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Original article

Environmental DNA metabarcoding with MiFish primer reveals marine fish fauna of Tsushima Island, Nagasaki for establishing a marine protected area

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Introduction

A local government has promoted the establishment of a marine protected area (MPA) for sustainable management of the marine ecosystem along the coast of Tsushima Island, Nagasaki Prefecture, Japan. Current and comprehensive information on the marine ecosystem should be utilized to establish an MPA; however, conventional methods of inventory surveying involve long time periods, incur significant costs, and require taxonomic expertise. In addition, conventional methods generally cannot cover an entire ecosystem for various reasons; for example, small fishes may remain concealed in shore reefs. This is where environmental DNA (eDNA) metabarcoding can be helpful, and the technique has been applied to reveal aquatic communities. In particular, MiFish primer is as a good tool to identify local fish fauna. The primer can amplify 12S rRNA gene of almost all fishes that contains sufficient information to identify fishes to taxonomic family, genus and species except for some closely related congeners.

In this study, eDNA metabarcoding with MiFish primer was used to reveal the current marine fish fauna of Tsushima Island for establishing an MPA.

Materials and methods

Water samples were collected along coastal area of Tsushima Island in October 2016 to assess the current marine fish fauna. The study area was separated into six water sampling areas to elucidate differences in fish fauna (Fig. 1). In each area, 10L volume of surface water was sampled and filtered using Sterivex^{TM}-HV Sterile Vented Filter Unit, 0.45μm (MERCK MILLIPORE). After filtering, RNA later® Stabilization solution (Thermo Fisher Scientific) was immediately inserted into the Sterivex filter to preserve eDNA.

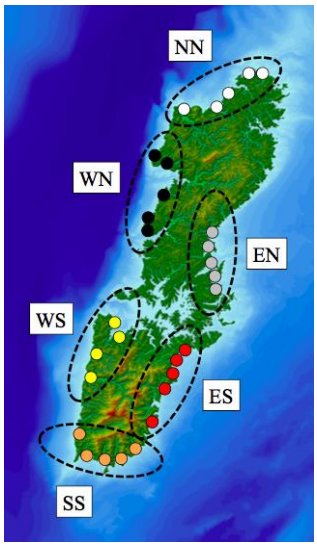

Fig. 1. Sampling sites of Tsushima Island. Each dot represents sampling sites. Six areas were optically established, that is; NN: Northern part of Northern Tsushima Island, WN: Western part of Northern Tsushima Island, EN: Eastern part of Northern Tsushima Island, SS: Southern part of Southern Tsushima Island, WS: Western part of Southern Tsushima Island, ES: Eastern part of Southern Tsushima Island.

Total DNA was extracted from each water sample using a DNeasy Blood and Tissue Kit (Qiagen) following manufacture's manuals. 12S rRNA gene was amplified with MiFish primer, and adaptor sequence and index sequences were add to the amplicon. Metabarcoding was conducted in MiSeq sequencing, and taxonomic assignment was performed following previous studies [1,2].

Hierarchical clustering analysis based on absence and presence of fish species was performed to reveal the relationship of species component among areas using the R version 3.3.3 software [3]. The analysis was conducted with ward method and euclidean distance.

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Sequences that considered as a contaminant were removed from the dataset.

Results and discussion

Over 150 species or similar species were identified from the water sample around Tsushima Island. This study found various species only from shore areas comparing with conventional method that reported around 380 species [4]. Some species were found in the study area that previous studies using conventional methods had not found. The utility of eDNA for inventory survey is demonstrated in the present study.

Some sequences could not identify as a species because several species had same sequence among species. Especially genus *Takifugu* shared common sequences due to the evolutionary process. They experienced explosive speciation [5], therefore, target regions of MiFish primer could not identify the species. It is recommended to use other primer to distinguish these species. The Freshwater and deep-sea fish also found in the results. This result can be explained by the possibility of contamination, the effect of influx from river, and Diurnal vertical migration. This study could not conclude the reasons. Continuous survey will clarify more detailed species components around Tsushima Island.

Clustering analysis using hierarchical methods revealed different fish fauna in northern and southern coastal areas of Tsushima Island, suggesting that each area may have different environmental factors, such as bottom conditions or the existence/absence of seaweed (Fig. 2). NN was clustered in to the same topology with Southern part of Tsushima Island. This result may means that NN and Southern part hold common environmental factors.

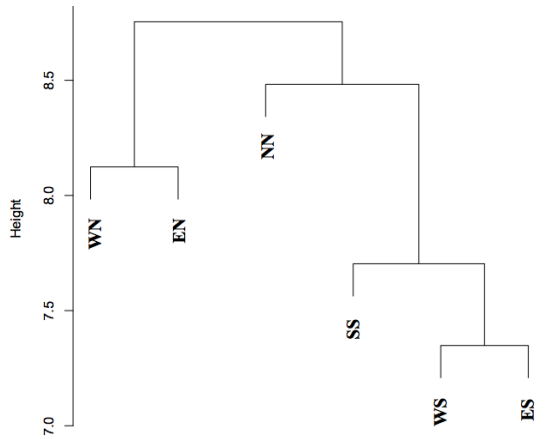

Fig. 2. Hierarchical Clustering analysis based on the absence and presence of fish species.

This study suggests that eDNA metabarcoding with MiFish primer offers sufficient resolution for inventory surveys and could potentially provide valuable information to more accurately understand the marine ecosystem near Tsushima Island. Continuous surveying is recommended in order to provide crucial information for establishment of an effective MPA.

Acknowledgements

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