

Systematic Status of Nine Mullet Species (Mugilidae) in the Mediterranean Sea

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Abstract

Systematic relationships among four genera and nine species (Mugil cephalus Linnaeus, 1758, Mugil soiuy Basilewsky, 1855, Liza ramada (Risso, 1827), Liza aurata (Risso, 1810), Liza abu (Heckel, 1843), Liza saliens (Risso, 1810), Liza carinata (Valenciennes, 1836), Chelon labrosus (Risso, 1827), Oedalechilus labeo (Cuvier, 1829)) of the Mugilidae family living in the Mediterranean Sea were investigated using morphological characters. Moreover the systematic relationship of M. soiuy and L. abu among other mullet species was investigated in the present study for the first time. Hierarchical cluster analyses of morphometric data were not concordant with the meristic data. Meristic characters in the present study were more discriminative than morphometric characters in terms of taxonomic classification of the mullets. According to meristic data in UPGMA tree, all nine species were grouped in two main branching. In the first branch, C. labrosus and O. labeo were clustered as closest taxa, and being the sister group to the L. aurata. The other four Liza species produced two sub-branching in this group; L. carinata was branched with L. saliens, which is neighbour to L. ramada. In the second branch two species, M. soiuy and L. abu were clustered together and highly isolated from others. M cephalus was clustered as a most differentiated species from all other Mugil species.

Keywords: Mugilidae, morphology, morphological characters, truss network system.

Akdenizde Bulunan Dokuz Barbun Türünün (Mugilidae) Sistematik Durumu

Özet

Akdeniz'de yaşayan Mugilidae familyasına ait dört cins ve dokuz kefal türü (*Mugil cephalus* Linnaeus, 1758, *Mugil soiuy* Basilewsky, 1855, *Liza ramada* (Risso, 1827), *Liza aurata* (Risso, 1810), *Liza abu* (Heckel, 1843), *Liza saliens* (Risso, 1810), *Liza carinata* (Valenciennes, 1836), *Chelon labrosus* (Risso, 1827), *Oedalechilus labeo* (Cuvier, 1829)) arasındaki sistematik ilişki morfolojik karakterler kullanarak incelenmiştir. Bu çalışmada ayrıca ilk olarak kefal türleri içerisinde *M. soiuy* ve *L. abu*'nun sistematik durumu birlikte incelenmiştir. Morfometrik verilere dayalı hiyerarşi küme analizi meristik verilerle aynı bulunmadı. Kefal türlerinin taksonomik sınıflandırılmasında meristik karakterler morfometrik karakterlere göre türleri daha ayırt edici özellikteydi. UPGM ağacına göre tüm türler iki ana branş altında toplandı. İlk branşta, *C. labrosus* ve *O. labeo* birlikte en yakın taksa olarak sınıflandırıldı ve *L. aurata*'da bu iki türe yakın en ilişki içerisinde sınıflandırıldı. Diğer dört *Liza* türü iki alt branş olarak bu grupta sınıflandırıldı; ilk branşta *L. carinat* ve *L. saliens* birbirine en yakın türler ve bunlara yakın olan *L. ramada* ile sınıflandırıldı, ikinci branşta iki tatlı su orijinli tür olan *L. abu* ve *M. soiuy* birlikte diğer türlerden çok farklı bir şekilde sınıflandırıldı. *M. cephalus* ise tüm türlerden çok farklı olarak sınıflandırıldı.

Anahtar Kelimeler: Mugilidae, morfoloji, morfolojik karakterler, hiyerarşi küme analizi.

Introduction

Mullets have worldwide distribution and inhabit tropical and temperate seas; a few spend their lives in freshwater (Nelsonv, 2006). The Mugilidae family includes 17 genera and 72 species in the world (Harrison and Senou, 1999; Nelson, 2006). Eight species of Mugilidae inhabit the Mediterranean Sea,

and originally classified as part of the single genus *Mugil*, under different names (*M. cephalus*, *M. ramada*, *M. labrosus*, *M. labeo*, *M. aurata*, *M. abu*, *M. saliens*, *M. carinata*). Lately, the systematic relationships of these species were revised and subdivided into four genera, *Mugil*, *Liza*, *Chelon*, *Oedalechilus*. After revision, the species names have been changed as *Mugil cephalus*, *Liza ramada*,

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Chelon labrosus, Oedalechilus labeo, Liza aurata, Liza abu, Liza saliens, Liza carinata, occurring along the Mediterranean Sea (Nelson, 2006) and one species, Mugil capurii, found at the Atlantic coast of Morocco and southward to Senegal (Nelson, 2006). Mugil soiuy has also recently been found in the Black Sea (Unsal, 1992). This is native to the Amur River estuary and brackish waters of the Sea of Japan (Zaitsev, 1991). Mugil soiuy was introduced into the Azov Sea during the early 1980s by Soviet scientists (Zaitsev, 1991). This species started to reproduce in the Sea of Azov and was reported to be very numerous along the South Crimean coast and was caught for the first time along the eastern Turkish Black Sea coast (Unsal, 1992). At the present Mugil soiuy are commonly fished in the Black Sea and rarely found in the Aegean Sea (Kaya et al., 1998; Golani et al., 2002). L. abu inhabit Asia: Irak and Pakistan and is reported from the Tigris, Europhrates and recently Orontes (connected to Mediterranean Sea) (Turan et al., 2004) river systems in Turkey. The geographic distribution of O. labeo is restricted to the Mediterranean Sea. C. Labrosus and L. carinata inhabit the Mediterranean, the Eastern Atlantic coast and Indo-Pacific areas (Tortonese, 1975). L. ramada is found Mediterranean and the Black Sea (Ben-Tuvia, 1986) and L. saliens inhabit Eastern Atlantic, Mediterranean Sea, Black Sea, Sea of Azov and Atlantic coasts from Morocco to France, Caspian Sea (Baltz, 1991). M. cephalus is the only worldwide species occurring on several continental waters (Crosetti et al., 1994).

To date limited number of studies on morphologic between species and genera in the Mugilidae family in the Mediterranean Sea has been found (Schultz, 1946; Corti and Crosetti, 1996; Trewavas and Ingham, 1972; Harrison and Howes, 1991; Stiassny, 1993; Turan et al., 2000). Turan et al. (2000) investigated four species (Mugil cephalus, Chelon labrosus, Liza aurata, Liza ramada) of the Mugilidae family with morphologic data. On the other hand, it appears that there is lack of systematic studies comprising all the species using morphometric and meristic data together in the Mediterranean Sea. Also the systematic position of M. soiuy and L. abu among

other mullet species was investigated in the present study for the first time.

The aim of this study is to contribute to the understanding of the systematic relationship of the Mediterranean grey mullet species using morphometric and meristic characters.

Material and Methods

Laboratory Procedures

Individual samples from each species were collected from Iskenderun Bay in North-eastern Mediterranean Sea and Trabzon Seaport in the Black Sea. Certain biological aspects of the samples are given in Table 1.

Morphometric

The truss network system described for fish body morphometrics (Strauss and Bookstein, 1982) was used to construct a network on mullet's body. Thirteen landmarks determining 24 distances were chosen and measured on the body, as illustrated in Figure 1. Fish were thawed, placed on their right side on acetate sheets, and body posture and fins were teased into a natural position. Each landmark was obtained by piercing the acetate sheet with a dissecting needle, and additional data such as eye diameter (ED), head width (HW), pectoral fin length (PFL) were also recorded with digital calliper. Only undamaged fish were included in the analyses. There were no significant correlation (P>0.05) between the standardized truss measurements and standard length, indicating that the size effect was successfully removed with the allometric transformation (Somers, 1986).

Meristic

Five meristic characters commonly used to describe mullets were examined using the number of: first dorsal fin rays (DFR1), second dorsal fin rays (DFR2), ventral fin rays (VFR), anal fin rays (AFR), pectoral fin rays (PFR), pyloric caeca (PC) under a binocular microscope.

Table 1. Location and biological features of mullet species. Standard deviations of mean stanfad length (STL) of samples are given brackets

Species	Locations	Collection	Gear	Sample	Mean STL (cm) (± SD)
	Locations	Time	Geal	size	(Min-Max)
Chelon labrosus	36°02' N 35°57' E	07.06.2007	Gill Net	25	13.75±3.03 (9.6-19.2)
Oedalechilus labeo	36°05' N 35°55' E	21.05.2007	Gill Net	25	21.50±2.24 (17.7-27.7)
Mugil cephalus	36°22' N 35°50' E	15.05.2007	Gill Net	25	15.70±0.90 (14.1-17.2)
Mugil soiuy	41°01' N 39°35' E	14.07.2007	Gill Net	10	39.33±1.51 (37.0-41.2)
Liza aurata	36°25' N 35°53' E	04.06.2007	Gill Net	25	14.03±3.05 (9.8-18.0)
Liza abu	36°04' N 36°01' E	16.07.2007	Gill Net	25	13.67±1.99 (11.5-17.0)
Liza carinata	36°36' N 35°30' E	24.11.2007	Gill Net	25	11.59±0.82 (10.1-13.2)
Liza ramada	36°49' N 36°09' E	09.07.2007	Gill Net	25	13.20±0.82 (12.0-14.7)
Liza saliens	36°45' N 36°10' E	14.07.2007	Gill Net	25	22.28±5.39 (15.4-29.7)

Multivariate Analyses

Morphometric and meristic characters were used separately in the multivariate analyses, though these variables are different both statistically and biologically (Allendorf et al., 1987; Ihssen et al., 1981). Body measurements strongly correlate with body size, while in most fish meristic characters do not change during growth beyond some threshold body size (Strauss, 1985). Therefore allometric growth can result in heterogeneity of shape without providing information on differences in body proportion among populations (Reist, 1985). In the present study, there were significant correlations in size only for morphometric characters between the samples. Therefore transformation of absolute measurements to size-independent shape variables was accomplished by using the formula by Elliott et al. (1995);

$$M_{adj} = M (L_s / L_o)^b$$

where M: original measurement, Madi: size adjusted measurement, Lo: standard length of fish, Ls: overall mean of standard length for all fish from all samples in each analysis. Parameter b was estimated for each character from the observed data as the slope of the regression of logM on logLo, using all fish in groups. Correlation coefficients between transformed variables and standard length were calculated to check if the data transformation was effective in removing the effect of size in the data. The effectiveness of size transformations was evaluated by testing the significance of correlation between transformed variables and standard length. A significant correlation indicated an incomplete removal of size effects from the data. Size adjusted data were standardized and submitted to a canonical discriminant function analysis (DFA), and discriminant function (DF) scores were used in cluster analyses using SPSSv13.0 hierarchical

statistical package program. The DFA combines a selection of body measures in a linear fashion to produce a mathematical function, which can be used to classify individuals into groups. In hierarchical cluster analyses, UPGMA dendogram based on Squared Euclidean distance was constructed to monitor taxonomic relationships among the species that does not plot actual distances but rescales the distance to numbers between 0 and 25.

Results

Morphometric

Univariate statistics (ANOVA) revealed highly significant (P<0.001) differences between species from all truss measurements. The canonical discriminant function analysis produced 8 functions (DFs). The first DF explained 49% and second and third DFs explained 20% and 12% of between-group variability respectively. The overall random assignment of individuals into their original group by the DFA was 100%. In order to illustrate which morphometric characters are playing role to differentiate species contribution of each variable to the canonical functions were examined, and high contribution from measurements 2_3, 3_12, 1_2, PFL, HW, ED were observed (Table 2).

The UPGMA cluster analysis of morphometric data did not cluster species on the bases of current meristic status of Mugilidae (Figure 2). Three main branching were produced: in the first branch, *C. labrosus* was clustered as a closest taxa to *L. ramada*, being the sister group to *O. labeo*, and *L. aurata* and *M. cephalus* were branched more divergently from these three species. In the second group, *L. carinata* was clustered together with *L. abu*, being sister to *M. soiuy. L. saliens* seen to be morphometrically most divergent from the other species and was branched as a third group.

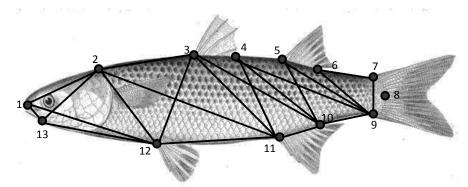


Figure 1. Locations of the 13 landmarks for constructing the truss network on fish (●) and morphometric lengths between dots. Landmarks refer to (1) anterior tip of snout at upper jaw, (2) most posterior aspect of neurocranium (beginning of scaled nape), (3) origin of dorsal fin, (4) insertion of dorsal fin, (5) origin of 2nd dorsal fin, (6) insertion of 2nd dorsal fin, (7) anterior attachment of dorsal membrane from caudal fin, (8) posterior end of vertebrae column, (9) anterior attachment of ventral membrane from caudal fin, (10) insertion of anal fin, (11) origin of anal fin, (12) insertion of pelvic fin, (13) posteriomost point of maxillary. Picture modified from Bauchot (1987).

Meristic

Observed meristic characters of nine mullet species (Table 3) were in the range of their description given by Nelson (2006). Highly

significant (P<0.001) differences between species were observed from all meristic characters. Dorsal (DFR1) and ventral fin rays (VFR) were constant in each group and could not be computed in the Univariate analysis.

Table 2. Contribution of morphometric and meristic variables to the canonical functions. Variables ordered by size of correlation within the functions *, indicate largest correlation between each variable and any discriminant function

-		Function								
Morphometric	1	2	3	4	5	6	7	8		
2_3	-0.065	0.416*	-0.126	0.076	-0.029	-0.051	0.097	0.022		
3_12	-0.232	-0.345*	0.137	0.258	0.129	-0.225	-0.068	0.128		
1_2	-0.125	-0.213*	0.129	0.095	-0.076	0.056	0.163	0.196		
PFL	0.008	0.177	0.438*	0.233	-0.026	-0.028	0.056	-0.070		
ED	-0.202	0.076	0.422*	0.302	-0.369	0.142	0.006	-0.030		
HW	-0.015	-0.234	0.268*	-0.134	-0.149	-0.019	0.121	0.031		
6_9	-0.163	0.008	-0.079	0.476*	-0.023	0.323	0.080	-0.058		
7_9	-0.028	-0.213	-0.297	0.372*	0.060	0.186	0.133	-0.247		
5_9	-0.146	-0.005	-0.107	0.313*	-0.134	0.280	-0.144	-0.084		
1_12	0.026	0.062	-0.064	0.251*	0.014	-0.002	0.212	0.227		
12_13	0.042	0.019	-0.178	0.225*	0.068	-0.107	0.137	0.005		
10_11	-0.163	0.118	0.055	-0.221*	-0.035	0.197	0.051	-0.179		
6_7	-0.026	0.025	-0.041	0.211*	0.183	0.105	0.046	-0.111		
2_12	0.047	0.083	-0.018	0.196*	0.176	0.011	0.152	0.014		
2_11	0.078	0.131	-0.071	0.189*	-0.131	-0.110	-0.044	0.104		
3_4	0.055	-0.311	-0.097	0.152	0.492*	0.058	-0.188	0.040		
4_10	0.073	-0.034	0.003	0.068	-0.402*	0.251	0.068	0.203		
4_11	0.026	-0.181	0.071	0.168	-0.308*	0.241	-0.149	0.129		
3_10	0.105	-0.159	0.032	0.169	-0.285*	0.020	-0.053	0.024		
4_9	0.064	0.018	-0.022	0.151	-0.285*	0.181	-0.050	0.216		
1_13	-0.064	0.018	0.198	0.011	-0.128	0.462*	0.299	-0.048		
5_10	-0.195	-0.146	-0.075	0.139	-0.332	0.343*	-0.051	-0.072		
3_11	0.126	-0.230	0.035	0.262	-0.103	0.160	-0.352*	0.273		
2_13	-0.072	-0.116	0.122	-0.064	0.017	-0.100	0.240*	0.035		
4_5	0.172	-0.017	0.088	-0.036	-0.298	0.197	0.097	0.309*		
11_12	-0.061	-0.029	-0.093	0.174	-0.104	0.002	-0.174	0.277*		
9_10	-0.051	0.046	-0.052	0.174	0.170	0.173	-0.203	0.238*		
Meristic										
PFR	0.950*	-0.177	0.100	-0.239						
PC	0.228	0.833*	0.381	-0.330						
DFR2	0.021	-0.273	0.896*	-0.349						
AFR	0.087	-0.042	0.570	0.816*						

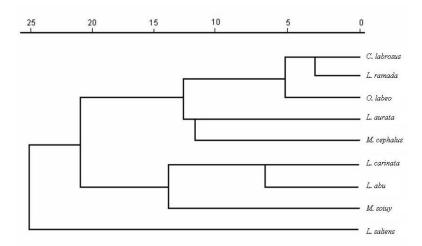


Figure 2. UPGMA tree of Squared Euclidiean distances based on morphometric data.

Species	DFR 1	DFR 2	VFR	AFR	PFR	PC
Chelon labrosus	IV	I 8	I 5	III 8-9	17	6-7
Oedalechilus labeo	IV	I 8	I 5	III 8-10	16-17	6
Mugil cephalus	IV	I 8	I 5	III 8-9	17	2
Mugil soiuy	IV	I 8-9	I 5	III 8-9	16	4-5
Liza aurata	IV	I 8	I 5	III 8-9	16	7-8
Liza abu	IV	I 7-8	I 5	III 8	11-12	3-5
Liza carinata	IV	I 7	I 5	III 7	15	5
Liza ramada	IV	I 7-8	I 5	III 8-9	16-17	6-8
Liza saliens	IV	I 7	I 5	III 7-8	16	7-9

Table 3. Observed meristic counts of the nine mullet species.

The first discriminant function explained 63% of between group variability and the second, third and fourth explained 29%, 6%, 2% respectively. Component loadings showed that PFR, PC, DFR2, AFR characters are playing key role to differentiate species respectively (Table 2).

Hierarchical cluster analyses of meristic data clustered *O. labeo* and *C. labrosus* as a closest taxa being the sister group to *L. aurata*. The neighbouring branch is made up of *L. carinata*, *L. saliens* and *L. ramada*. *M. cephalus* was most divergently clustered in the dendogram and close to its sister species *M. soiuy* and *L. abu* (Figure 3).

Discussion

Taxonomic description of fishes has commonly relied on the description of unique sets of morphological characters. Meristic characters in the present study were rather more discriminative than morphometric characters in terms of taxonomic classification of the mullets. Segmented rays in the dorsal (DFR2) and anal fins and pectoral fins, and also pyloric caeca were most discriminative characters in the present classification of mullets. Such characters are phylogenetically informative to distinguish between species of the Mugilidae.

The present meristic analysis within the family Mugilidae did not reveal similar pattern of morphologic results among the species. On the other hand, morphometric data were not congruent with meristic data. Based on meristic data UPGMA tree splited Liza and Mugil genera species into two main clusters. In the first group, M. cephalus was clustered with its sister species M. soiuy, supporting monophyletic status of Mugil genera. The results revealed that M. cephalus was morphologically more divergent than M. soiuy from the other Mediterranean taxa. On the other hand, L. abu is grouped within the Liza genera and sister group into three Liza species (L. ramada, L. saliens, L. carinata). However O. labeo and C. labrosus species was clustered together and included within the Liza genera. Similar controversy was also reported in connection with chromosome analysis by Cataudella et al. (1974) who did not find significant differences in the karyotype of C. labrosus compared to the three Mediterranean species of the Liza genus. Several studies have also questioned the present systematic status of the Mugilidae. Turan et al. (2000) analyzed by using Truss network system and 10 meristic characters of four grey mullets and all mullet species (M. cephalus, C. labrosus, L. aurata, L. ramada) were clearly different from each other. Antovic and Simonovic (2006) investigated interspecific variability and phenetic relationships in six southern Adriatic mullet species (M. cephalus, C. labrosus, L. aurata, L. ramada, L. saliens and O. labeo), and as a result M. cephalus was clearly separated from the other species and the species of the genus Liza were phenetically most similar.

Caldara et al. (1996) compared DNA sequences of mitochondrial cytochrome b and 12S rRNA genes for six mullets species and observed lowest genetic distance between C. labrosus and L. saliens. Papasotiropoulos etal. (2002)investigated phylogenetic relationship of five mullet species (M. cephalus, C. labrosus, L. aurata, L. ramada and L. saliens) with PCR-RFLP of mtDNA gene segments and found that L. saliens and C. labrosus were the closely related species while M. cephalus was the most distinct one. A similar result reported by Rossi et al. (2004) using allozyme data and 16s mt-rRNA, reffered to M. cephalus as being the most divergent species and the existence of a main cluster including all the Mediterranean species of Liza and C. labrosus. Also, Rossi et al. (2004) indicated that the Mediterranean species of Liza did not form a monophyletic group exclusive of Chelon and thus, the monophyly of the whole genus should be reconsidered.

In addition to several previous studies (Harrison and Howes, 1991; Caldara *et al.*, 1996; Murgia *et al.*, 2002; Gornung *et al.*, 2001, 2004), and this study is consistent in not identifying the Mediterranean Liza species as a monophyletic group. This is in agreement with our previous studies based on allozyme data (Turan *et al.*, 2005). Morever, Turan *et al.* (2005) reported that *C. labrosus* and *O. labeo* clustered as closest taxa and were sister group to *L. ramada. M. cephalus* and *M. soiuy*, clustered together and were clearly isolated from the other three genera. It is

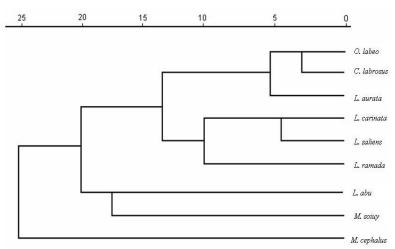


Figure 3. UPGMA tree of Squared Euclidiean distances based on meristic data.

reported in a recent phylogenetic study by Papasotiropoulos *et al.* (2007) using three (12s rRNA, 16s rRNA, and COI) mtDNA segments that the greatest genetic differentiation was observed between *M. cephalus* and all the other species studied, while *C. labrosus* and *L. aurata* were the closest taxa.

Our present study, indicated that monophyly were not supported, allowing room for the plea to get more data to resolve the systematics of mullets. M. cephalus and M. soiuy supported monophyletic status of Mugil genera, which had higher morphologic compared to other Mediterranean taxa. However, Chelon and Oedalechilus genera species were clustered within the Liza genera. The separation of Liza, Chelon and Oedalechilus might be unnatural, and that the monophyletic origin of the genus Liza is questionable. However the existence of such differences in morphologic and phylogenetic studies of Mugilidae in the literatures is not uncommon (Cataudella et al., 1974; Menezes et al., 1992; Caldara et al., 1996; Rossi et al., 1998; Fraga et al., 2007). Therefore, more studies are needed to integrate not only more species but also different genetic data (mtDNA, nDNA vs.).

In conclusion, we did not detect any appreciable degree of morphologic differentiation between *Liza*, *Chelon* and *Oedalechilus* species. This was strongly supported by our previous genetic study (Turan *et al.*, 2005) based on allozymic data. At the same time, other similar studies based on mtDNA and allozyme data (e.g., Caldara *et al.*, 1996; Rossi *et al.*, 2004; Papasotiropoulos *et al.*, 2007) seem to be in agreement with our current findings, leaving room for reconsidering the modern systematic classification of those species. The lack of congruence in the morphologic results of mullet species suggests us scrutinizing the present status of *Mugil* species with more comprehensive investigations using molecular genetic and morphologic markers together.

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