



Genetic Relationship Between Two Species of Genus *Dicentrarchus* Based on SCoT Markers and SDS-PAGE

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ABSTRACT

In the present study, start codon targeted (SCoT) technique was used for measuring the genetic variability between two fish species of family Moronidae (*Dicentrarchus punctus* and *Dicentrarchus labrax*), these species were collected from the Mediterranean Sea, Port Said, Egypt. Eleven SCoT primers were used in the study of generating different lengths of amplicons that ranged from 150bp to 1800bp (SCoT-2, SCoT-3, SCoT-4, SCoT-6, SCoT-7, SCoT-8, SCoT-9, SCoT-10, SCoT-28, SCoT-35 and SCoT-46). The polymorphism percent ranged from 18% with SCOT-28 primer to 75% with SCOT-6. The genetic similarity between the two species was 75.5%. Protein analysis using Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was also performed in this study producing a total of 22 bands that ranged in size from 19KD to 200KD. Each of the two species produced 22 bands.

INTRODUCTION

The importance of genetic data increases, it can help in conserving and managing of endangered and threatened species (Allendorf and Luikart, 2007). These data also can be used for identification of species and population which have a reduced genetic diversity (Saccheri, *et al.*, 1998). Recently, more complex conservation issues have been addressed by genetic data including effective population size estimation (Peterson and Ardren, 2009; Small *et al.*, 2009).

Morphological characters are not a robust method in identification. In fish especially, morphological plasticity among individuals of the same species is connate. For example, individuals of the same species may differ in the body color depending on habitat, diet regime and season (Price, *et al.*, 2008).

Studying of population structure is done using molecular techniques by analyzing colonization patterns, dispersal and gene flow among populations over a different of geographical scales. Deductions made from datasets can be impacted by the use of variable molecular techniques that based on nuclear DNA such as SCoT markers and

microsatellites or protein analysis such as Sodium Dodecyl Sulfate Polyacrylamide (SDS-PAGE) gel electrophoresis (Duran, *et al.*, 2004).

Molecular analysis has considered as a viable alternative in species identification and taxonomy. Species fitness for surviving in a niche environment is controlled by a group of biological attributes and any changes in the niche environment may lead to genetic drift, polymorphism and mutation as fish adaptation; at molecular level, molecular markers such as DNA marker can be used to detect species or individual uniqueness where individuals of the same species have their own DNA sequence that vary, to some extent, from individuals that belong to other species (Sbordoni, 2010).

In fish identification, the common applied DNA analyses are simple sequence repeat (SSR), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), expressed sequence tag (EST), length polymorphism (RFLP) and single nucleotide polymorphism (SNP) markers (Sampaio, *et al.*, 2003; Teletchea, 2009; Chauhan and Rajiv, 2010; Khoo, *et al.*, 2011; Kress, *et al.*, 2015).

SCoT is a benefit method for assessment of structure, DNA fingerprinting and genetic diversity of different species; this is because this technique was used successfully in a wide range of plant species studies such as grape, potato, rice and mango (Luo, *et al.*, 2010; Gorji, *et al.*, 2011; Xiong, *et al.*, 2011; Amirmoradi, *et al.*, 2012; Gorji, *et al.*, 2012; Guo, *et al.*, 2012; Que, *et al.*, 2014; Vivodík, *et al.*, 2016). SCoT technique uses a single reverse and forward primer (Bhattacharyya, *et al.*, 2013). Advantages of SCoT technique are simple primer design, simple operation, highly effective polymorphism, low cost and good reproducibility (Chen, *et al.*, 2009).

Proteins of the animal cells are species-specific macromolecules; sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a molecular biomarker in which proteins are separated according to their molecular weight; it is an effective technique used for studying variation between species and illustrate the metabolic level of species under the study (Muhammad, *et al.*, 2018)

Studying of the genetic variability of fish species using SCoT technique is limited; so, the present study was aimed to using SCoT for measuring the genetic relationship between a very closely related species of family Moronidae (*Dicentrarchus punctus* and *Dicentrarchus labrax*) and analysis the tissue proteins of the two fish species using SDS-PAGE.

MATERIALS AND METHODS

Samples collection

Two fish species of family Moronidae (*Dicentrarchus labrax* and *Dicentrarchus punctus*) (Fig.1) were obtained from the Mediterranean Sea. Appropriate size of muscle tissues were obtained from the four fish species and were frozen at -20°C.

DNA Extraction and SCoT-Technique

DNeasy Mini Kit (Qiagen) was used in DNA extraction from muscles of the two fish species. NanoDrop was used for measuring the genomic DNA concentration. Eleven SCoT primers (SCoT-2, SCoT-3, SCoT-4, SCoT-6, SCoT-7, SCoT-8, SCoT-9, SCoT-10, SCoT-28, SCoT-35 and SCoT-46) as shown in Table (1) were used in PCR reaction. Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) was used for performing PCR amplification. It was programmed to fulfill 40 cycles after an initial

denaturation cycle for 5 min at 94°C. Each cycle programmed to three steps; a denaturation step for 1 min at 94°C, an annealing step for 1 min at 50°C, and an elongation step for 1.5 min at 72°C.

Detection of the PCR Products

1.5% agarose gel with ethidium bromide was used for running the amplified products and the buffer used was 1X TBE buffer; the run was at 95% volts. UV light was used for visualizing PCR products and a Gel Documentation System (BIO-RAD 2000) was used for photographing.

Data Analysis

The banding patterns resulted from SCoT primers were compared to evaluate the genetic relatedness of fish samples under the study. Products of the amplification were scored as '1' for presence and '0' for absence of bands. The cluster analysis was made through the similarity matrix. The cluster analysis was used for organizing the resulted data into meaningful structures developing the taxonomies (**Sneath and Sokal, 1973**). The distances between accessions are showed by Dice coefficient through PAST program after each accession represented its own cluster.

SDS-PAGE analysis

Muscular proteins were separated based on their molecular weight by Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins of muscle tissue were extracted according to **Fadda et al., (1999)**. one gram of muscular tissue was homogenized with 9 ml PBS (phosphate buffer solution), and then the sample was centrifuged for 15 min at 10000 rpm; 4°C. The supernatant contained proteins and was transferred to a clean eppendorf. Treatment buffer (1% SDS, 10% glycerol, 10 mM Tris-HCL PH 6.8, 1mMEDTA, DTT (dithiolthreitor) and pinch of bromophenole blue) was added to the protein sample and boiled for 5 min at 90°C. Protein solution and protein marker were loaded into polyacrylamide gel; the run was carried out at 100 volt in 1x Tris/glycine- SDS-running buffer. After electrophoresis, staining of the gel was occurred using 50ml of staining solution (10% acetic acid, 0.125% coomassie blue R-250 and 50% methanol), and then gel was dried and photographed. SDS-PAGE was performed according to (**Laemmli, 1970**). Molecular weight of protein patterns were stated according to **Weber et al., (1972)**.

Table 1. The sequence of SCoT primers, A: Adenine, T: Thymine, G: Guanine, C: Cytosine

Primer Name	Sequence
SCoT-02	5'-ACCATGGCTACCACCGGC-3'
SCoT-03	5'-ACGACATGGCGACCCACA-3'
SCoT-04	5'-ACCATGGCTACCACCGCA-3'
SCoT-06	5'-CAATGGCTACCACTACAG -3'
SCoT-07	5'-ACAATGGCTACCACTGAC -3'
SCoT-08	5'-ACCATGGCTACCACGGCA-3'
SCoT-09	5'-ACAATGGCTACCACTGCC -3'
SCoT-10	5'-ACAATGGCTACCACCAGC-3'
SCoT-28	5'-CAACAATGGCTACCACCA-3'
SCoT-35	5'-AACCATGGCTACCACCAC-3'
SCoT-46	5'-ACCATGGCTACCACCGCC-3'

RESULTS

Start codon targeted analysis

In this study, *Dicentrarchus punctatus* and *Dicentrarchus labrax* (Fig.1) of family Moronidae were collected from Mediterranean Sea for Studying the genetic variability between them by using eleven SCoT primers. All eleven primers generated strong amplification profiles with distinct bands that revealed DNA polymorphism between the two species under study as shown in (Figures 2, 3 and 4). Gene profiler computer software program was used for analyzing the banding patterns of the DNA fragments. The eleven SCoT primers detected a total of 122 DNA fragments (Table 3), with an average of 11 fragments per primer. The total number of amplified fragments varied from 7 (SCoT-9) to 16 (SCoT-3) primers. Of the 122 amplified bands, 74 were monomorphic bands and 48 unique bands with polymorphism ranged from 18% to 75% as shown in Table (2).



Fig. 1. Photographs of *Dicentrarchus punctatus* (a) and *Dicentrarchus labrax* (b)

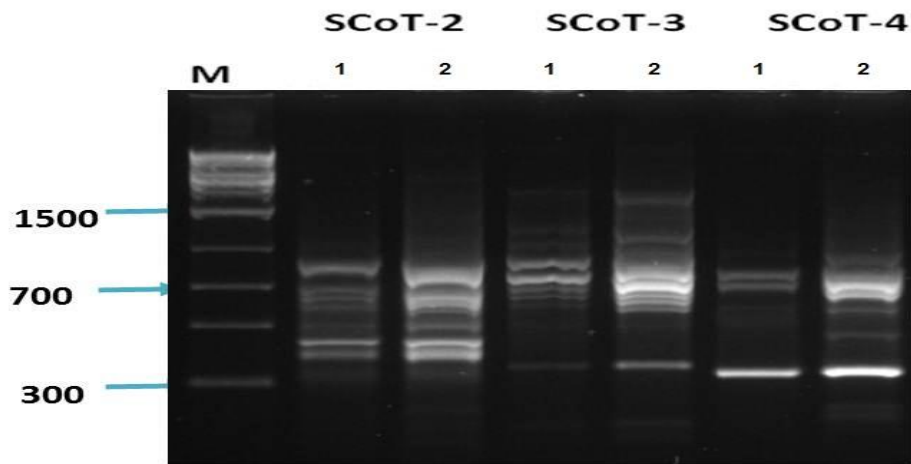


Fig. 2. SCoT profile of two fish species using SCoT primers (SCoT-2, SCoT-3 and SCoT-4). M refers to DNA ladder marker (1- *Dicentrarchus punctatus* and 2- *Dicentrarchus labrax*).

Table 2. Percentage of polymorphism, molecular weight and number of total bands, monomorphic bands, polymorphic and unique bands generated by eleven SCoT primers with two fish species (1- *Dicentrarchus punctus* and 2- *Dicentrarchus labrax*).

Primer code	No. of amplified bands		Total amplified bands	Size of amplified bands	NO. of monomorphic bands	No. of unique bands	Polymorphism%
	1	2					
SCOT-2	10	12	13	250-850bp	9	4	31%
SCOT-3	12	13	16	150-1800bp	9	7	44%
SCOT-4	6	9	10	200-1000bp	5	5	50%
SCOT-6	4	6	8	250-750bp	2	6	75%
SCOT-7	10	9	12	200-1400bp	7	5	42%
SCOT-8	8	7	10	230-800bp	5	5	50%
SCOT-9	6	6	7	250-850bp	5	2	28%
SCOT-10	9	8	11	300-900bp	6	5	45%
SCOT-28	10	10	11	180-500bp	9	2	18%
SCOT-35	9	12	12	170-700bp	9	3	25%
SCOT-46	9	11	12	190-550bp	8	4	33%
Total	93	103	122	150-1800bp	74	48	39%

Following are the amplification results of the two fish species obtained by the examined primers:

Dicentrarchus punctus

SCoT primers with this species produced different band patterns of 93 bands ranged in size from 170 bp in the primer (SCoT-35) to 1800 bp in (SCoT-3). The generated bands ranged in number from 4 in (SCoT-6) to 12 in (SCoT-3).

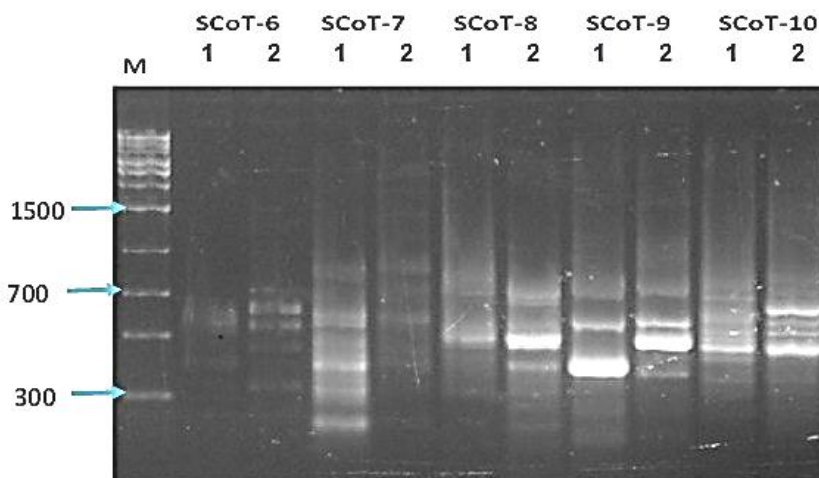


Fig. 3. SCoT profile of two fish species using SCoT primers (SCoT-6, SCoT-7, SCoT-8, SCoT-9 and SCoT-10). M refers to DNA ladder marker (1- *Dicentrarchus punctus* and 2- *Dicentrarchus labrax*).

Dicentrarchus labrax

In this species, SCoT primers produced band patterns of 103 bands ranged in size from 150 bp to 1800 bp in primer (SCoT-3). The generated bands ranged in number from 6 in (SCoT-6 and SCoT-9) to 13 in (SCoT-3). Genetic similarity between the two species was 75.5% as shown in Table (3).

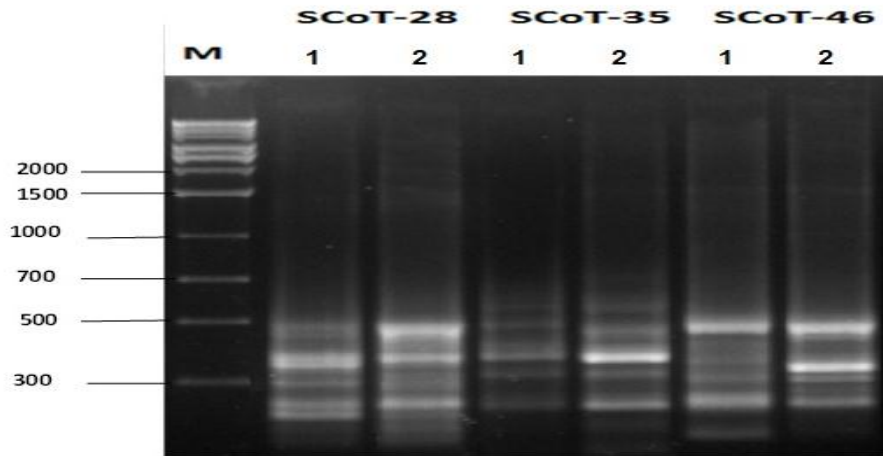


Fig. 4. SCoT profile of two fish species using SCOT primers (SCoT-28, SCoT-35 and SCoT-46). M refers to DNA ladder marker (1- *Dicentrarchus punctus* and 2- *Dicentrarchus labrax*).

Table 3. Averages of genetic similarities (%) estimated by molecular SCoT primers, adopting the arithmetic complement of Jaccard coefficient for two fish species of family moronidae. (1- *Dicentrarchus punctus* and 2-*Dicentrarchus labrax*).

	1	2
1	100	
2	75.5	100

SDS-PAGE analysis

Protein analysis using Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) generated a total of 22 bands that ranged in size from 19KD to 200KD. Each of the two species produced 22 bands as shown in Table (4) and (Fig.5).

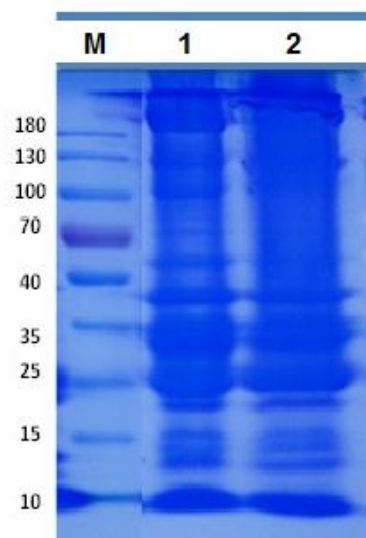


Fig. 5. Protein banding patterns of SDS-PAGE (1- *Dicentrarchus punctus* and 2- *Dicentrarchus labrax*).

Table 4. Bands of SDS-PAGE protein for two fish species of Moronidae (1- *Dicentrarchus punctus* and 2- *Dicentrarchus labrax*).

MW	1	2
200	1	1
178	1	1
169	1	1
137	1	1
127	1	1
124	1	1
109	1	1
99	1	1
92	1	1
85	1	1
71	1	1
59	1	1
46	1	1
42	1	1
36	1	1
33	1	1
32	1	1
30	1	1
26	1	1
24	1	1
22	1	1
19	1	1

DISCUSSION

DNA bands and Muscles protein profile of two species of genus *Dicentrarchus* (*Dicentrarchus punctus* and *Dicentrarchus labrax*) were investigated in this study using SCoT markers and SDS-PAGE. The genetic relationship between two species was indicated.

With the development of DNA-based molecular markers; Start codon target (SCoT) technique was developed based on the conserved sequences that flank ATG start codon of genes. SCoT is a reproducible marker and it can be used for bulk segregation analysis, genetic analysis and quantitative trait loci mapping SCoT is similar to ISSR in using a single primer that acts as the reverse and the forward primer (Collard and Mackill, 2009). Using start codon targeted technique was applied to determine genetic diversity and for quantitative trait loci mapping and DNA fingerprinting in different species (Cabo *et al.*, 2014).

According to Mar'ie and Allam, (2017) there were not studies in polymorphism in fishes based on Start Codon Targeted; at least in Egypt. They began the investigation of the efficiency of this technique on tissues of fishes through estimation of relationships and genetic diversity between three of closely related species in family Carangidae (*Caranx melampygus*, *Carangoides bajad* and *Caranx sexfasciatus* collected from Red Sea of Hurgada, Egypt).

Assessment of polymorphism between fish species is commonly studied through electrophoresis of muscle protein (Haniffa *et al.*, 2017). Proteins in complex extracts are widely analyzed by SDS-PAGE (Sodium dodecyl sulfate- polyacrylamide gel electrophoresis) which is first described by Laemmli, (1970).

The genetic similarity and muscles protein bands among four species of fishes (*Puntius tetrazone*, *Pethia nigrofasciatus*, *Brachydanio rerio* and *Barbonymus schwanenfeldii*) were concluded using molecular marker and SDS-PAGE (Abu Almaaty *et al.*, 2020)

This study indicates the possibility of usage of SCoT technique as a tool for differentiating between the closely related species. The genetic similarity between the two species was 75.5% by using eleven SCoT primers. SDS-PAGE analysis produced 22 bands with molecular weight ranging from 19 to 200 KD.

CONCLUSION

SCoT technique is a famous tool for differentiating between plant species, but it is rarely used in fish species differentiation. This study proved that this technique is a successful tool for measuring the genetic relationship between the closely related species and the author recommends studying the genetic relationship among Moronidae species using more SCoT primers and SDS-PAGE technique.

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