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Molecular Taxonomic Studies on Some Helminth Parasites Infecting Certain Marine Fish

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بسم الله الرحمن الرحيم

"وَهُوَ الَّذِي سَخَّرَ الْبَحْرَ لِتَأْكُلُوا مِنْهُ لَحْمًا طَرِيًّا وَتَسْتَخْرِجُوا مِنْهُ حِلْيَةً تَلْبَسُونَهَا وَتَرَى الْفُلْكَ مَوَاخِرَ فِيهِ وَلِتَبْتَغُوا مِنْ فَضْلِهِ وَلَعَلَّكُمْ تَشْكُرُونَ"

صدق الله العظيم

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List of contents

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

This study was conducted on three different levels, the first level was to study some of the factors affecting the prevalence of the helminthes parasites such as fish type, fish gender and fish body weight. This investigation included fifteen different helminth parasites which were belongs to Nematoda, (six types), Cestodes, (six types) and Acanthocephala, (three types).

The second level was to distinguish between these fifteen helminthes parasites under investigation morphologically using the light and scanning electron microscopes.

The third level was to discriminate between some of these fifteen helminthes parasites under investigation which collected from Egyptian and Libyan coasts on the molecular level using amplified fragment length polymorphism (AFLP).

The results indicated that in General, fish infection with helminthes was greater in the Egyptian coast than in the Libyan coast of the Mediterranean Sea.

In relation between the fish gender and the prevalence of the helminth parasites, the results indicated that, the infection ratio over all the twelve different fish types under investigation was higher in males than in females in both of Egypt and Libya.

In respect of the relation between fish body weight and the prevalence of helminthes parasites, the results indicated that, the infection ratio over all the twelve different fish types under investigation was higher in high fish body weight than in low fish body weight in both of Egypt and Libya.

The results of helminthes morphological investigation using light and scanning electron microscope, this part of the study could help to know the characteristic features of each one of them, which can aid in the differentiation between the similar species.

On the molecular level, six AFLP-primer pairs were used to detect the genetic variation among twelve helminthes collected from Egyptian coasts and twelve helminthes collected from Libyan coasts and to draw the consensus tree which revealed the genetic relationships among these twenty four helminthes parasites on the base of their banding patterns in AFLP protocol. The results of this part of the investigation revealed that, a total of 1023 bands were developed in the twelve helminthes parasite which collected form Egyptian costs and 614 bands were developed in the twelve helminthes parasite which collected form Libyan costs. Among these developed bands 160 and 141 different markers were detected among the Egyptian samples and Libyan samples, respectively. Each marker was specific for a particular helminthes genotype. Also, 863 and 141polymorphic bands were developed among the twelve Egyptian and the twelve Libyan samples, respectively.

The consensus tree for the twelve helminthes under investigation from Egypt and the twelve helminthes from Libya under investigation revealed that, the helminthes isolated from Egypt were completely diverged than those which isolated from Libya.

Similarity indexes results showed that, the two most similar genotypes i.e. the two most closely related genotypes were *Pseudoteranova decipiens* genotype$_2$ and *Pseudoteranova decipiens* genotype$_3$ (both were from Egypt) with similarity value of 0.52012. On the other hand, the two most distantly related genotypes were *Philometra* genotype$_2$ from Egypt and *Pseudoteranova decipiens* from Libya with similarity value of 0.20578.

CHAPTER I

INTRODUCTION

The importance of fishes as a rich source of protein, especially with the rapidly increasing Human populations and the animal protein shortage problem all over the World cannot be denied **(Osman, 2000).** With the ever increasing need for cheap sources of protein , more and more attention is being focused on fish , both from natural and fish farming, **(Khalil and polling, 1997)**.

The major component of fish is protein that has a big biological value besids, it contains variable quantities of calcium, phosphate, fat and other nutrients important for human health and growth. Moreover, fish provides the worlds" prime source of high quality protein, as 14-16% of the animal protein consumed worldwide, over one billion people rely on fish as their primary source of animal protein **(Hamilton, 1971).**

Parasitism, a form of symbiosis, is one of the most successful modes of life **(palm and klimple 2007)**. More than half of all plant and animal species on earth are parasites, and probably no organism avoids parasitic infection during its lifetime **(palm and Klimpel 2007)**, and fish parasitology is an important field in a quantic sciences. Fish parasites play a major role in marine biodiversity **(palm, 2004)**. Parasites are of interest for ecological studies because they can indicate environmental conditions such as eutrophication **(palm and Dobberstein, 1999)** and the ecology of the fish hosts, Besids fish parasites can be used as biological indicators for fish stock separation **(Mackenzie, 1983)** as well as for the fish feeding ecology **(Camphell *et al*., 1980, Willims *et al*., 1992 and Palm, 1999)**. Of special interest for the fish consumers are zoonotic parasites, which can cause diseases and devaluate the fish, **(Hoffman, 1967)**.

The helminthic parasites of fish have received a good deal of attention by various parasitologists before the last century (**Wedl, 1861; Fritsch, 1886**

and Looss, 1899& 1986) to understand biological, ecological aspects and host-parasite relationship. There is a usual concept that extensive mortality in the natural environment occurs from the infestation of larger parasites, such as trematodes, nematodes, Cestodes and Acanthocephala that are common in fish (**Bauer, 1958; Bullock, 1961; Williams, 1967; Banhawy *et al*., 1975; Chabaud, 1975; Palm and Ruckert, 2009 and Abdel-Ghaffar *et al*., 2013**). In crowded hatchery conditions, infestation of these larger parasites may often cause death. The host reaction may by expressed in tissue proliferation, degeneration, inflammation and probably in the development of immunity as described by **Davis (1953), Bauer (1958) , Williams and Jones (1967) and Smith and Wootten (1978).**

In the field of molecular genetics there have been major technological advance in recent years providing the ability to sequence multiple markers or whole genomes in a short time span with low costs, e.g. next generation sequencing (**Schuster, 2007; Mardis, 2008; Quail *et al*., 2012**). In fisheries science, multiple molecular markers such as allozymes, mitochondrial DNA, microsatellite and minisatellite loci, random amplified polymorphic DNA (RAPD) and single nucleotide polymorphisms (SNPs), have all been used to analyse stock structure of marine organisms directly (**Carvalho and Hauser, 1994; Thorpe *et al*., 2000; Baldwin *et al*., 2012; Ovenden *et al*., 2013).**

Molecular techniques have the advantage that they allow analyses of the parasite DNA, securing species identification and providing data for phylogenetics. Genomic DNA sequences evolve at different rates, with non-coding, non-transcribed sequences of ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) evolving faster than those that encode essential proteins or nuclear DNA (nDNA), respectively. Molecular anisakid nematode identification started with allozyme analyses including restriction fragment length polymorphism techniques (PCR-RFLPs of

ITS-DNA, as described by **D'Amelio *et al*. (2000); Kijewska *et al*. (2002); Pontes *et al*. (2005)**.These advances have subsequently helped to unveil previously unrecognised patterns of geographic genetic structure in marine organism (**Sala-Bozano *et al*., 2009**).

This investigation aimed to work on Helminthic parasites infecting some of the economically important fish from the Mediterranean Sea coasts of Egypt and Libya. Morphological and morpho-metrical characterization of the recovered parasites will go with the determination of the intensity of parasitic infections as well as the seasonal interaction with the morphological description of the recovered parasites based mainly on the use of light and scanning electron microscope to know the characteristic features to each one of them , which can aid in the differentiation between similar species from diferrent habitats. In addition to clarify the taxonomy of these parasites on the molecular level using nuclear DNA analyses and compare them all together using molecular comparative and phylogenetic tools, could aid in the detection of the exact taxonomy of these parasites .