Biometrics Characters, Length-Weight Relationships and Genetic Properties of Damselfish, *Chromis chromis* (Linnaeus, 1758*)* (Osteichthyes: Pomacentridae) from the Black Sea

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Research Article

Abstract

The damselfish *Chromis chromis* is typically the most common fish species in the rocky shores of the Mediterranean Sea and the Black Sea. Despite having no commercial value, *C. chromis* was considered as a key species for the rocky littoral environment. In this study, the biometric properties and length-weight relationships were examined in 112 specimens of *C. chromis* sampled from the Black Sea in 2018. Fifteen morphometric and six meristic characteristics were measured. We also used a molecular approach employing mitochondrial genes (16S rRNA and COI) for the first time to further characterize *C. chromis* specimens collected from the Black Sea. Morphometric and meristic characters of male and female specimens were similar and there were no differences between sexes. The average length and weight were calculated as 99.54 ± 11.49 mm (72.0-115.1) and 18.35 ± 5.95 g (5.96-26.56), respectively. The length-weight relationship was calculated as $W = 0.0127TL^{3.117}$ ($R^2 = 0.834$) and the growth indicates a positive allometric growth (b>3). 16S rRNA and COI partial sequences analysis indicated a close genetic relationship with *C. chromis* specimens from the Aegean Sea and the Mediterranean Sea.

Keywords: *Chromis chromis*, morphometric and meristic characteristics, mtDNA

Karadeniz'de Yayılım Gösteren Papaz balığının, Chromis chromis, Biyometrik Karakterleri, Boy-Ağırlık İlişkisi ve Genetik Özelliklerinin Belirlenmesi

Papaz balığı, Chromis chromis, Akdeniz ve Karadeniz'in kayalıklı kıyılarında görülen en yaygın balık türlerinden bir tanesidir. Ticari bir değeri olmayan C. chromis, kayalıklı litoral bölge için önemli bir türdür. Bu çalışmada, 2018 yılında Karadeniz'den örneklenen 112 C. chromis örneğinin biyometrik özellikleri ve boy-ağırlık ilişkisi incelenmiştir. Bu amaçla toplam 15 morfometrik ve 6 meristik karakter ölçümü yapılmıştır. Ayrıca C. chromis örneklerinin mtDNA'larının 16S rRNA ve COI gen bölgeleri analiz edilerek tür genetik olarak da karakterize edilmiştir. Çalışma sonunda ölçülen erkek ve dişi bireylerin morfometrik ve meristik karakterlerin benzer olduğu ve istatistiksel olarak cinsiyetler arasında fark olmadığı belirlenmiştir. Balıkların ortalama boy ve ağırlıkları sırasıyla 99,54 + 11,49 mm (72,0-115,1) ve 18,35 + 5,95 g (5,96-26,56) olarak ölçülmüştür. Balıkların boy-ağırlık ilişkisi W=0.0127〚TL〛^3.1172 (R^2=0.8341) olarak hesaplanmış ve pozitif algoritmik büyüme (b>3) tespit edilmiştir. 16S rRNA ve COI gen bölgelerinin kısmi analizi sonucunda Karadeniz'deki örneklerin Ege ve Akdeniz'deki örnekler arasında genetik benzerlik olduğu tespit edilmiştir.

Anahtar Kelimeler: Chromis chromis, morfometrik ve meristik karakter, mtDNA

INTRODUCTION

Genus *Chromis* is globally distributed in temperate and tropical waters and represented with 100 species (Cooper, Smith, & Westneat, 2009; Quenouille, Bermingham, & Planes, 2004; Tang, 2001). The damselfish *Chromis chromis* (Linnaeus, 1758) is among the most common species of the genus C*hromis* in the eastern Atlantic Ocean, the Mediterranean Sea (Domingues, Bucciarelli, Almada, & Bernardi, 2005), and the Black Sea (Bilecenoğlu, Kaya, Cihangir, & Çiçek, 2014) which inhabits rocky reefs of shallow waters in small flocks (Jardas, 1996). It is also reported as the most abundant fish species in the rocky littoral environment of the Mediterranean Sea (Pinnegar, 2018).

C. chromis reach sexual maturity at 6.8-7.3 cm (Total length) and fecundity increases with the age (Dulcic & Kraljevic, 1994). Age, growth (Dulcic & Kraljevic, 1995), sex-ratio, and body condition (Bracciali, Piovano, Sarà, & Giacoma, 2014) were previously described. The absence of sexual dimorphism was reported in most of the species of genus *Chromis* (Calado, Olivotto, Oliver, & Holt,

2017) and spawning takes place in summer (Dulcic & Kraljevic, 1994). Despite having no commercial value, *C. chromis* is represented as a key species in a rocky littoral environment (Pinnegar, 2018). Previous studies suggested *C. chromis* as an indicator species for human disturbances (Bracciali, Campobello, Giacoma, & Sarà, 2012), sewage contamination (Azzurro et al., 2010), and fishing pressure on other species (Kruschel, Schultz, Bakran-Petricioli, & Petricioli, 2012; Pinnegar, 2018). Due to their ecological importance, *C. chromis* was subjected to numerous scientific investigations including biology of the species (Dulcic, 2005; Dulcic & Kraljevic, 1995), morphologic characterizations (Dulcic, 2005; Kapiris & Klaoudatos, 2011), and phylogenetic studies (Cooper et al., 2009; Domingues et al., 2005). However, there are no studies except the faunal studies (Bilecenoğlu et al., 2014) in the Black Sea. In this study, morphometric, meristic, and mtDNA-based genetic characters of *C. chromis* were characterized. This study is important for being the first genetic characterization study carried out in the Black Sea.

MATERIAL and METHODS

A total of 112 *C. chromis* specimens (59 female and 53 male) from the southeastern Black Sea (Coasts of Ordu) (Figure 1) were morphologically and genetically analyzed. *C. chromis* specimens were monthly sampled from discard fish caught by commercial trammel nets between January and December 2018.
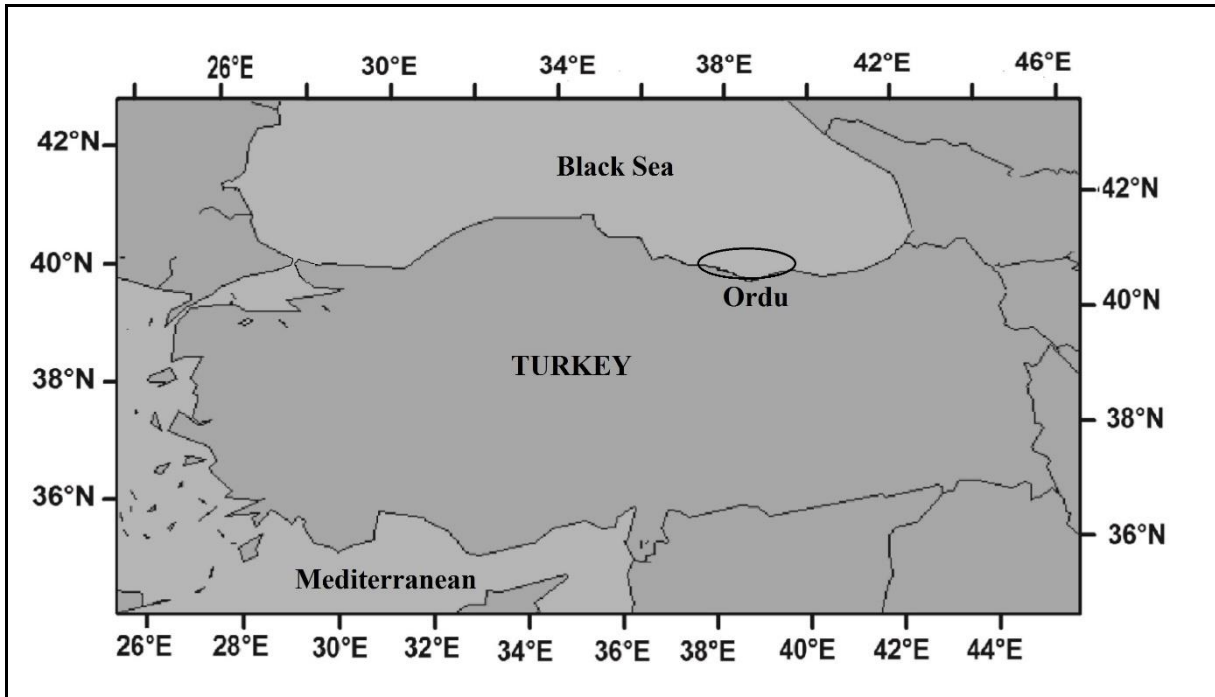

Figure 1. Sampling area of *C. chromis* in the Black Sea

All length measurements were made using a digital compass with 0.01 mm sensitivity and weight measurements were taken with an electronic scale with a sensitivity of 0.01 g. Forty-four specimens of different sizes were used for metric and meristic measurements and these measurements were performed while fish were fresh. There were no differences in metric and meristic characters between sexes. Therefore, morphometric characters were measured without separating sex. All morphometric characters used in this study are schematically presented in Figure 2.
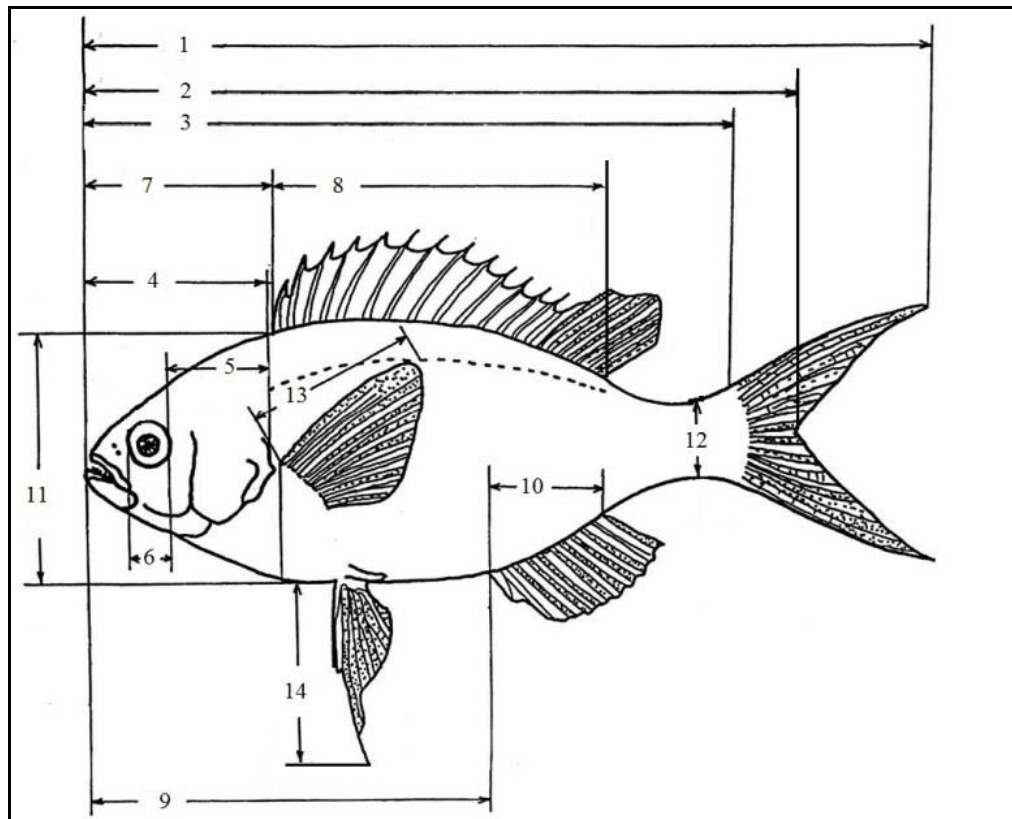
Figure 2. Overview of the morphometric measurements used in this study (modified from Dulcic, 2005) (1:total length, 2:fork length, 3: standard length, 4: head length, 5: post orbital length, 6: eye diameter, 7: predorsal fin distance, 8: dorsal fin base length, 9: preanal fin distance, 10: anal fin base length, 11: body depth, 12: minimal caudal peduncle depth, 13: pectoral fin length, 14: ventral fin length).

Total length and weight relations were calculated according to the exponential equation $W = aTL^b$, where W is the total weight of the fish (g), TL is the total length (mm), *a* and *b* are the parameters of the equation (Bagenal & Tesch, 1978). The t-test employed to test whether the slopes (b) were significantly different from 3, indicating the growth type: isometric (b=3), positive allometric (b>3), or negative allometric (b<3) (Pauly, 1986).

Molecular Identification and Characterization

Molecular identification and characterization of the *C. chromis* specimens (n=5) were conducted using universal primer sets that amplify the 16S rRNA and COI gene region of the mitochondrial DNA. Genomic DNA was extracted from fin tissue using Wizard SV Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's instructions. PCR amplification was performed using WizPure Pfu 2x Master Mix (Wizbiosolutions, Korea) in a total volume of 25 µl containing 12.5 µl 2X PCR Master Mix, 1 µM of each primer, 100 ng template DNA, and water. The universal primers of 16sbr-H (5'- CCG GTC TGA ACT CAG ATC ACG T -3') and 16sar-L (5'-CGC CTG TTT ATC AAA AAC AT-3') (Palumbi, 1996) were used to amplify the 16S rRNA gene. The PCR protocol is as follows; Initial denaturation at 95 ºC for 5 min followed by 35 cycles of denaturation at 95ºC for 30s, annealing at 54ºC for 30s, extension at 72ºC for 30 s followed by a final extension at 72ºC for 5 min. The universal primers of FishF1 (5′- TCA ACC AAC CAC AAA GAC ATT GGC AC-3′) and FishR2 (5′-ACT TCA GGG TGA CCG AAG AAT CAG AA -3′) (Ward et al. 2005) were used to amplify cytochrome c oxidase subunit 1 (CO1) gene. The PCR protocol is as follows; Initial denaturation at 95ºC for 5 min followed by 35 cycles of denaturation at 95ºC for 30s, annealing at 49ºC for 40s, extension at 72ºC for 40 s followed by a final extension at 72ºC for 5 min. PCR products were visualized on 1% agarose gel and sequenced on ABI PRISM 3730x1 genetic analyzer (Applied biosystem) using BigDye Terminator 3.1 cycle sequencing kit at Macrogen Inc.

Generated raw sequences were trimmed and aligned using ClustalW algorithm (Thompson, Higgins, & Gibson, 1994) in BioEdit v. 7.0.5.3 (Hall, 1999). Reference 16S rRNA sequence data for *Chromis chromis* (EF489733, EF489734, EF489735, EF489731, EF489732, FJ616425), *C. atrilobat*a

(EF489728, EF489729, EF489730), *C. multilineata* (EF489727) *C. fumea* (KC767733), *C. albicauda* (KC767731), *C. xanthura* (JF457405), and *C. mirationis* (KF957467); reference COI sequence data for *Chromis chromis* (KT003887, KF564300), *Chromis cadenati* (CQ341589), *Chromis alta* (GU440280), *Chromis degruyi* (EU358588), *Chromis earina* (AB689677), and *Chromis bowesi* (MH170487) were obtained from NCBI GenBank database. *Altrichthys curatus* (COI: KY963993; 16S rRNA: FJ616408) and *Acanthochromis polyacanthus* (COI: KP194731; 16S rRNA: FJ616407) were selected as outgroups. Genetic distances were calculated in MEGA X (Kumar et al. 2018) using the Kimura two−parameter model (K2P) (Kimura, 1980) with 1000 bootstrap replications. The best fit nucleotide substitution model was determined based on the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). Phylogenetic trees were generated with the maximum likelihood method using Kimura 2 parameter with 5000 randomized bootstrap iterations.

RESULTS

There was no difference in terms of morphometric and meristic characters between male and female specimens. Thus, morphological data were analyzed as a whole. The mean length of 112 specimens in the study was determined as 99.54 ± 11.49 mm (min: 72.0−max: 115.1 mm) and the average weight was determined as 18.35 ± 5.95 g (min: 5.96−max: 26.56 g). Detailed morphometric characteristics and measurements were given in Table 1.

Standard length/total length ratio (TL%) was calculated as 74.83 ± 1.38% (min: 72.58−max: 78). The ratio between head length and standard length is 29.51 ± 0.83% (min: 28.26−max: 31.29). Almost 90% of the 112 specimens consist of individuals within the length group of 9−10 cm (Figure 3).

Table 1. Some metric and meristic properties of *C. chromis* from the Black Sea.

| Measurements	Number	Mean+SE	Minimum	Maximum	TL%
Total length (mm)	44	99.85 ± 9.14	72.00	115.10	---
Fork length (mm)	44	82.85 ± 7.62	57.38	95.29	82.98
Standard length (mm)	44	74.57 ± 6.65	53.60	83.93	74.77
Weight (g)	44	18.23 ± 4.92	5.96	26.56	18.43
Body depth (mm)	44	31.54 ± 4.59	21.00	38.66	32.37
Head length (mm)	44	21.66 ± 2.12	15.58	25.59	22.08
Post orbital length (mm)	44	9.51 ± 1.15	5.83	11.58	9.66
Eye diameter (mm)	44	7.39 ± 1.06	4.71	8.80	7.49
Predorsal fin distance (mm)	44	28.69 ± 2.45	21.18	31.78	28.69
Dorsal fin base length (mm)	44	41.79 ± 4.59	28.19	49.21	41.94
Preanal fin distance (mm)	44	49.09 ± 5.51	35.13	55.78	49.43
Anal fin base length (mm)	44	14.76 ± 1.61	11.75	19.21	15.10
Minimal caudal peduncle depth (mm)	44	10.02 ± 0.97	7.26	11.55	10.08
Pectoral fin length (mm)	44	22.62 ± 2.82	16.66	28.31	23.10
Ventral fin length (mm)	44	22.13 ± 2.47	15.96	26.95	22.52
Meristic characters					
Dorsal fin	XIV/9-10				
Anal fin	II/9-11				
Pectoral	17				
Ventral	I/5				
Caudal	23				
Lateral line	26				
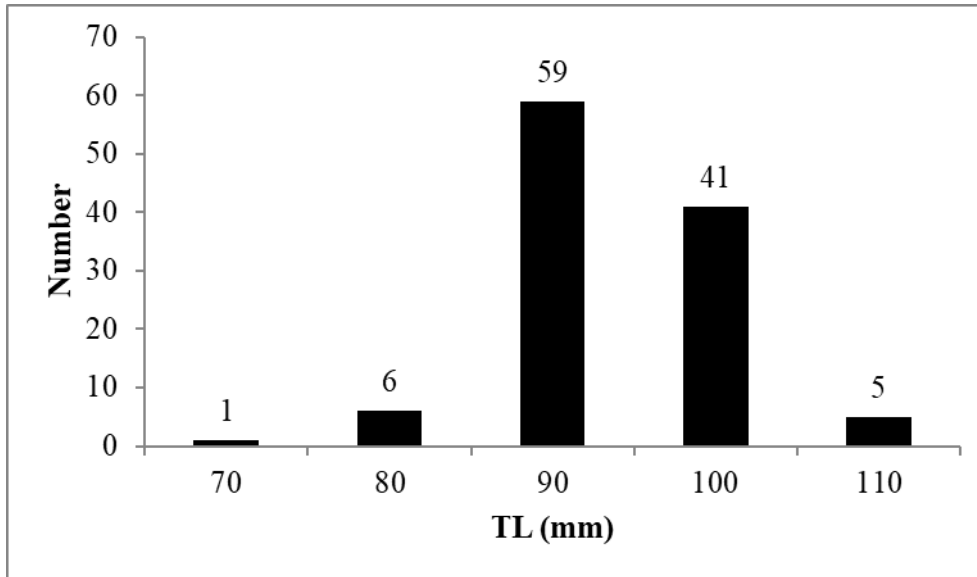
Figure 3. Length frequency distribution of *C. chromis* from the Black Sea

The TL-W relationship between the sampled individuals was presented in Figure 4. The "b" value in this study indicated a positive allometric growth (b=3.12) (p<0.05) (Table 2).

Table 1. Regression parameters of the TL-W relationship ($W=aTL^b$) of *C. chromis* from the Black Sea

N	a	b	SE(b)	CI(b)	R^2	Pauly t-test	P
112	0.0127	3.1172	0.132	2.854-3.379	0.8341	t = 0.884	P<0.05

N: number, *a* and *b:* intercept and slope of the regression line *SE*: standard error, *R^2*: coefficient of determination, *CI*: 95% confidence interval
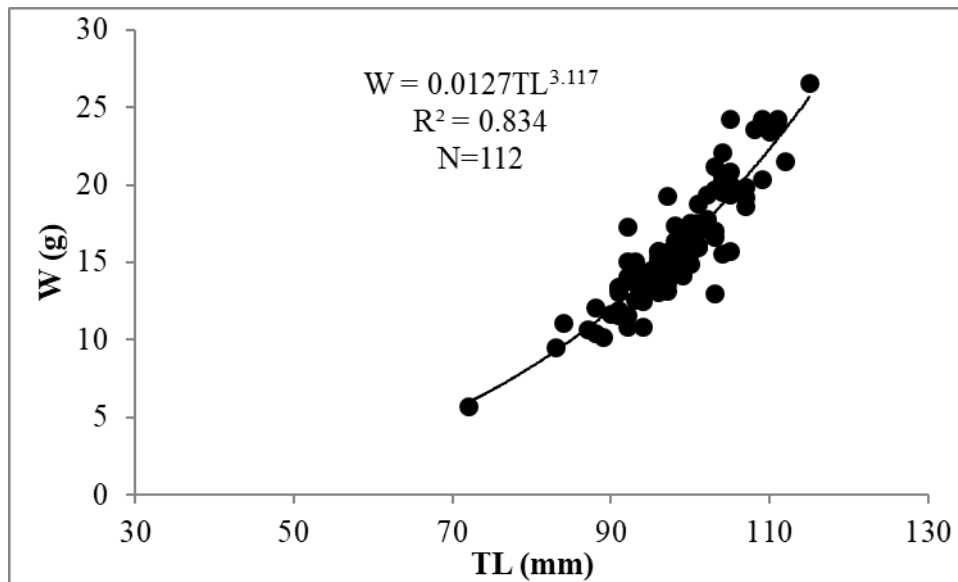

Figure 4. Length-weight relationship of *C. chromis* from the Black Sea

Molecular Identification and Characterization

DNA extracted from each specimen were successfully amplified, sequenced, and analyzed. Generated COI (a single haplotype) and 16S rRNA sequences (two haplotypes; H1 and H2) were submitted to GenBank database (accession numbers: MN401300, MN401301 and MN528744) Sequences of partial COI and 16S rRNA genes were 606 and 587 bp long, respectively. BLAST provided a species match with higher than 99% sequence similarity for 16S rRNA gene, 99.83% and 100 % sequence similarity for COI gene. Neighbor–joining trees generated based on 16S rRNA and

COI sequences produced well-defined clusters with available reference sequences (Figure 5-6). Specimens were clustered with reference taxa in the same manner as the BLAST results. Analysis of 606 bp of COI sequences showed that our specimens are closely related to *C. chromis* sampled from the Adriatic Sea and the Levantine Sea with low genetic distance (d=0.001). On the other hand, analysis of 516 bp of 16S rRNA sequences revealed that genetic distance between our specimens and *C. chromis* specimens sampled from Aegean and the Tyrrhenian Sea is lower than 0.002.
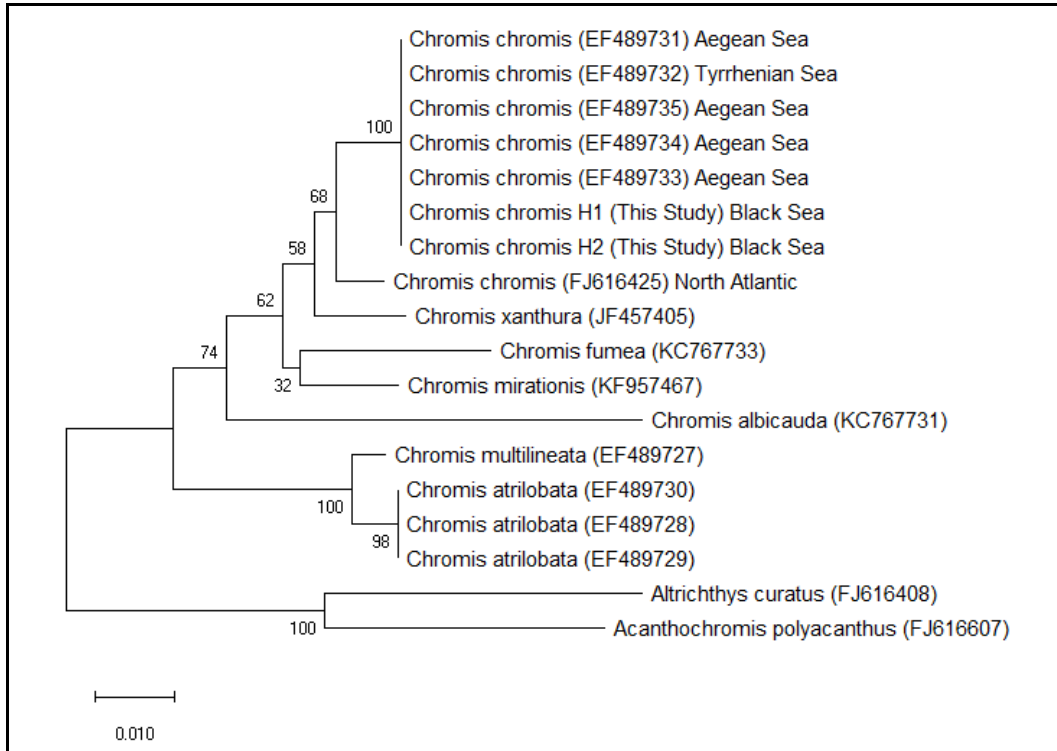

Figure 5. Neighbor–joining tree constructed by the 16S rRNA sequences of *C. chromis* with reference sequences of specimens belonging to genus *Chromis* and outgroups.
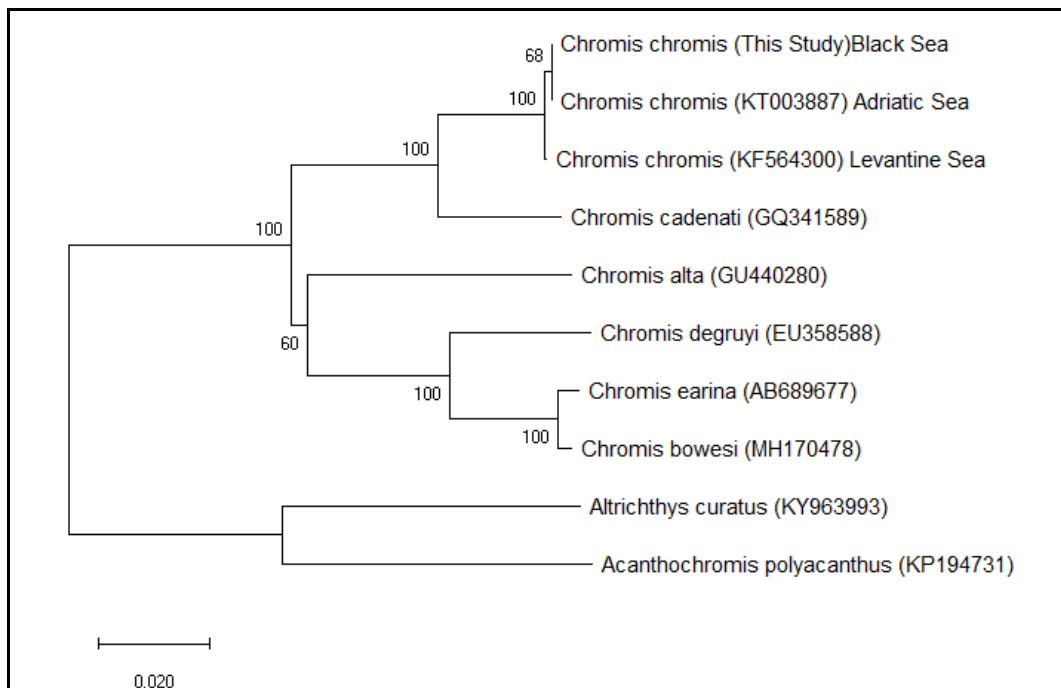

Figure 1. Neighbor-joining tree constructed by the COI sequences of *C. chromis* with reference sequences of specimens belonging to genus *Chromis* and outgroups

DISCUSSION

In the present study, biometric properties and mtDNA based genetic characters of *C. chromis* sampled from the southern Black Sea were determined. The damselfish population of the sampled region is relatively homogeneous and morphological characters appeared to be similar between sexes. The total length of the specimens was varied between 72 mm and 115 mm with an average length of 99.54±11.49 mm. Similarly, Dulcic (2005) reported similar length and weight data for *C. chromis* population in the Adriatic Sea. Whereas Duka and Shevchenko (1980) carried out the study on the morphology of *C. chromis* in which the length and weight range of the species was given as 61−120 mm, 3.0−27.0 g for the Mediterranean Sea population and 7-100 mm and 6.0-17.7 g for the Black Sea population. The mean standard length of males and females of the *C. chromis* sampled from the central Mediterranean Sea were reported as 66.5 mm and 62.3 mm, respectively (Bracciali et al., 2014). Dulcic and Kraljevic (1995) determined the length ranges of 40 mm−134 mm, the weight ranges of 3.24 g−40.21 g in the Adriatic Sea. In this study, length and weight values were measured as 72.0−115.1 mm and 5.96−26.56, respectively. According to the results obtained in this study, it can be said that the Black Sea population composes of comparatively smaller individuals. Ecological differences, environmental conditions, and nutritional differences in the environment might be one of the reasons behind morphological differences. The "b" values (3.08 - 3.12) calculated by Dulcic and Kraljevic (1995) and ''b" value obtained in this study (b = 3.11) were similar. Positive allometric growth was found in both studies. Duka and Shevchenko (1980) calculated this value as 2.945 in the Mediterranean Sea and 2.867 in the Black Sea and they stated that there is an isometric growth. These differences are also closely related to gender, length, age, and reproductive period. *C. chromis* reach sexual maturity at 68-73 mm in length. In the present study, the length of individuals ranged from 72 mm to 115.1 mm. Therefore, it can be said that analyzed specimens in the present study were possibly sexually mature.

The ratio between total and standard length was found as 75.0−76.9% in the Azores (Re & Gomes, 1982) and 72.58−75.83% in the Adriatic Sea (Dulcic, 2005). Similarly, the ratio between total and standard length was determined as 74.77% in this study. Based on these ratios (body proportions), both studies have similar values. The ratio between head length/standard length is calculated as 30.0−32.5% by Banarescu (1964), as 27−27.4% by Duka and Shevchenko (1980), 30.17−34.06% by Dulcic (2005). In this study, this ratio was found similar (29.53). In several studies, meristic characters were found as D XIV / 10, A II / 10, P 17, V I / 5 (Dulcic, 2005), D XIV / 9-11, A II / 10-11 (Svetovidov, 1964), D XIV / 10-11 and A II / 10-12, P17, V I / 5 (Bini, 1967), D XIV / 10, A II / 10, P 18 (Arruda, 1977) and, D XIII-XIV / 10-11, A II / 10-11by (Quingnard & Pras, 1986). The meristic characters of the *C. chromis* analyzed in the present study were compared with data generated in previous studies (Table 3).

Table 3. Meristic characters of damselfish from the Adriatic, Mediterranean, and Black Seas

Author	Dorsal fin	Anal fin	Pectoral	Ventral	Lateral line	Location
Dulcic, (2005)	XIV / 10	II / 10	17	I / 5	27	Adriatic
Bini, (1967)	XIV/10-11	II/10-12	17	I/5	24-30	Mediterranean
Tortonese, (1975)	XIV/10-11	II/10-12			18-19	Mediterranean
Wood, (1977)	XIV/10	II/10	18(17)		19	Mediterranean
Arruda, (1977)	XIV / 10	II /10	18	---	25	Mediterranean
Quingnard & Pras, (1986)	XIII-XIV/ 10-11	II/10-11			24-30	Mediterranean
Banarescu,(1964)	XIV/9-10	II/10-11	I/15-16	I/5	26-29	Black Sea
Svetovidov, (1964)	XIV/9-11	II/10-11	---	---	19	Black Sea
This study	XIV/9-10	II/9-11	17	I/5	26	Black Sea

Chromis is a diverse genus of Pomacentridae represented more than 100 species. Systematic status and genetic diversification of damselfish including genus *Chromis* are well documented (Cooper et al., 2009; Hubert et al. 2011). *C. chromis* is the only representative species of the genus *Chromis* in the Black Sea and only a handful of genetic studies are available for the species worldwide. In the present study, *C. chromis* sampled from the Southeastern Black Sea were genetically characterized based on two mtDNA gene regions. *C. chromis* specimens sampled from the Black Sea appeared to be closely related to *C. chromis* specimens sampled from the Aegean Sea, the Levantine Sea, the Tyrrhenian Sea, and the Adriatic Sea. This pattern is congruent with the phenotypic similarity found among different

geographic locations. Phylogenetic analyses indicate that genus *Chromis* is polyphyletic consisting of 2 major clades. Domingues et al. (2005) evaluated population structure and gene flow in *C. chromis* within the Mediterranean basin. While high levels of gene flow were detected between the Atlantic Ocean and the Mediterranean Sea, gene flow between eastern and western Mediterranean Sea was comparatively lower. Whereas lack of genetic divergence between the Black Sea specimens and available reference sequences from different parts of the Mediterranean Sea, including the Aegean Sea and the Levantine Sea, indicates genetic homogeneity and possible gene flow between populations. It is worth mentioning that the reference 16S rRNA and COI sequences of *C. chromis* are limited which restricts location-based genetic comparisons. Despite being one of the most abundant fish species, there is no study on the biology or genetic properties of *C. chromis* is the Black Sea possibly for not being economically valuable. This study is important for being the first study focused on the length-weight relationship, biometric, and genetics properties of the Black Sea *C. chromis* population. 16S rRNA and COI sequence data were generated for the first time from the Black Sea which would be useful for the population and phylogenetic studies.

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