PHYLOGENETIC RELATIONSHIP AMONG THE BLACK SEA *ALOSA* SPECIES FROM MTDNA ND5/6 SEQUENCES

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Abstract
Phylogenetic relationships among five species of *Alosa* (*Alosa maeotica, A. tanaica, A. caspia, A. immaculata* and *A. fallax nilotica*) was examined with sequence analyses of mtDNA ND5/6 region. In pairwise comparision, the highest genetic differences were observed between *A. caspia* and *A. tanaica* (0.691), and lowest between *A. maeotica* and *A. tanaica* (0.491). In minimum evolution tree, two phylogenetic nodes were detected; in the first node, *A. maeotica* and *A. tanaica* grouped together which were sister group to *A. f. nilotica*. In the second node, *A. caspia* and *A. immaculata* grouped together.
Keywords: Genetics, Teleostei, Black Sea

Introduction
Shad (*Alosa spp.*) is one of the most important fish resources and have high economic value in the world [1]. Shad species are commonly found in the Black Sea and represented with four species and one subspecies [1,2]. *Alosa maeotica, Alosa tanaica, Alosa immaculata, Alosa caspia* are distributed in the Black Sea. *Alosa fallax nilotica* is distribution in the Black, Marmara, Aegean and Mediterranean Seas. Knowledge on phylogenetic relationship among these species is important to elucidate evolutionary history and genetic relationships among these species. In the present study it is aimed to elucidate phylogenetic relationships among *Alosa* species with sequence analyses of mtDNA ND5/6 region.

Material and Methods
Speciments of *A. maeotica, A. tanaica, A. caspia, A. immaculata* and *A. f. nilotica* were collected from the Black Sea. The samples were placed individually in plastic bags, and kept frozen at -40°C until the molecular analyses. Total genomic DNA was extracted from a peace of fin tissue (approximately 2 mm^2) using Midi DNA isolation Kits. The amplification of the mitochondrial ND5/6 gene was performed using PCR with a profile of 94°C for 4 min, followed by 35 cycles of 94°C/30s strand denaturation, 52° C/20s annealing and 72 °C/1 min 30 sec primer extension, and a final 7 min elongation at 72°C. Purified DNA (3-5 µl) was sent to IONTEK for sequencing, using primers (F: 5'-AAC AGT TCA TCC GTT GGT CTT AGG-3' and R: 5'-TAA CAA CGG TGG TTC TTC AAG TCA-3'). Sequences were aligned and ambiguous bases resolved by eye using Sequencer v.4.5 (Gene Codes Corp.). The initial alignments of partial 16S rDNA sequences were performed with Clustal W program [3] and final alignment was completed manually with BioEdit [4]. MtDNA sequence data were analyzed to assess levels of pairwise nucleotide variation and to determine nucleotide composition for each taxon using Mega 4 [5]. The molecular phylogenetic tree was constructed using the three distinct phylogenetic approaches: a distance-based method using neighbor joining (NJ), [6] a cladistic approach using the maximum parsimony (MP) criterion, and minimum evolution (ME). The reliability of the inferred phylogenies was evaluated using the bootstrap method [7] with 1000 replicates. One species, *Engraulis encrosicolusrom*, were included as an out group taken from GenBank (NC003097).

Results and Discussion
There were 888 variable and 16 conservative nucleotides of which 857 were parsimony informative over 904 bp. Examination of the gene fragment reveals a lack of guanin (T; 18.1%) and abundance of adenine (A; 30.3%). The mean nucleotide diversity (*Pi*) was found to be 0.516. For congeneric comparisons of genetic distance (Kimura two parameter), the highest genetic differences were observed between *A. caspia* and *A. tanaica*, and lowest between *A. maeotica* and *A. tanaica* (Table 1). The three different phylogenetic approaches (NJ, MP, ME) resulted in similar tree topologies and the clades are well supported (Fig. 1). In minimum evolution tree, two phylogenetic nodes were detected; in the first node, *A. maeotica* and *A. tanaica* grouped together which were sister group to *A. f. nilotica.* In the second node, *A. caspia* and *A. immaculata* grouped together. Similarly Faria et al [8] also clustered *A. caspia* and *A. immaculata* together and found close relationship for these two *Alosa* species in the Black Sea using ND1 and Cytochrome b genes.
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Tab. 1. Pairwise genetic distance between the species

Species	*A.maeotica*	*A.caspia*	*A.immaculata*	*A.f.nilotica*	*A.tanaica*
A.maeotica	-				
A. caspia	0.690	-			
A.immaculata	0.655	0.519	-		
A.f.nilotica	0.610	0.690	0.668	-	
A. tanaica	0.491	0.691	0.689	0.650	-

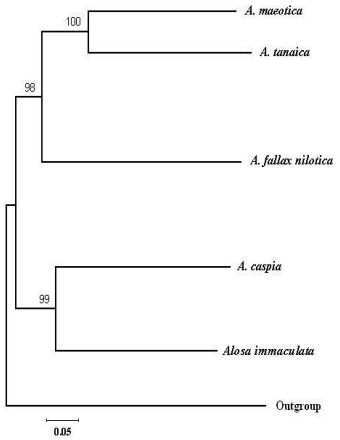

Fig. 1. Minimum evolution phylogenetic tree for ND5/6. Bootstrap values are shown on the tree. *E. encrosicolus*, seq. (NC003097) was used as outgroup species

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