Genetic Variation of Sand Smelt Fish in Egyptian Fisheries

A. Eladl [1,*], M. Hussein [1], S. D. Ibrahim [2], M. F. Abd-Elghany [1]

[1] *Fish Production, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.*

[2] *Agricultural Genetic Engineering Research Institute, Giza, Egypt.*

*Corresponding author E-mail: abdelbaseteladl@azhar.edu.eg (A. Eladl)

ABSTRACT

Molecular markers were used as indicators in assessing the genetic diversity of fish. They allow direct observation of genetic information and estimation of genetic relationships between the population and species. In the present study, five SCoT primers and five ISSR primers were used to estimate the genetic diversity. Fifteen samples of sand smelt fish species (three replicates for each species) were used in the present investigation. Sand smelt fish were collected from five different locations: (Fayoum, Burullus, Suez, Idiko and Port Said). The total number of DNA bands generated by ten primers 139 with an average of 13.9 amplicons /primer. The highest number of amplicon was 17 generated by ISSR-04 and the lowest number was 10 generated by SCoT-03. The total number of polymorphic amplicons was 63 with an average 6.3 / primer. The polymorphic amplicons ranged from 3 to 13 amplicons. Therefore, the ten primers expressed different levels of polymorphism, ranging from 20% with the primer ISSR $_{-2}$ to 81 % with the primer ISSR $_{-01}$. The frequency varied from 0.5 to 0.9 values where primer ISSR$_{-1}$ had the lowest frequency while primers ISSR$_{-02}$ and ISSR$_{-4}$ had the highest value with average 0.74. The polymorphic information content (PIC) varied from 0.24 to 0.37 values where primer ISSR$_{-03}$ had the lowest PIC value while primer ISSR$_{-01}$ had the highest value with average 0.32.

Keywords: Sand Smelt, Deoxyribnuleic Acid (DNA), Polymerase Chain Reaction (PCR), Inter Simple Sequence Repeats (ISSR), Start Codon Targeted (SCOT).

INTRODUCTION

Fish as one of an important source of animal protein, is a component of the traditional Egyptian diet. Fish as one of the main sources of food security will be a major issue facing mankind in the new millennium (Ashraf *et al.,* 2010). Freshwater fish are the most vertebrate groups exploited by humans. In this respect marine water fish species are of the main and important sources for food security (Moralee *et al*., 2000).

Fish production plays a great role in the nutritional foods of the human because of its high source polyunsaturated fatty acids (PUFAs) especially omega-3, omega-6, vitamins and minerals, and relatively low caloric content. These properties could limit atherosclerosis and thrombosis. Also, fishes have different minerals, i.e., iron (Fe), Calcium (Ca), Zinc (Zn), Phosphorus (P), Selenium (Se), Fluorine (F) and Iodine (I). These minerals are with high bioavailability; they can easily be absorbed by the body (Pal *et al.,* 2018) Aquaculture output will need several folds to be increased in order to meet the rising demands for fish in coming years. Biotechnology can provide the means to increase the intensity and capacity of the operation (Sabry *et al.,* 2015).

Atherinidae is represented by two native species (Mediterranean sand smelt, *Atherinahepsetus Linnaeus*, 1758 and Big-scale sand smelt, *Atherina boyeri* Risso, 1810), and one alien species, Red Sea hardy head silverside, *Atherino morusforskalii* (Rüppell, 1838) in the Mediterranean Coast of Turkey1. *A. boyeri* is the most frequent Atherinid species, while the other species are sparse.

Due to the low dispersal capability of these small fishes, the percentage of animals re-entering the native lagoons is probably very high, as is also confirmed by morphological studies (Berrebi and Britton-Davidia, 1980). The significant correlation between genetic and geographical distances among the populations was found, thus suggesting the presence of some migratory move mints along

the coastal line. Occasional exchanges of individuals among populations could be favored by the annual migration of adults toward the coastal sea during the cold season, establishing a pattern of isolation by distance along the coasts.

So aquaculture outputs are needed many aspects to be increased in order to meet the rising demands for fish in the coming years. Biotechnology can provide the means to increase the intensity and capacity of the operation (Sabry *et al.*, 2015). Molecular markers are useful tools for assessing the genetic diversity for individuals, populations and species. The genetic diversity data have varied applications in research on evolution, conservation and management of natural resources and the genetic improvement programs (Frankham *et al.*, 2004; Mahrous *et al.,* 2011). Al-Soudy (2018) studied the genetic diversity, relationships and population structure of sixty Egyptian camels derived from four breeds using ISSR (Inter Simple Sequence Repeat) and SCoT (Start Codon Targeted) primers. The results of the genetic relationship based on both markers (microsatellites and SCoT) confirmed the close relationship between the two breeds. The molecular markers research has made wide possible genetic characterization and biodiversity studies in fish populations (Sugama *et al.*, 2012; El-Tarras *et al.*, 2017). Different molecular markers have been used for studying the genetic diversity for fish such as ISSR, SCoT and barcoding. Moreover, the molecular markers were used to identify sex-associated genomic regions (Luzio *et al*., 2015; Ibrahim *et al*., 2018). The sequence targets of ISSR are available in a eukaryotic genome, thus it's revealed a much higher number of polymorphic fragments per primer than the RAPD markers (Esselman, 1999). ISSRs markers are very useful tools for population differentiation because of its longer length of the primer, highly reproducibility and easy quick handling (Bornet and Branchard, 2001). Genetic diversity has been evaluated using the SCoT among female fish in early development stages (Ibrahim, 2018). SCoT polymorphism has been developed as a new functional marker system depended on the short conserved region flanking the ATG start codon. SCoT markers are generally highly reproducible, targeted marker and could be used for genetic diversity searches, quantitative trait loci (QTLs) mapping and bulk segregation analysis (Nolte *et al.*, 2005; Mehta and Sahani.,. 2014).The microsatellite markers are targeting repetitive sequences, while the SCoT markers were employed to target the polymorphism in sequences near the genes (Penedo *et al.*, 1999; Nouairia *et al.,* 2015). The SCoT Polymorphism technique is similar to RAPD (Randomly Amplified Polymorphic) DNA and ISSR because it uses a single forward and reverses primer. So the SCoT markers are expected to be linked to functional genes and corresponding traits, (Bhattacharyya *et al.*, 2013).The present study was focused on the molecular variability among the five sand smelt species (Fayoum, Burullus, Suez, Adiko, Port Said,) using two molecular markers (SCoT and ISSR). Also, to assess the genetic relationship among the five genotypes, in addition, to identify the unique markers related to some species traits which can differentiate among the studied genotypes by bioinformatics tools.

MATERIALS AND METHODS

Materials:

Fifteen samples of sand smelt fish species (three replicates for each species) were used in the present investigation that collected from five different locations which be: Fayoum, Burullus, Suez, Idiko and Port Said.

Methods:

DNA Isolation and PCR analysis

Total DNA isolation was conducted using the DNeasy Plant Mini Kit (Qiagen, Germany) in compliance with the manufacturer's protocol. A Nano Drop 2000 Spectrophotometer (Thermo Scientific, Germany) was used to estimate the amount and purity of DNA in samples.

ISSR and SCoT marker analysis

Genetic diversity was investigated using ISSR five primers and SCoT Five primers between the studied sand smelt fish species. The PCR amplification of ISSR and SCoT and cycling parameters (Table 3) were carried out

as described by (Ibrahim *et al.*, 2019). The SCoT primers have been developed as (Collard and Mackill 2009) have described previously. The PCR amplification products were separated on 1.5% agarose gels in 1xTBE buffer stained with EtBr. The PCR products were visualized on UV light and photographed using a Gel Documentation System (Bio-Rad, USA).

Data Analysis

The banding patterns generated by SCoT and ISSR markers were compared to determine the genetic relationships among the five sand smelt fish species. Clear and distinct amplification products were scored as (1) for present and (0) for absent bands for all samples. Then, a binary statistic matrix was constructed. Dice's similarity matrix coefficients were then calculated between genotypes using the unweighted pair group method with arithmetic averages (UPGMA). This matrix was used to construct a phylogenetic tree (dendrogram) and Principal coordinate analysis (PCA) was performed according to Euclidean similarity index using the PAST software Version 1.91 (Hammer et al., 2001). The polymorphism information content (PIC) was calculated using the Power Marker software (Liu and Muse, 2005).

RESULTS

Assessment of the genetic diversity and relationship among the five sand smelt species by molecular markers:

The molecular markers research has made wide possible to genetic characterization and biodiversity studies in fish populations (El-Tarras *et al*., 2017). Many properties can be obtained with different molecular marker platforms uses including reliability, reproducibility, coverage, cost and automation (Agrawal *et al*., 2008).

Characterization of the capability of SCoT and ISSR primer to detect polymorphism

The percentage of polymorphism detected by the ten primers (Five ISSR and Five SCoT) among five sand smelt species was shown in Table (2) and Fig. (1). Burullus exhibited the highest value of polymorphism 25% for ISSR-05, while 22.2% among location Fayoum with SCoT-03 as compared to the other primers. The location of Fayoum five primers (ISSR-01, ISSR-03, SCoT-02, SCoT-03 and SCoT-04) produced the value of polymorphism. Also, the location of Suez five primers (ISSR-02, ISSR-03, ISSR-05, SCoT-03 and SCoT-05) produced the value of polymorphism. While, the location of Port Said one primer (ISSR-02) produced the value of polymorphism. SCoT-03 highest value of polymorphism as four locations (Fayoum, Burullus, Suez and Idiko), While, ISSR-04 produced the lowest of polymorphism (0).

Genetic relationships as revealed by ISSR and SCoT markers

In this study, ten primers Five ISSR and Five SCoT were used to assess the genetic diversity among the five groups Fifteen samples sand smelt species. As generated shown in table (3) and Fig. (2 and 3), the total number of DNA bands by ten primers was 139 with an average of 13.9 amplicons /primer. The highest number of amplicon was 17 generated by ISSR-04 and the lowest number was 10 generated by SCoT-03. The total number of polymorphic amplicons was 63 with an average 6.3 / primer. The polymorphic amplicons ranged from 3 to 13 amplicons. Therefore, the ten primers expressed different levels of polymorphism, ranging from 20% with the primer ISSR-02 to 81 % with the primer ISSR-01. The frequency varied from 0.5 to 0.9 values where primer ISSR-01 had the lowest frequency while primers ISSR-02 and ISSR-4 had the highest value with average 0.74. The polymorphic information content (PIC) varied from 0.24 to 0.37 values where primer ISSR-03 had the lowest PIC value while primer ISSR-01 had the highest value with average 0.32. (Fig. 1).

SCoT and ISSR molecular markers have been proven to be an efficient tool to assess genetic diversity within and between species and populations. An efficient number of unique markers differentiating between the different Egyptian sole species was revealed using SCoT and ISSR molecular markers. These markers could be used in fish breeding programs in order to save both time and money. On the other, hand using SCoT for studying sole genetics, suggests it usefulness in studying other fish species.

Finally, the mean polymorphism information content (PIC) represents another measure of DNA polymorphism that reflects the genetic variation. In addition, the PIC is a measure of the marker informativeness and it ranges from 0 to 1. The markers with a PIC higher than 0.5 are highly informative, while, a PIC value between 0.5 and 0.25 implies a locus of moderately informativeness (Bromley, P.J. (2003). The SCoT PIC values ranged from 0.936 to 0.947 with a mean of 0.945, while major allele frequency (MAF) ranged from 0.05 to 0.1 with a mean of 0.064, respectively of SCoT. On the other hand, The PIC and major MAF for ISSR markers were 0.945 and 0.064, respectively.

Genetic similarities matrix of send smelt as revealed by ISSR and SCoT markers

To investigate the genetic similarity among the Fifteen sand smelt species based on ISSR and SCoT results, the scored data obtained from ten primers were analyzed using the Dice coefficient to compute the similarity matrix. This similarity matrix was used to generate a dendrogram using the UPGMA method. As shown in Table (4), the estimated similarities among the Fifteen sand smelt species ranged from 77 to 99%. The highest genetic similarity (99%) was between species 4 and 5 (from Burullus), 11 and 12 (from Adiko), 13 and 14, 13 and 15, 14 and 15 (from Port Said), while the lowest genetic similarity (77) was between genotypes 4 and 14, 4 and 15, 5 and 14, 5 and 15 (4, 5 from Burullus and 14, 15 from Port Said).

Cluster analysis based on ISSR and SCoT markers

The dendrogram of fifteen sand smelt species based on ISSR and SCoT markers using UPGMA and similarity matrix computed according to Dice coefficient (Fig. 4). The dendrogram comprised two main clusters; the first main cluster contained three groups; group one contains (7, 8 and 9) from Suez, group two contains (10, 11 and 12) from Idiko, group three contains (13, 14 and 15) from Port Said. The second main cluster divided into two groups; group one contains (4, 5 and 6) from Burullus. While group two contains (3, 2 and 1) from Fayoum.

Principal coordinates analysis

The relationship observed in the principal coordinate analysis (PCoA) was in agreement with the UPGMA analysis of Fifteen sand smelt species; where group A contains Suez and Idiko, group B comprised Fayoum and Burullus, and group C contains Port Said as shown in (Fig. 5). Whereas the PCoA resulted from ISSR and SCoT marker was classified. The populations in this study were clustered according to the degree of similarity of their habitats and had nothing to do with their geographical location; the results of PCoA analysis also support this habitat-specific genetic clustering model.

CONCLUSION

In the present study, the relationship observed in the principal coordinate analysis (PCoA) was in agreement with the UPGMA analysis of 15 sand smelt species; where group A contains Suez and Idiko, group B comprised Fayoum and Burullus, and group C contains Port Said, whereas the PCoA resulted from ISSR and SCoT marker was classified. The populations in this study were clustered according to the degree of similarity of their habitats and had nothing to do with their geographical location; the results of PCoA analysis also support this habitat-specific genetic clustering model. However, further studies are needed to elucidate this relationship at the molecular level.

REFERENCES

Agrawal, M., Shrivastava, N., Padh, H. 2008. Advances in molecular marker techniques and their applications in plant sciences. Plant Cell Rep., 27(4):617-631.

Al-Soudy, A., El-Sayed, A., El-Itriby, H.A., Hussein, E.H.A. 2018. Assessment of the Genetic Diversity, Breeds Structure and Genetic Relationships in Four Egyptian Camel Breeds using Microsatellite and Start Codon Targeted (SCoT) Markers. Journal of Biodiversity & Endangered Species, 61:S2 DOI: 10.4172/2332-2543.S2-001

Ashraf, I.A., Mariam, M.S., Hebutallah, L. 2010. Reproduction of the Egyptian Sole, *Solea Aegyptiaca*, from Port Said, Egypt,

Mediterranean Sea. ActaIchthyologica Piscatoria. 420 (2): 161–166.

Berrebi, P., Britton-Davidian, J. 1980. Enzymatic survey of four populations of Atherinaboyeribased on electrophoresis and the occurrence of microsporidiosis. J Fish Biol 16:149–157.

Bhattacharyya, P., Kumaria, S., Kumar, S., Tardon, P. 2013. Start Codon Targeted (SCoT) marker reveals genetic diversity of DendrobiumnobilLindl., an endangered medicinal orchid species. Gene, 529(1): 21-26.

Bornet, B., Branchard, M. 2001. Non anchored Inter Simple Sequence Repeat (ISSR) Markers: Reproducible and Specific Tools for Genome Fingerprinting, Plant Molecular Biology Reporter, 19: 209–215.

Bromley, P.J. 2003. The use of market sampling to generate maturity ogives and to investigate growth, sexual dimorphism and reproductive strategy in central and south-western North Sea sole (*Soleasolea* L.). *ICES Journal of Marine Science* (60): 52-65.

Carvalho, G.R., Hauser, L. 1994. Molecular genetics and the stock concept in fisheries, Rev. Fish Biol. Fish, 4: 326-350.

Collard, B.C.Y., Mackill, D.J. 2009. Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. Plant MolBiol Rep 27:86–93.

Congiu, L., Rossi, R., Colombo, G. 2002 Population analysis of the sand smelt Atherinaboyeri (Teleostei Atherinidae), from Italian coastal lagoons by random amplified polymor-phic DNA. Mar Ecol Prog Ser 229:279–289

Durand, J., Shen, K., Chen, W., Jamandre, B., Blel, H., Diop, K., Nirchio, M., García, D., Whitfield, A., Chang, C., Borsa, P. 2012. Systematics of the grey mullets (Teleostei: Mugiliformes:Mugilidae): Molecular phylogenetic evidence challenges two centuries of morphology-based taxonomy, Molecular Phylogenetics and Evolution, 64: 73-92.

El-Tarras, A.E., Shahaby, A., Mohamed, A.A., Hassan, M.M., Gaber, A. 2017. Isolation of safe tissue glue and molecular fingerprinting of Hammour (Greasy grouper) fish. World J. Pharmaceut.life sci., 3: 94-100.

Esselman, E.J., Jianqiang, L., Crawford, D.J., Winduss, J.L., Wolfe, A.D. 1999.Genetic variation and its relationship to population size in reintroduced populations of pink sand verbena, Abroniaumbellata subsp. breviflora (Nyctaginaceae). Ecol. 8: 443-451.

Frankham, R., Ballou, J.D., Briscoe, D.A. 2004. A Primer of Conservation Genetics. Cambridge University Press, London, 220pp.

Healey, A., Mc-Keown, N., Potts, W., de Beer, C., Sauer, W., Shaw, P. 2017. Phylogeny of the *Sepia officinal* is species complex in the eastern Atlantic extends the known distribution of Sepiavermiculata across the Benguela upwelling region, African Journal of Marine Science: 39: 307–313.

Hillis, D.M., Mable, B.K., Larson, A., Davis, S.K., Zimmer, E.A. 1996. Nucleic acids IV: Sequencing and closing, In: D.M. Hillis, C. Moritz, B. Mable (Eds.), Molecular systematics 2nd edn., pp. 342-343. Sunderland,

Ibrahim, S.D., Alsamman, A.M., Abd-Elghany, M.F., Hassan, M.M. 2018. Development of Specific Molecular Marker for Sex Differentiation in Egyptian Fish Solea Using SCOT and ISSR Markers. International Scientific Conference "Agriculture and Futuristic Challenges Faculty of Agriculture-Cairo, Al-Azhar University, Nasr City, Cairo, Egypt April 10th – 12th, 2018, 1(I): 227-239.

Luzio, A., Coimbra, A.M., Benito, C., Fontanhas, A.A., Matos, M. 2015. Screening and identification of potential sex-associated sequences in Daniorerio. Molecular reproduction and development, 82(10):756-764.

Maheswaran, M. 2004. Molecular Marker: History, Features and Applications (Department of Plant Molecular Biology and Biotechnology, Centre for Plant Molecular Biology Tamil Nadu Agricultural University, Coimbatore, Tamil.

Mahrous, K.F., Ramadan, H.A.I., Abdel, A.S.H., Mordy, M.A., Hamdan, D. 2011.Genetic variations between camels breed using microsatellite markers and RAPD techniques. J. Appl. Biosci., 39: 2626-2634.

Martin, M., Michael, M., Ole, K.T., Kjetill, S.J., Sissel, J. 2017. Whole genome sequencing data and de novo draft assemblies for 66 teleost species PO Box 422, 4604 Kristiansand, Norway. Correspondence and requests for materials should be addressed to M.M.).

Mehta, S.C., Sahani, M.S. 2014. Microsatellite markers for genetic characterization of Bikaneri camel. Indian J. Anim. Sci., 77: 509-512.

Moralee, R.D., Vander Bank, F.H., Vander Waal, B.C.W. 2000. Biochemical genetic markers to identify hybrids between the endemic Oreochromis Mossambicus and alien species, O. niloticus (Pisces: Cichlidae Water S.A. (26)2: 263-268.

Nolte, M., Kotzé, A., Bank, F.H.V.D., Grobler, J.P. 2005. Microsatellite markers reveal low genetic differentiation among southern African Camelusdromedarius populations. South African J. Anim. Sci., 35: 152-161.

Nouairia, G., Kdidi, S., Ben, S.R., Hammadi, M., Khorchani, T. 2015. Assessing genetic diversity of three Tunisian dromedary camel (*Camelusdromedarius*) sub populations using microsatellite markers. Emir. J. Food Agric., 27: 362-366.

Pal, J., Shukla, B.N., Maurya, A.K., Verma, H.O., Pandey, G., Amitha, A. 2018. A review on role of fish in human nutrition with special emphasis to essential fatty acid. *International Journal of Fisheries and Aquatic Studies*, *6*(2): 427-430

Penedo, M.C.T., Caetano, A.R., Cordova, K. 1999. Six microsatellite markers for South American came lids. Animal Genet., 30: 161-168.

Pharmawati, M., Yan, G., McFarlane, I. 2004. Application of RAPD and SSR markers to analyze molecular relationships in Grevillea (Proteaceae). Australian Systematic Botany, 17: 49–61.

Saad, Y.M., Mohamed, J.S., El-Domyati, F.M. 2019. Molecular phylogeny of mullet fishes using Sox14 and COI gene sequence variations Research Journal of Biotechnology,14 (4): 130.

Sabry, A.M., Hassan, M.M., Mohamed, A.A., Awad, N.S. 2015. Molecular characterization of some popular fish species in Saudi Arabia. Inter. J. Appl. Sci. Biotechnol., 3: 359-366.

Sugama, K.J., Benzie, A.H., Ballment, E. 2002. Aquaculture Mitochondrial DNA variation in Indo-Pacific populations of the giant tiger prawn, Penaeus monodon 205: 37-48.

Table 1: The ISSR and SCoT PCR reaction parameters**.**

Steps	Temperature		Time		Cycles
	ISSR	SCoT	ISSR	SCoT	
Initial denaturation	94 °C	94 °C	5 min	5 min	1
Denaturation	94 °C	94 °C	40 Sec	40 Sec	40
Annealing	45°C	50°C	50 Sec	50 Sec	
Extension	72 °C	72 °C	1 min	1 min	
Final extension	72 °C	72 °C	7 min	7 min	1

Table 2: The percentage of polymorphism recorded using ten primers (5 ISSR and 5 SCoT) sand smelt species collected from five different locations.

PC	Location	TB	MB	PB	%P
ISSR - 01	Fayoum	10	8	2	20.00
	Burullus	10	9	1	10.00
	Suez	8	8	0	0.00
	Idiko	7	6	1	14.29
	Port Said	8	8	0	0.00
ISSR - 02	Fayoum	14	14	0	0.00
	Burullus	15	15	0	0.00
	Suez	15	14	1	6.67
	Idiko	14	12	2	14.29
	Port Said	15	14	1	6.67
ISSR - 03	Fayoum	13	11	2	15.38
	Burullus	13	12	1	7.69
	Suez	12	10	2	16.67
	Idiko	11	11	0	0.00
	Port Said	12	12	0	0.00
ISSR - 04	Fayoum	15	15	0	0.00
	Burullus	15	15	0	0.00
	Suez	14	14	0	0.00

	Idiko	15	15	0	0.00
	Port Said	14	14	0	0.00
ISSR - 05	Fayoum	4	4	0	0.00
	Burullus	8	6	2	25.00
	Suez	10	9	1	10.00
	Idiko	10	10	0	0.00
	Port Said	9	9	0	0.00
SCOT - 01	Fayoum	10	10	0	0.00
	Burullus	11	11	0	0.00
	Suez	12	12	0	0.00
	Idiko	11	9	2	18.18
	Port Said	7	7	0	0.00
SCOT - 02	Fayoum	11	10	1	9.09
	Burullus	11	11	0	0.00
	Suez	8	8	0	0.00
	Adiko	11	11	0	0.00
	Port Said	9	9	0	0.00
SCOT - 03	Fayoum	9	7	2	22.22
	Burullus	8	7	1	12.50
	Suez	8	7	1	12.50
	Idiko	8	7	1	12.50
	Port Said	7	7	0	0.00
SCOT - 04	Fayoum	11	10	1	9.09
	Burullus	11	11	0	0.00
	Suez	11	11	0	0.00
	Idiko	10	10	0	0.00
	Port Said	10	10	0	0.00
SCOT - 05	Fayoum	8	8	0	0.00
	Burullus	9	9	0	0.00
	Suez	10	9	1	10.00
	Idiko	9	9	0	0.00
	Port Said	9	9	0	0.00

Table 3: The list of primers sequence, Total Number of Bands (TB), Monomorphic Bands (MB), Polymorphic Bands (PB), Percentage of Polymorphism (%P), Frequency (F) and Polymorphism Information Content (PIC) as revealed by SCoT and ISSR analysis of sand smelt species.

Primer	Sequence	TB	MB	PB	% P	F	PIC
SCoT-01	5'-CAACAATGGCTACCACCA-3'	15	5	10	67	0.7	0.35
SCoT-02	5'-CAACAATGGCTACCACCC-3'	14	8	6	43	0.7	0.33
SCoT-03	5'-CAACAATGGCTACCACCG-3'	10	6	4	40	0.7	0.31
SCoT-04	5'-CAACAATGGCTACCACCT-3'	12	9	3	25	0.9	025
SCoT-05	5'-CAACAATGGCTACCACGA-3'	12	6	6	50	0.7	0.31
ISSR-01	5'-AGAGAGAGAGAGAGAGYC-3'	16	3	13	81	0.5	0.37
ISSR-02	5'-TCTCTCTCTCTCTCTCA-3'	15	12	3	20	0.9	0.32
ISSR-03	5'-ACACACACACACACACYT-3'	14	10	4	29	0.8	0.24
ISSR-04	5'-ACACACACACACACACYG-3'	17	13	4	24	0.9	0.35
ISSR-05	5'-GTGTGTGTGTGTGTGTYG-3'	14	4	10	71	0.6	0.37
Total		139	76	63	-	-	-
Average		13.9	7.6	6.3	45	0.74	0.32

Table 4: Genetic similarity matrix computed from ISSR and SCoT data for the Five send smelt species.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	100														
2	98	100													
3	97	96	100												
4	92	91	93	100											
5	91	90	93	99	100										
6	92	91	92	98	98	100									
7	86	84	87	87	88	88	100								
8	85	86	86	87	86	87	98	100							
9	85	84	85	85	86	86	98	97	100						
10	81	82	80	82	81	81	88	90	89	100					
11	80	82	79	82	82	81	88	88	88	98	100				
12	79	80	78	80	80	79	87	88	88	98	99	100			
13	80	79	78	78	78	79	88	87	89	89	89	90	100		
14	79	79	78	77	77	78	87	87	88	88	90	90	99	100	
15	79	79	78	77	77	78	87	87	89	89	89	90	99	99	100

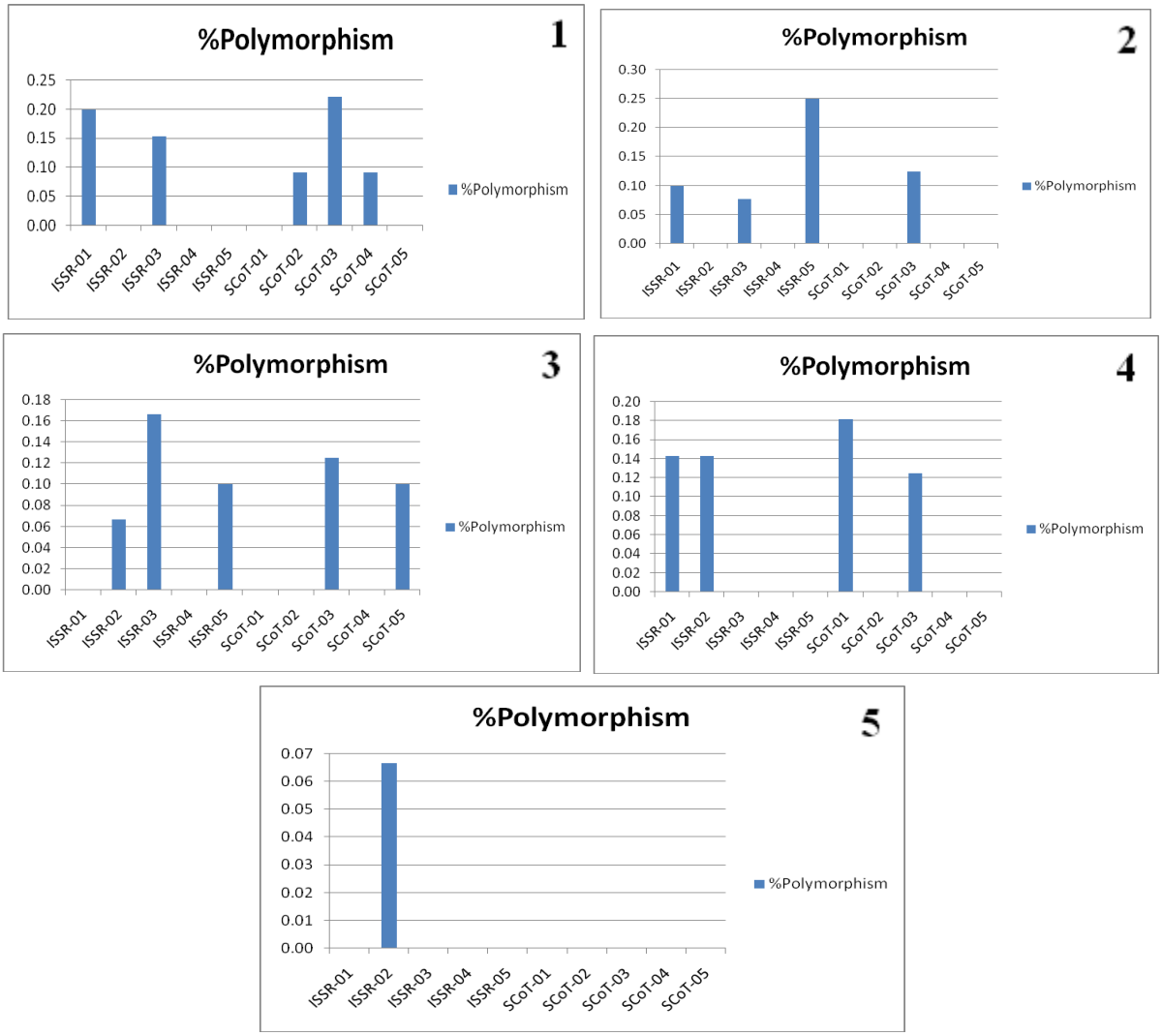

Figure 1: Polymorphism values detected using Five ISSR and Five SCoT primers for sand smelt species gathered from 1- Fayoum; 2- Burullus; 3- Suez; 4- Adiko and 5- Port Said.

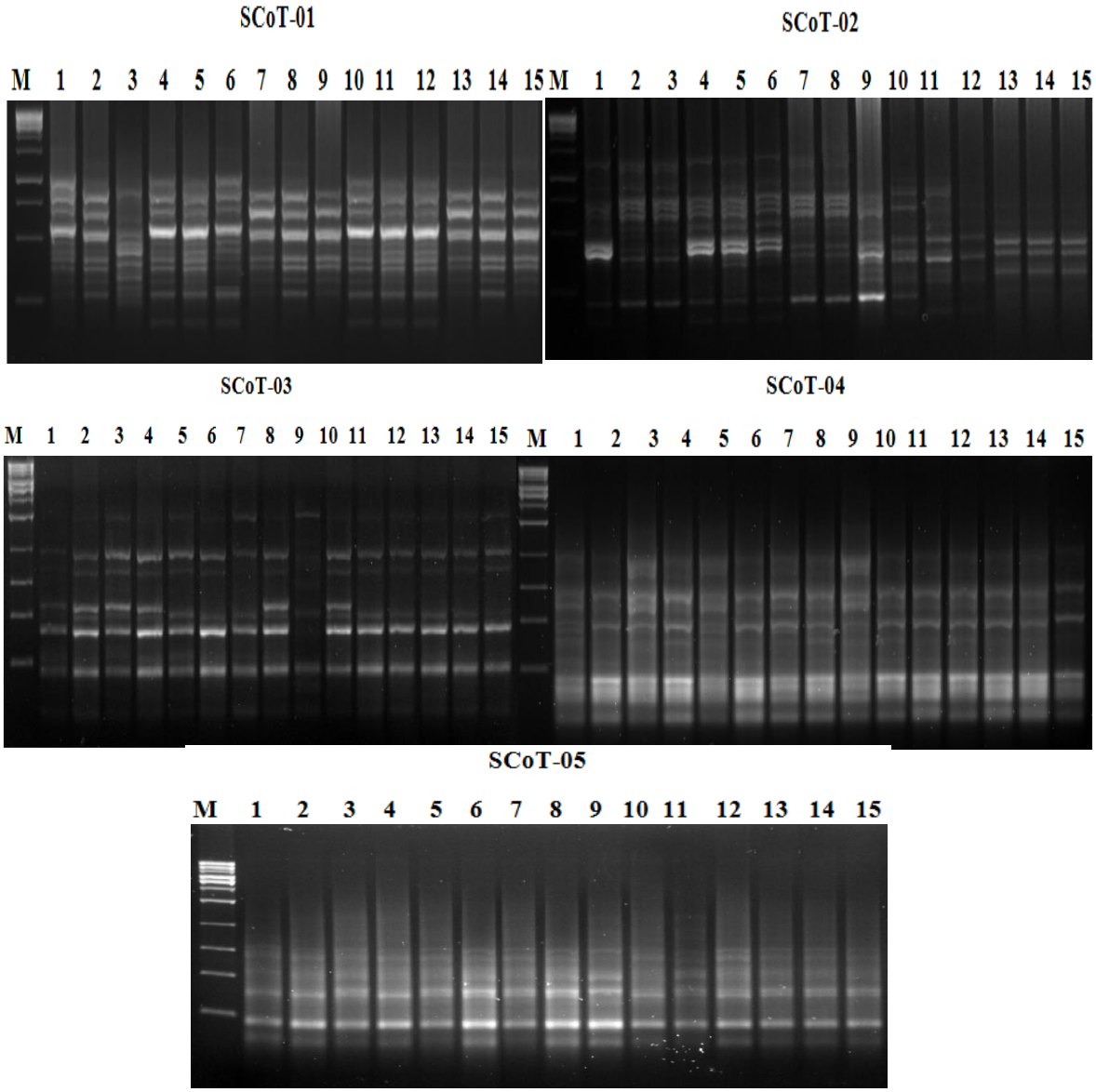

Figure 2: SCoT profiles, the PCR patterns of the sand smelt species using the Five SCoT Primers; SCoT-01, SCoT-02, SCoT-03, SCoT-04 and SCoT-05. M: 1kb DNA ladder (Fermentas, Germany). Lanes 1 to 15 sample sand smelt

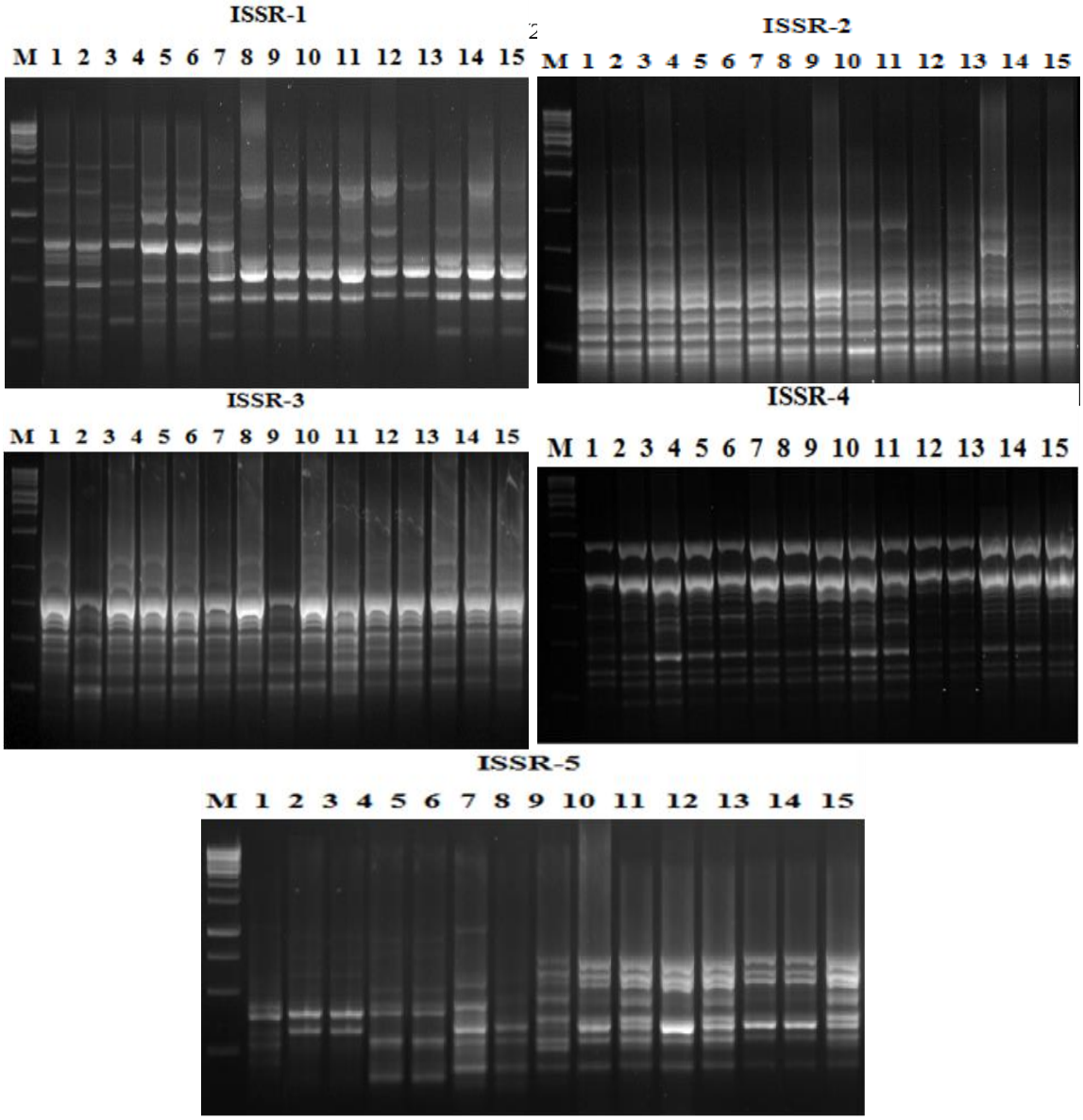

Figure 3: ISSR profiles, the PCR patterns of the sand smelt species using the Five ISSR Primers; ISSR-1, ISSR-2, ISSR-3, ISSR-4 and ISSR-5. M: 1kb DNA ladder (Fermentas, Germany). Lanes 1 to 15 sample sand smelt

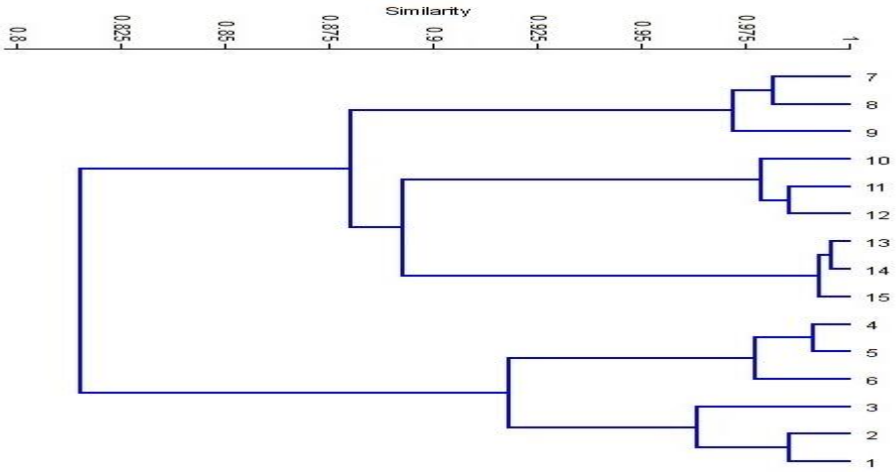

Figure 4: Dendrogram for the 15 send smelt species constructed from SCoT and ISSR data using UPGMA and similarity matrix computed according to Dice coefficient.

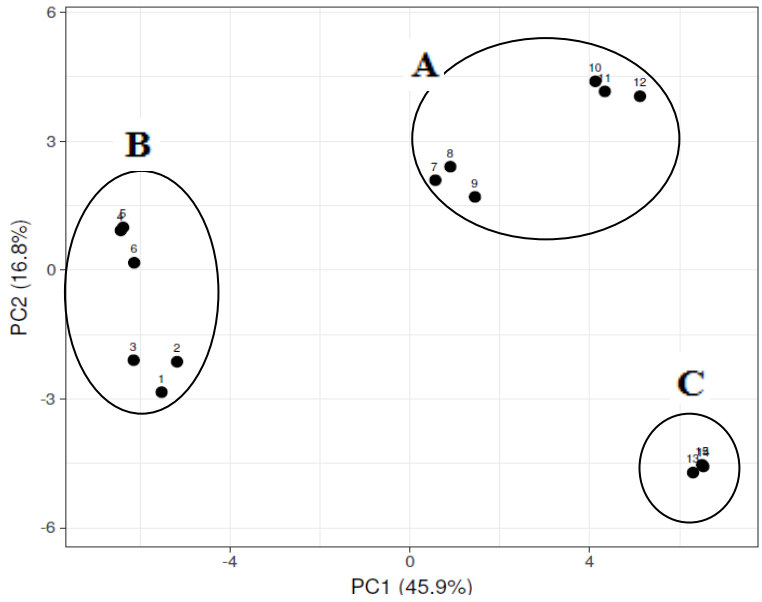

Figure 5: Scatter plot of principal coordinate analysis (PCoA) for 15 send smelt species based on ISSR and SCoT marker data: A. contain (7, 8, 9, 10, 11 and 12); B. contain (1, 2, 3, 4, 5 and 6); C. contain (13, 14 and 15).

الأختلافات الوراثية لأسماك البساريا في المصايد المصرية .

عبدالباسط العدل السيد [1,*]., محسن صالح حسين [1], محمد فتحى محمد عبد الغنى [1], شفيق درويش ابراهيم [2]

[1] قسم الإنتاج السمكي، كلية الزراعة، جامعة الأزهر، القاهرة، مصر.

[2] معهد الهندسة الوراثية، قسم الخرائط الوراثية، مركز البحوث الزراعية.

* البريد الإلكتروني للباحث الرئيسي:abdelbaseteladl@azhar.edu.eg

الملخص

تم استخدام العلامات الجزيئية كمؤشرات في تقييم التنوع الجيني للأسماك ، فهي تسمح بالمراقبة المباشرة للمعلومات الجينية وتقدير العلاقات الجينية بين السكان والأنواع. في هذه الدراسة ، تم استخدام خمسة بادئات SCoT وخمسة بادئات ISSR لتقدير الجينات. تم استخدام خمسة عشر عينة من أنواع الأسماك ذات الرائحة الرملية (ثلاثة مكررات لكل نوع) في هذا البحث. تم جمع مصهر الرمل من خمسة مواقع مختلفة هي: الفيوم والبرلس والسويس وأديكو وبورسعيد. كان العدد الإجمالي للحمض النووي الناتج عن عشرة بادئات 139 بمتوسط 13.9 أمبليكون / برايمر. كان أكبر عدد من amplicon 17 تم إنشاؤه بواسطة ISSR-04 وكان أقل رقم 10 تم إنشاؤه بواسطة SCoT-03. كان العدد الإجمالي للأمبليكون متعدد الأشكال 63 بمتوسط 6.3 / أساس. تراوحت أمبليكون متعددة الأشكال من 3 إلى 13 أمبليكون. لذلك ، عبّرت البادئات العشرة عن مستويات مختلفة من تعدد الأشكال ، تراوحت من 20٪ مع البادئة ISSR-02 إلى 81٪ مع البادئة ISSR-01. تباين التردد من 0.5 إلى 0.9 حيث كان للبادئ ISSR-01 أقل تردد بينما كان للبادئات ISSR-02 و ISSR-4 أعلى قيمة بمتوسط 0.74. تراوح محتوى المعلومات متعدد الأشكال (PIC) من 0.24 إلى 0.37 قيمًا حيث كان للبادئ ISSR-03 أقل قيمة PIC بينما كان للبادئ ISSR-01 أعلى قيمة بمتوسط 0.32.

الكلمات الاسترشادية: سمك البساريا,DNA, PCR, ISSR, SCOT