Phylogenetic Differentiation of Wild and Cultured Sea Bream (*Sparus aurata*) Populations: 1. Phenotypic Analysis

El-Zaeem S. Y.[1], Khouriaba, H. M.[2], El-Sherif, M. S.[2] and Shahin, M. M.[2*]

[1]Animal and Fish Production Dept., Faculty of Agriculture, (Saba-Bacha), Alexandria University, Alexandria, Egypt

[2]Animal Production and Fish Resources Dept., Faculty of Agriculture, Suez Canal University, 41522 Ismailia, Egypt

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Abstract: Phenotypic variation based on both morphometric character indices and meristic counts between wild and cultured Sea bream (*Sparus aurata*) populations were phylogenetically analyzed aiming to identify and measure the amount of phenotypic differences and also to help assess the degree of phenotypic plasticity presented by these populations. 100 individuals from both sexes were randomly collected from 3 wild populations and one cultured population. The results revealed a significant difference ($P \leq 0.05$) in most of morphometric character indices between wild and cultured Sea bream populations. Suez Canal population presented a significant superiority in most of morphometric character indices as its individuals achieved mean values of 0.4039±0.002, 0.3829±0.002, 0.1486±0.001, 0.0879±0.002, 0.3062±0.001, 0.0948±0.001, 0.1077±0.001, 0.0761±0.001, 0.0532±0.001 and 0.0999±0.001 respectively for the indices HW/HL, BD/SL, BW/SL, CPW/SL, Dist. Pel-An/SL, Min. BD/SL, LLoDFR/SL, LLoAFR/SL, LLaAFR/SL and LSCFR/SL with a significant differences ($P \leq 0.05$) from the other populations. The hierarchical cluster analysis based on quantitative phenotype (morphometric, landmark and meristic character indices) grouped the four populations of Gilthead Sea bream (*Sparus aurata*) into two major category groups; Suez Canal, Bardawil and cultured populations group, and Alexandria population group.

Keywords: Sea Bream, *Sparus aurata,* Phylogenetic Differentiation, Phenotypic Analysis

INTRODUCTION

The gilthead sea bream (*Sparus aurata*) is a marine teleost fish. Morphological analysis has been useful for fish stock identification. Environmentally induced phenotypic variation provides rapid information on stock or sub population identity (Clayton, 1981). Identification of fish species plays a key role in the behavioral study. Different methods are used for identification, but phenotype based on morphometric and meristic are considered as earliest and authentic methods for the identification of fish species in fish biology to measure discreteness and relationships among various taxonomic categories, and provide useful results used to differentiate fish populations in particular (Zafar *et al*., 2002; Costa *et al*., 2003; Barriga-Sosa *et al*., 2004; Doherty and McCarthy, 2004; Naesje *et al*., 2004). Phenotypic differences among specific populations cannot be taken as an evidence of genetic diversity since the phenotypic adaptations do not necessarily cause genetic changes in the population (Swaine *et al*., 1991 and Turan, 1999). Phenotypic plasticity can be inclusively defined as the production of multiple phenotypes from a single genotype, according to environmental circumstances (Hutchings, 2004; Miner *et al*., 2005). The aim of this study was to analyze and compare the differences in phenotype based on morphometric character indices, landmark, and meristic counts between wild (Bardawil, Suez Canal and Alexandria populations), and culture population (El-Deeba zone) of sea bream to help assess the degree of phenotypic plasticity shown by these populations.

MATERIALS AND METHODS

The current study was carried out at the laboratory of Fish Production, Animal Production & Fish Resources Department, Faculty of Agriculture, Suez Canal University.

*Corresponding author: monayshahin@agr.suez.edu.eg

Collecting Samples:

From four different populations of sea bream (*Sparus aurata*), randomly hundred individuals from both sexes were collected from different environments; wild populations including [Mediterranean Sea (Alexandria beach), Suez Canal (Ismailia beach) and Bardawil lake] and cultured population (El-Deeba zone).

Studied traits:

A total of 35 morphometric, 19 landmarks were based on morphometric, and 11 meristic measurements were measured for each fish within each population of Sea Bream (*Sparus aurata*) as described by (Doherty and McCarthy, 2004; Hossain *et al*., 2010; El-Zaeem 2011 and El-Zaeem *et al*., 2012; El-Zaeem *et al*., 2017).

The morphometric characteristics measured are listed in figure (1) and Table (1). While, figure (2) and Table (2) representing the landmarks based on morphometric. The meristic counts are listed in Table (3). All morphometric and landmarks based on morphometric characters measured were transformed by dividing the measurement by the standard length or head length of each fish to minimize the effect of fish size.

Phenotype analysis

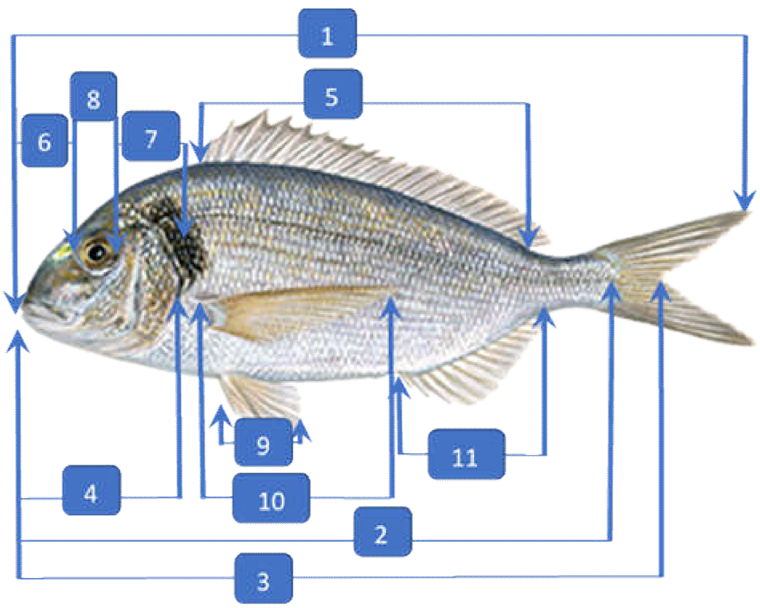
Phenotype differentiation between different populations of Sea Bream (*Sparus aurata*), based on morphometric and landmarks based on morphometric character indices and meristic counts was analyzed by means of hierarchical cluster analysis of the SPSS 22.0 (2013) software package. The cluster analysis using average linkage between-groups method (Sneath and Sokal, 1973) was performed on the matrix of Euclidian distances in order to depict hierarchically the shape differences. The results were presented as a dendrogram.

Statistical analysis

Data were statistically analyzed using the following model (SPSS, 2013):

$$Y_{ij}=\mu+T_i+B_j+E_{ij}$$

Where, Y_{ij} is the observation of the ijth parameter measured; μ is the overall mean; T_i is the effect of ith different population; B_j is the effect of jth block; E_{ij} is the random error. Significant differences ($P\leq0.05$) among means were tested by Duncan's multiple range test (Duncan, 1955).



1 Total length

2 Standard length

3 Forked length

4 Head length

5 Dorsal fin base length

6 Pre-orbital space

7 post-orbital space

8 Eye diameter

9 Pelvic fin length

10 Pectoral fin length

11 Anal fin length

Figure (1): Morphometric character measurements (see Table 1)

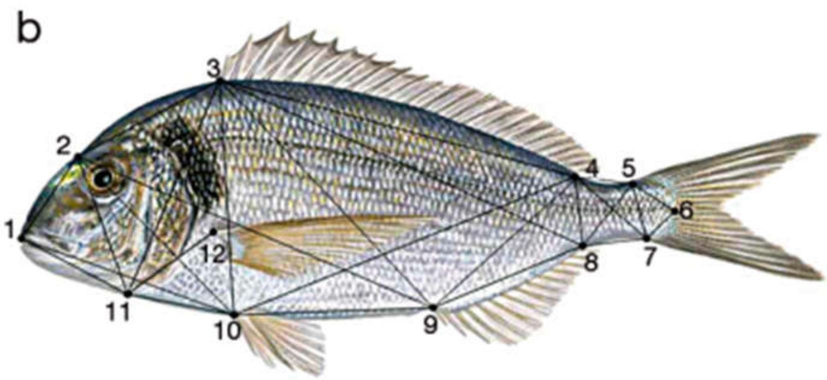


Figure (2): Landmarks based on morphometric measurements (see Table 2)

Table (1): Quantitative phenotype traits based on morphometric characters used for differentiation analysis among different population of sea bream (*Sparus aurata*)

	Characters	Acronyms
1	**Total Length**	TL
2	**Standard Length**	SL
3	**Fork Length**	FL
4	**Head Length**	HL
5	**Head Depth**	HD
6	**Head Width**	HW
7	**Body Depth**	BD
8	**Body Width**	BW
9	**Eye diameter**	ED
10	**Caudal peduncle Length**	CPL
11	**Caudal peduncle Depth**	CPD
12	**Caudal peduncle Width**	CPW
13	**Pre-Orbital Length**	Pr-OL
14	**Post-Orbital Length**	Po-OL
15	**Pre-Dorsal Fin Base Length**	Pr- DFL
16	**Post-Dorsal Fin Base Length**	Po- DFL
17	**Snout Length**	SnL
18	**Pre-Pectoral Fin Length**	Pre-PecFL
19	**Pre-Pelvic Fin Length**	Pre-PelFL
20	**Pre-Anal Fin Length**	Pre-AFL
21	**Caudal Height**	C.H.
22	**Dorsal Fin base Length**	DFL base
23	**Anal Fin base Length**	AFL base
24	**Pectoral Fin Length**	Pec- FL
25	**Pelvic Fin Length**	Pel- FL
26	**Distance between Pelvic and Anal Fin**	Dist. pel.& anal
27	**Caudal Fin Length**	CFL
28	**Minimum Body Depth**	Min BD
29	**Trunk Length**	TrL
30	**Length of longest Dorsal Fin ray**	LLoDFR
31	**Length of Last Dorsal Fin ray**	LLaDFR
32	**Length of Longest Anal Fin ray**	LLoAFR
33	**Length of Last Anal Fin ray**	LLaAFR
34	**Length of longest Caudal Fin ray**	LLoCFR
35	**Length of shortest Caudal Fin ray**	LSCFR

Table (2): Quantitative phenotype traits as landmarks based on morphometric characters used for differentiation analysis among different strains of Sea bream (*Sparus aurata*)

	Description
1	Forehead
2	Tip of Snout
3	Origin of dorsal fin
4	Termination of dorsal fin
5	Dorsal side of caudal peduncle at the nadir
6	Ventral side of caudal peduncle at the nadir
7	Termination of caudal peduncle at the nadir
8	Termination of anal fin
9	Origin of anal fin
10	Origin of pelvic fin

Table (2) *Continued*: Quantitative phenotype traits as landmarks based on morphometric characters used for differentiation analysis among different strains of sea bream (*Sparus aurata*)

	Description
1	1-2
2	1-10
3	2-3
4	2-9
5	2-10
6	3-4
7	3-8
8	3-9
9	3-10
10	4-5
11	4-7
12	4-8
13	4-9
14	5-6
15	5-7
16	6-7
17	7-8
18	8-9
19	9-10

Table (3): Quantitative phenotype traits based on meristic characters used for differentiation analysis among different strains of Sea bream (*Sparus aurata*)

	Characters	Acronyms
1	**Pelvic Fin spines count**	Pel FSC
2	**Pelvic Fin rays count**	Pel FRC
3	**Pectoral Fin Spines count**	Pec FSC
4	**Pectoral Fin rays count**	Pec FRC
5	**Anal Fin spines count**	AFSC
6	**Anal Fin rays count**	AFRC
7	**Caudal Fin rays count**	CFRC
9	**Dorsal Fin spines count**	DFSC
10	**Dorsal Fin rays count**	DFRC
11	**Total number of vertebrates**	TVN

RESULTS AND DISCUSSIONS

Considering a comparison between the four studied populations for the mean Values of morphometric character indices, the highest mean were (0.4039±0.002, 0.3829±0.002, 0.1486±0.001, 0.0879±0.002, 0.3062±0.001, 0.0948±0.001, 0.1077±0.001, 0.0761±0.001, 0.0532±0.001 and 0.0999±0.001) respectively for the indices HW/HL, BD/SL, BW/SL, CPW/SL, Dist. Pel-An/SL, Min. BD/SL, LLoDFR/SL, LLoAFR/SL, LLaAFR/SL and LSCFR/SL were achieved by Suez Canal population with a significant differences ($P \leq 0.05$) from the other populations. Suez Canal's population recorded also the highest mean values for CH/SL (0.4084±0.003) and CFL/SL (0.2641±0.002) with a significant difference ($P \leq 0.05$) from those populations of El-Deeba and El-

Bardawil, and for Pre-AFL/SL (0.6436±0.001) with a significant difference (P≤0.05) from Alexandria and El-Bardawil populations. Besides, Suez Canal Population differs significantly from only Alexandria population in CPD/SL mean value (0.1382±0.001). Alexandria population presented a significant superiority over all populations in HL/SL, ED/HL, CPL/SL, SNL/HL, Pr-PecFL/SL and Pr-PeLFL/SL, respectively as (0.2989±0.001, 0.0838±0.001, 0.1778±0.001, 0.8313±0.004, 0.3169±0.001 and 0.3632±0.002). While the same population showed a highest mean value in HD/HL (0.8745±0.004), Pec-FL/SL (0.3311±0.002) and LLaDFR/SL (0.0658±0.001) with a significant difference (P≤0.05) only from both El-Deeba and El-Bardawil populations. El-Bardawil population revealed a highest mean value in Po-OL/HL, DFL base/SL, AFL base/SL and Pel-FL/SL. While the highest mean values of Pr-OL/HL, TrL/SL and DFSDF/SL were achieved by El-Deeba population (Table 4).

Moreover, Suez Canal population showed the highest mean values for most of Landmark indices (Table 5). No differences were found between those individuals of the studied populations in all meristic counts mean values (Table 6).

Phylogenetic based on morphometric traits:

The hierarchical cluster analysis based on quantitative phenotype (morphometric, landmark and meristic character indices) grouped the four populations of Gilthead Sea bream (*Sparus aurata*) into two major category groups; Suez Canal, Bardawil and cultured populations group, and Alexandria, population group. Within these major grouping, Suez Canal, Bardawil and cultured populations were grouped close together and they were close to the major group of Alexandria population were grouped close. A dendrogram also showed that Suez Canal population is more phenotypically similar to that of Bardawil and cultured populations (Figure 3).

In fishery biology, phenotypic morphometric character and meristic counts are widely used to distinguish and measure the relationship between different taxonomic categories (Turan, 1999; Anene, 1999; North *et al.,* 2002; Costa *et al.,* 2003; Barriga-Sosa *et al.,* 2004). Environment has been reported as one of main effectors on body shape in fish (Beacham, 1990; Robinson and Wilson, 1995).Plasticity in fish body shape was classified as adaptive (Robinson and Parsons, 2002). Such phenotypic adaptations do not necessarily result in genetic modification in the population (Ihssen *et al.,* 1981; Allendorf, 1988), and consequently the appearance of phenotypic variations between populations cannot be reported as evidence of genetic differentiation.

Supporting the previous findings, results of the present study were found to be agreed with Clayton (1981), who reported that phenotypic variability is controlled by the environmental conditions and not directly under genetic control. Also, with Hutchings (2004) revealing that phenotypic plasticity occurs as different phenotypes is generated from the same genotype in different environments.

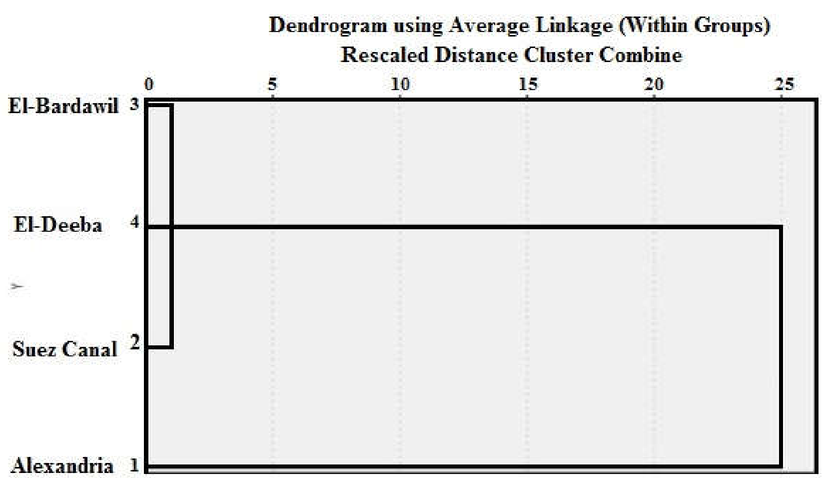


Figure (3): Average linkage of different populations of Gilthead Sea bream (*Sparus aurata*) as shown by hierarchical cluster analysis of the variables based on morphometric, landmark-morphometric, and meristic index measurements

Table (4): Mean ± SE of morphometric indices of different Gilthead Sea bream (*Sparus aurata*) population

Morphometric Indices	Deepa (cultured)	Bardawil	Alexandria	Suez Canal
HL/SL	0.2933±0.001^{b}	0.2859±0.001^{c}	0.2989±0.001^{a}	0.2911±0.001^{b}
HD/HL	0.8419±0.005^{b}	0.8417±0.003^{b}	0.8745±0.004^{a}	0.8684±0.004^{a}
HW/HL	0.3922±0.002^{b}	0.3901±0.003^{b}	0.3885±0.002^{b}	0.4039±0.002^{a}
BD/SL	0.3683±0.001^{bc}	0.3666±0.002^{c}	0.3715±0.002^{b}	0.3829±0.002^{a}
BW/SL	0.1385±0.001^{c}	0.1385±0.001^{c}	0.1431±0.001^{b}	0.1486±0.001^{a}
ED/HL	0.0693±0.001^{c}	0.0700±0.001^{c}	0.0838±0.001^{a}	0.0804±0.001^{b}
CPL/SL	0.1690±0.001^{bc}	0.1663±0.001^{c}	0.1778±0.001^{a}	0.1696±0.001^{b}
CPD/SL	0.1376±0.001^{a}	0.1361±0.001^{a}	0.1328±0.001^{b}	0.1382±0.001^{a}
CPW/SL	0.0764±0.001^{c}	0.0800±0.001^{b}	0.0798±0.001^{b}	0.0879±0.002^{a}
Pr-OL/HL	0.4326±0.002^{a}	0.4233±0.002^{b}	0.4317±0.002^{a}	0.4289±0.002^{ab}
Po-OL/HL	0.4566±0.004^{c}	0.4762±0.002^{a}	0.4645±0.001^{b}	0.4756±0.001^{a}
SNL/HL	0.6997±0.006^{c}	0.6830±0.005^{d}	0.8313±0.004^{a}	0.7829±0.007^{b}
Pr-PecFL/SL	0.3126±0.001^{b}	0.3023±0.001^{d}	0.3169±0.001^{a}	0.3086±0.001^{c}
Pr-PeLFL/SL	0.3537±0.001^{b}	0.3490±0.001^{c}	0.3632±0.002^{a}	0.3559±0.001^{b}
Pre-AFL/SL	0.6402±0.001^{a}	0.6309±0.002^{b}	0.6343±0.002^{b}	0.6436±0.001^{a}
CH/SL	0.3740±0.002^{b}	0.3751±0.003^{b}	0.4074±0.003^{a}	0.4084±0.003^{a}
DFL base/SL	0.5196±0.001^{b}	0.5305±0.001^{a}	0.5112±0.002^{c}	0.5284±0.001^{a}
AFL base/SL	0.1972±0.001^{b}	0.2065±0.001^{a}	0.1973±0.001^{b}	0.2039±0.001^{a}
Pec-FL/SL	0.3234±0.001^{b}	0.3227±0.002^{b}	0.3311±0.002^{a}	0.3224±0.001^{ab}
Pel-FL/SL	0.1842±0.001^{c}	0.1890±0.001^{a}	0.1860±0.001^{bc}	0.1874±0.001^{ab}
Dist. Pel-An/SL	0.2997±0.001^{b}	0.2956±0.001^{c}	0.2855±0.002^{d}	0.3062±0.001^{a}
CFL/SL	0.2357±0.002^{c}	0.2495±0.001^{b}	0.2593±0.002^{a}	0.2641±0.002^{a}
Min. BD/SL	0.0879±0.001^{c}	0.0897±0.001^{bc}	0.0911±0.001^{b}	0.0948±0.001^{a}
TrL/SL	0.5855±0.001^{a}	0.5686±0.002^{c}	0.5801±0.002^{b}	0.5842±0.001^{ab}
LLoDFR/SL	0.0827±0.001^{c}	0.1024±0.001^{b}	0.1013±0.001^{b}	0.1077±0.001^{a}
LLaDFR/SL	0.0605±0.001^{b}	0.0613±0.001^{b}	0.0658±0.001^{a}	0.0645±0.001^{a}
LLoAFR/SL	0.0685±0.001^{c}	0.0677±0.001^{c}	0.0722±0.001^{b}	0.0761±0.001^{a}
LLaAFR/SL	0.0508±0.001^{bc}	0.0493±0.001^{c}	0.0513±0.000^{b}	0.0532±0.001^{a}
LSCFR/SL	0.0941±0.001^{b}	0.0940±0.001^{b}	0.0940±0.001^{b}	0.0999±0.001^{a}
DFSDF/SL	0.2064±0.002^{a}	0.1947±0.001^{b}	0.1592±0.002^{d}	0.1752±0.002^{c}

Mean values in the same row having the same letters did not differ significantly ($P \leq 0.05$)

Table (5): Mean ± SE of Landmark indices of different Gilthead Sea bream (*Sparus aurata*) population

Morphometric Character	Deepa (cultured)	Bardawil	Alexandria	Suez Canal
LM1-LM2/SL	0.2051 ± 0.002^{c}	0.1952 ± 0.002^{d}	0.2485 ± 0.002^{a}	0.2278 ± 0.002^{b}
LM1-LM10/SL	0.3537 ± 0.001^{b}	0.3490 ± 0.001^{c}	0.3632 ± 0.002^{a}	0.3559 ± 0.001^{b}
LM2-LM3/SL	0.2064 ± 0.002^{a}	0.1947 ± 0.001^{b}	0.1592 ± 0.002^{d}	0.1752 ± 0.002^{c}
LM2-LM9/SL	0.5791 ± 0.001^{a}	0.5646 ± 0.001^{b}	0.5470 ± 0.002^{c}	0.5679 ± 0.002^{b}
LM2-LM10/SL	0.3533 ± 0.002^{a}	0.3369 ± 0.001^{c}	0.3487 ± 0.001^{b}	0.3535 ± 0.001^{a}
LM3-LM4/SL	0.5196 ± 0.001^{b}	0.5305 ± 0.001^{a}	0.5112 ± 0.002^{c}	0.5284 ± 0.001^{a}
LM3-LM8/SL	0.5653 ± 0.001^{b}	0.5650 ± 0.002^{b}	0.5541 ± 0.001^{c}	0.5711 ± 0.002^{a}
LM3-LM9/SL	0.4812 ± 0.001^{b}	0.4714 ± 0.002^{c}	$0.4679^{c} \pm 0.002$	0.4909 ± 0.001^{a}
LM3-LM10/SL	0.3699 ± 0.002^{b}	0.3666 ± 0.002^{b}	0.3715 ± 0.002^{b}	0.3829 ± 0.002^{a}
LM4-LM5/SL	0.1703 ± 0.001^{b}	0.1663 ± 0.001^{c}	0.1778 ± 0.001^{a}	0.1696 ± 0.001^{b}
LM4-LM7/SL	0.2016 ± 0.001^{bc}	0.1993 ± 0.001^{c}	0.2043 ± 0.001^{b}	0.2105 ± 0.002^{a}
LM4-LM8/SL	0.1491 ± 0.002^{a}	0.1361 ± 0.001^{bc}	0.1328 ± 0.001^{c}	0.1382 ± 0.001^{b}
LM4-LM9/SL	0.2822 ± 0.002^{b}	0.2888 ± 0.001^{a}	0.2809 ± 0.001^{b}	0.2896 ± 0.001^{a}
LM5-LM6/SL	0.0711 ± 0.001^{a}	0.0681 ± 0.001^{b}	0.0665 ± 0.001^{b}	0.0706 ± 0.001^{a}
LM5-LM7/SL	0.0879 ± 0.000^{c}	0.0897 ± 0.001^{bc}	0.0911 ± 0.001^{b}	0.0948 ± 0.001^{a}
LM6-LM7/SL	0.0757 ± 0.001^{b}	0.0760 ± 0.001^{b}	0.0792 ± 0.001^{a}	0.0792 ± 0.001^{a}
LM8-LM9/SL	0.1972 ± 0.001^{b}	0.2065 ± 0.001^{a}	0.1973 ± 0.001^{b}	0.2039 ± 0.001^{a}
LM9-LM10/SL	0.2997 ± 0.001^{b}	0.2956 ± 0.001^{c}	0.2855 ± 0.002^{d}	0.3062 ± 0.001^{a}

Mean values in the same row having the same letters are not differ significantly ($P \leq 0.05$)

Table (6): Mean of meristic counts of different of Gilthead Sea Bream (*Sparus aurata*) population

Meristic indices	Strains			
	Deepa (cultured)	**Bardawil**	**Alexandria**	**Suez Canal**
DFSC	11	11	11	11
DFRC	22	22	22	22
PelFRC	10	10	10	10
PecFRC	14	14	14	14
AFSC	3	3	3	3
AFRC	13	13	13	13
CFRC	16	16	16	16
No. of Ver.	21	21	21	21

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التمييز في شجرة النشوء والتطور لعشائر أسماك الدنيس البرية والمستزرعة: 1. تحليل صفات الشكل المظهري

سامى يحيى الزعيم[1] ، حافظ محمد خريبة[2] ، محمد سعد الدين الشريف[2] ، مناى محمد شاهين[2]
[1] قسم الإنتاج الحيواني والسمكي - كلية الزراعة (سابا باشا) - جامعة الإسكندرية - الإسكندرية - مصر
[2] قسم الإنتاج الحيواني والثروة السمكية - كلية الزراعة - جامعة قناة السويس - 41522 الإسماعيلية - مصر

تهدف هذه الدراسة إلى التعرف على حجم الفروق المظهرية وتقديرها وتحديد درجة ليونة الشكل المظهري لعشائر أسماك الدنيس البرية والمستزرعة عن طريق تحليل النشوء والتطور للتباين المظهري المعتمد على الاختلافات في الصفات الشكلية والـ meristic counts بين هذه العشائر. تم تمثيل كل عشيرة بعدد 100 عينة جمعت بشكل عشوائي من كلا الجنسين لهذا الغرض من ثلاثة عشائر برية (بحيرة البردويل - قناة السويس - الإسكندرية) بالإضافة إلى عشيرة من الأسماك المستزرعة (منطقة الديبة). أظهرت النتائج وجود فروق معنوية ($P\leq0.05$) بين عشائر أسماك الدنيس البرية والمستزرعة للصفات الشكلية. أظهرت عشيرة قناة السويس تفوق مقارنة بباقي العشائر لصفات HW/HL، BD/SL، BW/SL، CPW/SL، Dist. Pel-An/SL، Min. BD/SL، LLoDFR/SL، LLoAFR/SL، LLaAFR/SL و LSCFR/SL بمتوسط قيم 0.4039±0.002، 0.3829±0.002، 0.1486±0.001، 0.0879±0.002، 0.3062±0.001 ، 0.0948±0.001 ، 0.1077±0.001 ، 0.0761±0.001 ، 0.0532±0.001 و 0.0999±0.001 على التوالي. أظهر التحليل الهرمي القائم على النمط الظاهري الكمي تقسيم عشائر الدنيس موضع الدراسة إلى مجموعتين، تضمنت المجموعة الأولى عشائر كل من قناة السويس، بحيرة البردويل، وعشيرة الأسماك المستزرعة، في حين ضمت المجموعة الأخيرة عشيرة الإسكندرية.