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Research Article

First and southern-most records of the American blue crab *Callinectes sapidus* Rathbun, 1896 (Decapoda, Portunidae) on the African Atlantic coast

Mariam Oussellam[1,2], Abdelaziz Benhoussa[1], Antoine Pariselle[1,3], Imane Rahmouni[1], Meryem Salmi[1], Jean-François Agnèse[3], Mohamed Selfati[4], Najib El Ouamari[2] and Hocein Bazairi[1,5,*]

[1]*Biodiversity, Ecology and Genome Laboratory, Plant and Microbial Biotechnology, Biodiversity and Environment Research Centre, Faculty of Sciences, Mohammed V University in Rabat, Rabat, Morocco*

[2]*National Institute of Fisheries Research, 13, Bd Zerktouni, BP 493, Nador, Morocco*

[3]*ISEM, CNRS, Université de Montpellier, IRD, Montpellier, France*

[4]*Laboratoire Santé et Environnement, Faculté des Sciences Ain Chock, Hassan II University of Casablanca, B.P 5366, Maarif, Casablanca, Morocco*

[5]*Natural Sciences and Environment Research Hub, University of Gibraltar, Europa Point Campus, Gibraltar*

**Corresponding author*

E-mail: *hocein.bazairi@fsr.um5.ac.ma*

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Abstract

The American blue crab, *Callinectes sapidus* Rathbun, 1896 is native to the western Atlantic. The present work reviews the spread of *C. sapidus* in the European Atlantic, the Mediterranean, Black Sea and Sea of Azov, and identifies its first occurrence in the Merja Zerga and Khnifiss lagoons on the Moroccan Atlantic coast. These first and southernmost records on the African Atlantic widen the current biogeographic distribution of the species worldwide. Genetic analysis of specimens from both the Mediterranean (Marchica lagoon) and Atlantic (Merja Zerga and Khnifiss lagoons) coasts of Morocco, based on COI (Cytochrome *c* oxidase subunit I), confirmed the species sequences as being those of *Callinectes sapidus.* Moroccan populations have low haplotypic diversity with only two haplotypes (haplotype 1 and haplotype 7). These two haplotypes correspond to the two different haplotypes already found in the Mediterranean. Haplotype 1 from Merja Zerga, Marchica and Khnifiss lagoons was 100% identical to the sequences ON248058 in GenBank, while haplotype 7 from the Marchica and Merja Zerga lagoons was 100% identical to the sequences ON248059. Very likely, the origin and pathway of colonisation of the populations established in the three Moroccan lagoons is the result of the expansion of the species along the Mediterranean Sea and now the Atlantic coast of Africa.

Key words: non-indigenous, invasive, coastal lagoon, haplotype, colonisation, Morocco

Introduction

Invasive alien species (IAS) pose a severe threat to marine biodiversity (Simberloff et al. 2013) due to their adverse impacts on marine and coastal ecosystems (Katsanevakis et al. 2014). As a global response, IAS are treated as a serious issue under many regional and international regulations (e.g. Convention on Biological Diversity, Barcelona Convention) (Turbelin et al. 2017; El Kamcha et al. 2020). Therefore, providing comprehensive data on their trends in abundance, spatial and temporal concurrence, and their

potential impacts on ecosystem functions and services, is crucial for their management (Katsanevakis et al. 2020).

Marine and coastal ecosystems worldwide, such as lagoons and estuaries, are among the most heavily invaded systems on Earth (Saccà 2016). They are being invaded at high rates because of human activities such as shipping (Carlton and Geller 1993; Ruiz et al. 2000), the opening of navigational corridors (Zenetos et al. 2012), aquaculture (Naylor et al. 2001), the aquarium trade (Padilla and Williams 2004), and the live seafood and bait trades (Chapman et al. 2003; Weigle et al. 2005). Therefore, studies focusing on biological invasions in marine and coastal environments are warranted (Chan and Briski 2017).

Invasive decapod crustaceans are being commonly reported in marine and coastal habitats (Ruiz et al. 2000). The American blue crab, *Callinectes sapidus* Rathbun, 1896 (Decapoda, Portunidae), is native to the estuaries and coastal waters of the western Atlantic (Nehring 2011, 2012), where it ranges from the intertidal zone to 90 m of depth (Hines et al. 1987). It was first introduced on the European Atlantic coast in France in the early twentieth century (Nehring 2011). In the Mediterranean Sea, the species was first reported in the Aegean Sea between 1935 and 1945, but its first confirmed and documented presence was reported from Italy in 1949 and was commonly ascribed to Giordani Soika (1951). In the Black Sea, the blue crab was recorded for the first time from Bulgaria in 1967 (Bulgurkov 1968). According to Öztürk et al. (2020), the species has been introduced to the Black Sea, most likely through migration from the Aegean Sea, and most of its records are recent episodic catches with limited numbers of collected specimens. To date, the species has been recorded almost ubiquitously in the Mediterranean and the Black Sea (Nehring 2011; Pashkov et al. 2012; Castejón and Guerao 2013), where it is classified as one of the 100 "worst invasive" species (Streftaris and Zenetos 2006).

In Morocco, the first occurrence of *C. sapidus* was reported in 2017 on the Mediterranean coast of the country, in the Marchica coastal lagoon (Oussellam and Bazairi *in* Chartosia et al. 2018). Here, we document the first and the southern-most records of the species on the African Atlantic coast. Additionally, genetic analyses were performed on specimens from the Mediterranean and the Atlantic coasts of Morocco to confirm their identification as *C. sapidus* and to understand their origin and pathway of colonisation.

Materials and methods

Study sites

The Marchica lagoon (Figure 1A) (35.156944°; −2.845278°), also called the lagoon of Nador, is one of the largest coastal lagoons in the Mediterranean (115 km², 25 km long and 7.5 km wide) and the only one on the Mediterranean

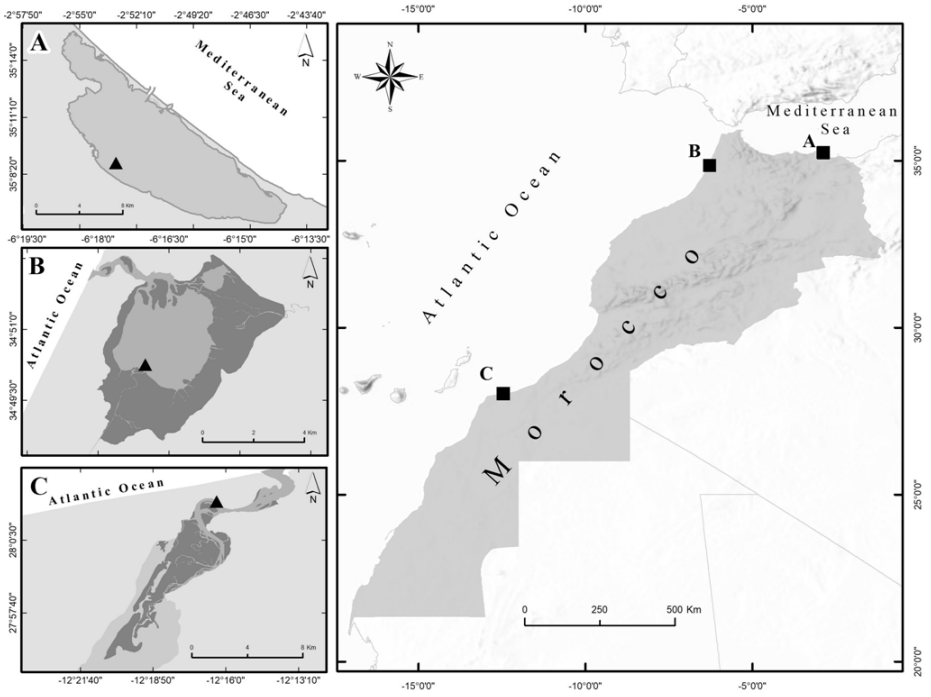

Figure 1. Map showing the localization of the study sites: (A) Marchica lagoon, (B) Merja Zerga lagoon, (C) Khnifiss lagoon. Black triangles: localities where the blue crab *Callinectes sapidus* was sampled.

coast of Morocco. The maximum depth is approximately 8 m, and the lagoon is separated from the Mediterranean Sea by a 25-km-long sandbar (Lido), with one artificial opening (300 m wide and 6 m deep) that allows water exchange. Despite its ecological (Site of Biological and Ecological Interest since 1996; RAMSAR site since 2005) and socio-economic (mainly artisanal fisheries) values, the lagoon is under pressure from a complex mixture of human-mediated stressors (urbanisation, pollution, overfishing, tourism, and wastewater, among others) (Selfati et al. 2017; El Kamcha et al. 2020).

The Merja Zerga lagoon (Figure 1B) (34.783333; −6.216667° and 34.866667°; −6.233333°) covers 45 km^2, with an average depth of 1.5 m. In addition to its tidal inflow, the freshwater is mainly received from the Drader River to the east, and the Nador artificial Channel, to the south of the lagoon. Due to substantial exchanges with the Atlantic Ocean, the downstream salinity is close to sea water's, at 34, while it does not exceed 2.4 in the southern sector, away from the "gullet" (Touhami et al. 2017). The temperature of the water shows a slightly decreasing gradient from the downstream to the upstream of the lagoon (Touhami et al. 2017). In 1980, it was declared a biological reserve and a RAMSAR site. Even so, the lagoon supports different aspects of human lives, such as economic, cultural and community relationships. Various human activities have been established in the region (farming areas surround the lagoon), and different marine activities, such as fishing, boating and tourist camping, have increased. Rapid urbanisation has occurred in recent years (Cheggour et al. 2001; Maanan et al. 2013).

The Khnifiss lagoon, also known as the National Park of Khnifiss, is located in the middle of the Moroccan Atlantic coast, at the northern limit of the coastal Sahara (28.041111°; −12.225833°) (Figure 1C). Considered the largest wetland in the desert bioclimatic zone (Beaubrun 1976), the lagoon covers a surface area of about 65 km^2, with a length of 20 km and a maximum depth of 8.7 m. The lagoon area is characterised by an arid climate with very low precipitation and a maximal annual rainfall of 50 mm (Lefrere et al. 2015). Water temperature and salinity increase from downstream to upstream, ranging from 16.1 °C to 17.2 °C and from 34 to 44.1, respectively (Lakhdar-Idrissi et al. 2004). As a result of the aridity of the area and the existence of the salinity gradient, the upper part of the lagoon is used as a salt marsh (Lakhdar-Idrissi et al. 2004). The air temperature in the Khnifiss lagoon fluctuates between 13 °C and 24 °C (Lefrere et al. 2015). This reserve shelters a diversity of desert fauna and flora. It was designated in 1980 as a RAMSAR site (Qninba et al. 2006). The National Park of Khnifiss is also the subject of increased exploitation, such as shellfish aquaculture, fishing and nature tourism (Lakhdar-Idrissi et al. 2004; Lefrere et al. 2015).

Crab identification and sampling

The American blue crab identification was based initially on morphological criteria (carapace and colouration) according to Williams (1974), as well as the shape of the gonopods in males (Williams 1974; Cuesta et al. 2015). Specimens of *C. sapidus* were collected in the above sites as bycatch by fishers using trammel nets.

The presence of *Callinectes sapidus* in the Merja Zerga lagoon was first documented by a specimen in June 2019 (Figure 2A). Overall, 222 specimens of *C. sapidus* were sampled monthly at the Merja Zerga lagoon between September 2019 and July 2020, caught at the same location, close to the fresh water artificial channel of Nador in the southern area of the lagoon (Figure 1). Only one specimen was collected from the Khnifiss lagoon in October 2020 (Figure 2B). In addition, 62 specimens from the Mediterranean Marchica lagoon (Figure 1) were collected in July 2020.

All specimens of *C. sapidus* were measured for their carapace length (CL), carapace width (CW) and fresh weight (W). Samples for genetic analysis were collected and deposited in the Biological Reference Collections of the Biodiversity, Ecology and Genomics Laboratory (Faculty of Sciences, Mohammed V University in Rabat, Morocco).

Literature review

A full literature search for all the first records by country of the blue crab was undertaken to evaluate and describe the spread of the introduced *C. sapidus* in the northeastern (European and African) Atlantic coastline and the Mediterranean, Black Sea and Sea of Azov.

Figure 2. *Callinectes sapidus* specimens (males, dorsal view) collected at Merja Zerga (A) and Khnifiss (B) lagoons, Atlantic coast of Morocco. Photographs by Abdelaziz Benhoussa.

DNA isolation, amplification and sequencing

To guarantee the identification of species collected along the Atlantic and Mediterranean coasts of Morocco, a leg of each specimen captured was dissected and transferred to an Eppendorf® tube containing ethanol (95%) to preserve DNA. The total genomic DNA was extracted from approximately 20 mg of muscle tissue preserved in ethanol using a rapid salt-extraction protocol (Aljanabi and Martinez 1997).

After several attempts to find the optimised protocol and annealing temperature, the primers showed a successfully amplified product. The amplification of the COI (Cytochrome *c* oxidase subunit I) gene was performed using the primers igLCOI 1490 (5′–GGT CAA CAA ATC ATA

Table 1. List of country and GenBank accession number for sequences used in the analysis.

Location	Accession numbers	Haplotypes
United states	MG462557	Hap 4
	MG462558	Hap 6
	MG462559	Hap 5
	MG462562	Hap 5
	MG462561	Hap 2
	MG462560	Hap 3
	KR030241	Hap 7
Colombia	MG462554	Hap 13
Nicaragua	MG462525	Hap 12
	MG462526	Hap 10
	MG462527	Hap 11
	MG462528	Hap 12
Spain	KT282079	Hap 1
Brazil	MG462242	Hap 8
	MG462243	Hap 8
	MH985984	Hap 9
Venezuela	MG462627	Hap 12
	MG462628	Hap 14
	MG462629	Hap 12
	MG462630	Hap 12
	MG462631	Hap 12
Turkey	MG462529	Hap 1
	MG462531	Hap 1
	MG462533	Hap 1
	MG462532	Hap 1
	MG462530	Hap 1

AAG ATA TTG G–3′) and igHCO2198 (5′–TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) (Folmer et al. 1994) in the genetic technical facility of the Biodiversity, Ecology and Genome Laboratory at the Faculty of Sciences, University Mohamed V in Rabat (Morocco). PCR was carried out in 25 µl volume containing 1 µl of DNA extract, 1 µl of each PCR primer (10 mM), 1.5 U Taq DNA polymerase, 5 µl PCR buffer (5X; 5 mM dNTPs, 15 mM $MgCl_2$), and 0.5 µl dNTPs (200 mM) and distilled water to the final volume. The amplification was achieved using the following steps: initial denaturation for 7 min at 94 °C followed by 40 cycles of 94 °C for 1 min, 46 °C for 1 min and 30 s and 72 °C for 2 min, and a final extension at 72 °C for 10 min. PCR products were examined on 1% agarose gel, stained with ethidium bromide. Successful PCR products were sent to the Genotyping Sequencing (GenSeq) platform (Montpellier, France) for purification and sequencing. The sequencing was carried out by using the same primers as for PCR. The obtained sequences were cleaned manually with MEGA version X (Kumar et al. 2018) and aligned using the computer program ClustalW, with multiple alignments running in MEGA version X. Sequences obtained were identified by the Basic Local Alignment Search Tool (BLAST). In addition to our samples, 26 sequences of *C. sapidus* were retrieved from GenBank and included in the analysis. Sources of sequences and Genbank accession numbers are given in Table 1. Haplotype network was computed in PopART version 1.7 (Clement et al. 2000) to estimate the relation between haplotypes using nexus input files produced by DnaSP version 5.

Table 2. Biometric measurements of both females and males of *Callinectes sapidus* specimens from the Mediterranean (Marchica, lagoon) and the Atlantic (Merja Zerga and Khnifiss lagoons) coasts of Morocco. CL: Carapace length; CW: Carapace width; W: Body weight; SD: standard deviation.

Females								
Lagoon	Sampling period	Total Individuals	W (g)		CW (cm)		CL (cm)	
			Min–Max	Mean ± SD	Min–Max	Mean ± SD	Min–Max	Mean ± SD
Marchica	July 2020	23	58.5–274.7	148.0 ± 57.3	9.0–18.5	13.7 ± 2.1	4.6–7.5	6.2 ± 0.7
Merja Zerga	Sep. 2019–July 2020	81	0.5–388.2	43.6 ± 71.4	2.1–21	7.8 ± 4.4	1.1–8.5	3.6 ± 1.8
Khnifiss	Oct. 2020	0	–	–	–	–	–	–
Males								
Lagoon	Sampling period	Total Individuals	W (g)		CW (cm)		CL (cm)	
			Min–Max	Mean ± SD	Min–Max	Mean ± SD	Min–Max	Mean ± SD
Marchica	July 2020	39	61.5–470.4	208.3 ± 79.7	9.4–18.7	14.5 ± 1.8	4.5–8.5	6.7 ± 0.7
Merja Zerga	Sep. 2019–July 2020	141	0.5–585.7	79.9 ± 158.3	2.1–22	7.6 ± 5.8	0.9–9.2	3.5 ± 2.4
Khnifiss	Oct. 2020	1	415.1		17.3		8.6	

Results

New data

Overall, 62 specimens of *Callinectes sapidus* were collected from the Marchica lagoon in July 2020. These were 23 females, with W ranging from 58.5 g to 274.7 g, CW ranging from 9.0 cm to 18.5 cm and CL ranging from 4.6 cm to 7.5 cm, and 39 males, with W ranging from 61.5 g to 470.4 g, CW ranging from 9.4 cm to 18.7 cm and CL ranging from 4.5 cm to 8.5 cm (Table 2).

Although the first specimen of the invasive crab *Callinectes sapidus* in the Merja Zerga lagoon, from Atlantic waters of Morocco, was caught in June 2019, local fishers mentioned that the species started appearing at the lagoon in late 2017–early 2018. Many other specimens were caught monthly in Merja Zerga from June 2019 to June 2020. They were all caught at the same location, close to the freshwater artificial channel of Nador in the southern area of the lagoon (Figure 1). In total, 222 specimens of *Callinectes sapidus* were collected from the Merja Zerga lagoon between June 2019 and June 2020. These were 81 females, with W ranging from 0.5 g to 388.2 g, CW ranging from 2.1 cm to 21 cm and CL ranging from 1.1 cm to 8.5 cm, and 141 males, with W ranging from 0.5 g to 585.7 g, CW ranging from 2.1 cm to 22 cm and CL ranging from 0.9 cm to 9.2 cm (Table 2).

The recorded specimen of *C. sapidus* in the Khnifiss lagoon was a male, with 415.1 g in W, 17.3 cm in CW and 8.6 cm in CL (Table 2). The local fishers mentioned that the species started to appear there in May 2020.

Genetic study

Sixteen COI sequences were generated from the three different localities: Khnifiss lagoon (one sequence), Marchica (eight sequences) and Merja Zerga lagoon (seven sequences). The alignment of fragments obtained resulted in 523 bp. The comparison of COI sequences with those already present in the GenBank database using BLAST search confirmed the identification of our species sequences as being those of *Callinectes sapidus*. In total, 14 different haplotypes were identified for COI gene (Figure 3).

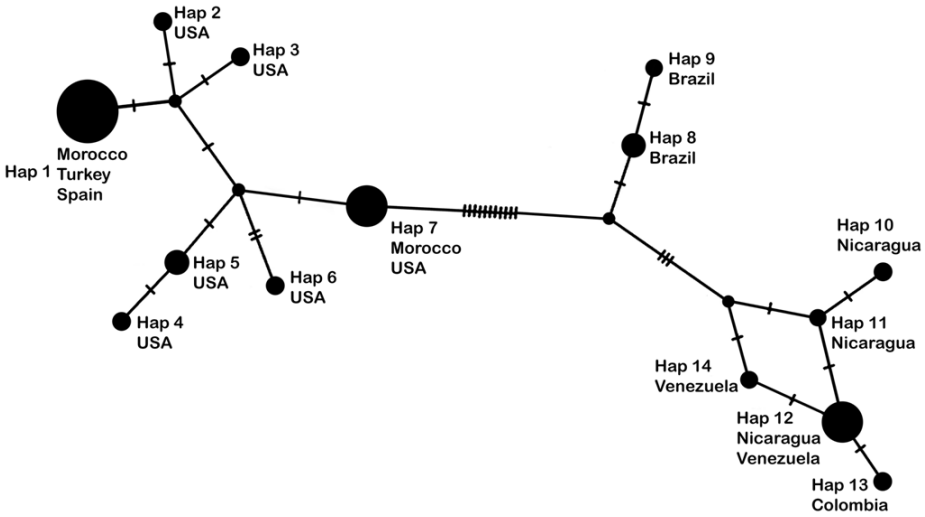

Figure 3. Haplotypes network of *Callinectes sapidus* using Cytochrome *c* oxidase subunit I marker. The area of each circle is proportional to the number of collections exhibiting that haplotype. Each bar in the network represented one mutational step.

Sequences of Moroccan specimens resulted in two haplotypes, haplotype 1 and haplotype 7 (Figure 3). These haplotypes differed at three nucleotide positions. Sequences of *Callinectes sapidus* from Merja Zerga lagoon, Marchica, and Khnifiss lagoon (haplotype 1) were 100% identical to the sequences from GenBank from USA (MG462571, Spain (KT282079) and Turkey (MG462571 and MN759041), which correspond to haplotype CSWM1 (Genbank ON248058, González-Ortegón et al. 2022). However, sequences of haplotype 7 from the Marchica and Merja Zerga lagoons were 100% identical to sequences from the USA (KR030241), which correspond to haplotype CSWM2 (Genbank ON248059; González-Ortegón et al. 2022).

Bibliographic data

Native to the Western Atlantic, *Callinectes sapidus* has been introduced into both Asia and Europe (Millikin and Williams 1984). The first occurrence of the blue crab *C. sapidus* on the northeastern Atlantic coast was recorded in France in 1900 (Bouvier 1901) (Figure 4, Supplementary material Table S1) and has since remarkably expanded along the Atlantic coasts of Europe (Figure 4). Additionally, the species has expanded northward from its native distribution (Mancinelli et al. 2021).

In the Mediterranean, *C. sapidus* was first reported in Venice in 1949. The species was established first in the central and eastern basins and has rapidly spread into the western basin. The species is currently present in 18 out of the 23 Mediterranean countries (Figure 4, Table S1).

Discussion

The first and southern-most records of *C. sapidus* on the Atlantic coast of Morocco (Merja Zerga and Khnifiss lagoons respectively) have been documented in the present study. The first published occurrence of *C. sapidus*

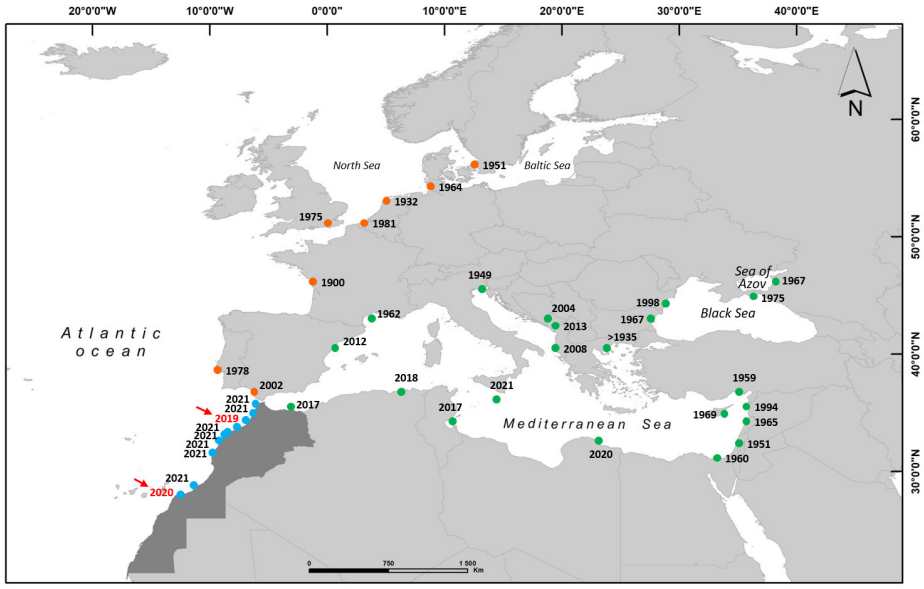

Figure 4. Spreading of *Callinectes sapidus* in the European North-Eastern Atlantic (orange dots) and in Mediterranean, Black Sea and Sea of Azov (green dots), based on the dates of first published records per country. The map shows also in detail the dates of records along the African Atlantic coast (blue dots), highlighting the first and southern-most records of *C. sapidus* reported in the present work (red arrows). Details in Table S1.

on the African Atlantic coast referred to records (165 specimens) made in 2021 on the Moroccan Atlantic coast (Chaouti et al. 2022). The species was reported from 20 localities (six estuaries, three lagoons and 11 offshore sites) along the Atlantic coast of Morocco. Nevertheless, our data reveal that the American blue crab was already well established there many years earlier, and its introduction to the African Atlantic coast may be dating back accordingly. Indeed, our record of the species at the Merja Zerga lagoon in 2019 is the first confirmed report of *Callinectes sapidus* on both the Moroccan and the African Atlantic, while the record from Khnifiss lagoon is the southern-most compared to the previous ones.

The first record in the Marchica Lagoon on the Mediterranean coast of Morocco dates only to 2017 (Oussellam and Bazairi in Chartosia et al. 2018). The present study confirmed that the blue crab is now well established there and rapidly becoming invasive. Moreover, it has expanded 20 km from both sides of the lagoon, to the Moulouya and Oued Kert estuaries (Taybi and Mabrouki 2020). The presence of the species in diverse environmental conditions supports the invasiveness of the species and its capacity to spread over various environmental conditions, including wide ranges of salinity (Stasolla and Innocenti 2014; Taybi and Mabrouki 2020; Shaiek et al. 2021). Moreover, the species can be considered a nuisance in Morocco because it clogs fishing gear, as in other localities in the Mediterranean (Kara and Chaoui 2021; Shaiek et al. 2021).

According to size classifications based on the CW (Cadman and Weinstein 1985; Harding 2003), females of the blue crab sampled at Merja Zerga lagoon were composed of immature and mature ovigerous individuals. Moreover, the presence of different length and width classes throughout

the year shows that the species is well established in the Merja Zerga Lagoon. This is in accordance with the species' ability for fast expansion and establishment reported worldwide (Fuentes et al. 2019; Shaiek et al. 2021). The single crab found in the Khnifiss lagoon was a mature male.

From a biogeographic point of view, *C. sapidus* is currently well established in the Mediterranean Sea, following an east-to-west colonisation pattern. As documented in the present study, the blue crab has started to invade the African Atlantic at least since 2019 and is now well established in many coastal and marine ecosystems of Morocco. The observed pattern of Mediterranean colonisation by *C. sapidus* is comparable to those observed for the tropical sea hare *Aplysia dactylomela* (Rang 1828) (Valdés et al. 2013) and the sea hare *Bursatella leachii* de Blainville 1817 (Selfati et al. 2017; Bazzicalupo et al. 2018), which were assumed to enter the Mediterranean from the Atlantic. The explanation of this counter-intuitive pattern in the Mediterranean, as proposed by Valdés et al. (2013) for the *Aplysia dactylomela* spread in the same location, could be related to the water circulation pattern in the Mediterranean, namely the Algerian current, which could transport the larvae entering the Mediterranean from the Atlantic directly into the Oriental and Central basins, where the species was first recorded. Another possible explanation, as suggested by Bazzicalupo et al. (2018) for the dispersal pattern of at least one part of the population of *B. leachii,* is that due to temperature conditions, the species was established first in the eastern and central Mediterranean and reached the western basin after the warming of water during the last decades.

In the Black Sea, *C. sapidus* was first recorded in 1967 in the western part of the Varna Bay in Bulgaria (Bulgurkov 1968). Its occurrence is now documented in many localities all around the Black Sea (see Öztürk et al. 2020; Stefanov 2021 and references therein). The present *C. sapidus* population in the Black Sea is most likely a result of the migration from the well-established, stable and reproductively successful population of *C. sapidus* in the Aegean Sea (Saros Bay) (Öztürk et al. 2020).

Genetic analyses showed that Moroccan populations of *C. sapidus* have low haplotypic diversity. Only two haplotypes were recognized, with 56% haplotype 1 and 44% haplotype 7, corresponding to those observed in the Mediterranean Sea. Windsor et al. (2019) highlighted the presence of three lineages for the American blue crab in their country of origin: lineage 1 predominated in the Atlantic coast of North America and the Gulf of Mexico, lineage 2 predominated in the Caribbean Sea, and lineage 3 is only present in the Brazilian water. Öztürk et al. (2020) showed that specimens of the Black Sea belong to lineage 1 and are probably of Northwest Atlantic Ocean origin. Moreover, according to Vecchioni et al. (2022), all Mediterranean blue crabs belong to lineage 1, which suggests that the introductions originated from a single source area. González-Ortegón et al. (2022) observed the presence of the same haplotypes (CSWM1 = haplotype 1 and

CSWM2 = haplotype 7) in the Gulf of Cadiz, Alboran Sea and the Mediterranean Sea and suggested a secondary introduction (*i.e.* from other Mediterranean populations) of the species into the studied areas. Our results suggest that the origin and pathway of colonisation of the populations established in the three lagoons of Marchica, Merja Zerga and Khnifiss is again the result of the expansion of the species along the Mediterranean Sea and now the Atlantic coast of Africa.

Conclusion

The distributional pattern of the American crab *C. sapidus* in Morocco provides evidence that the species is thriving through the coastal ecosystems of Morocco, both on its Mediterranean and Atlantic coasts. The present records confirm that *C. sapidus* is well established in Morocco and has become a serious bycatch problem, as it clogs fishing gear. Therefore, in-depth studies of the bio-ecological traits of the species, its spread, and its potential ecosystem and socio-economic impacts are desirable for elaborating an action plan to manage this invasive crab, including assessing its potential as a new exploitable marine resource. In addition, advanced genetic analysis based on a large-scale sampling of native and introduced populations worldwide will provide relevant insights into the invasion history of the species, its pathways of introduction, and the evolutionary genetic changes following its establishment in a new environment.

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Authors' contribution

Mariam Oussellam (investigation and data collection, data analysis and interpretation, roles/writing – original draft), Abdelaziz Benhoussa (investigation and data collection, writing – review and editing), Antoine Pariselle (investigation and data collection, writing – review and editing), Imane Rahmouni (data analysis and interpretation, roles/writing – original draft), Meryem Salmi (investigation and data collection), Jean-François Agnèse (data analysis and interpretation, writing – review and editing), Mohamed Selfati (investigation and data collection, writing – review and editing), Najib El Ouamari (writing – review and editing), Hocein Bazairi (research conceptualization, roles/writing – original draft; writing – review and editing).

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Supplementary material

The following supplementary material is available for this article:

Table S1. Published first records per country of *Callinectes sapidus* Rathbun, 1896 (Decapoda: Brachyura: Portunidae) from the Mediterranean Sea, Black Sea, Sea of Azov and north-eastern Atlantic Ocean as well as the up-to-day records on the African Atlantic.

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