Short communication

On the occurrence of the invasive Atlantic blue crab *Callinectes sapidus* Rathbun 1896 (Decapoda: Brachyura: Portunidae) in Sicilian inland waters

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Abstract - The Atlantic blue crab *Callinectes sapidus* Rathbun 1896 is included among the worst invasive alien species in the Mediterranean Sea. Here we report the finding of the species in two Sicilian rivers, the Irminio and the Imera Meridionale, where it was collected up to 6 km distant from the river mouths. Although several records of the species are already available from Italy, this is the first evidence of the occurrence of this invasive crab this far from the coastline throughout the Country. In the light of the well-known impact of the Atlantic blue crab on the invaded water bodies, the monitoring of the species and appropriate mitigation strategies should be implemented in order to protect the threatened native biota of the Sicilian inland waters.

Key words: biological invasions, COI gene, crustaceans, DNA barcoding.

Riassunto - Sulla presenza del granchio blu atlantico *Callinectes sapidus* Rathbun 1896 (Decapoda: Brachyura: Portunidae) nelle acque interne siciliane.

Il granchio blu atlantico *Callinectes sapidus* Rathbun 1896 è incluso tra le peggiori specie aliene invasive per il mar Mediterraneo. Qui riportiamo il ritrovamento della specie in due fiumi siciliani, l'Irminio e l'Imera Meridionale, dove la specie è stata raccolta fino a 6 km dalle loro foci. Sebbene diversi record della specie siano già disponibili per l'Italia, questa è la prima prova della presenza di questo granchio invasivo così lontano dalla linea di costa in tutto il Paese. Alla luce del ben noto impatto del granchio blu dell'Atlantico sui corpi idrici invasi, auspichiamo l'implementazione del monitoraggio della specie e di appropriate strategie di mitigazione al fine di proteggere il biota nativo delle acque interne siciliane.

Parole chiave: crostacei, DNA barcoding, gene COI, invasioni biologiche.

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Callinectes sapidus Rathbun 1896 (Decapoda: Brachyura: Portunidae) is one of the 100 worst invasive alien species that occur in the Mediterranean Sea (Streftaris & Zenetos, 2006). The species is native to the western Atlantic Ocean, but it has now been introduced nearly worldwide (Windsor *et al*., 2019; Mancinelli *et al*., 2021). The Atlantic blue crab was introduced in the European seas in the 20th century, and it is currently widely distributed throughout the Mediterranean and Black Sea (Mancinelli *et al*., 2017; 2021, and reference therein) with its distribution range continuously expanding both westward (Lipej *et al*., 2018) and eastward (Snigirev *et al*., 2021). In Italy, *C. sapidus* is widespread in the Adriatic and Ionian Seas (Cerri *et al*., 2020), and recent reports indicate the occurrence of the species in the Tyrrhenian Sea and the Strait of Sicily as well (Mancinelli *et al*., 2021). In Sicily, the occurrence of the Atlantic blue crab is reported from open sea, brackish coastal lagoons, and estuaries (Falsone *et al*., 2020; Di Martino & Stancanelli, 2021; Mancinelli *et al*., 2021).

In the frame of a sampling campaign aimed at investigating the decapod crustacean fauna of Sicilian inland waters, 11 adult *C. sapidus* were collected on 22nd July 2021 and 1st September 2021 in the Imera Meridionale river (Licata, province of Agrigento; WGS84 geographical coordinates: 37.138833 N, 13.916907 E, 8 m a.s.l., four individuals) and in the Irminio river (Marina di Ragusa, province of Ragusa; 36.775803 N, 14.596793 E, 1 m a.s.l., seven individuals) (Fig. 1); moreover, several other individuals were observed but not captured in both localities. Individuals were collected by hand or using hoop traps baited with beef liver and mackerel fillet and identified *in situ* based on Williams (1974). One leg was excised from each caught individual and fixed *in situ* in 96% ethanol and then stored in freezer at -20 °C.

To confirm the morphology-based identification of the collected crabs and to assess their geographical origin, we amplified a fragment of the gene encoding the *cytochrome oxidase subunit I* (COI) in four specimens following the procedure described in Vecchioni *et al*. (2019a). The obtained PCR products were sequenced with an ABI 3130xL sequencer (Applied Biosystems®) by Macrogen Inc. (Madrid, Spain; https://dna.macrogen.com/eng/) and uploaded to the public database GenBank® under Accession Numbers (A.N.) OK513030-OK513033.

Overall, four new COI sequences belonging to *C. sapidus* were produced. In addition to our novel sequences, 228 *C. sapidus* sequences were downloaded from GenBank® and included in the analyses (see Fig. 2 and Table S1 for their A.N.). Following Vecchioni *et al*. (2019b), a distance-based tree was built to identify the putative geographical origin of the Sicilian *C. sapidus* individuals. The Sicilian sequences correspond to two publicly available haplotypes (i.e., A.N. KR030241, from the USA, and a haplotype found in Spain, A.N. KT282079, and Turkey, A.N. MG462529); both haplotypes belong to the "Lineage 1" described by Windsor *et al*. (2019), i.e., the one originating from the USA Atlantic coast and the Gulf of Mexico.

Interestingly, all the COI haplotypes to date observed in the Mediterranean area belong to the "Lineage 1" described by Windsor *et al*. (2019) and Öztürk *et al*. (2020) (see also Fig. 2 and Table S1). Therefore, the Mediterranean populations of the species, albeit likely subject to multiple independent introductions, originated from a single source area. However, the occurrence of several haplotypes suggests that the Mediterranean populations of the invasive Atlantic blue crab experienced a scarce founder effect, if any, which could be ascribed to the introduction of a high number of individuals. In order to better assess the diversity and origin of the Mediterranean invasive populations of the species, it is desirable to obtain more sequences from throughout its native distribution range.

The finding of several individuals of *C. sapidus* in the Imera Meridionale river, 6 km from the river mouth, was quite unexpected. The upstream occurrence of the species might be linked to the environmental features that characterize the river itself, namely its natural high salinity (ranging from 2000 to over 75000 µS/cm, at 20 °C, see Rapti-Caputo, 2010 and pers. obs.) and/or the short-term and severe floods during the rainy season followed by long periods of drought during spring and summer (Amore *et al*., 2002). Conversely, the occurrence of *C. sapidus* individuals in Irminio river just 400 m from the river mouth is in accordance with the evidence to date available for the species in Sicily (e.g., Falsone *et al*., 2020; Di Martino & Stancanelli, 2021).

Based on Mancinelli *et al*. (2021), this is the first evidence of the occurrence of the Atlantic blue crab this far upstream in Italian inland waters, where to date, its presence was known to occur only along the coastlines (Manci-

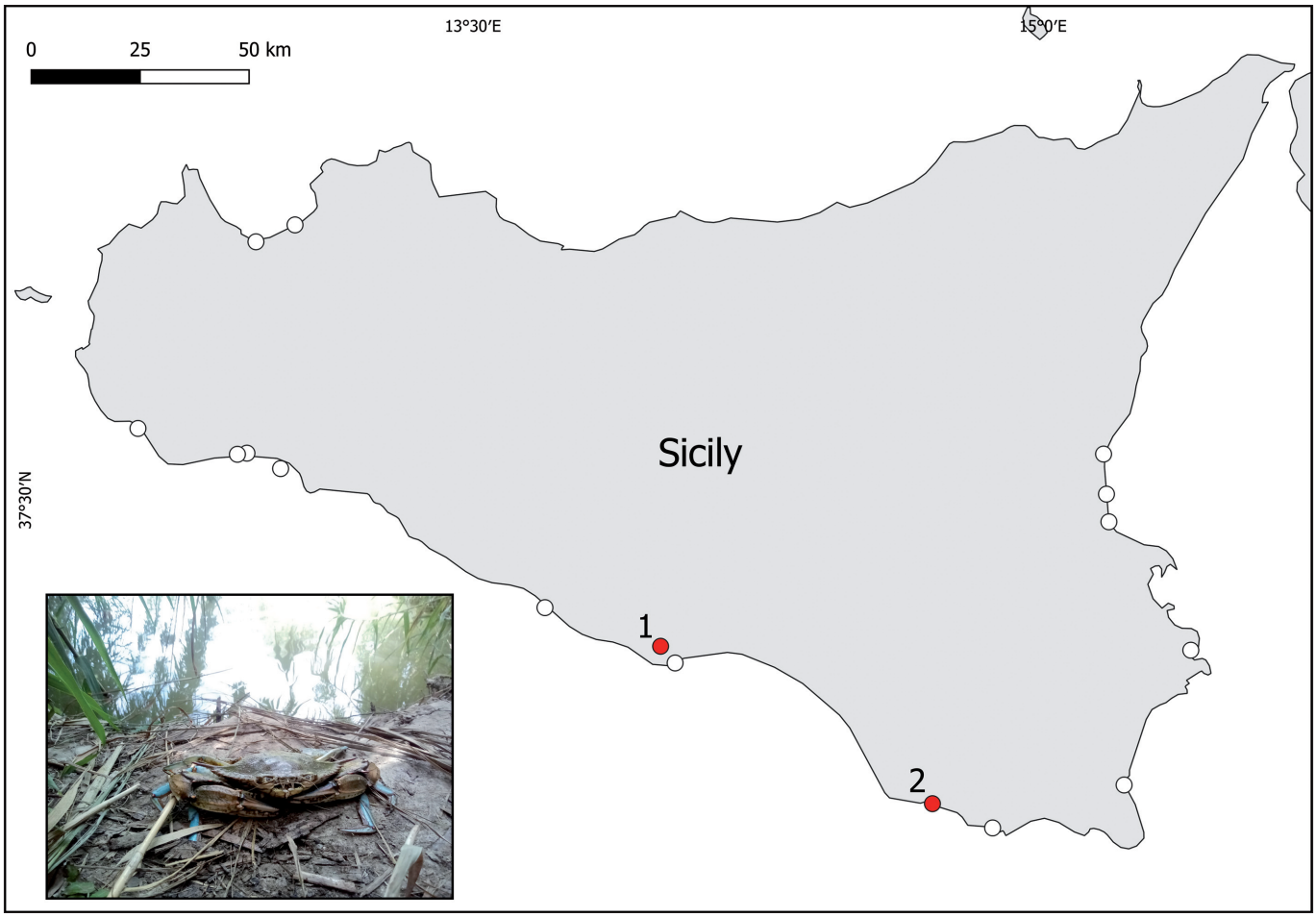

Fig. 1 - Location of the sampling sites. Red circles indicate the new Sicilian sites where *Callinectes sapidus* (inbox) was sampled; 1) Imera Meridionale river. 2) Irminio river. White circles indicate previous records of the species (see Mancinelli *et al.,* 2021 for further information). / Localizzazione dei siti campionati. I cerchi rossi indicano i nuovi siti siciliani dove è stato campionato *Callinectes sapidus* (nel riquadro); 1) fiume Imera Meridionale. 2) fiume Irminio. I cerchi bianchi indicano precedenti record della specie (vedi Mancinelli *et al.,* 2021 per ulteriori informazioni).

nelli *et al*., 2021). *Callinectes sapidus* is an opportunistic and aggressive predator and represents a serious threat to the marine native taxa (Falsone *et al*., 2020 and reference therein). The occurrence in inland waters, up to 6 km from the coast in the Imera Meridionale river, suggests that the impact of the species could now also be exerted on the biota of Sicilian inland water, which is already at risk due to environmental alterations and the occurrence and spread of other alien species (Marrone & Naselli-Flores, 2015; Naselli-Flores & Marrone, 2019; Vecchioni *et al*., 2022).

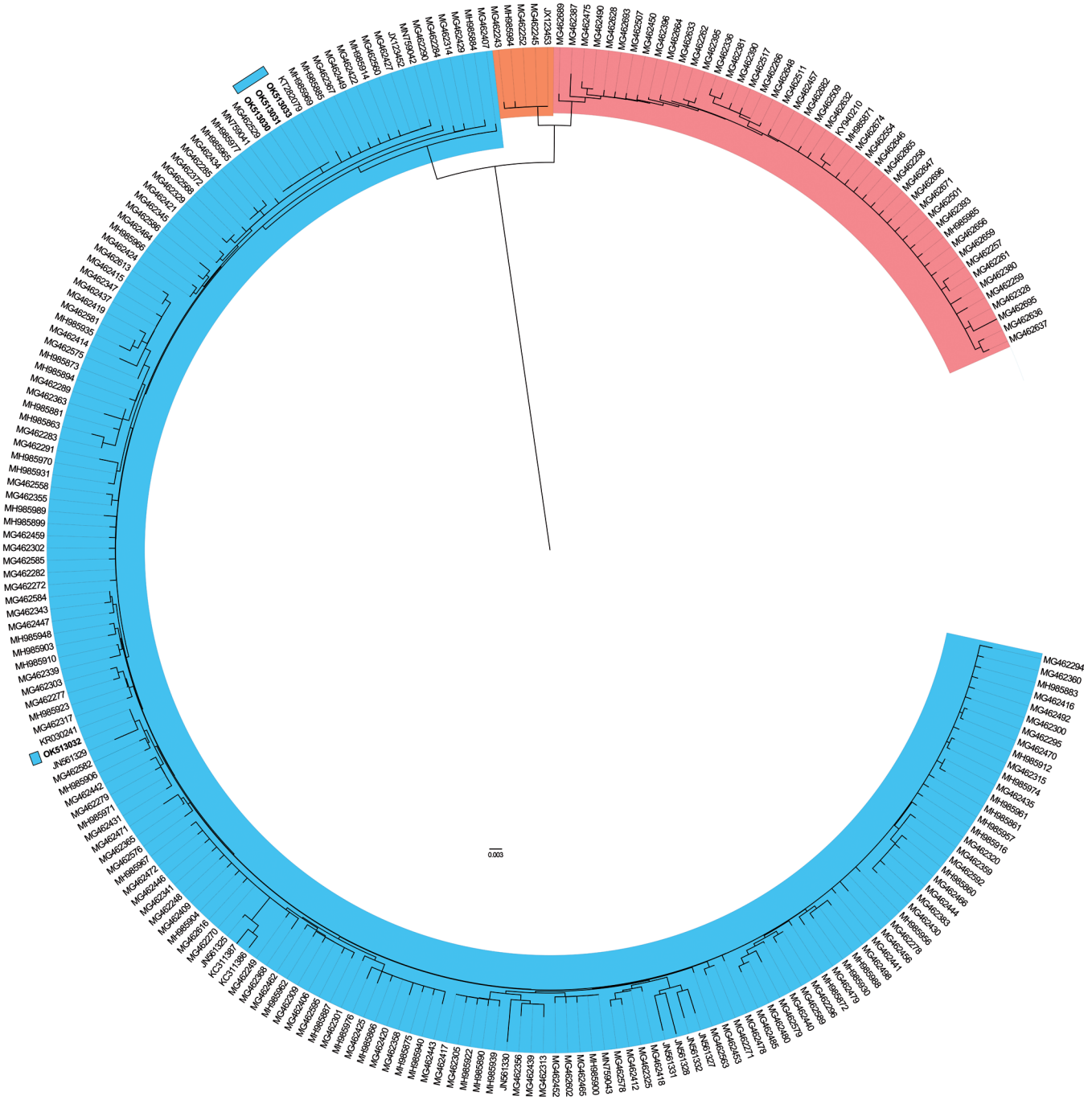

Fig. 2 - Neighbor-Joining tree based on a 659-bp long fragment of the mtDNA COI gene of *Callinectes sapidus* using Kimura-2-parameter distance model. The different clades are coloured according to their lineage as described by Windsor *et al.,* 2019: clear blue, Lineage 1 (northwestern Atlantic and Gulf of Mexico); pink, Lineage 2 (Caribbean region); orange, Lineage 3 (Brazil). Novel sequences are reported in bold. The analysed specimens are reported using the GenBank® Accession numbers listed also in Table S1. / Albero Neighbor-Joining basato su un frammento lungo 659-pb del gene mtDNA COI di *Callinectes sapidus*, utilizzando il modello di distanza "Kimura-2-parameter". I diversi cladi sono colorati secondo il loro lignaggio come descritto da Windsor *et al.,* 2019: blu chiaro, "Lineage 1" (Atlantico nord-occidentale e Golfo del Messico); rosa, "Lineage 2" (regione caraibica); arancione, "Lineage 3" (Brasile). Le sequenze nuove sono riportate in grassetto. Gli esemplari analizzati sono riportati utilizzando i numeri di accesso GenBank® elencati anche nella Tabella S1.

However, the absence of dedicated studies and the paucity of information available to date on the status of Sicilian inland water biota make an assessment of the impact of *C. sapidus* invasion difficult. Its possible impact on endemic species, e.g. the Sicilian pond turtle *Emys trinacris* Fritz et al. 2005 (Vecchioni *et al*., 2020), or on those with declining populations at regional level, e.g. the native river crab *Potamon fluviatile* (Herbst 1785) (Vecchioni *et al*., 2017; Marrone *et al*., 2020) is a major concern. Accordingly, it is crucial to identify the areas where mitigation strategies aimed at preventing impacts on the endangered native Sicilian inland water biota should be carried out.

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SUPPORTING INFORMATION / INFORMAZIONI SUPPLEMENTARI

Additional supporting information may be found online for this article. / Per questo articolo sono disponibili informazioni supplementari online.

S1 - List of the COI sequences used in the frame of this study with their GenBank® Accession Numbers. / Elenco delle sequenze COI utilizzate nell'ambito di questo studio con i rispettivi numeri di accesso GenBank®.