UNIVERSITI PUTRA MALAYSIA

GENETIC IDENTIFICATION OF ACETES SHRIMP FROM ESTUARY TANJUNG DAWAI, KEDAH USING CYTOCHROME OXIDASE I (COI) SEQUENCES

SITI NAQIBAH BT MAKTAR

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This project report is submitted in partial fulfillment of the requirements for the degree of Bachelor of Agriculture (Aquaculture)

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CERTIFICATION OF APPROVAL

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This is to certify that I have examined the final project report and all corrections have been made as recommended by the panel of examiners. This report complies with the recommended format stipulated in the AKU4999 project guidelines, Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia.

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ABSTRACT

Species identification by using mitochondrial DNA is a convenient method for species identification. In this study, mitochondrial DNA (mtDNA) partial Cytochrome oxidase I (COI) gene was used to identify Acetes shrimps from Tanjung Dawai, Kedah. DNA was extracted by Promega kit protocols. Out of 15 samples from 18 samples show the presence of DNA bands. However, due to some troubleshooting, the PCR optimization of the Acetes samples failed to be obtained from this study. The unsuitable protocol kit use, the condition and ways of preservation samples as well as amount of Proteinase K use are the major causes that lead to poor yield of DNA product during DNA extraction process. Meanwhile in polymerase chain reaction method, the failures in obtaining PCR products are because of the contamination problems in samples itself, the laboratory surfaces and devices and also the reagent and step apply during the processes. Overall, this study did not fulfill its objective in genetically identifying the shrimp of genus Acetes.

Keyword: species identification, *Acetes* shrimp, Cytochrome oxidase I (COI)

ABSTRAK

Identifikasi spesis dengan mengunakan DNA mitokondrial adalah satu cara yang mudah untuk mengenalpasti spesis. Dalam kajian ini, DNA mitokondrial gen cytochrome oksida I (COI) telah digunakan untuk mengenalpasti identiti udang Acetes dari Tanjung Dawai, Kedah. DNA telah diekstrak dengan mengunakan kaedah Promega. 15 sampel daripada 18 sampel menunjukkan kehadiran band DNA. Walau bagaimanapun, disebabkan beberapa masalah, optimum PCR bagi sampel Acetes gagal diperolehi dalam kajian ini. Penggunaan kit protokol yang tidak sesuai, keadaan dan cara pengawetan sampel selain jumlah penggunaan Proteinase K adalah sebab utama yang membawa kepada hasil produk DNA yang lemah semasa proses pengekstrakan. Sementara itu, di kaedah tidak balas rantai polimerase, kegagalan mendapatkan produk PCR adalah kerana masalah kontaminasi di dalam sampel itu sendiri, permukaan makmal dan peralatan dan juga reagen serta langkah yang digunakan semasa proses. Secara keseluruhannya, kajian ini tidak memenuhi objektifnya dalam pengenalpastian secara genetik udang dari genus Acetes.

Kata kunci: identifikasi spesis, udang Acetes, cytochrome oksida I (COI)

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LIST OF ABBREVIATIONS/ SYMBOLS

BLAST	Basic local alignment search tool
COI	Cytochrome Oxidase I
Cyt b	Cytochrome b
ddH2O	Deionised distilled water
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleotide triphosphate
g	Gram
kb	Kilobases
mtDNA	Mitochondrial DNA
MgCl2	Magnesium chloride
PCR	Polymerase chain reaction
TBE	Tric Borate EDTA
cm	Centimeter
µL	Microliter
°C	Degree Celcius

CHAPTER 1

INTRODUCTION

Acetes is a genus of shrimp from family Sergestidae. It mainly called as a group of small planktonic shrimps with a body length of adults ranging between 1 and 4 cm. The females Acetes are usually larger than the males Acetes in size (Omari, 1975). Their body appearances are translucent or semi-translucent, with several pairs of red pigment spots (chromatophores) on the bases of uropods and with black eyes. They are extensively caught by push nets, bag nets, and seines. Genus Acetes habitat are mainly in the estuaries and coastal waters of the tropical and subtropical regions (Holthuis, 1980).

Generally, there are fourteen species and five subspecies have been recognized; *Acetes americanus* Ortmann, 1893 - aviu shrimp (*Acetes americanus* ssp. Americanus and *Acetes americanus* ssp. Carolinae); *Acetes binghami* Burkenroad, 1934 ; *Acetes chinensis* Hansen, 1919 – northern mauxia shrimp; *Acetes erythraeus* Nobili, 1905 – tsivakihini paste shrimp; Acetes indicus H. Milne-Edwards, 1830 – Jawala paste shrimp; *Acetes intermedius* Omori, 1975 – Taiwan mauxia shrimp; *Acetes japonicus* Kishinouye, 1905 – akiami paste shrimp; *Acetes johni* Nataraj, 1947; *Acetes marinus* Omori, 1975; *Acetes natalensis* Barnard, 1950; *Acetes paraguayensis* Hansen, 1919; *Acetes serrulatus* (Krøyer, 1859) – southern mauxia shrimp; *Acetes sibogae* Hansen, 1919 – alamang shrimp

(*Acetes sibogae* ssp. Australis, *Acetes sibogae* ssp. sibogae, *Acetes sibogae* ssp. Sibogalis) and *Acetes vulgaris* Hansen, 1919 – jembret shrimp.

There are ten species of genus Acetes that have distributed well in the Indo-West Pacific, and the Indo Malayan region. Only one species is restricted to Pacific America with two species are found in Atlantic America and no species are known either from East Atlantic Mediterranean or from the islands of Central Pacific (Omari, 1975). According to Amin *et al.,* (2009), the main uses of the shrimp Acetes are as fermented food (shrimp paste) and as a dried product. It is also known for commercial importance for human food (Holthuis, 1980; Omari, 1978) and became potential used as food organisms in aquaculture (Deshmukh, 1991; Job *et al*., 2006) as well as important in food webs of coastal waters (Xiao and Greenwood, 1993).

Genetic identification is useful in identifying Acetes shrimp since the species are mostly look alike. While most the study on genus Acetes are toward identifying the biology, distribution and abundance of these commercially important where reported briefly in Peninsular Malaysia and East Malaysia (Omari, 1975; Amin *et. al.,* 2009; Amin *et. al.,* 2008; Arshad *et. al.,* 2008 and Amin *et. al.,* 2011), little is known about their genetic diversity level and patterns and population structure. In order to overcome and to conserve their existing resources for long term sustainable yields, the information on genetic diversity and population structure of Acetes species will be sought for assessment and

management of stocks (Allendorf and Luikart, 2006; Carvalho and Hauser, 1994; Thorpe *et al.,* 2000; Ward and Grewe, 1994).

The result of the analysis will be referring to the Genetic Species Concept as discussed by Baker and Bradley (2006). The Genetic Species Concept was applied by using genetic data from mitochondrial and nuclear genomes to identify species and species boundaries where it can give accurate information on different genome and population of the species. In addition, based on research by Baker and Bradley (2001) stated that genetic distance values lower than 2% were indicative of intra-specific variation; values between 2% to 11% had a high probability of being indicative of con-specific populations or valid species and merit additional study concerning specific status; and values higher than 11% were indicative of specific recognition.

Thus, the objective of this study is:

1. To genetically identify shrimp of the genus Acetes from the estuary of Tanjung Dawai, Kedah, Peninsular Malaysia using Cytochrome Oxidase I (COI) sequences.

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