Framework application of e-DNA metabarcoding as a variable to evaluate mangrove ecosystem

M Basyuni1,2*, A Susilowati1,2, I E Susetya2,3, T Kajita4

1Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara, Jl. Tri Dharma Ujung No. 1 Medan, North Sumatera 20155, Indonesia
2Center of Excellence for Mangrove, Universitas Sumatera Utara, Medan, North Sumatera 20155, Indonesia
3Department of Aquatic Resource Management, Faculty of Agriculture, Universitas Sumatera Utara, Medan 20155, Indonesia.
4Iriomote Station, Tropical Biosphere Research Center, University of the Ryukyus, Taketomi, Okinawa, 907-1541, Japan.

*Email: m.basyuni@usu.ac.id

Abstract. Indonesia has the largest mangrove forests in the world, about 22.6% of the global total area. Mangrove forests are known to provide great benefits to the community as an ecosystem service and globally. Mangrove forests are threatened with loss due to anthropogenic and non-anthropogenic activities. Our previous research results show the important role of mangroves in the context of climate change for reducing greenhouse gas emissions and the diversity of terpenoids for salinity defense. The term metabarcoding of environmental DNA as a key indicator for evaluating mangrove ecosystems was introduced. This work proposed framework to study environmental DNA metabarcoding applications that can provide accurate information for evaluating mangrove ecosystems using an easy and fast method. The rich mangrove forest is getting disappeared without being recognized by its true diversity, function, and potential services to human beings. Application of conservation activities based on the species diversity in the mangrove ecosystem is almost impossible, because it is too difficult to study, in term of time and effort, despite the importance of acquiring the biodiversity information. However, DNA metabarcoding can be a "dream-like" tool and will contribute to obtain the species diversity data of the mangrove ecosystem, rapidly, globally, and massively.

1. Introduction

Mangrove forests are known to provide great benefits to communities as ecosystem services [1,2] and globally, mangrove forests are threatened with loss due to anthropogenic and non-anthropogenic activities [3-5]. Our previous research results show the important role of mangroves in the context of climate change for reducing greenhouse gas emissions and terpenoid diversity for salinity defense [3, 6-7].

Each type of mangrove has different adaptations to various kinds of biotic and abiotic stresses. Species collection and composition can correlate with the health and function of mangrove ecosystems
which can be detected biochemically and molecularly [8-9]. Recent developments use environmental DNA metabarcoding technology to evaluate the biodiversity of an ecosystem [10,11].

Environmental DNA (eDNA) metabarcoding is a novel method of assessing biodiversity wherein samples are taken from the environment via sediment, water, and air, including whole cells, extracellular DNA and potentially whole organisms. eDNA may come from skin, mucous, saliva, sperm, secretions, eggs, feces, urine, blood, roots, leaves, fruit, pollen and rotting bodies of larger organisms, while microorganisms may be obtained in their entirety. Environmental DNA metabarcoding will show differences in species composition. Developing variables to evaluate the function of mangrove ecosystems by metabarcoding environmental DNA is a very important endeavor. Meta-barcoding can provide information for evaluating mangrove ecosystems using an easy and fast method, among those developed by Miya et al. [11], namely MiFish. The MiFish and related methods are non-invasive methods with excellent capabilities, simple, fast, and economical. Using this technique, we can perform biodiversity analysis based on environmental DNA data [12-14]. This work was aimed to emphasize the framework into an e-DNA metabarcoding application to mangrove ecosystems.

2. Literature framework

When we buy something at a store, we will look at a barcode to identify it. To sequence a portion of DNA that is unique to a species, the sequence can be used to identify the species known as DNA barcode [11]. DNA barcode is DNA barcoding usually consisting of a short sequence of DNA that can be amplified with the help of PCR (polymerase chain reaction) and then sequenced to analyze species [13]. The identification of a specimen using a barcode is usually obtained by a DNA sequence barcode from the genome of the species. Then the unknown species sequence barcode is compared to the known species sequence barcode reference library. If the sequence of unknown species closely matches the order in the reference library, a species is identified [14-16]. The new barcoding sequence implies the discovery of a new species. DNA barcoding provides great support for countless scientific domains such as epidemiology, ecology, biomedicine, evolutionary biology, biogeography, and conservation biology as well as in the bio-industry [14].

Environmental DNA (eDNA) refers to genetic material found in ecosystems. eDNA has significantly enhanced our ability to identify diverse taxonomic metabarcoding groups [17,18]. eDNA not only has the potential to assess phylogenetic biodiversity, more reliably and rapidly than before but also provides a cost-effective tool for evaluating community structures relevant to the function of biota in their habitat [17,18]. The advancement of these techniques also produces an opportunity to overcome some of the limitations of traditional ecotoxicological research, which focuses on the detrimental impacts of chemicals on individual organisms, and to assess responses from individual to community and ecosystem levels [19].

3. Implementation

Mangrove forests are typical ecosystems with a specific combination of flora and fauna but are very vulnerable to disturbance due to human activities as well as biotic and abiotic stresses. Like mangrove forests in other regions, the condition of mangrove forests in Indonesia, especially North Sumatra, is also quite affected by human activities. Land conversion to oil palm, ponds, and settlements is a major problem in mangrove forest management in North Sumatra. Whereas, the results of our previous research indicate the important role of mangroves in the context of climate change for reducing greenhouse gas emissions and the diversity of terpenoids for the defense of salinity [3].

The group and species composition can correlate with the health and function of mangrove ecosystems that can be detected biochemically and molecularly. Recent developments using environmental DNA metabarcoding technology to evaluate the biodiversity of an ecosystem enable us to be able to detect the effects of anthropogenic and non-anthropogenic activities on the biodiversity of mangrove ecosystems. Based on the consideration of developing variables to evaluate the function of mangrove ecosystems with environmental DNA metabarcoding is very important. Metabarcoding can
provide information for evaluating mangrove ecosystems using easy and fast methods. By knowing the condition of mangrove ecosystems in North Sumatra, the diversity of mangrove forests in North Sumatra can be a source of ecological dynamics and new knowledge and also potential sources of mangrove forests, which can open up other possible benefits of mangrove forests. The research framework is depicted in Figure 1.

Many literature reviews have summarized how environmental DNA (eDNA) can be used to detect biodiversity [11-16]. Miya et al [11] developed MiFish, a universal set of PCR primers for metabarcoding environmental DNA from fish: able to detect more than 230 subtropical marine species. Environmental DNA has successfully identified the distribution of mackerel in Japan [12]. Suriya [13] summarizes research results in his review of usage to barcode various organisms including freshwater meiothems, soil meiofauna, extinct birds, marine organisms, fish, and insects. In Malaysia, DNA barcoding was reported to be able to differentiate catfish identifications [19].

Fig. 1. Research framework application of e-DNA metabarcoding in mangrove ecosystem

It has previously been reported using environmental DNA to determine the source of organic carbon in seagrass beds [16]. Environmental DNA metabarcoding supports community assessments of environmental stress in field-based sediment microcosms studies [15]. In the forestry field, DNA barcoding methods are well known, our previous studies were able to distinguish styrax species [20-21]. The most recent is the monitoring of marine nematode communities via 18S rRNA metabarcoding as a sensitive alternative to morphological characters [17]. Another Environment DNA application also have been reported as potentially powerful tool in fields such as modern biodiversity assessment [22], environmental sciences [23], marine eucaryotic communities [24].

4. Conclusions
The diversity of mangrove forests can be a source of ecological dynamics and new knowledge as well as potential sources of mangrove forests, which can open up the possibility of other benefits from mangrove forests. Implementation of the eDNA metabarcoding methods to mangrove ecosystem to obtain preliminary information on species diversity of fish and macrobenthos.
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