

Genetic Identification and Taxonomic Relationship of Mediterranean Mugilid Species Based on Mitochondrial 16S rDNA Sequence Data

Deniz Erguden, Mevlut Gurlek, Deniz Yaglioglu and Cemal Turan
Laboratory of Fisheries Genetics, Faculty of Fisheries, Mustafa Kemal University,
31220, Iskenderun, Hatay, Turkey

Abstract: This study examines eight mugilid species: *Mugil cephalus*, *Chelon labrosus*, *Oedalechilus labeo*, *Liza abu*, *Liza aurata*, *Liza saliens* and *Liza ramada* from the Mediterranean Sea and *Mugil soiu* Black Sea on the basis 16S rDNA gene of mitochondrial DNA. The 16S rDNA dataset contained 121 variable and parsimony informative sites and the mean nucleotide diversity (Pi) was found to be 0.05. Haplotype diversity was found to be 0.88 and 7 different haplotypes were observed. Species specific haplotypes were detected and only *C. labrosus* and *L. ramada* shared the same Haplotype (H₃). Sequencing analysis revealed that *M. cephalus* was clearly separated from the other species. For inter-generic comparisons, there was no genetic difference between *C. labrosus* and *L. ramada* and *C. labrosus* and *O. labeo* should be considered within the genus *Liza*. Moreover *M. soiu* and *L. abu* should be considered under the genus *Liza*, or new genus name should be given for these two species.

Key words: Phylogenetic relationship, mugilidae, mugilid species, mitochondrial DNA, 16S rDNA, sequence

INTRODUCTION

Mugilid species are distributed worldwide and inhabit marine, estuarine and freshwater environments in all tropical and temperate regions. Various Mugilid species play an important role in the fisheries and aquaculture of many regions of the world (Nash and Shehadeh, 1980), especially in aquacultural practices based on natural food webs.

The family Mugilidae includes 17 genera and >75 species (Nelson, 2006) in the world. Mugilid species are commonly found along the Mediterranean and Black Seas and represented with four genera and nine species. *Mugil cephalus*, *Liza aurata*, *Liza ramada*, *Liza saliens*, *Chelon labrosus* and *Oedalechilus labeo* have Atlanto Mediterranean distribution. *L. carinata* has recently spread to the South-Eastern Mediterranean from the Red Sea through the Suez Canal (Thomson, 1997). *Mugil soiu* was initially introduced into the Azof Sea and now has been reported in the Black Sea and more recently in the Aegean Sea (Kaya *et al.*, 1998). *Liza abu* is a freshwater species and found in the Orontes River, Turkey connected to Mediterranean Sea (Turan *et al.*, 2004).

Due to the very conservative morphology displayed by mugilids many investigations based on various

morphological characters did not elucidate their systematic problem (Schultz, 1946; Trewavas and Ingham, 1972; Stiassny, 1993), while the use of the pharyngobranchial organ as a key character to address the identification and taxonomy of mugilids also provided poor results (Harrison and Howes, 1991). Thomson (1997) revised Mugilidae family and reported 14 genera and a total of 64 valid species, most of these species are representatives of *Liza* and *Mugil* genera. Despite the fact that the Mugilid species have been revised many times, genetic identification and the taxonomic status of some species and genera within the family is still confused (Rossi *et al.*, 1998; Turan *et al.*, 2005).

Nowadays, mitochondrial DNA analysis is a very useful tool for molecular genetic studies because of its special features (Meyer *et al.*, 1990; Billington and Hebert, 1991; Normark *et al.*, 1991; Meyer, 1992). In most species mitochondrial DNA (mtDNA) is highly variable and is therefore a good marker for detecting possible genetic differentiation. Therefore, mtDNA variation has been widely investigated among several fish species including mugilidae (Papasotiropoulos *et al.*, 2002; Caldara *et al.*, 1996; Turan, 2008; Turan *et al.*, 2009).

Although, the systematic relationships among these species have been investigated with the use of

nonmorphological characters, such as biochemical and cytogenetic markers (Delgado *et al.*, 1992; Rossi *et al.*, 1996, 1997, 2000; Gornung *et al.*, 2001, 2004; Nirchio *et al.*, 2003), allozyme electrophoresis (Autem and Bonhomme, 1980; Papasotiropoulos *et al.*, 2001; Rossi *et al.*, 2004; Turan *et al.*, 2005) and mtDNA analyse (Caldara *et al.*, 1996; Murgia *et al.*, 2002; Papasotiropoulos *et al.*, 2002, 2007; Rossi *et al.*, 2004; Fraga *et al.*, 2007) they did not result in a clear taxonomic perspective (Cataudella *et al.*, 1974; Delgado *et al.*, 1992; Crosetti *et al.*, 1993; Rossi *et al.*, 1997, 2000).

In this study, taxonomic description and status of 8 mugilid species (*Mugil cephalus*, *M. soiuy*, *Liza ramada*, *L. aurata*, *L. abu*, *L. saliens*, *Chelon labrosus*, *Oedalechilus labeo*), living in the Mediterranean Sea, were investigated with mitochondrial 16S rDNA gene sequence data.

MATERIALS AND METHODS

Samples from eight mugilid species (*Mugil cephalus*, *Liza ramada*, *Liza aurata*, *Liza abu*, *Liza saliens*, *Chelon labrosus*, *Oedalechilus labeo*) were collected from Iskenderun Bay in North-eastern Mediterranean Sea and only one species *Mugil soiuy* was sampled from Trabzon in the Black Sea.

The number and location of the samples used in the sequence analysis are given in Table 1. The samples were placed on ice and kept frozen at -40°C in the laboratory. In the laboratory fin clips and muscle tissue were collected and preserved in 95% ethanol for DNA extraction.

Total genomic DNA was extracted from a piece of fin tissue (approximately 2 mm²) using AGOWA mag Midi DNA isolation Kits (AGOWA, Berlin, Germany). The amplification of the mitochondrial 16S rDNA gene was performed using PCR with a profile of 94°C for 4 min, followed by 35 cycles of 94°C/30 sec strand denaturation, 52°C/20 sec annealing and 72°C/1 min 30 sec primer extension and a final 7 min elongation at 72°C.

The 16S rDNA amplification conditions were: 1.5 µL 10x polymerase buffer, 0.5 µL dNTP (10 mM), 0.3 µL Tg DNA polymerase (3 U µL⁻¹) equivalent to Taq DNA polymerase, 0.05 µL 16Fi1 40 primer (100 µM) (5'-CG(CT)AAGGGAA(ACT)GCTGAAA-3'), 0.05 µL 16Fi1 524 primer (100 µM) (5'-CCGGTCTGAACTCAGATCACGT AG-3'), 3-5 µL DNA from AGOWA purification and water for a total reaction volume of 15 µL.

Amplified DNA was purified with Exo/Sap enzymes (Cleveland, Ohio, USA) following the manufacturer's instructions.

Finally, all the samples were sequenced in both directions using 16Fiseq1463 (5'-TGCACCAT TAGGATGTCCRGATCC AAC-3') and 16sarL (5'-CGCCTG TTTAACAAAAACAT-3') primers. The sequencing products were loaded onto an ABI3730 (Applied Biosystems) automated sequencer.

Sequences were aligned and ambiguous bases resolved by eye using Sequencer v.4.5 (Gene Codes Corp) and final alignment was done manually with BioEdit (Hall, 1999). MtDNA sequence data were analysed to assess levels of pairwise nucleotide variation and to determine nucleotide composition for each taxon using MEGA 3.1 (Kumar *et al.*, 2004).

The molecular phylogenetic tree was constructed using the two distinct phylogenetic approaches: a distance-based method using Neighbor Joining (NJ) (Saitou and Nei, 1987) and a cladistic approach using the Maximum Parsimony (MP). Bootstrap resampling was applied to assess the relative stability of NJ trees produced with different substitution models.

Nucleotide diversity and DNA total divergence (Dxy; were estimated using MEGA v.4 (Tamura *et al.*, 2007). In all models, phylogenetic trees were rooted using out group species *Carangoides armatus*, which belongs to the family Carangidae and its sequence is published in GenBank under accession number NC_004405. The sequences have been deposited in the GenBank with accession numbers given in Table 1.

Table 1: Sampling coordinates and GenBank accession numbers for the 16S rDNA segment sequenced in this study

Species	Sampling location	n-S	GenBank
<i>M. soiuy</i>	Black Sea (Trabzon)	2	GU449119
			GU449120
<i>O. labeo</i>	Northeastern Mediterranean Sea (Iskenderun Bay)	2	GU449121
			GU449122
<i>C. labrosus</i>	Northeastern Mediterranean Sea (Iskenderun Bay)	2	GU449123
			GU449124
<i>L. aurata</i>	Northeastern Mediterranean Sea (Iskenderun Bay)	3	GU449125
			GU449126
			GU449127
<i>L. abu</i>	Northeastern Mediterranean Sea (Iskenderun Bay)	2	GU449128
			GU449129
<i>L. ramada</i>	Northeastern Mediterranean Sea (Iskenderun Bay)	3	GU449130
			GU449131
			GU449132
<i>M. cephalus</i>	Northeastern Mediterranean Sea (Iskenderun Bay)	2	GU449133
			GU449134
<i>L. saliens</i>	Northeastern Mediterranean Sea (Iskenderun Bay)	3	GU449135
			GU449136
			GU449137

n-S: Number of specimens used in the sequencing

RESULTS AND DISCUSSION

After alignment, the partial 16S rDNA gene sequences consisted of 788 bp. Examination of the gene reveals a moderate amount of guanine (G; 21.4%) and abundance of adenine (A; 31%). The 16S rDNA dataset contained 121 variable and parsimony informative sites and the mean nucleotide diversity (*Pi*) was found to be 0.05. Haplotype diversity was found to be 0.88 and 7 different haplotypes were observed. Variable nucleotide positions and frequencies of haplotypes are given in Fig. 1. Species specific haplotypes were detected and only *C. labrosus* and *L. ramada* shared the same Haplotype (H₃) (Table 2).

Pairwise genetic distances between the species were given in (Table 3). For inter-generic comparisons, there was no genetic difference between *C. labrosus* and *L. ramada* and highest genetic divergences were observed between *M. cephalus* and *O. labeo*.

The three different phylogenetic approaches resulted in same tree topologies and the clades are well supported (Fig. 2). *C. labrosus* and *L. ramada* clustered together on the same branch and were sister group to *L. saliens*, *O. labeo* clustered with *L. aurata* and *M. soiyuy* branched with *L. abu*. On the other hand, *M. cephalus* highly divergently clustered outside of this group.

The present study based on 16S rDNA gene sequencing data of four genera and eight species of the family Mugilidae revealed different systematic classification from the current classification and question existence of *Chelon* and *Oedalechilus* genera. Mugilid species are generally morphologically uniform; the genus *Oedalechilus* by the different pattern of the jaw structure, that features a very broad upper lip, with the maxillare extending vertically backwards under the preorbitale. In

other Mediterranean mugilid species (*L. abu*, *L. saliens*, *L. ramada*, *L. aurata* and *M. cephalus*), the upper lip is not broad and the mouth is rather horizontal, with the same extending maxillare. However, *M. cephalus* shows at least some features that make it diagnosable compared to *Liza*, *Chelon* and *Oedalechilus* and all the genetic and molecular studies (Caldara *et al.*, 1996; Cataudella *et al.*, 1974; Autem and Bonhomme, 1980; Rossi *et al.*, 1998; Papatotiroopoulos *et al.*, 2001, 2002; Turan *et al.*, 2005; Fraga *et al.*, 2007; Semina *et al.*, 2007) reported that *M. cephalus* shows the highest degree of genetic divergence among the other three genera. The present study is not congruent with the present monophyletic status of *Liza* and *Mugil* genera. There was no enough genetic differentiation of *Chelon* and *Oedalechilus* genera from *Liza* genus and *C. labrosus* and *O. labeo* seems to be belongs to the genus *Liza*.

The close relationship between *L. saliens* *C. labrosus* and *O. labeo* suggest that the separation of *Chelon*, *Oedalechilus* and *Liza* in three separate genera might be unnatural, making the monophyletic origin of the genus *Liza* questionable. In the previous studies, similar controversy was also reported. Papatotiroopoulos *et al.* (2002) investigated the phylogenetic relationship of five

Table 2: Distribution and frequency of 16S rDNA haplotypes of mugilid species

Species	H ₁	H ₂	H ₃	H ₄	H ₅	H ₆	H ₇
<i>M. soiyuy</i>	2	-	-	-	-	-	-
<i>O. labeo</i>	-	2	-	-	-	-	-
<i>C. labrosus</i>	-	-	2	-	-	-	-
<i>L. aurata</i>	-	-	-	3	-	-	-
<i>L. abu</i>	-	-	-	-	2	-	-
<i>L. ramada</i>	-	-	3	-	-	-	-
<i>M. cephalus</i>	-	-	-	-	-	2	-
<i>L. saliens</i>	-	-	-	-	-	-	3

H: Haplotypes

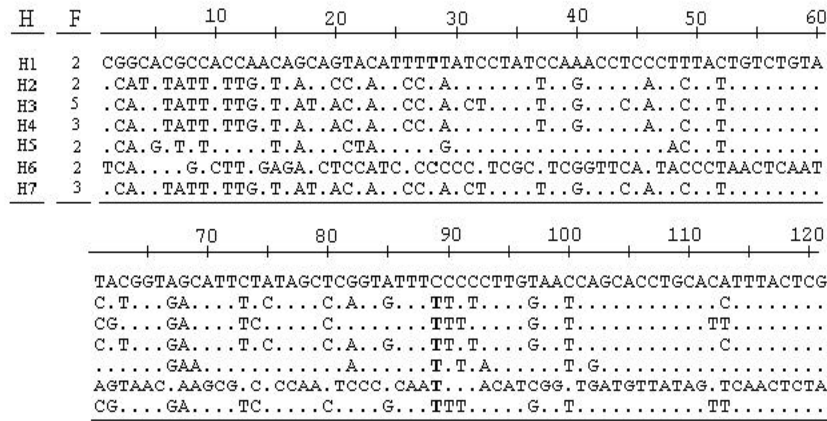


Fig. 1: Variable nucleotide positions and frequencies of 16S rDNA haplotypes in Mugilid species. For all haplotypes variable nucleotides are indicated, while identity is shown by dashes

Table 3: Total genetic distance between the species

Species	1	2	3	4	5	6	7	8	9
<i>C. armatus</i>	-	-	-	-	-	-	-	-	-
<i>M. soiyuy</i>	0.1610	-	-	-	-	-	-	-	-
<i>O. labeo</i>	0.1671	0.0459	-	-	-	-	-	-	-
<i>C. labrosus</i>	0.1580	0.0486	0.0181	-	-	-	-	-	-
<i>L. aurata</i>	0.1640	0.0446	0.0026	0.0155	-	-	-	-	-
<i>L. abu</i>	0.1610	0.0260	0.0379	0.0379	0.0366	-	-	-	-
<i>L. ramada</i>	0.1580	0.0486	0.0181	0.0000	0.0155	0.0379	-	-	-
<i>M. cephalus</i>	0.1900	0.1299	0.1357	0.1284	0.1343	0.1212	0.1284	-	-
<i>L. saliens</i>	0.1595	0.0499	0.0168	0.0013	0.0142	0.0392	0.0013	0.1299	-

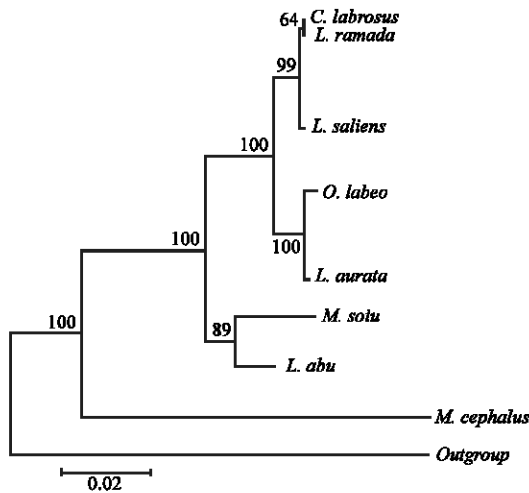


Fig. 2: Neighbour joining phylogenetic tree for 16S rDNA. Bootstrap values are shown on the tree. *C. armatus* was used as an outgroup

mugilid species by means of mtDNA PCR-RFLP and found that *L. saliens* and *C. labrosus* were the most closely related species. Turan *et al.* (2005) investigated phylogenetic relationships of the nine species of the family Mugilidae based on allozyme data and suggested that *C. labrosus* and *O. labeo* should be placed in the genus *Liza*. Rossi *et al.* (2004) and Papatotiroopoulos *et al.* (2007) investigated phylogenetic relationships of six mugilids (*M. cephalus*, *C. labrosus*, *O. labeo*, *L. aurata*, *L. ramada* and *L. saliens*) and five mugilid species (*M. cephalus*, *C. labrosus*, *L. aurata*, *L. ramada* and *L. saliens*) and found low level of sequence divergence between *Chelon labrosus* and *Liza aurata* using allozyme data and three segments of mtDNA. Investigations based on nuclear and mtDNA data provide good support for the hypothesis that *C. labrosus* and *O. labeo* should be placed in the genus *Liza*. The existence of such disorder in phylogenetic studies of Mugilidae in the study is not uncommon (Cataudella *et al.*, 1974; Menezes *et al.*, 1992; Caldara *et al.*, 1996; Papatotiroopoulos *et al.*, 2002).

The present study also revealed close relationship between *M. soiyuy* and *L. abu*. The molecular phylogenetic

position of *M. soiyuy* and *L. abu* was considered in this study first time. *L. abu* is a freshwater species and *M. soiyuy* is in fact a freshwater fish originating from Amu Darya River Basin in Far East Asia (Berg, 1965). This species was later introduced to the Sabolat (Hacibey) Lagoon, 60 km from Odessa in the Northeastern Black Sea. *M. soiyuy* found a suitable environment on the eastern Black Sea coast of Turkey, after leaving the Sea of Azov and following the Northeastern coast of the Black Sea. In time, the species migrated to the west, reaching the Sea of Marmara and Aegean Sea via the Istanbul Bogazi (Kaya *et al.*, 1998). The present study gives new hypothesis that *M. soiyuy* and *L. abu* should be considered in the genus *Liza*, or new genus name should be given for these two species.

16S rDNA gene sequencing data revealed a high level of genetic variability among the species examined. Only *C. labrosus* and *L. ramada* shared the same Haplotype (H_3) and the taxonomic tree support structuring of haplotypes by species designation. Any species-specific haplotypes could serve as a strict diagnostic marker for taxonomic and aquaculture practice purposes, which is especially, important at the larval and fingerling stages since the morphological and physiological characters do not show significant differences. The identification of several fish species by use of molecular markers proves a reliable tool facilitating discrimination of morphologically similar species. For example, McDowell and Graves (2002) used different mitochondrial and nuclear markers and concluded that the mitochondrial ND 4 and the nuclear locus unambiguously identified marlins, spearfishes, sailfishes and swordfishes; xiphiid billfishes that represent an important commercial and recreational fisheries resource.

CONCLUSION

The two genera *Chelon* and *Oedalechilus* should be considered together within the genus *Liza*. The present data also first time report the taxonomic revision the species of *M. soiyuy* and *L. abu*, which should be considered together under the same genus.

ACKNOWLEDGEMENT

Thanks to Turkish Academy of Sciences (TUBA) for financial support.

REFERENCES

- Autem, M. and F. Bonhomme, 1980. Biochemical systematics elements of the mediterranean mugilidae. *Biochem. Syst. Ecol.*, 8: 305-308.
- Berg, L.S., 1965. *Freshwater Fishes of The U.S.S.R. And adjacent Countries*. 4th Edn. Israel Program for Scientific Translations Ltd. (Russian version published in 1949), Jerusalem, pp: 510.
- Billington, N. and P. Hebert, 1991. Mitochondrial DNA diversity in fishes and its implications for introductions. *Can. J. Fish. Aqua. Sci.*, 48 (1): 80-94.
- Caldara, F., L. Bargelloni, L. Ostellari, E. Penzo, L. Colombo and T. Patarnello, 1996. Molecular phylogeny of grey mullets based on mitochondrial DNA sequence analysis: Evidence of differential rate of evolution at the intrafamily level. *Mol. Phylogenet. Evol.*, 6: 416-424. DOI: 10.1006/mpev.1996.0090. PMID: 8975696.
- Cataudella, S., M.V. Civitelli and E. Capanna, 1974. Chromosome complement of the Mediterranean mullet (Pisces, perciformes). *Caryologia*, 27: 93-105.
- Crosetti, D., J.C. Avicé, F. Placidi, A.R. Rossi and L. Sola, 1993. Geographic variability in the grey mullet *Mugil cephalus*. Preliminary results of mtDNA and chromosome analyses. *Aquaculture*, 111: 95-101. DOI: 10.1016/0044-8486(93)90028-W.
- Delgado, J.V., A. Molina, J. Lobillo, A. Alonso and M.E. Camacho, 1992. Morphometrical study on the chromosomes of three species of mullet (Teleostei: Mugilidae). *Caryologia*, 45: 263-271. <http://cat.inist.fr/?aModele=afficheN&cpsid=4522489>.
- Fraga, E., H. Schneider, M. Nirchio, E. Santa-Brigida, L.F. Rodrigues-Filho and I. Sampaio, 2007. Molecular phylogenetic analyses of mullets (Mugilidae, Mugiliformes) based on two mitochondrial genes. *J. Appl. Ichthyol.*, 23: 598-604. DOI: 10.1111/j.1439-0426.2007.00911.x.
- Gornung, E., C.A. Cordisco, A.R. Rossi, S. De Innocentiis, D. Crosetti and L. Sola, 2001. Chromosomal evolution in mugilids: Karyotype characterization of *Liza saliens* and comparative localization of major and minor ribosomal genes in the six Mediterranean grey mullets. *Mar. Biol.*, 139: 55-60. DOI: 10.1007/s002270100576. <http://www.springerlink.com/content/rhrqbjdf2120pc3q>.
- Gornung, E., M.E. Mannarelli, A.R. Rossi and L. Sola, 2004. Chromosomal evolution in Mugilidae (Pisces, Mugiliformes): FISH mapping of the (TTAGGG)_n telomeric repeat in the six Mediterranean mullets. *Hereditas*, 140: 158-159. DOI: 10.1111/j.1601-5223.2004.01784.x.
- Hall, T.A., 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.*, 41: 95-98.
- Harrison, I.J. and G.J. Howes, 1991. The pharyngobranchial organ of mugilid fishes: Its structure, variability, ontogeny, possible function and taxonomic utility. *Bull. Br. Mus. Nat. Hist. (Zool.)*, 57: 111-132.
- Kaya, M., S. Mater and A.Y. Korkut, 1998. A new grey mullet species *Mugil souiy* Vasilevsky (Teleostei: Mugilidae) from the Aegean coast of Turkey. *Turk. J. Zool.*, 22: 303-306.
- Kumar, S., K. Tamura and M. Nei, 2004. MEGA 3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform.*, 5: 150-163. DOI: 10.1093/bib/5.2.150. PMID: 15260895.
- McDowell, J.R. and J.E. Graves, 2002. Nuclear and mitochondrial DNA markers for specific identification of istiophorid and xiphiid billfishes. *Fish. Bul.*, 100: 537-544.
- Menezes, M.R., M. Martins and S. Naik, 1992. Interspecific genetic divergence in grey mullets from the Goa region. *Aquaculture*, 105: 117-129. DOI: 10.1016/0044-8486(92)90124-4.
- Meyer, A., T.D. Kocher, P. Basasibwaki and A.C. Wilson, 1990. Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature*, 347: 550-553. DOI: 10.1038/347550a0. PMID: 2215680. <http://www.nature.com/nature/journal/v347/n6293/abs/347550a0.html>.
- Meyer, A., 1992. Cichlid fishes: Behaviour, Ecology and Evolution. In: Keenleyside, M.H.A. (Eds). *Q. Rev. Biol.*, 67: 221-222.
- Murgia, R., G. Tola, S.N. Archer, S. Vallerga and J. Hirano, 2002. Genetic identification of grey mullet species (Mugilidae) by analysis of mitochondrial DNA sequence: Application to identify the origin of processed ovary products (Bottarga). *Mar. Biol.*, 4: 119-126. DOI: 10.1007/s10126-001-0074-6. PMID: 14961270.
- Nash, C.E. and Z.H. Shehadeh, 1980. Review of the breeding and propagation techniques for grey mullet, *Mugil cephalus*. *Stud. Rev.*, 3: 87.
- Nelson, J.S., 2006. *Fishes of the world*. 4th Edn. In: John Wiley and Sons, New York, pp: 262-263. ISBN: 0471250317.

- Nirchio, M., F. Cervigon, J.I.R. Porto, J.E. Perez, J.A. Gomez and J. Villalaz, 2003. Karyotype supporting *Mugil curema* Valenciennes, 1836 and *Mugil gaimardianus* Desmarest, 1831 (Mugilidae: Teleostei), as two valid nominal species. *Sci. Mar.*, 67 (1): 113-115.
- Normark, B.B., A.R. McCune and R.C. Harrison, 1991. Phylogenetic relationships of neopterygian fish, inferred from mitochondrial DNA sequences. *Mol. Biol. Evol.*, 8: 819-834. PMID: 1663569. <http://mbe.oxfordjournals.org/cgi/content/abstract/8/6/819>.
- Papastotiropoulos, V., E. Klossa-Kilia, G. Kiliias and S.N. Alahiotis, 2001. Genetic divergence and phylogenetic relationships in grey Mulletts (Teleostei: Mugilidae) using allozyme data. *Biochem. Genet.*, 39 (5/6): 155-168. DOI: 10.1023/A:1010289224573. PMID: 11530853.
- Papastotiropoulos, V., E. Klossa-Kilia, G. Kiliias and S.N. Alahiotis, 2002. Genetic divergence and phylogenetic relationships in grey mullets (Teleostei: Mugilidae) based on PCR-RFLP analysis of mtDNA segments. *Biochem. Genet.*, 40: 71-86. DOI: 10.1023/A:1015162304934. PMID: 12017509.
- Papastotiropoulos, V., E. Klossa-Kilia, N. Stamatis and K. Alahiotis-George, 2007. Molecular phylogeny of grey mullets (Teleostei: Mugilidae) in Greece: Evidence from sequence analysis of mtDNA segments. *Biochem. Genet.*, 45: 623-636. DOI: 10.1007/s10528-007-9101-2.
- Rossi, A.R., D. Crosetti, E. Gornung and L. Sola, 1996. Cytogenetic analysis of global populations of *Mugil cephalus* (striped mullet) by different staining techniques and fluorescent *in situ* hybridization. *Heredity*, 76: 77-82. DOI: 10.1038/hdy.1996.10. <http://www.nature.com/hdy/journal/v76/n1/abs/hdy199610a.html>.
- Rossi, A.R., E. Gornung and D. Crosetti, 1997. Cytogenetic analysis of *Liza ramada* (Pisces, Perciformes) by different staining techniques and fluorescent *in situ* hybridization. *Heredity*, 79: 83-87. DOI: 10.1038/hdy.1997.125. <http://www.nature.com/hdy/journal/v79/n1/abs/hdy1997125a.html>.
- Rossi, A.R., M. Capula, D. Crosetti, D.E. Campton and L. Sola, 1998. Genetic divergence and phylogenetic inferences in five species of Mugilidae (Pisces: Perciformes). *Mar. Biol.*, 131: 213-218. DOI: 10.1007/s002270050313.
- Rossi, A.R., E. Gornung, D. Crosetti, S. De Innocentiis and L. Sola, 2000. Cytogenetic analysis of *Oedalechilus labeo* (Pisces: Mugilidae), with report of NOR variability. *Mar. Biol.*, 136: 159-162. DOI: 10.1007/s002270050018. <http://www.springerlink.com/content/eqf7u7y8t3t992c4>.
- Rossi, A.R., A. Ungaro, S. De-Innocentiis, D. Crosetti and L. Sola, 2004. Phylogenetic analysis of Mediterranean mugilids by allozymes and 16S mt rRNA genes investigation: Are the Mediterranean species of *Liza* monophyletic? *Biochem. Genet.*, 42: 301-313. DOI: 10.1023/B:BIGI.0000039806.12756.81. PMID: 15524309.
- Saitou, N. and M. Nei, 1987. The neighbour-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, 4: 406-425. PMID: 3447015. <http://www.ncbi.nlm.nih.gov/pubmed/3447015>.
- Semina, A.V., N.E. Polyakova, M.A. Makhotkin and V.A. Brykov, 2007. Mitochondrial DNA divergence and phylogenetic relationships in mullets (Pisces: Mugilidae) of the Sea of Japan and the Sea of Azov revealed by PCR-RFLP-analysis. *Russ. J. Mar. Biol.*, 33 (3): 187-192. DOI: 10.1134/S1063074007030078.
- Schultz, L.P., 1946. A revision of the genera of mullets, fishes of the family Mugilidae with description of three new genera. *Proc. U.S. Nat. Mus.*, 96: 377-395.
- Stiassny, M.L.J., 1993. What are grey mullets? *Bull. Mar. Sci.*, 52 (1): 197-219.
- Tamura, K., J. Dudley, M. Nei and S. Kumar, 2007. MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.*, 24: 1596-1599. PMID: 17488738. <http://www.ncbi.nlm.nih.gov/pubmed/17488738>.
- Thomson, J.M., 1997. The Mugilidae of the World. *Mem. Queensland Mus.*, 41 (3): 457-562.
- Trewavas, E. and S.E. Ingham, 1972. A key to species of Mugilidae in the Northeastern Atlantic and Mediterranean, with explanatory notes. *J. Zool. Lond.*, 167: 15-29.
- Turan, C., D. Erguden, F. Turan and M. Gurlek, 2004. Genetic and morphologic structure of *Liza abu* (Heckel, 1843) populations from the river Orantes, Euphrates and Tigris. *Turk. J. Vet. Anim. Sci.*, 28: 729-734.
- Turan, C., I. Gunduz, M. Gurlek, D. Yaglioglu and D. Erguden, 2009. Systematics of Scorpaenidae species in the Mediterranean Sea inferred from mitochondrial 16S rDNA sequence and morphological data. *Folia Biologica, (Krakow)* 57: 219-226. DOI: 10.3409/fb57. <http://www.ingentaconnect.com/content/isez/fb/f0020003/art00017?crawler=true>.
- Turan, C., 2008. Molecular systematics of Capoeta (Cyprinidae) species complex inferred from mitochondrial 16S rDNA Sequence Data. *Acta Zool. Cracov.*, 51A (1-2): 1-14. DOI: 10.3409/azc.51a. <http://www.ingentaconnect.com/content/isez/azca/2008/00000051/f0020001/art00001>.
- Turan, C., M. Caliskan and H. Kucuktas, 2005. Phylogenetic relationships of nine mullet species (Mugilidae) in the Mediterranean Sea. *Hydrobiologia*, 532: 45-51. DOI: 10.1007/s10750-004-8772-5.