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THE INTERSPECIFIC VARIABILITY AND PHENETIC RELATIONSHIPS OF SIX EURO-MEDITERRANEAN MULLET SPECIES (MUGILIDAE) OBTAINED BY ANALYSIS OF THE GILLS LID ELEMENTS

Abstract

The interspecific variability and phenetic relationships of six mullet species from South Adriatic Sea (*Mugil cephalus* Linnaeus, 1758; *Liza aurata* Risso, 1810; *Liza saliens* Risso, 1810; *Liza ramada* Risso, 1826; *Chelon labrosus* Risso, 1826; *Oedalechilus labeo* Cuvier, 1829) were analyzed on base of 10 continuous characters of gills lid elements. In analyze of variability of gills lid characters (Principal Component Analyze, PCA), the species *Mugil cephalus* clearly separated from the other five Adriatic mullet species. Also, the species *Oedalechilus labeo* showed significant specificity of continuous characters variability of gills lid related to the other mullet species. In discriminative analyze (DA), the characters on opercular and preopercular bones had the greatest discriminative power. The most clearly was discriminated the *Mugil cephalus*, and then the *Oedalechilus labeo*. The distances between the *Mugil cephalus* and the *Oedalechilus labeo* were the biggest.

Keywords: Mugilidae, skeleton, interspecific variability, phenetic relationships

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INTERSPECIJSKA VARIJABILNOST I FENETIČKI ODNOSI ŠEST EVRO-MEDITERANSKIH VRSTA CIPOLA (MUGILIDAE) DOBIJENI ANALIZOM ELEMENATA ŠKRŽNOG POKLOPCA

Izvod

Interspecijska varijabilnost i fenetički odnosi šest vrsta mugilida iz južnog Jadrana (*Mugil cephalus* Linnaeus, 1758; *Liza aurata* Risso, 1810; *Liza saliens* Risso, 1810; *Liza ramada* Risso, 1826; *Chelon labrosus* Risso, 1826; *Oedalechilus labeo* Cuvier, 1829) analizirani su na osnovu 10 kontinuiranih karakteristika škržnog poklopca. Analiza varijabilnosti kontinuiranih karakteristika (Analiza Glavnih Komponenti, PCA) jasno je odvojila vrstu *Mugil cephalus* od ostalih pet vrsta mugilida. Takodje, vrsta *Oedalechilus labeo* pokazala je značajnu specifičnost varijabilnosti kontinuiranih karakteristika škržnog poklopca u odnosu na ostalih pet vrsta mugilida. U diskriminantnoj analizi (DA), najveću diskriminantnu moć imaju karakteri koji opisuju operkularnu i preoperkularnu kost. Najjasnije se odvaja vrsta *Mugil cephalus*, a zatim *Oedalechilus labeo*. Izmedju ove dvije vrste ustanovljene su najveće distance.

Ključne riječi: Mugilidae, skelet, interspecijska varijabilnost, fenetički odnosi

1. INTRODUCTION

Six Adriatic mullet species (*Mugil cephalus* Linnaeus, 1758; *Liza aurata* Risso, 1810; *Liza saliens* Risso, 1810; *Liza ramada* Risso, 1826; *Chelon labrosus* Risso, 1826; *Oedalechilus labeo* Cuvier, 1829) are Euro-Mediterranean faunistic elements. The interspecific relationships and the phylogenetic relationships within the family Mugilidae were studied using the analysis of DNA sequences (Caldara *et al*., 1996; Papasotiropoulos *et al*., 2002), chromosomes (Cataudella *et al*., 1974; Gornung *et al*., 2001), allozymes (Autem & Bonhomme, 1980; Papasotiropoulos *et al*., 2001; Turan *et al*., 2005) hemoglobin (Rizzotti, 1993) and pharyngobranchial organ (Harrison & Howes, 1991). The problems in distinguishing the genera *Liza* and *Chelon* on the basis of chromosomes (Cataudella *et al*., 1974) were resolved by allozyme (Autem & Bonhomme, 1980) and hemoglobin (Rizzotti, 1993) analysis.

The osteological analysis in mullets related to the relationships between taxa were accomplished by Burdak (1957), who compared functionally related elements of the jaw skeleton among three Black Sea mullet species (*Mugil cephalus*, *Liza aurata* and *Liza saliens*), and by Schultz (1946), who accomplished the revision of mullet genera and constructed the diagram of possible relationships between them on the basis of the form of preorbital, maxillar and premaxillar bones.

The results of this paper as well as the phenetic relationships between Euro-Mediterannean mullet species (family Mugilidae) from four cosmopolite genera from South Adriatic Sea: *Mugil* (Linnaeus, 1758), *Chelon* (Rose and Walbaum, 1793), *Liza* (Jordan and Swain, 1884) and *Oedalechilus* (Fowler, 1904), instruct on corresponding use of this taxonomic analysis form in a clearer determining phylogenetic relationships in the frame of this group of marine fish.

2. MATERIALS AND METHODS

The material was collected in 2000. and 2001. in the South Adriatic Sea, along the coast of Montenegro (Bar and Tivat mainly, but also Petrovac and Budva), using the trawl net. The analysed sample contained 12 *Mugil cephalus*, 14 *Liza aurata*, 15 *Liza saliens*, 24 *Liza ramada*, 15 *Chelon labrosus,* and 16 *Oedalechilus labeo*.

The measurement was accomplished by millimeter caliper (of the precision of 0.1 mm), under the binocular, with an applying four-times amplification. The complex of gills lid encompassed 10 continuous characters on branchiostegal I, opercul, preopercul, interopercul bones (Fig. 1).

The data analysis was accomplished using the Statistica package, Release 4.5 (StatSoft, Inc. 1993), and comprised Analysis of Variance (ANOVA) and Multivariate Analysis of Variance (MANOVA) with the species as factors. The multivariate analysis of both intra- and interspecific variability in size (PC1) and shape (PC2 & PC3) was accomplished using the Principal Component Analysis (PCA), whereas the discrimination between species on the most discriminative characters was analysed by Cannonical Discrimination Analysis using the PROC CANDISC module relationships between centroids of OTUs constructed by UPGMA Cluster Analysis on the Mahalanobis D^2 distances $D^2_{(i,j)} = (X_i - X_j) - 1\ COV\ (X_i - X_j)$ (Sneath & Sokal, 1973) using the NTSYS-pc package V. 1.50 (Rohlf, 1988).

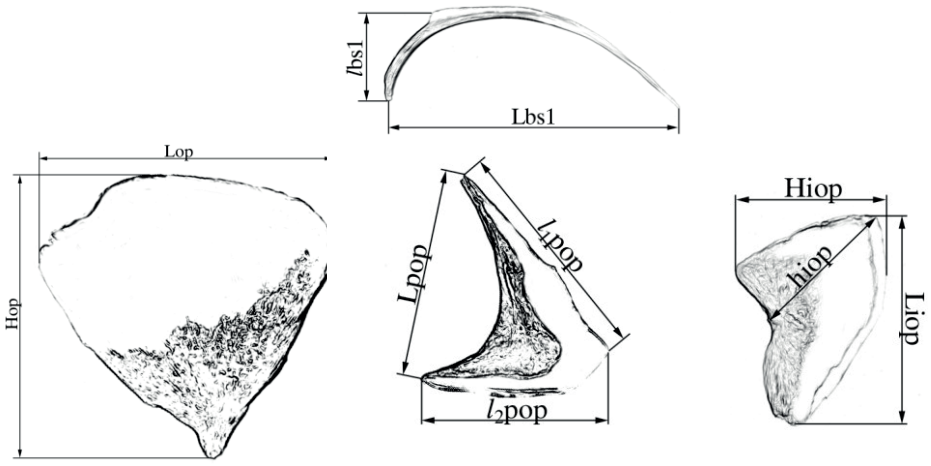

Figure 1: The model of continuous characters of gills lid elements. The list of abbreviations: Hop - opercul height; Lop - opercul length; Lpop - preopercul length; $l_1$pop - length of ventral continuation of preopercul; $l_2$pop - length of dorsal continuation of preopercul; Liop - interopercul length; Hiop - interopercul heigth; hiop - interopercul height at level joint which attaches for interhyal; Lbs_1 - branchiostegal I length; lbs_1 - length of the branchiostegal I cutting.

3. RESULTS

Both univariate (ANOVA) and multivariate (MANOVA, R = 25.929) analysis of variance of the continuous osteological characters revealed significant differences ($p < 0.001$) among the species (Table I and II).

Characters	MSge	MSerr	F(df1,2)	P<
Hop	864.305	4.804	179.882	0.000
Lop	958.822	6.423	149.270	0.000
Lpop	548.378	4.264	128.603	0.000
$l_1$pop	741.246	6.059	122.337	0.000
$l_2$pop	478.198	4.814	99.322	0.000
Liop	699.581	4.426	158.032	0.000
Hiop	274.807	1.451	189.349	0.000
hiop	304.992	2.139	142.538	0.000
Lbs1	2011.764	12.206	164.804	0.000
lbs1	280.210	2.610	107.319	0.000

Table I: One-factor Analysis of Variance (ANOVA) for species (6) on continuous characters of the gills lid elements (MSge - MS of the main effect; MSerr – MS of the error; F - ANOVA test value; P< - statistic significance level)

Test	Test value	P<
Wilks' Lambda	0.001	
Rao R (50,372)	25.929	0.000
Pillai-Bartlett Trac	2.920	
V (50,425)	11.935	0.000

Table II: Multivariate Analysis of Variance (MANOVA) for species (6) for continuous characters of the gills lid elements (P< - statistic significance level)

In analyzing the gills lid elements, the first Principal Component PC1 (λ_1 = 9.596; 95.958 %) separated the species *Mugil cephalus* from the other mullet species (Fig. 2). The characters Hiop, $l_2$pop and Lpop had the greatest loadings on the first Principal Component (Table III). The second Principal Component PC2 (λ_2 = 0.160; 97.570 %) distinguished by its variability *Liza ramada* from the other two species of the genera *Liza* (Fig. 2), with Lbs_1 as the character with the greatest loading on it (Table III). The third Principal Component PC3 (λ_3 = 0.097; 98.546 %) explained the best variability of the species *Oedalechilus labeo* (Fig. 2), with the l_1bs_1 as the character with the greatest loading on it (Table III).

Characters	PC1	PC2	PC3
Hop	0.536	0.661	0.512
Lop	0.557	0.667	0.484
Lpop	0.728	0.510	0.442
$l_1$pop	0.698	0.537	0.454
$l_2$pop	0.742	0.401	0.509
Liop	0.651	0.501	0.556
Hiop	0.763	0.544	0.330
Hiop	0.686	0.544	0.463
Lbs_1	0.519	0.738	0.414
lbs_1	0.487	0.534	0.683

Table III: Loadings of continuous characters of gills lid elements of six mullet species on the first, second and third Principal Component (PC1 - PC3)

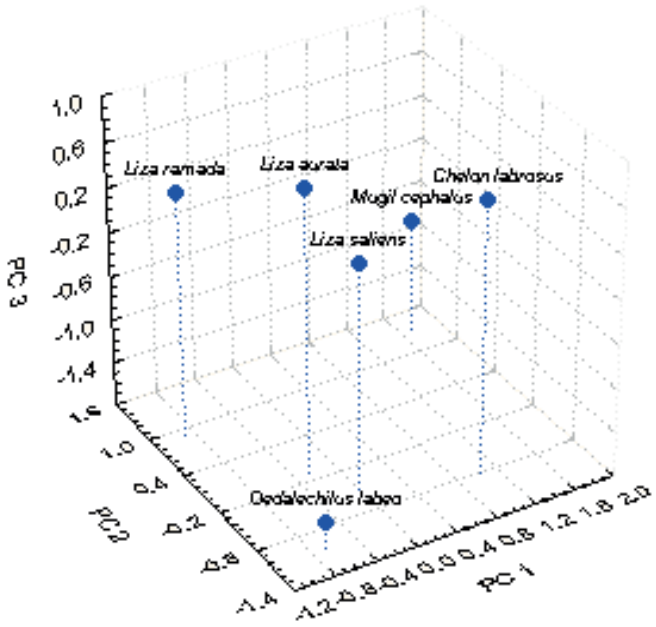

Figure 2: The scatterplot of centroids of six mullet species on the first, second and third Principal Component (PC1 - PC3) based on the continuous characters of gills lid elements

In discriminative analyze of gills lid elements, each of the first three Discriminant Components in the Cannonical Discriminant Analysis of six mullet species was bipolar. The first one, ROOT1 (λ_1 = 12.827), explained 49.348 % of the total discrimination between species (Table IV). The second Discriminant Component ROOT2 (λ_2 = 8.927) explained 34.343 % of the total discrimination between species (Table IV). The third Discriminant Component ROOT3 (λ_3 = 3.866) explained 14.875 % of the total discrimination between species (Table IV). The prominent descriptors on discriminative axes, which separated the genera *Oedalechilus* from the other five mullet species (Fig. 3), were Hop and Lpop (Table IV). The characters which somewhat separated the *Mugil cephalus* (Fig. 3) were Hiop and hiop (Table IV).

Characters	ROOT1	ROOT2	ROOT3
Hop	1.298	1.298	- 0.691
Lop	0.121	0.121	- 0.164
Lpop	- 1.049	- 1.049	0.176
$l_1$pop	- 0.375	- 0.375	0.044
$l_2$pop	0.155	0.155	0.021
Liop	0.261	0.261	2.968
Hiop	0.387	0.387	- 0.325
hiop	- 0.301	- 0.301	- 0.957
Lbs_1	0.555	0.555	1.045
lbs_1	- 0.169	- 0.169	0.209
Eigenval	12.827	12.827	3.866
Cum.Prop	0.493	0.493	0.985

Table IV: Loadings of continuous characters of gills lid elements of six mullet species on the first, second and third discriminative axes (ROOT1 - ROOT3)

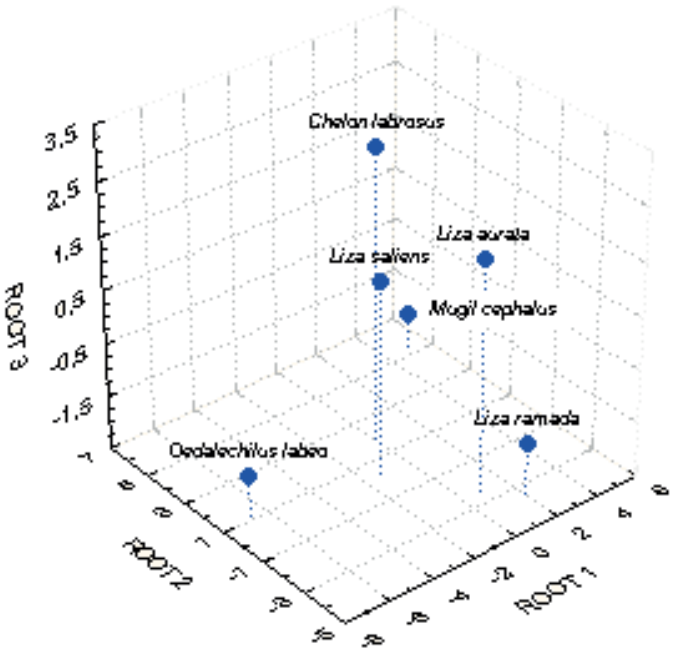

Figure 3: The scatterplot of centroids of six mullet species on the first, second and third discriminative axes (ROOT1 - ROOT3) based on the continuous characters of gills lid elements

The Mahalanobis distances among centroids of individuals of six mullet species, in the analyze of gills lid elements (Table V) were the biggest between centroids of *Mugil cephalus* and *Oedalechilus labeo* individuals, as well as between centroids of both *Oedalechilus labeo* and *Liza ramada* and *Mugil cephalus* and *Liza aurata* individuals. That also showed UPGMA clustering scattered individuals of the species, obtained by the Mahalanobis distances among their centroids (Fig. 4).

	Liza aurata	*Liza ramada*	*Liza saliens*	*Chelon labrosus*	*Oedalechilus labeo*	*Mugil cephalus*
L. aurata	0.000	16.493	16.585	24.234	89.023	100.099
L. ramada	16.493	0.000	35.037	56.805	104.207	94.738
L. saliens	16.585	35.037	0.000	9.287	42.253	82.789
C. labrosus	24.234	56.805	9.287	0.000	72.575	65.934
O. labeo	89.023	104.207	42.253	72.575	0.000	166.200
M. cephalus	100.099	94.738	82.789	65.934	166.200	0.000

Table V: Squared Mahalanobis distances among six mullet species on their continuous characters of gills lid elements

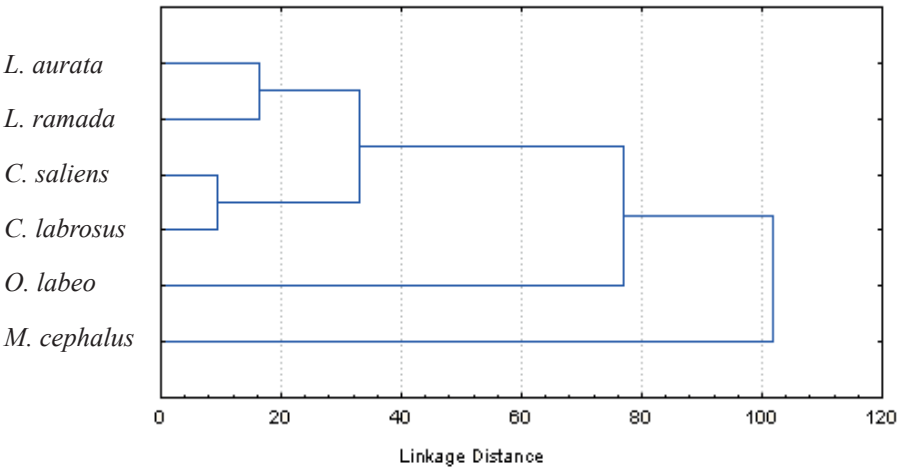

Figure 4: The phenetic relationships among six mullet species generated by UPGMA clustering of Mahalanobis distances among them

4. DISCUSSION

The greatest interspecific difference that was found between the species *Mugil cephalus* and the other five adriatic mullet species, as

well as smaller differences between both the three *Liza* species and the *Chelon labrosus* and the three *Liza* species and the *Oedalechilus labeo* are in concordance with the report of Caldara *et al*. (1996), who considered the phylogenetic position of the *Oedalechilus labeo* for the first time. Both the cytochrome b and 12s rRNA analysis revealed that the *Oedalechilus labeo* is in the sister group with the *Liza*-*Chelon* lineage.

In the analyze of the mtDNA sequences (12s rRNA, 16s rRNA and CO1) of five mullet species (Papasotiropoulos *et al*., 2002) the greatest amount of genetic differentiation was observed at the interspecific level, while little variation was observed at the intraspecific level. The highest values of nucleotide sequence divergence were observed between the *Mugil cephalus* and the other four species, while the lowest was found between the *Chelon labrosus* and the *Liza saliens*. The most distinct species is *Mugil cephalus*, while the other species are clustered in two separate groups, the first one contains the *Liza aurata* and the *Liza ramada*, and the other *Liza saliens* and the *Chelon labrosus*. A detachment of the *Mugil cephalus* and contiguity of the genera *Chelon* to phyletic lineage of the genera *Liza*, have been also obtained by the analyze of ten enzymic systems corresponding to 22 genetic loci (Papasotiropoulos et al., 2001), while the researching of phylogenetic relationships of nine mullet species by allozyme elektrophoresis using seven enzyme systems (Turan *et al.*, 2005), is shown that the *Chelon labrosus* and the *Oedalechilus labeo* are in one branch, which is in the sister group with the *Liza ramada*. The other four species of the genera *Liza* formed two subbranches, while the *Mugil cephalus* is more distinct species.

Cataudella *et al*. (1974) on the analysis of karyotypes did not find significant differences between the *Chelon labrosus* and three species of the genera *Liza* (*Liza aurata*, *Liza ramada,* and *Liza saliens*). On the contrary, Autem & Bonhomme (1980) found appreciable genetic differences between the *Chelon* and the *Liza*, as well as the significant defference between the *Mugil cephalus* and the other Mediterranean mullet species. That was corroborated by hemoglobine analysis on mullets (Rizzotti, 1993).

Our analyze shows existence of differences in the form of opercular bone among the gills lid elements. The opercular bone of mullets is very evolved, which is related to developing pharyngobranchial structure (Thomson, 1966). It is important to notice that, in a view of variability of continuous characters of the gills lid, the species *Liza ramada* detaches from the other two Adriatic species of the genera *Liza,* and it is closer to the *Mugil cephalus*. The analyze of phenetic relationships

based on continiuous characters of the gills lid shows a detachment of the genera *Mugil* from the other three genera of Adriatic mullets, but in the frame of the genera *Liza*, the degree of discrepancy is such that the species *Liza saliens* approaches the *Chelon labrosus*. That is different in compare to the phenetic relationships established by the analyze of skull continuous characteristics (Antović & Simonović, 2003) and the study of the visceral and dermal skeleton (Antović & Simonović, 2006), as well as in compare to the data obtained by Caldara *et al.* (1996). A compatibility can be seen over the highest interspecific differences between the *Mugil cephalus* and the *Oedalechilus labeo*.

5. CONCLUSION

The phenetic analyze is based on comparasions of morphologic expresion of phenotypes and indicates mutual similarity – differences of the species in the frame of genus politypes and affirms degree of these differences. In that way, the range and mode of variability of characters in the group is recognized. That is necessary for a presentation of taxon mutual relationships based on states variety of their characters during evolutive developing the group they appertained (for phylogenetic analyze).

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