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SHORT COMMUNICATION

Additional information on the blue runner, *Caranx crysos* (Mitchill, 1815), from the northern Adriatic Sea: meristic and molecular characterizations

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In October 2014, a blue runner (Caranx crysos) *was caught by a professional fisherman in waters off St. Eufemija Point (west Istrian Coast, northern Adriatic Sea, Croatia). This is the second recorded blue runner in the northern Adriatic Sea, which represents the northernmost biogeographic sector of the Mediterranean Sea. Recently, blue runners were recorded in the southern and central Adriatic Sea, suggesting that the central Mediterranean* C. crysos *is expanding its area of distribution throughout the Adriatic Sea. As a point of interest, this most recent specimen presented an unusual meristic feature: the number of spines of its first dorsal fin was VI, whereas it is generally recognised that the number of such spines characteristic for this species is VIII. Species identity of the specimen was confirmed by DNA sequence analysis of the mitochondrial cytochrome c oxidase subunit I (COI) gene. In addition, we provided the first report of* C. crysos *mitochondrial 16S ribosomal RNA (16S rRNA) partial sequence.*

Key words: *Caranx crysos*, meristic analysis, climate change, northern Adriatic Sea, cytochrome c oxidase subunit I gene (COI), 16S ribosomal RNA gene (16S rDNA)

INTRODUCTION

The blue runner, *Caranx crysos* (Mitchill, 1815), (Perciformes: Carangidae), is a thermophilic tropical and subtropical medium size marine fish of the family Carangidae inhabiting both sides of the Atlantic Ocean and the Mediterranean Sea (SMITH-VANIZ *et al.*, 1990). It is predominantly a fish predator (SLEY *et al.*, 2009), but it also eats shrimps, crabs and other invertebrates (SMITH-VANIZ *et al.*, 1990). The main diet of juveniles consists of plankton (McKENNEY *et al.*, 1958), primarily meso- and macro-zooplankton (KEENAN & BENFIELD, 2003). The species is distributed in both onshore and offshore environments, occurring in loose shoals in the neritic region (LONGHURST & PAULY, 1987) and in the vicinity of reefs (HOLLAND *et al.*, 1996). Additionally, *C. crysos* tends to be associated with fish aggregating devices (ANDALORO *et al.,* 2007), with the *Sargassum* community (DOOLEY, 1972) and with large manmade offshore structures such as oil platforms (HASTINGS *et al.,* 1976; BROWN *et al.,* 2010).

Recent findings show that the species is able to migrate to higher latitudes, such as Newfoundland (DEVINE & FISHER, 2014) as well as Argentina (DELPIANI *et al.,* 2011) and southwest

England (SWABY *et al.,* 1996). Previous studies suggest that the abundance and the range of distribution of the blue runner in the Mediterranean Sea are increasing over time. DODERLEIN (1878) considered *C. crysos* as very rare around Sicily (central Mediterranean) whereas TORTONESE (1952, 1961, 1975) described the population of the eastern and southern Mediterranean as relatively scarce. Moreover, the sparse records that exist for the northern coast of the western basins were considered to be unreliable by TORTONESE (1975). FISHER *et al.* (1987) reported that *C. crysos* can be found throughout the Mediterranean, except in the Adriatic Sea. However, recent investigations have revealed that *C. crysos* is now regularly caught in northwest Sicily and central-southern Tyrrhenian Sea up to the Gulf of Naples (PSOMADAKIS *et al.,* 2011). The species has been also recorded in the northern Tyrrhenian Sea and in the Ionian Sea (PSOMADAKIS *et al.,* 2011).

In the Adriatic Sea, the species was recorded for the first time in August 2008 (DULČIĆ *et al.,* 2009) when a specimen was caught along the west Istrian Coast in the northern Adriatic Sea, which represents the northernmost sector of the Mediterranean Sea (BIANCHI & MORRI, 2000; BIANCHI, 2007). Subsequently, five specimens were caught in the southern Adriatic (Montenegrin Coast): four were reported in the Ulcinj region in March 2013 (DULČIĆ *et al.,* 2014a); and one additional specimen was reported in the Boka Kotorska Bay in December 2013 (DULČIĆ *et al.,* 2014b). Two specimens were caught in the central Adriatic: a juvenile specimen was reported from the Pelješac Channel in October 2013 (DULČIĆ *et al.,* 2014b); and the second specimen was reported from the estuary of the River Neretva (Dalmatia) in January 2014 (PAVIČIĆ *et al.,* 2014).

The aim of this study is to provide additional information on the most recent record of *C. crysos* from the northern Adriatic Sea. The specimen was caught in the vicinity of Rovinj (West Istrian Coast, Croatia). The morphometric and meristic characteristics of the specimen have been compared to the aforementioned specimens found in the Adriatic Sea, as well as to literary data. In addition, species identity described in this work has been supported by the analysis of the two mitochondrial DNA markers, cytochrome c oxidase subunit I (COI) and 16S ribosomal RNA (16S rDNA).

MATERIAL AND METHODS

On the 1st of October 2014, a specimen of blue runner *Caranx crysos* (Fig. 1) was caught in a gill net by Mr. Danijel Despotović, a professional fisherman, in the vicinity of Rovinj, off St. Eufemija Point (45°07'17"N; 13°36'50"E) at approximately 25 m in depth. The specimen was caught together with seasonal fish that are regularly found in gill nets, predominantly *Sparus aurata* Linnaeus, 1758. Also present in the catch was the migratory fish, *Seriola dumerili*

*Fig. 1. The blue runner (*Caranx crysos*) caught in the northern Adriatic Sea (45°07'17"N; 13°36'50"E) on the 1st October 2014*

Table 1. Caranx crysos. *Morphometric measures (mm) for the present record and for other Adriatic specimens. %Lt = percent of a given morphometric measure to the total length of the fish*

Morphometric measures	DULČIĆ *et al.*, 2009		PAVIČIĆ *et al.*, 2014		Present record	
	Measure	%Lt	Measure	%Lt	Measure	%Lt
Total length	368	100	185	100	336	100
Standard length	295	80.2	114	61.6	262	77.97
Fork length	328	89.1	163	88.1	296	88.95
Head length	82	22.3	41	22.2	75	22.321
Eye diameter	14	3.8	10	5.4	12.78	3.803
Preorbital length	20	5.4	11	5.9	19.87	5.91
Postorbital length	44	12	17	9.2	35.08	10.44
Predorsal length	111	30.2	52	28.1	95	28.27
Pectoral fin length	120	32.6	40	21.6	88.20	26.70
Maximum depth	101	27.4	49	26.5	89.72	26.70
Minimum depth	10	2.7	6	3.2	9.30	2.76

(Risso, 1810), which is frequently found in the central Adriatic Sea.

C. crysos was identified according to SMITH-VANIZ *et al.* (1990) and DULČIĆ *et al.* (2009). Morphometric variables of this specimen were determined with an ichthyometer (total length, standard length, fork length and head length) with an accuracy of 0.1 cm and a digital calliper with an accuracy of 0.1 mm (other morphometric measures have been reported in Table 1). The specimen is stored in the Ichthyological Collection of the Centre for Marine Research in Rovinj, Croatia.

A small sample of muscle, used for molecular analysis, was separated from the caudal region of the fish. Genomic DNA was extracted from approximately 25 mg of muscle tissue by using the Qiagen DNeasy Blood & Tissue Kit, following the manufacturer's protocol. From the mitochondrial genome, we amplified a 704 bp fragment of the cytochrome c oxidase subunit 1 (COI) gene using the primers Fish-BCL (5'-TCA ACY AAT CAY AAA GAT ATY GGC AC-3′) and Fish-BCH (5′-ACT TCY GGG TGR CCR AAR AAT CA-3′) (BALDWIN *et al.*, 2009). We also amplified a 640 bp fragment of the mitochondrial 16S ribosomal RNA (16S rRNA) gene using the primers 16S-ar (5′-CGC CTG TTT ATC AAA AAC AT-3′) and 16S-br (5′-CCG GTC TGA ACT CAG ATC ACG T-3′) (PALUMBI *et al.*, 2002). Standard PCR reactions contained 20 ng DNA, 1xPCR buffer, 2.5 mM $MgCl_2$, 200 µM each dNTP, 0.5 µM each primer, 2 U GoTaq DNA polymerase (Promega) and Milli-Q H_2O to a 40 µL final volume. Cycling parameters included initial denaturation at 95 °C for 3 minutes; followed by 35 iterations of denaturation at 95 °C for 20 seconds, annealing at 55 °C (COI) or 52 °C (16S) for 20 seconds, and extension at 72 °C for 40 seconds; and a final extending step at 72 °C for 10 minutes. Double-stranded PCR products were electrophoretically checked for amplification of a single band in 1% agarose gel, and subsequently purified using the Qiagen QIAquick PCR Purification Kit. The PCR products were sequenced by Macrogen Europe Laboratory (Amsterdam, The Netherlands) with the same primers used in PCR amplifications, and both strands were sequenced to ensure accuracy. After primer trimming, the obtained sequences were deposited in the NCBI GenBank database under the accession numbers KP273457 (COI) and KP273458 (16S).

Using NCBI BLAST web service, *C. crysos* COI and 16S sequences were subjected to BLAST analyses using a MegaBLAST module optimized for highly similar sequences (MORGULIS *et al.*, 2008). The Barcode of Life Data Systems (BOLD Systems) web platform was utilised to compare the COI sequence of *C. crysos* to the COI reference library (RATNASINGHAM & HEBERT, 2007). The 16S sequence of *C. crysos* was compared to the carangid GenBank records under the accession numbers: AB096007, DQ427055, EF613269, EU848433, HQ127636, JQ939037, KC603532, KF649842, and KF649843, belonging to the species from the family Carangidae that inhabit the Adriatic Sea, according to JARDAS (1996) and MILIŠIĆ (2003). DNA sequence evolution models for the 16S sequence alignment were tested in MEGA6 (TAMURA *et al.*, 2013). The Maximum Likelihood tree was reconstructed based on the K2P+G (Kimura-2 parameter with gamma distribution) model selected as the best-fit model with the lowest Bayesian Information Criterion score. Statistical testing of the robustness of the tree topology was performed by bootstrap resampling of 1000 replications. Bayesian analysis was performed in MrBayes v.3.1.2. (HUELSENBECK & RONQUIST, 2001) using reversible jump Markov Chain Monte Carlo (MCMC) method with four parallel Markov chains; 2.000.000 generations; sampling frequency one in every hundred trees; consensus tree constructed based on the trees sampled after burn-in. The convergence of Markov chains was checked through standard deviations of split frequencies and log-likelihood scores for each run.

RESULTS

The colour of the fresh specimen (Fig. 1) was golden-bluish with green shading dorsally and with golden nuances behind the eyes and on the pectoral region. A black spot was evident on the upper margin of the operculum. The belly was silver-white with pale purple shadowing on the flanks. The fins were yellowish with dusky tips on the caudal fin and the first dorsal fin. The number of scutes along the straight part of the lateral line was 52 (Table 2). The first spiny section of the dorsal fin was lower than the second soft section. The terminal rays of the dorsal and anal fins were closely positioned to the last but ones. An irregular series of moderate canines was flanked by an inner band of small villiform teeth in the upper jaw, which reached to under the mid-eye (Fig.1). Caudal paired keels were particularly evident.

However, one meristic measure that differed from the described characteristics for *C. crysos* was the number of spines of the first dorsal fin. For the considered specimen, the number of spines of the first dorsal fin was VI (Fig. 2). All morphometric and meristic measures assessed

Table 2. Caranx crysos, *Meristic features of the present record compared to available data for Adriatic specimens and FishBase values*

Meristic features	DULČIĆ *et al.,* 2009	PAVIČIĆ *et al.,* 2014	FishBase	Present record
First dorsal fin	VIII	VIII	VIII[a], VIII[b]	VI
Second dorsal fin	I + 23	I + 25	I + 22-25[a], I +23 [b, c]	I + 22
Anal fin	II + I + 19	II + I + 25	II + I + 19-21[a], II + I + 19 [b, c]	II + I + 20
Pectoral fin	I + 21	I + 21	23 [c]	I + 20
Ventral fin	I + 5	I + 5	not given	I + 5
Lateral line scales	87	93	86-98 [c]	63
Scutes on lateral line	48	48	45-46 [c]	52

[a]Smith-Vaniz *et al.,* 1990, [b]Smith, 1997, [c]Uyeno *et al.*, 1983

for the present record are reported in Tables 1 and 2, respectively. In Table 1, these measures

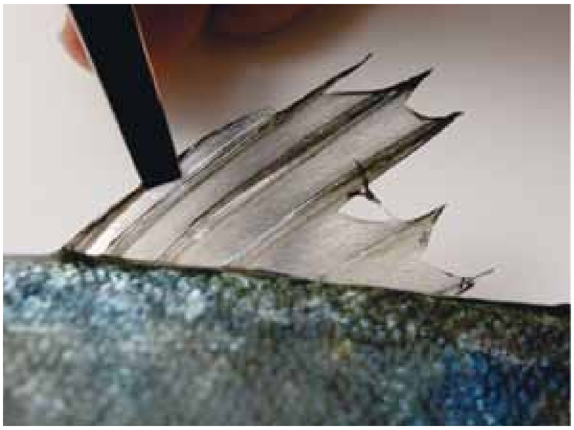

Fig. 2. The first dorsal fin of the Caranx crysos *shown in Fig. 1. Six spines (VI) composed the first dorsal fin*

were compared with available values for *C. crysos* recorded in the Adriatic Sea. In Table 2, the meristic features were compared with data for the species recorded in the Adriatic Sea as well as those available in FishBase.

To confirm species identity of this specimen, its mitochondrial DNA markers were analysed. Partial sequences of the COI and 16S rRNA genes were PCR-amplified generating 704 bp and 640 bp long fragments, respectively (Fig. 3). The PCR products were sequenced, and the obtained sequences were matched against NCBI GenBank sequence database. All the GenBank entries that matched our COI sequence under highly stringent criteria (>99% identity, >99% query coverage, and E-value 0.0) have been assigned to the species *C. crysos*. In addition, based on the COI sequence, our specimen was also identified as *C. crysos* in BOLD Systems, by sharing 100% identity with 35 previously registered and COI-barcoded *C. crysos* specimens.

Given the fact that there have been no GenBank records of *C. crysos* 16S rRNA gene sequence to date, it is not surprising that our 16S sequence did not match any of GenBank entries with 100% identity. However, it is significant that it shows the highest scores (97% identity, covering 100% sequence by E-value 0.0) with records belonging to the congeneric species *Caranx ignobilis* (DQ427054, KF649842),

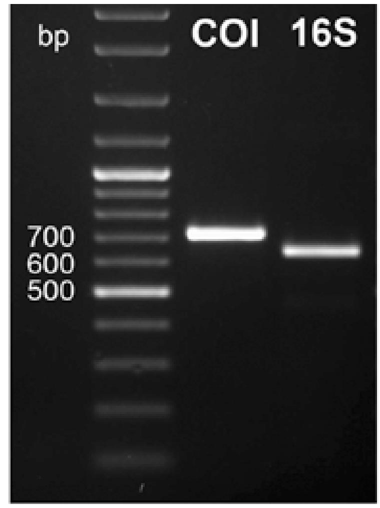

Fig. 3. The electrophoretic separation of PCR products amplified from Caranx crysos *using the primers specific for partial sequences of the mitochondrial cytochrome c oxidase subunit I gene (COI) and 16S ribosomal RNA gene (16S)*

C. melampygus (AP004445, DQ427053, KF649843), and *C. sexfasciatus* (DQ427055). To test the classification of our specimen within the genus *Caranx*, we broadened the 16S assay and performed phylogenetic analysis by including available 16S sequences from the GenBank database for other representative species from the family Carangidae that inhabit the Adriatic Sea. In the Maximum Likelihood tree (Fig. 4), the four *Caranx* species group together, while the other carangid sequences are more distantly related. Based on the topology of the tree, the two species from the tribe Naucratini, *Naucrates ductor* and *Seriola dumerili*, form a clade being separated from the Carangini tribe species with 100% bootstrap support. Bayesian interference analysis supported the ML tree topology, and confirmed association of our specimen with the *Caranx* group (Fig. 4).

DISCUSSION

Due to climate change, south Mediterranean thermophilic species are spreading northward. This process could affect the structure of marine assemblages in the northern sectors of the Mediterranean Sea, particularly if immigrant species interact with resident species. Starting in the

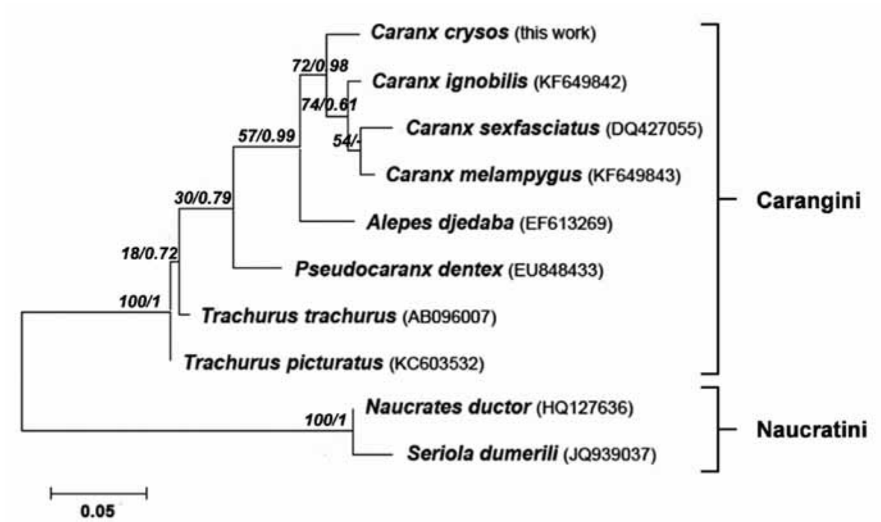

Fig. 4. The ML/Bayesian phylogenetic tree of 16S rDNA sequences from Caranx crysos *and related carangids. The accession numbers of the sequences from carangid representatives retrieved from the GenBank database are indicated in the parenthesis. Numbers on branches indicate ML bootstrap support / Bayesian posterior probabilities*

1990s, the abundance of predatory fish from warmer regions of the Mediterranean Sea has markedly increased in the northern Adriatic Sea (e.g., DULČIĆ *et al.,* 2005), which is a highly productive region (DEGOBBIS *et al.,* 2000) and, thus, rich in prey.

Predators such as the bluefish *Pomatomus saltatrix* (Linnaeus, 1766) (Pomatomidae) and *S. dumerili* (Carangidae), which were previously unrecorded (the former) or rare (the later) in the northern Adriatic Sea, can now be regularly purchased in Istrian fish markets during the summer and autumn months (authors' personal observation). While visiting Istrian fish markets, we observed that the number of *Lichia amia* (Linnaeus, 1758) (Carangidae) on offer has also increased. Occasionally, *Trachinotus ovatus* (Linnaeus, 1758) (Carangidae) and *Xiphias gladius* Linnaeus, 1758 (Xiphiidae) were also caught; in the majority of cases, the former species was unknown to fishermen. The impact of these predators on the northern Adriatic fish assemblages could be relevant; for instance, large shoals of bluefish have been observed to prey on mullets (Mugilidae) in the Cove of Tar (West Istrian Coast) (DULČIĆ *et al.,* 2005). Additionally, an increased number of thermophilic predators may out-compete the indigenous predator, *Dicentrarchus labrax* (Linnaeus, 1758) (Moronidae), which aggregate in shallow west Istrian waters during the November – December spawning season.

The biometric, meristic and morphological characteristics of the considered specimen of *Caranx crysos* corresponded to those provided in the descriptions of the species (SMITH-VANIZ *et al.,* 1990; DULČIĆ *et al.,* 2009).

The thermophilic carangid *C. crysos* has been recorded in the Adriatic Sea (DULČIĆ *et al.,* 2009; DULČIĆ *et al.,* 2014a,b; PAVIČIĆ *et al.,* 2014), even in the northern Adriatic (DULČIĆ *et al.*, 2009; this study). To date, all records of *C. crysos* are reported for the east Adriatic coast, suggesting that the eastern Adriatic coastal current could play a relevant role in the migration of this species from southern Mediterranean regions. The entrance into the Adriatic of rare thermophilic species could also be facilitated by the process of Levantine Intermediate Water ingressions i.e., the input of warm and highly saline waters from the Ionian Sea into the Adriatic Sea (DULČIĆ *et al.,* 2003, 2004; DULČIĆ & GRBEC, 2000).

The northern Adriatic is principally delimited by the February isotherm of 11° C, and even lower seawater temperatures are measured in its northernmost parts (BIANCHI, 2007). The thermal preference of *C. crysos* is typically around 20°C (McKENNEY *et al.,* 1958) and, in newly-colonized regions, it has been shown to avoid temperatures below 14°C (PSOMADAKIS *et al.*, 2011). Thus, *C.*

crysos can be expected in the northern Adriatic Sea only during the warmer seasons along with the migration of *S. dumerili* and other thermophilic predators, as in our case. The February isotherms of approximately 13°C and 14°C delimit the southern borders of the central and southern Adriatic, respectively (BIANCHI, 2007). Therefore, accounting for the effects of global warming, it can be suggested that stable populations of *C. crysos* could be established in these biogeographic sectors in the future.

The total length of the two *C. crysos* specimens caught in the northern Adriatic Sea was 368 mm (DULČIĆ *et al.*, 2009) and 336 mm (this study), which are both markedly below the officially-recorded maximum length assessed for the species in the Mediterranean Sea, that is 505 mm (PSOMADAKIS *et al.,* 2011). However, in a *C. crysos* sample of 130 specimens from the central Mediterranean Sea, the total length ranged from 95 to 370 mm (PSOMADAKIS *et al.,* 2011). Hence, in comparison with the size distribution of the fish in the central Mediterranean, both northern Adriatic specimens are located in the higher end of the length spectrum. In contrast, the other two fish reported from Dalmatia (PAVIČIĆ *et al.,* 2014) and the Montenegrin coast (DULČIĆ *et al.,* 2014b) were much smaller. A comparison of other morphometric variables expressed as percent of the total length among Adriatic specimens suggested that these proportions tended to vary with the size of the fish (Table 1). However, given the low sample size, further investigations including a larger number of individuals are needed to elucidate this point.

All meristic features determined for the specimen caught in of Rovinj correspond with those reported in literature, except for the number of spines of the first dorsal fin (Table 2, Fig. 3). In contrast to literary data, the number of spines of the first dorsal fin was VI. Therefore, we suggest further investigation to determine if this characteristic is also present in other Mediterranean specimens to ascertain if the diagnosis of the species should be modified. In our specimen, the first dorsal fin did not show any deformity (Fig. 3); hence, we exclude that this property was a fluke or that it was caused by an accident.

To complement meristic characterization of the specimen, we carried out the analyses of two mitochondrial DNA markers. Given the fact that *C. crysos* specimens that were previously caught in the Adriatic Sea (DULČIĆ *et al.,* 2009; DULČIĆ *et al.*, 2014a, b; PAVIČIĆ *et al.,* 2014) have not been DNA-barcoded, this work also represents the first mitochondrial DNA characterization and molecular identification of Adriatic *C. crysos* specimens. By analyzing the COI sequence, we confirmed the species identity of the described specimen through 100% matches with the *C. crysos* sequence data recorded in the NCBI GenBank and BOLD Systems databases. In addition to COI characterization, we also reported data for the first time on the mitochondrial 16S rDNA sequence of *C. crysos*. Comparing the *C. crysos* 16S sequence to 16S sequences of other species of the family Carangidae present in the Adriatic Sea, we revealed phylogenetic relationships that are in accordance with those obtained by the cytochrome b gene (REED *et al.*, 2002) and the COI gene (MAT JAAFAR *et al.*, 2012) studies from species of family Carangidae. Based on these results, 16S rDNA could be a useful marker in species identification as well as in molecular systematics of the marine fish of the family Carangidae. However, a larger number of carangid species needs to be analyzed in order to prove this hypothesis.

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Dodatne informacije o plavom trkaču, *Caranx crysos* (Mitchill, 1815), u sjevernom Jadranu: meristička i molekularna karakterizacija

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SAŽETAK

U listopadu 2014. godine, ribu plavi trkač (*Caranx crysos*) je ulovio profesionalni ribar nekoliko stotina metara van rta Sv. Eufemija (zapadna obala Istre, sjeverni Jadran, Hrvatska). Ovo je drugi po redu nalaz ribe plavi trkač koja je ulovljena u sjevernom Jadranu, što predstavlja najsjevernije biogeografsko područje Sredozemnog mora. Nedavno ova vrsta je zabilježena u južnom i srednjem Jadranu što ukazuje da se populacija *C. crysos* iz središnjeg Sredozemnog mora proširuje u Jadranskom moru. Razmatrani primjerak je pokazao neobičnu merističku značajku: broj bodlji u prvoj leđnoj peraji je VI, dok je općenito za ovu vrstu prihvaćeno (priznato) VIII bodlji. Identitet vrste potvrđen je analizom mitohondrijske DNA sekvence za pod-jedinicu I gena citokrom c oksidaze (COI). U ovom radu također je po prvi put analiziran dio mitohondrijske DNA sekvence odgovorne za kodiranje 16S ribosomske RNA (16S rDNA) kod vrste *C. crysos*.

Ključne riječi: *Caranx crysos*, meristička analiza, klimatske promjene, sjeverni Jadran, gen za citokrom c oksidazu podjedinica I (COI), gen za 16S ribosomsku RNA (16S rDNA)