SPECIES-SPECIFIC eDNA MARKERS FOR RAPID DETECTION OF INVASIVE FISHES IN TASIK RABAN, MALAYSIA

NURUL FIZATUL NABILAH BINTI OSMAN

SULTAN IDRIS EDUCATION UNIVERSITY

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SENIOR LECTURER
DEPARTMENT OF BIOLOGY
FACULTY OF SCIENCE AND MATHEMATICS
SULTAN IDRIS EDUCATION UNIVERSITY

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ABSTRACT

The objective of this study is to develop species-specific environmental DNA (eDNA) markers for Cichla spp. based on mitochondrial *cytochrome oxidase I* (*COI*) gene sequences. Validation of the developed species-specific eDNA markers were done *ex-situ* for environmental sample from Tasik Raban. New digital PCR (dPCR) method was also employed to detect the presence of *Cichla ocellaris*, and *Cichla kelberi* in environmental water samples. Result from this study showed that *COI* gene can be used to develop species-specific markers for species *Cichla*. *Ex-situ* validation results also showed that the developed markers CO1 amplified specifically to specimen *C. ocellaris* while CK1 amplified directly to specimen of *C. kelberi*. In addition, dPCR supported the specificity analysis as absolute fluorescent quantification detected the presence of *C. ocellaris* and *C. kelberi* eDNA in water sample at 90% detection confidence. In conclusion, CO1 and CK1 markers were successfully developed and specific to be used as eDNA markers for *C. ocellaris* and *C. kelberi* respectively. As an implication, eDNA analysis with species-specific markers suitable to be used as new rapid monitoring tool in detection of invasive species for biodiversity management and conservation purposes.

PENANDA eDNA SPESIFIK UNTUK PENGESANAN PANTAS IKAN SPESIS INVASIF DI TASIK RABAN, MALAYSIA

ABSTRAK

Objektif kajian ini adalah untuk membangunkan penanda DNA persekitaran (eDNA) yang spesies-spesifik bagi Cichla spp. berdasarkan jujukan gen *cytochrome oxidase I* (*COI*) mitokondria. Pengesahan penanda eDNA spesies-spesifik ini dijalankan secara *ex-situ* terhadap sampel persekitaran daripada Tasik Raban. Kaedah baharu PCR digital (dPCR) digunakan untuk mengesan kehadiran *Cichla ocellaris* dan *Cichla kelberi* di dalam sampel air persekitaran. Dapatan kajian ini menunjukkan gen *COI* boleh digunakan untuk membangunkan penanda spesies-spesifik untuk spesies *Cichla*. Dapatan pengesahan secara *ex-situ* juga menunjukkan penanda CO1 yang dibina boleh mengamplifikasi terus kepada spesimen *C. ocellaris* manakala CK1 mengamplifikasi spesifik kepada *C. kelberi*. Sebagai tambahan, dPCR menyokong analisis spesifisiti apabila penilaian fluorescen sebenar mengesan kehadiran eDNA *C. ocellaris* dan *C. kelberi* dalam sampel air pada 90% keyakinan pengesanan. Kesimpulannya, penanda CO1 dan CK1 telah berjaya dibangunkan dan spesifik untuk digunakan sebagai penanda eDNA bagi masing-masing *C. ocellaris* dan *C. kelberi*. Implikasinya, analisis eDNA dengan penanda spesies-spesifik sesuai digunakan sebagai alat pemantauan pantas baharu untuk pengesanan spesies invasif bagi tujuan pengurusan dan konservasi biodiversiti.

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LIST OF ABBREVIATIONS

AGE	Agarose Gel Electrophoresis
AIS	Aquatic Invasive Species
BOLD	Barcode of Life Data system
bp	Base pair
CDS	Coding sequence
°C	Degree celcius
CBD	Convention of Biological Diversity
CI	Confidence Interval
CK	*Cichla kelberi*
CM	*Cichla monoculus*
CO	*Cichla ocellaris*
COI	Cytochrome oxidase subunit 1
cytB	Cytochrome b
ddH_2O	Double distilled water
DNA	Deoxyribonucleic acid
DoF	Department of Fisheries
dNTPs	Deoxynucleotide
dPCR	Digital Polymerase Chain Reaction
eDNA	Environmental DNA

EUS	Epizootic Ulcerative Syndrome
GenBANK	Genetic sequence database
g	Gram
IAS	Invasive Alien Species
L	Liter
M	Mini Amazon
M	Molar
mA	Milliampere
mg	Milligram
mL	Milliliter
µg	Microgram
µL	Microliter
µM	Micromolar
mt	*Mitochondria gene*
NaCl	Sodium chloride
ng	Nanogram
NJ	Neighbour-Joining
nm	Nanometer
NWF	National Wildlife Federation
PB	Peacock Bass
PCR	Polymerase Chain Reaction
pH	Potential of hydrogen
RE	Restriction enzyme
RNA	Ribonucleic acid

rpm	Revolutions per minute
rRNA	Ribosomal RNA
sdH_20	Sterile distilled water
SMS	Sequence Manipulation Suite software
sp.	Species
TBE	Tris base/ Boric acid/ EDTA
Tm	Melting temperature
TR	Tasik Raban
UV	Ultraviolet
V	Voltage
W	Watt
WSSV	White Spot Syndrome Virus

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Aquatic invasive species (AIS) spread around the globe being the second biggest threat to diversity in our planet's ecosystems, particularly pervasive by causing food web disruption, biodiversity loss, and economic harm (Thomaz et al., 2014). Non-native or alien species is globally defined as an organism that is translocated from its natural or historical habitat, either accidentally or on purpose, and subsequently successful in residing in its new environment (Rahim et al., 2013). Most of the species introduced in Malaysia have been brought in from the Amazon River (South America) and introduced as popular game fishes usually in lakes (DoF, 2007). The commonly known invasive alien fish species in our local water system are *Arapaima gigas, Oreochromis mossambicus, Pygocentrus nattereri, Claria gariepinus, Belone belone* and *Cichla ocellaris* (Zakaria, 2017).

1.2 Research Background

Major reason for the introduction and establishment of alien fish species in Malaysia is due to recreational fishing. The most prominent example is the peacock bass (Cichla spp.) which was intentionally released into rivers or lakes made from former mining areas by irresponsible anglers for sport fisheries (Rahim et al., 2012). The peacock bass is known as a good sport fish (Neal et al., 2017) but the uncontrolled spread and unintentional release of this species had caused negative impact globally towards the declining of native fish species (Rahim et al., 2013). Thus, the peacock bass was also labelled as alien invasive species (AIS) due to the threat it caused to local freshwater biodiversity.

1.3 Problem Statement

It is an urgent need to monitor AIS in order to preserve native fish species but local authorities still depend on a common method of long-term surveys and large fish catchment. This so-called traditional field capture technique is often considered to be costly and labor intensive (Farley et al., 2018). Moreover, detection probabilities typically are low due to the AIS behavior that prefers to be hidden beneath the water's surface. Such fish surveillance programs also employ nets or electrofishing gear but these tools often lead to major false sightings as the target AIS species is unable to be predicted underwater (Gu & Swihart, 2004).

1.4 Objective of the Study

This thesis covers three areas of investigation in which each is focused on resolving specific issues. The objectives of current study are as follows:

1. To design novel species-specific environmental DNA markers for *Cichla sp* based on mitochondrial DNA (*mt*DNA) *COI* gene.

2. To validate designated species-specific eDNA markers in ex-situ from Tasik Raban using eDNA approach.

3. To apply and test a new digital PCR (dPCR) assay to detect the presence of environmental DNA (eDNA) for *C. kelberi*, and *C. ocellaris* in water samples.

1.5 Study Limitation

However, the extended invasion status of alien species in Malaysia is still not known. Since eDNA is still new and has not been implemented frequently in tropical environments especially in Malaysia, it is a challenging effort to identify as correct as possible unknown specimen firstly by morphology. Development strategy to produce species-specific primers can be utilized to detect the peacock bass fishes by eDNA-PCR method.

1.6 Importance of Research

Currently, an alternative and rapid new technique known as environmental DNA (eDNA) has gained much attention by AIS researchers as it enables detection of organisms in the environment using the analysis from water samples (Bohmann et al., 2014). Environmental DNA (eDNA) is defined as DNA that has been released by an organism into the environment, via feces, hair, urine, skin or gametes (Valentini et al., 2016). This DNA can be extracted from environmental samples such as soil, water or feces without having to isolate the target organism (Dejean et al., 2011). This DNA can be amplified by polymerase chain reaction (PCR) technology and delimit organisms which were present in a given water sample. eDNA also permits early detection of AIS even at very low densities, and at any life stage (Ficetola et al., 2008; Jerde et al., 2011; Dejean et al., 2011). This has been proven in several studies, of which the best-known examples are on invading Asian carp in Europe water systems (Jerde et al., 2011; Mahon et al., 2013). Positive feedback has been vastly reported upon application of this method as it strongly aids in AIS monitoring and management effort (Valentini et al., 2016).

1.7 Conclusion

Overall, the information on the strategy for developing species-specific eDNA primers for invasive *Cichla* species in current research will benefit researchers and AIS (alien invasive species) monitoring authorities as it permits early detection of *COI*-eDNA with an absolute quantitative Digital Polymerase Chain Reaction (dPCR) molecular method is a highly successful technique to identify species that are expanding rapidly. dPCR manages to provide data on species abundance and organism presence of ecologically invasive and rare species.