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First record of the Atlantic blue crab *Callinectes sapidus* (Crustacea: Brachyura: Portunidae) in the Segura River mouth (Spain, southwestern Mediterranean Sea)

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Abstract: A single male individual of *Callinectes sapidus* (Crustacea, Decapoda, Portunidae) was collected on 6 December 2014 in the Segura River mouth in southeastern Spain. Information on collection site, DNA barcoding, and its present distribution and dispersal strategies are provided.

Key words: Blue crab, invasive species, DNA barcoding, *COI* gene, Segura River, southeastern Spain

The blue crab *Callinectes sapidus* Rathbun, 1896 is a species native to the western Atlantic coasts. Its geographical distribution ranges from Nova Scotia in Canada down to northern Argentina, including Bermuda and the Antilles (Williams, 1974; Castriota et al., 2012). However, it started to spread to the Atlantic European coasts, being recorded in France in 1900 (Bouvier, 1901), and later northward in the Baltic Sea and southward along the Atlantic coasts of Spain and Portugal, including recent records in the Sado Estuary, western Portugal (Nehring, 2011; Ribeiro and Veríssimo, 2014). It has also been reported in the Pacific Ocean, colonizing Japan since 1975 and Hawaii since 1985 (Eldredge, 1995). The first occurrence of the blue crab in the Mediterranean (Marina di Grado, Italy) was on 4 October 1949 (Mizzan, 1993); later *C. sapidus* was reported in many localities from the Mediterranean and the Black Sea, including Israel (Holthuis and Gottlieb, 1955), Turkey (Holthuis, 1961; Tureli Bilen and Yesilyurt, 2014; Yağlıoğlu et al., 2014), Greece (Kinzelbach, 1965), northern Albania (Beqiraj and Kashta, 2010), southern Croatia (Onofri et al., 2008; Dulčić et al., 2011), northern Italy (Manfrin et al., 2015), eastern Italy (Castriota et al., 2012), southern Italy (Florio et al., 2008; Mancinelli et al., 2013), northwestern Italy (Stasolla and Innocenti, 2014), and northeastern Spain (Ebro Delta) (Castejón and Guerao, 2013) (Figure 1). Recently, a specimen of *C. sapidus* was found in the Albufera lagoon (eastern Spain, October 2014) and in close sites such as Gandía (May 2015), although these last sightings have not been confirmed formally yet (https://cienciagandia.webs.upv.es/en/2015/07/). In Turkey, *C. sapidus* has been reported in 15 lagoonal systems, supporting important commercial fisheries with 46 t of catches in 2010 (TÜİK, 2010), although in 2013 the catches decreased to 3 t (TÜİK, 2013).

The species is euryhaline and inhabits estuaries and marine bays from 0 m to 90 m depth on muddy and sandy bottoms (Hill et al., 1989). In its native habitats, *C. sapidus* carries out several ecosystem functions, playing an important role in energy transfer within estuaries and lagoons (Johnson and Eggleston, 2010). Blue crabs are mainly omnivores, acting as dominant, opportunistic benthic predators and scavengers, although some cases of cannibalism have been also registered (Hill et al., 1989; Douglass et al., 2011). In addition to its ecological importance, the blue crab is a target species for commercial and recreational fisheries throughout its geographical distribution (Tureli Bilen and Yesilyurt, 2014). Its settlement process in new geographical areas is probably favored by global climate change and/or ship traffic between seas; it is considered that *C. sapidus* was transferred from the Atlantic coasts of America to the European shores and the Mediterranean Sea by ballast waters of vessels (Nehring, 2011).

Considering the background of colonization of this invasive species, the aims of this paper are to record, for the first time, the occurrence *C. sapidus* in the Segura River mouth (southeastern Spain), and to discuss the potential geographical origin of the colonization in this area.

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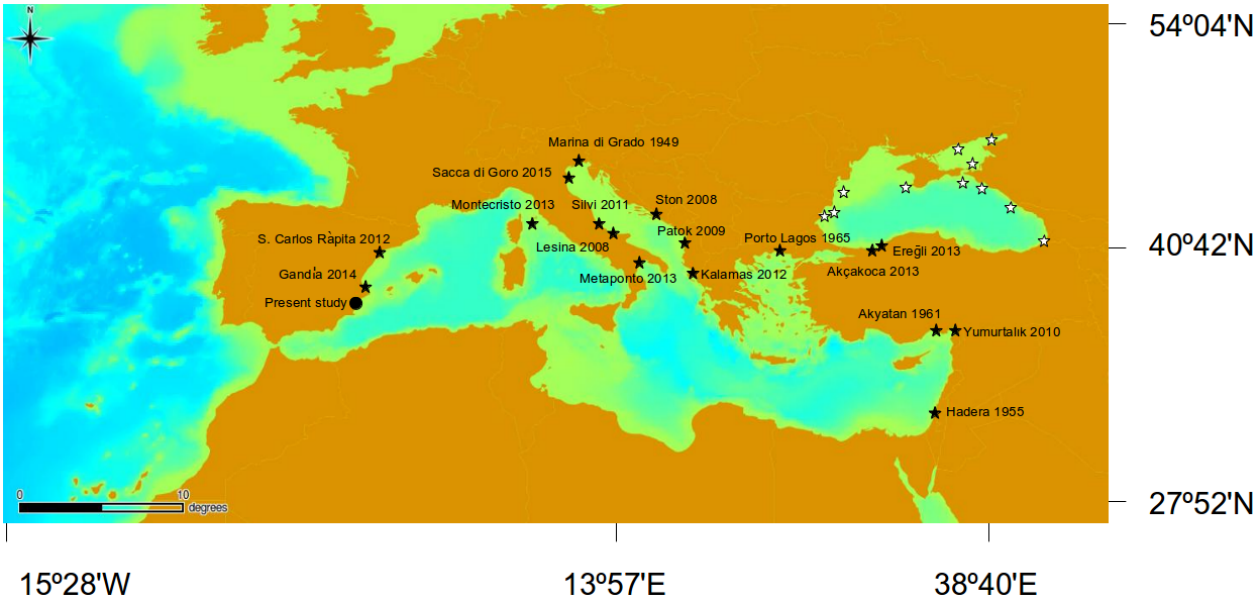

Figure 1. Locations and years (black stars) of previous records of blue crab in the Mediterranean and Black Sea. (Marina di Grado 1949: Mizzan, 1993; Hadera 1955: Holthuis and Gottlieb, 1955; Akyatan 1961: Holthuis, 1961; Porto Lagos 1965: Kinzelbach, 1965; Ston 2008: Onofri et al., 2008; Lesina 2008: Florio et al., 2008; Patok 2009: Beqiraj and Kashta, 2010; Yumurtalık 2010: Tureli Bilen and Yesilyurt, 2014; Silvi 2011: Castriota et al., 2012; Montecristo 2013 and Metaponto 2013: Stasolla and Innocenti, 2014; San Carlos de la Ràpita 2012: Castejón and Guerao, 2013; Akçakoca 2013 and Ereğli 2013: Yağlıoğlu et al., 2014; Gandía 2014: https://cienciagandia.webs.upv.es/en/2015/07/; Sacca di Goro 2015: Manfrin, 2015). White stars indicate records of *C. sapidus* in the Black Sea from 1968 to 2012 (see references in Yağlıoğlu et al., 2014). Black circle shows the location of the Segura River mouth (SE Spain) where *C. sapidus* was registered (present work).

A single male individual of *C. sapidus* (Figure 2) was collected on 6 December 2014 in the Segura River mouth (38°6′37.137″N, 0°38′53.804″W) in southeastern Spain. Fishermen caught it by chance because it was tangled in a fishing line.

The Segura River covers a surface area of about 18,870 km^2. It flows through the driest region of the Iberian Peninsula from the northwest to the southeast over a distance of 325 km. At its terminal part, the mean annual temperature is around 18 °C and the mean annual rainfall is below 300 mm (Bazin et al., 2014). At the river mouth, the water is hyperhaline (36 psu at surface), and the area located 2–3 km upstream from the mouth is polyhaline (19–23 psu) (Bazin et al., 2014).

The specimen was identified in situ as *C. sapidus* (Figure 2) based on its external morphology (carapace morphology and coloration pattern) according to Williams (1974). Subsequently this identification was confirmed using barcoding, amplifying the cytochrome c oxidase I (*COI*) gene according to the protocol of Folmer et al. (1994), comparing the result with the sequences recorded in the GenBank database using the Basic Local Alignment Search Tool (BLAST) available at http://www.ncbi.nlm.nih.gov/genbank/. BLAST finds regions of local similarity between sequences. The program compares nucleotides to sequence databases and calculates the statistical significance of matches. BLAST can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families.

The individual was weighed with a digital scale with an accuracy of ±0.1 g, and the carapace width and length were measured using a caliper with ±0.1 cm of precision. The carapace length (distance between the tip of the frontal teeth and the posterior margin of the carapace) was 86 mm, and carapace width (distance between the tips of the longest lateral carapace spines) was 200 mm. This size is close to the maximum carapace width registered for this species until now (225 mm), with a wet weight of 550 g (Mancinelli et al., 2013). The dorsal carapace surface was brownish-green, with small white spots scattered mainly on its anterior side; the ventral surface was whitish with blue walking legs and bright blue cheliped fingers (Williams, 1974).

The amplification of the *COI* mitochondrial gene, a sequence 634 bp in length, and the BLAST in GenBank (http://www.ncbi.nlm.nih.gov) identified the specimen from the Segura River mouth as *Callinectes sapidus* with a 99% maximum identity with JX123452 (specimen from São Paulo, Brazil), showing only one mutation at position

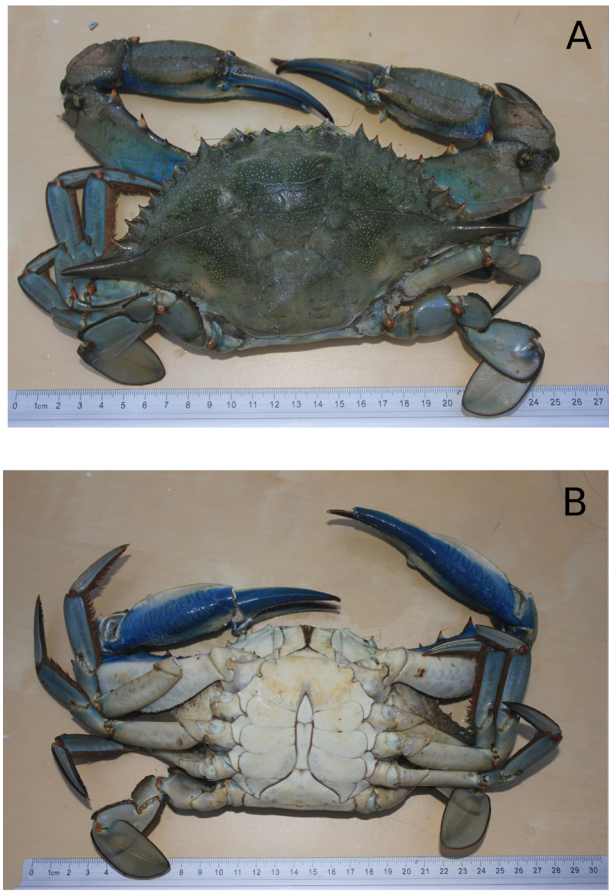

Figure 2. *Callinectes sapidus* Rathbun, 1896, adult male (A: dorsal view; B: ventral view) from the Segura River, SE Spain.

269 (632 bp were compared, 631 bp were identical), and with JQ435813 (specimen from Romania), having two mutations at positions 11 and 611 (625 bp were compared, 623 bp were identical). Therefore, the specimen from the Segura River showed a different haplotype, which was recorded in GenBank as KT282079.

The blue crab is a species that shows high tolerance to extreme variations in water conditions (temperature and salinity), high fecundity (females produce 2 to 8 million eggs per spawn; Jivoff et al., 2007), large body size, and aggressive behavior competing with other crabs for food and space (Mancinelli et al., 2013); all these features allow its invasive success (Nehring, 2011). The Segura River mouth harbors the right conditions for the settlement and spread of this invasive species with available food resources, right temperature and salinity ranges, and suitable habitat to provide refuge for its larvae. It has been described that blue crab growth is strongly temperature-dependent, with growth ceasing below temperatures of approximately 11 °C. Therefore, this species could grow year-round in the Segura River mouth, considering the temperatures described by Bazin et al. (2014) (minimum temperatures around 11.6 °C). The presence of seaweeds in this area also favors the settlement of *C. sapidus* larvae and juveniles: the sea grass meadows and macroalgal beds are considered critical nursery habitats for this invasive species, though its habitat preferences change with age, size, and sex (Hines, 2007; Castejón and Guerao, 2014).

The origin of the *C. sapidus* individual found in the Segura River is uncertain, but there are several factors that could explain the presence of this specimen. First, it is important to consider that adults are excellent swimmers and migrate from seawater to rivers and vice versa at different stages of their life cycle. Several studies established a high mobility of male adults (averaging 24 m/h in channel areas), this speed being even higher in inseminated females that migrate long distances into high salinity areas to incubate the eggs (Hines et al., 1995; Castriota et al., 2012 and references therein). The species also shows great larval dispersal ability. It has been described that the *C. sapidus* larval development occurs in 30–50 days; during this period the larvae remain in surface waters and may be transported considerable distances (30 to 80 km) (Olmi, 1994). Therefore, the individual found in the Segura River could be the result of a direct dispersal of larvae or adults from any close population of *C. sapidus*. Recently individuals of this species were also recorded in Gandía and Albufera, both located close to Guardamar (eastern Spain; https://cienciagandia.webs.upv.es/en/tema/crab/), which would imply that *C. sapidus* is creating permanent populations in this area such as it was registered in other regions from the Mediterranean Sea (Mancinelli et al., 2013). The indirect dispersal of *C. sapidus* larvae in ballast waters or on hulls of fishery and recreational vessels from stable populations in the central Mediterranean Sea (Mancinelli et al., 2013) or western European coasts (Ribeiro and Veríssimo, 2014) must also be considered. Very close to the location where the *C. sapidus* was found, there are the fishing port of Guardamar del Segura (SE Spain) and a large harbor for recreational craft (Marina de las Dunas, Guardamar, SE Spain; http://www.marinadunas.es/marina-de-las-dunas/) with 500 berths that can shelter ships of 15 m in length.

The introduction of blue crabs can have significant and negative consequences to the ecology of the invaded environments. *C. sapidus* has been defined as a highly aggressive species and was selected among the 100 worst invasive species in the Mediterranean Sea with impact on both biodiversity and economy (Streftaris and Zenetos, 2006; Castejón and Guerao, 2013). Further studies on this region (SE Spain), monitoring the presence of this invasive species and assessing its potential dispersal, will provide valuable information on population structure and dynamics of the blue crab on the Spanish coast. These data

would be useful for further management of this invasive species.

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