Republic of the Philippines
**Department of Environment and Natural Resources**
Visayas Avenue, Diliman, Quezon City, 1128
Tel. Nos. (632) 8929-66-26 to 29 • (632) 8929-62-52
8929-66-20 • 8929-66-33 to 35 • 8929-70-41 to 43
Email: web@denr.gov.ph; Website: https://denr.gov.ph

12 SEP 2023

**MEMORANDUM**

**FOR** : **MARCIAL C. AMARO, JR.,** *CESO II*
Assistant Secretary for Policy, Planning and
Foreign Assisted and Special Projects

**ARLEIGH J. ADORABLE,** *CESO III*
OIC-Assistant Secretary for Field-Operations
Westerns Mindanao and Director, in concurrent
Capacity, Forest Management Bureau

**GWENDOLYN C. BAMBALAN, DPA,** *CESO III*
Regional Executive Director, DENR Region 2

**ENGR. PAQUITO T. MORENO, JR.,** *CESO III*
Regional Executive Director, DENR Region 3

**ARTURO E. FADRIQUELA**
Regional Executive Director, DENR Region 8

**NONITO M. TAMAYO**
Regional Executive Director, DENR Region 13

**MARIA LOURDES G. FERRER**
Director, Ecosystems Research and Development Bureau

**CHERYL LOISE T. LEAL**
Director, Policy and Planning Service

**FROM** : **THE DIRECTOR**
Foreign Assisted and Special Projects Service

**SUBJECT** : **PROJECT COMPLETION REPORT OF THE PROJECT TITLED: "RESEARCH ON PHILIPPINE IRONWOODS FOR GENETIC CONSERVATION AND ECOSYSTEM RESTORATION"**

This refers to the completion of the Project titled, "Research on Philippine Ironwoods for Genetic Conservation and Ecosystem Restoration". This Project was funded under the Special Projects Fund (101.A.02.d) and implemented by the University of the Philippines Los Baños Foundation, Inc. (UPLBFI) through the College of Forestry and Natural Resources from April 29, 2021 to January 29, 2023.

MEMO NO. 2023 - 793

The Project was able to accomplish 100% of its physical outputs and activities and disbursed 100% of the total project cost amounting PhP2,000,000.

We would like to put forward the following implications for conservation and policy guidelines as indicated in the Project Completion Report (PCR):

1. Importance of assessing the genetic diversity of ironwoods in order to reflect the availability of genetic resources necessary for the short-term ecological adaptation and for long-term evolutionary change of population. Hence, low levels of genetic diversity within the population can threaten their low-term persistence by reducing their ability to adapt to the changing environment;

2. In terms of spatial genetic structure, if the genetic diversity is found evenly distributed over a population, the loss of individuals has negligible effect on the population. However, if the genetic diversity is spatially distributed (fragmented or in patched) over a population, the loss of individuals will have large effect on the population. In addition, in small surviving populations of a species these aspects of genetic diversity are very important to prioritize assessments as they may suffer rapid loss of genetic diversity, reducing their biological fitness and increasing extinction risk.

3. Knowledge on the distribution of genetic diversity within and among populations is critical in the formulation of management strategies. For species with high population differentiation values, more populations are needed to be conserved or sampled to ensure that allelic and genotypic diversity can be retained. This is also essential for monitoring the effects of in situ or ex situ conservation on the maintenance of genetic diversity.

4. Given the generally low number of adults observed in natural populations, this poses an alarming rate of population decline. While a few populations were found to have abundant regenerations, the potential risk of inbreeding in regenerations would be high. With generally moderate amount of genetic diversity of populations revealed in this study (average heterozygosity or He at three loci = 0.320; specifically lowest among X. bracteatus = 0.279), high priority 85 | **Research on Philippine Ironwoods for Genetic Conservation and Ecosystem Restoration: for conservation using both in situ and ex situ methods is warranted for the species.**

We would like to commend the participating regions and field offices for their valuable contribution to the Project and in providing needed administrative support prior and during the conduct of the research activities.

Lastly, the results of this study would serve as a guide by the DENR in its policy-making and program formulation related to Forestry Programs., i.e., intensifying tree planting activities of ironwoods seedlings/ saplings to increase its population and to improve the level of genetic biodiversity of ironwoods. Specifically, the Forest Management Bureau (FMB) should consider inclusion of ironwoods as one of the priority

species for reforestation. Also, awareness campaign relative to its protection and biodiversity conservation should be strengthened. On the other hand, the Biodiversity Management Bureau (BMB) should consider the Malapiga/ Palawan Mangkono in the updating of the DENR Administrative Order No. 2017 – 11 National List of Threatened Philippine Plans and their Categories. Currently, said ironwood species is categorized as vulnerable.

Attached is a copy of the Project Completion Report submitted by the Project for your reference.

For your information and consideration.

**ALO. OROLFO, *Ph.D.***

Cc:

**Atty. Jonas R. Leones,** *CESO I*
Undersecretary for Policy, Planning and
International Affairs

**Atty. Juan Miguel T. Cuna,** *CESO I*
Undersecretary for Field Operations- Luzon, Visayas and Environment

**Joselin Marcus E. Fragada,** *CESO III*
Undersecretary for Field Operations-Mindanao

**Jeslina B. Gorospe**
Chief, FASPS Project Monitoring and Evaluation Division

**Conrado A. Bravante, Jr.**
Chief, FASPS Project Preparation Division

**Maybelle N. Mangubos**
OIC-Chief, FASPS Project Accounts Management Division

Department of Environment and Natural Resources
Foreign-Assisted and Special Projects Service
**Project Completion Status Report**

## I. PROFILE

Project Title: **Research on Philippine Ironwoods for Genetic Conservation and Ecosystem Restoration**

Fund Source/s: DENR (II.d. Conduct of Special Studies, Design and Development in Support of Biodiversity, Forestry, Mining and Environmental Management Operations)

Total Project Cost: PhP2,000,000
PhP1,100,000 (1st Contract)
PhP900,000 (2nd Contract)

Signing Date: September 23, 2019 (Secretary approval)

Duration: 18 months

Effectivity Date: April 29, 2021 (1st Contract)
September 8, 2022 (2nd Contract)

Closing Date: July 31, 2022 (1st Contract)
January 29, 2023 (2nd Contract)
Note: It was agreed that under the 2nd contract the imposition of Cash-Based Budgeting will be deferred indefinitely.

Implementing Agency: UP College of Forestry and Natural Resources through the UPLBFI

Sector: Forestry

Region: 2, 8 and CARAGA (13)

Beneficiaries: Researchers/ scientists and other academic institutions, POs and DENR

**Description:**

The "Research on Philippine Ironwoods for Genetic Conservation and Ecosystem Restoration" will be implemented by the UPLB-CFNR through a Contract between the DENR and UPLBFI. It is composed of two (2) component studies as follows: 1) GIs-Based Resource Mapping of Philippine Ironwoods and 2) Genome Profiling and Analysis of the Genomic Basis of Adaptation of Philippine Ironwoods Trees.

The first component study aims to generate resource distribution maps that will serve as guide in developing strategies for genetic resource conservation of the Philippine ironwoods tree species. On the other hand, the second component study aims to elucidate the genomic mechanisms underpinning the localization of adaptation of the natural populations of the Philippine ironwoods species. This will involve: 1) characterization of the genetic structure of populations of Philippine ironwoods species; 2) generation of DNA barcodes of the Philippine ironwoods species; 3) assessment of the genetic and evolutionary relationships of the Philippine ironwoods species; 4) determination of the genetic basis of adaptation of selected ironwoods species using population genetics approach; 5) analysis of the pattern of genetic structure of populations of a Philippine ironwoods species in relation to their spatial distributions (habitats); and 6) provision of baseline genetic data for the establishment of genetic guideline. These studies are expected to generate patterns of endemicity and elucidate the mechanism(s) underpinning the endemicity of the Philippine ironwoods species.

**Objective:**

The overall objective of the study is to conserve and restore the genetic resources of native ironwoods in the Philippines.

Specifically, the study aims to:

a. Generate maps of spatial distribution range of selected Philippine ironwoods;
b. Evaluate the genetic structure of selected ironwood species based on their genotype profiles
c. Generate the DNA barcodes of the ironwoods; and
d. Develop a policy guidelines in the conservation of Philippine ironwoods

**Expected Outputs:**

1. Genetic resource maps/ spatial distribution maps of Philippine ironwoods generated
2. Genotype profiles of natural Philippine ironwoods population generated
3. DNA barcodes for Philippine ironwoods deposited in the database
4. A policy brief or draft policy recommendation on the conservation of Philippine ironwoods

## II. Project Performance

### 1. Physical Performance

The Project accomplished 100% against its overall target. The Project has a total cost of PhP2,000,000.00 and the duration of this project is 18 months. However, the FY 2021 Contract of the project was revised to cover only the first year of the project implementation amounting to PhP1,100,000 due to the current implementation of cash-based budgeting. Another contract was executed in FY 2022 to cover the remaining deliverables of the Project with a total cost of PhP900,000.

- **FY 2021 Contract**
  - **First request for extension of submission of deliverable**

UPLBFI in its letter dated June 27, 2021 requested to extend the submission of Progress Billing No. 2 until November 29, 2021 due to the delay in the issuance of a resolution by the Northern Sierra Madre Natural Park Protected Area Management Board as a requirement in securing the Gratuitous permit to be filed at the DENR Regional Office. The said request was approved by the Director, Administrative Service on October 15, 2021

- **Second request for extension of submission of deliverables**

In anticipation of possible further delays on the issuance of PAMB and the approval of the Gratuitous Permit, UPLBFI in its letter dated December 20, 2021 requested for another extension of submission of Progress Billing Nos. 2 and 3. The 2nd request was approved by the Director, Administrative Service on January 25, 2022. With this, the FY 2021 Contract concluded on December 31, 2021

**Completed deliverables under the 1st Contract:**

| Progress Billing | Deliverables | Updates/ Outputs |
|---|---|---|
| Progress Billing No. 1 15% (PhP165,000) | Inception Report | **COMPLETED** |

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|---|---|---|
| Progress Billing No. 2 40% (PhP440,000) | The 1st Progress Report comprised of the following milestones/outputs:<br>1. Result on coordination and consultation with concerned DENR officials and other stakeholders for site survey, field validation, and sampling in Isabela-Baler; Completed application for GP/FPIC and other related documents for the sampling in Isabela-Baler<br>2. Report on survey/field data on the identity and preliminary spatial distribution map of Philippine ironwoods in Isabela-Baler<br>3. Report on genotype profiling of ironwoods using trial samples from CFNR arboretum<br>- Optimized DNA extraction and purification<br>- Optimized conditions for amplification of screened molecular markers or loci (minimum of 8) for genotyping<br>- Fragment analysis of DNA using trial samples (4 - 8 trees) *<br>4. Report on DNA barcoding of ironwoods using trial samples from CFNR arboretum<br>- Optimized DNA extraction and purification<br>- Screening and amplification for sequencing two (2) barcoding genes (rbcl and matK)<br>- DNA sequencing reactions using trial samples (4 – 8 trees)* | **COMPLETED** |
| Progress billing No. 3 (PhP495,000) | The 2nd Progress Report comprised of the following:<br>1. Result on coordination and consultation with concerned DENR officials and other stakeholders for site survey, field validation, and sampling in Samar; Completed application for GP/FPIC and other related documents for the sampling in Samar<br>2. Report on survey/field data on the identity and preliminary spatial distribution map of Philippine ironwoods in Samar<br>3. Report on genotype profiling of ironwoods using samples from Isabela-Baler<br>- Optimized DNA extraction and purification<br>- Optimized conditions for amplification of screened molecular | **COMPLETED** |

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| | markers or loci (minimum of 8) for genotyping<br>- Fragment analysis of DNA using trial samples (4 - 8 trees) *<br>4. Report on DNA barcoding of ironwoods using trial samples from Isabela-Baler<br>- Optimized DNA extraction and purification<br>- Screening and amplification for sequencing two (2) barcoding genes (rbcl and matK)<br>- DNA sequencing reactions using trial samples (4 - 8 trees)* | |
| **PhP1,100,000** | **TOTAL** | |

- **FY 2022 Contract**
  - **Request for extension of submission of deliverable**

    The Project reported that there were delays on the Project due to the following reasons: a) delays in securing Gratuitous Permit in DENR R13 (CARAGA); b) molecular analysis for the last collected samples are not yet complete; and c) suspension of work during and after typhoon Paeng, with consequent brownouts and holidays until the first few days of November hampered the timely conduct of the laboratory experiment. With this, UPLBFI in its letter dated November 7, 2022 requested for extension of submission of the 1st progress report and succeeding reports and subsequently approved by the Director, Administrative Service on November 24, 2022.

**Completed Deliverables under 2nd Contract**

| Progress Billing | Deliverables | Updates/ Outputs |
|---|---|---|
| 15% of the total project cost (PhP135,000) | **Progress Billing No. 1:**<br><br>The 1st Progress Report comprised of the following:<br><br>1. Result on coordination and consultation with concerned DENR officials and other stakeholders for site survey, field validation, and sampling in **Surigao**<br>2. Report on survey/field data on the identity and preliminary spatial distribution map of Philippine ironwoods in Surigao<br>3. Report on genotype profiling of ironwoods using samples from Samar<br>- Optimized DNA extraction and purification<br>- Optimized conditions for amplification of screened molecular markers or loci (minimum of 8) for | **COMPLETED** |

| | | |
|---|---|---|
| | genotyping<br>- Fragment analysis of DNA using trial samples (4 - 8 trees) *<br>4. Report on DNA barcoding of ironwoods using samples from Samar<br>- Optimized DNA extraction and purification<br>- Screening and amplification for sequencing two (2) barcoding genes (rbcl and matK)<br>- DNA sequencing reactions using trial samples (4 - 8 trees)* | |
| 50% of the total project cost (PhP450,000) | **Progress Billing No. 2**<br><br>1. The 2nd Progress Report comprised the following:<br>• Complete report on survey, field validation, and sampling from the 3 locations and all species of ironwoods<br>• Complete report on genotype profiling of ironwoods of all samples from the 3 locations<br>• Complete report on DNA barcoding of ironwoods of all samples from the 3 locations and all species of ironwoods<br>2. Draft Technical Report on GIS-based spatial distribution map/s of Philippine ironwoods<br>3. Draft Technical Report on Genetic resource conservation strategies based on their spatial and genetic diversity distribution<br>4. Draft Technical Report on Bioinformatics analysis (population genetic diversity and structure) of all samples of a selected ironwood species (*X. verdugonianus*) from 3 locations<br>5. Draft Technical Report on Bioinformatics analysis (i.e. phylogenetic analysis of ironwoods)<br>6. Draft Policy Brief<br>7. Draft Project Completion Report | **COMPLETED** |
| 35% of the total project cost (PhP315,000) | **Progress Billing Report No. 3**<br><br>1. Final Technical Report on GIS-based spatial distribution map/s of Philippine ironwoods<br>2. Final Technical Report on Genetic resource conservation strategies based on their spatial and genetic | **COMPLETED** |

| | | |
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| | diversity distribution<br>3. Final Technical Report on Bioinformatics analysis (i.e., population genetic diversity and structure) of all samples of a selected ironwood species (*X. verdugonianus*) from 3 locations<br>4. Final Technical Report on Bioinformatics analysis (i.e., phylogenetic analysis of ironwoods and DNA barcodes application)<br>5. Policy Brief<br>Project Completion Report | |
| **PhP900,000** | **TOTAL** | |

**2. Financial Performance**

The fund utilization of the project are as follows:

| Contract | Budget Allocation | Amount Released | Utilization | %Utilization |
|---|---|---|---|---|
| 1st Contract | PhP1,100,000 | PhP1,100,000 | PhP1,100,000 | 100% |
| 2nd Contract | PhP900,000 | PhP900,000 | PhP900,000 | 100% |

## III. Major Findings of the Study

This study was conducted to provide information on the extent of existing genetic resources of the Philippine Ironwoods and its genetic variability. The research has two (2) components activities: 1) GIS-based resource mapping of the Philippine Ironwoods; and 2) genome profiling and analysis of the genetic basis of adaptation of the Philippine Ironwoods. Except for Xanthostemon Speciosus (Malapiga/ Palawan Mangkono), five distinct locations of X. Bracteatus (Mapilig), six of X. Philippinensis (Bagoadlau), 1 of X. Fruticosus (Sierra Madre Mangkono) and 3 of X. Verdugonianus (Mangkono) were surveyed. X. Bracteatus (Mapilig) yielded a tree density of as small as 1 tree/ha to 183/ha (predominantly represented by seedlings). Due to limitation in access, only one population of X. Fruticosus (Sierra Madre Mangkono) was surveyed in Divilacan, Isabela.

X. Philippinensis (Bagoadlau) was mostly represented in the sampling (6 distinct locations) because the species was generally co-occurring in areas where other species are present. X. Verdugonianus (Mangkono) yielded a few adult tree surveyed in two places in Surigao del Sur, while a large number of regenerations were recorded in Babatngon, Leyte. Their spatial distribution maps per region were generated.

### A. Field Validation and Species Survey

The three (3) species that are widely distributed are Mapilig, Bagoadlau, and Mangkono in Luzon (Aurora and Isabela Provinces), Visayas (Western Samar and Leyte) and Mindanao (Surigao del Sur). While, Sierra Madre Mangkono and Malapiga/ Palawan Mangkono are the two (2) rarely distributed species which are found in Isabela and Palawan, respectively. However, Malapiga/ Palawan Mangkono was not included in target species to be surveyed, and only an individual from the CFNR Arboretum was sampled for the genetic analysis. A GIS-based spatial distribution maps of Philippine Ironwoods per location was established to determine the actual location and density of tree populations of the surveyed area.

Among the surveyed distribution of Mapilig, the largest Diameter at Breast Height (DBH) recorded per site ranged from 60 to 100 cm and a density of as low as 1 to 183 trees/ha. Although it was not included in the proposed sites, the Mapilig in Babatngon, prolific number of regenerations were found in the area. Among the surveyed distribution of Bagoadlau, the larget DBH recorded per site ranged from 75 to 223 cm and a density of 17 to 221 trees/ha. Large number of adult trees and regenerations were recorded at Baler, Aurora. Similar to Mapilig, a large number of adults and regenerations Mangkono was observed in Babatngon, Leyte. Largest DBH per site ranged from 10.2 to 84.3 cm.

**A.1 Aurora and Isabela**

A total of four site two provinces (Aurora and Isabela) were included in the survey and collection of various Xanthostemon species (Bagoadlau and Mapilig). In Aurora, three municipalities were surveyed; Baler, Maria Aurora and Casiguran. On the other hand, only the municipality of Dinapigue was surveyed in Isabela.

In Baler, a total of 749 individuals were documented, which includes a total of 131 trees, 606 saplings, and 12 seedlings majority of which are Bagoadlau. With a total surveyed area of 3.376 hectares, Bagoadlau has a density of 221 trees/ hectare. In Casiguran, a total of 21 individuals were observed comprising of 3 trees, 15 saplings and 3 seedlings. With total surveyed area of 1.05 hectares, Bagoadlau has a density of 22 trees/ hectare. In Maria Aurora, 19 individuals were recorded this consists of 14 trees and 5 saplings. With total surveyed area of 0.75 hectare, Mapilig has a density of 25 trees/ hectare.

In province Isabela Province, a total of 5 populations of Sierra Madre Mangkono were recorded with 35 individuals, consisting of 5 trees and 30 saplings. With a total surveyed area of 0.879 hectare, Bagoadlau has a total density of 40 trees/ hectare.

**A.2 Samar and Leyte**

A total of five sites in three provinces in the Visayas (Western Samar, Eastern Samar and Leyte) were included in the survey and collection of various Xanthostemon species (Mangkono, Mapilig, and Bagoadlau). In Western Samar, two municipalities were surveyed; Paranas and Hinabangan. Additionally, two municipalities were surveyed in the province of Eastern Samar, namely; Taft and Llorente. Lastly, only the municipality of Babatngon in Leyte.

In Paranas, Western Samar, within the surveyed transect, a total of 8 individuals were observed, comprising of 4 seedlings, 3 saplings and 1 tree and with a total of 9.410 hectares, it has a density of 1 tree/hectare. While in Hinabangan, with a total surveyed aread of 4.023 hectares, Mangkono has a total density of 20 trees/ hectare. In Llorente, Eastern Samar, a total of 18 individuals, comprising 5 seedlings and 13 tree individuals were recorded. In Taft, a total of 6 populations of Bagoadlau were surveyed. In Leyte, specifically in the municipality of Babatngon, a total of 5 populations were recorded with approximately 310 individuals. From this, there were 7 trees and 303 seedlings.

**A.3 Surigao del Sur**

A total of two sites in Surigao del Sur Province (Bislig and Lianga) were included in the survey and collection of various Xanthostemon (Bagoadlau, Mapilig and Mangkono). In Bislig, two municipalities were surveyed; Maharlika and Borboanan). While one municipality was surveyed in Lianga which is Manyayay. In Maharlika, a total of 3 populations of Bagoadlau and 1 population of

mangkono were surveyed. A total of 12 individuals were documented. In the municipality of Borboannan, 3 populations of Mapilig were surveyed. Similarly, Bagoadlau and Mangkono were surveyed and collected in the municipality of Manyayay. A total of 5 Bagoadlau trees were surveyed.

**B. Molecular Characterization**

DNAs were extracted from all the possible samples collected per species, but only representative samples were analyzed for sequencing and fragment analysis.

**B.1 DNA Barcoding**

DNA barcoding, a system that makes use of codes similar to the Universal Product Code system, is an efficient method for species level identifications. It has, for more than a decade, contributed greatly to taxonomic and biodiversity research. It has complemented current research both in molecular phylogenetics and population genetics by providing background information helpful in the selection of taxa for further analyses.

Based on the DNA Barcoding of the Philippine Ironwoods, considerable nucleotide variation was only detected by matK and ITS1 genes, and two genes were concordant in clustering the species and populations of ironwoods, **with X. Bracteatus (Mapilig) and X. Philippinensis (Bagoadlau) phylogenetically related, while X. Verdugonianus (Mangkono) was found distinct**. Although one population of X. Philippinensis (Bagoadlau) in Lianga, Surigao del Sur clustered with X. Verdugonianus (Mangkono) samples collected in the same area.

## IV. Implications for conservation and policy guidelines

- In plants, natural variation refers to heritable variation within and between populations, and it is encoded in the sequence of the four nucleotide bases that make up the DNA;
- There are two important aspects of genetic diversity for conservation and management of forest genetic resources; levels and structure of genetic diversity. First, it is important to assess the genetic diversity in a species because the levels reflect the availability of genetic resources necessary for the short-term ecological adaptation and for long-term evolutionary change of a population. Hence, low levels of genetic diversity within the population can threaten their long-term persistence by reducing their ability to adapt to the changing environment conditions. Second, in terms of spatial genetic structure, if the genetic diversity is found evenly distributed over a population, the loss of individuals has negligible effect on the population. However, if the genetic diversity is spatially distributed (fragmented or in patches) over a population, the loss of individuals will have a large effect on the population. In addition, in small surviving populations of a species these aspects of genetic diversity are very important to prioritize assessments as they may suffer rapid loss of genetic diversity, reducing their biological fitness and increasing extinction risk;
- Knowledge on the distribution of genetic diversity within and among populations is critical in the formulation of management strategies. This is also essential for monitoring the effects of in situ or ex situ conservation on the maintenance of genetic resources;
- Given the generally low number of adults observed in natural populations, this poses an alarming rate of population decline. While a few populations were found to have abundant regenerations, the potential risk of inbreeding in regenerations would be high. With generally moderate amount of genetic diversity of populations revealed in this study (average heterozygosity) or He at three loci = 0.320; specifically lowest among X. Bracteatus (Mapilig)