosum*). These native species were collected during flora exploration and some were obtained by contribution. Meanwhile, the foreign species were all obtained by contribution. CBG also maintains some hybrids of *Paphiopedilums*.

**Keywords:** Cibodas Botanic Gardens, Conservation, Indonesia, Orchidaceae, *Paphiopedilum*

### INTRODUCTION

*Paphiopedilum* has become a fascinating orchid genus since the middle of nineteenth century among botanists and orchids lovers. The botanists are endlessly fascinated by its classification and evolution, and the orchid's growers love them because of the showy and exotic flower's look. *Paphiopedilum* is characterized by its curious flower and the slipper shaped of the lower petal where the name came from. This orchid also called lady's slipper orchid (Cribb 1997).

*Paphiopedilum* can be found as terrestrial, epiphyte, or lithophyte, small to large sized; leaves surface tessellate or non-tessellate, lower surface in some species with dark marking (purple), green or bluish green, elliptic, ligulate, or oblong. Inflorescence terminal, bears single to multiflower. Flower with fused lateral sepal (synsepalus), petals linear, spatulate, sometimes twisted and hairy along the margin, labellum pouch shaped or deeply saccate (Cribb 1987; Pridgeon 1999).

*Paphiopedilum* belong to subfamily Cypridoideae consists of *Cypripedium*, *Phragmipedium*, *Mexipedium*, *Selenipedium*, and *Paphiopedilum* as a representative genus in Tropical Asia region (Pridgeon et al. 1999). Cypridoideae has lateral anther, united lateral sepal (sysepalus), the labellum deeply saccate (Dressler 1993). Cribb (1987) mention 60 species within *Paphiopedilum*, but new discoveries still continued. Meanwhile, on the list of WCSP (World Checklist of Selected Plant families) there are 130 species including the variety and 35 are natural hybrid (apps.kew.org).

Habitat destruction and over exploitation are the major problems in orchid existence in their natural habitat (Koopowitz 2001). *Paphiopedilum* with their beauty and unique flower become a problem on its own existence, due to over collection.

The taxonomy of *Paphiopedilum* has become an interesting subject for Orchidologist, and sometimes leads to conflict. Recent study regarding to classification has divided this genus into

three subgenus and four sections (Cox *et al.* 1997), slightly different with Cribb (1987) with three subgenus and several sections. This paper is to inform about *Paphiopedilum*'s collection in CBG, classification and their conservation.

## MATERIALS AND METHODS

The plant materials for this observation were *Paphiopedilum* spp collected in the orchid's house of Cibodas Botanic Garden. Method used was descriptive method by observing *Paphiopedilum* collection in CBG and conducting literature studies.

## RESULTS AND DISCUSSION

Until now, CBG already collected 12 species of *Paphiopedilum*, from Indonesia region and foreign countries. Based on Cribb (1987) and updated by Cox *et al.* (1997) *Paphiopedilum* collected in CBG was divided into three subgenuses i.e. *Brachypetalum*, *Parvisepalum*, and *Paphiopedilum* with 4 sections. The first two subgenuses were distributed mostly in Tropical China region, India, Thailand, Myanmar, and subgenus *Paphiopedilum* mostly was found in Indonesia, Peninsular Malaysia, and Borneo.

Inflorescence in *Paphiopedilum* ranges from single flowered to multiple flowered. *Paphiopedilum* section Barbata mostly has single flower (*P. bulenianum*, *P. javanicum*, *P. tonsum*). *Paphiopedilum lowii*, *P. villosum*, and *P. wilbelminae* have multiple flowers which open simultaneously, different from *P. glaucophyllum* and *P. victoria regina*. These two species also have multiple flowers which open successively (one after another), once a plant bears inflorescence (Cribb 1997, 1987); the flower will continue to bloom through several months ahead as seen in our collection, up until 30 flowers from single inflorescence. Some species of *Paphiopedilum* in orchid house of CBG are shown in Figure 1.

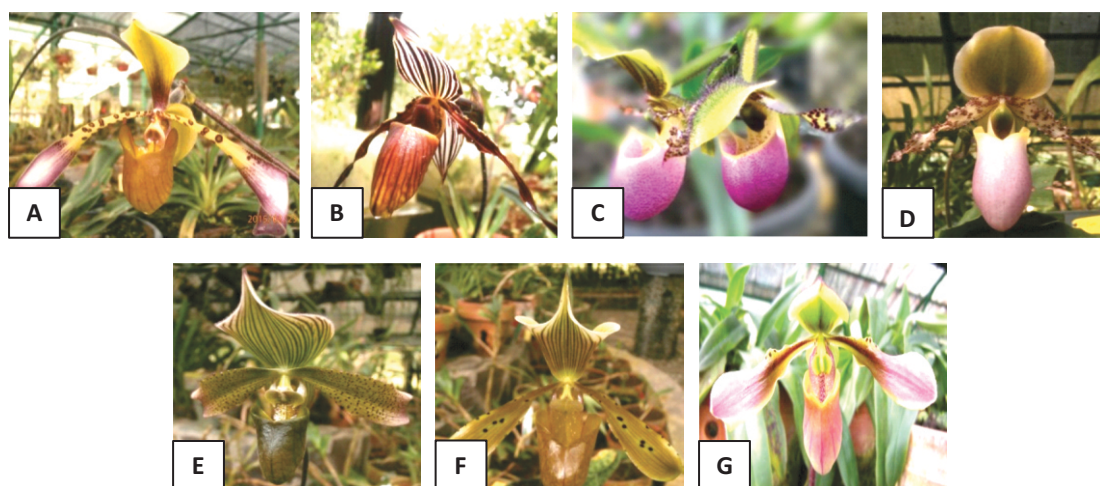


Figure 1 Species of *Paphiopedilum* spp. Collected in Cibodas Botanic Garden: A. *Paphiopedilum lowii*, B. *Paphiopedilum wilbelminae*, C. *Paphiopedilum victoria-regina*, D. *Paphiopedilum glaucophyllum*, E. *Paphiopedilum javanicum*, F. *Paphiopedilum tonsum*, G. *Paphiopedilum bulenianum*

### Identification key to *Paphiopedilum* spp. collection of CBG

1. a. Leaves mottled ..... 2  
b. Leaves without mottled ..... 7
2. a. Leaves mottled on upper surface and purple underside or purple streaks ..... 3  
b. Leaves mottled on the upper surface but not on the undersurface ..... 5
3. a. Leaves oblong-elliptic, mottled with dark green and bluish green on the surface and purple on entire under surface, mostly 1-flowered, 5.5-8 cm, tepal creamy with maroon spots ..... **1. *P. bellatulum***  
b. Leaves mottled with dark green and bluish green or dark green and light green, purple streaks on underside ..... 4
4. a. Leaves 6-12 cm, oblong, fleshy, 1-flowered, bright yellow with inflated labellum ..... **2. *P. armeniacum***  
b. Leaves very long elliptic, up to 40 cm, ciliate at the base of margin, shiny, flower unknown ..... **3. *Paphiopedilum* sp.**
5. a. Inflorescence 1-flowered, petal spatulate, pink with brown spots near the base, ciliate at basal part, staminode semi orbicular, incised at lower part to the middle ..... **4. *P. bullenianum***  
b. Inflorescence 1-flowered, green on almost overall tepal, staminode bidenticulate ..... 6
6. a. Staminode reniform, cover by fine hair on the surface, petal spatulate, olive, green with black spotted, margin glabrous ..... **5. *P. tonsum***  
b. Staminode reniform, bilobed at upper part, petal green flushed with pink, margin ciliate ..... **6. *P. javanicum***
7. a. Inflorescence with single flower ..... 8  
b. Inflorescence with multiple flower ..... 9
8. a. Leaves linear, ligulate, petal broad, spatulate, pink toward the apex, undulate at the base of the margin ..... **7. *P. hirsutissimum***  
b. Leaves linear, petal broad, spatulate, yellow and brown, shiny, staminode semicircular, with small projection in the middle (wart) ..... **8. *P. villosum***
9. a. Flower open simultaneously ..... 10  
b. Flower open successively ..... 11
10. a. Peduncle long, bring 3-4 flower, petal spatulate, once twisted in the middle, staminode obovate ..... **9. *P. lowii***  
b. Peduncle not very long, 1-2 flower, petal linear, deflexed, ciliate along the margin, staminode sub quadrate ..... **10. *P. wilbelminae***
11. a. Inflorescence up to 60 cm, rachis zigzag with up to 32 flowers, dorsal sepal yellow green or white, staminode deltoid ..... **11. *P. victoria* -regina**  
b. Inflorescence less than 60 cm, rachis with internode, bring up to 20 flowers, dorsal sepal white or cream, staminode ovate ..... **12. *P. glaucophyllum***

## Enumeration of the species

1. *Paphiopedilum bellatulum* (Rchb.f.) Stein (Orchid.-Buch: 456 (1892)).  
Subgenus *Brachypetalum*. A terrestrial herb and considered to be closely related to *P. godefroye*. *P. bellatulum* is distributed in Western Burma, Thailand, and probably south-west China (Cribb 1987).
2. *Paphiopedilum armeniacum* S.C Chen & F.Y. Liu (Acta Bot. Yunnan. 4:163 (1982)).  
Subgenus *Parvisepalum*, first described on 1982. A lithophytic or terrestrial herbs, on limestone hills. *P. armeniacum* is endemic to China.
3. *Paphiopedilum* sp.  
Collection was from Sumatra. Leaves shiny and pendulous mottled, probably belong to subgenus *Paphiopedilum* section *Cochlopetalum* based on the purple streak on under surface as in *P. liemianum*. This species remains unidentified because it never bears the flower since it was collected in CBG (less than two years).
4. *Paphiopedilum bullenianum* (Rchb.f.) Pfitzer (Bot. Jahrb. Syst. 19: 40 (1894)).  
Subgenus *Paphiopedilum* section *Barbata*. A terrestrial herb, found from sea level to 1,850 m above sea level (asl). Cribb (1987) explained that *P. bullenianum* altogether with its allies, become taxonomist problem, therefore this species has so many synonyms. *P. bullenianum* is also widespread and may found in different types of habitat. This species was recorded from Java, Sumatra, Borneo, Peninsular Malaysia and Sulawesi. The collection was from Tana Toraja, South Sulawesi.
5. *Paphiopedilum tonsum* (Rchb.f.) Stein (Orchid.-Buch:488 (1892)).  
Subgenus *Paphiopedilum*, section *Barbata*. Terrestrial herbs on very thick humus in lower montane forest from 1,000-1,800 m asl. *P. tonsum* is distributed in north and central Sumatra.
6. *Paphiopedilum javanicum* (Reinw.ex Lindl.) Pfitzer. (Jahrb. Wiss. Bot.19:165 (1888)).  
Javan paphiopedilums, a terrestrial herb on montane forest at 900-2,500 m asl, under shade. The variety was found in Borneo (*P. javanicum* var. *virens*) (Cribb, 1997).
7. *Paphiopedilum hirsutissimum* (Lindl.ex Hook.) Stein (Orchid.-Buch: 470 (1892)).  
Subgenus *Paphiopedilum*, section *Paphiopedilum*. A terrestrial or epiphytic herb, found at 700-1,200 m asl. The distribution area is North East India (Assam, Manipur, Lushai, and Naga Hills).
8. *Paphiopedilum villosum* (Lindl.) Stein (Orchid.-Buch: 490 (1892)).  
Subgenus *Paphiopedilum* section *Paphiopedilum*. An epiphytic or rarely lithophytic herb, widespread with variable species, found from 1,100-2,000 m asl. The distribution area is North East India (Lushai hills, Assam), Burma, and Thailand (Cribb, 1987).
9. *Paphiopedilum victoria-regina* (Sander) M.W.Wood. (Orchid Rev.84: 134 (1976)).  
Queen Victoria's paphiopedilums. Subgenus *Paphiopedilum* section *Cochlopetalum*. A lithophytic herbs, found at 800-1,600 m asl. This species is Endemic to Sumatra (Cribb 1987). The collection was from West Sumatra.
10. *Paphiopedilum glaucophyllum* J.J. Sm. (Bull. Inst. Bot. Buitenzorg 7:1 (1900)).  
Subgenus *Paphiopedilum*, section *Cochlopetalum*. A lithophytic herbs, found at 200-700 m asl. *P. glaucophyllum* is endemic to Java, and it looks similar to *P. victoria-regina* but the flower is slightly larger with shorter inflorescence. The collection was from Java.
11. *Paphiopedilum lowii* (Lindl.) Stein (Orchid.-Buch.: 476 (1892)).  
Subgenus *Paphiopedilum* section *Pardalopetalum*. This orchid is epiphyte, rarely lithophyte, found at 250-1,600 m asl. This pretty species is distributed in Java, Sumatra and Borneo. The collection was from Java.
12. *Paphiopedilum wilhelminae* L.O. Williams (Amer. Orchid Soc. Bull. 10: 373 (1942)).  
Subgenus *Paphiopedilum* section *Coryopedilum*. Terrestrial herbs in montane grassland on

limestone, found at 1,200-1,800 m asl. This species is distributed in Papua (www.orchids.naturalis.nl). Most literature cited in this paper do not mention this species, but it is on the list of WCSP. The collection was from Papua (Irian Jaya).

## Conservation

Extinction is the problem for most Orchidaceae, especially slipper orchid. The major causes of extinction are habitat destruction, over exploitation, and impact of climate change (Koopowitz *et al.* in Dixon *et al.* 2003; Swarts & Dixon 2009). Based on IUCN Red List 2017, the conservation status of *Paphiopedilum* spp. in CBG is show in Table 1.

Table 1 Conservation status of *Paphiopedilum* collected in CBG based on IUCN Red List 2018

No.	Species	Conservation status	Threats
1	<i>P. victoria-regina</i>	Critically endangered	Collection, human intrusion and disturbance
2	<i>P. bullenianum</i>	Endangered	Residential development, climate change, collection, human disturbance, etc.
3	<i>P. glaucophyllum</i>	Endangered	Residential and commercial development, collection, plantation, human disturbance
4	<i>P. javanicum</i>	Endangered	Climate change, agriculture, collection, human disturbance
5.	<i>P. lowii</i>	Endangered	Residential and commercial development, climate change, collection, plantation, human disturbance, pollution
6.	<i>P. tonsum</i>	Endangered	Collection, human disturbance, fire suppression
7.	<i>P. wilhelminae</i>	Endangered	Collection, human disturbance
8.	<i>P. armeniacum</i>	Endangered	Agriculture, collection, fire suppression
9.	<i>P. bellatulum</i>	Endangered	Residential and commercial development, collection, climate change, agriculture, human disturbance, pollution, etc.
10.	<i>P. hirsutissimum</i>	Vulnerable	Mining, collection, human disturbance, fire suppression
11.	<i>P. villosum</i>	Vulnerable	Agriculture, mining, collection, human disturbance, fire suppression, etc.
12.	<i>Paphiopedilum</i> sp.	Unknown	Unknown

*Paphiopedilum* is one of the important genus for conservation, but so far only 30% of *Paphiopedilum* spp. native to Indonesia have been conserved in CBG. Meanwhile, the population tends to decrease following over exploitation and habitat destruction, not to mention the endemism. Based on IUCN red list, *Paphiopedilum*'s collections in CBG are categorized as vulnerable to critically endangered (www.iucnredlist.org). Quick action needs to be done to save this fascinating genus from extinction. Therefore, Botanical Garden plays an important role for this action through ex situ conservation (Swarts & Dixon 2009).

Until now, most *Paphiopedilum* spp in CBG were collected through flora expedition project and some were contributions from nursery around CBG. Exploring to natural habitat is a method to collect new species or duplicate of the existing species. Propagation is crucial to ensure the existence of a species. Propagation of *Paphiopedilum* spp. in CBG should become a long term project in order to support its conservation. Generative propagation of *Paphiopedilum* spp. in CBG is in early stage at present time, but the vegetative propagation through splitting the clump of this orchid has been conducted. Even, this method only duplicate the number of plants, but not the genetic variety.

## CONCLUSION

Until now, Cibodas Botanic Garden already collected 12 species of *Paphiopedilum*, eight of them are native to Indonesia and the rest are from other countries (China, Vietnam, Thailand). The conservation status of *Paphiopedilum* in CBG ranges from vulnerable to critically endangered (*Paphiopedilum victoria-regina*). Focus of conservation effort in CBG is propagation through vegetative method by splitting the plant from the clump.

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## GALL MORPHOTYPES CAUSED BY CECIDOMYIIDS IN SOME AREAS OF WEST JAVA

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### ABSTRACT

Gall midges (Cecidomyiidae) is one of the largest groups of gall-inducing insects. Many gall midges become important pests on agricultural crops. The gall morphotypes is poorly studied in Indonesia. This study aimed to describe the gall morphotypes on several plants caused by gall midges in West Java. The research was conducted from August 2017 to May 2018 with purposive sampling in Bogor, Cianjur and Sukabumi Districts. Sixteen morphotypes closed galls were found on leaves, stems, and petioles in 11 plant species of 8 families. Eight gall morphotypes have been reported in the earlier studies, while eight others were never been described.

**Keywords:** Agriculture, gall midges, insect, pests

### INTRODUCTION

A gall is abnormal growth on plants (hyperplasia or hypertrophy). Gall midges (Cecidomyiidae) is one of the largest groups of gall-inducing animals in the world (Mani 1964). Some of the cecidomyiids are fungus feeders, predators but most are plant feeders (Shorthouse *et al.* 2005). Most of gall midges are specific, but some of them are oligophagous or polyphagous. Leeuwen-Reijnvaan and Leeuwen (1926) described and illustrated gall and its causes in Indonesia. Gall midges can induce parts of plant such as flowers, leaves, apical roots to apical shoots, stems, and roots. Gall midges infestation causes gall. The structure of galls can be very simple to complex. Gall can decrease photosynthesis, inhibits plant growth, and become pest on crop plant. Some plants, however, can increase chemical compound that can be beneficial for pharmaceutical industries. This study aimed to describe the gall morphotypes on several areas in West Java.

### MATERIALS AND METHODS

The research was conducted from August 2017 to May 2018. Gall sampling was done using purposive sampling on several plants in Bogor, Cianjur and Sukabumi, West Java. Observation of gall morphology was done in the field to observe the shape, color, and gall location on the plant. Galls were documented using camera Fujifilm XA3. Galls were removed from the host plant, transported to the laboratory, and observed under stereo microscope Leica M250 C to determine the type of galls and its size.

### RESULTS AND DISCUSSION

A total of 16 different symptoms of gall caused by Cecidomyiids were found on 11 plant species and 8 families (Fig. 1). The gall symptoms were found mostly in leaves, petiole, and stem. The pattern of galls on leaves happened because the leaves have abundant and constant nutrient

resources for the gall-inducing insects (Whitham 1979; Maia 2001). The size ratio of insects to plant parts also affects infestation success, for example Cecidomyiidae has a very small body (1-5 mm), fragile ovipositor, and can only lay eggs on soft tissues (Gagne 1994), such as young leaves. Thus, it is possible that the insect chooses to lay eggs on young leaves and forms gall on the leaves.

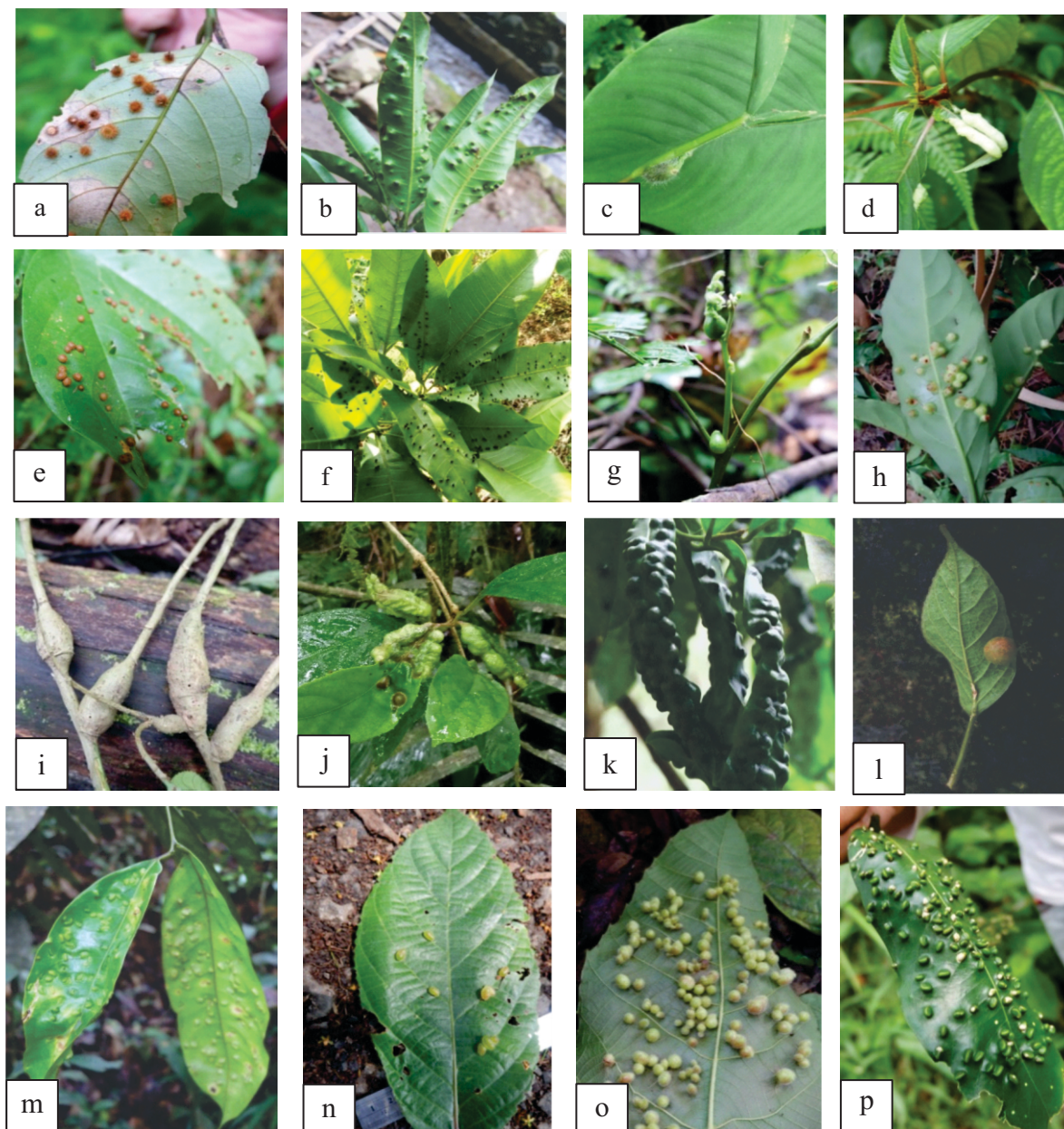


Figure 1 Cecidomyiid galls (a) leaf gall *Millettia* sp. (b) leaf gall *M. indica* (c) leaf gall *Panicum* sp. (d) *L. platyptala*: (e) leaf gall *Derris* sp. leaf gall indica; (g) stem and petiole gall *L. leucocephala*; (h) leaf gall *Psychotria* sp.; (i) stem gall *V. rubescens*; (j) leaf gall *V. rubescens*; (k) leaf gall *Antidesma* sp.; (l) leaf gall *V. rubescens*; (m) leaf gall *Xanthophyllum* sp.; (n) leaf gall *V. rubescens*; (o) leaf gall *M. triloba*; (p) leaf gall *Xanthophyllum* sp.

The shape and structural complexity are affected by feeding behavior and oviposition site of gall midges. There are two kinds of gall i.e. close and open galls. In this study there were only closed gall found. Insects that caused gall can modify host plant, vegetative tissue structure, encourage plants to produce more nutrient and habitable food sources, and form defense or protective structures from natural enemies (Price 1986).

Characteristics of galls on several plants are different and unique (Table 1). The hair is a modification of epidermal cells with additional trichomes as an effective protection against parasitoid larvae (Abrahamson *et al.* 1991). In addition, the galls have a hard layer and different thickness. Some of these anatomical structures indicate a larval defense system from interference. The hard layer of the observed gall is a hardened sclerenchyma layer that serves as a mechanism to protect parasitoids and prevent oviposition by micro hymenopterans (Brooks & Shorthouse 1998; Vandevyvere & De Bruyn 1998). However, this mechanism does not occur in galls which do not have a layer of sclerenchyma and gall shaped pouch (Rohfritsch 1992).

Table 1 Characteristics of gall on several plants caused by Cecidomyiids in some areas of West Java

Host Plant (Species: Family)	Characteristics	Fig 1
<i>Millettia</i> sp. (Fabaceae)	Closed gall and globular. The color is red to brown, became darker and deflated if the insect come out. Unilocular, indehiscent, epiphyllous, leaf upper side like normal leaf. Gall size 2-3 mm. There are hairs that cover the entire surface of gall, the hair color is darker than the gall's color.	a
<i>Mangifera indica</i> (Anacardiaceae)	Closed gall. Unilocular. Sometimes gall developed on upper side of leaf, but often on underside surface of leaf. Gall color is green, tube shape, there is a brown circle in the middle. Diameter 2.1-2.3mm, high 1-1.2 mm. Spread through surface of leaf.	b
<i>Panicum</i> sp. (Poaceae)*	Closed gall. Indehiscent. Gall develop on petiole and leaf base, covered by white hair	c
<i>Impatiens platypetala</i> (Balsaminaceae)*	The margin of the leaf is rolled up for a small part. Part of infected leaf swell, turn white, sometimes slightly yellow. Gall size determine the number of larvae in there. High attack of insect makes the shoot not grow.	d
<i>Derris</i> sp. (Fabaceae)	The color is brown and sturdy, when the insect come out, the color change became brown and shrink, measuring diameter 3-4 mm. Globular to oval shaped, in certain parts of gall form hollow and holes for the insect that will come out. Diameter 3-4 mm, Glabrous but a little bit rough. Epiphyllous. Indehiscent. Unilocular. Gall symptoms are performed only on adaxial, or part of adaxial part of the leaf, like normal leaf.	e
<i>Mangifera indica</i> (Anacardiaceae)*	Closed gall. The gall's outer part is very hard and the surface is dark brown or glossy. Epiphyllous, cylindrical swelling on the leaf upper side, on the underside scarcely visible. Unilocular, there is longitudinal larval chamber.	f
<i>Leucaena leucocephala</i> (Fabaceae)	Closed gall. Gall development on all organ especially petiole. Petiole become swollen into big globular and glabrous. Unilocular. Gall colour is green. Diameter about 5-7 mm. Indehiscent.	g
<i>Psychotria</i> sp. (Rubiaceae)	Gall color is light yellow. Closed gall. Unilocular. Hypophyllous, leaf upper side only visible brown spot. The galls formed single or in groups. Gall shape is globose with upper surface, diameter 1-2 mm.	h
<i>Villebrunea rubescens</i> (Urticaceae)	Wood tissues are distended, the bark remain unaltered, irregular spindle shaped or more cylindrical gall is formed. There are great many irregular longitudinal larva chamber or canals inside. Total gall may reach a length from 20 -150 mm and diameter of 10-20 mm.	i
<i>Villebrunea rubescens</i> (Urticaceae)*	Closed gall. Gall develop on base of leaf. Irregular shaped. There are great many irregular longitudinal larva chamber or canals inside. Indehiscent. Gall color is light green. Mature gall makes the leaves not develop.	j

Table 1 (Continued)

Host Plant (Species: Family)	Characteristics	Fig 1
<i>Antidesma</i> sp. (Euphorbiaceae)*	Closed gall. Epiphyllous. Gall developed on both side of the leaf. Gall shape in upperside surface leaf is semi globular dark green and glabrous swellings, measuring 4-5mm across. On the underside of leaf, the gall is a circular discoid swelling on the leaf blade. Larva chamber is circular. Unilocular. Indehiscent.	k
<i>Villebrunea rubescens</i> (Urticaceae)*	Closed gall. Gall develop on the underside of leaf. Ball shaped, 5-12 mm. Gall color is light green. There is a small larva chamber. Unilocular.	l
<i>Xanthophyllum</i> sp. (Polygalaceae)	Closed gall. Gall develop on upper side surface of leaf, irregular shape, hard. The colored tip of gall is light green to yellow. Spread throughout the leaf.	m, p
<i>Villebrunea rubescens</i> (Urticaceae) *	Closed gall. Gall can develop on the both of leaf side but often on the upperside of the leaf. Mostly on the mid rib or offspring on the leaf. Galls are oval, 7-9 mm long, 4-5 mm broad and 3-4 mm high. The color of gall is green, yellow, red. Gall surface was covered with white hair.	n
<i>Macaranga triloba</i> (Euphorbiaceae)*	Hypophyllous, on the upper side there is only a small rounded thickening, covered with white hair. Variable in size and form, ball shaped to oval with diameter 4 -6 mm. Unilocular, there is a long and narrow larva chamber perpendicular to the leaf blade. Indehiscent. The color of gall is yellow to light red.	o

Note: \*gall symptoms were reported by Leeuwen-Reijnvaan and Leeuwen (1926)

## CONCLUSION

Sixteen gall morphotypes caused by the cecidomyiid gall midges were collected from West Java. Eight of them were reported by Leeuwen-Reijnvaan and Leeuwen (1926), while eight others have never been reported. Most of the galls caused by the cecidomyiid gall midges were formed in the leaves.

## ACKNOWLEDGEMENT

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## GALLS ON THE WHITE EUCALYPTUS, *Eucalyptus alba* Reinw. IN SOE SUBDISTRICT, EAST NUSA TENGGARA PROVINCE

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### ABSTRACT

Galls in eucalyptus occurs due to abnormal growth of plant tissues. This study aimed to obtain information of gall types in *Eucalyptus alba* that grows in Soe Subdistrict, East Nusa Tenggara Province. Sampling was carried out by direct retrieval method on 50 sample plants. Observations were made directly to the symptomatic part of plant. The galls were characterized morphologically according to their shapes, colors, and plant parts. The percentage of each gall type was calculated. The results showed that there were 10 types of galls on the leaves, buds, twigs, and stems. The gall shapes were characterized as globoid, ovoid, conical, fusiform, spherical, and lenticular. The percentage of gall was different for each gall type, in the range of 4-90%.

**Keywords:** Cecidomyiidae, Eulophidae, Morphotype

### INTRODUCTION

*Eucalyptus* is native to Australia, Papua New Guinea, Philippines and Indonesia (Davidson 1993). *Eucalyptus* grows naturally in the eastern part of Indonesia i.e *E. deglupta* from Sulawesi, *E. urophylla* and *E. alba* from East Nusa Tenggara (NTT), and *E. pellita* from West Papua (Pramono & Pudjiharta 1996). *Eucalyptus* is also one of the cultivated plants in Industrial Plantation Forest in Indonesia as raw material of pulp and paper industry. One of the obstacles in the cultivation of *Eucalyptus* is the presence of gall-forming insects. Plant galls are structures which may appear in any host-plant organ in response to the feeding activity of galling arthropod, commonly an insect. The relationship between host plants and their herbivores is highly specific, and the galls are considered the extended phenotype of their inducing organisms (Abrahamson & Weis 1997). From this point of view, it seems plausible to consider gall morphotypes as a reliable representation of the Biodiversity of the Insects in a given area, even if the involved taxa are not scientifically described yet (Carneiro *et al.* 2009). In Indonesia, especially NTT, information about these insects and gall types on *Eucalyptus alba* are still limited. The aim of this study was to determine types and percentage of galls occurrences on *E. alba* plant in Soe Subdistrict, East Nusa Tenggara Province.

### MATERIALS AND METHODS

The galls were collected at the Buat Reforestation (altitude:  $\pm$  829 m asl; latitude: 9°50'59''S; longitude: 124°16'20''E) Soe Subdistrict of East Nusa Tenggara Province. The observations were conducted on 50 plant samples. The galls were characterized morphologically according to their shapes, colors, plant organs of occurrence (Isaias *et al.* 2013). Percentage of gall occurrences from each of the galls symptoms were analyzed.

## RESULTS AND DISCUSSION

Gall is an abnormal form change in the form of swelling of cells or plant tissue that looks enlarged beyond its normal size. The symptoms of galls on *E. alba* plants were found in the leaves, buds, twigs, and stems. Ten gall morphotypes were found on *E. alba*. The gall shapes were characterized as globoid, ovoid, conical, fusiform, spherical, and lenticular. The most commonly reported is globoid. In addition, galls presenting green, brown, and red colors were observed (Fig. 1, Table 1). Therefore, it can be assumed that they are distinct species and probably endemic. A detailed ecological and taxonomic study are needed to analyze each gall.

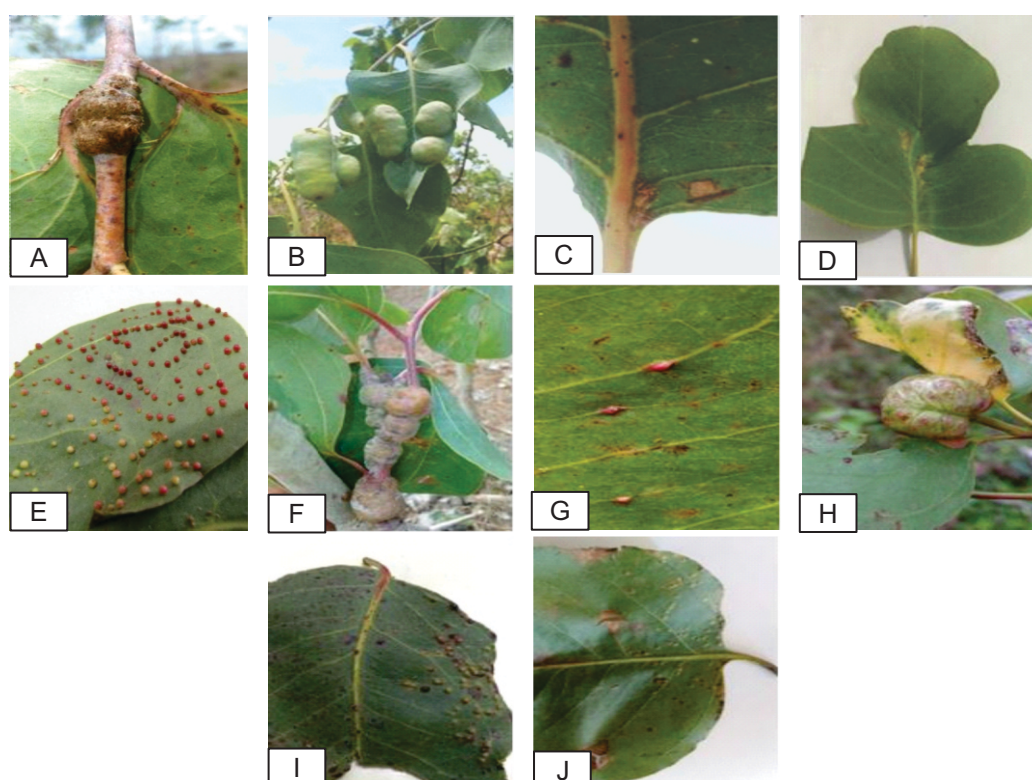


Figure 1 Galls found on *E. alba*, in Soe Subdistrict of East Nusa Tenggara Province A. Fusiform; B. Globoid/avoid; C. Globoid; D. Spherical; E. Globoid; F. Swelling (Fusiform); G. Avoid; H. Globoid; I. Conical; J. Lenticular

Symptoms of galls on *E. alba* plants were caused by some insects from Family of Eulophidae, Hymenoptera and Family of Cecidomyiidae, Diptera. The percentage of gall occurrences were different for each symptom. On *E. alba* the percentage was in the range of 4-90%. All of galls found in *E. alba* were closed types. In many of these cases, it is essential to conduct phenological and anatomical analyses to avoid misinterpretations. Gall shapes and colors may vary during their development, which could lead to the accounting of the same gall as different morphotypes. Some of the collected insects must be further identified. Some of the insects may have never been reported for existence elsewhere.

Table 1 Gall characteristics and occurrences on *E. alba*

Plant part	Foliar surface	Description	Occurrence	Percentage of Gall Occurrences (%) <sup>*</sup>	Figure 1
Twig	-	Gall is round in length on twigs. The shape is fusiform. The color of gall is green or brown. Length $\pm 0.5-5$ cm.	Aggregate	64	A
Leaf	Both	The oval gall extends in the center of the leaf enlarged to the entire surface of the upper and lower leaves. The shape is globoid/ovoid and color is green. Length $\pm 0.6-6$ cm.	Aggregate	69	B
Leaf	Adaxial/abaxial	Color is brown, hardened like wood, on the top or bottom surface of the leaf. The shape is fusiform and color is brown. Length $\pm 0.2-1$ cm.	Isolated or Aggregate	20	C
Leaf	Both	The gall on the edge of leaf causes malformation of the leaves. The shape of the gall is rounded covered at the top and bottom surfaces of the leaves. The shape is spherical. The color of gall is yellowish green. Length $\pm 0.2$ cm.	Isolated or Aggregate	28	D
Leaf	Abaxial	The small round gall on the surface of the lower leaves are green and then turned red. The shape is globoid. The color of gall is red or green. Diameter $\pm 0.5$ mm.	Isolated	17	E
Stem	-	Malformation of the stem. The shape is swelling (Fusiform) and color is green or brown. Length $\pm 0.5-8$ cm.	Aggregate	64	F
Leaf	Both	The oval gall covered in the leaf bone on the top surface and the bottom surface of the leaf. The shape is ovoid and color is red or green. Diameter $\pm 2$ mm.	Isolated	90	G
Bud	-	Gall is globoid on tip a leaf. The color is green. Diameter $\pm 2$ cm.	Isolated	4	H
Leaf	Both	Round gall with red color spreads in the middle and the edge of the leaves. Gall that formed is closed on the top surface and the bottom surface of the leaf. The shape is conical and color is red or green. Diameter $\pm 2$ mm.	Isolated	85	I
Leaf	Adaxial	Gall is small blister, greenish on the surface of the leaf. The shape is lenticular. Diameter $\pm 1$ mm.	Isolated	76	J

Note: <sup>\*</sup>Percentage of infestation incidences from each gall; n = 50



## CONCLUSION

There were 10 types of galls recorded on the leaves, buds, twigs, and stems. The percentage of gall was different for each gall type, in the range of 4-90%. The gall can seriously threaten the eucalyptus industry in Indonesia.

## ACKNOWLEDGEMENTS

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## MORPHOLOGICAL IDENTITY OF *Intsia palembanica* Miq. AND *Intsia bijuga* (Colebr.) Kuntze (FABACEAE)

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### ABSTRACT

Recently the Ministry of Environment and Forestry has established a Ministerial Regulation NOMOR P.20/MENLHK/SETJEN/KUM.1/6/2018 that set the protected species of plants and animals. This regulation allows the government to supervise and control the protected species from extraction or exploitation. *Intsia palembanica* Miq. is one of the valuable timber species, known as *Merbau*, and is currently included in the list of protected species. The species looks similar and often is confused with *Intsia bijuga* (Colebr.) Kuntze, another known *Merbau*. The research aimed to eliminate the confusion in identifying both species. Species identification is critical, especially for timber industry and local people to use the species sustainably. Studies of herbarium specimens and taxonomic reference were carried out to understand the species morphology and to find key characters for the two species. The results showed that key characters that can be used to distinguish *Intsia palembanica* from *Intsia bijuga* are the states of leaflets, petiole and hypanthium.

**Keywords:** *Intsia bijuga*, *Intsia palembanica*, *Merbau*

### INTRODUCTION

*Intsia palembanica* Miq. and *Intsia bijuga* (Colebr.) Kuntze, both known as *Merbau*, are high quality timbers that are known for their durability. These timbers have an important role in industry and is widely used in building construction, and as raw material for furniture, floor and door panels. Both *Merbau* species have morphological characters that are similar and difficult to distinguish. The proper characterization for each species is very important because both species of *Merbau* have different protection status. *Intsia palembanica* is included in the list of plant species protected under the recently set Ministerial Regulation NOMOR P.20/MENLHK/SETJEN/KUM.1/6/2018, which is a revision of the previous list of protected species in Government Regulation, PP NO. 7/1999, while *Intsia bijuga* is not included in the Ministerial Regulation. As a consequence, the extraction and transportation of *Merbau* species of *Intsia palembanica* is under supervision and controlled by the Government of Indonesia. In other words, extraction of *Intsia palembanica* is not possible except by submitting a permit application to the Minister of Environment and Forestry, while the extraction of *Intsia bijuga* is still possible. In this situation, proper characterization between the two species of *Merbau* is very critical for both the sustainability of *Merbau* industry and *Merbau* species itself. Therefore, the research aimed to characterize each *Merbau* species and understand the distinction between the two species.

## MATERIALS AND METHODS

Observation of herbarium specimens of *Intsia palembanica* and *Intsia bijuga* was done at the Herbarium Bogoriense (BO). Taxonomic reference study was also carried out to consult and clarify the identity of the species through their protologs and type specimen references. Characters and character states observed were noted and analyzed to determine key characters to differentiate the two species. Species descriptions were created based on observation and/or following Hou (1996) when needed.

## RESULTS AND DISCUSSION

Study on the specimens of both *Intsia* species at the Herbarium Bogoriense (BO) showed that recognizing the two *Intsia* species is not easy, because some characters were mixed within the two species. Several characters, such as leaflet texture, thickness, shape, size, venation, shape of apex and base, cannot be used to differentiate *Intsia palembanica* from *Intsia bijuga* because they are somewhat similar or occur in both species. Every state of those characters can interchangeably be found in each species, e.g. both rough and smooth upper surface of leaflets occurred in both species; the apex was mixed from obtuse or rounded to acute or shortly acuminate, the same case occurred for the leaflet base. The leaflet shape and size also overlapped in both species.

Further observation on the herbarium specimen found that some characters can be useful for distinguishing *Intsia palembanica* from *Intsia bijuga*. State characters of leaf, petiole and hypanthium can be used to differentiate, i.e. *Intsia palembanica* commonly has 4-jugate leaf, shorter hypanthium and longer petiole meanwhile *Intsia bijuga* has 2-jugate leaf, rarely 3-jugate, longer hypanthium and shorter petiole. The petiole is puberulous and glabrescent in *Intsia palembanica*, but glabrous in *Intsia bijuga* (Table 1, Fig. 1).

Table 1 Key characters for *Intsia palembanica* and *Intsia bijuga*

Characters	<i>Intsia palembanica</i>	<i>Intsia bijuga</i>
Leaves	commonly 4-(rarely 3-) jugate	usually 2- (or 3-) jugate
Petiole/rachis	(9.5-)9.5-17.5 cm long	5-6.5(-11.5) cm long
Petiole/rachis	puberulous and glabrescent	glabrous
Hypanthium	3-4 mm long	6-10(-16) mm long

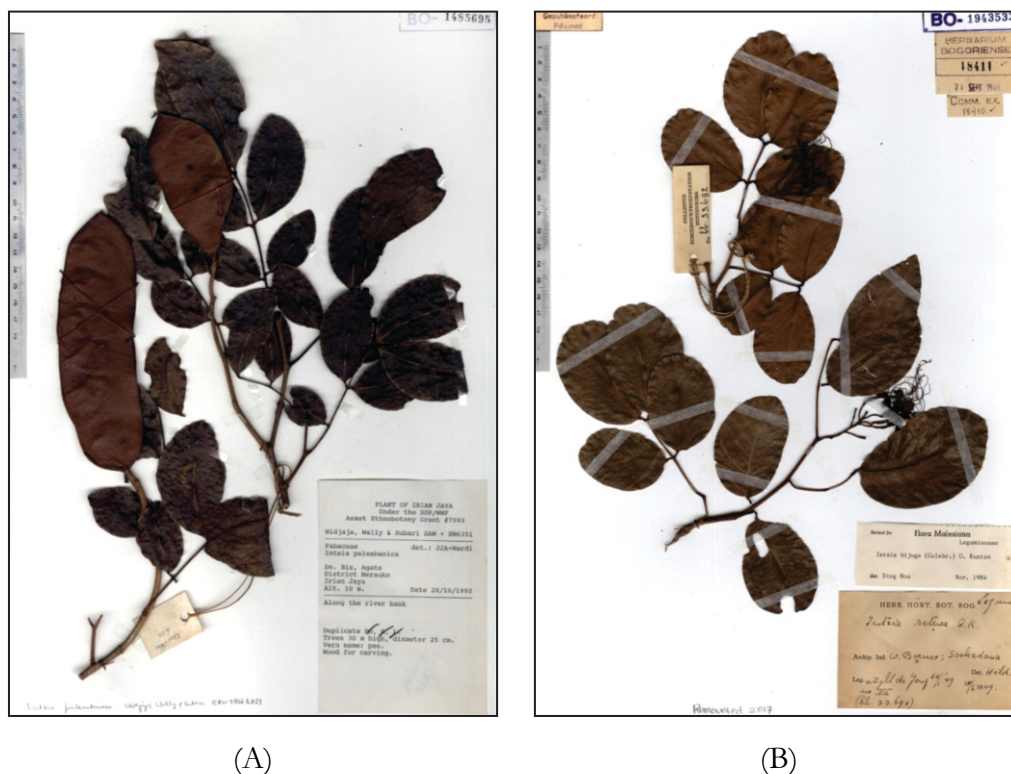


Figure 1 A. *Intsia palembanica* Miq., showing 4-jugate leaves with young pod (from Widjaja *et al.* 6351, BO); B. *Intsia bijuga* (Colebr.) Kuntze, showing 2-jugate leaves with inflorescences and flowers (from de Jong bb. 33692, BO)

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## DIVERSITY OF RATTANS IN WATUWILA MOUNTAIN, SOUTHEAST SULAWESI, INDONESIA

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### ABSTRACT

Rattans flora of Sulawesi, especially Southeast Sulawesi, is poorly known. Only few rattans scientist made rattans explorations there, including Heyne in early 1900 and Mogeia in around 1980. After almost one hundred years, another field work in Mt. Watuwila had been done in 2011. The objective of this study is to inventory and to update rattans collection deposited at Herbarium Bogoriense (BO). This field work recognized ten rattans species including *Daemonorops beguinii*, one new record for the island.

**Keywords:** Diversity, Mt. Watuwila, new record, rattans, Sulawesi,

### INTRODUCTION

Rattans (derived from the Malay word *rotan*) are spiny climbing palm belonging to subfamily *Calamoideae*. The *Calamoideae* is the second largest palm subfamily, containing around 650 species in 22 genera distributed throughout the wet tropical regions of the world. This subfamily is particularly diverse in Western Malesia and neighbouring parts of Southeast Asia. Member of this subfamily can easily be recognized by having overlapping scales covering the fruit. This fruit scale is arranged regularly in the vertical line such as in *Salacca* fruit (Dransfield *et al.* 2008).

Most species of rattans are used for a great variety of purposes. Rattans' strength and their great flexibility are ideal for binding purposes. People living near the forest may have used almost all rattans species encountered and only few species enter the world trade in rattan's furniture industry (Dransfield 1992). Other uses range from thatch to medicine. The red resin of *Daemonorops draco* and its allied species are reported as useful natural coloring material, medicine and other domestic uses (Rustiemi *et al.* 2004).

Palm flora of Sulawesi has been known since Rumphius in 1741, and the first record was published by Giseke in 1792. Those two former researchers had never visited Sulawesi. Beccari was the first palm specialist who works in Sulawesi since 1886. He conducted palms exploration from South Sulawesi, South East Sulawesi, North Sulawesi and some remote islands near North Sulawesi. Most of his collection were deposited at Herbarium Florence (FI), and only small portions were deposited at Herbarium Bogoriense (BO). After Beccari, Heyne also conducted some palm explorations, especially in Southeast Sulawesi in early 1900 (Heyne 1949). After almost one hundred years, in 2011 another field work had been made in Mt. Watuwila.

This research aimed to understand the diversity of rattans in Mt. Watuwila, to inventory and update rattans collection at BO, especially herbarium specimen from Sulawesi and also to provide specimen data information for taxonomic study and species conservation status.

Mt. Watuwila area is situated in Sanggona Village, Uluiwoi District, Kolaka Regency Southeast Sulawesi Province (latitude 3°46'60" S and longitude 121°34'0" E). The mountain is located in the northern part of the capital city of Kolaka Regency, extended from north to

northeast. Uluiwoi District is located in the inland of Kolaka with Morowe River stream down in the area for irrigation and household needs (BPS Kolaka Regency 2008). Unfortunately, recent situation reported that rattans habitat in Mt. Watuwila was under severe threat because of illegal logging, cacao and coffee plantation and also slash and burning activities by local people. These irresponsible activities could harm the rattans species as well as other species. In the end, the forest will be lost forever, even before they are fully described. Results of this study can be used as basic information for local stakeholders and government to conserve the forest for the next generation.

## MATERIALS AND METHODS

Field work was done in Mt. Watuwila, Southeast Sulawesi and its adjacent areas to collect herbarium specimens. Herbarium specimens preparation was conducted following standard procedure of Dransfield (1986). Data or information recorded from the field included location, general habitat, altitude, association with other plant, vernacular name, uses, habit (solitary/clustered), stem (height, diameter with/without leaf sheath, internode length, color), leaves (length, leaflets arrangement, number of leaflets, length and width of leaflets), inflorescence (length, number of rachilla, color), flower (color, scented/not scented), fruit and seed (length and width, color). Field work data combined with herbarium data of each taxon resulted in comprehensive data set for morphological data comparison. Herbarium study was done in BO following de Vogel (1987) and Rifai (1976) using comparative morphology data as the main source, especially in developing species concept (Davis & Heywood 1963; Dransfield 1999).

## RESULTS AND DISCUSSION

### General Morphology of the Rattan

#### Habit

All species of Sulawesi rattans are climbing plants; some species climb high into the forest canopy, while others climb rather weakly or scramble in the forest undergrowth. Some rattans are solitary-stemmed (such as in *Calamus symphysisipus*), while others clustered (such as in *C. minabassae*).

#### Stems

Most *Calamus* in Sulawesi have multiple, long, woody, flexible, unbranched stems arising from a single rootstock. In some species the stem forms a short erect trunk. The rattan stems range from a few millimeters to over 6 cm in diameter.

#### Leaves

Each section of stem (or internode) ends in a ring (or node) bearing a large compound leaf. The leaf bases are though tubes which overlap, referred to as the leaf sheath, and conceal the upper part of stem (Evans *et al.* 2002). At its upper end, the sheath narrows into the petiole that continues into the rachis or leaflet-bearing portion of the leaf. The petiole of some *Calamus* in Sulawesi is sometimes very short or absent. In the field, the absence of petiole is a useful character for distinguishing species. The rachis of some *Calamus* in Sulawesi is extended beyond the terminal leaflets into a barbed whip (cirrus) which acts as a climbing organ (Dransfield 1992b).

Rattan's leaf sheath armature is the most striking feature. In almost all species the sheath bears spines. The spine arrangement is extraordinarily diverse and has diagnostic importance character. Within Sulawesi, it is possible to identify almost all 28 different species based on the mature leaf sheath. In a few species the leaf sheaths lack of spines such as in some forms of *Calamus ornatus*, but this species is not totally spineless because the leaf rachis, cirrus and flagellum are still heavily armed. In between the spines there are a wide range of hairs, scales or indumentum and/or wax (Dransfield 1992b).

At the tip of the sheath (leaf sheath mouth) where the sheath narrows into the petiole or leaf rachis, there is a prolongation of the main part of the sheath and known as an ocrea (Dransfield 1992a). All *Calamus* in Sulawesi have papery, short ocrea. *Calamus rosetus* has a pair of distinctive spine at the border of leaf sheath mouth and petiole areas which can also be used as diagnostic character to differentiate rattans species.

The leaf rachis is part of the leaf axis which bears the leaflets and sometimes bears reflexed grapnel-like spines on the lower surface and these contribute to the climbing process by locking on to support (Dransfield 1992b). The rattans leaf is basically feather-like rather than palmate or fan-like. The commonest arrangement of the leaflets in *Calamus* of Sulawesi is arranged regularly, that is evenly spaced along the rachis. Only few species have irregular leaflets which grouped and fanned within the group such as in *Calamus rosetus* which has beautiful paired leaflets.

### Climbing organs

There are two types of organs related with climbing in *Calamus*. They look superficially similar but they differ completely in their morphological origin i.e. the cirrus and the flagellum. The former form is an extension of the leaf rachis beyond terminal leaflets, whereas the latter is a sterile inflorescence borne on the leaf sheath near the knee. Both of climbing organs bear groups of short, grapnel-like spines (Dransfield 1992b). *Calamus* found in Sulawesi is mostly climbing using their cirrus instead of using their flagellum. The result of this study showed that *Calamus perpendiculatus* was the only species having vestigial flagellum up to 20 cm long and bearing slightly 5 peduncular bracts.

### Inflorescence

The pistillate inflorescence bears dyads (pairs of flowers), consisting of female flower and sterile male flower along each side of the rachilla in the axils of the rachilla bracts, whereas staminate inflorescence bears male flowers only. In the axil of each bract on the female rachilla there are two bracteoles, i.e. the involucre and involucrophore (Dransfield 1984).

### Infructescence

*Calamus* fruits, as the other rattan fruits are covered in vertical rows of reflexed scales. There is often a beak at the top of the fruit, tipped by the remains of the stigmas. The remains of the sepals and petals are found at the base of the fruit (Dransfield 1992b). Inside the fruit there is usually single seed, but some species in Sulawesi also have two seeds such as in *Calamus didymocarpus* and *C. koordersianus*.

### Diversity of Rattans in Mt. Watuwila

The information of Sulawesi rattans is very limited. The rattans of Sulawesi are represented by three different genera *Calamus*, *Daemonorops* and *Korthalsia*. In Sulawesi, *Calamus* is the largest rattan genus with more than 23 species (Mogea 2002; Rustiami *et al.* 2017). This genus is very

common tropical genus with the centre of diversity in Southeast Asia. In Sulawesi, *Daemonorops* consists of 8 species, whereas *Korthalsia* has one species, *K. celebica* is endemic species of Sulawesi. Considering large area of study to be covered, the possibility to find new species and new record is tremendous.

Based on field work in 2011, ten rattans species were found in Mt. Watuwila: *Calamus bonienseis*, *C. inops*, *C. kandariensis*, *C. minabassae*, *C. ornatus*, *C. symphysipus*, *C. zollingeri*, *Calamus* sp. 1, *Daemonorops beguinii* and *Korthalsia celebica* (Fig. 1). *Daemonorops beguinii* is new record for Sulawesi. Additional herbarium specimen from this field work can be used for updating specimen data deposited at BO as well as a baseline data provided for taxonomic study and species conservation purposes.



Figure 1 Clockwise from top to left: *C. symphysipus*, *C. minabassae*, *C. ornatus*, *C. zollingeri*, *C. bonienseis*, *C. inops*, *D. beguinii*, *K. celebica*

### Identification Key to the Rattans of Mt. Watuwila and Its Adjacent Areas

- |     |  |                             |
|-----|--|-----------------------------|
| 1a. | Leaflets praemorse   | <i>Korthalsia celebica</i>  |
| 1b. | Leaflets entire  | 2                           |
| 2a. | Entire inflorescences enclosed by the first bract, splitting along their length to the very base | <i>Daemonorops beguinii</i> |
| 2b. | Inflorescences not enclosed by the first bract, tubular, if splitting then persistent            | 3                           |
| 3a. | Alternate diminutive leaflets on its summit of leaf rachis present                               | <i>Calamus ornatus</i>      |
| 3b. | Alternate diminutive leaflets on its summit of leaf rachis absent                                | 4                           |
| 4a. | Leaves subcirrate  | 5                           |
| 4b. | Leaves cirrate   | 7                           |
| 5a. | Leaflets lanceolate, leaf sheath flagellate  | <i>Calamus symphysipus</i>  |



- |  |                             |
|--|-----------------------------|
| 5b. Leaflets broadly linear, leaf sheath eflagellate   | 6                           |
| 6a. Petiole up to 1 cm, basal leaflets swept back across the stem  | <i>Calamus minabassae</i>   |
| 6b. Petiole more than 1 cm, basal leaflets not swept back across the stem  | <i>Calamus kandariensis</i> |
| 7a. Primary bracts elongate and tubular; rachilla sessile  | <i>Calamus zollingeri</i>   |
| 7b. Primary bracts tubular, tattering in age; rachilla pedicellate   | 8                           |
| 8a. Primary spatha very closely sheathing  | <i>Calamus</i> sp.          |
| 8b. Primary spatha loosely sheathing and often more or less inflate  | 9                           |
| 9a. Leaf sheath covered with scattered, solitary spines; leaf sheath surface shiny yellow, horizontal ridges present | <i>Calamus bonienseis</i>   |
| 9b. Leaf sheath covered with densely series of spines; leaf sheath surface green, horizontal ridges absent           | <i>Calamus inops</i>        |

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## UTILIZATION OF LIQUID ORGANIC FERTILIZER AND SOIL COMPOST FOR ENGINE OIL WASTE BIOREMEDIATION

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### ABSTRACT

Minimizing the risk of inorganic waste such as engine oil, can be done by bioremediation processes. The purpose of this research was to determine the effectiveness of soil compost and liquid organic fertilizer for waste degradation. The parameters of this research were: pH, water content, ash content, water holding capacity, and total petroleum hydrocarbons (TPH). The results showed that TPH levels reached 81% and more in two treatments. The other treatments showed different TPH level between 40-60%. Environmental conditions in bioremediation process also showed good results. The average pH range was 6-7. Combination of soil compost and liquid organic fertilizer for TPH degradation showed good results, although not all treatments showed efficient results.

**Keywords:** Bioremediation, degradation, liquid organic fertilizer, Total Petroleum Hydrocarbon (TPH), soil compost

### INTRODUCTION

Waste is a material from a production process that generally cannot be utilized anymore. If waste is not managed properly, it will negatively affect the community. One of the rarely used wastes is engine oil. Chemistry, physics and biology elements can be used to process oil waste. Biologically, oil waste can be degraded by bioremediation process, using microorganisms' activity.

In this research, the bioremediation process was conducted by means of composting technique, using natural liquid organic fertilizers made from environmentally friendly ingredients, such as a mixture of rotten fruits, banana roots, plain rice water, coconut water, and sugar (Parnata 2004). Other experiment was also conducted in a laboratory scale by combining rice straw and natural microorganisms found in compost soils in a pot or polybag.

Total degradation of Total Petroleum Hydrocarbons (TPH) content in engine oil waste reached 81%, using composting method. Difference in total degradation of engine oil waste was also influenced by adding 10% of oil in this research. The oil addition caused the group of bacteria found in liquid fertilizer and composted soil unable to efficiently degrade oil waste. Environmental factors in the form of pH indicated normal condition for microbial growth and metabolism, i.e. pH range 6-8.

### MATERIALS AND METHODS

Tools used in this research were electric oven, furnace, petri dish, desiccator, analytical balance, polybag, pH indicator, stirrer, shaker, filter paper, Whattmann paper, Beaker glass, Erlenmeyer, and other glasswares. Materials used in this research were composted soil obtained from sellers of various types of local flowers, rice straw obtained from local farmers, waste oil

from surrounding garages, rotten fruits, banana roots, rice water, coconut water, brown/granulated sugar, aquadest, and n-hexane.

This research was an examination of oil waste biodegradation using combination of concentrated liquid organic fertilizer and composted soil. Bioremediation technique implemented was composting method using polybag as small scale composting medium. Two variables were observed in this study. The first variable was the dosage of liquid organic fertilizer (0 mL, 2 mL, 5 mL, 8 mL, and 11 mL) and the second variable was the dosage of composted soil (100 g, 300 g, and 500 g). The amount of rice straw used was 50 g for each treatment. The amount of oil used was 10 mL for each treatment. Every 7 days, the sample of the treatments was sprayed three times with liquid fertilizer.

## RESULTS AND DISCUSSION

### pH

pH measurement results indicated that the average pH of treatment samples was in a normal pH range, i.e. 6-7 (Fig. 1). The first result of this research concluded that bacteria were able to degrade engine oil waste.

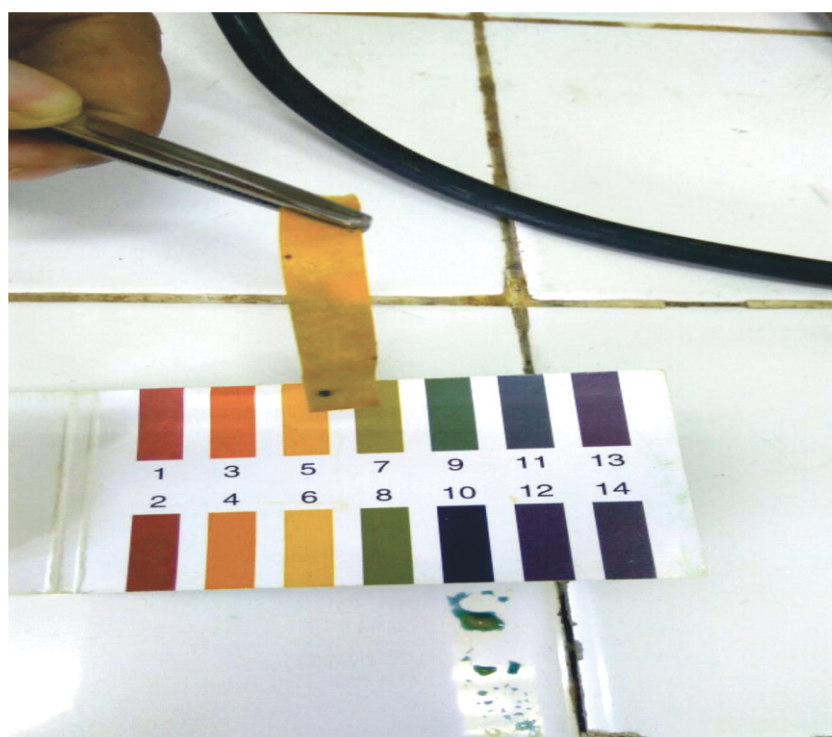


Figure 1 The result of pH analysis

### Total Petroleum Hydrocarbon (TPH)

TPH measurements were performed to find out the percentage of hydrocarbon remaining in the oil waste after going through composting process. This analysis was conducted so that the harmful compounds in the oil waste can be minimized and that the oil waste is safe to be used as fertilizer.

Overall, each treatment showed different results (Table 1). Treatments 8 and 12 showed the highest total TPH degradation of 81%. Treatment 8 consisted of 5 mL of liquid fertilizer and 300 g of composted soil with TPH total degradation reached 81.17%. On the other hand, treatment 12 consisted of 2 mL and 500 g of composted soil with total degradation of TPH reached 81.52% (Table 1). The addition of composted soil can accelerate degradation process of oil waste by microbes. A minimum amount of oil (i.e. 10 mL) added to each treatment also affected TPH degradation of oil waste. Research results indicated that larger combination of liquid fertilizer and composted soil may not necessarily reduce the level of oil TPH (Table 1), due to differences in bacterial composition contained in liquid fertilizer and composted soil. Basuki (2011) stated that environmental condition was very useful for metabolic rate of microbial decomposers. As seen in Table 1, Total TPH degraded by treatment 15 (11 mL of liquid fertilizer + 500 g of compost) was 62.29% (Table 1).

Table 1 Result of TPH level analysis

Treatments	First Weight	End Weight	% Degradation
P.1	5.0083	3.7463	25.20
P.2	5.0023	2.9302	41.42
P.3	5.0059	2.5365	49.33
P.4	5.0034	2.3889	52.25
P.5	5.0059	1.8609	62.83
P.6	5.0029	2.8159	43.71
P.7	5.0038	2.4735	50.57
P.8	5.0050	0.9423	81.17
P.9	5.0026	2.5068	49.89
P.10	5.0054	1.7060	65.92
P.11	5.0014	2.0254	59.50
P.12	5.0089	0.9254	81.52
P.13	5.0071	2.0703	58.65
P.14	5.0062	2.9905	40.26
P.15	5.0008	1.8858	62.29

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## **ZUCCHINI YELLOW MOSAIC POTYVIRUS (ZYMV) ON CUCUMBER (*Cucumis sativus* L.) IN JAVA, INDONESIA**

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### **ABSTRACT**

Mosaic symptoms with variations were found in several cucumber cultivations in Java, Indonesia. The study aimed to characterize the causal virus of typical mosaic symptoms on cucumber. A total of 543 typical symptomatic leaf samples were collected from cucumber cultivation area in Java. Disease frequency was determined serologically by dot blot immunobinding assays (DIBA) test using specific antiserum of *Zucchini Yellow Mosaic Virus* (ZYMV) and *Watermelon Mosaic Virus* (WMV). Furthermore, the Potyvirus was detected by RT-PCR, then cloned into TA-cloning vector, and the DNA was sequenced. Disease frequencies of ZYMV in West Java, Central Java, Yogyakarta, and East Java Provinces were up to 42.86%, 63.63%, 61.66%, and 54.44%, respectively, while the disease frequencies of WMV were 23.62%, 38.84, 13.33, and 47.77, respectively. Nucleotide sequences analysis of samples which gave positive reaction against ZYMV and WMV antisera were identified as ZYMV. The homology of either nucleotide or amino acid sequences analysis of ZYMV Java isolates ranged from 92.3-98.3% and 99.0-100%, respectively. The phylogenetic tree analyses showed that ZYMV Java isolates were in the same cluster with those of Reunion Island (L29569) and Timor Leste (KY225545) isolates.

**Keywords:** Cucurbit, cylindrical inclusion, mosaic, Potyvirus

### **INTRODUCTION**

Cucumber is one of important vegetables in Indonesia. Production of cucumber fluctuates every year. Many factors affect cucumber production, among them is virus diseases. Mosaic symptoms with variations were found in several cucumber cultivations in Java, Indonesia. It is difficult to diagnose virus disease and to determine the causal virus based merely on phenotypic symptoms. Only a limited amount of information is available on virus causing mosaic symptoms on cucumber in Java. The study aimed to characterize the causal virus of typical mosaic symptoms on cucumber.

### **MATERIALS AND METHODS**

A total of 543 typical symptomatic leaf samples were collected from cucumber cultivation area in Subang, Indramayu, and Bogor (West Java Province), Brebes and Klaten (Central Java Province), Kulon Progo (Yogyakarta Province), Nganjuk, Kediri, and Tulungagung (East Java Province). Disease frequency was determined serologically by *Dot Blot Immunobinding Assays* (DIBA) test using specific antiserum of *Zucchini Yellow Mosaic Virus* (ZYMV) and *Watermelon Mosaic Virus* (WMV). Based on DIBA test, the total RNA of samples positively against ZYMV and WMV antisera were extracted, and used as template for cDNA synthesis and Reverse

Transcription (RT) PCR. RT-PCR was conducted using either virus specific or a pair of universal primer of cylindrical inclusion (CI) gene of Potyvirus (Ha *et al.* 2008). DNA PCR products were cloned into TA-cloning vector, then the plasmid DNA clones were sequenced and analyzed. Nucleotide sequences of the CI gene were aligned and analyzed using BioEdit version 7.05 software, then compared with corresponding sequences deposited in Genbank. The phylogenetic tree was constructed using MEGA 6.0 software with the neighbor-joining algorithm using 1.000 bootstrap as replications (Tamura *et al.* 2013).

## RESULTS AND DISCUSSION

Mosaic symptoms found varied such as light green mosaic, yellow mosaic, dark green mosaic, and green mosaic (Fig. 1). Mixed infection between ZYMV and WMV occurred on cucumber with percentage of mixed infection up to 40.88%. Disease frequency of ZYMV in West Java, Central Java, Yogyakarta, and East Java Provinces was up to 42.86%, 63.63%, 61.66%, and 54.44%, respectively, while disease frequency of WMV was 23.62%, 38.84%, 13.33% and 47.77%, respectively. These percentages suggested that ZYMV and WMV infections were evenly distributed on cucumber plants in Java.

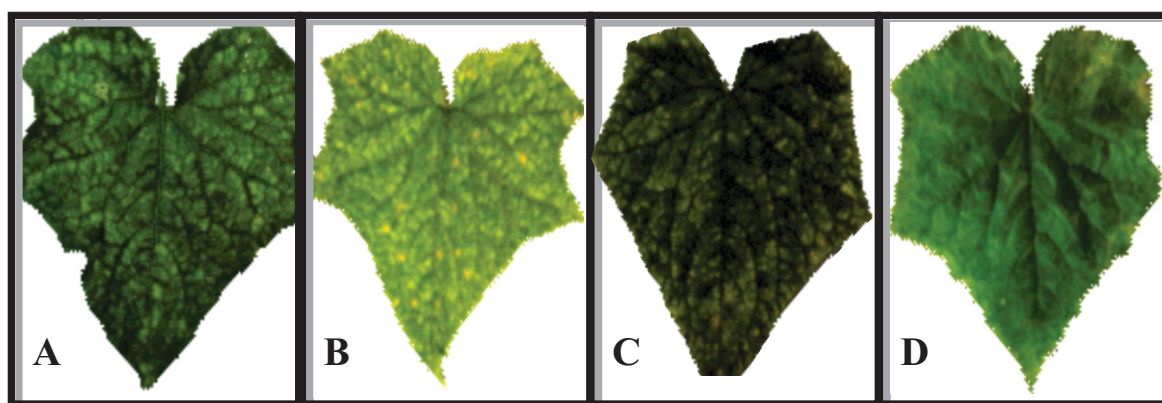


Figure 1 Typical mosaic symptoms on cucumber found in fields. A = light green mosaic (Kdr isolate), B = yellow mosaic (KP isolate), C = dark green mosaic (Brbs isolate), and D = green mosaic (Bgr isolate)

Amplification of ZYMV and WMV using specific primers was unsuccessful. Successful amplification, however, was conducted using universal primer of CI gene of *Potyvirus* with size of  $\pm 650$  bp. Nucleotide sequences analysis of samples which gave positive reaction against ZYMV and WMV antisera were identified as ZYMV. This indicated the cross reaction of WMV antisera to the samples infected by ZYMV on DIBA test.



Table 1 Homology of nucleotide (nt) and amino acid (aa) of ZYMV Java Isolates with corresponding isolates from other countries deposited in GenBank

Isolate source	Homology (%)								Accession number
	Kdr		Brbs		Bgr		KP		
	nt	aa	nt	aa	nt	aa	nt	aa	
ZYMV_IDN_Kdr			92.5	99.5	93.2	99.5	98.3	99.5	LC383936
_Brbs	92.5	99.5			97.9	100	92.3	99.0	LC383937
_Bgr	93.2	99.5	97.9	100			92.8	99.0	LC383938
_KP	98.3	99.5	92.3	99.0	92.8	99.0			LC383939
TUR	80.3	97.7	82.7	97.2	82.3	97.2	80	97.2	KP828394
TLS	84.1	98.1	84.8	97.7	84.8	97.7	83.3	97.7	KY225545
RU	85.7	99.0	86.3	98.6	87.4	98.6	85.3	98.6	L29569
KOR	80.2	97.2	82.1	96.8	81.7	96.8	80.5	96.8	AJ429071
JPN	80.5	96.8	82.3	96.3	81.8	96.3	80.5	96.3	AB188116
AUS	79.6	96.8	82.1	96.3	81.4	96.3	79.9	96.3	KY225547
USA	80.2	97.2	82.6	96.8	81.8	96.8	80.2	96.8	KJ875865
IRN	80.2	97.7	82.7	97.2	82.1	97.2	80.2	97.2	KU198853
ISR	79.4	97.7	81.8	97.2	81.1	97.2	79.4	97.2	EF062582
SVK	79.9	97.7	82.3	97.2	81.5	97.2	79.9	97.2	KF976713
WMV_CHN*	68.1	77.4	67.6	77.0	68.2	77.0	68.1	77.9	KX926428
PRSV_CHN*	61.0	66.3	61.6	65.9	61.5	65.9	61.2	66.3	MF085000

Notes: *Watermelon Mosaic Virus* (WMV) and *Papaya Ring Spot Virus* (PRSV) as out group; nt (nucleotida) and aa (amino acid); Indonesia (IDN), Turki (TUR), Reunion Island (RU), Timor Leste (TLS), South Korea (KOR), Japan (JPN), Australia (AUS), Amerika Serikat (USA), Iran (IRN), Israel (ISR), Slovakia (SVK), China (CHN), Kediri (Kdr), Brebes (Brbs), Bogor (Bgr), Kulon Progo (KP)

The homology of either nucleotide or amino acid sequences analysis of ZYMV Java isolates: Bogor (LC383938), Brebes (LC383937), Kulon Progo (LC383939), and Kediri (LC383936) ranged from 92.3-98.3% and 99.0-100%, respectively (Table 1). The nucleotide and amino acid sequences homology of ZYMV Java isolates were closely related to Reunion Island isolate (L29569) ranging from 85.3-87.4% and 98.6-99.0%. The homology of the CI gene among ZYMV Java isolates were high, indicating low genetic diversity.

The phylogenetic tree analyses showed that ZYMV Java isolates belongs to one cluster which were separated from corresponding isolates in the other countries (Fig. 2). ZYMV Java isolates were in the same cluster with that of Reunion Island (L29569) and Timor Leste (KY225545) isolates. The ZYMV was detected in most cucumber cultivation areas in Java. It may impact on cucumber production and mitigation attempts needs to be explored in the future.

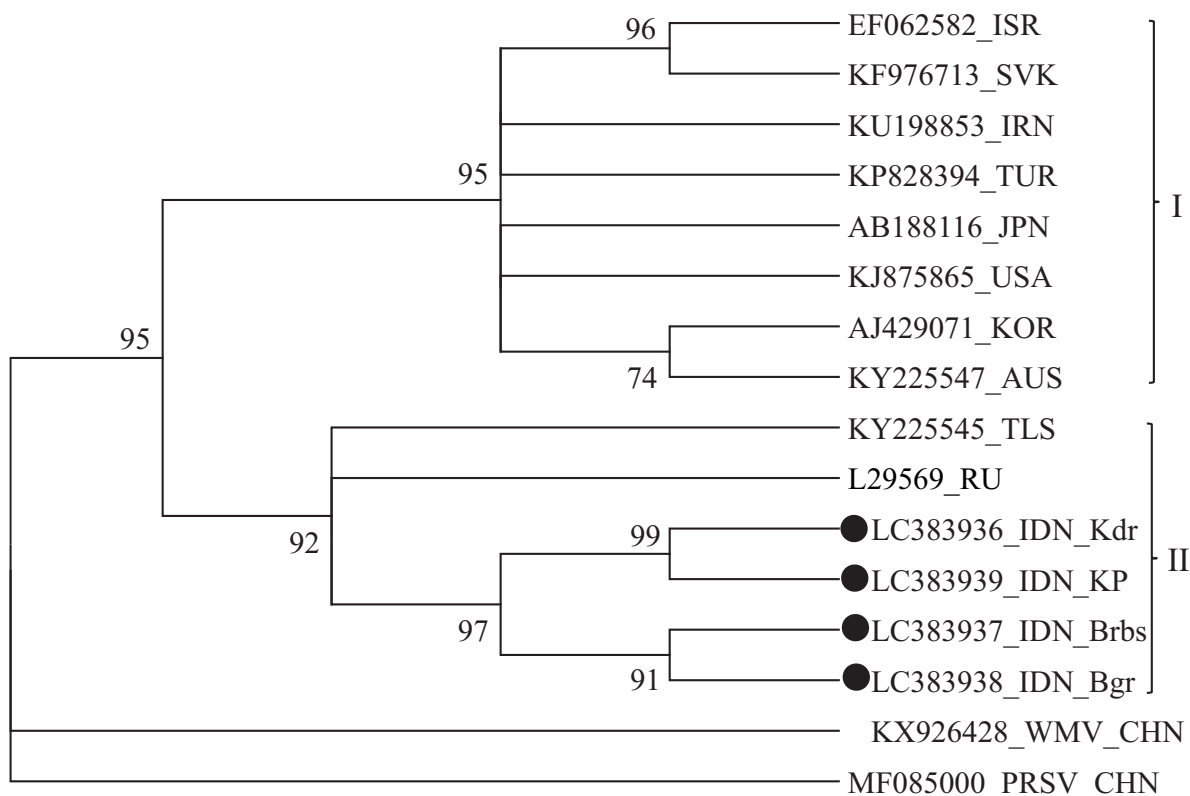


Figure 2 A phylogenetic tree based on nucleotide sequences of cylindrical inclusion gene of ZYMV Java Isolates and other isolates. The number at the branch node indicated the confidence values (%) from bootstrap analysis using 1.000 replicates. Outgroup: *Watermelon Mosaic Virus* (WMV) and *Papaya Ring Spot Virus* (PRSV). IDN-Indonesia, TUR-Turki, RU-Reunion Island, TLS-Timor Leste, KOR-South Korea, JPN-Japan, AUS-Australia, USA-Amerika Serikat, IRN-Iran, ISR-Israel, SVK-Slovakia, CHN-China

## CONCLUSION

ZYMV was found in cucumber cultivation and distributed evenly in Java. The homology of the CI gene among ZYMV Java isolates were high and showed the highest homology to Reunion Island isolate.

## ACKNOWLEDGMENTS

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## TABANIDAE IN SUMATRAN RHINO SANCTUARY AND ITS POTENTIAL AS DISEASE VECTOR IN SUMATRAN RHINOCEROS

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### ABSTRACT

Sumatran rhinoceros is an herbivorous mammal species from Indonesia which has already been in critically endangered (CR) status in the IUCN Red List. One of diseases suffered by Sumatran rhinoceroses is surra disease (trypanosomiasis) transmitted through *Tabanus* sp. from Tabanidae family. Because of its role as disease vector, further knowledge about Tabanidae flies can certainly improve health management of Sumatran rhinoceros in captivity. The purpose of this study was to identify various species of Tabanidae flies and their potency to act as a disturbers and diseases vector in Sumatran rhinoceros conserved in Sumatran Rhino Sanctuary (SRS), Way Kambas National Park (WKNP). Tabanidae fly specimens were collected using sweeping net and Nzi trap methods. The collection of specimens was done from 29 July to 07 August 2017 in SRS, WKNP. The collected Tabanidae flies were then identified. This study collected 408 Tabanidae flies which were then identified into 36 different species. The identified species belong to genera *Tabanus* and *Chrysops*. The highest dominating species caught using Nzi trap method was *C. fasciata* (17.87%) and using sweeping net was *T. incultus* (11.85%). Tabanidae flies in SRS, WKNP was potential as disturbers and disease vectors on Sumatran rhinoceros. Transmission of diseases from cattles and buffaloes around WKNP to SRS with the help from Tabanidae flies as vectors is quite possible because Tabanidae flies can fly quite far. Tabanidae flies can also cause health problems, such as anemia. Besides, Tabanidae fly infestation may also cause decrease in body weight and milk production, as well as increase in stress and uneasiness. However, it is still unclear if the infestation of Tabanidae flies in SRS is already severe and can transmit disease. Therefore, surveillance and further research about the severity of infestation is needed.

**Keywords:** Sumatran rhinoceros, Sumatran Rhino Sanctuary, Tabanidae flies, Way Kambas National Park

### INTRODUCTION

Sumatran rhinoceros is an herbivorous mammal species from Indonesia which is endangered (Zahari *et al.* 2005). Sumatran rhinoceros is also a species that is difficult to be conserved *ex situ* because it is sensitive to environmental changes and vulnerable to diseases (Nardelli 2014). One of the diseases suffered by Sumatran rhinoceroses is surra disease (trypanosomiasis). It was known that in 2004, in Malaysia there was surra disease in Sumatran rhinoceros with *Tabanus* sp. flies acting as the disease vector (Ahmad *et al.* 2013). *Tabanus* sp. is a genus of blood-sucking flies from the Tabanidae family. Tabanidae flies can be a vector for numerous diseases, such as trypanosomiasis, hog cholera and anthrax (Hadi & Soviana 2010).

Because of its role as disease vectors, further knowledge about Tabanidae flies can certainly improve health management of Sumatran rhinoceros in captivity.

Sumatran Rhino Sanctuary (SRS), Way Kambas National Park (WKNP) is an ex situ conservation program with main purpose to be a breeding center of Sumatran rhinoceroses. SRS as an ex situ conservation program can be considered to have achieved its goal (Riyanto *et al.* 2003). However, until now there has not been any studies that specifically describe Tabanidae flies and their potency to act as a disturbers and disease vector in Sumatran rhinoceroses conserved in SRS.

## MATERIALS AND METHODS

Collection of specimens was done from 29 July to 07 August 2017 in SRS, WKNP. Tabanidae fly specimens were collected using sweeping net and Nzi trap methods. The collection using sweeping net was done between 07.00 and 11.00 AM local time in rhinoceros observation cage, while the collection using 2 Nzi traps were conducted at 5.00 PM local time everyday. The collected specimens were identified in Entomology Laboratory, Faculty of Veterinary Medicine, Institut Pertanian Bogor using identification key according to Stekhoven (1926). Data on collected blood-sucking fly species was descriptively processed and analyzed.

## RESULTS AND DISCUSSION

This study collected 408 Tabanidae flies which were identified into 36 different species. The identified species belong to the genera *Tabanus* and *Chrysops*. The highest dominating species caught using Nzi trap method was *C. fasciata* (17.87%) and using sweeping net was *T. incultus* (11.85%) (Table 1).

Tabel 1 Tabanidae fly species collected in SRS TNWK

Species	Nzi trap domination (%)	Sweeping net domination (%)
❖ Tabanidae family		
a. <i>Tabanus</i> genus		
1 <i>T. albitriangularis</i>	0.19	0.89
2 <i>T. angustitriangularis</i>	1.76	2.61
3 <i>T. atripilosus</i>	0.04	0.09
4 <i>T. atrobirtus</i>	0.02	2.41
5 <i>T. basalis</i>	0.08	0.36
6 <i>T. brunneus</i>	0.08	0.36
7 <i>T. brunnipes</i>	2.34	3.57
8 <i>T. brunniventer</i>	0.02	0.00
9 <i>T. ceylonicus</i>	7.32	0.27
10 <i>T. chloropis</i>	0.02	0.00
11 <i>T. chrysanter</i>	0.00	0.27
12 <i>T. flavipenis</i>	0.00	0.02
13 <i>T. flavistriatus</i>	0.73	0.02
14 <i>T. flavitriangularis</i>	0.52	4.17
15 <i>T. flavothorax</i>	0.02	0.00
16 <i>T. fulvissimus</i>	0.00	0.02
17 <i>T. fumifer</i>	0.02	0.09

Table 1 (Continued)

	Species	Nzi trap domination (%)	Sweeping net domination (%)
18	<i>T. fumipennis</i>	0.00	0.00
19	<i>T. fusciventer</i>	3.91	1.07
20	<i>T. griseithorax</i>	0.00	0.09
21	<i>T. immanis</i>	0.08	4.28
22	<i>T. incultus</i>	1.84	11.85
23	<i>T. malayensis</i>	0.94	5.61
24	<i>T. megalops</i>	0.19	0.00
25	<i>T. minimus</i>	0.02	0.00
26	<i>T. nexus</i>	0.31	0.67
27	<i>T. ochroater</i>	0.00	0.02
28	<i>T. oviventris</i>	0.02	0.09
29	<i>T. perakiensis</i>	0.02	0.04
30	<i>T. rubidus</i>	0.00	0.02
31	<i>T. rufiventris</i>	0.02	0.00
32	<i>T. striatus</i>	0.13	0.00
33	<i>T. tintothorax</i>	0.00	0.02
34	<i>T. tristis</i>	0.02	0.89
b. <i>Chrysops</i> genus			
1	<i>C. fasciata</i>	17.87	0.00
2	<i>C. fixissima</i>	1.84	0.00

Genuses *Tabanus* and *Chrysops* can act as disease vector causing trypanosomiasis, hog cholera, and anthrax (Hadi & Soviana 2010). Results from a study by Andriansyah *et al.* (2008) showed that Sumatran rhinoceroses in SRS did not suffer from trypanosomiasis, however, there were cattles and buffaloes living around TNWK which were infected by *Trypanosoma*. It is possible that the spread of trypanosomiasis disease from cattles and buffaloes around TNWK to the Sumatran rhinoceros in SRS occurred with the help from Tabanidae flies as disease vectors, because Tabanidae flies can fly quite far (Cooksey & Wright 1989; Sheppard & Wilson 1976).

Tabanidae flies can also cause health problems, such as anemia (Raut *et al.* 2008). Besides, Tabanidae fly infestation may also cause decrease in body weight and milk production, as well as increase in stress and uneasiness (Keawrayup *et al.* 2012). Problems caused by major infestation is possible to happen to Sumatran rhinoceros population in SRS.

This research indicated that SRS has various species of Tabanidae flies which has a potency to act as a disturbers and disease vector in Sumatran rhinoceros. However, it is unclear if the infestation of Tabanidae flies in SRS is already severe and can transmit disease. Therefore, surveillance and futher research about infestation is needed.

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## UTILIZATION OF LEACHATE FROM COMPOSTING PROCESS AS PLANT NUTRIENTS SOURCE

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### ABSTRACT

Increased population growth in urban areas resulted in increasing solid waste production. Piles of garbage disposed by the community produce leachate which has a negative impact on the surrounding environment. The objectives of the research were to analyze leachate quality from the composting process and to analyze the quality of water spinach where leachate was applied as nutrients source. Water spinach was planted in a floating raft hydroponic system with leachate application as source of nutrients. Applied leachate concentrations were 30%, 40%, 50%, and 60%. Leachate were divided according to age of leachate harvesting consisted of 1-7, 8-14, 15-21, and 1-21 days. Leachate test result showed that its quality did not comply with the standard based on Regulation of the Minister of Agriculture No. 70 issued in 2011, pertaining to organic fertilizer, biological fertilizer and soil conditioner. Water spinach grown with the application of leachate were also cultivated using soil media consisted of Alluvial, Andisol, Latosol red yellow Podzolic, Regosol, as well as Latosol. The water spinach products grown in this experiment were inconsumable based on fresh weight, heavy metal content, and physical appearance of the water spinach. This research indicated that leachate resulted from this composting process was not feasible as nutrients source for water spinach.

**Keywords:** Composting, hydroponic, leachate, nutrition, water spinach

### INTRODUCTION

Waste is a material from human activities or unused by products of human activity (Hidayat 2006 in Salawati *et al.* 2008). Sources of waste can come from households, industries, offices, and others. The amount of waste produced by human increases with the increase of human population (Suthar *et al.* 2016). Solid waste generation continues to increase, resulting in solid waste disposal affecting the environment and sustainable development (Samad *et al.* 2017). Waste has great potential as a resource (recyclable materials, energy sources, etc.), but nowadays most of the waste is still a source of environmental pollution or disease sources. Waste discharged by the community produces leachate consisted of various pollutants that have a negative impact on the surrounding environment (Arunbabu *et al.* 2017). Leachate formed can cause diseases, so it needs to be further processed. Based on the above explanation, this research was carried out to try utilizing leachate as plant nutrients source. This study aimed to analyze water quality of leachate produced by composting process of organic waste and quality of water spinach grown using leachate as nutrients source.

### MATERIALS AND METHODS

This research was conducted from February to May 2018. The research was carried out in a greenhouse located in Margajaya Village, Bogor Barat District, Bogor City. Analyses of leachate

water quality and water spinach quality were conducted in greenhouse location and at Soil Laboratory of SEAMEO BIOTROP, Bogor. Materials used in this research were hydroponic water spinach, rockwool, AB Mix nutrients, and leachate produced by composting process of organic waste. Leachate water quality from the composting process was tested, based on Regulation of the Minister of Agriculture No. 70 issued in 2011. The tested quality parameters of water spinach grown with hydroponics media included number of dominant leaves, fresh weight of water spinach, heavy metal content in water spinach, rate of hydroponic water level drop. Tested quality parameters of water spinach grown with soil media included number of dominant leaves and fresh weight of water spinach.

## RESULTS AND DISCUSSION

### Quality of Leachate Produced by Organic Waste Composting Process

Wastes originated from different places or areas and their different types allow different characteristics (Nasir 2013). Different compost materials will produce different compost quality. In this study, the main ingredients of compost were leaves, banana leaves, fruit, food scraps, vegetables, grass, and additional manure of 20 kg for each compost. Composting bin used had dimensions of 210 x 150 x 80 cm<sup>3</sup>. Compost was left in the open space (natural static pile), allowing leachate to be collected in a special leachate container. Reduction of compost materials volume up to 22 days were 50% in the first composting bin and 41% in the second composting bin. Reduced compost materials affected the quality of collected leachate. The collected leachate was then compiled in a drum container before being applied to the water spinach. The leachate was previously tested for pH, electrical conductivity (EC), and total dissolved solids (TDS) values. Leachate pH tended to fluctuate with a pH range of 5.5-8.2. The pH value presented agreed with the quality standard requirement of 4-9 based on Regulation of the Minister of Agriculture No. 70 issued in 2011. EC values in leachate ranged from 2666-3593  $\mu$ S/cm. Range of TDS values obtained was 1370-1688 ppm. Minimum TDS of water spinach was 700 ppm (Budiana 2007). Therefore, leachate had to be diluted before it was applied to the water spinach. Leachate water was also tested for several chemical parameters in Soil Laboratory of SEAMEO BIOTROP, which results are presented in Table 1.

Table 1 Chemical parameters of leachate quality

No.	Testing Parameters	Unit	Quality Standards	Leachate			
				Day 1-7	Day 8-14	Day 15-21	Day 1-21
1	C Organic	%	Min 6	0.009	0.018	0.012	0.018
2	Total N	%	3-6	0.006	0.007	0.004	0.003
3	Total P <sub>2</sub> O <sub>5</sub>	%	3-6	0.0035	0.0038	0.0039	0.0037
4	Total K <sub>2</sub> O	%	3-6	0.19	0.24	0.26	0.27
5	Total Cu	ppm	250-5000	<0.006	<0.006	<0.006	<0.006
6	Total Zn	ppm	250-5000	0.10	0.17	0.17	0.24
7	Total Mn	ppm	250-5000	1.2	1.6	1.0	1.1
8	Total Fe	ppm	90-900	1.4	3.4	2.9	3.8
9	Total Cd	ppm	Max 0.5	<0.009	<0.009	<0.009	<0.009
10	Total Pb	ppm	Max 12.5	<0.007	<0.007	<0.007	<0.007
11	Total Mo	ppm	2-10	1.9	1.7	1.8	1.7
12	Total B	ppm	125-2500	0.36	0.76	0.72	0.65



### Quality of Water Spinach Grown using Leachate as Nutrients Source

Quality parameters tested for water spinach were number of dominant leaves, fresh weight, heavy metal content, and physical condition of water spinach.

Comparison of water spinach quality was conducted between water spinach grown using leachate as nutrients source and water spinach using AB mix as nutrients source. Water spinach using leachate as nutrients source showed significant difference in number of dominant leaves from the 6<sup>th</sup> day until the 21<sup>st</sup> day for all leachate concentrations compared to water spinach using AB mix as nutrients source. On the 21<sup>st</sup> day, the leaves of water spinach grown using leachate concentrations of 40%, 50% and 60% exhibited decay at the shoot (tip of stem) resulted in leaf decay, which indicated the occurrence of deficiency symptoms in water spinach. Deficiency symptoms occur due to lack of certain nutrients in plants that are marked by changes in the physical condition of the plant.

Fresh weight of water spinach grown using all leachate concentrations showed underweight symptom. Generally, fresh weight of water spinach by treatment of manure on average 25.20 g/plant (Polyi 2009 in Moerhasrianto 2011).

Heavy metals concentrations tested on water spinach were total Cu, total Fe, total Cd, and total Pb. Heavy metals tests showed that water spinach grown using leachate as nutrients source had lower total Cu concentration. Total Fe in water spinach grown using leachate as nutrients source was than that in water spinach grown in AB Mix. Total Cd and Pb in water spinach grown both using leachate and AB Mix were very small, i.e. <0.009 ppm (total Cd) and <0.007 ppm (total Pb). Based on SNI 7387 of 2009, AB Mix and leachate concentrations (30%, 40%, 50% and 60%) were below the safe threshold for consumption.

A 30% leachate concentration was also applied to soil media. Soil samples used consisted of: (1) Alluvial from Karawang, (2) Andisol from Kuningan, (3) Latosol red yellow Podzolic from Sumber Jaya, Lampung Regency, (4) Regosol from Pelabuhan Ratu, and (5) Latosol from Sumber Agung Village, Bandar Lampung. Fresh weight comparisons of water spinach grown in soil media using well water and leachate as nutrients sources are presented in Figure 1.

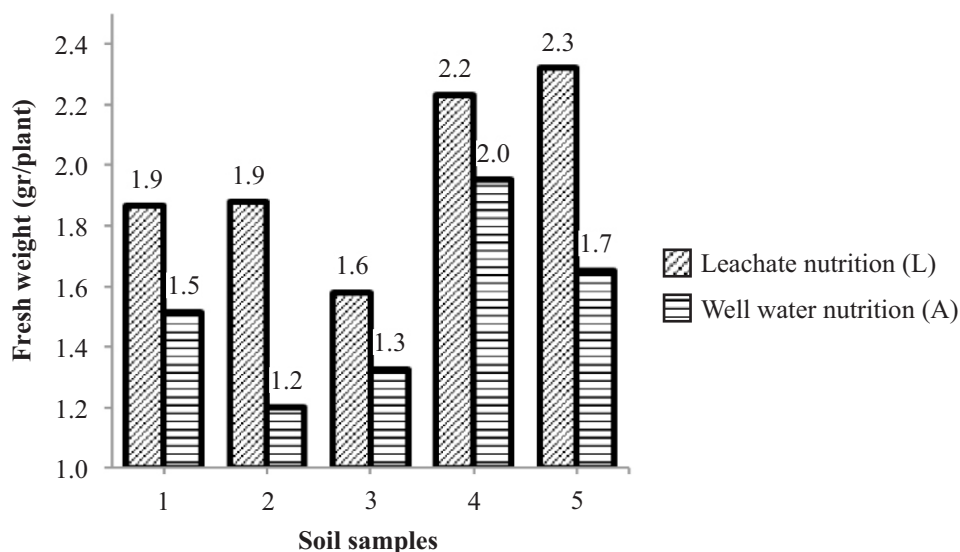


Figure 1 Fresh weight of water spinach grown in several types of soil media

## CONCLUSION

Leachate harvested on the 1-7, 8-14, 15-21 and 1-21 days from organic waste composting process did not qualify as liquid organic fertilizer. Water spinach products grown using leachate as nutrients source were inconsumable.

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## SELECTION OF DROUGHT-STRESS-TOLERANT FOXTAIL MILLET (*Setaria italica* (L.) P. Beauv) COLLECTED FROM EAST NUSA TENGGARA (ENT) PROVINCE, INDONESIA

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### ABSTRACT

Foxtail millet (*Setaria italica* (L.) P. Beauv) is a cereal plant that has a potency to be developed as a functional food. This study aimed to determine the adaptability of several local accessions of foxtail millet from East Nusa Tenggara (ENT) to be developed as agricultural commodity in dry land area. The research was done using Factorial Completely Randomized Design with 2 factors of treatments, i.e. number of local accessions of foxtail millet from ENT and watering period treatments. The number of local accessions from East Nusa Tenggara (ENT) used in this study were 7 accessions, while the watering period treatments consisted of 3 treatments i.e. watered every day (P1), watered every 3 days (P3), and watered every 7 days (P7). The results showed that JW14 accession grown in drought stress condition (P3) and (P7) had similar results on plant height and panicle weight with that grown in normal condition. Root length of JW14 accession grown in drought stress condition showed better growth compared to that grown in normal condition. Shoot and root biomass of JW14 under drought stress showed no significant difference compared to normal condition.

**Keywords:** Drought stress, foxtail millet (*Setaria italica* (L.) P. Beauv), plant growth

### INTRODUCTION

Drought stress is a major environmental constraint hampering agricultural production. It is reported that the intensity of drought stress increased over the past decades (Vurukonda *et al.* 2016) and it is expected to occur in more than 50% of the agricultural land by 2050 (Kasim *et al.* 2013). This condition will ensue a dramatic impact on crop growth and yield, which will in turn affect food security and human welfare in the world (Lobell *et al.* 2008). Efforts to generate crop plant having a tolerance for drought stress might be a promising approach in supplying food demand (Farooq *et al.* 2009).

Plants experience water stress either when the soil moisture becomes lower and the soil dryness occurred, thereby reducing water supply to the root, or when the loss of water from plant through transpiration process becomes intense (Anjum *et al.* 2011). Plants have evolved mechanisms to tolerate drought stress which vary among plant species. These mechanisms are a complex phenomenon involving arrays of physiological, biochemical, and molecular processes occurred at cellular and whole-organism levels which affect plant growth and productivity (Bhattacharjee & Saha 2014; Osakabe *et al.* 2014). Drought stress has been reported to severely reduce the yield of many crops, such as in barley (Samarah 2005), maize (Atteya *et al.* 2003; Kamara *et al.* 2003), wheat (Balla *et al.* 2011) and potato (Kawakami *et al.* 2006).

Foxtail millet (*Setaria italica* (L.) P. Beauv) is a type of cereals having a small seed (millet) and is thought to come from the highlands of China (Oelke *et al.* 1990). During its long domestication and improvement process, this plant has adapted well to dry areas, arid and semi-arid regions, or less fertile areas. *S. italica* is a diploid ( $2n = 2x = 18$ ). It has a short life cycle (~ 12 weeks), self-

pollination and C4 photosynthesis mechanisms (Tang *et al.* 2017), and a high genetic diversity (~6.000 varieties) (Devos *et al.* 1998; Wang *et al.* 1998). Foxtail millet has been used as a staple food in some countries in the world before the cultivation of rice is known. Until present, some countries such as China, India, Russia, Africa, and USA, keep cultivating this plant in large-scale, especially for various form of processed foods (Baltensperger 1996). In Indonesia, foxtail millet is grown in some areas such as in West Sulawesi, South Sulawesi, and Central Java.

East Nusa Tenggara (ENT) is one of the provinces in the eastern region of Indonesia. This province has a comparative advantage of quite extensive dry land areas. These areas have a great opportunity to be developed to improve the welfare of local community, especially the dryland farmers. One of the local bioresources that can be developed as a functional food in ENT Province is foxtail millet due to its high nutritional value. Unfortunately, foxtail millet has been cultivated in small-scale using a simple agricultural technique due to its low prestige compared to rice or corn as staple food of the people there.

A better understanding of plant responses under drought stress is required to improve crop management and breeding techniques (Chaves *et al.* 2003). This research was conducted to test growing ability of some accessions of foxtail millet in dry land area. The potential foxtail millet accession will be grown in large-scale as one of agricultural commodities in Indonesian dry land area, as an effort to substitute rice as a functional food.

## MATERIALS AND METHODS

The experiment was arranged as Factorial Completely Randomized Design with 2 factors of treatments, i.e. number of local accessions of foxtail millet from ENT and watering period treatments. The first factor was 7 types of local accession of foxtail millets collected from East Nusa Tenggara (ENT) Province, namely JW1, JW3, JW6, JW9, JW10, JW13, and JW14. The second factor consisted of 3 treatments of watering period i.e. watered every day (P1), watered every three days (P3) and watered every seven days (P7). Each treatment consisted of 2 plants and three replications.

Seeds of foxtail millet plant used in this study were germplasm collection of Plant Physiology Laboratory, Division of Botany, Research Center for Biology, Indonesian Institute of Sciences (LIPI). To start the experiment, seeds of foxtail millet plants were germinated in the germination trays. The seedlings were then cultivated in a pot-based system (P<sub>sys</sub>) and maintained in the glasshouse with temperature of  $30 \pm 5$  °C, relative humidity of  $60 \pm 20\%$ , and a natural photoperiod. In this study, growth medium consisted of soil mixed with basal fertilizer of compost at a ratio of soil: compost (2:1, v/v) and NPK fertilizer at 10 g/10 kg growth medium. Watering period treatments were started at 6 weeks after planting (WAP) and finished at 18 WAP. Each watering period treatment was applied to growth medium until reaching field capacity of the medium.

Observation was performed to the growth medium experiencing a drought stress condition and to the plants. Observation on growth medium was conducted by measuring soil temperature, soil pH and soil water potential in every watering period treatments. Several parameters observed on plant growth were plant height, root length, weight of panicle per plant, and also root and shoot biomass.

## RESULTS AND DISCUSSION

Soil pH increased with the increase of watering period (Fig. 1A). The initial soil pH was 4.62, but the watering period treatments of P3 and P7 induced soil pH until it reached 5.1 and 6.93, respectively. This result also corresponded with data of increasing rhizosphere soil pH in the pot with corn hybrids at tasseling growth stage exposed to water stress compared to the pot with a well-watered condition (Song *et al.* 2012).

Soil temperature increased with the increase of watering period (Fig. 1B). In this study, the highest soil temperature was 31.95 °C generated by P7 treatment. Drought stress is known to reduce water potential of growth medium at both vegetative and anthesis stages (Shiddique *et al.* 1999). Watering period treatments P3 and P7 exhibited soil water potential of -16.69 Mpa and -7.37 MPa, respectively (Fig. 1C).

In most types of local accessions of foxtail millet used in this study, the plant height in treatment P7 was lower than that in treatment P1 and P3. However, plant height of local accession JW14 showed similar results in each watering period treatment (Fig. 2A). Observation on root length showed that most types of local accessions of foxtail millet exhibited an increased root length in all watering period treatments compared to that in normal condition (Fig. 2B). Local accessions of foxtail millet JW1, JW3, JW6, and JW14 produced higher panicle weight per plant in all watering period treatments as compared to that in normal condition (Fig. 2C).

Local accessions of foxtail millet JW3, JW6, and JW13 showed significant decrease in shoot biomass compared to that in normal condition (Table 1). Only JW9 accession showed a significant reduction in root biomass under drought stress condition compared to that in normal condition (Table 1).

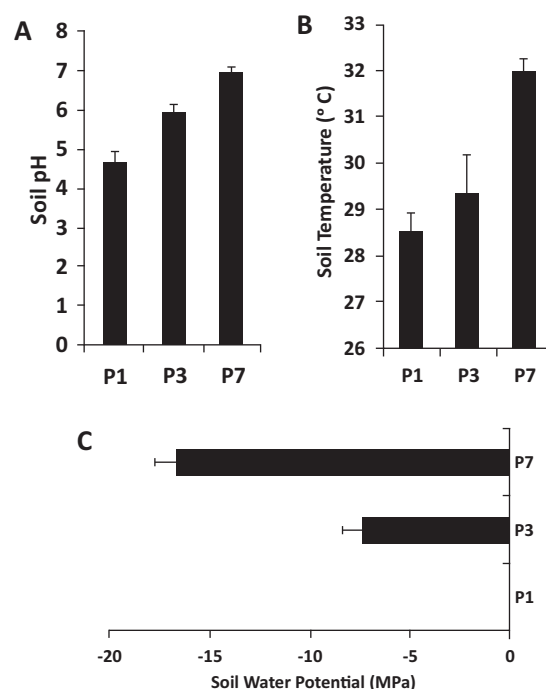


Figure 1 Soil status of growth medium. A. Soil pH, B. Soil temperature (°C), C. Soil water potential (MPa). All parameters were measured at each watering period treatment, i.e. watered every day (P1), watered every three days (P3), and watered every seven days (P7). Means are value  $\pm$  SD ( $n = 7$ )

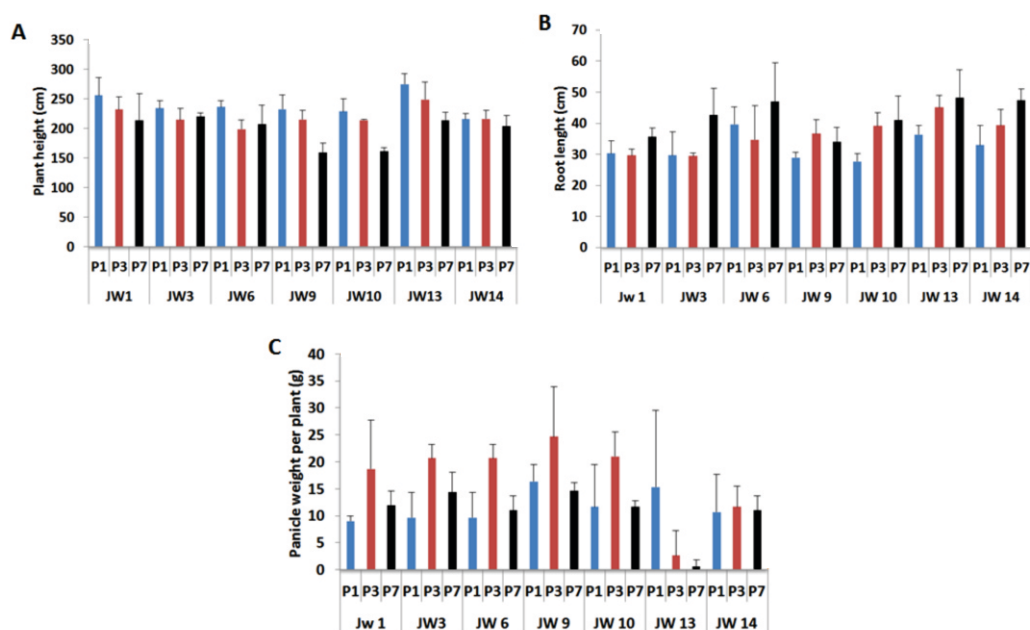


Figure 2 Morphological data of foxtail millet plants. A. Plant height (cm), B. Root length (cm), and C. Panicle weight per plant (g). The measurement was performed at 18 WAP (week after planting) in all local foxtail millet accessions (JW1, JW3, JW6, JW9, JW10, JW13, and JW14) collected from ENT (East Nusa Tenggara) Province to obtain morphological characters data of plants in each watering period treatment, i.e. watered every day (P1), watered every three days (P3), and watered every seven days (P7). Means are value  $\pm$  SD ( $n = 3$ )

Table 1 Shoot and root biomass of several accessions of foxtail millet under drought stress

Treatment		Shoot biomass	Root biomass
Accessions	Treatments		
JW 1	P1	45.33 $\pm$ 4.16 CD	4.00 $\pm$ 1.00 AB
	P3	54.33 $\pm$ 7.77 CD	3.00 $\pm$ 2.65 AB
	P7	43.33 $\pm$ 18.34 CD	2.33 $\pm$ 1.15 AB
JW 3	P1	46.00 $\pm$ 14.80 CD	2.67 $\pm$ 2.31 AB
	P3	41.33 $\pm$ 2.31 CD	2.33 $\pm$ 1.53 AB
	P7	33.33 $\pm$ 1.15 D	2.33 $\pm$ 0.58 AB
JW 6	P1	63.67 $\pm$ 8.62 BC	3.06 $\pm$ 0.26 AB
	P3	40.67 $\pm$ 12.70 CD	2.62 $\pm$ 0.64 AB
	P7	31.33 $\pm$ 5.13 D	1.87 $\pm$ 0.17 AB
JW 9	P1	48.00 $\pm$ 6.93 CD	5.33 $\pm$ 1.15 A
	P3	56.33 $\pm$ 1.53 CD	5.00 $\pm$ 1.00 AB
	P7	35.00 $\pm$ 3.61 CD	1.33 $\pm$ 0.58 B
JW 10	P1	43.33 $\pm$ 4.93 CD	2.36 $\pm$ 0.76 AB
	P3	41.33 $\pm$ 6.81 CD	1.93 $\pm$ 0.66 AB
	P7	42.67 $\pm$ 9.24 CD	1.57 $\pm$ 0.48 AB
JW 13	P1	93.00 $\pm$ 20.07 A	3.69 $\pm$ 1.15 AB
	P3	95.00 $\pm$ 10.58 AB	3.49 $\pm$ 1.23 AB
	P7	48.33 $\pm$ 5.03 CD	3.16 $\pm$ 0.81 AB
JW 14	P1	46.00 $\pm$ 14.8 CD	2.67 $\pm$ 2.31 AB
	P3	41.33 $\pm$ 2.31 CD	2.33 $\pm$ 1.53 AB
	P7	35.00 $\pm$ 1.73 CD	2.33 $\pm$ 0.58 AB

Note: Means followed by the same letter are not significantly different from each other by LSD test ( $p > 0.05$ )

Cell growth is thought to be one of the most drought-sensitive physiological responses of plants (Taiz & Zeiger 2006). Impaired cell growth causes reduction of growth and yield traits during drought affecting decreased soil water potential. Functional equilibrium of plant growth is generated by a complex interplay between sources and sink limitation of the main two plant organs, the root system and the shoot (Fang *et al.* 2017). As the plant organ which firstly perceives a decrease of soil humidity by drought, the development of root system during water deficit conditions is one of the mechanisms of root adaptation relating to its function to absorb water and nutrient which further affects growth and biomass allocation to all of plant organs (Liu *et al.* 2017). Drought-tolerant foxtail millet plant has the character of the deepest-rooting accessions presented in the longest-rooting plant to absorb water in the deep soil layers which is not normally available (Araus *et al.* 2002).

## CONCLUSION

Drought stress affected growth and productivity of foxtail millet accessions. JW14 is determined as a drought-resistant foxtail millet accession having growth stability of plant height, panicle weight/plant, and also shoot and root biomass under the increasing soil water potential. JW14 also exhibits increasing root length under drought stress of -16.69 MPa.

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## POTENTIAL OF SEVERAL BACTERIAL FILTRATES TO SUPPRESS THE INFECTION OF *BEAN COMMON MOSAIC VIRUS* ON YARD LONG BEAN

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### ABSTRACT

Endophytic bacteria and plant growth promoting rhizobacteria (PGPR) are widely used as potential biocontrol agents of plant pathogens and as plant growth inducer. However, study of utilization of those bacteria against plant virus is limitedly known in Indonesia. This research aimed to examine the effectiveness of endophytic bacteria and PGPR's filtrates to suppress *Bean Common Mosaic Virus* (BCMV) infection on yard long bean. Eleven bacteria isolates were screened on indicator plant and among them, four bacteria isolates (E1, E2, P1, P2) were selected to test their effectiveness to suppress BCMV on yard long bean infection in greenhouse trial. The bacteria filtrate was applied as seed treatment (B), leaf spray (S), and combination of those treatments (BS). Disease assessment (incubation period, disease severity, virus titer) and growth parameters were measured. Effect of bacterial treatments on disease assessment and growth parameters varied, depending on bacteria isolate and application. Bacteria's filtrate treatments showed ability to prolong the incubation period, reduced either disease severity or virus titer compared with untreated control. Among those treatments, E1 isolate as seed treatment (E1B) was the best treatment in reducing the disease severity up to 68.25% and virus titer up to 75.10%. This result indicated the potency of isolate E1 as biocontrol agent for BCMV.

**Keywords:** Endophyte, ISR, mosaic disease, Rhizobacteria

### INTRODUCTION

*Bean Common Mosaic Virus* (BCMV) is one of production constraints of legumes in the world. The virus is transmitted mechanically, seed-borne, and its transmission in the field is facilitated by aphid and causes varying mosaic symptoms. BCMV is difficult to mitigate due to its characters. Up to now, there is no chemical viricide to suppress plant virus infection. However, biological control approach is one of disease management methods which is environmentally friendly. This method must be developed by exploration and evaluation on its effectiveness against many types of pathogens intensively.

Endophytes and *Plant Growth Promoting Rhizobacteria* (PGPR) are widely utilized as potential biocontrol agents of plant pathogens and as plant growth inducer (Backman & Sikora 2008; Ahemad & Kibret 2014). However, studies of utilization of those bacteria against plant virus(es) are limitedly known in Indonesia. Plant virus infection can not be affected directly using many control methods, because it is obligate intercellular pathogens. Utilizing beneficial bacteria will assist in reducing virus titer indirectly by enhancing plant health and inducing plant systemic resistance. This study reported the role of endophytic bacteria and PGPR to suppress BCMV infection on *Yard Long Bean* (YLB).

## MATERIALS AND METHODS

Four endophytic (E1-E4) bacteria and seven PGPR isolates (P1-P7) were screened for their ability to suppress BCMV infection on indicator plants *Chenopodium amaranticolor*. Potential isolates which produced the lowest lesion local necrotic (LLN) was selected to test their effectiveness to suppress BCMV infection in greenhouse trial.

Bacteria were cultured in liquid media, and the filtrates were extracted. The filtrates were then applied as seed treatment (B), leaf spraying (S) and combination of B and S treatments. BCMV was inoculated mechanically one day after treatment. Disease assessment and growth parameters were measured. The experiment was arranged in completely randomized design, in which each treatment consisted of fifteen plants as replications.

## RESULTS AND DISCUSSION

Either endophytes bacteria or PGPR treatments significantly showed the decrease of the *Lesion Local Necrotic* (LLN) formation of BCMV on indicator plants compared with untreated control plants. This indicated the ability of those bacterial isolates to suppress BCMV infection. Bacterial treatment showed ability to inhibit LLN formation, ranging from 52.80% up to 100% (Fig. 1) depending on bacterial isolates. E1, E2, P1, and P2 were selected to evaluate their effectiveness against BCMV on yard long bean in greenhouse trial.

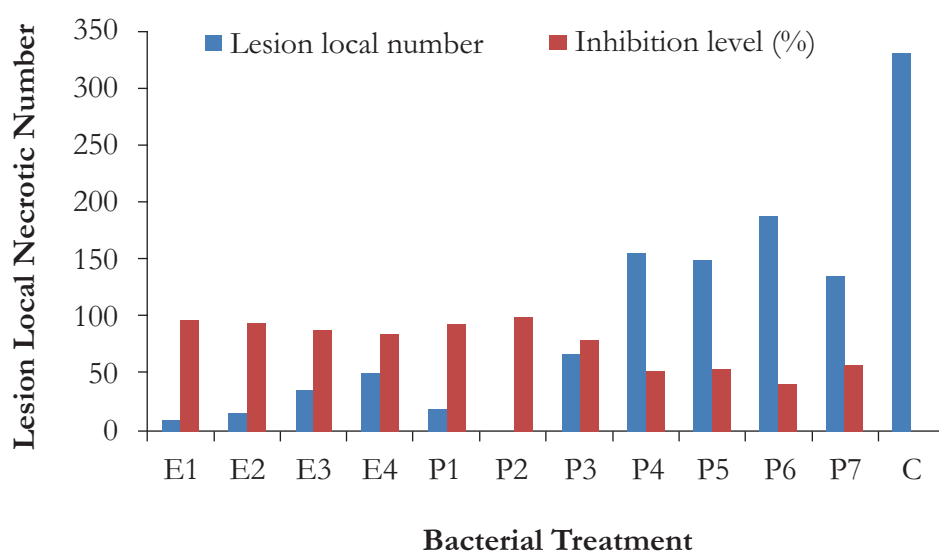


Figure 1 Screening of potential bacterial isolates based on lesion local necrotic number of BCMV (blue diagram) on indicator plants *Chenopodium amaranticolor* and its inhibition level (red diagram)

Effect of bacterial filtrates varied depending on bacteria isolates and treatments. Generally, bacterial treated plants showed significantly higher seed germination, vigor index, root and shoot length compared with control (Table 1).

Table 1 Effect of potential filtrates on seed germination, vigor index, length of root and shoot

Treatment	Germination (%)	Vigor Index (%)	Root Length (cm)	Shoot Length (cm)
E1	90.0 ± 7.07b <sup>1</sup>	87.50 ± 3.54c	10.21 ± 1.02a	14.10 ± 1.98b
E2	96.3 ± 3.54ab	95.00 ± 0.00ab	11.42 ± 2.58a	16.80 ± 1.42a
P1	100.0 ± 0.00a	100.00 ± 0.00a	10.49 ± 1.46a	16.60 ± 1.80a
P2	97.5 ± 7.07a	97.50 ± 3.54a	10.83 ± 1.28a	13.40 ± 1.70b
Control	90.0 ± 0.00b	90.00 ± 0.00b	7.37 ± 0.91b	9.97 ± 2.14c

Note: <sup>1</sup>Numbers followed by different letters in the same column indicate significant difference based on DMRT at  $\alpha=0.05$

Effect of bacterial treatments on disease assessment parameters showed that the treatments were significantly able to prolong incubation period, reduce disease severity and virus titer with the highest virus inhibition on E1B treatment. The E1B treatment was able to reduce disease severity and virus titer up to 68.27% and 75.10%, respectively (Table 2).

This indicated that the bacteria had potency to suppress BCMV with possible mechanism such as through antiviral activity, enhance plant systemic resistance, and induce plant growth. Further study related with mechanism is necessary in the future.

Table 2 Effect of bacterial treatment on disease parameters

Treatment	Incubation period (day)	Severity	Virus Titer <sup>2</sup>	Virus Inhibition (%)
E1 B	9.80 d	0.87 b <sup>1</sup>	0.57 a	75.10 a
E2 B	9.33 d	3.07 bc	1.24 ab	51.32 ab
P1 B	13.00 ab	1.73 bc	2.09 bc	15.26 b
P2 B	9.27 d	1.60 bc	2.60 c	-2.59 b
E1 S	13.93 ab	1.13 b	1.24 ab	48.16 ab
E2 S	10.93 cd	2.47 bc	1.89 bc	2 28.63 ab
P1 S	11.91 bc	1.40 b	1.54 abc	4 41.96 ab
P2 S	9.47 d	1.47 bc	2.31 bc	10.18 b
E1 BS	10.97 cd	1.93 bc	1.61 abc	3 38.58 ab
E2 BS	13.20 ab	2.07 bc	1.38 ab	3 39.60 ab
P1 BS	14.20 a	1.80 bc	2.01 bc	18.77 b
P2 BS	10.00 cd	2.47 bc	2.59 bc	-1.02 b
C-infected	4.40 e	2.74 c	2.61 bc	0.00 b
Healthy		0.00 a	0.11 d	

Notes: <sup>1</sup>Numbers followed by different letters in the same column indicate significant difference based on DMRT at  $\alpha=0.05$

<sup>2</sup>Virus titer was determined serologically by ELISA test. Virus titer is positive if virus titer > 0.22

## CONCLUSION

Endophyte and PGPR treatments were able to induce seed germination, vigor index, root and shoot length. E1S, P1S, P2B, P2S and P2BS were able to enhance plant growth parameters better than other treatments. E1B and E1S showed the best ability to suppress BCMV infection, indicating its potential use to protect YLB from BCMV infection.

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## OPTIMIZATION OF GROWTH CONDITIONS OF *Pleurotus djamor* ON COCONUT WASTES AS SUBSTRATE

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### ABSTRACT

Coconut wastes such as coconut saw dust, coconut coir dust and coconut water were used in this study. Growth performance of *Pleurotus djamor* on coconut water gulaman, different physical conditions (pH, aeration, illumination and temperature), coconut wastes starter formulations and coconut based substrate formulations were evaluated. *P. djamor* produced luxuriant mycelial growth on mature coconut water gulaman at pH 8.0, incubated on either sealed or unsealed, lighted or dark conditions at room temperature. Coconut from Aurora Province produced the shortest incubation period with thick mycelial density. A combination of 25% waste grated coconut meat and 75% coconut coir dust recorded the best combination for starter formulation. Substrate consisting of 75% rice straw and 25% coconut sawdust produced the highest yield (143 g) with the highest biological efficiency (28.60%). The best substrate formulation of rice straw: coconut coir dust was noted in 100% coconut coir dust which recorded the highest yield (134.40 g) with the highest biological efficiency (26.88%).

**Keywords:** Coconut, efficacy, *Pleurotus*, waste

### INTRODUCTION

In the Philippines, there is a growing interest on mushroom production as a source of additional income and nutritious food. Mushrooms are considered as nutraceutical and functional food because of their unique nutritional attributes and medicinal properties. Fruiting bodies of mushrooms have been reported to contain bioactive compounds that can provide health benefits such as antibacterial, antitumor, anti-inflammatory, antioxidant and antihypercholesterolemic properties. The Philippines is the second largest producer of coconut in the world, next to Indonesia with an annual production of 19.5 million tons per annum. However, there is a large accumulation of waste such as coconut water, coconut coir dusts and other biomass. Coconut waste is generated during processing of this crop into high value products. The use of agroindustrial wastes has been in significant interest, where numerous processes have been developed based on these waste materials, such as substrates in bioprocess for production of single cell protein, organic acids, ethanol, mushrooms, enzymes and biologically important secondary metabolites. With this arising concerns and problems in dumping waste from coconut, our research team has conceptualized the feasibility of using these coconut waste to be used in the field of mushroom production. This study provided the information on whether coconut waste would improve yield performance, its biological efficiency and its optimum physical and nutritional conditions needed for growing *Pleurotus djamor*.

## MATERIALS AND METHODS

### Revival of Pure Culture

Ten (10) mm mycelial block from the pure culture of *Pleurotus djamor* was aseptically transferred into a previously prepared PDA plate. The culture was incubated at room temperature to allow the growth of secondary mycelia.

### Evaluation of the Best Media for Secondary Mycelial Growth

The mycelial growth performance of *P. djamor* was evaluated using young and mature coconut water agar as treatments. pH level was not adjusted to maintain its natural pH level. One (1) L of coconut water from mature and young coconut added with 20 g agar was separately boiled until homogenous mixture was attained and sterilized in an autoclave at 15 lbs/in<sup>2</sup>, 121 °C for 20 minutes.

### Collection and Evaluation of Substrate from Different Geographical Locations

Coconut water samples were collected from five different provinces in the Philippines with the highest production of coconut namely Aurora, Batangas, Laguna, Quezon and Romblon, which also served as treatments. Coconut water was extracted from the mature coconut fruit and was subjected to evaluation as an effective indigenous coconut water medium for the mycelial growth of *P. djamor*.

### Evaluation of Coconut Based Spawn Starter Formulation

The probability of using coconut waste as spawn starter was evaluated in this study. Different combinations of waste grated coconut meat and coconut coir dust were formulated to serve as treatments (100% waste grated coconut meat; 100% coconut coir dust; 75% WGCM + 25% CCD; 50% WGCM + 50% CCD; 75% CCD + 25% WGCM; unmilled rice grains (control)). In this segment, waste grated coconut meat and coconut coir dust were used to evaluate which combination would further support the luxuriant mycelial growth of *P. djamor*.

### Evaluation of Substrate for Fruiting Body Production

Nine (9) combinations from coconut sawdust + rice straw and coconut coir dust + rice straw was prepared and used as treatments i.e. 70% rice straw + 30% saw dust; 75% rice straw + 25% coconut saw dust; 50% rice straw + 50% coconut saw dust; 25% rice straw + 75% coconut saw dust; 100% coconut saw dust; 75% rice straw + 25% coconut coir dust; 50% rice straw + 50% coconut coir dust; 25% rice straw + 75% coconut coir dust; 100% coconut coir dust (CSD – coconut saw dust; CCD – coconut coir dust; RS – rice straw). These treatments were compared with control.

### Experimental Design and Statistical Analysis

Analysis was laid out using Completely Randomized Design (CRD). The data were subjected to SAS (Statistical Analysis System) version 9.1 for the analysis of variance (ANOVA). All treatment means were compared using T-test and Post hoc Procedures (LSD) at 5% level of significance.

## RESULTS AND DISCUSSION

### Influence of Coconut Water Media

It was recorded that *Mature Coconut Water Agar* (MCWA) had a significantly wider mycelial diameter with a mean of 88.66 mm, while *Young Coconut Water Agar* (YCWG) recorded a smaller mycelial growth diameter with mean of 15.72 mm. Moreover, MCWG produced thick mycelial density, while YCWG exhibited very thin mycelial density.

Table 1 Influence of physical factors on the growth of *Pleurotus djamor*

Physical Factors	Mycelial diameter (mm)	Incubation period (days)	Density
pH			
6.0	51.72 <sup>c</sup>	12.67 <sup>a</sup>	+++
6.5	54.80 <sup>c</sup>	12.00 <sup>a</sup>	+++
7.0	81.54 <sup>b</sup>	8.67 <sup>b</sup>	+++
7.5	75.45 <sup>b</sup>	10.33 <sup>c</sup>	+++
8.0	90.00 <sup>a</sup>	7.00 <sup>d</sup>	+++
Aeration			
Sealed	89.48 <sup>a</sup>	6.00 <sup>a</sup>	+++
Unsealed	89.30 <sup>a</sup>	6.00 <sup>a</sup>	+++
Illumination			
Light	88.86 <sup>a</sup>	6.00 <sup>a</sup>	+++
Dark	88.75 <sup>a</sup>	6.00 <sup>a</sup>	+++
Temperature			
Room Temperature	90.00 <sup>a</sup>	6.00 <sup>b</sup>	+++
Air conditioned	35.42 <sup>b</sup>	10.00 <sup>a</sup>	+++
Refrigerated	10.00 <sup>c</sup>	NA	NA

Notes: Means followed by the same superscript letter in the same column are insignificantly different from each other at 5% level of significance using DMRT

Mycelial density was evaluated as: very thin (+), thin (++), thick (+++), very thick (++++); \*NA – Not applicable

Among the five (5) coconut varieties, Aurora strain recorded the largest mycelial diameter with mean of 86.50 mm with thick mycelial density and the shortest incubation period of 7.33 days. On the other hand, Quezon and Romblon varieties recorded the smallest mycelial diameter, having mean of 60.89 mm and 63.47 mm, respectively with thick mycelial density after seven (7) days of incubation. Aurora variety recorded the highest mean diameter of 86.50 mm after 7 days of incubation. Coconut water being the best indigenous medium for *P. djamor* can be attributed to the nutritional component of coconut water. Among different treatments evaluated, the largest mycelial diameter was recorded in the 25% WGCM + 75% CCD treatment with mean diameter of 89.71 mm, which was significantly different among treatments and had the shortest incubation period with mean of 7.33 days and thick mycelial density. On the other hand, the smallest mycelial diameter was recorded in 100% WGCM treatment with mean of 11.33 days incubation period with thick mycelial density. In this segment of the study, comparison among means revealed that 100% CCD, 50% WGCM + 50% CCD and rice grains treatments were not significantly different. After each substrate was fully colonized with mycelium, the top portion of polypropylene bags was removed to produce basidiocarps of *P. djamor*. Three (3) flushes were considered in the study, whereas, the weight of freshly harvested fruiting bodies for each treatment was recorded for every flushing. The highest total yield with a total of 143 g was recorded from 75% RS:25% CSD substrate formulation, while the lowest total yield was recorded on 75% RS:25% CCD substrate

formulation. Results of this segment showed that the highest biological efficiency (28.60%) was recorded in 75% RS:25% CSD, while the lowest biological efficiency (23.12%) was observed in 75% RS:25% CCD.

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## ANTIBACTERIAL ACTIVITY AND BIOCONTROL POTENTIAL OF ENDOPHYTIC FUNGI ISOLATED FROM *Plectantrus amboinicus* Lour. LEAVES

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### ABSTRACT

Endophytic fungi reside internally and asymptotically in plant tissues and play significant role in the ecosystem. Medicinal plants, such oregano (*Plectantrus amboinicus* Lour.), are valuable sources of important endophytic fungi as endophytes are known to confer several benefits to their host. In this study, endophytic fungi from the leaves of *P. amboinicus* were isolated and morphologically identified. Fungal endophytes were subjected to antibacterial screening and biocontrol potentiality assessment. Three fungal endophytes were isolated from the leaves of Mexican oregano, all of which belong to genus *Aspergillus*. Fungal endophytes were morphologically identified as *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus terreus*. In the antibacterial assay, preliminary screening using agar plug diffusion method revealed that the endophytes were able to impressively suppress the growth of both *Escherichia coli* and *Staphylococcus aureus*. Meanwhile, the biocontrol activities of the endophytic fungi were tested against *Fusarium verticillioides* using the dual culture method. Results showed that the *Aspergillus* endophytes could restrict the growth of *F. verticillioides* effectively. Observations indicated that *A. flavus* and *A. niger* antagonized *F. verticillioides* through overgrowth mechanism, which could be a result of antagonism due to parasitism or antibiosis as lytic activity. In the case of *A. terreus*, zone of inhibition was clearly observed indicating the production of antibiotic substances, either by the pathogen against antagonistic fungi or vice versa. This study showed that the endophytes could be further exploited to provide a novel source of bioactive compounds.

**Keywords:** Antibacterial, biocontrol, endophyte, oregano, *Plectantrus amboinicus*

## POTENTIAL OF INDONESIA'S INDIGENOUS DARK SEPTATE ENDOPHYTIC FUNGI TO CONTROL *Fusarium* WILT IN VITRO

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### ABSTRACT

Research on Dark Septate Endophytic (DSE) fungi in Indonesia is still limited. Therefore, efforts should be made to investigate the potential of indigenous DSE fungi from Indonesia that can be used as a biological control. The isolation of DSE fungi from tomato root samples was performed using direct isolation and soil baiting methods. Tomato root samples were obtained from tomato farms in Cisarua, Citeupuh, Cianjur, Sukabumi, and Garut, West Java. The soil baiting method was conducted using organic forest soil and cabbage farm soil to grow the tomato plants. DSE fungi from those tomato roots were isolated. The best DSE fungal isolates were selected using *in-vitro* treatments by conducting pathogenicity test of DSE fungal candidates to tomato plant, hemolytic reaction test of selected DSE fungi, antagonistic test to *F. oxysporum* f.sp. *lycopersicii*, and metabolite test of volatile compound. There were 49 DSE fungal candidates isolated from tomato root samples. The pathogenicity test showed that 20 DSE fungal isolates had ability to promote tomato seed germination by 97%-100%. Hemolytic reaction test of 20 DSE fungal isolates using blood agar showed that all isolates were negative. Antagonistic test performed on DSE fungal isolate DS08 ic and DS08 ib showed a significant different ability to inhibit *F. oxysporum* f.sp. *lycopersicii* with percentage of inhibitory zone of 76.11% and 71.07%, respectively. The results of metabolite test showed that the highest volatile compound was obtained by DS08 ib (23.70%), followed by DS06 iib (22.96%) and DS06 iia (22.96%). This research indicated that DSE fungi isolates from tomato roots can be used as Indonesia's indigenous dark septate endophyte fungi in suppressing *Fusarium* wilt disease.

**Keywords:** Dark septate endophytic fungi, dual culture, *Fusarium oxysporum* wilt, tomato

### INTRODUCTION

Dark septate endophytic fungi (DSE) are a fungal group capable of colonizing plant roots without causing any disease symptoms. The characteristics of DSE is dark colonies in agar media, have melanized hyphae, septate hyphae, conidial or sterile ascomycetous fungi (Jumpponen & Trappe 1998) and sometimes forming microsclerotia (Gomes 2017). DSE can be found in all ecosystems and wide range of plants species. There are 144 families and 587 plant species reported to have been colonized by DSE (Jumpponen & Trappe 1998). The symbiosis between DSE fungi and its host plants includes mutualism. Potential DSE is known to improve plant performance and promoting plant growth under biotic or abiotic stress. Several studies have reported that DSE fungi have the potential as a biological control agent for pathogenic fungi, such as DSE fungi that suppressed *Verticillium dahliae* in *chinese cabbage* (Narisawa *et al.* 2004), and *Fusarium* on *chinese cabbage* (Khastini *et al.* 2012). Surono and Narisawa (2018) reported *Phialocephala fortinii* suppressed *Fusarium* disease in *Asparagus officinalis*.

Researches on the DSE fungi have been conducted and developed in many sub-tropical countries, while in tropical countries especially in Indonesia, research on DSE fungi is still limited, whereas Indonesia is one of the countries with a very high biodiversity in the world. Therefore, it is necessary to explore indigenous DSE fungi from Indonesia that have the potency to be used as biological control to plant disease. The aim of this research were to obtain DSE fungal isolate from tomato plant root and to investigate the ability of selected DSE fungal isolate to inhibit *Fusarium oxysporum* f.sp. *lycopersicii* growth under *in vitro* assay. *F. oxysporum* f.sp. *lycopersicii* was used as a pathogen target in this study because this pathogen can cause wilt disease in tomato plants, and as one of which is the most important diseases in tomato, both in nursery and field in Indonesia.

## MATERIALS AND METHODS

### Isolation and Cultivation of DSE Fungi

In this study, isolation of DSE fungi from tomato root samples collected from field was performed using direct isolation and soil baiting methods. Tomato root samples were obtained from tomato farms in Cisarua, Citeupuh, Cianjur, Sukabumi, and Garut, West Java. Soil baiting method was conducted using organic forest soil and cabbage farm soil to grow the tomato plants. DSE fungi from those tomato roots were isolated. Surface sterilization of root samples was performed using tween 20, NaOCl and sterile distilled water. Root samples were surface-sterilized, air-dried with sterile tissue, and then plated into 50% corn meal agar (CMA) medium in 9 cm plastic petri dishes. DSE fungi in this study were considered to be dark and slow-growing fungal isolate. The growing DSE fungal isolates were cultivated on potato dextrose agar (PDA) media.

### Early Detection Test of DSE Presence in Tomato Roots Samples

Early detection test was carried out to observe the presence of DSE in the roots of both intercellular or intracellular. The early detection test was done using staining technique of the DSE colonization method (Zhang 2013).

### Selection of Isolate with In Vitro Assay

#### *Pathogenicity Test*

Pathogenicity test was carried out to determine whether or not the candidate is pathogenic to the host plant. Pathogenicity test was determined according to methods described by Surono and Narisawa (2017) with modification. Tomato seeds surface sterilization was conducted with 1% NaOCl for 3 minutes, followed by washing with sterile water for three times. The sterilized seeds were soaked for three hours in sterile water. Afterwards, they were dried and placed on candidate DSE fungal isolate that have been grown for 7 days and incubated for two weeks.

#### *Hemolytic Reaction Test*

Hemolytic reaction test of selected DSE fungi was performed to make sure that the selected DSE fungi are not pathogen to human and animals. The test was conducted using blood agar media according to Beutin (1991). An agar plug (5 mm diameter) of DSE fungal isolate was placed on blood agar media and incubated for one week.

#### *Antagonistic Test*

Antagonistic test was carried out using the dual culture test method referring to Dwiastuti *et al.* (2016) with modifications. Dual culture test was carried out using PDA media in 90 mm plastic

petri dishes. Due to the slow growth of DSE fungi, the fungi were firstly grown for 2 weeks. Subsequently, the fungal pathogen was grown in the same petri dish as the DSE fungal isolates for dual culture testing. After 2 weeks, the inhibition zone and inhibition of the radial growth of the pathogen were measured.

#### *Test of DSE Volatile Isolate Metabolites*

Testing of volatile metabolites was carried out by following the methods of Dennis and Webster (1971) i.e. taking pieces of 5 mm diameter of pure culture from each DSE isolate and FOL pathogen, then placed on PDA media in a separate petri dish. The two petri dishes were then cupped against each other, so that the DSE isolate was on top and the FOL isolate was below. Observations were made on the growth of FOL pathogenic colonies by measuring the diameter of colonies 7 days after inoculation (dai).

## RESULTS AND DISCUSSION

DSE fungi were isolated from the tomato roots in West Java, Indonesia. Figure 1 shows DSE fungi isolated from tomato roots, stained by coloring as an initial detection.

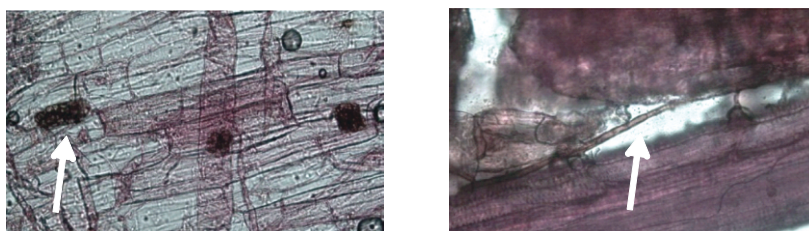


Figure 1 Color stained DSE fungal isolate in tomato root tissue, observed by 400x magnification: (a) microsclerotia; (b) melanin hyphae

Existence percentages and various sample locations of DSE fungi were presented in Table 1. DSE fungal isolates found from this exploration were 49 candidate DSE isolates.

Table 1 Percentage of DSE existence based on sampling location

Isolation Method	Location	Percentage (%)
Direct	Sukabumi	10.23
	Cianjur	7.45
	Citeupuh	4.76
	Garut	4.44
	Cisarua	4.00
Indirect (Soil Baiting)	Organic forest soil	6.67
	Cabbage farm soil	6.19

Selection of successfully isolated DSE fungi was conducted, followed by pathogenicity test. At this stage, isolates selected were those which did not cause death or inhibit the growth of tomato seeds. These isolates were not potential as pathogenic fungi, which would not kill or inhibit seeds' growth. Characteristics of pathogenic fungi in the selection of endophytic fungi included seeds which were not developing, were slow-growing compared to other fungi isolates; were not able to germinate and to grow, and eventually died. Characteristics of non-pathogenic fungi included seeds that were able to germinate well with well-grown roots and stems (Fig. 2).

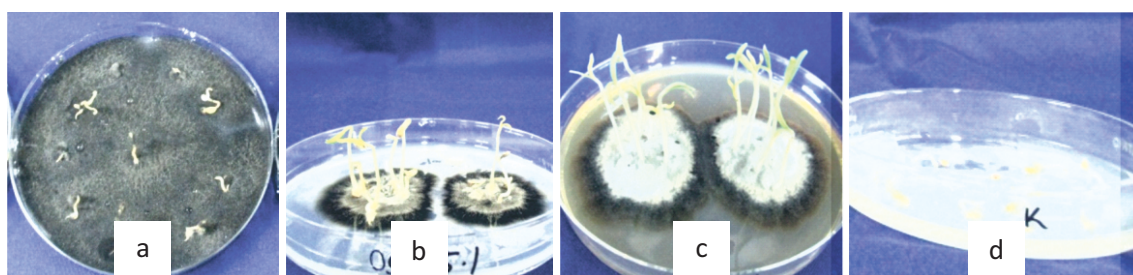


Figure 2 The effect of fungal isolate candidates on tomato seed germination in the pathogenicity test. (a) DSE isolate candidate inhibits seed germination (abnormal germination); (b) DSE isolate candidate shows seed germination (germination 70-85%); (c) DSE isolate candidate shows seed germination (germination 90-100%); (d) control (germination 70%)

Results of pathogenicity test from 49 candidates of DSE isolate obtained 21 DSE fungal isolates having the ability to promote tomato seed germination by 97%-100%.

Hemolytic reaction test of selected DSE fungi was conducted to ensure that the selected DSE fungi are not pathogen to human and animals. Hemolytic reaction is divided into three types (Beutin 1991), i.e. alpha hemolysis ( $\alpha$ -hemolysis), beta hemolysis ( $\beta$ -hemolysis), and gamma hemolysis ( $\gamma$ -hemolysis). If Alpha hemolysis ( $\alpha$ -hemolysis) is present, the agar under the colony is dark or greenish and the organism is called partial or incomplete hemolysis. Beta hemolysis ( $\beta$ -hemolysis) is a complete lysis of red cells in the media around and under the colonies: the area appears lightened (yellow) and transparent. Gamma hemolysis ( $\gamma$ -hemolysis) is unchanged in blood agar media and the organism is called non-hemolytic.  $\alpha$ -hemolysis and  $\gamma$ -hemolysis existence on blood agar indicates the growth of normal flora (negative as human or animal pathogen).  $\beta$ -hemolysis existence on blood agar indicates the presence of pathogen (positive as human or animal pathogen). Hemolytic reaction test of 20 selected DSE fungal isolates using blood agar showed that all isolates were negative and 1 positive. Therefore, those isolates are not pathogen to human and animals (Fig. 3).

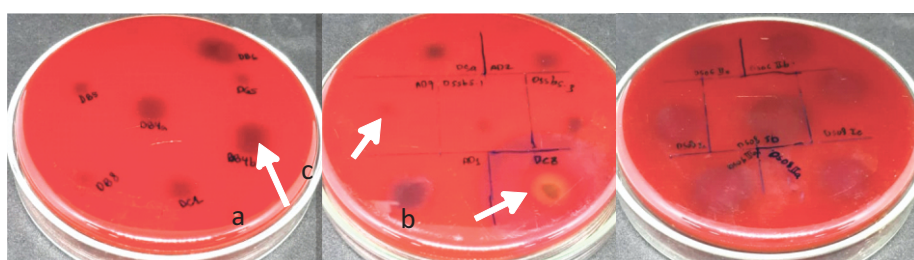


Figure 3 Hemolytic reaction test of selected DSE fungal isolate on blood agar media. (a) Alpha hemolysis ( $\alpha$ -hemolysis); (b) beta hemolysis ( $\beta$ -hemolysis); (c) gamma hemolysis ( $\gamma$ -hemolysis)

Nine (9) of the 20 selected DSE fungal isolates inhibited the growth of *F. oxysporum* (Table 2). The inhibition levels by DSE fungal isolate DS08 ic and DS08 ib on *F. oxysporum* were 76.11% and 71.07%. The negative control treatment was not given control formulation, so it did not show inhibitory activity as indicated by the maximum growth of *F. oxysporum* filling the entire surface of the petri dish (Fig. 4).

Tabel 2 Growth diameter and inhibition zone of *F. oxysporum* resulted from antagonism test for 14 days after inoculation (dai) in vitro

DSE Fungal isolate	Inhibition Percentage of pathogen growth rate (%)
DS08-1c	76.11a
DS08-1b	71.07ab
DS08-1a	66.67abc
DS06-IIa	66.67abc
AD1	66.04abc
DS06-IIIa	65.00abc
DS06-IIb	64.45abc
DS08-IIa	60.38bc
DG5	56.11c
AD2	36.97d
DB5	18.34e
DB8	16.11e
AD7	15.56ef
DC1	13.33efg
DB6	8.33efg
DB4a	6.33efg
DB4b	1.11gf
DSA	0.00g
DSSB 5.1	0.00g
DSSB 5.3	0.00g
CONTROL	0.00g

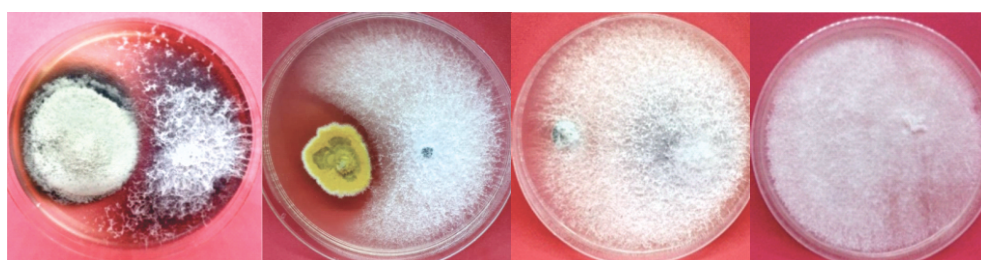


Figure 4 Antagonism between *F. oxysporum* and DSE fungal isolates. (a) DS08-1c; (b) DS08-1b; (c) not inhibit the growth of *F. oxysporum*; (d) control

Result of the volatile organic compounds (VOC) test showed diameter of pathogenic colony that had been inoculated with DSE isolate was smaller than that of control (Fig. 5). This indicated that the presence of volatile organic (VOC) compounds produced by DSE fungi can function as antifungal.

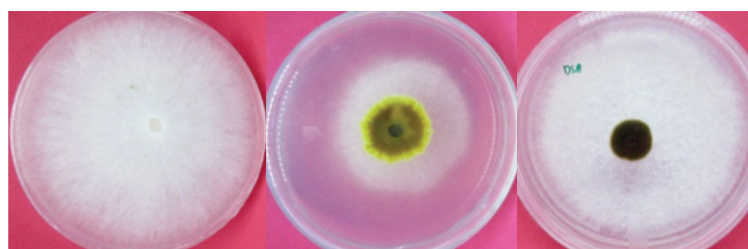


Figure 5 Effect of VOC from DSE fungi isolated from tomatoes plant on *Fusarium* diameter growth: (a) control; (b) showing growth inhibition of *F. oxysporum*; (c) showing no inhibition of growth of *F. oxysporum*

## CONCLUSION

DSE fungi can be isolated from the roots of tomato plants in several sample locations on West Java. Twenty (20) candidates of DSE fungi increased tomato seed germination up to 96.67-100% compared to 29 other fungus candidates. Hemolysis reaction test on 20 fungi isolates showed negative and 1 fungi isolate showed positive. DSE isolate DS08-Ic and DS08-Ib had the highest inhibition in suppressing *Fusarium* growth. DSE isolates from Indonesia are potential to be used as biocontrol agents in suppressing *Fusarium* growth.

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## INVENTORY AND IDENTIFICATION OF FUNGUS CAUSATIVE LEAF SPOT IN JABON (*Anthocephalus* sp.) IN INDONESIA

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### ABSTRACT

Jabon (*Anthocephalus* sp.) is the one of the most economically forestry crops. Jabon has several advantages compared to other woody plants. Among benefits of jabon include a very straight stem, has rapid growth, and has a self-deceased branch (self-pruning), so it does not require pruning. However, the development of jabon seeds faces obstacles in the form of pathogenic infection causing leaf disease on the seeds of red jabon in the nursery. Information related to the causes of leaf spots disease and its pathogenic symptoms has not been studied, which hinders the determination of the disease. Objectives of this research were to inventory the types of leaf spot symptoms and identify the pathogenic fungi causing the symptoms. Results of isolation and identification of pathogenic fungi showed six genus of pathogenic fungi i.e. *Pestalotia* sp. (from Yogyakarta, Maluku and Bogor), *Botryodiplodia* sp. (from Yogyakarta), *Curvularia* sp. (Kuningan and Bogor), *Rhizoctonia* sp. (Cianjur and Maluku), *Colletotrichum* sp. (Bogor), and *Botrytis* sp. (Bogor).

**Keywords:** Disease symptoms isolation, forest plant, fungal pathogen

### INTRODUCTION

Jabon (*Anthocephalus* sp.) is one of the most economical forestry crops. Jabon has several advantages compared to other woody plants such as sengon (*Albizia chinensis*), acacia (*Acacia* sp.), pine (*Pinus* sp.), and eucalyptus (*Eucalyptus* sp.). In addition to its rapid growth, the benefits of Jabon include a very straight stem and has a self-deceased branch (self-pruning), so it does not require pruning. In Indonesia, there are two types of jabon namely white Jabon (*Anthocephalus cadamba* Roxb.) and red jabon (*A. macrophyllus* Roxb.). Prospects for developing Jabon crops implicate the supply of Jabon seeds. However, the development of Jabon seeds faces obstacles in the form of pathogenic infection causing leaf disease on the seeds of red jabon in the nursery. Information related to the causes of leaf spots disease and its pathogenic symptoms has not been studied, which hinders the determination of the disease. Objectives of this research were to inventory the types of leaf spot symptoms and identify the pathogenic fungi causing the symptoms.

### MATERIALS AND METHODS

#### Sampling of Symptomatic Leaves

Samples of symptomatic Jabon leaves were collected from Yogyakarta, Cianjur, Kuningan, and Bogor for white Jabon species, and in Maluku for red Jabon species. The sample leaves taken



were leaves with symptomatic spots. The samples were then documented and taken to the laboratory for pathogen isolation.

### **Postulate Koch**

Koch Postulate Test. The stages of Koch postulate test conducted in this study consisted of isolation, inoculation, and the reisolation of pathogenic fungi.

### ***Isolation of Fungi***

Jabon leaves having spot and blight symptoms were isolated by taking part of the leaves between infected and uninfected tissues. The leaf pieces were then surface-sterilized by soaking them in 70% alcohol solution, followed by rinsing 3 times with sterile distilled water. The sterilized pieces of leaves were then placed on potato dextrose agar (PDA) media and incubated for 7 days. The hyphae growing from the leaf pieces were then purified in new PDA media. All stages of isolation were carried out in laminar air flow to maintain the working process.

### ***Inoculation***

Pathogenic fungi isolates obtained were then inoculated on 2-month-old white Jabon seedlings. The inoculation technique was carried out by attaching the agar block to Jabon leaves which had previously been injured. Inoculated seeds were then covered using plastic to maintain moisture. Observations were made for seven (7) days to find out the symptoms that occurred in Jabon seeds.

### ***Reisolation***

Reisolation was done after the inoculated leaves showed symptoms. Method used for the reisolation was the same method used in the pathogen isolation activity. Inoculated cobs will be considered as pathogenic and regarded as pathogens infecting jabon samples, if the symptoms of pathogen inoculation are identical to the symptoms in the sample leaves.

### **Identification of Pathogenic Fungi**

The purified fungi colonies were then identified. Identification fungi were carried out by observing macromorphology and micromorphology. Macromorphological forms consist of colony colors on PDA media and colony growth speed. Micromorphological forms consist of the form of hyphae, conidia, conidiophores, and conidial size. Identification refers to identification key book of Barnett and Hunter (1998).

## **RESULTS AND DISCUSSION**

Based on the results of observations in all observation locations, various leaf spot symptoms were found. Jabon leaves with symptomatic spots were characterized by the occurrence of necrosis (tissue death) causing the appearance of yellow, brown, and black color. In severe infections, the disease can cause leaves to perforate and fall. There were ten types of leaf spot symptoms found on leaves from Yogyakarta, Cianjur, Kuningan, Maluku and Bogor (Fig. 1).

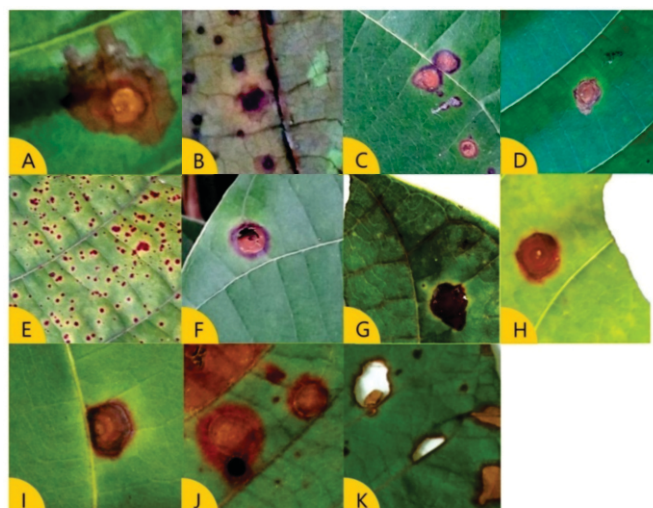


Figure 1 Types of leaf spot symptoms in Jabon (*Anthocephalus* sp.) A. YB1 (Yogyakarta), B. YK3 (Yogyakarta), C. BGB01 (Bogor), D. BGB02 (Bogor), E. BGB03 (Bogor), F. BGB04 (Bogor), G. C21 (Cianjur), H. C6 (Cianjur), I. K6 (Kuningan), J. AC (Maluku), K. AD (Maluku)

There were two types of leaf spot symptom from Yogyakarta, two types from Cianjur, one type from Kuningan, two types from Maluku, and four types from Bogor. Each type of the leaf spot symptoms had different characteristics from each other. In general, size ranges of the leaf spot symptoms were from <math><0.1\text{ mm}</math> to 1 cm. The leaf spot color found were light brown, dark brown, and black, indicating the death of leaf tissue. Some of the leaf spot symptoms also contained a yellow *hollo*. On a severe infection, the spots can spread evenly on the surface of the leaf and cause leaf holes. According to Manik *et al.* (2016) the leaf spot symptoms are indicated by the presence of necrosis in round, oval, and irregular leaves. In some spots there were also commonly formed yellow *hollo* around dead tissue.

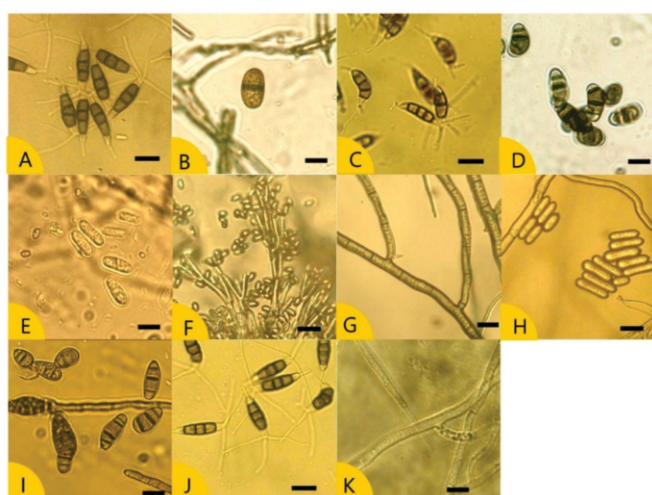


Figure 2 Pathogenic fungi causing leaf spot symptoms in Jabon (*Anthocephalus* sp.) A. *Pestalotia* sp. YB1 (Yogyakarta), B. *Botryodiplodia* sp. YK3 (Yogyakarta), C. *Pestalotia* sp. BGB01 (Bogor), D. *Curvularia* sp. BGB02 (Bogor), E. *Colletotrichum* sp. BGB03 (Bogor), F. *Botrytis* sp. BGB04 (Bogor), G. *Rhizoctonia* sp. C21 (Cianjur), H. *Colletotrichum* sp. C6 (Cianjur), I. *Curvularia* sp. K6 (Kuningan), *Pestalotia* sp. J. AC (Maluku), K. *Rhizoctonia* sp. AD (Maluku)

Spots and blight diseases on Jabon leaves can kill leaf tissue, so that it can decrease photosynthesis and can turn plants into having severe infections. There are several fungi that can cause blight and spotting on the leaves of forestry plants, including *Pestalotia* sp., *Lasiodiplodia* sp., *Cercospora* sp., *Curvularia* sp., *Helminthosporium* sp., *Gloeosporium* sp., *Cylindrocladium* sp., and *Colletotrichum* sp. (Anggraeni 2009).

Table 1 Leaf spot symptoms characteristics of Jabon (*Anthocephalus* sp.)

Region/ Code	Spot Characteristics				Pathogen
	Color	Diameter (cm)	Shape	Hollo	
<i>Yogyakarta</i>					
YB1	Light brown and dark brown in the edges	1 – 1.5	Circular with irregular shape in the edges	✓	<i>Pestalotia</i> sp.
YK3	Dark brown – black	0.1 – 0.3	Circular	-	<i>Botryodiplodia</i> sp.
<i>Bogor</i>					
BGB01	Light brown and dark brown in the edges	0.3 – 1	Circular	-	<i>Pestalotia</i> sp.
BGB02	Light brown and dark brown in the edges	0.3 – 0.5	Irregular	✓	<i>Curvularia</i> sp.
BGB03	Light brown	0.1 – 0.2	Irregular	✓	<i>Colletotrichum</i> sp.
BGB04	Light brown and dark brown in the edges	0.3 – 0.4	Circular	-	<i>Botrytis</i> sp.
<i>Cianjur</i>					
C6	Light brown	0.75 – 1	Circular	✓	<i>Colletotrichum</i> sp.
C21	Dark brown	0.5 – 0.7	Circular	✓	<i>Rhizoctonia</i> sp.
<i>Kuningan</i>					
K6	Light brown	0.6 – 0.75	Circular	✓	<i>Curvularia</i> sp.
<i>Maluku</i>					
AC	Light brown and reddish in the edges	0.3 – 1	Circular	-	<i>Pestalotia</i> sp.
AD	Light brown and dark brown in the edges	0.3 – 0.5	Irregular	-	<i>Rhizoctonia</i> sp.

*Curvularia* sp. is reported to be the cause of yellowish spots and *Pestalotiopsis* sp. is reported to be the cause of brownish yellow spots on Tembesu leaves (*Fragraea fragrans*) in southern Sumatra (Asmaliyah *et al.* 2015). *Curvularia* sp. is also known to cause leaf spot symptoms on nutmeg (Rachmawati & Soekarno 2016) and jatropa (Laksono *et al.* 2010). *Gloeosporium* sp. and *Colletotrichum* sp. each was reported as the cause of blotches and blight on Damar Siput plants. Leaf spots on Meranti Luang plants are caused by *Marssonina* sp. (Malaysia *et al.* 2005). Leaf spots caused by *Curvulria* sp. in the palm oil is shown by the initial symptoms of translucent yellow spots that can be seen on both leaf surfaces, round, spotting and then slowly becomes light brown and forms a circular pattern. Spots will then turn out to be more beautiful by being surrounded by a yellowish orange halo (Lalang *et al.* 2016).

## CONCLUSION

Results of isolation and identification of pathogenic fungi showed six genera of pathogenic fungi, i.e. *Pestalotia* sp. (from Yogyakarta, Maluku, and Bogor), *Botryodiplodia* sp. (from Yogyakarta), *Curvularia* sp. (Kuningan and Bogor), *Rhizoctonia* sp. (Cianjur and Maluku), *Colletotrichum* sp. (Bogor), and *Botrytis* sp. (Bogor). Each of the fungi causes different symptoms at different locations, although it is caused by the same genus of fungi.

## ACKNOWLEDGEMENTS

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## MORPHOMETRIC VARIATION OF *Pentalonia nigronervosa* Coquerel (HEMIPTERA: APHIDIDAE) INHABITING DIFFERENT BANANA CULTIVARS IN JAVA

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### ABSTRACT

Banana aphid, *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae), is known as the vector of *Banana Bunchy Top Virus* (BBTV) that threatens banana production worldwide. Some studies showed that the aphids exhibited phenotypic change when inhabiting different host plants. The objective of this study was to assess the morphometric variation of *P. nigronervosa* inhabiting different banana cultivars in Java. Aphid collection was conducted on several locations in West Java, Central Java and East Java. Eleven morphometric characters were examined to assess the morphometric variation of *P. nigronervosa*. Principle component analysis was performed to assess the morphometric variation of *P. nigronervosa*. The result showed that *P. nigronervosa* inhabiting different banana cultivars exhibited the variation in their morphometric characters, i.e. body length, head width, and length of antennal segment VI. However, *P. nigronervosa* showed low variation on the length of ultimate rostrum segment. Body length was the character that contributed most in morphometric variation of *P. nigronervosa*. Linear discriminant analysis indicated that the morphometric variation of *P. nigronervosa* was not influenced dominantly by banana cultivars.

**Keywords:** Banana aphid, morphology, phenotypic, principle component analysis

### INTRODUCTION

*P. nigronervosa* is the most important pest on banana and known as vector of *Banana Bunchy Top Virus* (BBTV). This species plays an important role on the spread of banana bunchy top disease (Hu *et al.* 1996). The infection of BBTV causes yield loss on banana up to 100%. Incidence of BBTV reached 100% and 96% on banana cv. Cavendish Williams and plantain hybrid variety PITA 23, respectively in Southern region of Cameroon, Africa (Ngatati *et al.* 2017). In contrast, incidence of BBTV in Indonesia ranged from 0% to 38.6% (Nurhayati 2003).

Variation in morphology of aphids have been reported on cabbage aphid (*Brevicoryne brassicae*) inhabiting two host species, *Brassica oleracea* and *Brassica campestris* (Ruiz-Montoya *et al.* 2005). Agarwala *et al.* (2009) also reported the morphological, ecological and biological variations in mustard aphid (*Liphaphis pseudobrassicae*) on three species of Brassicae. In recent study, Bhadra and Agarwala (2010) reported the differences in the fitness characters of *P. nigronervosa* and *P. caladii* inhabiting different host plants. The nutrient quality of a host plant and aphid nutritional biology affects the fitness of aphid (Awmack & Leather 2002; Powell *et al.* 2006). Aphids reared on poor nutrient quality of host plants were smaller than those reared on rich nutrient quality (Dixon & Kindlmann 1994). Genetic and morphological differentiation in relation to the use of different host plants may lead to ecological specialization (Agarwala *et al.* 2009). A good and proper study of banana aphid's morphometric is needed to get an appropriate controlling strategy of BBTV and its vector.

## MATERIALS AND METHODS

The research was conducted from August 2017 to February 2018. Aphid sampling was done using purposive sampling on several locations in West Java, Central Java and East Java. Aphid specimens were mounted using Canada balsam. Eleven morphometric measurements were performed according to Footitt *et al.* (2010), i.e. length of body (BL), head width (HW), antennal length (AL), length of antennal segment I-II (A1-2), length of antennal segment III-V (A3-5), length of antennal segment VI (A6), length of ultimate rostrum segment (URS), length of siphunculus (Sph), length of hind femur (Fem), length of hind tibia (Tib) and length of caudal (Cau). Morphometric variation of banana aphids was analyzed with *Multivariate Analysis of Variance* (MANOVA), *Principle Component Analysis* (PCA) with correlation matrix, *Unweighted Pair Group Method with Arithmetic Mean* (UPGMA) using Euclidean distance, and *Linear Discriminant Analysis* (LDA). Analysis was performed using Paleontological statistics (PAST) version 3.18 (Hummer *et al.* 2001).

## RESULTS AND DISCUSSION

As many as 101 specimens of *P. nigronervosa* collected from eleven different banana cultivars in Java were examined. Multivariate analysis of variance (MANOVA) showed a significant difference on morphometry of *P. nigronervosa* collected from eleven banana cultivars (Wilks' lambda = 0.0147, F = 4.21,  $p \leq 0.001$ ). The first two principle components showed eigenvalues greater than 1 and explained 76.2% of variance on the morphometry of *P. nigronervosa* (Table 1). Therefore, PC1 and PC2 were proper variable to analyse the morphometric variation of banana aphid (Peppe & Lomonaco 2003; Ruiz-Montoya *et al.* 2005). All morphometric characters had positive loadings on PC1. The loading values on PC1 were associated with the variation in general size of the characters. Hind tibia, hind femur, antennal dimensions (AL, A1-2, A3-5), and siphunculus had loading value a little bit higher than other characters on PC1. On the other hand, the length of ultimate rostrum segment showed the lowest contribution for the morphometric variation among *P. nigronervosa* collected from different banana cultivars on PC1. This situation indicated that the characters of URS among *P. nigronervosa* have no length variation. Body length showed higher positive contribution on PC2 with value of 0.5268, whereas length of antennal segment VI showed higher negative contribution to PC2 with value of -0.5239. In addition to body length and length of antennal segment VI, head width showed a bit higher positive contribution on PC2 with loading value 0.442 whereas antennal length also showed a bit higher negative contribution on PC2 with loading -0.3172.

The scatter plot of two principle components (PC1 and PC2) showed eleven projection plots of the morphometric characters of *P. nigronervosa* collected from eleven banana cultivars in Java (Fig. 1). The scatter plot of PC1 and PC2 indicated morphometric variation on *P. nigronervosa* inhabiting different banana cultivars. The scatter plot of PC1 and PC2 showed some projection plots which were overlapped and other projection plots were separated. The overlapping projection plot explained the close similarity in the morphometry, whereas the separated plots explained high differentiation in morphometry. Scatter plot of PC1 and PC2 exhibited the projection plot of *P. nigronervosa* collected from cv. Raja Sajen were separated from other plots. It indicated that colonies of *P. nigronervosa* collected from cv. Raja Sajen was morphologically different from other colonies. In general, most of the projection plots were overlapping with each other, except some specimens of *P. nigronervosa* which showed differentiation on their morphometric characters.

Table 1 Loading value of first two principle components of eleven morphometric characters of *P. nigronervosa* inhabiting different banana cultivars in Java

Variable	PC1	PC2
Body length (BL)	0.2599	0.5268
Head width (HW)	0.2856	0.4442
Antennal length (AL)	0.3394	-0.3172
Length of antennal segment I-II (A1+2)	0.3185	0.1461
Length of antennal segment III-V (A3-5)	0.3335	-0.2194
Length of antennal segment VI (A6)	0.2559	-0.5239
Length of URS (URS)	0.1912	-0.2021
Length of hind femur (Fem)	0.3448	0.1186
Length of hind tibia (Tib)	0.3506	-0.0243
Length of siphunculus (Sph)	0.3207	-0.0809
Length of cauda (Cau)	0.2745	0.1353
Eigenvalue	7.33	1.06
Proportion of variance (%)	66.6	9.6
Cumulative variance (%)	66.6	76.2

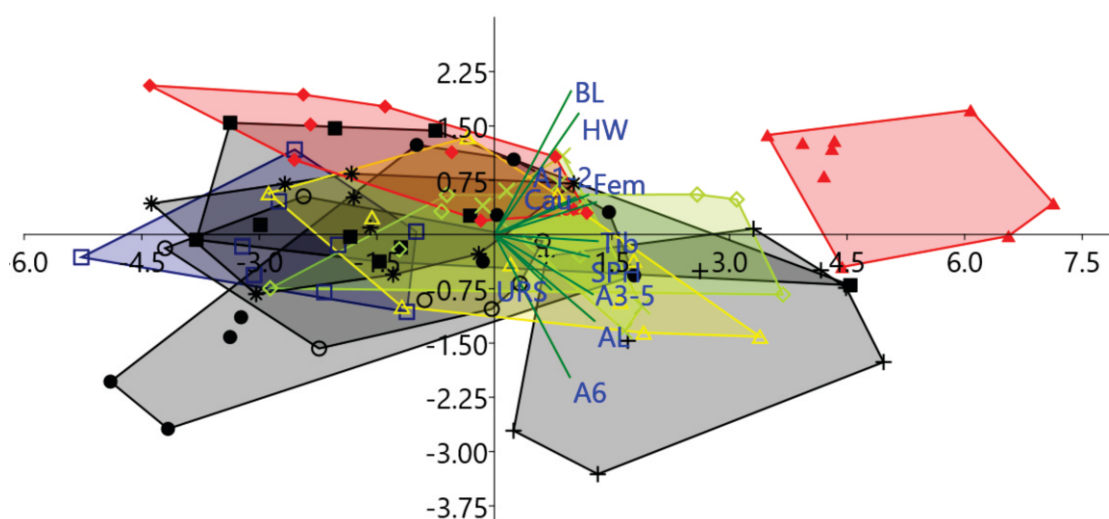


Figure 1 Scatter plot of two principle components of morphometry of *P. nigronervosa* collected from eleven banana cultivars; Five cultivars collected from Bogor (●: 'cv. Ambon', +: 'cv. Mas', ■: 'cv. Tanduk', ○: 'cv. Siem', \*: 'cv. Lampung'); two cultivars collected from Sukabumi (×: 'cv. Barangan', ◇: 'cv. Cavendish'); two cultivars collected from Malang (▲: 'cv. Raja Sajen', ◆: 'cv. Sobo'); one cultivar collected from Magelang (□: 'cv. Raja Nangka'); one cultivar collected from Banyuwangi △: 'cv. Kepok')

Hierarchical clustering with unweighted pair group method with arithmetic mean (UPGMA) of *P. nigronervosa* had cophenetic correlation coefficient of 0.864. Cophenetic correlation coefficient defined a fine clustering of *P. nigronervosa*. *P. nigronervosa* collected from cv. Raja Sajen was located on single leaf at the dendrogram and had the longest dissimilarity distance (Fig. 2). *P. nigronervosa* collected from cv. Raja Sajen indicated the dissimilarity in morphometry from other colonies of *P. nigronervosa* examined. The dendrogram also denoted that *P. nigronervosa* collected from one area were more similar in morphometry than *P. nigronervosa* collected from different area. All samples of *P. nigronervosa* collected from Bogor Regency were grouped in one cluster; similar to all samples collected from Sukabumi Regency which were grouped in one cluster. Linear discriminant analysis showed that only 38.61% of *P. nigronervosa* was correctly

classified based on banana cultivars. It indicated that morphometric variation of *P. nigronervosa* was not dominantly influenced by the banana cultivars. Further study needs to be carried out to evaluate the other factors contributing in the morphometric variation of *P. nigronervosa*.

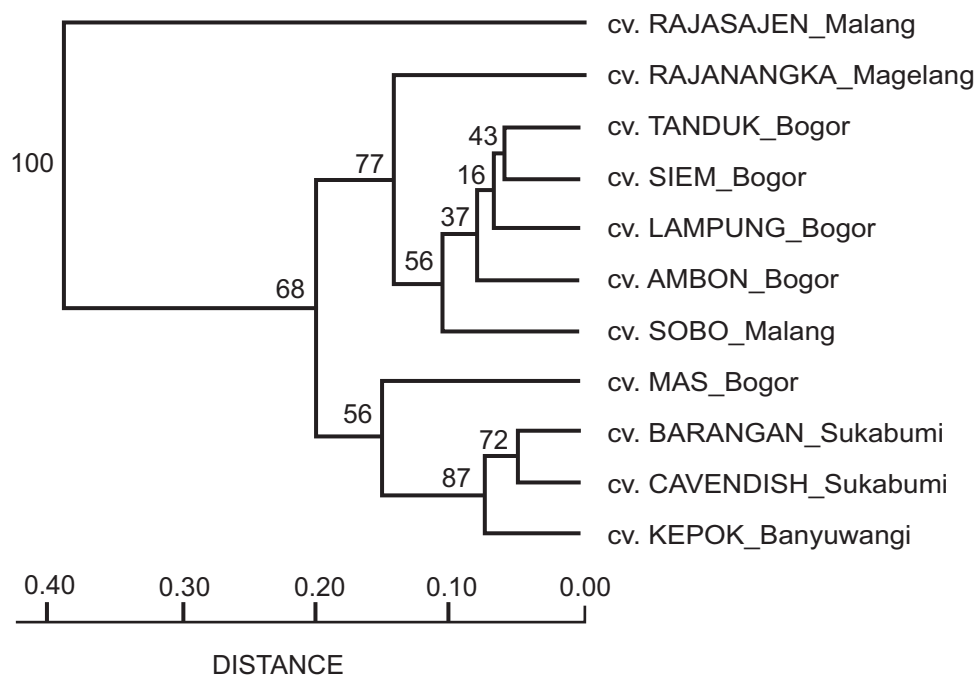


Figure 2 Dendrogram of cluster analysis based on UPGMA between colony of *P. nigronervosa* collected from eleven cultivars of banana in Java (bootstrap= 1000x, cophenetic correlation coefficient = 0.864)

### CONCLUSION

Principle component analysis showed the morphometric variation on *P. nigronervosa* which inhabited different banana cultivars in Java. *P. nigronervosa* showed the variation on the body length, head width and length of antennal segment VI. However, *P. nigronervosa* inhabiting different banana cultivars showed low variation on the length of ultimate rostrum segment. The variation in the morphometry of *P. nigronervosa* was not dominantly influenced by the banana cultivars.

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## DIVERSITY OF SCALE INSECTS (HEMIPTERA: COCCOIDEA) ON VARIOUS PLANTS IN BOGOR BOTANICAL GARDENS

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### ABSTRACT

Scale insects (Hemiptera: Coccoidea) are group of polyphagous insects that have a wide range of host plants. Coccoidea can cause direct or indirect damage to plants. This group of insects can also act as virus vectors. Bogor Botanical Gardens has a high level of plant diversity, so it is a good location to study the diversity of scale insects. The objective of this research was to study the diversity of scale insect from the Order Hemiptera, Superfamily Coccoidea in Bogor Botanical Gardens. Samples in this study were taken by purposive sampling from various plants. Collected samples were mounted as slide preparation and identified until species level, based on morphological characters. There were 17 Coccoidea species identified on 36 species of plants. The scale insect families found were Coccidae, Diaspididae, Margarodidae, Ortheziidae, and Pseudococcidae. *Dysmicoccus lepelleyi* (Betrem) was the scale insect mostly found during the study. This species is known to infest 10 host plants. Arecaceae (palmae) was the plant mostly infested by the scale insects.

**Keywords:** Armored scale insect, margarodidae, mealybugs, ortheziidae, soft scales

## MARASMIOID FUNGI (BASIDIOMYCOTA, AGARICALES, MARASMIEAE AND OMPHALOTACEAE) OF INDONESIA

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### ABSTRACT

Marasmioid fungi is a kind of mushroom which is morphologically similar to genus *Marasmius*, and it consists of several genera, namely *Marasmius*, *Marasmiellus*, *Micromphale*, *Collybia*, *Crinipellis*. In the framework of diversity study of the marasmioid fungi in Indonesia, the research concentrated on the *Marasmius* and *Marasmiellus*. Both are cosmopolitan in tropical and subtropical areas. Method of the research involves collection, description, identification, preservation, and documentation of specimens of *Marasmius* and *Marasmiellus*. Fieldworks to several different locations in Java, Bali and Kalimantan were done in 2000-2015, and it yielded 351 specimens. All specimens were preserved dried, stored in protective boxes in airtight herbarium cabinets and maintained against insect infestation. Comprehensive notes on the macromorphology of collections must be made immediately after collecting, before their basidiomes begin to dessicate. Microscopic analyses entailed the description and illustration of all tissues and cell types of dried collections which were examined under microscope in laboratory. Accurate species diagnoses were based on comparison of macro- and microdescriptions with the previously described taxa from all published accounts and other pertinent herbarium specimens. Identification process produced a total of 43 species of *Marasmius* and 35 species of *Marasmiellus* with the presence of 9 new species of *Marasmius*, 16 species of *Marasmiellus*, and 3 new varieties of *Marasmius*. Two species of genus *Marasmius* were transferred to genus *Marasmiellus* as a new combination.

**Keywords:** Agaricales, Indonesia, *Marasmiellus*, *Marasmius*

### INTRODUCTION

Marasmioid fungi is a group of mushrooms which looks like the genus *Marasmius* with small, white-spored, tough sporocarps, especially in the stipe, lack partial and universal veils, pilules vary from white, through shades of pale-yellow brown, to dark reddish brown, to black, a very few are purplish, and a few are burgundy-red. Generally, marasmioid fungi are simply small, tough, little brown mushrooms. This group contains several genera, namely *Marasmius*, *Marasmiellus*, *Micromphale*, *Collybia*, *Crinipellis*, and this research focuses on the genera *Marasmius* and *Marasmiellus*.

*Marasmius* (Marasmieae) consists of approximately 500 species and *Marasmiellus* (Omphalotaceae) comprises 250 species (Kirk *et al.* 2008). Both are widespread in tropical and subtropical areas, and they play an important role in the ecosystem as a litter decomposer, nutrient recycling and retention, and soil genesis.

Several reports of marasmioid fungi from Indonesia, particularly *Marasmius*, had been documented by Levéillé (1844), Moritzi (1845-1846), Zollinger (1854), Hennings (1900), Overeem-de Haas (1922), Boedijn (1940) and Desjardin *et al.* (2000). On the contrary, there are only a few reports on *Marasmiellus* from Indonesia (Desjardin *et al.* 2000; Retnowati 2018). To explore and to fill the information on the diversity of marasmioid fungi from Indonesia, intensive fieldwork had been done in several locations in Indonesia, and the results are provided here.

## MATERIALS AND METHODS

Specimens of marasmioid fungi were collected in several areas in Java, Bali and Kalimantan by Purposive Random Sampling. Basidiocarps were collected representing as many growth stages as were available in order to examine variability within the species. Photographs of fresh materials, such as suitable basidiocarps were taken. Each specimen was wrapped in aluminium foil or put in plastic boxes. Then the specimens were placed in plastic boxes with moist tissue paper, dried on portable oven, and identified tentatively in the field station. Many taxonomically important characters on habit of growth, odor, taste, substrate type, and plant associates were observed only in the field. Macro- and micromorphological characters were described and illustrated based on fresh and dried fungal specimens collected from the locations. Microscopic observation was made on material mounted in 3% KOH. Color notation was determined using Kornerup and Wanscher (1978). Specimens examined were deposited in Herbarium Bogoriense (BO) and the Harry D. Thiers Herbarium (SFSU) at San Francisco State University, USA.

All line drawings of the micro-characters were made with the aid of a camera lucida attached to a compound microscope using 40x or 100x (oil immersion) objectives. Spore range was obtained by measuring 25 mature basidiospores.

## RESULTS AND DISCUSSION

A total of 351 specimens were collected in 2000-2015 (Table 1), and identification process produced a total of 43 species of *Marasmius* and 35 species of *Marasmiellus* with the presence of 9 new species of *Marasmius* (*M. berambutanus*, *M. coklatus*, *M. dicandinus*, *M. balimunensis*, *M. kembangus*, *M. luteomarginatus*, *M. perplexus*, *M. persicinus*, *M. schizochaetus*, *M. gypseus*, and *M. xenopellis*), 16 species of *Marasmiellus* (*Marasmiellus bisporus* Retn., *M. cibodasensis* Retn., *M. cikanikiensis* Retn., *M. clavatus* Retn., *M. desjardini* Retn., *M. diverticulatus* Retn., *M. haurbentensis* Retn., *M. javanicus* Retn., *M. longisiccus* Retn., *M. pipericola* Retn., *M. pruinusosus* Retn., *M. reniformis* Retn., *M. rifaii* Retn., *M. subglobosus* Retn., *M. tamblinganensis* Retn. and *M. zingibericola* Retn.), and 3 new varieties of *Marasmius* (*M. cladophyllus* var. *tjibodasensis*, *M. coklatus* var. *mentarangensis* and *M. caryote* var. *pa'rayensis*) (Desjardin *et al.* 2000, Retnowati 2008, 2010, 2018) (Table 2). Two species of genus *Marasmius* were transferred to genus *Marasmiellus* as new combinations (Retnowati 2018), namely *Marasmiellus pangerangensis* (Henn.) Retnowati comb. nov. and *Marasmiellus nugatorius* (Corner) Retnowati comb. nov.

Table 1 The number of specimens of marasmioid fungi from Indonesia in different islands

Location	<i>Marasmius</i>	<i>Marasmiellus</i>	Total
Java	173	89	262
Bali	25	35	60
Kalimantan	27	2	29
Total	225	126	351

Table 2 Result of the research

	<i>Marasmius</i>	<i>Marasmiellus</i>
Described species	43	35
New species	9	16
New varieties	3	-
New combination	-	2

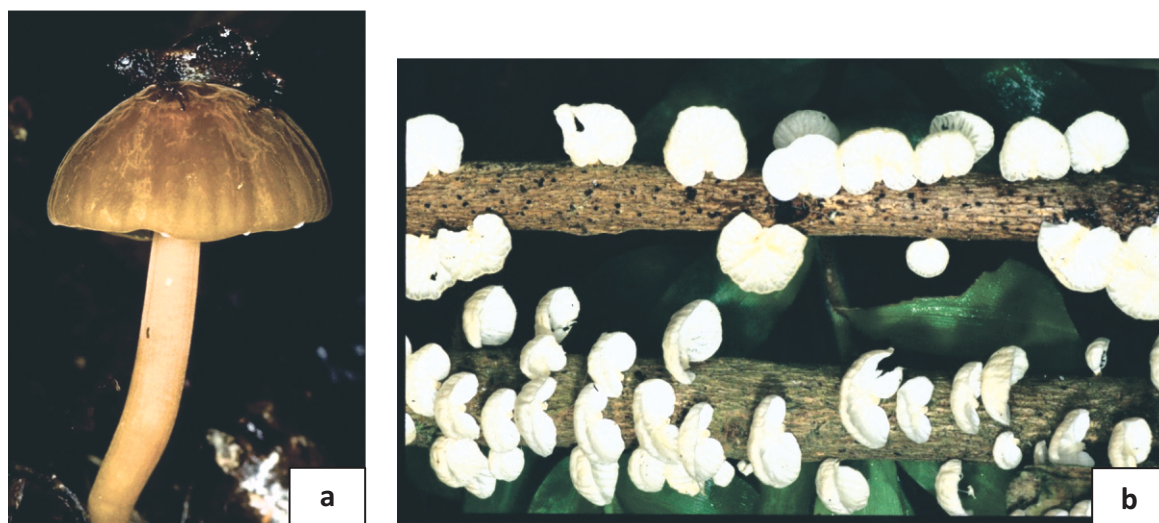


Figure 1 a. *Marasmius coklatus* Desjardin, Retnowati & E. Horak; b. *Marasmiellus reniformis* Retnowati (Photo: Dennis E. Desjardin)

The high number of new taxa in this study (approximately 35% of described species) indicated that Indonesia is a suitable area for discovering new species of marasmioid fungi, and there are still many undescribed species of *Marasmius* and *Marasmiellus* in Indonesia. Therefore, more fieldworks in Java and Bali or other islands in Indonesia will yield more species of *Marasmius* and *Marasmiellus*.

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## SIALANG HONEY PRODUCTION: BALANCING NATURE AND WELL-BEING (CASE STUDY IN TESSO NILO NATIONAL PARK)

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### ABSTRACT

Sialang Honey is produced naturally in which bees use Sialang trees as their hive. In Tesso Nilo National Park, this kind of honey production becomes an integral part of the local culture. The production is believed to provide benefit in economic, conservation, and social aspect. Using literature review and discussion, this research analyzed traditional practice in managing Sialang Honey and provided information regarding challenges in maintaining its existence. Some findings of this research were farmers adopting sustainability aspect in harvesting Sialang Honey. They applied methods which are safe for the environment and support communities. There was also customary law protecting the trees from danger. However, Sialang Honey production has been gradually decreased due to land conversion, lack of interest, low market, and availability of alternative income. Therefore, related stakeholders must jointly collaborate to enforce the law, create market, and massively promote traditional knowledge as a pride.

**Keywords:** Conservation, Sialang honey, traditional knowledge

### INTRODUCTION

Tesso Nilo National Park was appointed as a national park based on the Decree of Ministry of Forestry Number: SK.255/Menhut-II/2004 dated 19 July 2004 and Decree Number: SK 663/Menhut-II/2009 dated 15 October 2009. The park is habitat of Sialang trees which are home of Forest Bee (*Apis dorsata*). In this area, honey collection becomes an integral part of local culture. It relates to local wisdom in maintaining sustainability of the forest (Arsyad *et al.* 2011). Research has shown that existence of traditional knowledge supports conservation (Popova 2013).

Therefore, this paper explores traditional knowledge used in management of Sialang Honey and provides information regarding challenges in its implementation. This information is very important to help related stakeholders formulating better extension and regulation in accommodating local people and park's conservation.

### MATERIALS AND METHODS

This research used literature review. Selected papers were used to acquire information regarding Sialang Honey production and its problems. Discussion with other party was also done to evaluate available information.

### RESULTS AND DISCUSSION

#### Sialang Trees Distribution

According to Malay Riau Tribe, sialang trees refer to trees used as bees' hive. Types of Sialang trees include Balau (*Shorea atrinervosa*), Ara (*Ficus* spp), Meranti (*Shorea* spp), Kempas

(*Koompasia malaccensis*), Rengas (*Gluta rengas*), etc (Anggraheni 2012). There are at least 490 sialang trees in Tesso Nilo National Park. Each tree has 10-15 hives and estimated to produce 80–500 kg of honey. Therefore, total potential honey production is around 7,500–25,000 kg in one-time harvest (Arsyad *et al.* 2011).

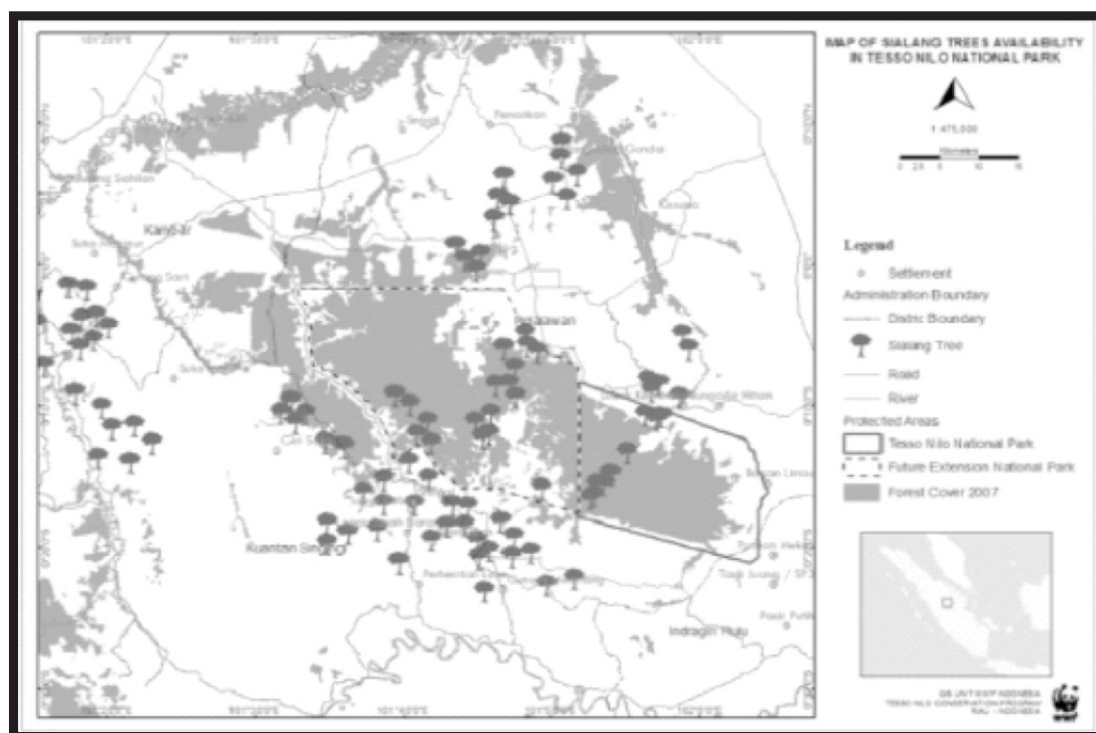


Figure 1 Distribution of Sialang trees in Tesso Nilo National Park (Arsyad *et al.* 2011)

### Traditional Knowledge in Managing Sialang Honey and Trees

Below is summary of traditional knowledge applied by local people in managing Sialang honey. This indigenous knowledge has been practiced for a long time and depicts sustainability.

Tabel 1 Traditional knowledge in managing Sialang honey

No	Aspect	Traditional Knowledge
1	Ecological Aspect	<ul style="list-style-type: none"> <li>Harvesting honey is done at certain period and criteria</li> <li>People believe that honey is inhabited by spirits, and therefore, it must be protected</li> <li>The protection of Sialang trees is done up to area of two hectares</li> </ul>
2	Community Involvement	<ul style="list-style-type: none"> <li>Involving groups of local people for collecting and sharing results (mutual cooperation)</li> </ul>
3	Institutional Support	<ul style="list-style-type: none"> <li>There is customary institution and law which protect Sialang trees and forest area. This customary law not only binds local communities, but also companies</li> </ul>
4	Sustainability Technique	<ul style="list-style-type: none"> <li>Torch materials to drive bees away is bark of <i>Dellenia exenia</i> tree which is safe and does not kill bee</li> <li>The stairs and ropes used for climbing last for a year</li> </ul>

Sources: Syafii and Yennita (2016); Sari *et al.* (2014); Anggraheni (2012); Arsyad *et al.* (2011)



## Ecological, Social and Economic Challenges

Sialang honey production has been well developed, especially after the formation of honey farmer group. However, Sialang trees still face some threats due to illegal logging, land conversion, and deforestation (Puspitasari 2016). This problem has caused change in micro climate which affects honey production (Perial *et al.* 2013). To overcome this problem, implementation of integrated natural resources management and law enforcement need to be well realized.

From economic perspective, market of Sialang honey is still limited and it must compete with other well-known honey, such as Sumbawa and Sentarum honey. Limited source of electrical energy has also caused delay in packaging (Anggraheni 2012). Improving brand image and gaining support from the government to create market is crucial.

Nowadays, some farmers harvest honey at daytime which drive bee away from Sialang trees. They also prefer to cultivate palm oil (Sari *et al.* 2014). The younger generation has begun to abandon the tradition of harvesting honey that may someday be lost (Anggraheni 2012). Therefore, local administration and government should promote their tradition as part of cultural identity.

## CONCLUSION

Preservation of Sialang trees and its adherent culture is important to protect the park and local culture. To achieve this, related stakeholder must jointly collaborate to formulate better regulation. To ensure the sustainability of bee production, market should be widened and cooperation needs to be done with more parties.

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## VISITORS' WILLINGNESS TO PAY FOR THE ENHANCED CONSERVATION OF THE MOUNT MAKILING FOREST RESERVE, PHILIPPINES

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### ABSTRACT

The Mount Makiling Forest Reserve (MMFR) hosts unique flora and fauna and provides various ecosystem services. It was declared in the 33<sup>rd</sup> ASEAN Heritage Park (AHP) in 2013. The Makiling Center for Mountain Ecosystems (MCME) has drawn up the MMFR Management Plan. However, the funds that MCME gets are not sufficient to fully finance the implementation of the plan. Visitors are major stakeholders of MMFR; therefore, MCME planned to propose an increase in the entrance fee of the park. This study was undertaken to estimate the value to visitors of the enhanced conservation of MMFR using the contingent valuation method. The results can provide MCME a basis in setting the appropriate entrance fee of the park. Out of 102 survey respondents, 63% expressed Willingness To Pay (WTP) for the enhanced conservation of MMFR, with mean WTP of Php110 on top of the current entrance fee of Php10.00. The only factor that was found to affect WTP of the visitors was the bid level, with the expected negative sign. If the visitors' mean WTP will be added to the current entrance fee (a total fee of Php120/visitor/day), the potential revenues from 2019-2025 can total Php35,391,720. This is about Php32 million more than revenues generated from the current entrance fee. This potential revenue source can be used to partly finance the activities of the plan, which requires Php258.76 million to implement. Hence, MCME needs to find other funding sources to fully finance the implementation of MMFR's management plan in the context of being an AHP.

**Keywords:** ASEAN Heritage Park, contingent valuation method, Mount Makiling Forest Reserve, willingness to pay

### INTRODUCTION

The Mount Makiling Forest Reserve (MMFR) hosts unique flora and fauna and provides various ecosystem services (Caparas 2013). It was declared in the 33<sup>rd</sup> ASEAN Heritage Park (AHP) in 2013 (University of the Philippines Los Baños [UPLB] 2015). With this recognition, comes the responsibility to enhance the conservation of the park following the context of an AHP. Therefore, the Makiling Center for Mountain Ecosystems (MCME), MMFR's managing agency, has drawn up the MMFR Management Plan. However, the funds that MCME gets are not sufficient to fully finance the implementation of the plan. Visitors are major stakeholders of MMFR; therefore, MCME planned to properly set the entrance fee of the park (J. Barile, personal communication, March 15, 2018).

With these conditions, the main objective of this study was to estimate the value of the enhanced conservation of the MMFR to its visitors. Specifically, the study aimed to: assess the level of awareness of visitors about the importance of the MMFR and its identity as an ASEAN Heritage Park; estimate the Willingness To Pay (WTP) of the visitors for the enhanced conservation of the MMFR; determine the factors influencing WTP of MMFR visitors; and

identify features or services that can be provided to visitors to enhance their experience at the MMFR. The results may be used to provide MCME a reference in setting the right entrance fee that can support the maintenance of the park without affecting its number of visitors. The rest of the information that will be gathered from MMFR visitors could also help the management in enhancing the visitors' value of the park.

## MATERIALS AND METHODS

### Study Site

The study was conducted at the Station 1 of MMFR's Mariang Makiling Trail. The MMFR, situated 14°8' north and 121°12' east, 65 km southeast of Metro Manila, is a 4,244-hectare mountain covering parts of Los Baños, Bay and Calamba City of Laguna province and Sto. Tomas of Batangas Province (Fig. 1). It is being managed by the MCME under the College of Forestry and Natural Resources-University of the Philippines Los Baños (CFNR-UPLB) (Makiling Center for Mountain Ecosystems [MCME] 2014).



Figure 1 Location and satellite maps of MMFR showing the neighboring cities and municipalities (Source: Google)

Mariang Makiling Trail, on the other hand, is the main trail of the MMFR (Fig. 2). It stretches 8.9 kilometers from the campus of CFNR in UPLB, and is also called “UPLB Trail”. Points of interests such as Mudspring, Flatrocks, Makiling Rainforest Park and Peak II can be accessed using this trail (Makiling Center for Mountain Ecosystems [MCME] 2013).

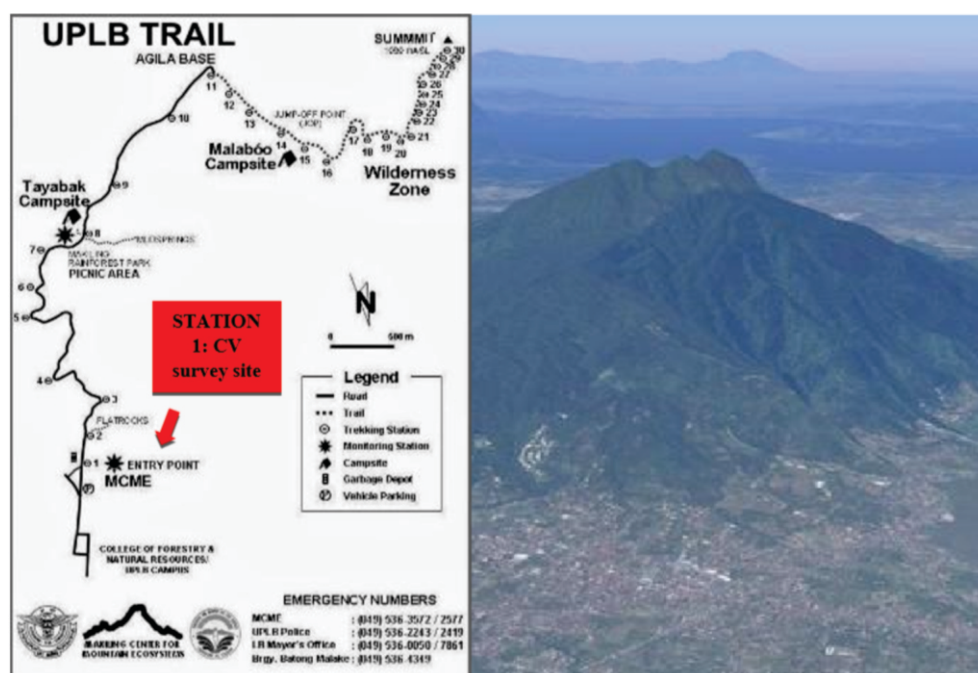


Figure 2 The site of CV survey at the Mariang Makiling Trail of MMFR (Source: MCME)

## Contingent Valuation Survey

With the main objective of estimating the value of enhanced conservation of the MMFR to its visitors, the researcher chose CVM. This is a widely-used method in environmental and natural resources valuation studies (Samdin 2008). It is a survey questionnaire-based technique under the stated preference method where the respondents are asked about their willingness to pay for a certain goods or services (Bruner *et al.* 2015).

The face-to-face method was applied in implementing the CV survey. This type of survey method is highly recommended by stated preference practitioners. According to Özdemiroglu and Pearce (2002), face-to-face surveys could be relatively expensive, but are highly flexible that complex questions and questionnaires are possible; permit probing and clarification; can collect larger quantity of data; have potential for extensive use of visual and demonstration aids; have greatest sample control; and have high response rate of 70% and above that allow high quality of contingent valuation study.

The interview was conducted before the visitor entered the park to avoid biases. According to Bruner *et al.* (2015), this will ensure that the WTP values revealed would reflect visitors' expectations prior to the visit (i.e. based on the criteria they used when they decided to visit), and not be influenced by their perception of their actual visit.

## Sampling

The target population for this study was the set of incoming Mariang Makiling Trail visitors of MMFR, who may have some value for the enhanced conservation of MMFR as they can be directly affected if the park deteriorates if not properly managed and conserved.

Sampling was employed because it was not possible, considering time and available resources, to interview all visitors to the MMFR. The sample should be highly representative of the entire population in a way that the sample could showcase variations in the perceptions and

preferences of the visitors related to the topic. Ideally, the sample should come from both peak and off-peak seasons. From MMFR's visitation data from 2014 to 2017 shown in Figure 3, the park's peak season is during the month where the Holy Week falls while the rest of the months can be considered off-peak season. The Holy Week for 2018 was from March 29 to April 1. The survey was thus, conducted from Thursday to Sunday, starting from March 18 to April 15 to cover one week of peak season (from March 26 to April 1) and three weeks to represent lean season (i.e. from Thursday to Sunday of the weeks of March 19 to March 25, March 31 to April 6, and April 7 to April 15) since there was no significant difference between the visitation rates of other months of the years as shown in Fig. 3 and 4.

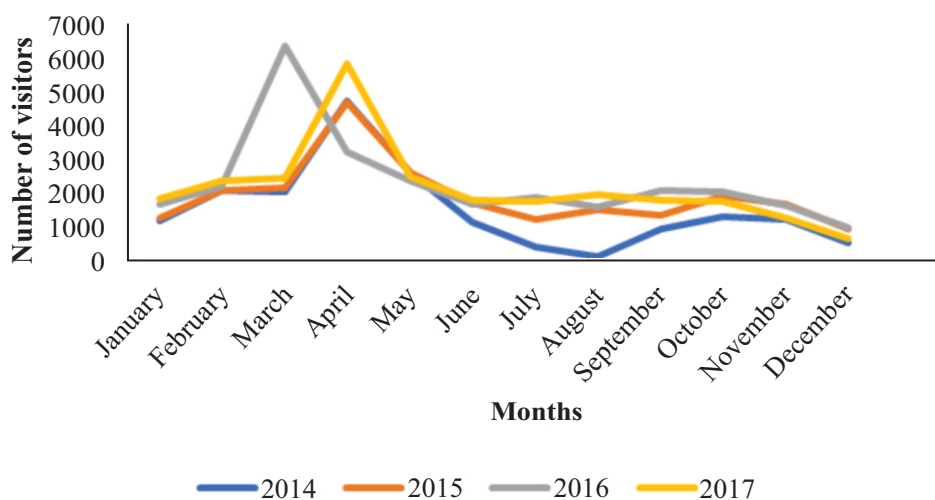


Figure 3 Trends of visitation in Mariang Makiling Trail per month from 2014 to 2017  
 Note: <sup>a</sup>MCME

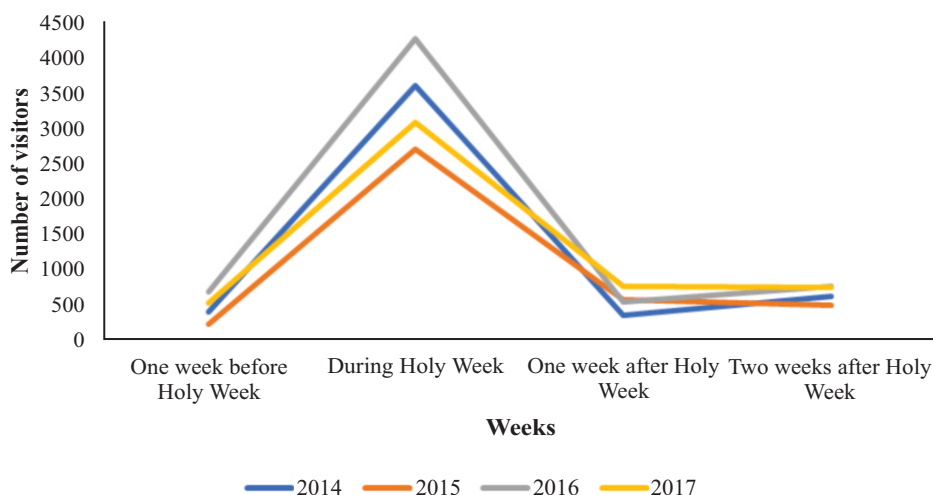


Figure 4 Visitation rates of the Mariang Makiling Trail during similar weeks as the survey period from 2014 to 2017  
 Note: <sup>a</sup>MCME

The sample size was determined using the Slovin's formula (1):

$$n = \frac{N}{1+\epsilon^2} \dots\dots\dots (1)$$

where: n = sample size  
 N = population size  
 e = 10% allowed margin of error at 95% confidence level

The Slovin's formula of finding the sample size for a particular study is used when behavior and other characteristics of the population are not known. In this study, the characteristics of visitors, the population of this research, are not known, thus the formula was used (Stephanie 2012).

In applying the formula, the population size was first determined based on the average of the total number of visitors from Thursday to Sunday during the Holy Week, one week before the Holy Week, and two weeks after the Holy Week, from year 2014-2017. This yielded an average population size of 4,442 visitors, from which a sample size of 98 respondents was computed. These 98 respondents were distributed over the four weeks the survey was implemented using the average proportion of respondents from previous years on those particular weeks. In the final survey implementation, however, 102 respondents were interviewed to have the same number of respondents per bid level (i.e. 17 respondents per bid level). The distribution of the respondents across the survey period is given in Table 1.

Table 1 Distribution of respondents across the survey period (March 22 to April 15, 2018)

Week	Number of Respondents	
	Original Sample	Actual Survey Implementation
One week before Holy Week	9	10
During Holy Week	69	70
One week after Holy Week	10	11
Two weeks after Holy Week	10	11
Total	98	102

The study used the systematic sampling method, one of the probability sampling techniques in which all the members of the target population have an equal probability of being chosen. Among the probability sampling techniques (i.e. Simple, Systematic, Stratified and Cluster), systematic sampling was selected because it is the most appropriate to use for the type of population in this study.

The complete list, characteristics and exact number of the elements of the MMFR visitors for 2018 were not known or predetermined. Thus, the application of random number generator, draw-lots or table of random numbers, which are examples of simple random sampling techniques, could not be used. Likewise, the assignment of labels or grouping that are needed in the stratified random and cluster sampling techniques was not possible (Sharma 2017).

Systematic sampling uses a fixed skipping pattern. The respondents of this study were obtained by selecting the first respondent with a random start, and then selecting the next respondent after skipping K number of visitors. K was calculated using Equation 2:

$$\underline{K} = \frac{N}{\frac{x}{n}} \dots\dots\dots (2)$$

which can also be expressed as (3)

$$\underline{K} = \frac{N}{nx} \dots\dots\dots (3)$$

where:  $K$  = sampling interval  
 $N$  = population size  
 $n$  = sample size  
 $x$  = average number of members per group

According to the MCME staff in charge of visitor registration, visitors to MMFR come in groups of 5-20 members. The skipping pattern was applied per group, and the value of  $K$  was obtained by dividing the population size used (4,442 visitors) by 13 (average size of a group), and then dividing again by 98 respondents, the sample size. This resulted in a  $K$  value of three (3), which means that one (1) respondent was interviewed for every three (3) groups of visitors. The first group of visitors from which the first respondent would be drawn was determined with a random start and then skipped the next two, and interviewed the fourth representative, and so on. This scheme was used until the target number of respondents per week was reached.

### Questionnaire Design

The survey questionnaire that was used in this study was structured according to the summary guide of Özdemiroglu and Pearce (2002). Figure 5 shows the components of the CV questionnaire of this study:

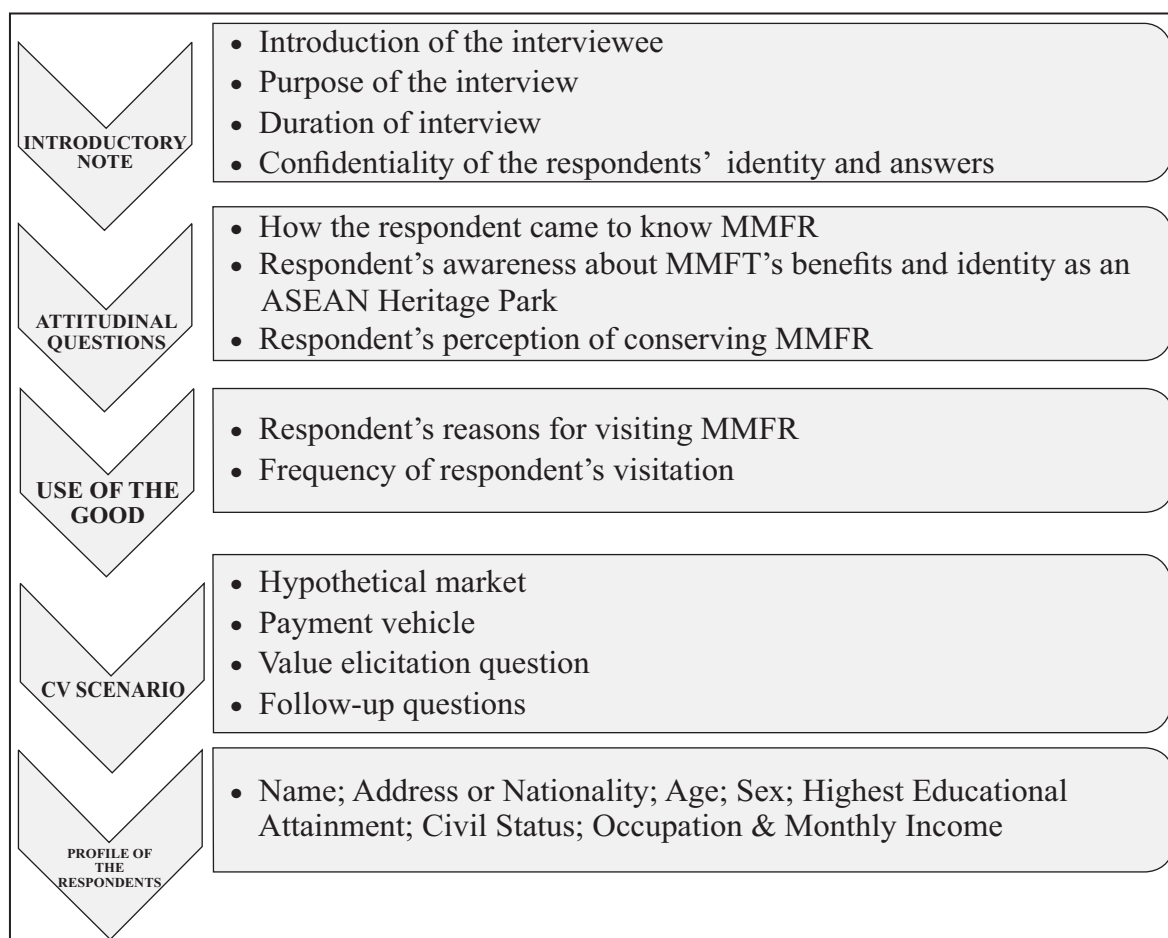


Figure 5 The components of the survey questionnaire

The introductory note in the survey questionnaire was included so that target respondents would be encouraged to participate in the survey and answer truthfully. The attitudinal questions, on the other hand, aimed to get the respondent's knowledge, attitudes, and opinion towards MMFR, which could be considered as factors affecting their WTP. The next part was the use of the good, which aimed to determine purpose of the respondent in visiting MMFR in order to test their familiarity with it and to distinguish users from non-users. The CV scenario was then introduced. The respondent was provided information about the MMFR, its benefits, threats, the management's efforts and plans, among other things. The CV question was then posed as follows:

Suppose a fund will be created where visitors and other stakeholders can contribute for the enhanced conservation of MMFR. This fund will be managed by MCME and will be used for the sole purpose of funding management interventions to ensure the enhanced conservation of MMFR. Considering your income, expenses, and the importance of conserving MMFR, are you willing to pay an additional amount of Php 10/25/50/75/100/200 for the enhanced conservation and maintenance of MMFR that will be added to the current entrance fee of P10/visitor/day?

A hypothetical market for the enhanced conservation of MMFR was created, i.e. a fund where visitors can give their contributions. The payment vehicle in this study was the entrance fee. Visitors' value of MCME's enhanced conservation of MMFR was elicited using the single-bounded dichotomous choice format, by asking the visitor whether or not (yes or no) they would still be willing to pay the entrance fee if it increased by a specified amount. According to Bruner *et al.* (2015), this approach is widely recommended because it addresses potential challenges/biases such as outliers and inaccurate bids to intentionally influence the study results and/or prominence bias. Moreover, according to Özdemiroglu and Pearce (2002), the referendum method:

...is thought to simplify the cognitive task faced by respondents. Respondents have to make a judgement only about a given price, in the same way as they decide whether or not to buy a supermarket goods at a certain price, while at the same time providing incentives for the truthful revelation of preferences under certain circumstances (incentive compatibility). That is, it is in the respondent's strategic interest to accept the bid if his WTP is greater than or equal to the price asked and to reject otherwise, i.e. ensuring that the respondent tells the truth... (p. 52).

Follow-up questions after the elicitation part were asked to determine the reasons why the respondents were not willing to pay if they answered no in the previous part, and also to determine their needs and/or wants to enhance their experience in the park. Lastly, the respondent's profile was asked to test the validity of theoretical relationships on WTP answers. The CV questionnaire used in the study is provided in Appendix A.

### **Pretest, Bid Amounts and Survey Implementation**

Similar to the CV studies that were conducted before, such as the study of the water user fee for households in Metro Manila, Philippines by Calderon, Camacho, Dizon, Rebugio and Tolentino (2004); Catugda's (2015) estimation on the beneficial effects of flood regulation function of forest in relation with the flood problems encountered in the City of Marikina; Kulapalanont's (1994) “Assessment of the Willingness To Pay for Water Quality Improvement in the Lower Chao Phraya River, Bangkok Metropolitan (BMR), Thailand; and Fuente's (n.d.) “Estimation of Household Willingness to Pay (WTP) for Solid Waste Collection and Disposal



Service in Poblacion, Davao City”, the questionnaire was pretested before the implementation of the main CV survey. Based on their studies, pretesting of the survey could serve as a review of the structure and quality of the questionnaire made and to give practice opportunity to the interviewers as well. The bid amounts to be used in the final CV survey were also generated from the pre-tests.

A total of six bid amounts were generated from the pretest. These are Php 10, 25, 50, 75, 100 and 200 that would be added to the current entrance fee of 10Php/visitor/day. These were distributed evenly among 102 survey questionnaires, four (4) questionnaires more than the initial 98 target number of respondents to ensure that the number of respondents per bid level was equal for all bid levels.

### Data Processing and Analysis

Right after the survey proper, the data obtained were tabulated and cross-checked for input errors (Özdemiroglu & Pearce 2002). Statistical instruments were employed in the descriptive analysis and estimation of the mean WTP of the visitors for MMFR enhanced conservation. The variables were first coded as shown in Table 2.

Table 2 Variables and their codes used for the descriptive analysis subjected

Variable	Variable Name	Code
Willingness to pay	WTP	0 = not willing to pay 1 = willing to pay
Age	age	Age in years
Sex	sex	0 = female 1 = male
Highest educational attainment	hieduc	0 = no formal schooling 1 = primary 2 = high school 3 = college or vocational 4 = advanced studies (MS/PhD)
Income	income	Monthly income in pesos
Awareness on the benefits of MMFR	benefits	0 = not aware 1 = aware
Awareness on MMFR's identity as an AHP	ahp	0 = not aware 1 = aware
Number of environmental services MMFR can provide as reason/s for conserving MMFR	es	0-4
Regular visitor	regvisitor	0 = does not visit MMFR per year 1 = visit MMFR per year
First time in MMFR	sttimer	0 = not first timer 1 = first timer
Number of reasons for visit	r4visit	0-11
Bid level	bidlvl	10, 25, 50, 75, 100, 200

### Estimating Mean WTP

Since this study used the dichotomous choice format in eliciting the visitors' WTP for the enhanced conservation of MMFR, the binomial logit model was selected to estimate the mean WTP of the visitors for the enhanced conservation of MMFR. From the WTP study of Calderon *et al.* (2004), Hanemann's formula expresses the willingness to pay for a change in environmental quality as:

$$\log \frac{\Pr(WTP=1)}{1 - \Pr(WTP=1)} = \alpha + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_m X_m + \beta_n A \dots \dots \dots (4)$$

The mean willingness to pay, on the other hand, is computed by:

$$MWTP = - \left[ \frac{\alpha + (\beta_1 \times M_{X_1}) + (\beta_2 \times M_{X_2}) + \dots + (\beta_m \times M_{X_m})}{\beta_n} \right] \dots\dots\dots (5)$$

where: WTP = 1 is equivalent to the “yes” response;  
 $X_1, X_2, \dots, X_m$  are the independent variables;  
 A is the bid amount;  
 $\alpha$  is the constant;  
 $\beta_1, \beta_2, \dots, \beta_m$  are the coefficients of the variables;  
 $\beta_n$  is the coefficient of the bid amount; and  
 $M_{X_1}, M_{X_2}, \dots, M_{X_m}$  are the means of the variables

### Cost-Benefit Analysis

A Cost-Benefit Analysis (CBA) was undertaken to compare the cost of managing MMFR and the potential revenues that could be generated if the WTP of visitors for the enhanced conservation of MFR would be captured. In doing the CBA, there should first be a list of all costs and benefits associated with the decision. In this study, the costs that were considered include the total amount needed for the enhanced conservation of MMFR, based on the “MMFR Management Plan in the context of an AHP for 2015 to 2025” located in the website of the MCME. The benefits, on the other hand, were based on the total revenue the management could generate if the mean WTP will be captured and added to the entrance fee (Php120) and the projected number of visitors. Linear regression using the visitation data from 2014 to 2017 was used to predict the number of visitors from 2018 to 2025.

## RESULTS AND DISCUSSION

### Socioeconomic Characteristics of the Visitors

The age of the respondents ranged from 15 to 61 years old. Many visitors came from the age bracket 20 and below, particularly 18 years old, which constituted 32.35% of the total sample size. This visitor characteristic can be considered positive because it can be inferred that the parks appeals to a wide range of age of Filipinos, and most especially to the youth, the future of this country.

Since MMFR is a mountain, it was hypothesized that there could be more male visitors who would go trekking. But the sample had more females (61 respondents) than males (41 respondents). This could be because MMFR offers many kinds of activities that visitors of any sex or gender can engage in.

Among the 102 respondents, 65% had college education, while 33% completed or reached secondary education. Only 1% of the respondents had no formal schooling or had advanced degrees. These imply that in general, the higher the education, the more a person tends to visit MMFR. Thus, the school can be an effective venue to showcase MMFR for the people to appreciate it more.

The income bracket Php10,000/month and below has the greatest proportion of respondents (69.61%). In fact, 58 in this group of respondents had no occupation and therefore had zero income. This is explained by the two previous factors, age and highest educational attainment, which revealed that most of the visitors are youth.

## General Level of Awareness about MMFR

Findings show that only 72 out of 102 respondents are familiar with the benefits that MMFR provides, while 58 respondents know that MMFR is an AHP. Most of the respondents (50%) said that the reason why they were familiar about the MMFR's benefits was because these were taught in school. Likewise, the awareness on MMFR's declaration as an AHP mainly came from school (34%).

The result on the awareness of these respondents on MMFR's benefits and identity as an AHP further supports the inference that school is a great venue for disseminating information about MMFR.

## Visitors' Willingness to Pay for the Enhanced Conservation of MMFR

### *Respondents who are Willing and Not Willing to Pay*

The following table shows the proportion of the respondents who are willing to pay and those who are not for an additional amount for the enhanced conservation of MMFR that would be added to the current entrance fee of Php10/visitor/day.

Table 3 Distribution of the respondents who are willing and not willing to pay for the enhanced conservation of MMFR

WTP	Frequency	Percentage (%)
Yes	64	63
No	38	37
Total	102	100

More than half of the visitors (63%) were willing to pay an additional amount for the enhanced conservation of MMFR through their entrance fee. On the other hand, 37% of the respondents said they were not willing to pay, and their reasons are shown in Figure 6.

The most common reason given by the visitors was that they could not afford to pay more. This reason can be supported by the study's data on the visitors' monthly income which is Php10,000 and below, and that most of the respondents in that income bracket were students with zero income. According to 21% of the respondents, the government should pay for the protection of MMFR, while another 21% of the respondents said that the amount asked was too high. Furthermore, 16% said that they believed that other visitors could not afford to pay the amount asked and 5% who disagreed with the additional amount and suggested that the increase in the entrance fee should be done gradually or the management should just look for other companies to sponsor the park's enhanced conservation. These reasons usually came from those respondents who were presented the higher bid levels of Php75, Php100, and Php200.

### *Factors Influencing Visitors' WTP*

Table 4 shows the results of the logistic regression. Based on the values of  $P > |z|$ , only one explanatory variable was found to have a significant effect on the willingness to pay of visitors for the enhanced conservation of MMFR, the bid level. Only the assumed negative relationship between the WTP and bid level was found significant. Figure 7 shows the number of visitors who said yes for each bid level. Each bid level (i.e., Php 10, 25, 50, 75, 100 and 200) was presented to 17 respondents.

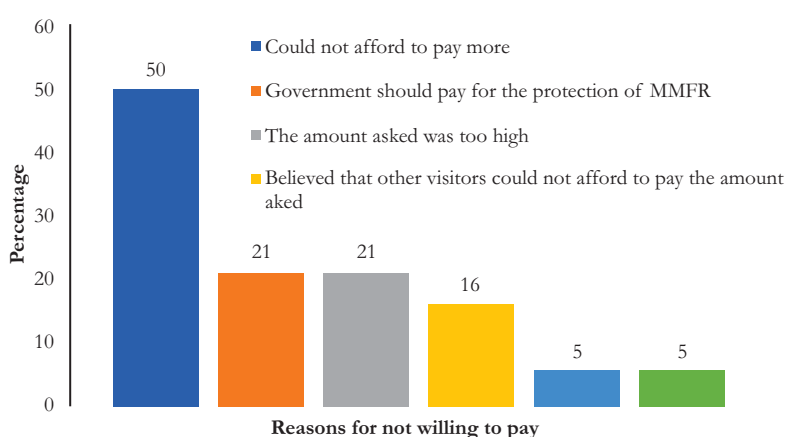


Figure 6 Reasons why respondents are not willing to pay for the enhanced conservation of MMFR

Table 4 Results of the Logistic Regression

Logistic regression					Number of obs = 102	
					LR chi <sup>2</sup> (11) = 29.43	
					Prob > chi <sup>2</sup> = 0.0019	
					Pseudo R <sup>2</sup> = 0.2185	
Log likelihood = -52.63557						
WTP	Coefficient	Standard Error	z	P>z	95% Confidence Level	Interval
Age	-0.03519	0.02468	-1.43	0.154	-0.08357	0.013178
Sex	-0.11125	0.506321	-0.22	0.826	-1.10362	0.881123
Highest educational attainment	0.18147	0.4907	0.37	0.712	-0.78028	1.143225
Income	2.75E-06	1.67E-05	0.16	0.869	-0.00003	3.55E-05
Benefits	-0.44288	0.577367	-0.77	0.443	-1.5745	0.688738
AHP	-0.04377	0.558828	-0.08	0.938	-1.13906	1.051508
Number of environmental services MMFR can provide as reason/s for conserving MMFR	0.180298	0.228915	0.79	0.431	-0.26837	0.628963
Regular visitor	0.790908	0.665504	1.19	0.235	-0.51346	2.095271
First Timer	-0.19932	0.667336	-0.3	0.765	-1.50728	1.108631
Reason for visit	-0.15496	0.233711	-0.66	0.507	-0.61302	0.303105
Bid level	-0.01814	0.004708	-3.85	0	-0.02737	-0.00891
_cons	2.251379	1.54951	1.45	0.146	-0.78561	5.288362

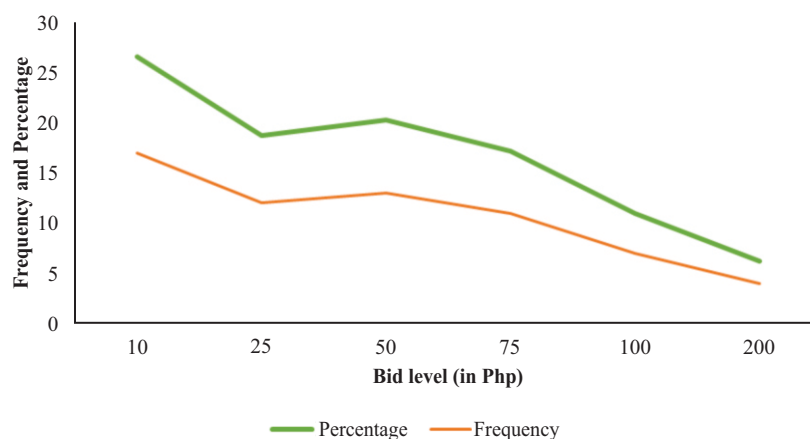


Figure 7 Distribution of respondents who said yes for each bid level

Table 4 also shows the marginal effect, i.e., the effect of change of each independent variable to the change in the dependent variable while holding all other variables constant. It is observed by looking at the coefficients. The predicted probability of visitors responding yes was found to be greater by 18.15% for those with higher educational attainment, 18.03% for those who know more ES, and 79.89% for regular visitors. Results show that those who have higher income are more likely to say yes to the WTP question, but with very small percentage than those who have less. Furthermore, WTP is less by 3.52% for older than younger ones, 11.13% less for males than females, 44.29% and 4.38% less for those who are familiar with MMFR's benefits and identity as an AHP respectively, 19.93% for first timers than those who are not, and 15.50% less for those who had more reasons for visit at the park. Lastly, it was found that the WTP of all visitors are less by 1.81% for higher bid levels.

### ***Estimated Mean WTP of the Visitors***

Through the substitution of the values generated using the logit model in Table 4 to Equation 5, the mean WTP of visitors for the enhanced conservation of the MMFR to be added to the current entrance fee of Php10/visitor/day was estimated at Php109.16 or approximately Php110 resulting in a total of Php120/visitor/day recommended entrance fee.

### **Cost-Benefit Analysis**

#### ***Management Costs of MMFR-AHP***

The Makiling Center for Mountain Ecosystems prepared the MMFR Management Plan in the context of an AHP for 2015 to 2025. The programs, projects and activities require Php131,849,400 for the first year (short term), Php302,381,200 for the first three years (medium term), and Php561,150,000 for the first five years and more (long term). These will cover the execution and completion of eight program themes for MMFR, namely Biodiversity Protection and Law Enforcement; Water Resources and Sustainable Use; Research, Development and Monitoring; Legal Concerns; Livelihood; Ecotourism; Communication, Education and Public Awareness; and Resource Generation (MCME, 2015).

#### ***Projection of Number of Visitors and Potential Revenues***

The potential revenues that may be generated if the visitors' WTP for the enhanced conservation of the MMFR will be captured by adding it to the entrance fee towards the creation of the conservation fund.

The number of visitors for the next five years was predicted through regression ..... (6):

$$Y = 16959.5 + 2797 (X)$$

where: Y = number of visitors per year  
X = time

Table 5 shows the number of visitors in the next seven (7) years (2019-2025) using visitation data from previous years.

Table 5 Projection of number of visitors in MMFR Mariang Makiling Trail within eight (8) years from 2019-2025

Year	Time (X)	Number of Visitors (Y)
2014	1	18,662
2015	2	23,019
2016	3	27,703
2017	4	26,424
2018	5	30,945
2019	6	33,742
2020	7	36,539
2021	8	39,336
2022	9	42,133
2023	10	44,930
2024	11	47,727
2025	12	50,524

The potential revenues from the visitors' entrance and conservation fees were estimated based on the projected number of visitors. Table 6 shows the projected revenue of the MMFR using the current entrance fee and the recommended entrance fee (current plus the mean WTP) from 2019-2025.

Table 6 Comparison of MMFR's projected revenue from 2019-2025 using current and recommended entrance fee

Year	Number of visitors (Y)	Revenue from current entrance fee (Php10)	Revenue from recommended entrance fee (Php120)
2019	33,742	337,420	4,049,040
2020	36,539	365,390	4,384,680
2021	39,336	393,360	4,720,320
2022	42,133	421,330	5,055,960
2023	44,930	449,300	5,391,600
2024	47,727	477,270	5,727,240
2025	50,524	505,240	6,062,880
TOTAL	294,931	2,949,310	35,391,720

If the recommended entrance fee of Php120 will be implemented in the year 2019, considering all the preparations of the management for this change, the MMFR can generate Php4.05 million compared to Php337,420 at the current entrance fee level. The potential revenue will increase with the expected increase in visitation rate and by 2025, the revenue will be Php6.06 million, compared to Php505,240 at the current entrance fee level. The estimated total revenue resulting from the integration of the WTP for enhanced conservation to the entrance fee is

Php35,391,720 from 2019 to 2025, which is about Php32 million more than the total revenue at the current entrance fee level.

However, Php35,391,720 is not sufficient to fund the Php258.76 million required to implement the MMFR Management Plan, assuming that all management interventions have been made in the first three (3) years. This assumes that Php302.38 million has been spent and deducted from Php561.15 million required to fund all conservation activities for 10 years.

### Respondents' Suggestions to Enhance Recreation Experience at MMFR

The last part of the survey asked the respondents to give suggestions of additional features and/or services that could enhance, either directly or indirectly, their experience at the MMFR (Table 7).

Table 7 Suggested features or services to enhance visitors' experience in MMFR

Suggestion	Percentage (%)
Addition of points of interest	36
Security and safety enhancement	33
Addition of amenities	15
Park maintenance	9
Access and registration enhancement	6

Most of the suggestions (36%) were the addition of points of interest such as zip-line, trails, view deck, cable car, catchy signage about the flora and fauna, hanging bridge at Mudspring, allowing pets, rent-a-bike program, and allowing biking at Peak 2. Visitors also suggested assignment of tour guides, monitors, ushers and medics, and improvement of arrows and signage for the enhancement of security and safety at the park. Additional amenities were also mentioned such as cleaner portalets, drinking stations, food stalls, pet area at Station 1, and dry-bags for garbage. Visitors provided ideas for the maintenance of the park like clean-up drives, tree planting, removal of the concrete cooking area at the camp sites, and complete restriction of vehicles inside the park. There were also suggestions regarding the access and registration like issuance of Camper's ID and increase of the entrance fee. The MCME may consider these suggestions to enhance the experience of visitors to MMFR, which may result in an increase in the visitation rate and consequently increase the revenue from the entrance fee that may be used in the enhanced conservation of the park.

## CONCLUSION

### Summary and Conclusion

A contingent valuation (CV) study involving 102 respondents was conducted to estimate the value of the enhanced conservation of the Mount Makiling Forest Reserve-ASEAN Heritage Park (MMFR) to its visitors.

The results of the study indicate that 100% of the respondents were familiar with MMFR with which 71 respondents know about the benefits given by MMFR while 58 respondents know about its identity as an ASEAN Heritage Park (AHP). This indicated that the general level of awareness of the visitors about MMFR, specifically their deep understanding of its importance for its benefits and identity as an AHP, is not that high.

More than half (63%) expressed willingness to pay for the enhanced conservation of MMFR, with a mean willingness to pay estimated to be Php109.16, or approximately Php110, in

addition to the current entrance fee of Php10/visitor/day. This means that the maximum entrance fee that can be collected from the MMFR visitors to continually visit the park is Php120/visitor/day. Only the bid level was found to be a significant factor influencing visitors' WTP.

The cost-benefit analysis revealed that the estimated total revenue resulting from the integration of the WTP for enhanced conservation to the entrance fee (recommended entrance fee) of Php35,391,720 from 2019 to 2025 is about Php32 million more than the total revenue at the current entrance fee level. However, this is not sufficient to fund the amount of Php258.76 million required to implement the MMFR Management Plan until 2025. Therefore, management should find other funding sources to fully finance the implementation of MMFR's management plan in the context of being an ASEAN Heritage Park.

Lastly, the respondents offered suggestions to enhance the recreation experience in MMFR, with addition of points of interest like zip-line and trails, improvement of signage for security and safety, and enhancement of amenities like cleaner portalets leading the list.

## Recommendations

Based on the results and conclusions, the following recommendations are offered:

1. Information, education and communication (IEC) about the Mount Makiling Forest Reserve (MMFR), its importance and benefits to the ecosystem, and identity as an ASEAN Heritage Park (AHP) should be heightened to enhance people's appreciation about the value of the park, forests, and nature. The IEC activities may be undertaken in schools, as these were found to be the main source of information of the respondents about the importance of MMFR. Other venues for EIC that were found from the results are the internet and social media.
2. The MCME may consider increasing the entrance fee to MMFR through the Mariang Makiling Trail to capture visitors' WTP for the enhanced conservation of MMFR. The conservation fund can finance the conservation and development activities needed to sustainably manage MMFR. The mean WTP derived in this study can be used as one of the bases for the increase in entrance fee. It was found in the study that the WTP of the respondents was less for a higher bid level. Therefore, a gradual increase in the entrance fee may be considered.
3. The potential revenue that can be generated from the entrance and conservation fees of the visitors is not enough to fund the management interventions under the MMFR Management Plan until 2025. Thus, the MCME should seek other fund sources to finance the management plan in the context of AHP.
4. The CV survey was done for only a month and was not able to cover other times or seasons of the year. A longer period of CV survey may be conducted by future studies.
5. The MCME may consider the suggestions offered by respondents to improve visitor services, such as additional points of interest, security and safety signage, and improved amenities.
6. Studies using revealed preference methods, such as Travel Cost Method, may also be conducted to analyze the demand for the recreation service that MMFR provides.

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## EXPLORATION OF ENDOPHYTIC BACTERIA FROM MANGROVE IN JAVA AND THE BIOCONTROL ACTIVITY AGAINST FUNGAL PATHOGEN *Phytophthora colocasiae*

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### ABSTRACT

Mangrove forest plays an important role as a buffer area of terrestrial and marine ecosystems, reduces abrasion by sea water, provides food and nutrients for several species of marine animals, and becomes source of microbes, including endophytic bacteria. The current decline in mangrove land area greatly affects the habitat of endophytic microbes that live in these plants. The objective of this research was to study the diversity and potentiality of endophytic bacteria from 2 genera of mangroves, *Avicennia* sp. and *Rhizophora* sp. as biocontrol agent and plant growth promoters. Samples of plant were taken from four different coastal areas of mangrove namely Jakarta, Indramayu-West Java, Yogyakarta, and Banyuwangi-East Java. Bacterial endophytes were isolated from the aerial roots of plant by surface sterilization method using sodium hypochlorite and alcohol, to be further cultured on three different types of growing media, Tryptic Soy Agar (TSA), Nutrient Agar (NA), Kings 'B (KB) medium. The results showed that more than 769 isolates of endophytic bacteria were obtained from four areas of mangrove plants. A total of 542 endophytic bacterial isolates (70% of 769 isolates) showed negative reaction after hemolysis test and 403 bacterial isolates (74% of 542 isolates) showed negative reaction after hypersensitive test. After screening test, a total of 13 selected isolates of endophytic bacteria were identified for their potentiality as biocontrol agents against plant pathogenic fungi *Phytophthora colocasiae* under in vitro test.

**Keywords:** Antibiosis, biodiversity, endophytic bacteria, mangrove

### INTRODUCTION

Indonesia is one of the eight mega biodiversity countries in the world with millions of species of plants, animals and microbes of various ecosystems. One of Indonesia's biodiversity is coastal ecosystems, especially mangrove forests. Indonesia has 27% of the world's total mangrove forest, equivalent to 4.25 million ha, while *Avicennia* and *Rhizophora* are the most common genera. Mangrove forest plays an important role as a buffer area of terrestrial and marine ecosystems, reduces abrasion by sea water, provides food and nutrients for several species of marine animals, and becomes source of microbes, including endophytic bacteria. Endophytic bacteria are microbes living in plant tissues with various roles. The role of endophytic bacteria includes plant growth promotion as part of its ability to synthesize and mobilize phosphates, producing growth hormones and enzymes. In addition, endophytic bacteria also play a role in supporting plant health as a biological control agent (Hallmann *et al.* 1997; Munif *et al.* 2012). Endophytic bacteria act as biological control agents by producing antimicrobial compounds that are antagonistic to pathogens, space and nutrition competition, micronutrient competitions such as iron, and producing siderophores, and causing host plants to be resistant to pathogen (Munif *et al.* 2013). In addition, some endophytic bacteria also produce antibiotic compounds such as phenazines,

pyrolnitrin, pycocyanin, and phloroglucianol and extracellular enzymes and pseudomonic acid. Previous research showed that endophytic bacteria originated from mangrove plants in India exhibited antibiotic activity, produced pectin enzymes, proteases, chitinases, lipases, exhibited nitrogen fixing capabilities, played a role as phosphate providers and auxin hormone producers (Gayathri & Saravanan 2010; Gayathri & Muralikrishnan 2014).

The objective of this research was to study the diversity and the potentiality of endophytic bacteria from 2 genera of mangroves, *Avicennia* sp. and *Rhizophora* sp. as biocontrol agent and plant growth promoters.

## MATERIALS AND METHODS

### Isolation of Endophytic Bacteria

Isolation of endophytic bacteria from mangrove plants was done using sterilization method. Two species of mangrove, *Avicennia* sp. and *Rhizophora* sp were taken from four areas: 1) Conservation center area at Karongsongan Indramayu, West Java, 2) Forest Mangrove Baros, Bantul Yogyakarta, 3) Taman Wisata Alam Pantai Indah kapuk, Jakarta, and 4) Taman Mangrove Bedul, Banyuwangi. The most dominant mangrove vegetation represents the best ability of the vegetation to survive among the mangrove species. Endophytic bacteria were isolated by following the method presented by Hallmann *et al.* (1997). The first step was sterilizing the plant roots. Plant roots was cut in small pieces of 1-2 cm and sterilized by dipping the roots in a solution of 1% natrium hypochlorit for 2 minutes, followed by 70% alcohol for 2 minutes. The roots were then rinsed with water for 3 times and then macerated and planted on media 20% Tryptic Soya Agar (TSA) and King's B medium.

### Hypersensitivity Test

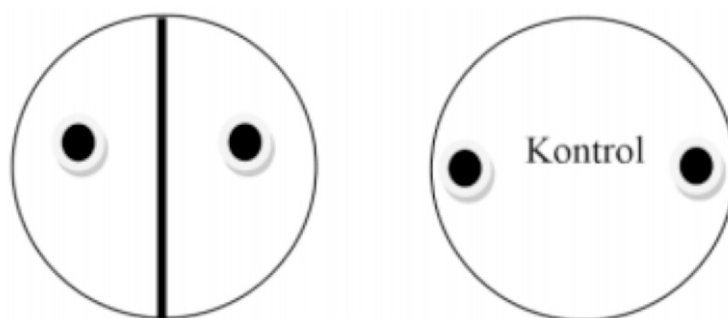
Hypersensitivity test was done using tobacco plants with rejuvenation of endophytic bacteria in 5 mL 100% TSB media for over 48 hours. An additional 2 mL of suspension was injected in tobacco plants. The test result is positive, if patches of necrosis occurs after 24-48 hours, and is negative if the patches of necrosis does not occur.

### Hemolysis Test

Hemolysis test was done by scraping bacteria on blood agar media consisting of 100% TSA coupled with the blood of goats with a concentration of 5%. If the bacteria can grow to form a clear zone, this means that the bacteria can produce alpha-toxin. If the bacteria form dark zones, this means that the bacteria produce beta-toxin, which is harmful to human and the test will not be continued.

### Antibiosis Test

Antibiosis test was done using “Dual Culture Method” on Potato Dextrose Agar (PDA) medium. This test aims to determine the potential of endophytic bacteria isolates as biological control against leaf blight pathogen *Phytophthora colocasiae*. Tests were done by growing the fungus *P. colocasiae* in conjunction with endophytic bacteria on PDA. Endophytic bacteria were grown at the center of a petri dish, and then the fungus was grown in ¼ part of the petri dish. This test was done in two replications. After 5 days of growth diameter measurement, the fungal hyphae development was leading toward the opposite direction of the bacteria. Illustration of dual culture test method is depicted as follows:



- █ = Endophytic bacteria  
● = Culture of pathogenic fungi *P. colocasiae*

The measurement results were then calculated to determine the inhibition percentage using the formula:

$$P = \frac{R1 - R2}{R1} \times 100\%$$

Description:

P = inhibition percentage

R1 = average diameter of pathogenic fungi colonies in the control treatment

R2 = average diameter of fungal pathogens in endophytic treatment

### Plant Growth Promoter Test

Plant growth promoter activity of endophytic bacteria was tested by growing bacterial isolate on 100% TSB medium. The isolate was then incubated for 48 hours. Rice seeds were surface-sterilized and dipped into the bacterial suspension and incubated for 24 hours. The rice seeds were grown in sterile soil. Pathogenic symptoms observed on the rice seeds were necrotizing pathogens, chlorosis, curly, rot, wilt, fall. Isolates showing these symptoms will not be proceeded to the next test (Vrbničanin *et al.* 2011).

## RESULTS AND DISCUSSION

The results showed that more than 769 isolates of endophytic bacteria were obtained from four areas of mangrove plants. A total of 163 isolates of endophytic bacteria were obtained from Karangsong, Indramayu, 229 isolates of endophytic bacteria from Baros Bantul, Yogyakarta, 197 isolates of endophytic bacteria were obtained from the Nature Park Angke Kapuk, Jakarta and 182 isolates of bacteria were obtained from the National Park Alas Purwo, Bayuwangi (Table 1). The population in colony forming unit (CFU) of endophytic bacteria on medium varied. The highest population of endophytic bacterial colonies was found in mangrove plant from Yogyakarta with total colonies of  $1.8 \times 10^5$  CFU/g, while the lowest population of bacterial colony was found in mangrove plants from Banyuwangi with total colony of  $2.6 \times 10^3$  CFU/g.

Table 1 Total isolates of bacterial endophyte isolated from *Avicennia* sp and *Rhizophora* sp originated from different mangrove forests and the results after hypersensitive and hemolysis test

Origin of Mangrove Forest	Number of Endophytic Bacterial Isolates	Number of Isolates with Negative Hemolysis Test	Number of Isolates with Negative Hypersensitive Test
Indramayu	163	118 (72.4%)	60 (50.8%)
Yogyakarta	229	170 (74.2%)	132 (77.6%)
Jakarta	195	130 (66.7%)	103 (79.2%)
Banyuwangi	182	124 (68.1%)	108 (87.1%)
TOTAL	769	542 (70.5%)	403 (74.4%)

A total of 769 mangrove endophytic bacterial isolates were obtained from four sampling regions, namely 168 isolates obtained from Karangsong, Indramayu, 229 isolates from Baros Bantul, Yogyakarta, 195 isolates obtained from the Angke Nature Park, Jakarta and 182 isolates from the Alas Purwo Bedul National Park, Banyuwangi. Morphologically, bacterial isolates obtained consisted of irregular edges (regular), mucoid and slimy colonies. On the other hand, the color of the colonies obtained were white, yellow, orange, red and transparent. A total of 542 endophytic bacterial isolates (70% of 769 isolates) showed a negative reaction after hemolysis test and 403 bacterial isolates (74% of 542 isolates) showed a negative reaction after hypersensitive test.

After screening test, a total of 24 selected isolates were identified for their potential antibiosis activities as biocontrol agent against *P. colocasiae* under in vitro test and plant growth promoter activity. The results showed that 13 isolates were able to have antibiosis against *P. colocasiae*. All endophytic bacteria from mangrove were able to promote and to increase plant growth of rice (Table 2).

Table 2 Antibiosis activity of selected endophytic bacteria from mangrove forest against fungal pathogen *Phytophthora colocasiae* on media PDA in vitro

Isolate of endophytic bacteria	Antibiosis activity (%) against <i>Phytophthora colocasiae</i>	Plant growth promoter activity to rice (cm)
BA2N2-4	26.7 de	31.7 ab
BA2T2-2	41.2 c	29.9 ab
BR1K5-4	41.2 c	29.1 ab
KAT5-1	19.2 ef	29.9 ab
YA1K2-5	24.7 de	31.8 ab
YA1K5-7	52.6 b	32.8 ab
YA1N2-7	18.8 ef	33.8 a
YA1T2-6	58.3 ab	30.3 ab
YA2K2-4	11.8 fg	30.0 ab
YA2K2-5	31.3 d	32.3 ab
YR1K5-1	33.3 cd	30.8 ab
YR1K5-3	33.3 cd	31.2 ab
YR1T2-9	65.6 a	33.7 a
BA2T5-12	0 h	30.9 ab
BA2T5-7	0 h	28.6 ab
BR1T2-4	0 h	32.6 ab
KAK5-21	0 h	31.5 ab
KAN5-1	0 h	32.2 ab
KAN5-19	0 h	30.6 ab
YA1K2-3	0 h	31.7 ab
YR1N2-3	0 h	32.6 ab
YR2K2-3	0 h	32.6 ab
YR2K2-4	0 h	29.8 ab
YR2N5-1	0 h	29.7 ab
Kontrol	0h	25 c

Bacteria can serve as growth promoter to a plant. Endophytic bacteria can also produce compound growth that can be used by plants in the form of 1-aminocyclopropane-1-carboxylate (ACC), hormones IAA, siderophore, solubility of phosphate, antibiotic and ammonia (Ali 2013). Sgroy *et al.* (2009) reported that endophytic bacteria isolated from plant *Prosopis strombulifera* was able to produce phytohormones and some siderophore. In addition, Eliza (2004) reported that the endophytic bacteria which was isolated from the roots of corn are able to support the growth of cucumber and banana plants. Physiologically, chemical compounds that can be produced by endophytic bacteria include growth hormones, extracellular enzymes, cyanide, solvents phosphate and fluorescence activity (Munif 2012). Some of endophytic bacteria are also known to be able to bind nitrogen nutrients and to dissolve phosphate, and thus, reducing the use of synthetic fertilizers (Pedraza *et al.* 2004).

## CONCLUSION

Endophytic bacteria isolated from mangrove have potentiality as biological control agent against plant pathogenic fungi *P. colocasiae* and are able to increase plant growth.

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## FORMULATION OF RURAL ECOSYSTEM HEALTH INDEX IN JAVA ISLAND AS A BASIS FOR NATIONAL STANDARDS

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### ABSTRACT

Development in various sectors in rural areas influenced the diversity of ecosystems. In Indonesia, there was no guidance for assessing rural ecosystem health, and therefore, quantitative analysis is required. This study aimed to formulate quantitative system and to calculate *Rural Ecosystem Health Index* (REHI) in Java Island. This study chose five regencies in Java Island (Malang, Madiun, Bantul, Kulonprogo, and Sleman) as the case study with six parameters observed, i.e. air pollution, water pollution, forest/vegetation coverage, biodiversity, public health, and environmental health. The study was conducted from February until July 2018. The study method used Microsoft Excel on computer to input data from local environmental management files. Results of this study showed that REHI was 69.2 and it meant that the rural ecosystem in Java Island was at a relatively healthy level and regions with climates D and E based on Schmidt-Ferguson had scores that were not significantly different. Quantitative analysis was conducted on 6 indicators by weighing each indicator with 16.7%.

**Keywords:** Ecosystem health index, environment, Java Island, pollution, rural



## UNLOCKING BOTANIC GARDENS TO UNDERPIN THE GREEN ECONOMY OF INDONESIA: A MULTI-DIMENSIONAL APPROACH

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### ABSTRACT

The lack of public awareness on the importance of biodiversity to human well-being is the biggest barrier to achieving the three main objectives of Convention on Biological Diversity (CBD). Indonesia has at least 32 botanical gardens, which are very potential to be leveraged for optimum sustainable management of biodiversity. We propose the implementation of five interconnected functions of botanic gardens i.e. conservation, education, bioeconomy, ecotourism, and research. We recommend the expansion of botanic gardens conservation to community-owned land, such as yard, forest, or farm (*Kalivu* system). Conservation by seed banking has a direct social impact by making the seeds easily available for local people to use them as food and medicine as well as commercialization. Capacity building workshop is an ideal education method to equip local communities with tools and skills required to monitor, manage, and profit from their natural capital. There is a promising opportunity to combine technological inputs with local wisdom to empower the public for taking practical steps towards developing their local industry, with activities centered in or around botanic gardens, such as in the nearby local villages. Ecotourism industry could potentially provide a very promising income-earning opportunities to local communities which will lead to the functioning of green economy. All biodiversity management strategies should be based on research to achieve continuous improvement following environmental and social dynamics. Together, these integrative natural capital research and development programs pave the way for unlocking the region's socio-economically important plants to boost the green economy of the poorest rural communities.

**Keywords:** Biodiversity, bioeconomy, botanic gardens, conservation, ecotourism, Indonesia

### INTRODUCTION

The lack of public awareness on the importance of biodiversity to human well-being is the biggest barrier to achieving the three main objectives of Convention on Biological Diversity (CBD). According to a review by Natural History Museum (2015), the public feel that they do not know a lot about biodiversity and even though they are aware that biodiversity is under threat, they do not fully understand what the implications of biodiversity loss are, and do not really give the loss of biodiversity much thought. Botanic Gardens Conservation International (BGCI) considers botanic gardens as the significant contributor to the implementation of the CBD. Indonesia has at least 32 botanical gardens, which are very potential to be leveraged for optimum sustainable management of biodiversity.

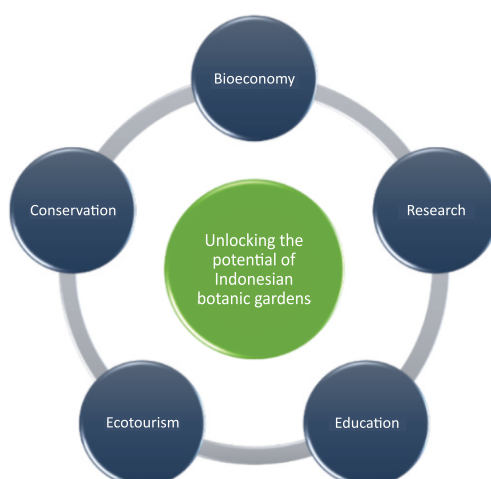


Figure1 The multi-dimensional approach towards Indonesia green economy and sustainable management of biodiversity (own figure)

## MATERIALS AND METHODS

This paper is a synthesis of idea and research agenda based on justified observations and reviews of the published article.

## RESULTS AND DISCUSSION

We propose the implementation of five interconnected functions of botanic gardens; conservation, education, bioeconomy, ecotourism, and research (Fig. 1).

### Conservation

We recommend the expansion of botanic gardens conservation to community-owned land, such as yard, forest, or farm. This system is also known as *Kalim*, which is based on the harmony and sustainability between utilization and conservation of biodiversity by local community. Community participation will also enhance the production of economically valuable plants to meet industrial demand (Njurumana *et al.* 2014). Conservation by seed banking method is also an attractive approach due to its direct social impact by making the seeds easily available for local people to use them as food and medicine as well as commercialization.

### Education

Capacity building workshop is an ideal education method to equip local communities with tools and skills required to monitor, manage, and profit from their natural capital. Given the unique rich culture of Indonesia, educating the public about biodiversity should use strategies customized to specific groups and contexts (Ham & Kelsey 1998). In this diversity-based education scheme, the message about biodiversity should not overwhelm the public with a sense of despair towards environmental issues, but rather it should try to emphasize the links between other species, habitats, and human needs; highlight responsibilities and opportunities to help; use specific facts through a language that speaks to the audience; and try to make biodiversity real by drawing attention to local issues that affect people personally (The Biodiversity Project 1999).

## Bioeconomy

Many environmental issues do not rank as a priority for people and may be easily undermined by the other concerns, especially economic needs, thus making it difficult to elicit public support (Coffin & Elder 2005). This issue is particularly a concern because many biodiversity hotspots, including those in Indonesia, are located in areas where the local communities are characterized by low human development and high poverty levels.

It is estimated that 40 million Indonesians living in rural areas rely on biodiversity for their subsistence needs. Indonesia has about 370 ethnic groups (Persoon & van Weerd 2006) associated with a long tradition of local knowledge systems of sustainable biodiversity utilization and nature conservation (BAPPENAS 2003). Therefore, there is a promising opportunity to combine technological inputs with local wisdom to empower the public for taking practical steps towards developing their local industry, with activities centered in or around botanic gardens, such as in the nearby local villages.

Some examples of Indonesian local industry that could be developed include foods, medicinal products, raw materials (biofertilizer, bioremediation, biopesticide, biomass for energy, timber, colorant, aromatic oils, cosmetics), ornamental products (orchid, aglonema), and local merchandise (fashion products, clutter, home furnitures, ornaments, seeds, woven fabric such as *kain tenun* Sumba). Local industry based on agrotourism is another potential approach to generate income while promoting local farms and nurseries.

However, given this big opportunity of biodiversity-based local industry, Indonesia has not developed a dedicated bioeconomy policy strategy yet. Thus, we suggest that this is one of the key policies Indonesian government has to put focus on.

## Ecotourism

Many of the stakeholders are unaware of how to derive economic benefits from ecotourism; unless local capacity is developed, there is a risk that only large businesses will benefit. In this sense, ecotourism industry could potentially provide a very promising income-earning opportunities to local communities which will lead to the functioning of green economy.

To enrich visitors experience in botanic gardens, we suggest the use of the public portal “Biodiversity Information System” for serving visitors of the botanic gardens. This interactive portal should ideally include digitalized plant information, such as species, conservation status, role in ecosystem services, economic value, traditional uses, cultivation method, habitat, and be linked with relevant research articles online and PROSEA (Plant Resources of South-East Asia).

## Research

All biodiversity management strategies should be based on research to achieve continuous improvement following environmental and social dynamics. Based on data gathered by Rintelen *et al.* (2017) Indonesia only ranks 6<sup>th</sup> among ASEAN countries in terms of the percentage of local authorship to the total number of publications per country. We suggest some essential research need to be undertaken are documenting the diversity and distribution of plant diversity and building an inventory of the region's natural capital; assessing the resilience of biodiversity to climatic and anthropogenic changes, such as mining and infrastructure development; and evaluating and developing the potential of natural capital to underpin its green economy.

Together, these integrative natural capital research and development programs pave the way for unlocking the region's socio-economically important plants to boost the green economy of the poorest rural communities.

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## 9. Synthesis

### About the Conference

The conference generally aimed to assess successes, opportunities, and gaps in current practices, research efforts and policies associated with the conservation, enhancement and sustainable use of flora and fauna in Southeast Asia.

Specifically, it was expected to generate consensus among participants to formulate practical and sustainable ways, based on current policies and research results, to strengthen participation and contribution of stakeholders in eliminating current problems and, at the same time, enhancing the conservation and sustainable use of the region's biodiversity and natural resources.

The expected outputs of this conference were to gain the consensus among the participants on an agenda for future research and advocacy actions on the conservation, enhancement and sustainable use of indigenous flora and fauna in Southeast Asia to support the effective implementation of existing national policies.

The conference covered 4 subthemes, namely: (1) Diversity and Resiliency of Indigenous Tropical Flora and Fauna and Their Ecosystems; (2) Approaches, Technologies, and Innovations in Conservation, Enhancement and Sustainable Use of Indigenous Tropical Flora and Fauna; (3) Socio-economic, Cultural, and Ethical Aspects in Conservation, Enhancement and Sustainable Use of Indigenous Flora and Fauna; and (4) Policies and Other Legal Frameworks in Conservation and Sustainable Use of Indigenous Flora and Fauna.

### Core Issue

Common core issue emerged in the conference was the loss of biodiversity in general, and specifically addressed the depletion and loss of native species of flora and fauna caused by human collective actions which led to habitat loss and destruction and/or direct pressure to populations.

Challenges emerged, among others were: (1) Climate Change; (2) Introduction of invasive alien species; (3) Limited knowledge on indigenous species pool; (4) Expansion of global market on natural resource-based products, including biotechnological based products; (5) Zero loss of natural habitat, both terrestrial and marine; (6) Discovery of new products, including medicine, food and fibre.

### State of the Art

Most of research results presented in the conference provided baseline information on many different species or species group or taxa and some also focused on ecosystems and current practices of conservation, ecosystem restoration and “sustainable” utilization, including local community as part of their customary wisdom. Only small part of research results concerned on management actions, innovation and best practices.

Governing the commons is a difficult task. Although conservation policy refined by time in all South Asia's Countries, accelerated depletion of the populations of native species of flora and fauna remain the core issue in the 21<sup>st</sup> century.

Biodiversity governance is a complex issue that requires strong political will and action, best fit policies and law enforcement. Figure 1 provides the example of complex issue in biodiversity governance in Indonesia.

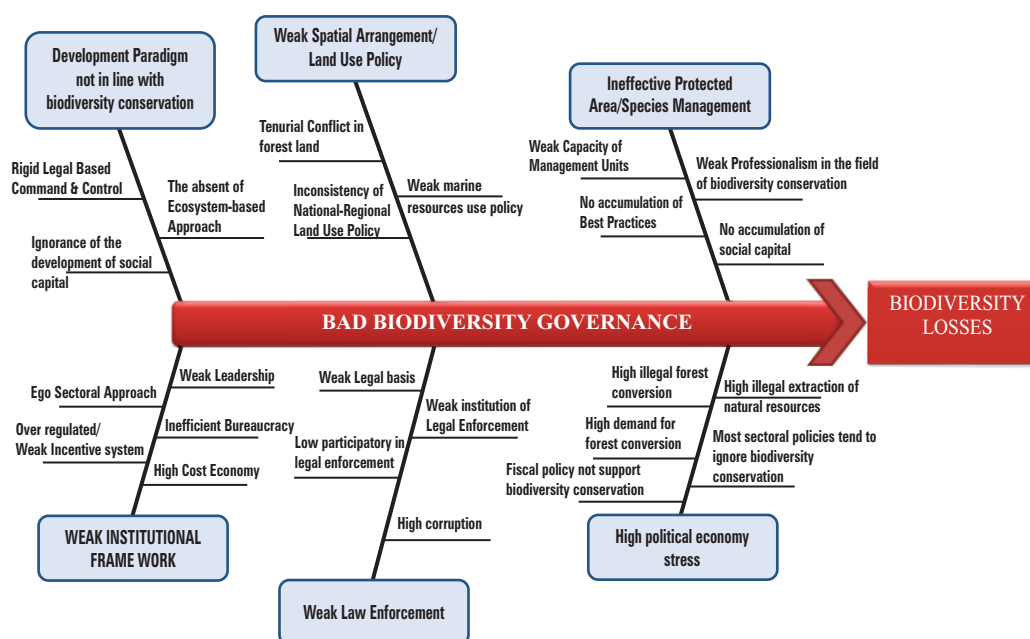


Figure 1 Example of Indonesian Context of Biodiversity Governance – Facing with Complexity

At present all native species of flora and fauna depend on all remaining natural habitat/ecosystem in the tropic, either in conservation and other protected areas and other land use, managed by many government institutions, private entities and local community, including but not limited, to adat community. Many of them are waiting for researchers to conduct baseline research before being decided as endangered species in the near future. Our old biodiversity strategy to “save-study-use” is still relevant to be kept in mind.

Intensive management of sanctuary and ex situ conservation strategy are important to save critically endangered species and enhance economic value of all native species of flora and fauna through green business market.

Although all research results presented here have significant value to conduct a right action in specific context, it seems that biological and/or ecological approach solely will not enough to cope with underlying causes of the depletion of native species of flora and fauna. Action research based on transdisciplinary approach to promote coexistence between human and native flora/fauna at a landscape scale is urgently required.

### Paradigm Shift

Coping with the underlying causes of native species loss of flora and fauna needs paradigm shift towards ecosystem-based approach as mandated by Convention on Biological Diversity (1990). Ecosystem-based approach basically explores ecosystem as a field of inquiry, bridging the two different points of view: natural science approach, which tends to look ecosystem as an empirical world and social science approach, in which human society look at ecosystem as their living space to share philosophy, value and knowledge.

Following the paradigm shift mentioned above, we need to change our mindset and value as introduced by Fritjof Capra (1996) as a system view of life (web of life) (Table 2).

Table 2 Illustration of mindset and value change as a system view of life

MINDSET CHANGE (From-To)		VALUE CHANGE (From-To)	
Assertive	Integrative	Self Existence	Integrative
Rational	Intuitive	Expansion	Conservation
Analytical	Synthesis	Competition	Cooperation
Reductionism	Holistics	Quantitative	Qualitative
Linear	Non Linear	Dominancy	Partnership

## Future Agenda

There are some future agenda based on the conference discussion, as follows:

1. Continue biological and ecological baseline research that cover all top priority species, species group and ecosystems. Focus on genetic research will be very valuable to save critically endangered species in the near future.
2. Develop innovation and best practices to conserve and enhance sustainable use and value of socio-economic important native species of flora and fauna, including but not limited to, biotechnology-based products. One extra ordinary example of human success story: chicken (we still have wild relative species in many natural habitats, but we use mostly from ex situ propagation system).
3. Promote and actively be involved in action research (transdisciplinary approach) to prevent massive loss or depletion of native species of flora and fauna in the remaining important and valuable natural ecosystem at a landscape scale.
4. Promote natural ecosystem restoration involving green market.
5. Promote socially inclusive approach on species conservation and sustainable use of native species of flora and fauna, especially where important natural habitat still exists. Human-wildlife coexistence at a landscape scale needs to be realized in the future.
6. Strengthen site level management of conservation/protected area and remaining natural ecosystem in other land uses based on ecosystem approach principles. In this context, adaptive collaborative management is important concept need to be adopted.
7. Develop science and technology on multi products management of tropical ecosystems.
8. Develop global partnership on Wildlife Trading Watch.
9. Develop knowledge management and information system based on species, species group and taxa and or site level management system.

## 10. Closing Remarks

Dr Jesus Corpus Fernandez  
SEAMEO BIOTROP Deputy Director for Program

Honorable members of SEAMEO BIOTROP Governing Board  
Distinguished speakers and moderators  
Generous Partner-Institutions  
Dear Participants  
Ladies and Gentlemen

Good afternoon!

Our 3<sup>rd</sup> International Conference on Tropical Biology is now about to end! One and a half days may not be enough to fully discuss the many facets of conserving, enhancing, and using sustainably our indigenous flora and fauna in our region. However, I believe that you would all agree that we had interesting and insightful discussions during the plenary and parallel sessions we had yesterday and this morning.

We, at SEAMEO BIOTROP, would not like to end our conferences with just a closing program like this. And that after all of you have gone back to your respective countries and institutions, nothing follows. On the other hand, we always like to explore the possibilities of collaboration with our participants' institutions in relation to the theme of our conference and also with our Centre's program thrusts.

Last night, you have witnessed the signing of Memorandum of Understanding with the Universitas Islam Indonesia and the extension of our collaboration with the Pampanga State Agricultural University in the Philippines. We would also like to extend the invitation to all universities and research institutions represented in this conference to establish collaboration with us specifically in conducting joint research projects towards making our biodiversity conservation efforts more relevant and effective. As BIOTROP's Deputy Director for Program, I would personally look into all the papers presented during the conference and see what follow up actions we can do together.

Ladies and gentlemen,

You have also witnessed last night the launching of two books. We thank Prof Renato Reyes from Central Luzon State University in the Philippines and Dr Sutomo from the Bali Botanical Garden in Indonesia for publishing their books with us. These books will not grace the libraries of our partner-institutions in the region for students' use. We certainly welcome other more researchers to write books and co-publish them with us.

With the interesting papers that were presented in our conference, we encourage the authors to finalize their full papers and submit them to our journal, BIOTROPIA, which we are proud to say that it has been Scopus-indexed since 2012 and also accredited by the Indonesian Ministry of Science and Technology since 2015. We will also be publishing the proceedings of the conference to include all extended abstracts of papers and posters.



The synthesis and the comments you all gave on the conference will surely serve as inputs for the planning of our next conference in 2020. We hope that you will all participate again.

Ladies and gentlemen,

On behalf of our Director and the rest of SEAMEO BIOTROP family, we thank you for contributing to the success of this conference. We always believe that the success of any learning activities like this depends much on the active involvements of the participants. Everyone of you deserves a big applause. Our heartfelt thanks also go to all our partner-institutions as well as to all the speakers and session moderators for making this conference possible.

Before I officially close this conference, allow me to recognize the people who have worked hard in organizing and implementing this Conference. As I call the conference coordinator, co-coordinator and all the conference committee members to come up on stage, let us show our appreciation to all of them by giving them a big round of applause.

I encourage all of us to keep communicating with each other even after this conference. I wish everyone a safe journey back to your respective places of abode.

Now, on behalf of SEAMEO BIOTROP Director, I now declare SEAMEO BIOTROP's 3<sup>rd</sup> International Conference on Tropical Biology officially closed.

Thank you.

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