Biodiversity monitoring using eDNA metabarcoding : A Snapshot of Pelagic microbiomes in Savu Sea, Indonesia

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Programme:BIFA
Project ID: BIFA6_021
Project lead organization:YAYASAN BIODIVERSITAS INDONESIA
Project implementation period:1/9/2021 - 28/2/2023
Report approved: 13/10/2022

Narrative Midterm report

Executive Summary

The overall objectives of this project are to
(1) Enrich existing metazoan species detected in Eastern Indonesia by using the eDNA Metabarcoding technique,
(2) To train conservationists to use molecular data, and
(3) To develop a practical guidebook to collect and analyze eDNA data for research and conservation purposes. Until the mid-term period, 80-90% of the objective (1) had been achieved, which includes:
a. Collection and analysis of seawater samples for eDNA Metabarcoding from the Savu Sea, Eastern Indonesia. A total of 20 seawater samples have been extracted and 10 of them have been sequenced. However, there was no single elasmobranch DNA detected from our samples, but we observed 788 taxa (631 bacterial taxa and 157 eukaryote) with total 21649 sequences associated with the taxa.
b. eDNA-Metabarcoding Dataset uploaded to the GBIF database
c. Two project members participated in the BIFA enhancement workshop
d. To ensure the project run as expected and planned, all the team members regularly meet and communicate on the progress of the project and solving technical and laboratory problems. In the middle of the project duration, we changed the genetic marker for the project due to technical problems. Instead of using Elamosbarch specific markers, we used metazoan COI universal metabarcoding marker to collect as much as possible marine biodiversity
d. We anticipate no changes in plan and deliverable for the remaining project duration

Progress against milestones

Has your project published at least one dataset through GBIF.org?: Yes

Dataset published:

Dataset	DOI
Savu Sea-Indonesia eDNA Dataset	DOI10.15468/hyxfc6

Has at least one member of your project team received certification following the BIFA capacity enhancement workshop?: Yes

Name of the workshop participant: Aji Wahyu Anggoro

Certification obtained: Basic Badge

Report on Activities

Activity progress summary

Until mid-term period, we had conducted several activities listed below:

1. Coordination meeting with all partners had been done to arrange lab work, data analysis and to share the progress of project implementation.
2. Two project members, i.e. Aji Wahyu Anggoro and Ni Kadek Dita Cahyani had participated the BIFA Enhancement Virtual Workshop and gained *Basic Level* certificates
3. We had conducted laboratory work to analyze the seawater samples for eDNA from the Savu Sea. DNA extractions were conducted in the BIONESIA Lab facility while Library Prep and Sequencing were performed by Genetika Science sequencing facility. All of 20 samples have been extracted of which 10 samples have been sequenced by using Illumina platform, while the rest will be done within next phase of the project (as proposed). A slight change in library prep method had to be done due to excessive sequencing cost. Library preps using universal COI primer (Leray, 2013 (https://doi.org/10.1186/1742-9994-10-34) were performed to target eukaryote DNA while, initially, we proposed to use MiFish Elasmobranch primer (Miya et al. 2015) which specifically targets Elasmobranch DNA. This modification had been consulted to GBIF secretariat representative.
4. The sequenced results were analyzed using metaworks pipelines for bioinformatics, that combine R and Phyton languages. The first batch of dataset had been published through the GBIF.org database

Completed activities

Activity name: Coordination meeting

Description: Regular meeting with all partners had been done to arrange lab work, data analysis and to share the progress of project implementation. Meetings were held virtually and hybrid in two occasions
Start Date - End Date: 1/9/2021 - 20/5/2022
Verification Sources: We did not documented every meeting. The attached file represented one of our internal meetings. File name : Coordination meeting 2022-05-10 at 09.28.19

Activity name: BIFA Capacity Enhancement Virtual Workshop

Description: Two project members participated the mandatory BIFA Capacity Enhancement Virtual Workshops and gained certification
Start Date - End Date: 1/11/2021 - 30/11/2021
Verification Sources: Links :
Aji Wahyu Anggoro
https://openbadgefactory.com/v1/assertion/8ee64864c822e8a734e250898580a287853b1ac8

Ni Kadek Dita Cahyani
https://openbadgefactory.com/v1/assertion/3c5a4ee16d61d09f9315b747088fe3f47bd45672

Activity name: Laboratory Work

Description: 20 eDNA samples were extracted in Bionesia lab. Then, 10 extraction samples were sent to Genetika Science Sequencing Facility to perform Library Prep using COI primer and NGS Illumina sequencing to detect Elasmobranch DNA
Start Date - End Date: 1/10/2021 - 31/12/2021
Verification Sources: Invoice for sample analysis (NGS sequencing) issued by Genetika Science sequencing facility.
File name: 29 des 2021-Invoice26136-WL.pdf

Activity name: Project Dissemination and Data Analysis (Phase 1)

Description: The sequenced results were analyzed using Metaworks pipelines for bioinformatics, that combine R and Phyton languages. The first batch of dataset was then published through the GBIF.org database.
Start Date - End Date: 1/1/2022 - 28/2/2023
Verification Sources: https://github.com/terrimporter/MetaWorks

Report on Deliverables

Deliverables progress summary

Within the mid-term period, we have completed sampling collection and species listing. Other deliverables will be fulfilled in the next project period. Following is the status of each deliverable mentioned above:

1. Sampling Collection Events
eDNA samples have been collected from four sites in the Savu Sea, Indonesia and completed within the period of 18 October 2018 - 9 April 2019. The fieldwork were part of other on-going project which is funded by other organizations. The project has already collected 75 eDNA samples with support by our site partners. For the current project, we worked on 20 subset samples. Since the samples were collected by other parties and stored for a considerable amount of time hence we do not have fully understanding of the pre-condition of the samples which we are using for the project project

2. Species List Data
20 eDNA samples have been extracted of which 10 samples have readily been sequenced using Illumina sequencing platform with COI primer to primarily target Elasmobranch DNA. There has been a modification in Library Prep method as initially we proposed to use a more specific primer i.e. MiFish Elasmobranch primer (Miya et al. 2015) but had to change to universal COI primer (Leray, 2013). The cause of the modification was due to the excessive sequencing cost for using MiFish. The modification was possibly impacting the resolution of taxa recovered. Following data analysis result using multiple pipelines, we observed DNA from other taxa besides Elasmobranch. Our results recorded bacteria and eukaryote for total 788 taxa (631 bacterial taxa and 157 eukaryote) with total 21649 sequences associated with the taxa. The first dataset has been published through the GBIF platform.

Progress towards deliverables

Title: Sampling Collection Event

Type: Dataset
Status update: completed
Dataset scope: The sampling collection event will summarize the collection event across 4 location in Savu Sea waters. We were collected surface seawater samples in 5 sites from each location
Expected number of records: 20
Data holder: Bionesia
Data host institution: Bionesia
Sampling method: eDNA associated seawater sample were collected across five regions, with ten sites in each location. Two liter of seawater sample were collected with sterile nalgene bottle from sea surface in every site. Water sample than filtered through a 0.22-133 micron Sterivex™ filter.
% complete: 100
DOI: DOI10.15468/hyxfc6
Expected date of publication:

Title: Species list Data

Type: Dataset
Status update: All samples have been extracted. 10 out of 20 eDNA samples have been analyzed using multiple pipelines combined with R and Python language. The dataset have been published to GBIF platform
Dataset scope: Savu Sea
Expected number of records: 788
Data holder: Bionesia and Misool Foundation
Data host institution: Bionesia
Sampling method: eDNA samples and blanks were extracted using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, 143 Germany). Extracted DNA was then sent to sequencing facility for NGS metabarcoding method using Illumina platform with COI primer, primarily targeting Elasmobranch DNA
% complete: 75
DOI: DOI10.15468/hyxfc6
Expected date of publication:

Communications and visibility

Currently we are just finishing our first batch dataset analysis and publishing the results through GBIF. Once we have our dataset published, we will share the project activities and results through Bionesia's website and social media. Until the mid-term period, we did not have any event to share. However, we are planning to disseminate our project results through an event of a training workshop and a peer-reviewed scientific publication prior to the final report in February 2023.

Monitoring and evaluation

Monitoring and evaluation findings

- As a part of the monitoring, precaution, and evaluation plan, as many as two water samples were pre-processed to evaluate sample condition and determine DNA concentration. These two samples were sent to a sequencing facility and were determined for their overall read compositions and the success of the sequencing process. This pre-screening process was meant to anticipate unnecessary sequence spending and was overall a successful step to mitigate downstreaming problems upon generating taxa assignment from sequencing results.

- During the course of the project we change the use of genetic marker for the lab metabarcoding process. We initially plan to use the Elasmobranch-specific genetic marker, however, due to a lab-related problem we switched to a metazoan-specific marker which compromise our ability to detect elasmobranch. Future implementation of a similar project should pay attention to this problem and as much as possible to detect the problem at the onset of project implementation

- Dr. Vijay was extremely useful in guiding us through the whole uploading process via IPT. Although there were hiccups during the process, we successfully uploaded the data and the data is now available in GBIF. Time planning is essential in this process as problems can't be predicted and there are always things that need to be fixed, thus we suggest other teams plan the data uploading process accordingly

- Continuous and adaptive project management is important to ensure the project runs smoothly and we enforce team communication on weekly bases.

- Although online meeting and communication cant be avoided, we as much as possible tried to also do offline meeting to increase team works, confidence and sense of project ownership.

Impact of COVID-19 pandemic on project implementation

Covid-19 had no specific impact on our project implementation. All project activities were conducted on schedule

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GBIF leads the Biodiversity Information Fund for Asia (BIFA), a programme funded by the Ministry of the Environment, Government of Japan. The programme provides supplementary support for activities addressing the needs of regional researchers and policymakers through mobilization and use of biodiversity data.

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