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Mitochondrial NADH Dehydrogenase gene (mtND2) Phylogeny of Egyptian *Tilapia* species

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Abstract

NADH dehydrogenase is a very important protein and is expressed by the mitochondrial NADH dehydrogenase gene (mtND2). Dehydrogenase enzyme is used to remove hydrogen from its substrate, which is used in the cytochrome (hydrogen carrier) system in respiration to produce a net gain of ATP. Also, it reversibly catalyses the oxidation of NADH to NAD and reduced acceptor. The size of mtDN2 of *Tilapia* species and their hybrids is ~1050 base pairs and was detected by using the polymerase chain reaction technique. To identify the molecular phylogeny and the physical characteristics of mtND2 gene of *Tilapia* species were done by using the restriction fragment length polymorphisms (RFLPs) with some restriction endonucleases(*Acc*I, *Ava*II, *Ava*I, *Sty*I, *Bg1*I and *Eae*I). The PCR-RFLPs of NADH dehydrogenase gene of *Tilapia* species and their hybrids may prove that the gene is quite evolution phylogenetic difference from one species to another. At the same time, This study investigated the feasibility of mitochondrial DNA (mtDNA) based approaches in addressing problems of identification of *Tilapia* species and their hybrids, isolated from the River Nile by using the PCR-RFLPs analysis of mtND2 gene.

Key words; ***Tilapia* spp., PCR-RFLP, mtND2, Polymorphism**

Introduction

Tilapias are a group of 40-50 species of cichlid fish (genus *Oreochromis*, *Sarotherodon* and *Tilapia*) native to Africa, in particular Egypt. The significance of *Tilapia* for human culture predates their modern scientific investigation, e.g., *Oreochromis niloticus*, a geographically widespread *Tilapia*, was already known to the early Egyptian cultures and played a significant role in their lives, as indica -ted by their presence in ancient Egyptian art (Fryer and Iles, 1972). Tilapias continue to have great econo -mic importance because several species are an important source of protein in human diets, particularly in Egypt, and increasingly, through aquacul-ture, worldwide (Pullin and Lowe-Mc Connell, 1982 and Trewavas, 1982). Widespread exploitation and aquaculture of *Tilapia* has lead to their introduction in all tropical regions around the globe, often adversely affecting the natural ichthyofauna.

The first genetic linkage map of the *O. niloticus* linked 162 microsatellite and AFLP markers (Kocher *et al.*, 1998). The second generation map is based on an F2 cross between *O. niloticus* and *O. aureus*, and contains over 500 microsatellite markers (Danley and Kocher, 2001).

Tilapia fish have received wide attention from evolutionary biologists for more than 100 years because of their extremely diverse morphology, behavior, and ecology (Fryer and Iles, 1972; Liem and Osse, 1975; Greenwood, 1978; Trewavas, 1983; Oliver, 1984; Meyer *et al.*, 1990; Keenleyside, 1991; Meyer, 1993; Meyer *et al.*, 1994; Stiassny and Meyer, 1999).

Phylogenies for *Tilapia* genera were inserted at positions suggested by Sodsuk (1993) and Schliewen *et al.* (1994), and Sturmbauer *et al.* (1994), respectively. Previous research on *Tilapia* focused on all aspects of their biology, including behavior, ecology, and evolutionary biology (Keenleyside, 1991). The recently gathered knowledge of *Tilapia* spp. phylogenetic relation -ships (e.g., Oliver, 1984; Stiassny,

1991; Kocher *et al.*, 1993; Meyer, 1993; Zardoya *et al.*, 1996; Kullander, 1998; Streelman *et al.*, 1998 and Farias *et al.*, 1999) has permitted the study of the evolution of their diverse ecology and varied mating and parental care behaviors (Goodwin *et al.*, 1998). Until quite recently, the investigation of phylogenetic relationships among cichlids was restricted to morphological characteristics (Stiassny, 1991 and Kullander 1998). However, although Tilapias are a morphologically extrem -ely diverse group, there are only few morphological characteristics which can be used to examine the intrafamilial relationships (Stiassny, 1991).

The phylogenetic validity of the morphological criterion and the conseq-uential subdivision of *Tilapia* fish have been repeatedly challenged during the last 80 years (Regan, 1920; 1922; Greenwood, 1978; Stiassny, 1991 and Kullander 1998). Even the Egyptian Tilapias are questioned by some modern scientists (Stiassny, 1991; Zardoya *et al.*, 1996; Kullander, 1998 Streelman *et al.*, 1998; Farias *et al.*, 1999; 2000 and 2001).

Tilapias, by Stiassny's definition, are a more reduced grouping, as compared with the taxonomic entity characterized by *Tilapia*-type apophysis by Regan (1920) and corresponds well with the traditional view of the genus *Tilapia*. *Tilapia* was split by Trewavas into several genera, including *Tilapia*, *Oreochromis*, and *Sarotherodon* (Trewavas, 1983).

It is known that dehydrogenase enzyme is used to remove hydrogen from its substrate, which is used in the cytoc-hrome (hydrogen carrier) system in respira-tion to produce a net gain of ATP. Also, it reversibly catalyses the oxidation of NADH (Nicotinamide Adenine Dinucleotide, redu-ced form to NAD (Nicotinamide Adenine Dinucleotide) and reduced acceptor.

The present study determined the DNA PCR-RFLPs of the mitochondrial NADH dehydrogenase subunit 2 (ND2) genes from a representative collection of some Egyptian *Tilapia* to further examine behavioral and morphological hypotheses concerning the evolution of the *Tilapia* spp. This gene was previously analyzed by Kocher *et al.*, (1995) in an effort to elucidate taxonomic relationships among the east African cichlids of Lakes Malawi and Tanganyika.

Material And Methods

Fish Collection*:*

Live *Tilapia* fish were fished from El-Tawfiqi Stream (a branch of the Nile river) and carried to the laboratory in Faculty of Science- Zagazig University-Benha- Egypt. By using the morphometric analysis and the meristic analysis, the *Tilapia* spp. were arduously identified into *T. zillii (Tilapia), O. niloticus* and *O. aureus (Oreochromis), S. galilaeus (Sarotherodon)* and two hybrids (H1 and H2; Azab, in press). The fish were killed and liver pieces were stored in the freezer until the DNA extraction started within one week.

Total DNA content Extraction*:*

Total DNA was extracted from the liver of the *Tilapia* species *(T. zillii, O. niloticus, O. aureus* and *S. galilaeus)* and their hybrids using the UNSET lysis solution (Hugo *et al.*, 1992 and El-Serafy *et al.*, in press). One µl of the total DNA content was checked by 0.8% gel electrophoresis for the presence of DNA, as in Figure 1.

Detection and Amplification of ND2 gene

The mitochondrial NADH dehydro-genase (ND2) gene was PCR amplified using published primers in the flanking methionine (''ND2Met'' 59-CATACCCC AAACATGTTGGT- 39, internal primer number 2, Kocher *et al.* 1995) and trypto-phan (''ND2Trp'' 59- GTSGSTTTTCACT CCCGCTTA- 39, Kocher *et al.*, 1995).

The standard polymerase chain reac-tion program for amplification of nuclear srRNA was: 30-35 cycles; one minute, at 94^0C; two to three minutes, at 45^0C; and three minutes, at 72^0C (El-Serafy *et al.*, in press).

Production and Evaluation of the Mitochondrial ND2 gene RFLPs Profiles*:*

The enzymes were tested including *Acc*I, *Ava*II (Boehringer Mannheim), *Ava*I, *Sty*I (Sigma-Aldrich), *Bg1*I (Amersham, Life Science) and *Eae*I (Roche Applied Science), to distinguish the ND2 gene of

all *Tilapia* species and their hybrids. One microlitre (10-12 units) was used for each digestion reaction, together with 1.2 µl of the respective enzyme buffer for a final volume of 12.2 µl. The digestion was performed for ~3.5 h at ~37°C, and the digestion products were evaluated on 2% TBE-agarose gels and stained with ethidium bromide. Bands were detected upon ultraviolet transillumination and photographed.

Results

Tilapia were identified by using morphometric and meristic analysis into four species and two hybrids. The genus *Oreochromis* included *Oreochromis niloticus* and *Oreochromis aureus*. The third species was *Tilapia zillii* and the fourth was *Sarotherodon galilaeus*. Another two individuals were identified as hybrids (H1 and H2). PCR-RFLPs tools were used in order to validate the evolution of mtND2 of these species taking into consideration the morphometric and meristic analysis. Total genomic DNA (nDNA and mtDNA) was extracted from liver of *Tilapia* species *(T. zillii, O. niloticus, O. aureus, S. galil-aeus* and their hybrids*)* and represented in Figure 1; lanes 1-6 represented *T. zillii, O. niloticus, O. aureus, S. galilaeus*, H1 and H2 DNA genome, in that order. The sizes of the PCR products of the mtND2 gene were ~1050 bp (Figure 2).

*Acc*I restriction endonuclease digested mtND2 gene of *O. auraeus* uniquely into two restriction patterns (~100 and ~ 950 bp, lane 3; Figure 3 and Table 1) and did not react with the mtDN2 gene of the others and their hybrids (Figure 3 and Table 1). The mtDN2 gene of *O. niloticus, O. aureus*, H1 and H2 was digested with *Ava*I into two restriction fragments (~150 and ~900 bp, lanes 2-3 and 5-6; Figure 4 and Table 2), without digesting the same gene of *T. zillii* and *S. galilaeus* (lanes 1 and 4; Figure 4 and Table 2). The restriction enzyme, *Ava*II fragmented mtDN2 gene of *O. aureus, S. galilaeus*, H1 and H2 into same size of two restriction fragments (~350 and ~700 bp, lanes 3-6; Figure 5 and Table 3) whenever did not cut the mtDN2 gene of *T. zillii* and *O. niloticus* (lanes 1 and 2; Figure 5 and Table 3). *O. niloticus, O. aureus* and H2 of *Tilapia* species, their mtND2 gene cut into two bands (~150 and ~900 bp, lanes 2-3 and 6; Figure 6 and Table 4) when treated with *Bg1*I restriction endonuclease. The same restriction endonuclease undigested the genes of *T. zillii, S. galilaeus* and H1 (lanes 1 and 4-5; Figure 6 and Table 4) . *Eae*I restriction enzyme differentiated the mtDN2 gene of *T. zillii* and *S. galilaeus* when digesting this gene into two restriction fragments (~50 and ~1000 bp, lanes 1 and 4; Figure 7and Table 5), whenever the same restriction endonuclease did not digest the same gene of the other species of *Tilapia* species (lanes 2-3 and 5-6; Figure 7and Table 5). *Sty*I restriction endonuclease digested and identified the mtND2 gene of *Tilapia* species and their hybrids into three groups (Figure 8 and Table 6). The restriction endonuclease, *Sty*I gathered *O. niloticus*, H1 and H2 in a group when digesting their mtND2 gene into three fragment patterns (~50, ~100 and ~900 bp, lanes 2 and 5-6), mtND2 of *T. zillii* and *O. aureus* in another group with two restriction fragments (~50 and ~1000 bp, lanes 1 and 3) while the same enzyme clustered the mtND2 of *S. galilaeus* in another different group with three different patterns (~50, ~400 and ~600 bp, lane 4).

Table 1: Shows the length of mtND2 genes fragments, resulted from digestion with *Acc*I enzyme in the six *Tilapia* species.

Tilapia spp.	Band 1	Band 2	Band 3
T. zillii	~1050	------	------
O. niloticus	~1050	------	------
O. aureus	~100	~ 950	------
S. galilaeus	~1050	------	------
H1	~1050	------	------
H2	~1050	------	------

Table 2: Shows the length of mtND2 genes fragments, resulted from digestion with *Ava*I enzyme in the six *Tilapia* species.

Tilapia spp.	Band 1	Band 2	Band 3
T. zillii	~1050	------	------
O. niloticus	~150	~900	------
O. aureus	~150	~900	------
S. galilaeus	~1050	------	------
H1	~150	~900	------
H2	~150	~900	------

Table 3: Shows the length of mtND2 genes fragments, resulted from digestion with *Ava*II enzyme in the six *Tilapia* species.

Tilapia spp.	Band 1	Band 2	Band 3
T. zillii	~1050	------	------
O. niloticus	~1050	------	------
O. aureus	~350	~700	------
S. galilaeus	~350	~700	------
H1	~350	~700	------
H2	~350	~700	------

Table 4: Shows the length of mtND2 genes fragments, resulted from digestion with *Bg1*I enzyme in the six *Tilapia* species.

Tilapia spp.	Band 1	Band 2	Band 3
T. zillii	~1050	------	------
O. niloticus	~150	~900	------
O. aureus	~150	~900	------
S. galilaeus	~1050	------	------
H1	~1050	------	------
H2	~150	~900	------

Table 5: Shows the length of mtND2 genes fragments, resulted from digestion with *Eae*I enzyme in the six *Tilapia* species.

Tilapia spp.	Band 1	Band 2	Band 3
T. zillii	~50	~1000	------
O. niloticus	~1050	------	------
O. aureus	~1050	------	------
S. galilaeus	~50	~1000	------
H1	~1050	------	------
H2	~1050	------	------

Table 6: Shows the length of mtND2 genes fragments, resulted from digestion with *Sty*I enzyme in the six *Tilapia* species.

Tilapia spp.	Band 1	Band 2	Band 3
T. zillii	~50	~1000	------
O. niloticus	~50	~100	~900
O. aureus	~50	~1000	------
S. galilaeus	~50	~400	~600
H1	~50	~100	~900
H2	~50	~100	~900

Fig. 1

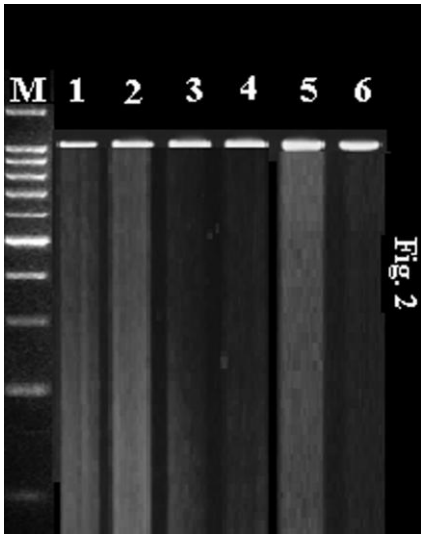

Fig. 2

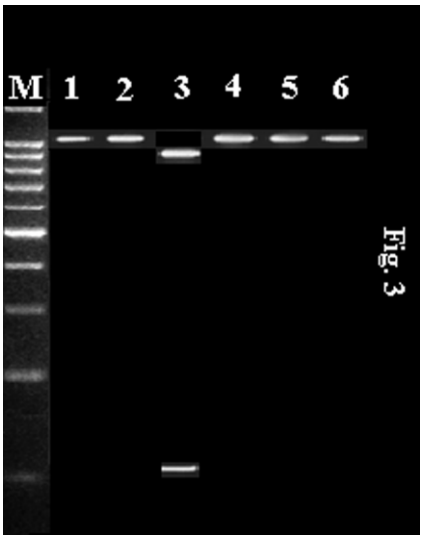

Fig. 3

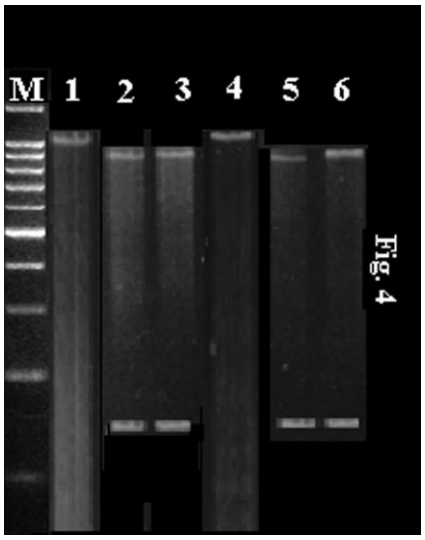

Fig. 4

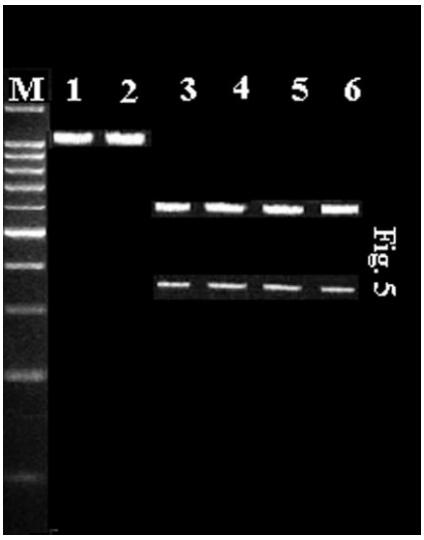

Fig. 5

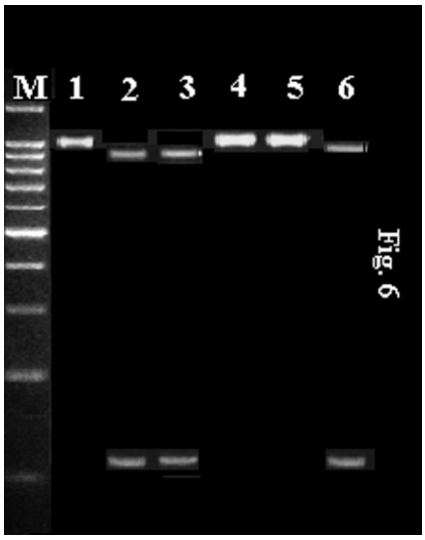

Fig. 6

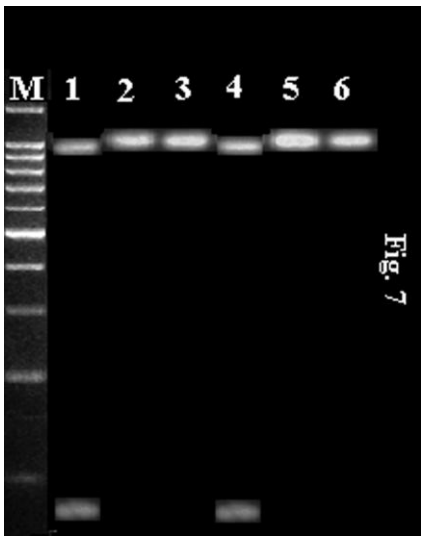

Fig. 7

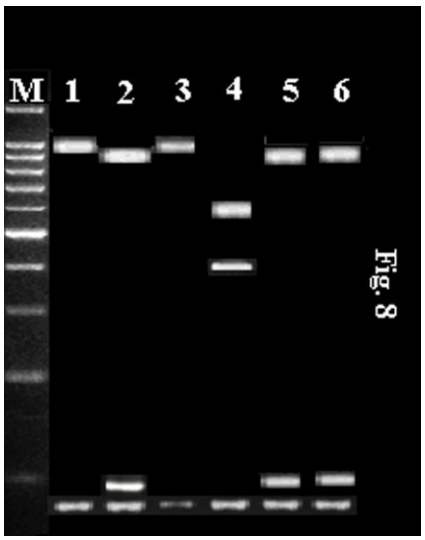

Fig. 8

Figure 1: DNA genome from *Tilapia* spp.. Lane M is the 1 kb DNA ladder. Lanes 1 – 6 represent the DNA genome of *T. zillii, O. niloticus, O. auraeus, S. galilaeus,* H1 and H2 respectively.

Figure 2: Symbolized full-segment mtND2 (~1050 bp) of *Tilapia* spp.. Lane M is the 1 kb DNA ladder. Lanes 1 – 6 represent srDNA of *T. zillii, O. niloticus, O. aureus, S. galilaeus,* H1 and H2 respectively.

Figure 3: shows *Acc*I restriction enzyme digested the mtND2 of *O. aureus* uniquely into two different band sizes (~100 and ~950 bp, lane 3) whereas *T. zillii, O. niloticus, S. galilaeus,* H1 and H2 genes were not digested at all by this restriction enzyme (lanes 1, 2 and 4-6).

Figure 4: shows the representative RFLPs patterns from *Tilapia* spp. with *Ava*I restriction endonuclease, which produced roughly the same fragments (two bands) with *O. niloticus, O. aureus,* H1 and H2 (~150 and ~900 bp, lanes 2-3 and 5-6) whereas *T. zillii* and *S. galilaeus* genes were not digested at all by this restriction enzyme (lanes 1and 4).

Figure 5: shows *Ava*II restriction enzyme digested the mtND2 of *O. aureus, S. galilaeus,* H1 and H2 to two different band sizes (~350 and ~700 bp, lanes 3-6) whereas *T. zillii* and *O. niloticus* genes were not digested at all by this restriction enzyme (lanes 1and 2).

Figure 6: shows the representative RFLPs patterns from *O. niloticus, O. aureus* and H2 (two bands; ~150 and 900 bp; lanes 2, 3 and 6); *T. zillii, S. galilaeus* and H1 were not digested at all (lanes 1, 4 and 5) with *Bg1*I restriction endonuclease.

Figure 7: shows *Eae*I restriction enzyme digested the mtND2 of *Tilapia zillii* and *S. galilaeus* into two restriction fragments (~50 and ~1000 bp; lanes 1 and 4) whereas, *O. niloticus, O. aureus,* H1and H2 mtND2 genes were not digested at all by this restriction enzyme (lanes 2, 3, 5 and 6).

Figure 8: shows *Sty*I restriction enzyme digested the mtDN2 of *O. niloticus, S. galilaeus,* H1 and H2 to three different band sizes (~50, ~100 and ~900 bp, lanes 2, 4, 5 and 6) whereas *T. zillii* and *O. aureus* mtDN2 genes cut into two restriction patterns (~50 and ~1000 bp; lanes 1and 3).

Discussion

Differences in rates of evolution between and within mtND2 gene regions have important implications for phylogeny reconstruction and understanding the morphological, physiological, behavioral and ecological characteristics of the organisms specially *Tilapia* species (Fryer and Iles, 1972; Liem and Osse, 1975; Greenwood, 1978; Trewavas, 1983; Oliver, 1984; Meyer *et al.*, 1990; Keenleyside, 1991; Meyer, 1993; Meyer *et al.*, 1994 and Stiassny and Meyer, 1999).

These differences have been well documented for several gene regions (Holmquist *et al.*, 1983; Li and Graur, 1991 and El-Serafy *et al.*, in press). Ideally, differences in rates of evolution within and between gene regions would be reflected in phylogenetic weighting schemes by giving more weight to relatively conserved sites and substitution types which occur less frequently, thus emphasizing sites and changes with a lower probability of homoplasy.

Comparisons of the evolution of mitochondrial gene regions (Jacobs *et al.*, 1988; Li and Graur, 1991; Mindell and Thacker, 1996 and Russo *et al.*, 1996) suggest considerable variation in rates of change within and between gene regions. Comparisons of widely divergent taxa suggest differences in the rate and mode of evolution of NADH dehydrogenase subunit 2 (ND2) between the different species (Jacobs *et al.*, 1988; Meyer, 1994 and Russo *et al.*, 1996). It is important to determine whether or not these differences in constraints are evident at all taxonomic levels and whether these differences cause sequences of the mtND2 gene regions to be phylogenetically incongruent (Johnson and Sorenson, 1998).

Englander and Moav, 1989; Wright, 1989; Franck *et al*., 1992; Seyoum and Kornfield, 1992; Agnese *et al*., 1997; Rognon *et al*., 1996; Farias *et al*., 1999 and El-Serafy *et al.*, (in press) used restriction fragment length polymorphisms of nuclear

and mitochondrial DNA PCR products (RFLPs\PCR) as a basis for examining relationships among *Tilapia* spp. and finding out if the species is monophylogenetic or polyphylogenetic species and discovering specific enzymes to identify individual subspecies.

The present study clarified the evolution phylogeny of the NADH dehydrogenease gene (mtND2) of the *Tilapia* species in Egypt by using PCR/RFLPs technique. Uniquely, AccI restriction endonuclease differentiated mtND2 of the *O. auraeus* of the *Tilapia* species. Thus, mtND2 gene of *O. auraeus* is polyphylogenetic relationship when compared with the other species gene and differ than the others in the sequence. Also, mtND2 of *O. niloticus*, *O. auraeus*, H1 and H2 is evolutional phylogeneric difference with *T. zillii* and *S. galilaus*, when reacted with *Ava*I restriction enzyme. The evolution phylogeny of mtND2 of *O. auraeus*, *S. galilaeus*, H1 and H2 is different than the gene of *T. zillii* and *O. niloticus* when their gene fragmented with *Ava*II restriction enzyme. The ristriction endonuclease *Bg1*I differentiated the mtND2 gene of *Tilapia* species into two groups ; *O. niloticus*, *O. auraeus* and H2 as a group and *T. zillii*, *S. galilaeus* and H1 as a group. While *Eae*I restriction enzyme differentiated the gene of *T. zillii* and *S. galilaeus* without cutting in the gene of the rest of *Tilapia* species gene. *Sty*I restriction endonuclease clustered the mtND2 gene of *Tilapia* species, evolution phylogenetically, to three clusters when grouped the gene of *O. niloticus*, H1 and H2 in one group, the gene of *T. zillii* and *O. auraeus* in another and the mtND2 gene of *S. galilaeus* only in a separate group.

The PCR-RFLPs of NADH dehydrogenase gene of *Tilapia* species and their hybrids may prove that the gene is quite evolution phylogenetic difference from one species to another. At the same time, This study investigated the feasibility of mitochondrial DNA (mtDNA) based approaches in addressing problems of identification of *Tilapia* species and their hybrids, isolated from the River Nile by using the PCR-RFLPs analysis of mtND2 gene.

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العلاقات التطورية لجين إنزيم الناده ديهيدروجينيز الخاص بالميتوكندريا فى أسماك البلطى المصرى

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إن أسماك البلطى تعتبر من أهم أنواع الأسماك على الإطلاق التى تعيش فى المياه العذبة فى مصر, ولهذا فإنها تستخدم فى التغذية وفى مجالات اقتصادية عديدة نظرا لجودة لحومها ومعدل تكاثرها السريع.

هذه الأسماك هى أسماك البلطى التى تتميز عن غيرها بالتنوع إلى درجة كبيرة مما دفع الباحثين إلى البحث فى تمييز هذه الأنواع بعضها من بعض وإيجاد خصائص مميزة لكل نوع منها عن النوع الآخر. لذا فقد استهدفت الدراسة الأربعة أنواع الرئيسية وهم *الأوروكروماس نيلوتيكس*, *الأوروكروماس أوريا*, *الساروثيرودون جالليوس والتيلابيا زيللى* ومعهم اثنين من الهجن (الهجين 1 و الهجين 2) وذلك بعد التعرف والتمييز على هذه الأنواع بالطرق التقليدية وهى تحديد الصفات المورفومترية والصفات الميريستمية.

يهدف هذا البحث دراسة العلاقات التطورية للجين الذى ينتج إنزيم الناده ديهيدروجينيز الخاص بالميتوكندريا هى هذه الأسماك وذلك لأهميته, حيث أن هذا الإنزيم وبالاشتراك مع السيتوكروم ب ينتج الطاقة وكذلك تكوين الأدنين ثلاثى الفوسفات وذلك من خلال عملية كريبس وبالتالى عملية التنفس للخلية والكائن الحى. . وعليه استخدمت تقنية التغاير فى طول القطع المحددة لجين الناده ديهيدروجينيز الخاص بالميتوكندريا للأنواع الست سالفة الذكر, والذى يختلف حسب الطراز الذى ينتمى اليه الكائن الحى.

وقد تم عن طريق الفرد أو الفصل الكهربى استخلاص جينات إنزيم الناده ديهيدروجينيز الخاص بالميتوكندريا بطريقة التفاعلات المتتابعة لإنزيم البلمرة. وقد وجد أن مورث إنزيم الناده ديهيدروجينيز الخاص بالميتوكندريا لجميع العزلات عبارة عن حوالى 1050 من أزواج القواعد النيتروجينية.

وقد وجد أنه عند هضم الجين بإنزيمى *Acc*I أن سلالة جنس *الأوروكروماس أوريا* هى التى قد هضم ولم تقطع جينات باقى السلالات وكذلك هجنهم أعطت قطعتان (حوالى 100 و 950 من أزواج القواعد). وعلى هذا فإن جين إنريم الناده ديهيدروجينيز الخاص بالميتوكندريا لسمكة *الأوروكروماس أوريا* له صفات تطورية خاصة ومختلفة عن جينات الأنواع الأخرى من أسماك البلطى.

كذلك فإن جين إنريم الناده ديهيدروجينيز الخاص بالميتوكندريا لنوعى *الساروثيرودون جالليوس والتيلابيا زيللى* لهما علاقات تطورية متشابهة فيما بينهما ومختلفة عن بلقى الأنواع. حيث أن إنزيم القطع AvaI قد قطع جين الأنواع الأربع (*الأوروكروماس نيلوتيكس*, *الأوروكروماس أوريا*, الهجين 1 والهجين 2) إلى قطعتين (حوالى 150 و 900 من أزواج القواعد) ولم يهضم الجين لنوعى *الساروثيرودون جالليوس والتيلابيا زيللى*.

وعند دراسة العلاقات التطورية لجين إنزيم الناده ديهيدروجينيز الخاص بالميتوكندريا لكل هذه الأنواع من الأسماك عن طريق هضم جينات هذه الأسماك بإنزيم القطع *Ava*II وجد أن هناك علاقات تطورية متشابهة لأنواع *الأوروكروماس أوريا*, *الساروثيرودون جالليوس*, الهجين 1 والهجين 2(حوالى 350 و 700 من أزواج القواعد) بالمقارنة عن نوعى *التيلابيا زيللى* و*الأوروكروماس نيلوتيكس* حيث لم يهضم جينهما بإنزيم القطع *Ava*II.

قد تبين أن هناك علاقات تطورية متشابهة بين جين إنزيم الناده ديهيدروجينيز الخاص بالميتوكندريا لثلاث أنواع من أسماك البلطى (*الأوروكروماس نيلوتيكس, الأوروكروماس أوريا* و الهجين 2) جين هضمت جينات هذه الأسماك بإنزيم القطع *Bg1*I وأعطى قطعتين (حوالى 150 و 900 من أزواج القواعد) ولم يؤثر هذا الإنزيم على جين إنزيم الناده ديهيدروجينيز الخاص بالميتوكندريا للثلاثة الأنواع الأخرى.

لقد أوضح إنزيم القطع *Eae*I عند هضمه لجين إنزيم الناده ديهيدروجينيز الخاص بالميتوكندريا لأسماك البلطى أن هناك علاقات تطورية متشابهة واضحة بين جين نوعى *التيلابيا زيللى و الساروثيرودون جاليليوس* حيث قطع إنزيم القطع جينهما إلى قطعتين (حوالى 50 و 1000 من أزواج القواعد) ولم يهضم هذا الإنزيم جين إنزيم الناده ديهيدروجينيز الخاص بالميتوكندريا للأنواع الأخرى.

إن إنزيم القطع *Sty*I قد قسم العلاقات التطورية لجين إنزيم الناده ديهيدروجينيز الخاص بالميتوكندريا لأسماك البلطى إلى ثلاثة مجموعات وتضم كل مجموعة على علاقات تطورية متشابهة لجين هذه الأفراد. تضم المجموعة الأولى أسماك *التيلابيا زيللى و الأوروكروماس أوريا* حيث هضم جينهما إلى قطعتين (حوالى 50 و 1000 من أزواج القواعد) وتضم المجموعة الثانية أسماك *الأوروكروماس نيلوتيكس*, الهجين 1 والهجين 2 حيث هضم جينهم إلى ثلاث قطع (حوالى 50 , 100 و 900 من أزواج القواعد) وأما المجموعة الثالثة فتضم *الساروثيرودون جاليليوس* حيث هضم جينها إلى ثلاث قطع (حوالى 50 , 400و 600 من أزواج القواعد).

وعلى هذا فإن العلاقات التطورية لجين إنزيم الناده ديهيدروجينيز الخاص بالميتوكندريا لأسماك البلطى ربما تكون واحدة الأصل أومتعددة الصفات الجينيه. كما يبدو أيضا أن التغاير فى طول القطعة المحددة لجين إنزيم الناده ديهيدروجينيز الخاص بالميتوكندريا يمكن استخدامه كمدخل للتعرف على الخصائص المعقدة لهذه الأسماك.

أيضا يمكن القول أنه من المعتقد أن استخدام طرق البيولوجيا الجزيئية يعطى صورة توضيحية عما إذا كان جين إنزيم الناده ديهيدروجينيز الخاص بالميتوكندريا لأسماك البلطى متشابهة فى الشكل والتركيب أم مختلفة وكذلك يمكن من خلال دراسة هذا الجين إعطاء صورة تصنيفية أكثر دقة عن استخدام الشكل التركيبى فى هذه الدراسات.