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

NURUL FIZATUL NABILAH BINTI OSMAN



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FISHES IN TASIK RABAN, MALAYSIA

NURUL FIZATUL NABILAH BINTI OSMAN

DISSERTATION PRESENTED TO QUALIFY FOR A MASTER IN
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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. Pengesanan 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 pengesanan secara *ex-situ* juga menunjukkan penanda *COI* 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 *COI* 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.





CONTENTS

	Page
DECLARATION OF OROGINAL WORK	ii
DECLARATION OF DISSERTATION	iii
ACKNOWLEDGEMENT	iv
ABSTRACT	v
ABSTRAK	vi
CONTENT	vii
LIST OF TABLES	xiii
LIST OF FIGURES	xv
LIST OF ABBREVIATIONS	xviii
LIST OF APPENDICES	xxi
CHAPTER 1 INTRODUCTION	1
1.1. Introduction	1
1.2. Research Background	2
1.3. Problem Statement	2
1.4. Objective of Study	3
1.5. Study Limitation	3



1.6. Importance of Research	4
-----------------------------	---

1.7. Conclusion	5
-----------------	---

CHAPTER 2 LITERATURE REVIEW	6
------------------------------------	----------

2.1. Introduction of invasive species	6
---------------------------------------	---

2.2. Introduction of invasive fish species	7
--	---

2.3. Threat of invasive fish species	16
--------------------------------------	----

2.3.1 Hybridization and introgression	17
---------------------------------------	----

2.3.2 Pathogenic transmissions	20
--------------------------------	----

2.3.3 Predation and competition	22
---------------------------------	----

2.3.4 Environmental changes	24
-----------------------------	----

2.4. Spread and establishment of invasive fish species	25
--	----

2.4.1 Global scenario	26
-----------------------	----

2.4.2 Malaysia scenario: Peacock Bass as an IAS	27
---	----

2.4.3 Aquarium Fish Industries	30
--------------------------------	----

2.5. Detection of AIS	31
-----------------------	----

2.5.1 Traditional method	31
--------------------------	----

2.5.2 Environmental DNA (eDNA) method	33
---------------------------------------	----

2.5.2.1 Environmental DNA (eDNA) application	34
--	----

2.5.2.2 Species specific marker	35
---------------------------------	----

2.5.2.3 Limitations of eDNA methods	36
2.5.3 Hybrid methods (Traditional and eDNA method combo)	37
2.6. Digital Polymerase Chain Reaction (dPCR)	38
CHAPTER 3 METHODOLOGY	42
3.1. Introduction	42
3.2. Experimental Design	43
3.3. Study area	44
3.4. Methodology for objective 1: To design species-specific markers	48
3.4.1 Introduction	48
3.4.2 Species-specific primer development	48
3.4.3 Species-specific dPCR probes design	49
3.5. Methodology for objective 2: To validate the designated species-specific markers	50
3.5.1 Introduction	50
3.5.2 Fish sampling	50
3.5.3 Verification morphological Species	53
3.5.4 Obtaining DNA samples	55
3.5.5 Agarose Gel Electrophoresis (AGE)	56
3.5.6 The use of PCR for fish identification	57
3.5.7 Identification of fishes through DNA sequencing	57

3.5.8 Primer validation using polymerase chain reaction (PCR)	59
3.5.9 The use of digital PCR to validate primers	59
3.6. Methodology for objective 3: To identify and validate eDNA	60
3.6.1 Introduction	60
3.6.2 eDNA sample collection	61
3.6.3 eDNA filtration	63
3.6.4 eDNA isolation	65
3.6.5 eDNA amplification by conventional PCR	66
3.6.6 dPCR analysis for eDNA detection	66

CHAPTER 4 RESULT

4.1. Development of species-specific primer	68
4.2. Sample collection	73
4.3. Morphology Identification	86
4.4. DNA extraction	86
4.5. Amplification of <i>COI</i> gene with conventional PCR	88
4.6. Molecular identification of <i>Cichla</i> spp.	88
4.7. Primer validation through PCR	91
4.8. Primer validation through digital PCR (dPCR)	104
4.9. Map location of water samples	108

4.10. eDNA extraction	112
-----------------------	-----

4.11. Multiplex PCR for eDNA	115
------------------------------	-----

4.12. dPCR for eDNA identification	127
------------------------------------	-----

CHAPTER 5 DISCUSSION	137
-----------------------------	-----

5.1. Development of species-specific primers	137
--	-----

5.2. Fish sampling	139
--------------------	-----

5.3. Integrity of the isolated fish DNA	141
---	-----

5.4. PCR for designated primer confirmation	143
---	-----

5.5. Factors tha influence quantity and quality of eDNA	144
---	-----

5.6. Integrity of eDNA	147
------------------------	-----

5.7. Multiplex scope and limitation on rare detection	148
---	-----

5.8. Advancement in digital quantification for rapid detection	150
--	-----

CHAPTER 6 CONCLUSION	152
-----------------------------	-----

6.1. Introduction	152
-------------------	-----

6.2. Designated species-specific markers for <i>C. ocellaris</i> and <i>C. kelberi</i>	153
--	-----

6.3. Validated species-specific primers and probes	153
--	-----

6.4. eDNA captured by species-specific CO1 and CK1 markers	154
--	-----

6.5. Future recommendation and precautions	154
--	-----

6.6. Conclusion	155
-----------------	-----

REFERENCE	157
------------------	-----

APPENDIX	183
-----------------	-----



LIST OF TABLES

Table		Page
2.1	List of invasive alien fish species present in Malaysia listed by Department of Fisheries, Malaysia (2007)	9
4.1	Table of variance analysis show the alignment of the mitochondrial DNA sequences.	70
4.2	List of Mitochondria COI sequence retrieved from Genbank for three <i>Cichla</i> sp. These sequences were used as reference to design species-specific primer sets in this research	71
4.3	List of designated species-specific COI sequence for <i>Cichla kelberi</i> labelled as CK where (F) is a forward sequences and (R) is reverse sequence with detail of melting temperature (T _m), GC content and the product size	71
4.4	List of designated species-specific COI sequence for <i>Cichla ocellaris</i> labelled as CO where (F) is a forward sequence and (R) is reverse sequence with detail of melting temperature (T _m), GC content and the product size	72
4.5	List of designated species-specific COI sequence for <i>Cichla monoculus</i> labelled as CM where (F) is a forward sequence and (R) is reverse sequence with detail of melting temperature (T _m), GC content and the product size	72
4.6	List of designated species-specific COI probes sequence for <i>Cichla kelberi</i> and <i>Cichla ocellaris</i> with detail of fluorescent label and channel	73
4.7	List and detail of the <i>Cichla</i> spp. collected from Tasik UniSEL, Kampung Kelawar, and Tasik Raban as identification reference	74
4.8	List and detail of the native fish samples that have been collected from Tasik Raban	81





4. 9 Summary of the amplification 18 designated primers with DNA of twelve PB samples for different *Cichla* spp. AGE picture shows that the presence of band which indicate the presence of amplified DNA at 300~ bp in size 92
4. 10 Digital PCR (dPCR) table shows the result of individual well have different reaction testing singleplex or multiplex amplification between PB12 and PB14 with primer-probe CK1 and CO1. NTC or non-template-control used as negative control in this reaction. The results were presented by the concentration of the copies/amplified product, valid partition and positive partition detected, percent of positive partition over the total valid partition and the CI value with confidence of 95% 105
- 4.11 List of water samples collected and replicated for each site. Depth of water for each collection together with water pH value and coordinate of the location were recorded as listed. The water samples were filtered for the range of 21 days 110
- 4.12 Table of the summary of the amplification of multiplex reaction. Eight reactions (A, B, C, D, E, F, G, H) assemble three primer pairs from CK, CO, and CM of the designated primer pairs amplify nine selected eDNA samples, TR1A, TR2A, TR3A, TR4A, TR5A, M1A, M2A, M3A, and M4A where in the column is the size of the amplified product (bp) 116
- 4.13 Digital PCR (dPCR) table shows the result of individual well have different reaction testing singleplex or multiplex amplification of 27 eDNA samples with primer-probe CK1 and CO1. Well labeled H2 uses PB4 and PB12 samples as positive control and H3 well is non-template control reaction. The results were presented by the concentration of the copies/amplified product, valid partition and positive partition detected, and the CI value with confidence of 95%. 128





LIST OF FIGURES

Figures		Page
2.1	(A) <i>Figure 2.1</i> (A) <i>Arapaima gigas</i> , source by Stephen Alvarez, 2020 (B) <i>Oreochromis mossambicus</i> , source by Khalaf, 2019 (C) <i>Pygocentrus nattereri</i> , source by Zell, H. 2019 (D) <i>Claria gariepinus</i> , source by Schafer, F., 2018 (E) <i>Belone belone</i> . source by Roberto, 2018 (F) <i>Cichla sp.</i> source by Karelj, 2010 (G) <i>Hypostomus plecostomus</i> source by Covain, R., 2016 (H) <i>Hemibagrus wyckioides</i> source by Fabian, 2020 (I) <i>Geophagus surinamensis</i> . source by Verkennis, 2020 (J) <i>Cichlasoma hybrid</i> . source by Aquadiction, 2021 (K) <i>Potamotrygon motoro</i> , source by Page, A., 2019 (L) <i>Polyodon spathula</i> . source by Dana, C. L., 2019	16
2.2	White Spot Syndrome Virus (WSSV) caused by <i>Ichthyophthirius multifiliis</i> , parasite. Photograph by Heiko, B., 2016	17
2.3	Collection of <i>Cichla</i> species. Photograph by Khaleel et al., 2021	29
2.4	QIAcuity Nanoplates for Digital PCR (dPCR). Photograph by QIAGEN	41
3.1	Illustration of experimental design for this research.	43
3.2	Maps of sampling location (1) Kampung Kelawar, Tanjung Malim (2) Tasik Universiti Selangor (3) Tasik Raban, Lenggong	46
3.3	Photograph of Tasik Raban	47
3.4	Photograph of Mini Amazon lake.	47





3.5	Photograph of fish samples (<i>Cichla</i> spp.) collected from Kg. Kelawar, Tanjung Malim	52
3.6	Photograph of characteristics of reference <i>cichla</i> spp. from the Sastraprawira et al., (2020) study	54
3.7	Van Dorn water sampler equipment, Photograph by Sciencefirst	62
3.8	pH meter used to take pH reading of water while water sampling	63
3.9	Filtration vacuum machine attached to the filter glass apparatus	64
3.10	QIAGEN's Qiacuity Instruments for digital PCR. Photograph by QIAGEN	67
4.1	Quality of DNA extraction in gel electrophoresis for (a) all 13 <i>Cichla</i> spp. and (b) for native fish samples	87
4.2	AGE results show band formation for all fish <i>Cichla</i> spp. samples labelled as PB and native sample labelled N, with estimated size 600 bp	89
4.3	The Neighbor-joining tree shows the clustering of PB samples together with reference sequence and five native species sequence	90
4.4	Photograph of fluorescence imaging for digital PCR reaction detects the fluorescence signal where partition of positive and negative partition was counted and separated with red line threshold (a) CO1 reaction with yellow channel (b) CK1 reaction with green channel	107
4.5	Maps that locate the sites of water and fish sampling area labelled as TR (Tasik Raban) and M (Mini Amazon). PB indicates the site where the fish samples were collected (PB 10 collected at M1 site while PB 11 was collected at TR 2 site and PB12 was collected at M4 site)	109
4.6	A photograph of water samples that have been filtered and incubated with a lysis buffer	112
4.7	AGE for 15 eDNA samples collected from Tasik Raban labelled as TR1A, TR1B, TR1C, TR2A, TR2B, TR2C, TR3A, TR3B, TR3C TR4A, TR4B, TR4C, TR5A, TR5B, and TR5C.	113





- 4.8 AGE for 12 eDNA samples collected from Mini Amazon labelled as M1A, M1B, M1C, M2A, M2B, M2C, M3A, M3B, M3C, M4A, M4B, and M4C. 114
- 4.9 Photograph of fluorescence imaging for digital PCR reaction detects the fluorescence signal where partition of positive and negative partition was counted and separated with red line threshold (a) CK1 reaction with green channel and (b) CO1 reaction with yellow channel, for TR1A, TR2A, TR2B, TR2C, TR2D, TR3A, TR3B while (C) CK1 (green) and (d) CO1 (yellow), for TR3C, TR4A, TR4B, TR4C, TR5A, TR5B, TR5C sample, (e) CK1 (green) and (f) CO1(yellow) channel for sample M1A, M1B, M1C, M2A, M2B, M3A, M3B, (g) CK1 (green) and (h) CO1(yellow) for M4A, PB4 & PB12, NTC (non-template control) reaction. 136





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	<i>Cichla kelberi</i>
CM	<i>Cichla monoculus</i>
CO	<i>Cichla ocellaris</i>
<i>COI</i>	Cytochrome oxidase subunit 1
<i>cytB</i>	Cytochrome b
ddH ₂ O	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
<i>mt</i>	<i>Mitochondria gene</i>
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 ₂ O	Sterile distilled water
SMS	Sequence Manipulation Suite software
<i>sp.</i>	Species
TBE	Tris base/ Boric acid/ EDTA
T _m	Melting temperature
TR	Tasik Raban
UV	Ultraviolet
V	Voltage
W	Watt
WSSV	White Spot Syndrome Virus





LIST OF APPENDICES

Appendix 1 List of Materials

Appendix 2 List of *Cichla* species reference sequences



CHAPTER 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 (*mtDNA*) *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.