



## A NEW SPECIES OF MULLET *Chelon caeruleum* (Family: Mugilidae) WITH DESCRIPTION OF ITS GENETIC RELATIONSHIP TO SOME MUGILIDS

Lamiaa Elsayed Mokhtar Deef

Department of Zoology, Faculty of Science, Damietta University, New Damietta, Damietta, Egypt

\*Corresponding Author, Email: lamiaadeef@du.edu.eg

### ARTICLE INFO

Received: 29 April 2018  
Received in revised form: 29 May 2018  
Accepted: 18 June 2018  
Online first: 10 August 2018

#### Keywords:

Mullet  
PCR  
Phylogeny  
Sequencing  
Taxonomy

### ABSTRACT

*Chelon caeruleum* sp. nov. is described as a new species of *Chelon* encountered in the Rashid coastal region of the Mediterranean Sea, Egypt. With this finding, the new species is the sixth member of the genus *Chelon*. *Chelon caeruleum* sp. nov. is distinguished from its congeners by the following characters: colouration of body is dark bluish grey dorsally and the sides are blue; head length 20.5 to 21.6% SL; head width 13 to 13.5% SL; pre-first dorsal fin length 50% SL; eye diameter 4.6 to 5.2% SL; postorbital length 10.7 to 11.1% SL; unbranched pyloric caeca are 2 short and 4 long; a pair of long (one longer than the other), spine-like neural postzygapophyse on the second vertebra. DNA of *Chelon caeruleum* sp. nov. and five mullet species (*Mugil cephalus*, *Liza carinata*, *Liza ramada*, *Liza aurata*, and *Chelon labrosus*) was extracted then amplified using polymerase chain reaction (PCR) and sequenced. The mtDNA cytochrome oxidase subunit I sequence analysis confirmed that *Chelon caeruleum* sp. nov. is distinct from other congeners of *Chelon* and it is a different species of *Chelon* which is new to science.

### How to Cite

Deef, L.E.M. (2018): A new species of mullet, *Chelon caeruleum* (Family: Mugilidae), with description of its genetic relationship to some Mugilids. Croatian Journal of Fisheries, 76, 107-114. DOI: 10.2478/cjf-2018-0014

### INTRODUCTION

Mugilidae or grey mullets contain 17 genera and 72 species, most of which are classified into three genera: *Mugil*, *Liza* and *Chelon* (Nelson, 2006; Durand et al., 2017). The taxonomy and phylogenetic relationships among the species of Mugilidae remain unresolved (Harrison et al., 2007). The reason for this is that most classical methods used for species identification and systematics, such as morphological characters, are greatly similar within the mullets (Schultz, 1946; Thomson, 1997; Liu et al., 2010).

*Chelon* (Rose, 1793) is the largest genus of the family Mugilidae and the most difficult to classify. The species of this genus are distributed in the Mediterranean Sea (Turan et al., 2011) and European seas, throughout the Indo-Pacific region and along the western coast of Africa (Senou et al., 1996). Schultz (1946) revised the genera of the Mugilidae on a worldwide basis; he considered *Chelon* to be a valid genus on the basis of the morphology of the mouth including maxilla and teeth, and other characters such as the form of the adipose eyelid and scales. He regarded *Liza* as synonyms of the genus. Thomson (1954) accepted Schultz's

classification, but stated that *Chelon* Röse is an unavailable name. He regarded *Liza* as the valid name for the genus. Thomson's invalidation of *Chelon* was rejected by Trewavas and Ingham (1972); they reported that *Chelon* is available name according to the International code of zoological nomenclature of 1961, Art. 68d (i).

In this study, specimens of an unidentified *Chelon* species were collected from the Rashid coastal region of northern Egypt. Moreover, COI sequence variation of different mullet species was analysed to determine the precise taxonomic status of the newly described species. A description of morphological features and a taxonomical study of a new species of the genus *Chelon* are given in this work.

## MATERIALS AND METHODS

### Study area and sample collection

Fifteen specimens of each mullet species were collected from the Rashid coast in the north of Egypt, in the south-eastern part of the Mediterranean Sea at 31° 32' N 30° 25' E. These are: *Mugil cephalus* (Flathead grey mullet), *Liza ramada* (Thinlip mullet), *Liza aurata* (Golden grey mullet), *Chelon labrosus* (Thicklip grey mullet) and *Chelon caeruleum* sp. nov. Samples of *Liza carinata* (Keeled mullet) were collected from the Red Sea (Safaga Coast) at 26° 51' N 34° 9' E (Fig. 1).



**Fig 1.** Map of Egypt showing localities of *Mugil cephalus*, *Liza ramada*, *Liza aurata*, *Chelon labrosus* and *Chelon caeruleum* sp. nov. specimens from the Rashid coastal region of the Mediterranean Sea and *Liza carinata* from the Safaga coastal region of the Red Sea

### Morphological data

Nineteen morphometric measurements were recorded with vernier calipers accurate to 0.05 mm. Body proportions were expressed in percentage of standard length (SL). All measurements are presented in Table 1 and abbreviations for measurements are as follows: Prepectoral fin length (PPF); Pre-first dorsal fin length (PDF); Presecond dorsal fin length (PSF); Prepelvic fin length (PVF); Preanal fin length (PAF); Caudal peduncle length (CPL); Head length (HL); Head width (HW); Snout length (SL); Eye diameter (ED); Postorbital length (PSL); Preorbital length (POL); Interorbital width (IOW); Pectoral-fin base length (PBL); First dorsal-fin base length (FDL); Second dorsal-fin base length (SDL); Pelvic-fin base length (VBL); Anal-fin base length (ABL); Caudal-fin base length (CBL).

**Table 2.** Morphometric data for a new *Chelon* sp. (*Chelon caeruleum* sp. nov.). Morphometric data (except standard length) are given as percentages of standard length.

Holotype	Paratypes	
	AFDZ 2008–123	AFDZ 2008–124 to AFDZ 2008–127
SL (mm)	682	273 - 678
	%SL	
PPF	22.7	22.5-22.8
PDF	50	50
PSF	76.1	76.0-76.4
PVF	39.8	39.4-39.9
PAF	75.5	75.2-75.6
CPL	17.4	17.2-17.7
HL	20.7	20.5-21.6
HW	13.2	13.0-13.5
SL	6.2	6.1-6.4
ED	4.8	4.6-5.2
PSL	10.9	10.7-11.1
POL	6.8	6.5-6.8
IOW	10.5	10.4-10.7
PBL	3.8	3.7-4.0
FDL	7.9	7.8-8.0
SDL	7.4	7.4
VBL	3.5	3.4-3.7
ABL	8.3	8.2-8.6
CBL	8.4	8.3-8.6

## Molecular data

### DNA extraction, polymerase chain reaction (PCR), amplification and sequencing

Muscle tissue was obtained from each fish. These samples were then transferred to laboratory and immediately frozen at  $-80^{\circ}\text{C}$ , where the fish's DNA was extracted using a GeneJET™ kit Genomic DNA Kit#K0721, following manufacturer's recommendations. Amplification of the COI gene fragment was carried out using the primers FF1 (5' TCA ACC AAC CAC ATA GAC ATT GGC TG 3') and FR1 (5' TAG ACT TCT GGG TGG CCA ACG AAT GC 3') (modified of Ward et al., 2005 by the present author (Deef L.E.)). The total volume of each polymerase chain reaction was 50  $\mu\text{L}$ , consisting of approximately 50 ng of template DNA, 0.5  $\mu\text{M}$  of each primer, 5  $\mu\text{L}$  of 10  $\times$  PCR reaction buffer, 4 mM of dNTP and 2 Units of Taq DNA polymerase. The thermal profile started with  $94^{\circ}\text{C}$  for 5 min, followed by 30 cycles of  $94^{\circ}\text{C}$  (30 sec),  $60^{\circ}\text{C}$  (30 sec) and  $72^{\circ}\text{C}$  (60 sec), finishing at  $72^{\circ}\text{C}$  for 10 min. Aliquots (5  $\mu\text{L}$ ) of amplicons were examined in 2% gels, stained with ethidium bromide and photographed under UV transillumination. The PCR product was purified using a GeneJET™ kit (Thermo K0701), according to the manufacturer's protocol. Purified PCR samples were sent for sequencing to a GATC Company in England which uses an ABI 3730xl DNA sequencer.

## Molecular analysis

The resulting sequences were confirmed as being derived from mullet DNA using the GenBank Blast algorithm. The DNADynamo software version 1.459 was used for editing the sequences and they were aligned using Clustal W. DNASTAR lasergene software version 15 was used for estimating genetic distances between studies species. Finally, the phylogenetic analyses used were Maximum Likelihood (ML) in MEGA 6.0 software (Tamura et al., 2013). Bootstrap values were used for estimating the support for tree nodes with 1000 replicates.

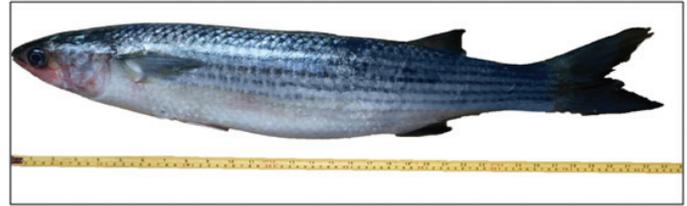
## RESULTS

### *Chelon caeruleum* sp. nov.

<http://zoobank.org/NomenclaturalActs/0B5DF860-C9704D95-BB0A-C67FB041DA13>

### Holotype

AFDZ 2008-123, adult male, 682 cm SL, northern coast of Egypt,  $31^{\circ} 32' \text{N } 30^{\circ} 25' \text{E}$ , 20 December 2016 (Fig. 2).



**Fig 2.** *Chelon caeruleum* sp. nov. 682 mm SL, caught from the Rashid coastal region of the Mediterranean sea

### Paratypes (4 specimens, all from the Mediterranean coast of Egypt)

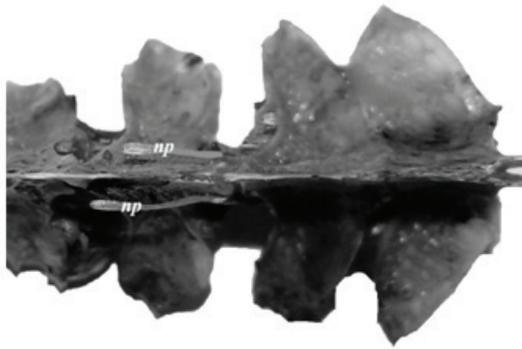
AFDZ 2008-124, male, 273 mm SL,  $31^{\circ} 51' \text{N } 30^{\circ} 46' \text{E}$ ;  
AFDZ 2008-125, male, 288 mm SL,  $31^{\circ} 44' \text{N } 30^{\circ} 33' \text{E}$ ;  
AFDZ 2008-126, male, 355 mm SL,  $31^{\circ} 44' \text{N } 30^{\circ} 33' \text{E}$ ;  
female, AFDZ 2008-127, 678 mm SL,  $31^{\circ} 41' \text{N } 30^{\circ} 30' \text{E}$ .  
Four fresh specimens were deposited in the archive of fish in the Department of Zoology, Faculty of Science, University of Damietta.

### Differential diagnosis

The following unique combination of characters further diagnoses this new species: adipose eyelid not thick, not extending onto iris, upper lip very deep, corner of mouth cleft not reaching to below front nostril and the unique presence of colouration of body is dark bluish grey dorsally and the sides are blue but abdomen is whitish and the fins are greyish, except pelvic and anal fins which are whitish. The new species is distinguished by dark longitudinal stripes present along scale rows and by the absence of dark axillary blotch.

*Chelon caeruleum* sp. nov. distinguished from all congeners by the body which is elongated and spindle-shaped, 2 dorsal fins are small and widely separated (anterior fin has 4 slender spines, while posterior fin is soft-rayed), and the head is flattened.

Five species of *Chelon* only have both long and short, unbranched pyloric caeca: *Chelon saliens* (Risso, 1810) from the Mediterranean; *Chelon dumerilli* (Steindachner, 1870) from West Africa and South Africa; *Chelon richardsonii* (Smith, 1849) from South Africa; *Chelon tricuspiciens* (Smith, 1935) from South Africa; and *Chelon persicus* (Senou, Randall & Okiyama, 1995) from the Persian Gulf (Senou et al., 1996). *Chelon caeruleum* sp. nov. is the sixth species bearing such pyloric caeca. However, this new species is different from the other 5 in having a pair of long (one longer than the other) spine-like neural postzygapophyse on the second vertebra (Fig. 3). In *Chelon saliens*, *Chelon dumerilli* and *Chelon richardsonii*, the postzygapophyseis is slightly compressed and hook-shaped. That of *Chelon tricuspiciens* is short and spine-like, but *Chelon persicus* has an equal pair of long spine-like neural postzygapophyse.



**Fig 3.** Neural postzygapophyse (np) on second vertebra of *Chelon caeruleum* sp. nov., 682 mm SL.

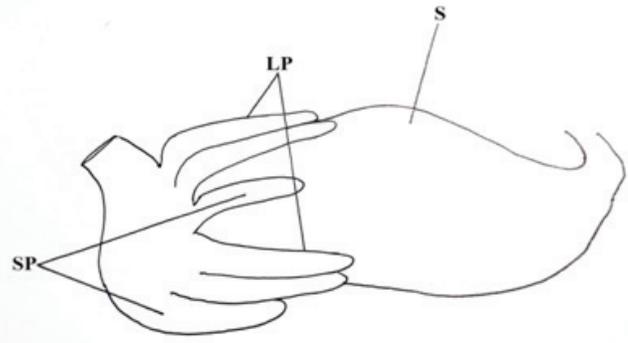
*Chelon persicus*, *Chelon dumerili* and *Chelon tricuspidens* were not different in either the number or composition of 3 short and 3 long caeca. However, *Chelon saliens* has 5 short caeca and 4 long caeca, and *Chelon richardsonii* has 4 short and 2 long caeca, subgenus of *Liza* (= *Chelon* in the present paper) on the basis of having both short and long pyloric caeca, and multiple grooves in the scales. These two conditions are classified *Liza saliens* and *Liza dumerili* in this subgenus. However, as mentioned above, *Chelon caeruleum*, *Chelon persicus*, *Chelon richardsonii* and *Chelon tricuspidens* also have short and long pyloric caeca, but there are no multiple grooves in their scales so the new species is included in the genus *Chelon*, not in the genus *Liza*.

*Chelon caeruleum* sp. nov. has 2 short and 4 long caeca which are significantly different from the aforementioned species. *Chelon caeruleum* sp. nov. is similar to *Chelon labrosus* (Risso, 1827) from the Mediterranean Sea in external appearance when fresh. However, the following colour character serves to distinguish the two: *Chelon caeruleum* sp. nov. has blue sides whereas *Chelon labrosus* has silver sides. The following measurements expressed as percentages of the SL are also differentiating (data for *Chelon caeruleum* sp. nov. given first): head length 20.5 to 21.6% (versus 24.9 to 28.8%); head width 13 to 13.5% (versus 14.9 to 17.7%); pre-first dorsal fin length 50% (versus 52.1-53.8); eye diameter 4.6 to 5.2% (versus 6.8 to 9.6%) and postorbital length 10.7 to 11.1% (versus 13.4 to 14.9%).

### Description

The following morphometric characters of *Chelon caeruleum* sp. nov. were registered: scales cycloid; head is small and dorsally flattened; eyes lateral; first dorsal fin rays IV; second dorsal fin rays I, 8; anal fin rays III, 9; 14 pectoral fin rays; standard length 682 mm; fork length 758 mm; snout length 42 mm; head length 141 mm; eye diameter 32 mm; interorbital 72 mm; upper lip height 8 mm; mouth length 41 mm; mouth width 49 mm. Body width at origin of first dorsal fin 153 mm; width of body at origin of anal fin 64 mm; length

of caudal peduncle from posterior end of base of anal fin to caudal flexure 119 mm; length from tip of snout to origin of first dorsal fin 341 mm; length from tip of snout to origin of second dorsal fin 519 mm; length from tip of snout to origin of pectoral fin 155 mm; length from tip of snout to origin of anal fin 514 mm; length from origin of first dorsal fin to the base of caudal fin 341 mm; length from origin of first dorsal fin to the origin of pelvic fin 234 mm. First dorsal origin is equal to snout as to caudal base. Base of pectoral fin 26 mm. Horizontal distance of base of second dorsal fin 51 mm. Horizontal distance of base of anal fin 57 mm. Caudal fin deeply forked. Morphometric data are given as percentages of standard length in Table 1. Gizzard-like stomach and unbranched pyloric caeca are 4 long and 2 short (Fig. 4). Colouration of body is dark bluish grey dorsally and the sides are blue. The abdomen is whitish and the fins are greyish, except pelvic and anal fins whitish (Fig. 2).



**Fig 4.** Stomach with long and short pyloric caeca of *Chelon caeruleum* sp. nov. LP: long pyloric caeca; S: stomach; SP: short pyloric caeca.

### Distribution

Only known from Rashid at the Mediterranean coast of Egypt.

### Etymology

The specific name "caeruleum" refers to the dark blue colour of its sides.

### Analysis of sequence characteristics

Six fish species were analysed, providing a total of 90 sequences. Approximately 663 bp of the mitochondrial DNA COI gene were amplified and sequenced. No insertions, deletions or stop codons were observed in any sequence. In total, 129 sequences (collected from GenBank) related to the studied fishes (Table 2) were obtained for the construction of a phylogram and for genetic distance estimation.

**Table 2.** List of Mugilidae members sequenced at mitochondrial DNA loci (COI).

Species	No. sequences	Accession numbers
Flathead grey mullet ( <i>Mugil cephalus</i> )	45	KT347598, KJ202179, KJ202180, KC500933, KC500934, KC500938, KC500939, KC500940, KC500950, KC500951, KC500952, KC500956, JQ623956, JQ060532, KP112323, KP112324, KP200024, JN242565, JN242566, JN242567, JN242568, JN242569, JN242570, JN242571, GU260664, GU260665, GU260666, GU260667, GU260668, GU260669, GU260670, GU260671, GU260672, GU260673, GU260674, GU260675, GU260676, GU260677, GU260678, HQ149083, HQ149714, HQ149715, HQ149082, JX559532, JX559533
Thicklip grey mullet ( <i>Chelon labrosus</i> )	23	KJ768226, KJ76827, KJ553192, KJ553183, KJ553145, KJ553132, KJ553284, KJ553053, KJ552931, KJ552871, KJ552858, KJ552826, KJ128451, KJ128452, JQ060484, JQ060411, JQ060412, HM208837, HM208838, EU715472, EU715473, EU715474, EU392233
Thinlip mullet ( <i>Liza ramada</i> )	18	KJ553231, KJ553122, KJ552955, KJ552776, KJ552761, KF676639, KC349864, JQ775055, JQ775056, JQ775057, JQ775058, JQ775059, JQ775079, JQ775080, HM208839, HM208840, EU715469, EU392240
Golden grey mullet ( <i>Liza aurata</i> )	22	KJ553106, KJ553023, KJ553076, KJ552920, KJ552923, KJ2859, KJ552886, KJ552773, JQ060456, JQ060457, JQ060458, JQ060459, JQ060460, HQ131882, HM208835, HM208836, EU392234, EU392235, EU715466, EU715467, EU715468, C114152
Keeled mullet ( <i>Liza carinata</i> )	21	KC500850, KC500851, KC500852, KC500833, KC500834, KC500835, KC500836, KC500837, KC500838, KC500839, KC500840, KC500841, KC500842, KC500843, KC500844, KC500845, KC500846, KC500847, KC500848, KC500849, JQ623947

### Genetic identification of Mugilidae

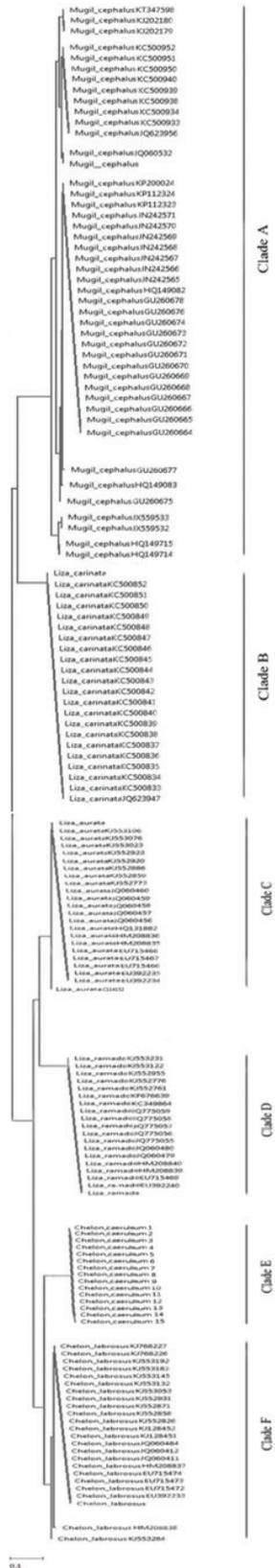
The COI sequences of the studied species were compared with COI sequences deposited in GenBank. For *Mugil cephalus*, *Chelon labrosus*, *Liza ramada*, *Liza aurata* and *Liza carinata* GenBank provided 45, 23, 18, 22 and 21 COI sequences, respectively (Table 2). When the *Chelon caeruleum* sp. nov. sequences were compared with records deposited in GenBank, the results did not reveal any entirely similar records. NCBI BLAST analysis revealed that *Chelon caeruleum* sp. nov. had a divergence level of 10 – 13% with some *Liza* spp. and some *Chelon* spp. nucleotide database.

### Genetic distance analysis

The genetic distance of the studied species of family Mugilidae was calculated using pair-wise distance via the ML method. *Mugil cephalus* has the largest genetic distance with the other species (0.550 – 0.691) in the present study (Table 3). Distances calculated between species showed that the smallest differences (0.323) existed between *Chelon labrosus* and *Chelon caeruleum* sp. nov., whereas the largest was between *Mugil cephalus* specimen and *Chelon labrosus* (0.691).

**Table 3:** Genetic distance between the studied mullet species.

	<i>Chelon labrosus</i>	<i>Liza aurata</i>	<i>Liza carinata</i>	<i>Liza ramada</i>	<i>Mugil cephalus</i>	<i>Chelon caeruleum</i> sp. nov.
<i>Chelon labrosus</i>	0					
<i>Liza aurata</i>	0.562	0				
<i>Liza carinata</i>	0.568	0.271	0			
<i>Liza ramada</i>	0.385	0.539	0.561	0		
<i>Mugil cephalus</i>	0.691	0.558	0.550	0.661	0	
<i>Chelon caeruleum</i> sp. nov.	0.323	0.610	0.620	0.365	0.678	0

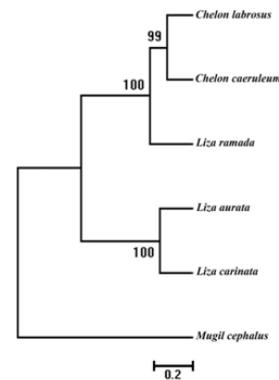


**Fig 5.** Kimura 2-parameter distance Maximum Likelihood (ML) tree of COI variation for 144 barcode sequences from 6 species belonging to family Mugilidae. Specimen numbers denote the accession number of GenBank.

*The phylogenetic relationships of the Mugilidae*

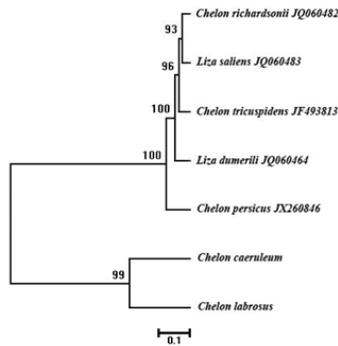
According to the COI dataset, haplotypes of the same species were always placed together in phylogenetic reconstructions. The ML tree (Fig. 5) is split into two independent lineages, *Mugil* and *Liza*. All haplotypes of *Mugil cephalus* formed a monophyletic cluster on the phylogenetic tree (clade A). The haplotypes of *Mugil cephalus* (clade A) and *Liza craniata* (clade B) were placed together and appeared as a sister group. Haplotypes of *Liza aurata* (clade C) and *Liza ramada* (clade D) clustered together. *Liza carinata* was intermediate between the *Liza* mullets and the *Mugil* cluster. *Chelon labrosus* (clade F) grouped with *Chelon caeruleum* sp. nov. (clade E) to form a monophyletic clade (Fig. 5).

Phylogenetic analysis performed in the current study using the Maximum Likelihood method revealed that *Mugil cephalus* was the farthest genetic distance with the other mullet species. The other studied mullet species included in a single branch. The ML tree places *Chelon caeruleum* sp. nov. on one branch with *Chelon labrosus*, with high nodal support (bootstrap value 99%) and *Liza ramada* on different branches next to each other (bootstrap value 100%). *Liza aurata* and *Liza carinata* were situated on another branch close to each other with high bootstrap support (100%) (Fig. 6).



**Fig 6.** Maximum Likelihood (ML) tree of 6 species belonging to family Mugilidae. Numbers on the branches refer to bootstrap values.

The ML tree of closely related *Chelon* species (all their sequences were obtained from GenBank except *Chelon labrosus*) with *Chelon caeruleum* sp. nov. revealed that *Chelon richardsonii*, *Chelon saliens* or *Liza saliens*, *Chelon tricuspidens*, *Chelon dumerili* or *Liza dumerili* and *Chelon persicus* were situated on another branch close to each other with high bootstrap support (100%). On the other hand, *Chelon caeruleum* sp. nov. was placed on one branch with *Chelon labrosus* with high nodal support (bootstrap value 99%) close to each other (Fig. 7).



**Fig 7.** Maximum Likelihood (ML) tree of closely related *Chelon* species with *Chelon caeruleum* sp. nov. Specimen numbers denote the accession number of GenBank. Numbers on the branches refer to bootstrap values.

## DISCUSSION

Classification of Mugilidae species into one of three genera, *Liza*, *Chelon* or *Mugil*, has been greatly studied because of the significant morphological and genetic resemblances of these fishes (Fraga et al., 2007; Semina et al., 2007). In this light, it is necessary to make additional genetic studies to find reliable genetic markers for species phylogeny description (Heras et al. 2009). Morphological analysis of specimens, and comparisons with similar species, has led to the detection of a new species of Mugilidae of the genus *Chelon*, which is described herein. The presence of morphological characters is suggesting a possible inclusion in the *Chelon* group. This new mullet is differentiated from other studied mullets by having dark bluish grey dorsally and the blue sides. It has a pair of long neural postzygapophyse on the second vertebra which is spine-like (one of neural postzygapophyse is longer than the other). These morphological differences are supported by a NCBI BLAST analysis of COI sequence variation of *Chelon caeruleum* sp. nov., which suggested that it should be considered as a new species of mullets.

The analysis of genetic distances based on COI variation showed that *Chelon caeruleum* sp. nov. is closely related to *Chelon labrosus*. In contrast, other species such as *Liza ramada*, *Liza aurata*, *Liza carinata* and in particular *Mugil cephalus* are more distantly related based on their greater pairwise genetic distances to *Chelon caeruleum* sp. nov. The ML tree of closely related *Chelon* species with *Chelon caeruleum* sp. nov. confirmed that *Chelon labrosus* is closely related to *Chelon caeruleum* sp. nov.

Conversely, compared with the other studied Mugilid species the greatest mean genetic distance was observed for *Mugil cephalus* in comparison to all other species that were tested. This result agrees with the findings of Martin (1995) who reported that this may be a result of faster substitution rate observed in this species, which could be described as a combined effect of nucleotide bias and saturation of signal.

Also this result is in line with Papatotiropoulos (2001, 2002, 2007) who utilized PCR-RFLP and allozyme, and sequenced 3 mtDNA genome.

A Neighbor-Joining phylogenetic tree constructed by Hillis and Bull (1993) located *Mugil cephalus* in a solely separate branch, a result reported by Caldara et al. (1996), Murgiaetal (2002) and Papatotiropoulos et al. (2001, 2002, 2007) as well. Papatotiropoulos et al. (2007) reported that both Neighbor-Joining and Bayesian topologies agree that *Mugil cephalus* lays into a separate phylogenetic branch being a sister group to all studied species. In agreement, this work placed *Mugil cephalus* in a completely distinct branch supporting the idea of re-examination of the taxonomy of mullet species.

In the present work, the Maximum Likelihood tree placed *Liza ramada* in a branch different from the other *Liza* species, and assigned *Liza carinata*, *Liza aurata* to a branch dissimilar to *Liza ramada* and *Chelon caeruleum* sp. nov. Thus, three species from the genus *Liza* did not sit together in a single group. This result is in agreement with the findings of Papatotiropoulos et al. (2002; 2007). These inadequate observations may be related to the differences in the methods applied leading to a better result due to the application of nucleotide sequencing, opposite to PCR-RFLP technique (Papatotiropoulos et al., 2007). The finding of this study is in agreement also with Rossi et al. (2004) who noted that 3 *Liza* species (*Liza aurata* and *Liza ramada*) from the Mediterranean and *Liza carinata* from the Red Sea did not locate in one branch.

## CONCLUSIONS

A new Mugilidae species has been identified and described in this study. The data obtained from this study reveal that *Chelon caeruleum* sp. nov. is the sixth member of the genus *Chelon* and that it possesses short and long unbranched pyloric caeca. The phylogeny and the genetic relationship of *Chelon caeruleum* sp. nov. based on DNA sequencing were also described. Furthermore, COI sequencing had a good phylogenetic signal and can be used for fish identification.

## SAŽETAK

### NOVA VRSTA CIPLA *Chelon caeruleum* (Mugilidae) S OPISOM GENETSKOG ODNOSA NASPRAM NEKIH OD OSTALIH VRSTA PORODICE MUGILIDAE

*Chelon caeruleum* sp. nov. opisana je kao nova vrsta cipla nađenog u Rashidovom obalnom području Sredozemnog mora u Egiptu. S ovim nalazom, nova vrsta je šesti član roda *Chelon*. *Chelon caeruleum* sp. nov. razlikuje se od njegovih srodnika sljedećim obilježjima: dorzalna boja tijela je tamnoplavo do siva, a lateralne strane su plave; duljina glave je od 20,5 do 21,6% od SD (standardne duljine); širina

glave 13 do 13,5% SD; duljina prva leđne peraje 50% SD; promjer oka 4,6 do 5,2% SD; postorbitalna duljina 10,7 do 11,1% SD; posjeduje 2 kratka i 4 duga nerazgranata pilorična nastavka; kralježnična neuralna postzigapofiza se nalazi na drugom kralješku. DNK *Chelon caeruleum* sp. nov. i ostalih pet analiziranih vrsta cipla (*Mugil cephalus*, *Liza carinata*, *Liza ramada*, *Liza aurata* i *Chelon labrosus*) je ekstrahirana, umnožena reakcijom lančane polimeraze (PCR) te sekvencionirana. Analiza sekvenci mtDNA citokrom oksidaze podjedinice I potvrdila je da se *Chelon caeruleum* sp. nov. razlikuje se od ostalih srodnika roda *Chelon*.

**Ključne riječi:** cipal, PCR, filogeneza, sekvencioniranje, taksonomija.

## REFERENCES

- Caldara, F., Bargelloni, L., Ostellari, L., Penzo, E., Colombo, L., Patarnello, T. (1996): Molecular phylogeny of grey mullets based on mitochondrial DNA sequence analysis: evidence of a differential rate of evolution at the intra-family level. *Molecular Phylogenetics and Evolution*, 6, 3, 416-424.
- Durand, J.D., Hubert, N., Shen, K.N., Borsa, P. (2017) DNA barcoding grey mullets. *Reviews in Fish Biology and Fisheries*, 27, 233-243.
- Fraga, E., Schneider, H., Nirchio, M., Santa-Brigida, E., Rodrigues-Filho, L.F., Sampaio, I. (2007): Molecular phylogenetic analyses of mullets (Mugilidae, Mugiliformes) based on two mitochondrial genes. *Journal of Applied Ichthyology*, 23, 5, 598-604.
- Harrison, I.J., Nirchio, M., Oliveira, C., Ron, E., Gaviria, J. (2007): A new species of mullet (Teleostei: Mugilidae) from Venezuela, with a discussion on the taxonomy of *Mugil gaimardianus*. *Journal of Fish Biology*, 71, 76-97.
- Heras, S., Roldan, M.I., Castro, M.G. (2009): Molecular phylogeny of Mugilidae fishes revised. *Reviews in Fish Biology and Fisheries*, 19, 2, 217-231.
- Hillis, D.M., Bull, J.J. (1993): An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology*, 42, 2, 182-192.
- Liu, J.Y., Brown, C.L., Yang, T.B. (2010): Phylogenetic relationships of mullets (Mugilidae) in China Seas based on partial sequences of two mitochondrial genes. *Biochemical Systematics and Ecology*, 38, 4, 647-655.
- Martin, A.W. (1995): Mitochondrial DNA sequence evolution in sharks: rates, patterns, and phylogenetic inferences. *Molecular Biology and Evolution*, 12, 6, 1114-1123.
- Murgia, R., Tola, G., Archer, S.N., Vallerga, S., Hirano, J. (2002): Genetic identification of grey mullet species (Mugilidae) by analysis of mitochondrial DNA sequence: application to identify the origin of processed ovary products (bottarga). *Marine Biotechnology*, 4, 2, 119-126.
- Nelson, J.S. (2006): *Fishes of the World*. John Wiley and Sons, New York.
- Papasotiropoulos, V., Klossa-Kilia, E., Kiliass, G., Alahiotis, S. (2001): Genetic divergence and phylogenetic relationships in grey mullets (Teleostei: Mugilidae) using allozyme data. *Biochemical Genetics*, 39, 5, 155-168.
- Papasotiropoulos, V., Klossa-Kilia, E., Kiliass, G., Alahiotis, S. (2002): Genetic divergence and phylogenetic relationships in grey mullets (Teleostei: Mugilidae) based on PCR-RFLP analysis of mtDNA segments. *Biochemical Genetics*, 40, 3, 71-86.
- Papasotiropoulos, V., Klossa-Kilia, E., Alahiotis, S., Kiliass, G. (2007): Molecular phylogeny of grey mullets (Teleostei: Mugilidae) in Greece: evidence from sequence analysis of mtDNA segments. *Biochemical Genetics*, 45, 7, 623-636.
- Rossi, A.R., Ungaro, A., De Innocentiis, S., Crosetti, D., Sola, L. (2004): Phylogenetic analysis of Mediterranean Mugilids by allozymes and 16S mtrRNA genes investigation: are the Mediterranean species of *Liza* monophyletic? *Biochemical Genetics*, 42, 9, 301-315.
- Schultz, L.P. (1946): A revision of the genera of mullets, fishes of the family Mugilidae, with descriptions of 3 new genera. *Proceedings of the United States National Museum*, 96, 377-395.
- Semina, A.V., Polyakova, N.E., Makhotkin, M.A., Brykov, V.A. (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. *Russian Journal of Marine Biology*, 33, 3, 187-192.
- Senou, H., Randall, J.E., Okiyama, M. (1996): *Chelon persicus*, a New Species of Mullet (Perciformes: Mugilidae) from the Persian Gulf. *Bulletin of the Kanagawa Prefectural Museum Natural Science*, 25, 71-78.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. (2013): MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 30, 2725-2729.
- Thomson, J.M. (1954): The Mugilidae of Australia and adjacent seas. *Australian Journal of Marine and Freshwater Research*, 5, 1, 70-131, pls. 1-2.
- Thomson, J.M. (1997): The Mugilidae of the World. *Memoirs of the Queensland Museum*, 43, 457-562.
- Trewavas, E., Ingham, S. E. (1972): A key to the species of Mugilidae (Pisces) in the northeastern Atlantic and Mediterranean, with explanatory notes. *Journal of Zoology London*, 167, 15-29.
- Turan, C., Gürlek, M., Ergüden, D., Yağlıoğlu, D., Öztürk, B. (2011): Systematic Status of Nine Mullet Species (Mugilidae) in the Mediterranean Sea. *Turkish Journal of Fisheries and Aquatic Sciences*, 11, 315-321.
- Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., Hebert, P.D.N. (2005): DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London. Series B, Biological sciences*, 360, 1847-1857.