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Genetic characterization of the invasive Blue crab, *Callinectes sapidus* (Rathbun, 1896), in the Black Sea

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ABSTRACT

Blue crab, *Callinectes sapidus* (Rathbun, 1896), is a commercially important crab species native to the Western Atlantic Ocean. *Callinectes sapidus* has invaded the Mediterranean Sea in 1930s and spread rapidly. It has become widely distributed and has been moving through adjacent basins including the Black Sea. In this study, mitochondrial 16S rRNA and COI sequences were generated for individuals captured from the Black Sea for the genetic characterization of the population for the first time. Additionally, available sequences of *C. sapidus* from their native and introduced populations were included in the analysis to evaluate phylogenetic affinities and the most probable origin of the population in the Black Sea. The Black Sea specimens were clustered with the sequences from the Northwest Atlantic Ocean on the basis of 16S rRNA sequence analysis and with those from the Northwest Atlantic Ocean and the Levantine Sea on the basis of COI sequence analysis. The Caribbean Sea and the Southwest Atlantic Ocean sequences were clustered together. Notable genetic divergence was observed between the populations of the Black Sea and the Levantine Sea. The present *C. sapidus* population in the Black Sea is most probably a result of the migration from the Aegean Sea (Saros Bay), where *C. sapidus* has established a stable, reproductively successful population. Patterns of the haplotype diversity and maximum likelihood trees indicate that the invasive population of *C. sapidus* in the Black Sea possibly originated from the Northwest Atlantic Ocean. Genetic divergence between populations of the Black Sea and the Levantine Sea were attributed to the limited sample size from the Black Sea.

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1. Introduction

Blue crab, *Callinectes sapidus* (Rathbun, 1896), is a recreationally and commercially important crab species (Mancinelli et al., 2017a) that inhabits muddy and sandy bottoms mainly in the shallows and in depths up to 90 m (Nehring, 2011). The blue crabs are important predators in benthic communities which affect the diversity and structure (Mancinelli et al., 2017b). *Callinectes sapidus*, characterized as an opportunistic benthic omnivore, eats whatever is available in the habitat (Kampouris et al., 2019; Mancinelli et al., 2017c, 2016).

The native range of *C. sapidus* covers the Western Atlantic extending down to Paraguay, abundant from the Massachusetts Bay to the Gulf of Mexico (Hill et al., 1989; Nehring, 2011). Disjointed populations of the species sprawl in USA south to Venezuela and Brazil to Argentina (Windsor et al., 2019). *Callinectes sapidus* has long been regarded as an invasive alien species for the Eastern Atlantic Ocean and Mediterranean Sea (Zenetos et al., 2005). The first record of the *C. sapidus* in Europe dates back to early 20th century on the Atlantic coast of France (Bouvier, 1901). In the following years *C. sapidus* specimens were reported from the Mediterranean Sea (Giordani Soika, 1951), the Aegean Sea (Kocatas, 1971), and the Black Sea (Bulgurkov, 1968). According to Artüz (1990) *C. sapidus* was intentionally introduced to the northern Aegean Sea, especially to the Gulf of Saros (Turkey) and the Gulf of Thessaloniki (Greece) between 1935–1945. A survey study among the fisherman revealed that the occurrence of the species in the Gulf of Thessaloniki goes back to 1935 (Georgiadis and Georgiadis, 1974) which is in accordance with (Artüz, 1990). The first report of the species in the Mediterranean Sea is 1949 (Giordani Soika, 1951), however it is assumed that the introduction of the species was early 1930's (Enzenross et al., 1997). Although the specimens of *C. sapidus* were reported since early 20th century, it is unclear how

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the species had arrived in European seas. This has been a matter of debate since the first record (Bouvier, 1901). The abundance in the major shipping routes and the ports where the ballast waters were discharged nearby caused the consideration of the ballast tanks as the primary pathway of introduction (Wolff, 1954; Holthuis and Gottlieb, 1955). Also three living specimens were reported in ballast water tanks by Gollasch (1996). On the other hand the species could be introduced intentionally or accidentally from the holding tanks since they had been imported alive for human consumption (ICES WGITMO, 2006). Somehow, *C. sapidus* is now distributed abundantly in the Mediterranean Sea and the Aegean Sea and their colonized populations form an important fishery resource (Mancinelli et al., 2017a; Kevrekidis et al., 2013; Kampouris et al., 2019). The exploitation and management of the established blue crab population started in the early 1990s in the Aegean and the Mediterranean Sea coasts of Turkey (Zaitsev and Ozturk, 2001).

The continental shelf of the Black Sea is very narrow because of the steep coastal morphology (Panin, 2005). Moreover, a hydrogen sulfide layer limits the aerobic life cycle down to approximately 170-meter depth (Jørgensen et al., 1991). The limited habitat of the Black Sea is very fragile and can be easily affected by the intruders. Anthropogenic activities have led to an enormous displacement of biota beyond their natural barriers leading to the establishment of invasive populations. Introduction of the invasive species into the Black Sea is accelerating at an unprecedented rate, effecting the biodiversity adversely and have became an environmental issue. Invasive species are causing ecological changes at different scales given their ability to cause habitat destruction, biodiversity decline, and known and unknown ecosystem changes. Previous experiences have shown that native species are affected by invasive species (Khvorov, 2010). For instance, *Rapana venosa* predation was identified as the key reason for the decline of commercially valuable *Mytilus galloprovincialis* in the Black Sea coast (Snigirov et al., 2013). The majority of the *C. sapidus* records in the Black Sea are recent episodic catches (Fig. 1) in which numbers of the collected specimens were limited. There are three possible ways of the introduction of *C. sapidus* to the Black Sea; (i) accidental introduction of *C. sapidus* larvae via ship ballast waters, (ii) intentional release, because of commercial value and, (iii) migration through the straits. Considering their ability to swim long distances with their paddle legs, *C. sapidus* is able to migrate from the Aegean Sea, the Gulf of Saros, where *C. sapidus* formed a commercially exploited and well established population. Invasion success in marine environment depends on the characteristics of the species, environmental parameters of the recipient environment, propagule pressure, and biotic interactions (Chan and Briski, 2017). *Callinectes sapidus* is an extremely euryhaline and eurythermal crab species (Guerin and Stickle, 1992) which enables them to occupy seas, brackish coastal lagoons, estuaries, and freshwater. Moreover, large body size, aggressive behavior (Reichmuth et al., 2011), high fecundity (Mancinelli et al., 2017a), and the ability of swimming long distances with paddle shaped legs (Carr et al., 2004) are ecological and biological determinants of its invasive success. Like all invasive species, *C. sapidus* not only has negative effects on the human activities like mutilating fish caught in traps and tearing nets but also has negative impact on benthic communities. Thus, the early identification of patterns and mechanisms of such invasions is very important to understand the risk level and develop strategies to manage or reduce the affects for the future (Pyšek and Richardson, 2010).

Understanding the origin of an introduced species is important for management and control of the invasive species (Bushar et al., 2015). Molecular markers have proven to be useful tools for genetic characterization of the invasive species, inferring the possible origin of the populations, and the pathways of range expansion (Sanz et al., 2013; Peñarrubia et al., 2016). May et al. (2008) revealed that introduced zebra mussel (*Dreissena polymoprha*) populations in North America and Europe were derived from the Caspian Sea basin based on COI analysis. Brown and Stepien (2009) determined the origin of round goby (*Neogobius melanostomu*) invasion based on mitochondrial cytochrome b gene sequence and nuclear microsatellite marker analysis. Mitochondrial COI gene has proven to be useful for determination of the geographic structure and species identification in decapods (Fratini and Vannini, 2002; Dudoit et al., 2018; Windsor et al., 2019). Moreover, Windsor et al. (2019) suggested that COI might be useful for detecting the geographic origin of *C. sapidus* based on the 3.34% mean distance found between Venezuelan and USA specimens.

The objective of this study was to genetically characterize *C. sapidus* specimens captured in the Black Sea by using mitochondrial DNA markers of COI and 16S rRNA. Additionally, sequences of *C. sapidus* from their native and introduced populations were included in the analysis to evaluate phylogenetic affinities and geographic origin of the invasion in the Black Sea.

2. Materials and methods

2.1. Specimens

In the present study, a total of six *C. sapidus* specimens, unintentionally captured by trammel nets off the coast of Ordu ($n = 5$) and Trabzon ($n = 1$) between 2017 and 2019, were used (Fig. 1). The external morphology of the specimens (Fig. 2) was characterized by color and carapace measurements. Carapace length (CL) and width (CW) including tips of the lateral spines were 189–201 mm (194.3 ± 4.03 mm) and 192–198 mm (189.12 ± 3.33 mm), respectively. *Callinectes sapidus* is a sexually dimorphic species. Sex of the specimens was identified based on inverted "T" shaped apron and blue tipped claws. Five of the specimens were identified as male, a single specimen from Ordu was identified as female. The maturity stage was determined according to characteristics described by Van Engel (1990). Specimens were designated as adults by size (>11 mm CW) and the second pleopods lay within the first pleopod. Weights of the specimens were between 269–458 g (406.77 ± 91.68 g).

2.2. DNA analysis

Total DNA was extracted from 20 mg of leg muscle tissue with Wizard SV Genomic DNA purification kit (Promega) according to the manufacturer's protocol. Polymerase chain reactions were performed in a total volume of 50 μl containing 10 μl of 5X Hot-FIREPol Blend Master Mix (Solis, Biodye), 10 μM each primer, 100–150 ng of DNA and nuclease-free water. The universal primers of LCO1490 (5′ GGT CAA CAA ATC ATA AAG ATA TTG G 3′) and HCO2198 (5′ TAA ACT TCA GGG TGA CCA AAA AAT CA 3′) were used to amplify the cytochrome oxidase subunit 1 gene (COI) (Flomer, 1994). Amplifications were carried out with the following protocol; initial denaturation at 94 °C for 12 min, followed by 30 cycles of denaturation at 95 °C for 50 s, annealing at 54 °C for 30 s, extension at 72 °C for 30 s and a final extension at 72 °C for 5 min. 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′) were used to amplify 16S rRNA gene (Palumbi, 1996). Amplifications were carried out with the following protocol; initial denaturation at 94 °C for 12 min, followed by 30 cycles of denaturation at 95 °C for 50 s, annealing at 49 °C for 40 s, extension at 72 °C for 40 s and a final extension at 72 °C for 5 min. An aliquot (2 μl) of each PCR product was

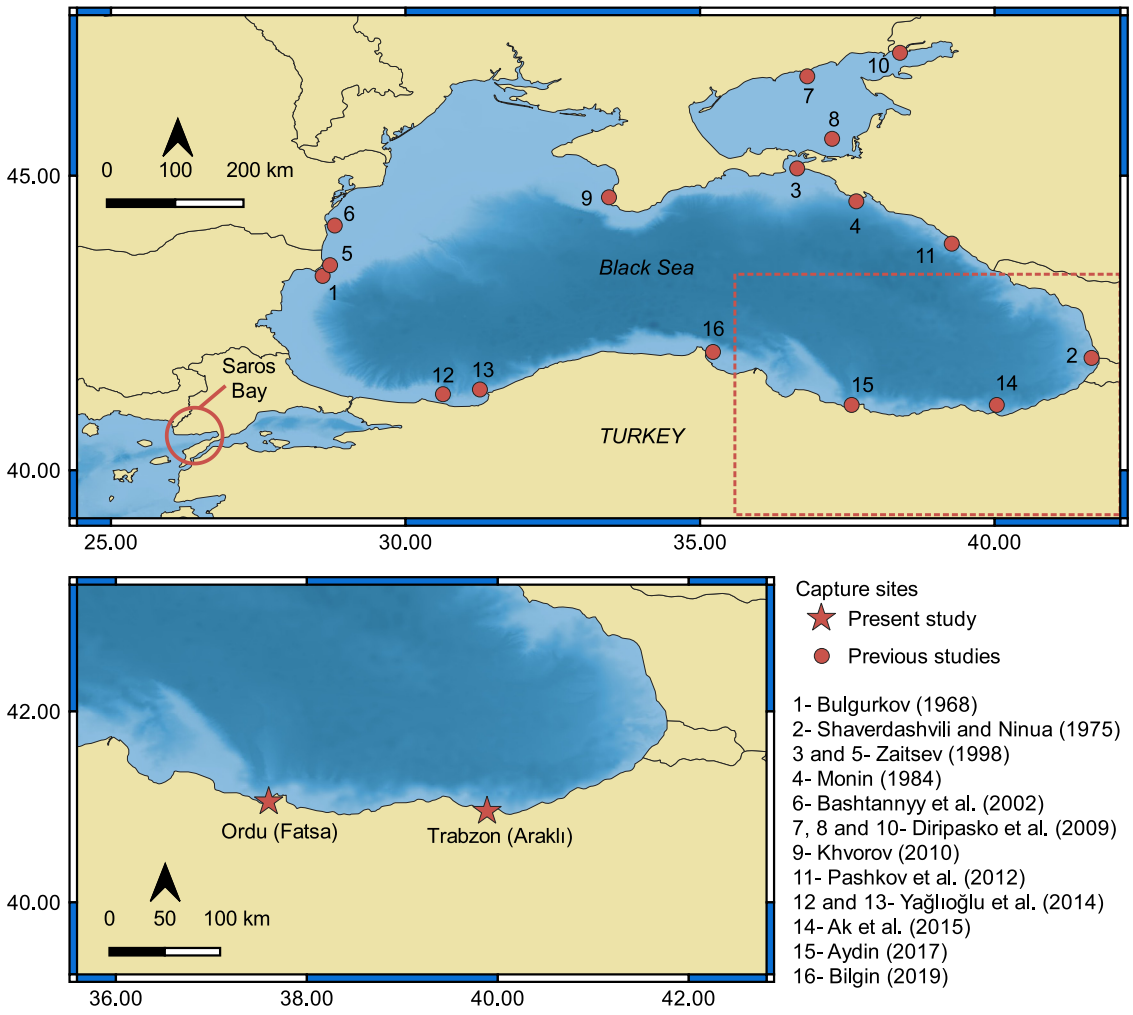


Fig. 1. Capture sites of *C. sapidus* specimens in the Black Sea (Bulgurkov, 1968; Shaverdashvili and Ninua, 1975; Monin, 1984; Zaitsev, 1998; Bashtannyy et al., 2002; Diripasko et al., 2009; Khvorov, 2010; Pashkov et al., 2012; Yağlıoğlu et al., 2014; Ak et al., 2015; Aydin, 2017; Bilgin, 2019).



Fig. 2. *Callinectes sapidus* specimen captured off the coast of Trabzon, Turkey in early May 2018. Photographs from dorsal and ventral aspects.

analyzed by electrophoresis on a 1% agarose gel stained with Red safe (Intron Bio Inc., South Korea) to confirm amplification quality prior to sequencing. Polymerase chain reaction products were purified with Wizard SV Gel and PCR Clean-Up Kit (Promega) following the manufacturer's protocol. Sequencing was performed on ABI3500 genetic analyzer (Applied Biosystems).

2.3. Data assembly and genetic analyses

Obtained raw sequences were edited and aligned with the reference sequences using BioEdit v. 7.0.5.3 (Hall, 1999). All of the sequences generated in the present study (a total of 6 sequences for each gene region) have been deposited in GenBank (NCBI) under the following accession numbers: 16S rRNA; MN699471 and MN696231, COI; MN759041, MN759042, and MN759043. Additionally, available sequences from the COI and 16S rRNA regions of *C. sapidus* were downloaded from GenBank from four different geographic regions; Northwest Atlantic Ocean (NWA) (Schubart et al., 2001; Sullivan and Neigel, 2017), Southwest Atlantic Ocean (SWA) (Zupolini et al., 2017; Windsor et al., 2019), Caribbean Sea (CS) (Schubart et al., 2001; Windsor et al., 2019), and Levantine Sea (LS) (Keskin and Atar, 2012). These GenBank sequences

were included to the dataset as ingroup terminals in molecular analysis. GenBank accession numbers of *C. sapidus* specimens, their sampling locations, and geographic regions are given in Table 1. *Callinectes danae* (COI: KY940215; 16S rRNA: AJ298185) and *Arenaeus cribrarius* (COI: MH087581; 16S rRNA: DQ407667) were included in the analyses and used as outgroup terminals.

The number of polymorphic sites, number of haplotypes, haplotype diversity, and nucleotide diversity were calculated with DnaSP v. 5.10.01 (Librado and Rozas, 2009). Haplotype networks were generated using PopArt (Leigh and Bryant, 2015). Phylogenetic trees were generated in MEGA with Maximum likelihood method with 1000 randomized bootstrap iterations in Mega X (Kumar et al., 2018). Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were used to determine the best fit nucleotide substitution model (COI: T92+G; 16S rRNA: T92). Pairwise genetic distances between geographic regions were also calculated in Mega X with 1000 bootstrap iterations (Kumar et al., 2018). Genetic variation between geographic regions were further evaluated with hierarchical analysis of molecular variance (AMOVA) in ARLEQUIN v.3.5 (Excoffier and Lischer, 2010). Significance of fixation indices were assessed with 10,000 permutations.

3. Results

Six partial 16S rRNA gene sequences in a length of 497 base pairs were generated from the *C. sapidus* specimens collected from the Black Sea (Trabzon and Ordu). A total of 12 variable sites with six parsimony informative sites were identified (Table S1). Haplotype assignment with the fifteen reference alignment sequences yielded 8 haplotypes with one haplotype (H1) shared by 12 individuals and 7 singleton haplotypes (Table S1). The haplotype diversity and nucleotide diversity were calculated as 0.6017 and 0.01454, respectively. Black Sea specimens ($n = 6$) were clustered within a single haplotype (H1). The haplotype network is given in Fig. 3b. Haplotype one (H1) was shared with the Black Sea and the Northwest Atlantic Ocean specimens. The phylogenetic tree constructed with the reference 16S rRNA gene sequences of the *C. sapidus* specimens in GenBank and with outgroups (*Callinectes danae* and *Arenaeus cribrarius*) revealed presence of two major clades; one consisted of the specimens from the Caribbean Sea (CS) and the other one consisted of the Black Sea (BS), the Northwest Atlantic Ocean (NWA), and the Southwest Atlantic Ocean (SWA) specimens. There was no available 16S rRNA sequence of *C. sapidus* from the Levantine Sea. The Black Sea specimens appeared to be more closely related to NWA sequences (Fig. 3a). Pairwise genetic difference between geographic regions were between 0.25%–1.46% (Table 2). Pairwise genetic distances between the Black Sea and the Northwest Atlantic Ocean was calculated as 0.25%. Based on the analysis of molecular variance, percentage of variation between the Black Sea and other geographic regions were insignificant and varied between −63.33 and 9.02% (Table 2). Negative values of variance indicates absence of genetic differentiation.

Six partial COI gene sequences in a length of 661 base pairs were generated from the *C. sapidus* specimens collected from the Black Sea (Trabzon and Ordu). A total of 67 variable sites with 44 parsimony informative sites were identified (Table S2). Haplotype assignment with the reference 28 alignment sequences yielded 27 haplotypes with 2 haplotypes shared by 3 individuals, 2 haplotypes shared by 2 individuals and 23 singleton haplotypes (Table S2). The haplotype diversity and nucleotide diversity were calculated as 0.9857 and 0.02179, respectively. The Black Sea specimens ($n = 6$) were clustered within three haplotypes; with a single haplotype (H1) shared by three individuals, a single haplotype (H2) shared by two individuals and a singleton haplotype (H3). The haplotypes of each geographic region were unique and there were no shared haplotypes (Fig. 4b). The haplotype network revealed two major structures. While the haplotypes from the Black Sea, the Levantine Sea, and the Northwest Atlantic Ocean were clustered together within the first structure, haplotypes from the Southwest Atlantic Ocean and the Caribbean Sea were clustered within the second structure. At least 16 base differences were detected between the haplotypes of these two major clusters (Fig. 4b). Most of the haplotypes had a relatively mixed distribution pattern in each cluster. The phylogenetic tree constructed with the reference COI gene sequences of *C. sapidus* specimens and with outgroups (*C. danae* and *A. cribrarius*) revealed presence of two major clades; first clade was consisted of the mixed specimens from the Black Sea (BS), the Levantine Sea (LS), the Northwest Atlantic Ocean (NWA), the Southwest Atlantic Ocean (SWA), and the Caribbean Sea (CS) and the other one was consisted of some of the specimens from the Levantine Sea (H4, H11, H12). Within the first clade, SWA and CS sequences were clustered together, meanwhile BS, LS, and NWA sequences were clustered together (Fig. 4a). The Black Sea specimens were appeared to be more closely related to LS and NWA sequences. Pairwise genetic difference between BS with LS-NWA was 1.5% and 0.76%, respectively. Pairwise genetic differences between geographic regions ranged between 0.76% and 3.76%. Based on the analysis of molecular variance, percentage of variation between Black Sea and other geographic regions were varied between −72 and 143.23% (Table 2). However, variations were not significant ($p>0.05$). Negative values of variance indicates absence of genetic structure.

4. Discussion

This study represents two mitochondrial gene based genetic characterizations of blue crab, *Callinectes sapidus*, from the Black Sea where its presence is defined as invasive. Furthermore, molecular phylogeny of *C. sapidus* across its distribution range was also presented using reference sequences in GenBank. Ingroup terminal reference specimens included in the present study represent the totality of the available *C. sapidus* sequence.

Distribution of *C. sapidus* in its natural ranges are disjointed and three main distribution ranges are recognized; the Northwest Atlantic Ocean, the Caribbean Sea, and the Southwest Atlantic Ocean (Rodrigues et al., 2017; Windsor et al., 2019). Phylogenetic evaluation of *C. sapidus* based on COI sequence analysis revealed presence of two lineages when the entire disjointed distribution (excluding the Caribbean Sea) was taken in to account; specimens from NWA (USA) and some from Brazil were nested within the first lineage, most of the specimens from SWA (Brazil) were nested within the second lineage (Rodrigues et al., 2017). Pairwise genetic distance between phylogenetic lineages were varied between 1.8–2.7%. Windsor et al. (2019) evaluated the origin of the blue crabs in the US market by analyzing the COI barcode region. Presence of three lineages and high level of genetic variation was detected across the geographic regions. The first lineage was included samples throughout the distribution range including the Caribbean Sea (CS) and Brazil (SWA) but mostly predominated with products from United States (NWA). The second lineage was consisted of the Caribbean Sea (CS) samples and the third lineage was consisted of Brazil (SWA) samples. Mean pairwise distance between lineages varied between 1.35–3.15%. In the present study, the analysis of COI gene sequence of *C. sapidus* with reference sequences formed well-supported clades which are consistent with previous studies. The Southwest Atlantic Ocean and the Caribbean Sea sequences were separated into different clusters. Meanwhile, the Black Sea sequences were nested with the Northwest Atlantic Ocean and the Levantine Sea sequences in another cluster. Similar geographic region-based

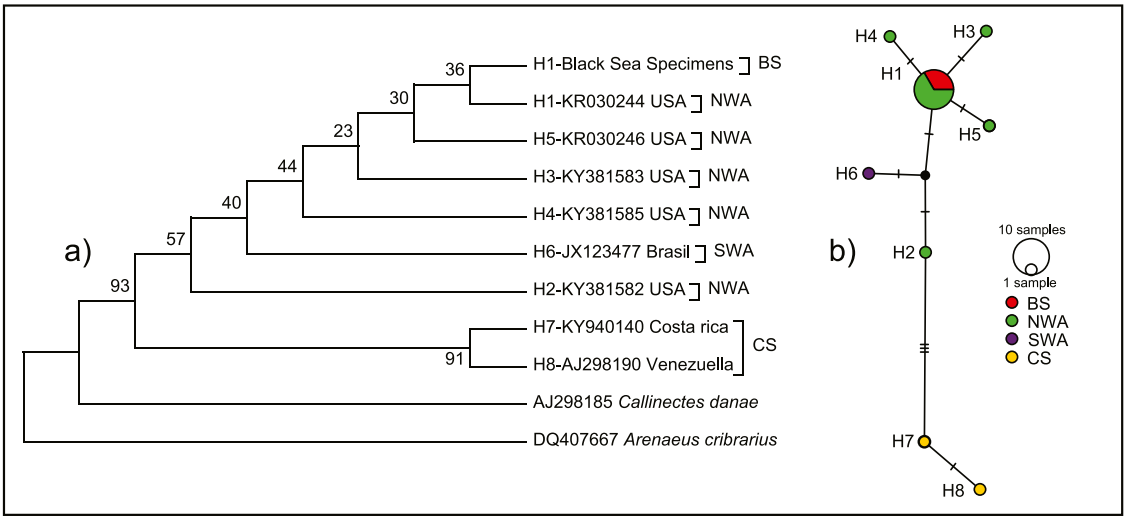


Fig. 3. a. Maximum-likelihood trees based on the 16S rRNA haplotypes of the *Callinectes sapidus* specimens along with their available sequences stored in GenBank and outgroups. The numbers in internal nodes are maximum-likelihood bootstrap support values produced with 1000 replicates. b. Haplotype networks based on the 16S rRNA sequences. Each circle represents a single haplotype, and the size of the circle corresponds to the number of individuals with that haplotype. The number of mutations between the haplotypes are presented with hatch marks and each hatch mark represents a single nucleotide mutation. The black circle represents hypothetical haplotypes automatically generated by POPART software based on the provided data. Number of the haplotypes in each sampling site are provided in Table S1.

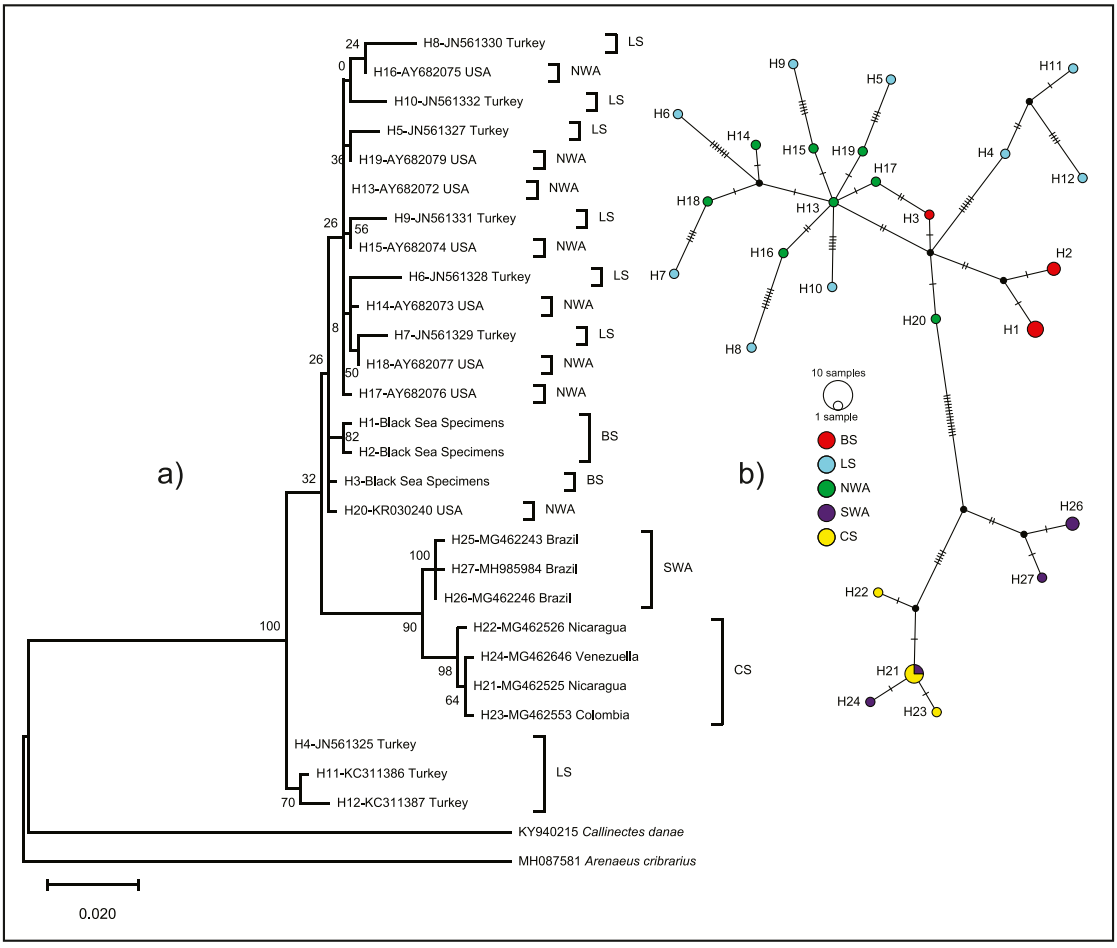


Fig. 4. a. Maximum-likelihood trees based on the COI haplotypes of *Callinectes sapidus* specimens along with their available sequences stored in GenBank. The numbers in internal nodes are maximum-likelihood bootstrap support values produced with 1000 replicates. b. The haplotype networks based on the COI gene sequences. Each circle represents a single haplotype, and the size of the circle corresponds to the number of individuals with that haplotype. The number of mutations between the haplotypes is represented with the hatch marks and each hatch mark represents one nucleotide mutation. The black circles represent hypothetical haplotypes automatically generated by POPART software based on the provided data. The number of haplotypes in each sampling site are provided in Table S2.

Table 1
Frequency distribution of COI and 16S rRNA haplotypes, geographic regions, collection sites, and GenBank accession numbers of *Callinectes sapidus* specimens included in this study. Accession numbers of sequences generated in this study are indicated with an asterisk.

Cytochrome c oxidase subunit I (COI)				16S rRNA			
Acc. Number	H. type	Sampling location	Geo. region	Acc. Number	H. type	Collection site	Geo. region
MN759041*	H1	Turkey: Ordu	BS	MN696231*	H1	Turkey: Ordu	BS
MN759042*	H2	Turkey: Ordu	BS	MN699471*	H1	Turkey: Trabzon	BS
MN759043*	H3	Turkey: Trabzon	BS	KY381582	H2	USA: Galveston	NWA
JN561325	H4	Turkey: Mersin	LS	KY381583	H3	USA: Galveston	NWA
JN561327	H5	Turkey: Mugla	LS	KY381585	H4	USA: Galveston	NWA
JN561328	H6	Turkey: Mugla	LS	KR030247	H1	USA: Maryland	NWA
JN561329	H7	Turkey: Hatay	LS	AJ298189	H1	USA: Florida	NWA
JN561330	H8	Turkey: Mersin	LS	KR030244	H1	USA: Maryland	NWA
JN561331	H9	Turkey: Antalya	LS	KR030245	H1	USA: Maryland	NWA
JN561332	H10	Turkey: Mugla	LS	KR030246	H5	USA: Maryland	NWA
KC311386	H11	Turkey: Antalya	LS	KR030247	H1	USA: Maryland	NWA
KC311387	H12	Turkey: Antalya	LS	KR030248	H1	USA: Maryland	NWA
AY682072	H13	USA: Connecticut	NWA	AJ298189	H1	USA: Florida	NWA
AY682073	H14	USA: Maryland	NWA	AJ130813	H1	USA: Louisiana	NWA
AY682074	H15	USA: Naples	NWA	JX123477	H6	Brazil: San Paulo	SWA
AY682075	H16	USA: PortCharlotte	NWA	KY940140	H7	Costa Rica:Limon	CS
AY682076	H17	USA: Apalachicola	NWA	AJ298190	H8	Venezuela: Zulia	CS
AY682077	H18	USA: O.Springs	NWA				
AY682079	H19	USA: Brownsville	NWA				
KR030240	H20	USA: Maryland	NWA				
MG462525	H21	Nicaragua	CS				
MG462526	H22	Nicaragua	CS				
MG462553	H23	Colombia	CS				
MG462549	H21	Colombia	CS				
MG462646	H24	Venezuela	CS				
MG462375	H21	Porto Rico: San Juan	CS				
MG462243	H25	Brazil: San Paulo	SWA				
MG462246	H26	Brazil: Santa Catarina	SWA				
MG462252	H26	Brazil: San Vicento	SWA				
MH985984	H27	Brazil: San Paulo	SWA				

NWA: Northwest Atlantic Ocean, SWA: Southwest Atlantic Ocean, CS: Caribbean Sea, LS: Levantine Sea, BS: Black Sea.

Table 2
Pairwise genetic divergence (uncorrected) and percentage of the genetic variation based on AMOVA between the *Callinectes sapidus* specimens from different geographic regions based on the mitochondrial 16S rRNA and COI genes.

Geographic regions		Pairwise genetic divergence		Percentage of variation (AMOVA)	
		16S rRNA	COI	16S rRNA	COI
Black Sea	Levantine Sea	–	0.0150	–	−10.46
Black Sea	Northwest Atlantic	0.0025	0.0076	−63.33	−72.02
Black Sea	Southwest Atlantic	0.0050	0.0294	−52.20	143.23
Black Sea	Caribbean Sea	0.0141	0.0355	9.02	122.27
Levantine Sea	Northwest Atlantic	–	0.0132	–	−7.77
Levantine Sea	Southwest Atlantic	–	0.0372	–	339.17
Levantine Sea	Caribbean Sea	–	0.0428	–	248.28
Northwest Atlantic	Southwest Atlantic	0.0066	0.0310	−103.09	126.13
Northwest Atlantic	Caribbean Sea	0.0146	0.0371	92.92	120.48
Southwest Atlantic	Caribbean Sea	0.0141	0.0141	89.72	140,80

clustering was also revealed by haplotype network analysis. Notable genetic divergence between the Black Sea and the Levantine Sea sequences was noted. High genetic diversity between close localities was reported in *C. sapidus* based on mitochondrial (Feng et al., 2017; McMillen-Jackson and Bert, 2004; Williams et al., 2017; Keskin and Atar, 2012) and nuclear markers (Yednock and Neigel, 2014). It is worth mentioning that while the Levantine Sea population is represented with more than 140 individuals (Keskin and Atar, 2012), the Black Sea dataset (present study) is limited and represented with only 6 individuals. Thus, the dataset is insufficient to conclude the magnitude of the divergence between Levantine Sea and Black Sea. Furthermore, there was no shared haplotype between the Levantine Sea and Black Sea sequences. One of the possible reasons behind not having shared haplotypes between these two regions might be the limited sample size from the Black Sea. Keskin and Atar (2012) evaluated the genetic divergence between *C. sapidus* specimens sampled from different localities in the Mediterranean Sea coast of Turkey. Based on COI sequence analysis, the genetic divergence between localities varied between 1.2–2.5% and considerable genetic divergence was reported between geographically close localities. In the present study genetic divergence between the Levantine Sea and Black Sea was found to be 1.5%. The analysis with 16S rRNA did not form well-supported clades. Meanwhile the Caribbean Sea specimens segregated from the rest of the geographic regions. Remaining sequences nested together and the Black Sea sequences appeared to be more closely related to the Northwest Atlantic Ocean sequences. Similar results were found based on haplotype network analysis. Compared to the COI sequence, the number of available 16S rRNA sequences of *C. sapidus* were limited which made data interpretation difficult and explains low resolution.

The adult *C. sapidus* prefers low salinity areas for establishing and the females migrate to estuaries to release larvae (Tankersley and Forward Jr., 2007). Although the Black Sea is suitable for the establishment and reproduction in terms of salinity (16–20%) and the importance of the low salinity for the juveniles is well documented (Posey et al., 2005; Rome et al., 2005; Tankersley and Forward Jr., 2007) no juveniles were found in the studies conducted in the Black Sea. All of the *C. sapidus* specimens

captured in the Black Sea consisted of mature individuals and there were not any ovigerous females among them. Although a positive correlation between salinity and the abundance of ovigerous females was reported, their abundance decreased in relatively low temperatures in spite of high salinity (Rodrigues et al., 2011). In this case, it can be assumed that temperature is the key factor affecting the establishment and reproduction of *C. sapidus* in the Black Sea rather than the salinity. Nehring (2011) also suggests that non-establishment of *C. sapidus* in the Black Sea is more likely because of the low water temperature. We presume that the adult individuals migrated from Saros Bay (a bay located in the Northern Aegean Sea) to the Black Sea where the species is well-established and formed a commercially exploited population. Considering this case, increased sampling in Black Sea and including samples from Aegean Sea could help us to better understand gene flow and possible population growth in depth.

5. Conclusions

In this study a molecular approach was used for the first time to characterize *Callinectes sapidus* in the Black Sea. Moreover, genetic divergence between different geographic regions was evaluated. The Black Sea specimens appeared to be more genetically related to the Northwest Atlantic Ocean population based on mtDNA analysis. High genetic variability was observed between the Levantine Sea and the Black Sea specimens. Considering the changing climate and increasing water temperature, the Black Sea can be a suitable habitat for the *C. sapidus* in the future. Therefore, further studies are needed to understand the lifecycle, growth, feeding position in a food chain and possible impacts or benefits of *C. sapidus* in the Black Sea.

CRediT authorship contribution statement

Rafet Çağrı Öztürk: Conceptualization, Methodology, Investigation, Writing - original draft, Data curation. **Yahya Terzi:** Writing, Visualization, Software. **Ali Muzaffer Feyzioğlu:** Conceptualization, Writing. **Ahmet Şahin:** Sample collection, Methodology. **Mehmet Aydın:** Sample collection.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.rsma.2020.101412.

References

Ak, O., Hasimoglu, A., Bayram, K., 2015. Southeastward expansion of the blue crab *Callinectes sapidus* (Rathbun, 1896) in the Black Sea. Cah. Biol. Mar 56, 397–399.

Artüz, I., 1990. Mavi yengecin serüvenleri. Cumhuriyet Bilim Teknik 148, 6.

Aydin, M., 2017. First record of blue crab *Callinectes sapidus* (Rathbun 1896) from the Middle Black Sea Coast (in Turkish). Turkish Journal of Maritime and Marine Sciences 3 (2), 121–124.

Bashtannyy, R., Webster, L., Raaymakers, S., 2002. First Black Sea conference on ballast water control and management. In: First Black Sea Conference on Ballast Water Control and Management, Odessa, p. 112.

Bilgin, S., 2019. An update list of crab species (Crustacea: Decapoda: Brachyura) with exotic crabs in the Black Sea Fauna (in Turkish). J. Anatol. Environ. Animal Sci. 4 (2), 211–215.

Bouvier, E., 1901. Sur un *Callinectes sapidus* M. Rathbun trouvé à Rochefort. Bull. Mus. Nat. His. Nat. 7, 16–17.

Brown, J.E., Stepien, C.A., 2009. Invasion genetics of the eurasian round goby in North America: Tracing sources and spread patterns. Mol. Ecol. 18, 64–79. http://dx.doi.org/10.1111/j.1365-294X.2008.04014.x.

Bulgurkov, K.I., 1968. *Callinectes sapidus* Rathbun in The Black Sea. Izvest. Niors 9, 97–99.

Bushar, L.M., Reynolds, R.G., Tucker, S., Pace, L.C., Lutterschmidt, W.I., Odum, R.A., Reinert, H.K., 2015. Genetic characterization of an invasive boa constrictor population on the Caribbean Island of Aruba. J. Herpetol. 49 (4), 602–610. http://dx.doi.org/10.1670/14-059.

Carr, S.D., Tankersley, R.A., Hench, J.L., Forward, R.B., Luettich, R.A., 2004. Movement patterns and trajectories of ovigerous blue crabs *Callinectes sapidus* during the spawning migration. Estuar. Coast. Shelf Sci. 60 (4), 567–579. http://dx.doi.org/10.1016/j.ecss.2004.02.012.

Chan, F.T., Briski, E., 2017. An overview of recent research in marine biological invasions. Mar. Biol. 164 (6), 121. http://dx.doi.org/10.1007/s00227-017-3155-4.

Diripasko, O.A., Izergin, L.V., Koshkalda, A.I., 2009. First finds of the blue crab *Callinectes sapidus* (Portunidae, Decapoda) in the Sea of Azov. Vestnik zoologii 43 (6), 529–532.

Dudoit, A., Iacchei, M., Coleman, R.R., Gaither, M.R., Browne, W.E., Bowen, B.W., Toonen, R.J., 2018. The little shrimp that could: Phylogeography of the circumtropical *Stenopus hispidus* (Crustacea: Decapoda), reveals divergent Atlantic and Pacific lineages. PeerJ 6 (1), e4409. http://dx.doi.org/10.7717/peerj.4409.

Enzenross, R., Enzenross, L., Bingel, F., 1997. Occurrence of blue crab, *Callinectes sapidus* (Rathbun, 1896)(Crustacea, Brachyura) on the Turkish Mediterranean and the adjacent aegean coast and its size distribution in the bay of Iskenderun. Turk. J. Zool. 21 (2), 113–122.

Excoffier, L., Lischer, H.E., 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10 (3), 564–567.

Feng, X., Williams, E.P., Place, A.R., 2017. High genetic diversity and implications for determining population structure in the blue crab *Callinectes sapidus*. J. Shellfish Res. 36 (1), 231–242. http://dx.doi.org/10.2983/035.036.0126.

Flomer, O., 1994. DNA Primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotech. 3, 294–299.

Fratini, S., Vannini, M., 2002. Genetic differentiation in the mud crab *Scylla serrata* (Decapoda: Portunidae) within the Indian ocean. J. Exp. Mar. Biol. Ecol. 272 (1), 103–116. http://dx.doi.org/10.1016/S0022-0981(02)00052-7.

Georgiadis, C., Georgiadis, G., 1974. Zur Kenntnis der Crustacea Decapoda des Golfes von Thessaloniki. Crustaceana 26 (3), 239–248. http://dx.doi.org/10.1163/156854074X00631.

Giordani Soika, A., 1951. Il Neptunus pelagicus (L.) nell'Alto Adriatico. Natura 42, 18–20.

Gollasch, S., 1996. Untersuchungen des Arteintrages durch den internationalen Schiffsverkehr unter besonderer Berücksichtigung nichtheimischer Arten. Kovač.

Guerin, J.L., Stickle, W.B., 1992. Effects of salinity gradients on the tolerance and bioenergetics of juvenile blue crabs (*Callinectes sapidus*) from waters of different environmental salinities. Mar. Biol. 114 (3), 391–396. http://dx.doi.org/10.1007/BF00350029.

Hall, T., 1999. BIOEDIT: A User-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. Nucleic Acids Symp. Ser. 41, 95–98.

Hill, J., Fowler, D.L., Avyle, M.J., 1989. Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates (mid-atlantic): Blue crab. In: Biological Reports, vol. 82. pp. 1–13.

Holthuis, L.B., Gottlieb, l.E., 1955. The occurrence of the american blue crab, *Callinectes sapidus* Rathbun. Israel waters. Bull. Res. Counc. Israel B 5, 154–156.

ICES WGITMO, 2006. Report of the Working group on Introductions and Transfers of marine Organisms. ICES CM 2006/ACME:05, International Council for the Exploration of the Sea, The Working Group on Introduction and Transfers of Marine Organisms, Oostende, Belgium.

Jørgensen, B.B., Fossing, H., Wirsen, C.O., Jannasch, H.W., 1991. Sulfide oxidation in the anoxic Black Sea chemocline. Deep Sea Res. A. 38, S1083–S1103. http://dx.doi.org/10.1016/S0198-0149(10)80025-1.

Kampouris, T.E., Porter, J.S., Sanderson, W.G., 2019. *Callinectes sapidus* Rathbun, 1896 (Brachyura: Portunidae): An assessment on its diet and foraging behaviour, Thermaikos Gulf, NW Aegean Sea, Greece: Evidence for ecological and economic impacts. Crustac. Res. 48, 23–37. http://dx.doi.org/10.18353/crustacea.48.0_23.

Keskin, E., Atar, H.H., 2012. Türkiye'nin Akdeniz Kıyısındaki Mavi Yengeç (*Callinectes sapidus*) Populasyonlarını Arasındaki Genetik Farklılığın COI Gen Dizileri Kullanılarak Değerlendirilmesi (in Turkish). J. Fish. Sci. 6 (2), 125–131.

Kevrekidis, K., Antoniadou, C., Avramoglou, K., Efstathiadis, J., Chintiroglou, C., 2013. Population structure of the blue crab *Callinectes sapidus* in Thermaikos Gulf (Methoni Bay). In: Proceedings of the 15th Pan-Hellenic Congress of Ichthyologists, Thessaloniki, Greece. pp. 113–116.

Khvorov, S.A., 2010. Decapods (decapoda). In: Matishov, G., Boltachev, A. (Eds.), Vselentsy v bioraznoobrazii i produktivnosti Azovskogo i Chernogo morei (Invaders in Biodiversity and Productivity of the Sea of Azov and Black Sea). Rostov-na-Donu, YUNC RAN, pp. 69–76.

Kocatas, A., 1971. Izmir korfezi ve civari yengeclerinin brachyura taksonomi ve ekolojisi uzerine arastirmalar. Ege Universitesi Fen Fakultesi Ilmi Raporlar Serisi 121, 1–77.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549. http://dx.doi.org/10.1093/molbev/msy096.

Leigh, J.W., Bryant, D., 2015. POPART: Full-feature software for haplotype network construction. Methods Ecol. Evol. 6 (9), 1110–1116. http://dx.doi.org/10.1111/2041-210X.12410.

Librado, P., Rozas, J., 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25 (11), 1451–1452. http://dx.doi.org/10.1093/bioinformatics/btp187.

Mancinelli, G., Chainho, P., Cilenti, L., Falco, S., Kapiris, K., Katselis, G., Ribeiro, F., 2017a. On the Atlantic blue crab (*Callinectes sapidus* Rathbun 1896) in southern European coastal waters: Time to turn a threat into a resource? Fish. Res. 194 (November 2016), 1–8. http://dx.doi.org/10.1016/j.fishres.2017.05.002.

Mancinelli, G., Chainho, P., Cilenti, L., Falco, S., Kapiris, K., Katselis, G., Ribeiro, F., 2017b. The atlantic blue crab *Callinectes sapidus* in southern European coastal waters: Distribution, impact and prospective invasion management strategies. Mar. Pollut. Bull. 119 (1), 5–11. http://dx.doi.org/10.1016/J.MARPOLBUL.2017.02.050.

Mancinelli, G., Glamuzina, B., Petrić, M., Carrozzo, L., Glamuzina, L., Zotti, M., Raho, D., Vizzini, S., 2016. The trophic position of the Atlantic blue crab *Callinectes sapidus* Rathbun 1896 in the food web of Parila Lagoon (South Eastern Adriatic, Croatia): A first assessment using stable isotopes. Mediterr. Mar. Sci. 17 (3), 634–643.

Mancinelli, G., Guerra, M.T., Alujević, K., Raho, D., Zotti, M., Vizzini, S., 2017c. Trophic flexibility of the Atlantic blue crab *Callinectes sapidus* in invaded coastal systems of the Apulia region (SE Italy): A stable isotope analysis. Estuar. Coast. Shelf Sci. 198, 421 – 431. http://dx.doi.org/10.1016/j.ecss.2017.03.013, ECSA 55 Unbounded boundaries and shifting baselines: estuaries and coastal seas in a rapidly changing world.

May, G.E., Gelembiuk, G.W., Panov, V., Orlova, M.I., Lee, C.E., 2008. Molecular ecology of zebra mussel invasions. Mol. Ecol. 15 (4), 1021–1031. http://dx.doi.org/10.1111/j.1365-294X.2007.03676.x.

McMillen-Jackson, A.L., Bert, T.M., 2004. Mitochondrial DNA variation and population genetic structure of the blue crab *Callinectes sapidus* in the eastern United States. Mar. Biol. 145, 769–777. http://dx.doi.org/10.1007/s00227-004-1353-3.

Monin, V.L., 1984. New find of the blue crab *Callinectes sapidus* (Decapoda, Brachyura) in The Black Sea. Zool. Zhurnal 63, 1100–1101.

Nehring, S., 2011. Invasion history and success of the American blue crab *Callinectes sapidus* in European and adjacent waters. In: Galil, B.S., Clark, P.F., Carlton, J.T. (Eds.), In The Wrong Place-Alien Marine Crustaceans: Distribution, Biology and Impacts. Springer, pp. 607–624.

Palumbi, S.R., 1996. Nucleic acids II: The polymerase chain reaction. In: Hillis, D.M., C., M., K., M.B. (Eds.), Molecular Systematics. Sinauer Associates, Inc, pp. 205–247.

Panin, N., 2005. The Black Sea coastal zone–an overview. GeoEcoMarina 11, 21–40. http://dx.doi.org/10.5281/zenodo.57388.

Pashkov, A.N., Reshetnikov, S.I., Bondarev, K.B., 2012. The capture of the blue crab (*Callinectes sapidus*, decapoda, crustacea) in the Russian sector of the Black Sea. Russ. J. Biol. Invasions 3 (1), 22–28. http://dx.doi.org/10.1134/s2075111712010067.

Peñarrubia, L., Vidal, O., Viñas, J., Pla, C., Sanz, N., 2016. Genetic characterization of the invasive zebra mussel (*Dreissena polymorpha*) in the Iberian Peninsula. Hydrobiologia 779, 227–242. http://dx.doi.org/10.1007/s10750-016-2819-2.

Posey, M.H., Alphin, T.D., Harwell, H., Allen, B., 2005. Importance of low salinity areas for juvenile blue crabs, *Callinectes sapidus* Rathbun, in river-dominated estuaries of southeastern United States. J. Exp. Mar. Biol. Ecol. 319 (1–2), 81–100. http://dx.doi.org/10.1016/J.JEMBE.2004.04.021.

Pyšek, P., Richardson, D.M., 2010. Invasive species, environmental change and management, and health. Ann. Rev. Environ. Res. 35 (1), 25–55. http://dx.doi.org/10.1146/annurev-environ-033009-095548.

Reichmuth, J.M., MacDonald, J., Ramirez, J., Weis, J.S., 2011. Fight or flight: An investigation of aggressive behavior and predator avoidance in two populations of blue crabs (*Callinectes sapidus* Rathbun) in New Jersey. Hydrobiologia 658 (1), 173–182. http://dx.doi.org/10.1007/s10750-010-0460-z.

Rodrigues, M.A., Dumont, L.F.C., dos Santos, C.R.M., D'Incao, F., Weiss, S., Froufe, E., 2017. Two distinct mtDNA lineages of the blue crab reveal large-scale population structure in its native atlantic distribution. Estuar. Coast. Shelf Sci. 197, 45–53. http://dx.doi.org/10.1016/j.ecss.2017.08.004.

Rodrigues, M.A., Heberle, M.F., D'Incao, F., Incao, F.D., 2011. Fecundity variation and abundance of female blue crab *Callinectes sapidus* Rathbun, 1896 (Decapoda, Brachyura, Portunidae) in the Patos Lagoon estuary, RS, Brazil. Atlântica, Rio Grande 33 (2), 141–148. http://dx.doi.org/10.5088/atl.2011.33.2.141.

Rome, M., Young-Williams, A., Davis, G., Hines, A., 2005. Linking temperature and salinity tolerance to winter mortality of Chesapeake Bay blue crabs (*Callinectes sapidus*). J. Exp. Mar. Biol. Ecol. 319 (1–2), 129–145. http://dx.doi.org/10.1016/J.JEMBE.2004.06.014.

Sanz, N., Araguas, R.M., Vidal, O., Diez-del Molino, D., Fernández-Cebrián, R., García-Marín, J.L., 2013. Genetic characterization of the invasive mosquitofish (*Gambusia spp.*) introduced to Europe: Population structure and colonization routes. Biol. Invasions 15, 2333–2346. http://dx.doi.org/10.1007/s10530-013-0456-5.

Schubart, C.D., Conde, J.E., Carmona-Suarez, C., Robles, R., Felder, D.L., 2001. Lack of divergence between 16s mtDNA sequences of the swimming crabs (*Callinectes bocourti* and *C. maracaiboensis* (Brachyura: Portunidae) from Venezuela. Fish. Bull. 99 (3), 475–481.

Shaverdashvili, R.S., Ninua, N.S., 1975. New find of crab *Callinectes sapidus* Rathbun, 1896 in the Black Sea. Nauch. Dokl. Vyssh. Shkol 9, 19–20.

Snigirov, S., Medinets, V., Chichkin, V., Sylantyev, S., 2013. Rapa whelk controls demersal community structure off Zmiinyi island, Black sea. Aquat. Invasions 8 (3), 289–297. http://dx.doi.org/10.3391/ai.2013.8.3.05.

Sullivan, T.J., Neigel, J.E., 2017. Misidentification of megalopae as a potential source of error in studies of population genetics and ecology of the blue crab *Callinectes sapidus*. Mar. Ecol. Prog. Ser. 565, 95–111. http://dx.doi.org/10.3354/meps12043.

Tankersley, R., Forward Jr., R., 2007. Environmental physiology. In: Kennedy, V.S., Cronin, L. (Eds.), The Blue Crab: Callinectes Sapidus. Maryland Sea Grant College University of Mary, pp. 451–483.

Van Engel, W.a., 1990. Development of the reproductively functional form in the male blue crab, *Callinectes Sapidus*. Bull. Mar. Sci. 46, 13–22.

Williams, E.P., Feng, X., Place, A.R., 2017. Extensive heteroplasmy and evidence for fragmentation in the *Callinectes sapidus* mitochondrial genome. J. Shellfish Res. 36 (1), 263–272. http://dx.doi.org/10.2983/035.036.0129.

Windsor, A.M., Moore, M.K., Warner, K.A., Stadig, S.R., Deeds, J.R., 2019. Evaluation of variation within the barcode region of cytochrome c oxidase I (COI) for the detection of commercial *Callinectes sapidus* Rathbun, 1896 (blue crab) products of non-US origin. PeerJ 7, e7827. http://dx.doi.org/10.7717/peerj.7827.

Wolff, T., 1954. Tre ostamerikanske krabber fundet i Danmark. Flora og Fauna 60 (1), 19–34.

Yağlıoğlu, D., Turan, C., Öğreden, T., 2014. First record of blue crab *Callinectes sapidus* (Rathbun 1896) (Crustacea, Brachyura, Portunidae) from the Turkish Black Sea coast. J. Black Sea/Mediterr. Environ. 20 (1), 13–17.

Yednock, B.K., Neigel, J.E., 2014. An investigation of genetic population structure in blue crabs, *Callinectes sapidus*, using nuclear gene sequences. Mar. Biol. 161, 871–886. http://dx.doi.org/10.1007/s00227-013-2387-1.

Zaitsev, Y., 1998. Samoe sinee v mire (Most Blue in the World), New York: Izd. OON.

Zaitsev, Y., Ozturk, B., 2001. Exotic Species in the Aegean, Marmara, Black, Azov and Caspian Seas. Turkish Marine Research Foundation, p. 267.

Zenetos, A., Çinar, M.E., Pancucci-Papadopoulou, M.A., Harmelin, J.G., Furnari, G., Andaloro, F., Bellou, N., Streftaris, N., Rowius, H.Z., 2005. Annotated list of marine alien species in the mediterranean with records of the worst invasive species. Mediterr. Mar. Sci. 6, 63–118. http://dx.doi.org/10.12681/mms.186.

Zupolini, L.L., Magalhães, T., Pileggi, L.G., Mantelatto, F.L., 2017. Taxonomic revision of the speckled crabs, genus *arenaeus* Dana, 1851 (Brachyura: Portunidae) based on morphological and molecular data. Zootaxa 4273 (3), 362–380. http://dx.doi.org/10.11646/zootaxa.4273.3.3.