

## Multivariate Discrimination among Three *Trachurus* Species from Turkey

Hakan Karaoglu and Ali Osman Belduz

Department of Biology, Faculty of Arts and Sciences,  
Karadeniz Technical University, 61080 Trabzon, Turkey

**Abstract:** *Trachurus mediterraneus* (Steindachner, 1868), *T. trachurus* (Linnaeus, 1758) and *T. picturatus* (Bowdich, 1825) are three closely related, morphologically similar horse mackerel species. Here multivariate analyses are used to study interspecific morphometric and meristic variation for these three species. The statistical analysis confirmed that three species were well differentiated. In particular, discriminant analysis revealed that a strong discriminating power of the morphometric variables and three *Trachurus* species was clearly distinguished due to measurements of preanal length, head length, eye diameter, length of lateral line and numbers of scale in lateral line.

**Key words:** *Trachurus* species, carangidae, morphology, multivariate analysis, Turkey

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### INTRODUCTION

The horse mackerel ranges widely throughout the tropical waters throughout temperate, tropical and subtropical waters of the world (Eschmeyer, 2003). The genus *Trachurus* which was first described its taxonomies by Nichols (1920) this genus is represented by three species: the Atlantic horse mackerel, *T. trachurus* (L.), the Mediterranean horse mackerel, *T. mediterraneus* (Steind.) and the blue jack mackerel, *T. picturatus* (Bowd.) in the Turkey waters. Multivariate analysis of morphometric and meristic characters is a standard tool for defining population units and differentiating between genera, species, sub-species and groups of animals (Fridriksson, 1958; Thorpe, 1976; Boetius, 1980; Pierce *et al.*, 1994; Tudela, 1999).

A recently published study has pointed morphological variation within rather than among species: Murta (2000), morphometric and meristic characters analyzed for stock identification of Atlantic horse mackerel *T. trachurus*; Turan (2004) studied morphologic differentiation among stocks of Mediterranean horse mackerel, *T. mediterraneus* throughout the Black, Marmara, Aegean and Northeastern Mediterranean seas from coasts of the Turkish waters; Belduz and Bektas (2009) were studied for *T. trachurus* populations from Turkey by multivariate analyses but the morphological differences among *Trachurus* species were not analyzed. Moreover, genetic relationships among *Trachurus* sp. from Turkey have mainly been examined by mtDNA genes (Bektas and Belduz, 2008) and they were suggested that

genetic distances among *T. mediterraneus* and *T. picturatus* are more closely related to each other more than any of *T. trachurus*.

The goal of the present research are to reveal interspecies variation in morphological and meristic characters of three *Trachurus* species and to identify the best set of characters for group separation with the use of the multivariate analysis technique.

### MATERIALS AND METHODS

The samples made up of 480 specimens of *Trachurus mediterraneus* (9.6-19.7 cm SL; N = 270), *T. trachurus* (10.7-26.2 cm, N = 150) and *T. picturatus* (19.5-26.5 cm, N = 60) were collected from nine different locations in the four different seas of Turkey (Aegean sea, Sea of Marmara, Black sea and Northeastern Mediterranean sea) (Fig. 1). All specimens were directly fixed in 20% formalin. Analysis took place at the Karadeniz Technical University. Particular measurements were always made by the same person to minimise errors.

Measurements and counts were taken from 30 individuals per population, comprising 11 morphometric: Lengths of First Dorsal Fins (DFL1), Second Dorsal (DFL2), Pectoral (PFL), Anal (AL), Lateral Line (LL), Preanal (PrAL) Lengths, maximum Body Heights (BH), minimum Body Heights (CPH), Head Length (HL) and Eye Diameter (ED) and 5 meristic characters: number of branched rays in First Dorsal (DFR1), Second Dorsal (DFR2), Pelvic (PFR), Anal (AFR) and number of pectoral rays (PFR) and number of scales on the lateral line (LS)

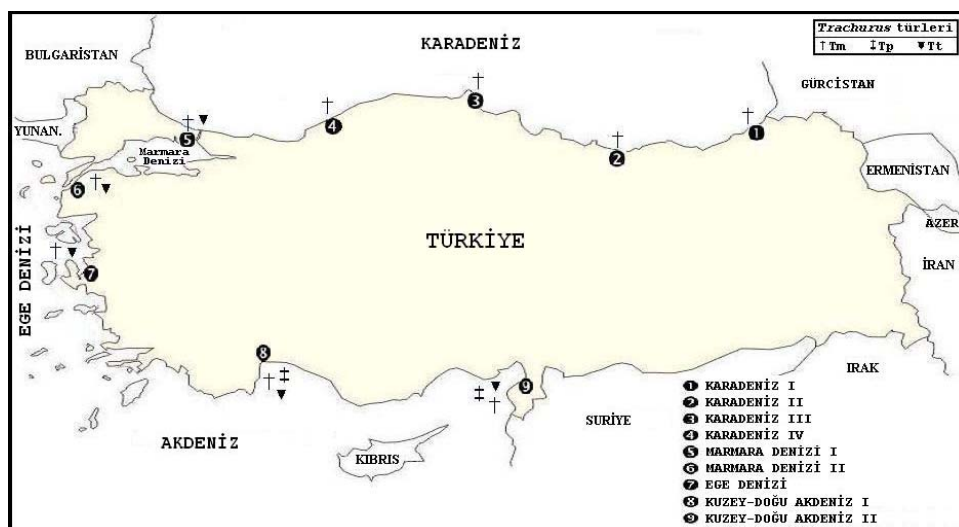


Fig. 1: Map showing the different sampled localities for three species from Turkey

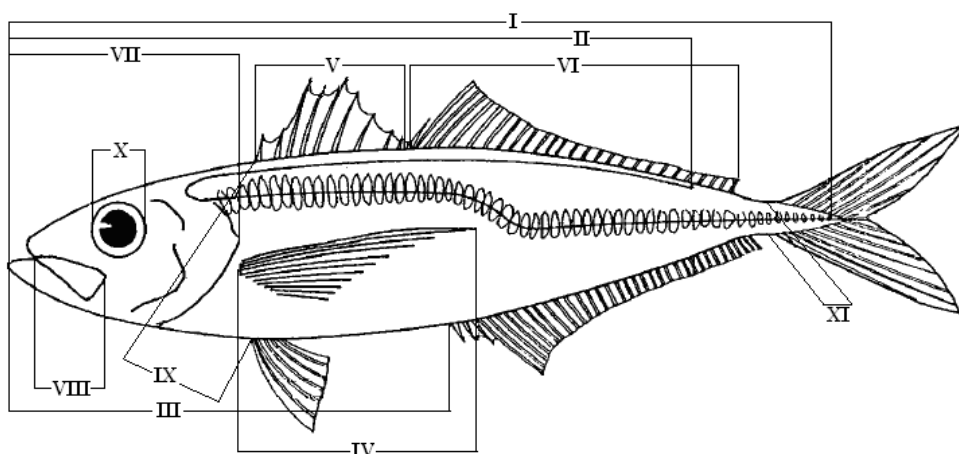


Fig. 2: Measurements on the body of Horse mackerel. I) SL: Standart Length; II) LL: Length of Lateral line; III) PrAL: Preanal Length; IV) PFL: Pectoral Fin Length; V) DFL1: First Dorsal Fin Length; VI) DFL2: Second Dorsal Fin Length; VII) HL: Head Length; VIII) ML: Maxilla Length; IX) BD: Body Depth; X) ED: Eye Diameter; XI) CPD: Caudal Peduncle Depth (Murta, 2000)

were selected based on previous research (Murta, 2000) (Fig. 2). The morphometric measurements were measured to the nearest 0.01 mm. Standard length was expressed as a percentage of the total length. Others metric measurements were expressed as percentages of the standard length. Eye Diameter (ED) was expressed as percentages of the head length (ED/HL) and the minimum body height was expressed as a percentage of the maximum body height (CPD/BD). All variables used for morphometric and meristic analysis were measured under a binocular microscope.

To determine interspecific variations among *Trachurus* species, morphometric and meristic characters were used separately in multivariate analyses since their

allocation abilities are different statistically. Because of the variation in size of fish from different areas, morphometric and meristic data were statistically adjusted to permit comparative analysis in terms of shape and counts independently of size (Thorpe, 1976). Accordingly, comparison of morphometric data among different populations should be preceded by a suitable correction to remove size-effect. Carangid fishes show allometric growth (Santic *et al.*, 2002). All individual morphometric data were thus corrected using the  $M_s = M (L_o/L_s)^b$  equation (Elliott *et al.*, 1995).

The pattern of discrimination between species was examined by canonical discriminant analysis. Discriminant functions for each species were worked out on the basis

of variables with a higher individual contribution, evaluated from standardized discriminant coefficients. To investigate the phenotypic relationships between populations a dendrogram was constructed based on Mahalanobis distances using UPGMA cluster analysis of arithmetic averages (Sneath and Sokal, 1973). Statistical analyses were carried out using STATISTICA Statsoft, Inc. Version 7.0 packages.

## RESULTS AND DISCUSSION

Derived discriminant functions in DFA using eleven of the size-corrected morphometric characters identified Head Length (HL), Length of Lateral Line (LL), PreAnal Length (PrAL), Body Depth (BD), Eye Diameter (ED) and Pelvic Fin Length (PFL) as significant six contributors that were found to be useful for distinguishing related species. The rest of the morphometrics also contributed significantly to heterogeneity but were not good identifiers of related taxa (Table 1). The Wilks test indicated a significant difference for mean vectors for the eleven distances among *T. mediterraneus*, *T. trachurus* and *T. picturatus* ( $L = 0.073$ ;  $F = 175.65$ ;  $p < 0.0001$ ) (Table 1). Discriminant analysis results revealed that there are clearly differentiating characteristics between these species, e.g., lateral line scale counts, termination point of dorsal accessory lateral line. *T. picturatus* has been low body depth and differences in the head characters of fish. This demonstrated that the total morphometric variation between species, considering 11 measures could be reduced to just six discriminants. Discriminant analysis based on these standardized

variables resulted in 100% success in classifying fish to the correct species. Despite this high degree of accuracy, it should be taken into account that these results applied to only the length classes analyzed for each species. No precision was shown for classes out of the analyzed range due to allometric changes in shape during growth. The projection of scores for *T. mediterraneus*, *T. trachurus* and *T. picturatus* indicated strong dependence on the first component rather than the second which was probably due to allometry. However, in the present study such influence might have been minimized by the reduction of allometry effects derived from the range of three species sizes which were very similar. It was showed that the three species were morphologically clearly distinct.

The plot of the canonical variables 1 and 2 shows a significant discrimination of species scores in three significant main groups along CV1 (Table 2). As revealed by the respective eigen-values, the first two Canonical Variates (CV) explained 100% of the variability. This demonstrated that it was proper to use them for morphological analyses, since they adequately explained the variability for the whole set of data (Johnson and Wichern, 1992). The first Canonical Variate (CV1) was responsible for 57.2% of the variation among-species, the second (CV2) for 43.8% (Table 2). Examination of the correlations revealed that PrAL and HL contributed mostly to CV1 and hence (This first axis was as expected, dominated by the effect of body size), accounted mostly for the variance in the data whereas LS contributed mostly to CV2. These 3 characteristics also showed the highest values of correlation with the first and second canonical

Table 1: Summary of the discriminant function analysis for morphometric and meristic characters

Variables	<i>Trachurus mediterraneus</i>				<i>Trachurus trachurus</i>				<i>Trachurus picturatus</i>				Turbot			
	Wilks	F-rem.	p-level	Toler.	Wilks	F-rem.	p-level	Toler.	Wilks	F-rem.	p-level	Toler.	Wilks	F-rem.	p-level	Toler.
<b>Morphometric variables</b>																
HL	0.065	2.462	0.023	0.899	0.078	1.102	0.370	0.252	0.490	0.804	0.379	0.670	0.001	47.55	0.000	0.657
LL	0.058	1.435	0.202	0.875	0.097	3.553	0.015	0.474	0.592	5.768	0.024	0.625	0.002	93.04	0.000	0.840
D1L	0.055	1.007	0.441	0.848	0.072	0.446	0.773	0.579	0.493	0.931	0.344	0.844	0.000	1.230	0.295	0.821
D2L	0.052	0.614	0.761	0.902	0.073	0.519	0.721	0.792	0.477	0.151	0.700	0.661	0.001	6.443	0.002	0.855
PrAL	0.051	0.476	0.867	0.834	0.087	2.341	0.074	0.327	0.474	0.005	0.940	0.516	0.002	122.7	0.000	0.740
BD	0.080	4.619	0.000	0.770	0.090	2.700	0.046	0.252	0.476	0.116	0.736	0.649	0.001	8.002	0.000	0.786
ED	0.103	7.913	0.000	0.925	0.100	3.880	0.000	0.284	0.481	0.344	0.563	0.594	0.001	10.03	0.000	0.615
AL	0.053	0.694	0.695	0.874	0.072	0.383	0.818	0.792	0.507	1.626	0.214	0.823	0.000	3.023	0.051	0.852
PL	0.059	1.632	0.136	0.835	0.094	3.129	0.026	0.822	0.487	0.671	0.420	0.660	0.001	12.40	0.000	0.935
CPD	0.059	1.550	0.161	0.821	0.096	3.366	0.019	0.295	0.474	0.001	0.975	0.826	0.000	1.401	0.249	0.853
ML	0.056	1.243	0.292	0.885	0.094	3.142	0.026	0.428	0.475	0.068	0.796	0.770	0.000	3.707	0.026	0.906
	F (88.370) = 2.473				F (44.135) = 3.115				F (11.23) = 2.320				F (22.29) = 418.25			
Total	0.048				0.069				0.473				0.0009			
<b>Meristic variables</b>																
P	0.638	1.753	0.086	0.995	0.287	0.726	0.578	0.953	0.875	0.142	0.725	0.658	0.074	1.251	0.287	0.996
D1	0.612	0.341	0.949	0.995	0.274	0.204	0.934	0.972	0.884	0.184	0.689	0.799	0.074	1.407	0.246	0.996
D2	0.681	4.00	0.000	0.988	0.383	4.740	0.000	0.870	0.937	0.437	0.544	0.504	0.093	45.06	0.000	0.921
A	0.798	10.24	0.000	0.987	0.383	4.714	0.000	0.855	0.887	0.201	0.676	0.655	0.093	44.04	0.000	0.923
L	0.634	1.53	0.146	0.997	0.305	1.491	0.220	0.966	0.847	0.008	0.930	0.881	0.570	1103.58	0.000	0.993
	F (40.112) = 3.4215				F (20.150) = 3.6342				F (5.4) = 0.1462				F (10.65) = 175.65			
Total	0.605				0.270				0.845				0.073			

$p < 0.0001$

Table 2: Structure matrix of discriminant loadings for each of morphometric and meristic variable selected by the backward stepwise Discriminant Function Analysis (DFA)

Variables	<i>Trachurus mediterraneus</i>			<i>Trachurus trachurus</i>			<i>Trachurus picturatus</i>	All species	
	CV1	CV2	CV3	CV1	CV2	CV3	CV1	CV1	CV2
<b>Morphometric variables</b>									
HL	0.4284	0.0709	0.5508	-0.3063	0.4337	-0.7811	-0.3903	0.5951	-0.5139
LL	0.1299	0.3508	-0.7081	0.7961	0.4456	-0.0976	-0.1214	-0.5236	0.6439
D1L	-0.1936	0.0318	0.2585	-0.1837	0.1706	0.4241	-0.2960	-0.0214	-0.1430
D2L	-0.0856	-0.3387	0.0434	0.1676	-0.2725	-0.0826	0.1373	0.1233	0.2879
PrAL	0.0234	0.2131	-0.0136	0.1846	-1.0410	0.2195	0.0302	0.7651	0.5357
BD	0.2937	0.9315	0.3209	0.8505	-0.7153	-0.6252	-0.2172	-0.2210	-0.2843
ED	0.7880	-0.3234	-0.2223	0.9202	-0.4610	0.9701	-0.7805	0.1939	0.4067
AL	0.2343	0.0057	-0.3842	-0.2071	-0.0817	-0.2557	-0.3095	-0.2119	0.0548
PL	-0.2402	-0.3782	0.4238	-0.5087	-0.3671	0.4276	-0.2858	-0.1531	0.3705
CPD	0.2787	-0.4488	-0.2127	0.8291	-0.8387	-0.0079	-0.0100	-0.1504	-0.0066
ML	0.1238	-0.5128	-0.1498	0.0765	1.0365	-0.2219	0.0853	0.1873	-0.1410
Eigenval	4.4230	0.9228	0.2869	3.7932	1.1076	0.3031	1.1097	36.4810	27.2012
Cum.prop	0.7254	0.8768	0.9238	0.7157	0.9247	0.9819	1.0000	0.5728	1.0000
<b>Meristic variables</b>									
P	-0.0512	-0.0626	0.9582	-0.2606	-0.2901	0.0260	-0.5808	0.0145	-0.1492
D1	0.0367	0.0401	0.1789	-0.0779	0.1113	-0.4227	-0.5979	0.0559	-0.1315
D2	0.3037	0.8281	-0.0723	-0.5922	-0.7270	0.4471	-1.1235	0.0500	-0.8340
A	-0.9827	0.1564	-0.0363	-0.6128	0.6813	-0.5302	0.6875	-0.1914	0.7686
L	0.0335	-0.4876	-0.2663	0.1637	-0.6733	-0.6791	0.1254	-0.9876	-0.1650
Eigenval	0.3330	0.1412	0.0572	1.8432	0.2543	0.0262	0.1828	8.0472	0.5015
Cum. Prop	0.5971	0.8504	0.9529	0.8631	0.9822	0.9945	1.0000	0.9413	1.0000

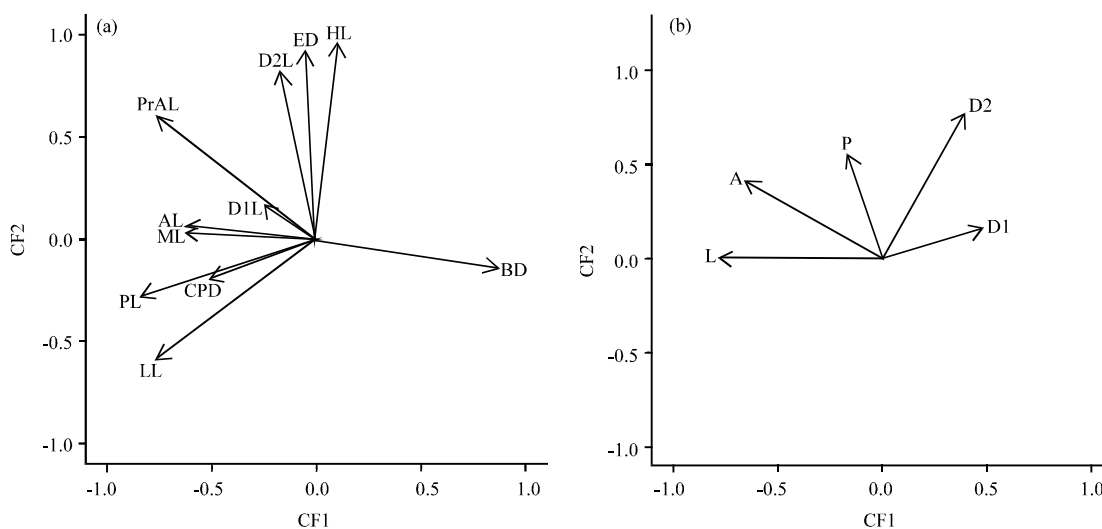


Fig. 3: Contribution of morphometric (a) and meristic (b) characters to the discriminant functions. Vectors indicate the loadings of the scores for each variable on the first and second discriminant functions

variables of the discriminant analysis (Table 2) which is useful for discriminating *Trachurus* species. There was good separation of *T. trachurus* from *T. mediterraneus* and *T. picturatus* along axis 1 and 2.

Analyses of the meristic characters revealed notable significant differences between the three *Trachurus* species. Derived discriminant functions in DFA using 5 meristic characters identified LS as significant contributors while PFR, DFR1, DFR2 and AFR were not significant (Table 1). Wilk's lambda tests showed small

differences among the three species when their meristic characters were compared with morphometrics. Moreover, the stepwise analysis revealed that only one meristic character contributed significantly to the multivariate discrimination of the three species of *Trachurus* (Table 2). Among the 5 characters that were found to be useful in population differentiation. In this case, the plot of the two first canonical variates (Fig. 3) which account for 94.13 and 5.87% of total variation (Table 2). The plots derived from both discriminant function analyses (Fig. 4) also

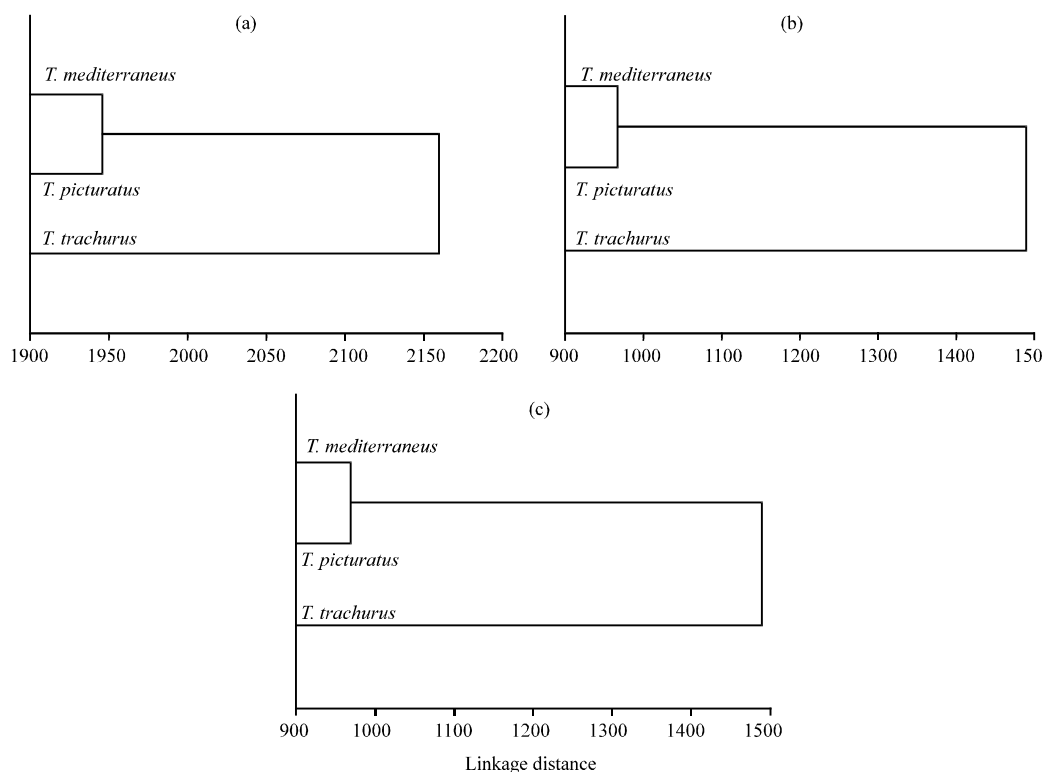


Fig. 4: UPGMA cluster analysis based on Mahalanobis  $D^2$  distances between the morphometric (a) meristic (b) and morphometric and meristic (c) characters

confirmed that morphometric characters are much more effective for discriminating between *Trachurus* species than meristic characters which is expressed lower distances among species. The dissimilarity between morphometric and meristic data showed that unlike meristic characters which are fixed early in life, morphometric characters typically show ontogenetic changes associated with allometric growth (Gould, 1966) and may be labile to environmental influences throughout life (Wainwright *et al.*, 1991). Discriminant function analyses indicated that morphometric and meristic differentiation among species was largely due to differences in the head characters of fish which may reflect differential habitat use.

Multivariate analysis of both morphological and meristic characters has been used successfully on *Trachurus* species as a tool for separating species from same geographical regions. Especially, *T. mediterraneus* and *T. trachurus* share the same areas and have similar appearances which make identification on the species level rather difficult. *T. trachurus* is usually discriminated by the presence of smaller eye has little than 74 scales in the lateral line. The observed differences in morphometric

characteristics of related *Trachurus* species have a genetic or environmental basis remains an important question. It is interesting to note that for several of the external morphometric characters which show significant variation, values for *T. picturatus* and for *T. trachurus* and *T. mediterraneus* tend to be at opposite extremes and standardized body depth is higher in *T. trachurus* and *T. mediterraneus* than in the deep seas form (*T. picturatus*). This could indicate the selective effect of interspecific competition, since it is only around the South Aegean sea and North-eastern Mediterranean sea that these three species overlap substantially in their range. Thus, *T. picturatus* tend to be more different from *T. trachurus* and *T. mediterraneus*.

To investigate the phenotypic relationships between the examined species a dendrogram was constructed based on Mahalanobis distances using UPGMA cluster analysis (Sneath and Sokal, 1973). For both morphometric and meristic characters, the dendrogram obtained by UPGMA cluster analysis was revealed 3 major groups containing *T. mediterraneus*, *T. mediterraneus* and *T. picturatus* populations from nine Turkish localities (Fig. 5). The results on species discrimination from the

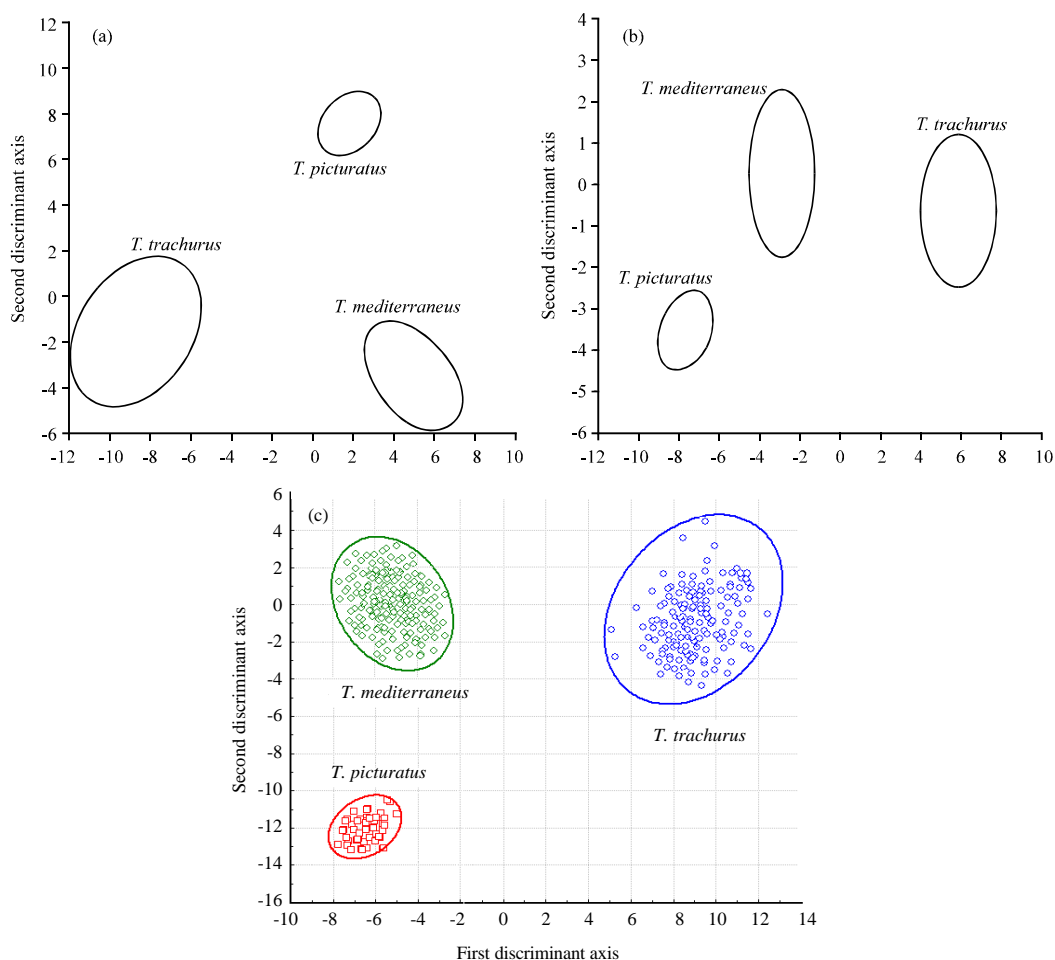


Fig. 5: Scatterplots of the Discriminant Function Analysis (DFA) scores along the first and second root for the nine collection sites of *Trachurus* species based on the morphometric (a) meristic (b) and morphometric and meristic characters (c) with 95% confidence ellipses

present study are consistent with results of genetic analysis by Karaïskou *et al.* (2003) and Bektas and Belduz (2008) who indicated that *T. mediterraneus* and *T. picturatus* are genetically more closely related to each other than either is to *T. trachurus*.

### CONCLUSION

The discriminant function analysis revealed that this technique was sensitive enough to separate the three *Trachurus* species since, the plot (Fig. 5) showed a high convergence for the three species.

### ACKNOWLEDGEMENTS

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