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

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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 Plasms, 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

Philippnes, 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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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 3rd 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 3rd 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 knoweldge, 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 currents 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 Plasms, 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 appeciation 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 warahmatulaahi wabarakaatuh.

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 form 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 natrium 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 form 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 spesies 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 hypoclorit 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

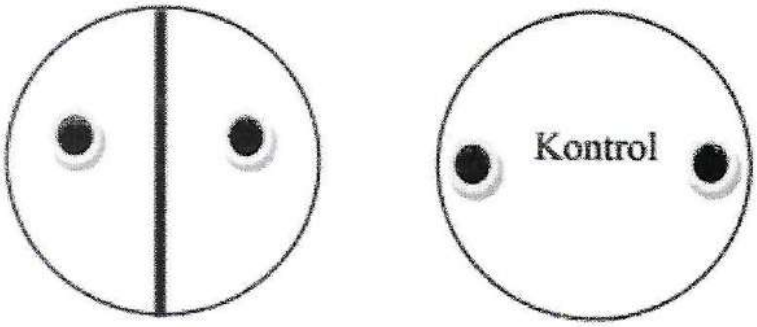
Hypersensitivity test was done using tobacco plants with rejuvenation of endophytic bacteria in 5 mL 100% TSB media for over 48 hours. An additional 2 mL of suspension was injected in tobacco plants. The test result is positive, if patches of necrosis occurs after 24-48 hours, and is negative if the patches of necroseis 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 form 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 1.8×10^5 CFU/g, while the lowest population of bacterial colony was found in mangrove plants from Banyuwangi with total colony of 2.6×10^3 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 *Phytophthora colocasiae*	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 1aminocyclopropane-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 sidorophore. 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 sinthetic 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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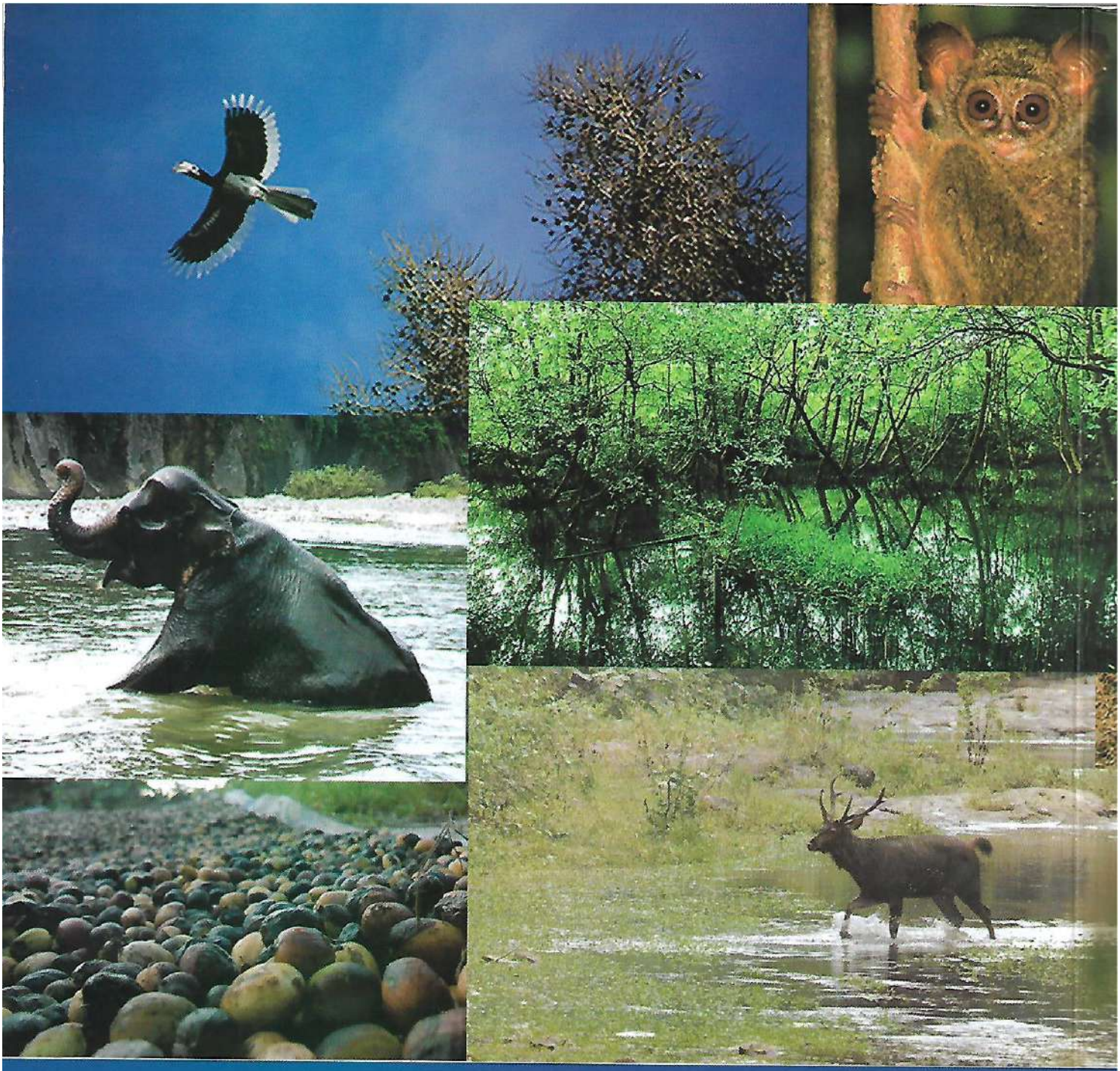

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