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Potential of Start Codon Targeted (SCoT) Markers and SDS-PAGE to Estimate Genetic Diversity and Relationships among Three Gastropods Species from The Mediterranean Sea, Port Said, Egypt

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ABSTRACT
Gastropods are one of the most diverse groups of animals, in form, habit, and habitat. Till now they are the largest group of molluscs. The present study was aimed to evaluate the genetic variability among three gastropods species; *Neverita josephinia*, *Hexaplex trunculus* and *Murex altispira* using molecular marker start codon targeted (SCoT) and Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). A total of ten SCoT primers produced 115 amplicons, their size ranged from 120 to 1500 bp and the percentage of polymorphism ranged from 20% to 78%. The results of the study revealed that *Neverita josephina* and *Hexaplex trunculus* have higher genetic similarity 76% while *Hexaplex trunculus* and *Murex altispira* exhibited lower genetic similarity 70%. The protein analysis by SDS-PAGE Produced 23 bands of molecular weight ranging from 8 to 149 KDa with polymorphism 9%. Considering all the gained data, it is evident that molecular detection of SCoT and SDS-PAGE are suitable tools in assessing genetic variation and relationships among gastropods species.

INTRODUCTION

Gastropods in marine environment are characterized by common morphological features such as; large aperture teeth and increased shell thickness for defense against shell-crushing predators. Several environmental factors such as extreme temperature, desiccation stress are also correlated with the shell pattern, color and morphology. The habitat of shells also play role for such changes. Most marine gastropods have simple shells and are deprived of easily identifiable morphological structure. Moreover, difficulty of identification due to the plasticity in shell morphology makes the identification more difficult **(Teske *et al*., 2011; Kumbhar and Rivonker 2012).**

Genetic diversity is the basic of species diversity and an important precursor **i**n a study of any species, because its quantity and distribution has an effect on the evolutionary potential of species **(Futuyma, 1986).** Genetic data help in conserving and managing of endangered and threatened species **(Allendorf and Luikart, 2007)**. Genetic

data also can be used for investigation of species and population which have a reduced genetic diversity **(Saccheri *et al*., 1998)**.

The development of DNA-based on genetic markers has a revolutionary impact on animal genetics. Through DNA markers, it is theoretically possible to determine genetic variation in the entire genome **(Liu and Cordes, 2004)**. DNA markers have many applications in genetic diversity research. These markers such as inter simple sequence repeat (ISSR) markers, sequence-related amplified polymorphism (SRAP) markers, and simple sequence repeat (SSR) markers **(Ma *et al*., 2008; Yan *et al*., 2010; Xie *et al*., 2015)**. In last years, new promising markers techniques have emerged such as start codon targeted (SCoT) marker. It is a dominant and a reproducible marker that targets the short-conserved region in the genes of species surrounding the ATG translation start (or initiation) codon by using a single primer in the polymerase chain reaction (PCR) assays and a high annealing temperature **(Collard and Mackill, 2009).**

A single primer is used with SCoT technique (**Bhattacharyya *et al*., 2013)**. SCoT technique has many advantages such as; simple primer design, simple operation, highly effective polymorphism, low cost and good reproducibility **(Chen *et al*., 2009)**.

SDS-PAGE technique was applied extensively to investigate and estimate the genetic characters and relationships among different species **(Kakaei and kahrizi, 2011; Maged and Shawkat, 2012)**. There are many recommendations to use SDS-PAGE protein as rapid method to identify and characterize species (**Freitas *et al*., 2004; Oppong - Konadu *et al*., 2005).** SDS-PAGE is a one of molecular marker in which proteins are separated according to their molecular weight. Sodium dodecyl sulfate polyacrylamide gel electrophoresis is an effective technique used for determination the variation between species and illustrates the metabolic level of species **(Muhammad *et al.*, 2018)**

There are few attempts to study the genetic variation among gastropods species using different molecular markers. Therefore, the objectives of this study are to consider and compare polymorphic SCoT and electrophoretic profiles of total muscles proteins with different molecular weights through SDS- PAGE with highly informative values and to determine the genetic variability and to evaluate genetic relationships among three gastropods species from the Mediterranean Sea, Port Said, Egypt.

MATERIALS AND METHODS

Collection of Samples

Samples of three gastropods species (*Neverita josephinia, Hexaplex trunculus* and *Murex altispira*) (Fig.1) were collected from the Mediterranean Sea, Port Said, Egypt. Appropriate size of muscle tissues were immediately separated from the three gastropods species and were frozen at -20˚C.

DNA Extraction and SCoT-Reaction

Genomic DNA was isolated from muscles of the three gastropods species according to **Porebski *et al*., (1997)** with some modifications. The DNA concentrations of three species were estimated by NanoDrop**.** A set of ten SCoT primers as shown in Table (1) was used in the detection of polymorphism**.** 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 94ºC. Each cycle programed to three steps; a denaturation step for 1 min at 94ºC, an annealing

step for 1 min at 50ºC, and an elongation step for 1.5 min at 72ºC. The amplified products were detected by 1.5% denaturing agarose gels with ethidium bromide. UV light was used for visualizing SCoT-PCR products and photographed using a Gel Documentation System (BIO-RAD 2000).

The amplified products resulted from SCoT-PCR primers were scored as absent (0) and present (1). The amplicon patterns were compared to evaluate the genetic relationship of three gastropods species under this study. Estimation of the genetic similarity coefficient (GS) among the three species was done according to Dice coefficient **(Sneath and Sokal, 1973)**. The cluster analysis of the accessions was made through the similarity matrix. The cluster analysis was used to organize the observed data into meaningful structures to develop species taxonomy.

SDS-PAGE analysis

Proteins were separated from muscles based on their molecular weight by (SDS-PAGE) technique. Proteins of muscle tissue of gastropods species were extracted according to **Fadda *et al.*, (1999).** SDS-PAGE technique was performed according to **(Laemmli, 1970).** Molecular weight of protein bands of three species were stated according to **Weber *et al*., (1972).**

Fig. 1. Colored photographs of (a- *Neverita josephinia*, b- *Hexaplex trunculus* and c- *Murex altispira***)**.

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

Primer Name	Sequence
SCoT-11	5'-ACAATGGCTACCACTACC-3'
SCoT-12	5'-CAACAATGGCTACCACCG-3'
SCoT-13	5'-ACCATGGCTACCACGGCA-3'
SCoT-16	5'-CCATGGCTACCACCGGCA-3'
SCoT-20	5'-CAACAATGGCTACCACGC-3'
SCoT-21	5'-CCATGGCTACCACCGGCC-3'
SCoT-28	5'-CAACAATGGCTACCACCA-3'
SCoT-35	5'-AACCATGGCTACCACCAC-3'
SCoT-41	5'-CAACAATGGCTACCAGCA-3'
SCoT-46	5'-ACCATGGCTACCACCGCC-3'

RESULTS

Three species of gastropods (*Neverita josephinia*, *Hexaplex trunculus* and *Murex altispira*) were molecule-genetically studied, using SCoT-PCR analysis. All species were examined at the same conditions. Ten single primers (SCoT-11, SCoT-12, SCoT-13, SCoT-16, SCoT-20, SCoT-21, SCoT-28, SCoT-35, SCoT-41 and SCoT-46) and SDS-PAGE technique were used in the present investigation to estimate the genetic differences and relationships among three species under study.

SCoT-Technique:

The DNA fragments generated by the ten SCoT primers from the genomic DNA of the three gastropods species were separated using agarose gel electrophoresis and illustrated in figures (2, 3 and 4). The patterns of these DNA fragments were analyzed by Gene profiler computer software program and summarized with each primer in Table (2).

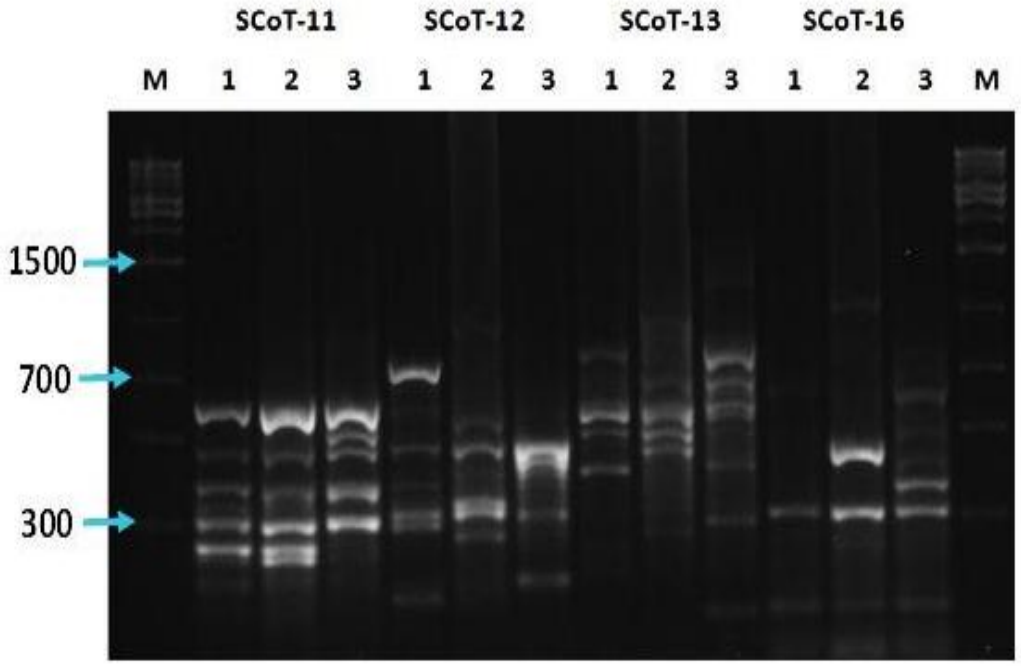

Fig. 2. Agarose-gel electrophoresis of SCoT product generated with the primers (SCoT-11, SCoT-12, SCoT-13 and SCoT-16) in species (1- *Neverita Josephina*, 2- *Hexaplex trunculus* and 3-*Murex altispira*). M refers to DNA marker.

Neverita josephinia

This sample produced with all SCoT primers 81 different band patterns. They were ranged in size from 120 bp in the primer (SCoT-16) to 1000 bp in (SCoT-16 and SCoT-46). The generated bands ranged in number from four in (SCoT-20) to ten in (SCoT-12 and SCoT-21).

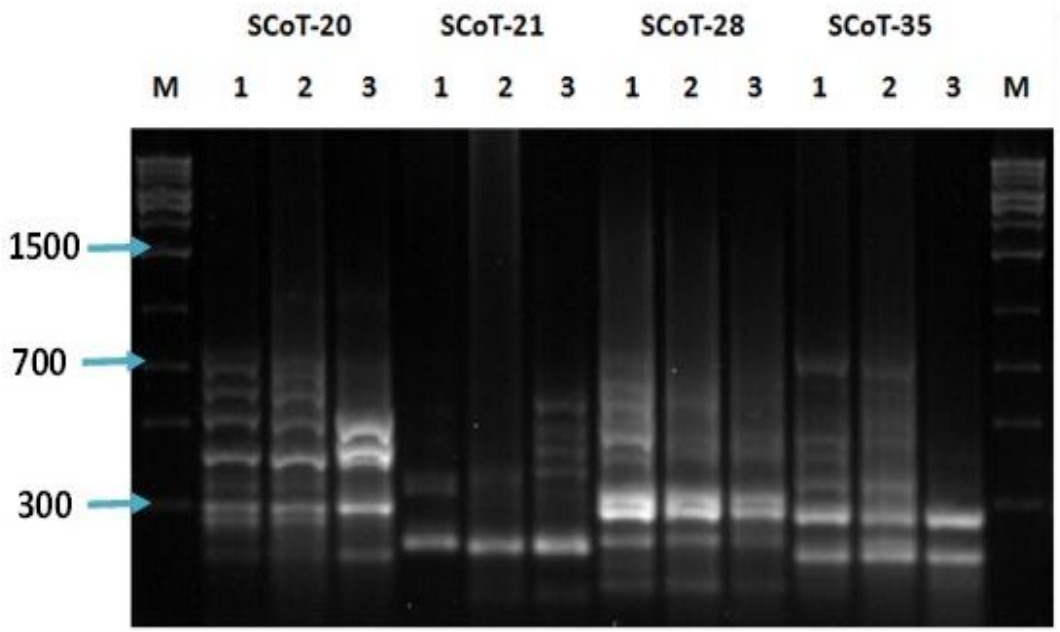

Fig. 3. Agarose-gel electrophoresis of SCoT product generated with the primers (SCoT-20, SCoT-21, SCoT-28 and SCoT-35) in species (1- *Neverita Josephina*, 2- *Hexaplex trunculus* and 3-*Murex altispira*).

Table 2. Number of total amplified bands, monomorphic bands, polymorphic and unique bands generated by ten SCoT primers with three gastropods species (1- *Neverita Josephina*, 2- *Hexaplex trunculus* and 3-*Murex altispira*).

No. of primers	Primer code	No.of amplified bands			Total amplified bands	Molecular weight of bands (bp)	No. of monomorphic bands	No. of polymorphic bands	No. of unique bands	Polymorphism %
		1	2	3						
1	SCoT-11	9	9	9	10	150-620	8	1	1	20%
2	SCoT-12	10	10	7	17	130-1000	4	2	11	77%
3	SCoT-13	8	10	12	16	120-1500	6	2	8	63%
4	SCoT-16	7	8	13	14	120-1000	5	4	5	64%
5	SCoT-20	4	3	7	7	120-550	2	3	2	71%
6	SCoT-21	10	10	9	12	150-1000	7	3	2	42%
7	SCoT-28	9	7	7	9	150-600	7	0	2	22%
8	SCoT-35	7	7	4	9	160-700	2	5	2	78%
9	SCoT-41	8	8	10	12	200-1100	5	4	3	58%
10	SCoT-46	9	5	5	9	150-1000	3	4	2	67%
Total		81	77	83	115	120-1500	49	28	38	57%

Hexaplex trunculus

Regarding the band patterns resulted from SCoT primers with this species was 77 different bands. They were ranged in size from 120 bp in the primer (SCoT-16 and SCoT-20) to 1500 bp in (SCoT-13). The generated bands ranged in number from three in (SCoT-20) to ten in (SCoT-12, SCoT-13 and SCoT-21).

Murex altispira

The ten SCoT primers with this species produced 83 different band patterns. They were ranged in size from 120 bp in the primer (SCoT-16 and SCoT-20) to 1100 bp in (SCoT-41). The generated bands ranged in number from four in (SCoT-35) to thirteen in (SCoT-16).

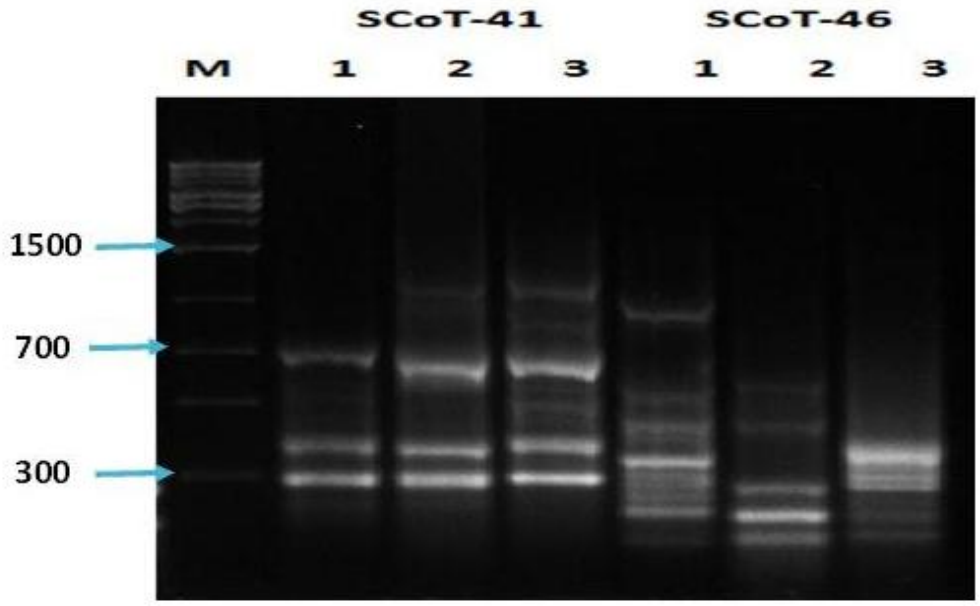

Fig. 4. Agarose-gel electrophoresis of SCoT product generated with the primers (SCoT-41 and SCoT-46) in species (1- *Neverita Josephina*, 2- *Hexaplex trunculus* and 3-*Murex altispira*).

Ten SCoT primers with three gastropods species were produced 115 DNA bands. From SCoT data 43% were monomorphic bands, 24% polymorphic ones and 33% unique bands were observed among three species. The number of total fragments amplified per primer varied between seven (SCoT-20) to 17 (SCoT-12) (Table 2). The accessions were separated into two major clusters using the cluster analysis. Genetic similarity was lowest between *Hexaplex trunculus* and *Murex altispira*, while was highest between *Neverita Josephina* and *Hexaplex trunculus*. Genetic similarity index was from 70% to 76% (Table 3), and dendrogram (Fig. 5).

Table 3. Similarity matrix UPGWA Dice Coefficient (1- *Neverita Josephina*, 2- *Hexaplex trunculus* and 3-*Murex altispira*).

	Similarity Matrix		
	1	**2**	**3**
1	**100**		
2	**76**	**100**	
3	**73**	**70**	**100**

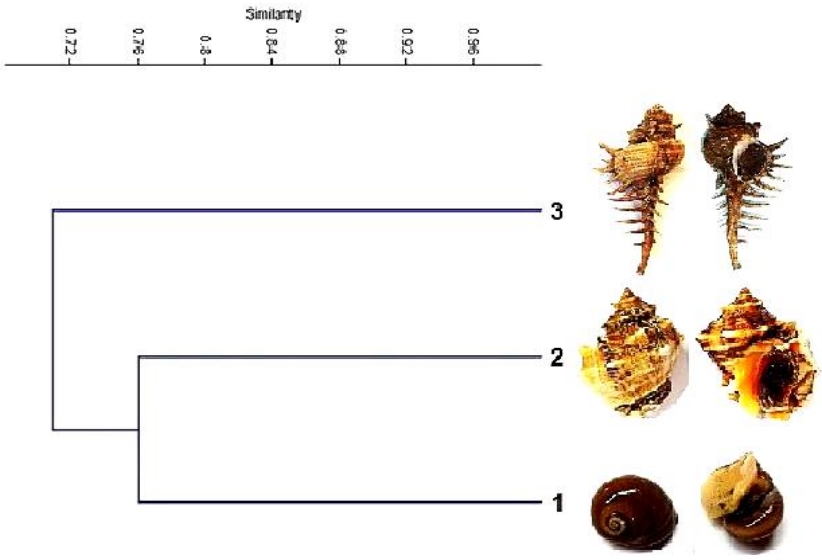

Fig. 5. Dendrogram for three species of gastropods constructed from the SCoT data using similarity matrices computed according to dice coefficients. (1- *Neverita Josephina*, 2- *Hexaplex trunculus* and 3-*Murex altispira*).

SDS-PAGE Technique

The protein profile bands resulted from three gastropods species with SDS-PAGE were 23 bands as shown in Table (4) and (Fig. 6). The molecular weights of bands ranging from 8 to 149 KDa. The monomorphic bands were 21. Only two polymorphic bands were recorded with a percentage of 9%. *Neverita josephinia* produced 21 bands ranged in size from 8 to 131 KDa, *Hexaplex trunculus* produced 21 bands ranged in size from 8 to 131 KDa and *Murex altispira* produced 23 bands ranged in size from 8 to 149KDa (Table 4).

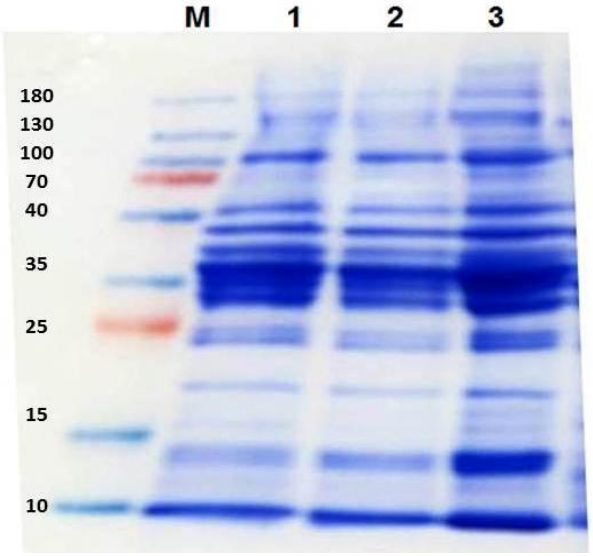

Fig. 6. SDS-PAGE protein banding patterns of three gastropods species (1- *Neverita Josephina*, 2- *Hexaplex trunculus* and 3-*Murex altispira*). M refers to protein marker.

Table 4. Molecular weight of SDS-PAGE protein bands of three gastropods species 1- *Neverita Josephina*, 2- *Hexaplex trunculus* and 3-*Murex altispira*).

Marker Molecular weight (KD)	1	2	3
149	0	0	1
131	1	1	1
110	1	1	1
106	1	1	1
85	1	1	1
83	1	1	1
68	0	0	1
58	1	1	1
54	1	1	1
48	1	1	1
46	1	1	1
37	1	1	1
35	1	1	1
33	1	1	1
30	1	1	1
27	1	1	1
24	1	1	1
23	1	1	1
21	1	1	1
17	1	1	1
11	1	1	1
9	1	1	1
8	1	1	1

DISCUSSION

The present study indicated that *Neverita josephinia* and *Hexaplex trunculus* are more genetically similarity than *Murex altispira.* The molecular genetic taxonomic relationship and variability among three species of gastropods was investigated using SCoT markers and SDS-PAGE for first time in Egypt.

SCoT technique includes a polymerase chain reaction (PCR) based DNA marker. It has many benefits such as low-cost, high polymorphism, simple operation and extensive genetic information **(Gou *et al.*, 2012; Chai *et al.*, 2017)**. The SCoT technique has been successfully used to estimate genetic variation, diversity and relationships among some fish species and some plant species **(Etminan *et al.*, 2018; Abu Almaaty *et al.*, 2020a).**

In population studies SCoT is an effective technique. SCoT technique is benefit in DNA finger printing, identifying cultivars and estimation of genetic variation and structure **(Collard and Mackill 2009; Poczai *et al.*, 2013; Etminan *et al.*, 2016)**.

Estimation of the molecular weight of protein via polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) is a universally used method in field of biomedical research; the electrophoresis of proteins (SDS-PAGE) can be economically used in determining the genetic variation and relationship among species, and it also can be used in differentiation between mutants and parent genotypes of a species **(Ranjan *et al*., 2013)**

The protein fingerprinting of three gastropods species of genus *Thais* was investigated by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) **(Ali *et al.*, 2018)**. Protein profiles of gonggong snail (*Strombus* sp) were

identified by using SDS-PAGE **(Viruly *et al*., 2019).** SCoT- technique and SDS-PAGE were used for estimation the genetic variation and relationship among some fish species **(Abu Almaaty *et al*., 2017; 2020a; 2020b)**

Polymorphism percentages of different used markers (SCoT and SDS-PAGE) were recorded as follow 57 % and 9 % respectively. The results indicated that molecular analysis by using SCoT and SDS-PAGE techniques may be good tools for estimating DNA fingerprinting, genetic variability, protein patterns and identifying of gastropods species.

CONCLUSION

Our findings resulted from SCoT-based DNA analysis bands and SDS-PAGE-based protein profile revealed a close genetic relationship between *Neverita Josephina*, and *Hexaplex trunculus* compared with *Murex altispira*. The results revealed also that SCoT technique and SDS-PAGE could be used for assessment of genetic relationships and genetic variation of other gastropods species. However, much specific molecular biomarker and more SCoT primers are required for understanding the relations of many other species of gastropods.

REFERENCES

Abu-Almaaty, A. H.; Abd-Alaty, H. E.; Abbas, O. A. and Hassan, M. K. (2020a). Genetic relationship between two species of genus *Dicentrarchus* based on SCoT markers and SDS-PAGE**.** Egyptian Journal of Aquatic Biology & Fisheries, 24(5): 393 – 402

Abu-Almaaty, A. H.; Bahgat, I. M. and Al-Tahr, Z. M. (2017). Genetic variability of three fish species of genus *Puntius*. Journal of Experimental and Applied Animal Sciences, 2(2): 153-164.

Abu-Almaaty, A. H.; Bahgat, I. M. and Al-Tahr, Z. M. (2020b). Using SDS-PAGE and ISSR as biochemical markers for assessment the genetic similarity and protein analysis of some Cyprinid fish species. Genetika, 52(1): 161-175.

Ali, S. A.; Humayun, F.; Munir, I.; Ahmad, S.; Ayub, Z.; Fatima, H. and Haider, M. S. (2018). Morphological, molecular, biochemical and nutritional characterization of three major Thais species from the Sindh coast of Pakistan. The Open Food Science Journal, 10(1).

Allendorf, F. W. and Luikart, G. (2007). Conservation and the genetics of populations 2nd ed. Blackwell publishing. Oxford U. K. 642pp.

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

Chai, X.; Dong, R.; Liu, W.; Wang, Y. and Liu, Z. (2017). Optimizing sample size to assess the genetic diversity in common vetch (Vicia sativa L.) populations using start codon targeted (SCoT) markers. Molecules, 22(4): 567.

Chen, H.; He, X.; Luo, C.; Gao, M. and Zhu, J. (2009). The optimization of SCoT-PCR system of Longan (*Dimocarpus longan*). Genomics and Applied Biology, 28(5): 970-974.

Collard, B. C. Y. and Mackill, D. J. (2009). Start Codon Targeted (SCoT) Polymorphism: a simple novel DNA marker technique for generating gene-targeted markers in plants. Plant Mol. Biol. Rep. 27: 86–93

Etminan, A.; Pour-Aboughadareh, A.; Mohammadi, R.; Ahmadi-Rad, A.; Noori, A.; Mahdavian, Z. and Moradi, Z. (2016). Applicability of start codon targeted (SCoT) and inter-simple sequence repeat (ISSR) markers for genetic diversity analysis in durum wheat genotypes. Biotechnology & Biotechnological Equipment, 30(6): 1075-1081.

Etminan, A.; Pour-Aboughadareh, A.; Noori, A.; Ahmadi-Rad, A.; Shooshtari, L.; Mahdavian, Z. and Yousefiazar-Khanian, M. (2018). Genetic relationships and diversity among wild Salvia accessions revealed by ISSR and SCoT markers. Biotechnology & Biotechnological Equipment, 32(3): 610-617.

Fadda, S.; Sanz, Y.; Vignolo, G.; Aristoy, M. C.; Oliver, G. and Toldra, F. (1999). Characterization of muscle sarcoplasmic and myofibrillar protein hydrolysis caused by *Lactobacillus plantarum.* Appl. Environ. Microbiol. 65: 3540-3546.

Freitas, R. L.; Teixeira A. R. and Ferreira R. B. (2004). Characterization of the proteins from *Vigna unguiculata* seeds. J. Agric. Food Chem., 52: 1682-1687.

Futuyma, D.J. (1986). Evolutionary Biology; Sinauer Associates: Sunderland, MA, USA; p. 2.

Guo, D. L.; Zhang, J. Y. and Liu, C. H. (2012). Genetic diversity in some grape varieties revealed by SCoT analysis. Mol. Biol.Rep. 39(5): 5307-5313.

Kakaei, M. and kahrizi, D. (2011). Evaluation of seed storage protein patterns of ten wheat varieties using SDSPAGE. Biharean Biol., 5: 116-118.

Kumbhar, J. V. and Rivonker, C. U. (2012). A new record of Morula anaxares with a description of the radula of three other species from Goa, central West Coast of India (Gastropoda: Muricidae). Turk J Fish Aquat Sci 2; 12: 189-97.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4', Nature, 227(5259): 680–685.

Liu, Z. J., and Cordes, J. F. (2004). DNA marker technologies and their applications in aquaculture genetics. Aquaculture, 238(1-4): 1-37

Ma, X.; Zhang, X. Q.; Zhou, Y. H.; Bai, S. Q.; and Liu, W. (2008). Assessing genetic diversity of Elymus sibiricus (Poaceae: Triticeae) populations from Qinghai-Tibet Plateau by ISSR markers. Biochemical Systematics and Ecology, 36(7): 514-522.

Maged, A. E. A. and Shawkat, M. A. (2012). Interspecific diversity of Egyptian and foreign new lines of chickpea based on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and isozyme markers. AJB. 6:135-139.

Muhammad, O. I.; Mahmoud, U. M.; Fazio, F., Sayed, A. H. (2018). SDS-PAGE technique as biomarker for fish toxicological studies. Toxicology Reports, 5: 905–909.

Oppong-Konadu, E. Y. R.; Akromah, I. K. and Okai E. (2005). Genetic diversity within Ghanaian cowpea germplasm based on SDS-PAGE of seed proteins. African Crop Science Journal, 13: 117-123.

Poczai, P.; Varga, I.; Laos, M.; Cseh, A.; Bell, N.; Valkonen, H.P.; and Hyvonen, J. (2013). Advances in plant gene-targeted and functional markers: a review. Plant Methods 9(1):6

Porebski, S.; Bailey, L. G. and Baum, B. R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Mol Biol Rep. 15: 8–15.

Ranjan, S.; Poosapati, A.; Vardhan, H.; and Matcha, R. (2013). Seed storage protein profile of few leguminous grains grown in India using SDS-PAGE. International Journal of Advanced Biotechnology and Research, 4(4): 505-510.

Saccheri, I.; Kuussaari, M.; Kankare, M.; Vikman, P.; Fortelius, W. and Hanski, I. (1998). Inbreeding and extinction in a butterfly metapopulation. Nature. 392(6675): 491-494.

Sneath, P. H. A. and Sokal, R. R. (1973). Numerical taxonomy; the principles and practice of numerical classification. San Francisco: Freeman Medical Reseach Council Microbial systematic Unit. Leicester, England and Dept. Ecology and Evolution State Univ. New York, Stony Brook, NY.

Teske, P. R.; Papadopoulos, I.; Mmonwa, K. L.; Matumba, T. G.; McQUAID, C. D.; Barker, N. P. and Beheregaray, L. B. (2011). Climate-driven genetic divergence of limpets with different life histories across a southeast African marine biogeographic disjunction: different processes, same outcome. Molecular Ecology, 20(23): 5025-5041.

Viruly, L.; Andarwulan, N.; Suhartono, M. T. and Nurilmala, M. (2019). Protein profiles and DNA isolation of hemolymph gonggong snail (*Strombus sp*.) from Bintan. In IOP Conference Series: Earth and Environmental Science, 278(1): p.012078. IOP Publishing.

Weber, K.; Pringle, J. R. and Osborn, M. (1972). Measurement of molecular weights by electrophoresis on SDS-acrylamide gel. Methods in enzymology, 26, 3-27.

Yan, J.; Bai, S.; Zhang, X.; You, M.; Zhang, C.; Li, D. and Zeng, Y. (2010). Genetic diversity of wild Elymus sibiricus germplasm from the Qinghai-Tibetan Plateau in China detected by SRAP markers. Acta Prataculturae Sinica, 19(1): 173-183.

Xie, W.; Zhao, X.; Zhang, J.; Wang, Y. and Liu, W. (2015). Assessment of genetic diversity of Siberian wild rye (Elymus sibiricus L.) germplasms with variation of seed shattering and implication for future genetic improvement. Biochemical Systematics and Ecology, 58: 211-218.