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ADDITIONAL RECORD OF THE PEA CRAB *PINNOTHERES BICRISTATUS* (BRACHYURA: PINNOTHERIDAE) IN THE ADRIATIC SEA

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ABSTRACT

The pea crab Pinnotheres bicristatus *lives in symbiosis with the saddle oyster* Anomia ephippium. *The first record in the Adriatic Sea dates to 2021, when one male specimen was observed in the Gulf of Trieste. This study reports additional findings from fouling samples collected in the port of Trieste as part of the Marine Strategy Framework Directive monitoring program. Seven specimens were identified based on morphological features, confirmed through CoxI DNA marker analysis, showing 100% similarity with GenBank reference sequences. This record represents the fourth occurrence of the species in the Mediterranean Sea and confirms its continued presence in the Adriatic. Due to the identification challenges in pinnotherids, its small sizes and the fact that its host, the saddle oyster, has no commercial value,* P. bicristatus *presence could have gone unnoticed in the past and its real distribution could be more widespread, resembling those of its host.*

Key words: *Pinnotheres bicristatus*, *Anomia ephippium*, DNA barcoding, CoxI, Gulf of Trieste

ULTERIORE SEGNALAZIONE DEL GRANCHIO PISELLO *PINNOTHERES BICRISTATUS* (BRACHYURA, PINNOTHERIDAE) NEL MARE ADRIATICO

SINTESI

Il granchio pisello Pinnotheres bicristatus *vive in simbiosi con l'ostrica a sella* Anomia ephippium. *La prima segnalazione nell'Adriatico risale al 2021, quando un esemplare maschio è stato osservato nel Golfo di Trieste. Lo studio riporta un'ulteriore segnalazione da campioni di fouling raccolti nel porto di Trieste durante il monitoraggio della Direttiva Quadro sulla Strategia Marina. Sette esemplari sono stati identificati tramite caratteristiche morfologiche, confermate dall'analisi del marcatore DNA CoxI, con una similarità del 100% rispetto alle sequenze di riferimento GenBank. Questa è la sua quarta segnalazione nel Mediterraneo e ne conferma la presenza nell'Adriatico. Per le difficoltà di identificazione nei Pinnotheridae, le sue piccole dimensioni e il fatto che l'ostrica ospite non ha alcun valore commerciale, la presenza di* P. bicristatus *potrebbe essere passata inosservata in passato e la sua reale distribuzione più ampia, simile a quella dell'ospite.*

Parole chiave: *Pinnotheres bicristatus*, *Anomia ephippium,* DNA barcoding, CoxI, Golfo di Trieste

INTRODUCTION

The family Pinnotheridae De Haan, 1833 consists of small brachyuran crabs that live in symbiosis/commensalism with other invertebrates, mainly bivalves (Schmitt et al., 1973). This family was originally represented by five species from European waters: *Nepinnotheres pinnotheres* (Linnaeus, 1758), *Pinnotheres ascidicola* Hesse, 1872, *Pinnotheres marioni* Gourret, 1887, *Pinnotheres pectunculi* Hesse, 1872, and *Pinnotheres pisum* (Linnaeus, 1767). However, Becker (2010) and Becker & Türkay (2010) established that *P. ascidicola* and *P. marioni* are synonyms of *N. pinnotheres*, reducing the number of recognized pinnotherid species in European waters to three: *N. pinnotheres*, *P. pisum*, and *P. pectunculi*. The African pea crab *Afropinnotheres monodi* Manning, 1993 was subsequently reported in European waters from the Gulf of Cádiz (SW Iberian Peninsula) (Subida *et al.,* 2011; Perez-Miguel *et al.,* 2019). More recently, *Pinnotheres bicristatus* Garcia Raso & Cuesta, 2019 was described from southern European waters (Cuesta *et al.*, 2019), bringing the number of known pinnotherid species in European waters back to five (Marco-Herrero *et al.*, 2020).

The common saddle oyster *Anomia ephippium* Linnaeus, 1758 appears to be the exclusive host of this species in the pallial cavity, although it has also been found once in the flat oyster *Ostrea edulis* Linnaeus, 1758 (Cuesta *et al.*, 2019; Marco-Herrero *et al.*, 2020; Cuesta *et al.*, 2024). The present record of the species from the port of Trieste follows its recent finding in the Adriatic Sea, documented in an underwater photograph by Mavrič & Vrezec (2024). Therefore, up to date, three species of the family Pinnotheridae are recorded in the Adriatic Sea: *N. pinnotheres*, *P. pisum* and *P. bicristatus*, whereas in the rest of the Mediterranean there are only two: *N. pinnotheres* and *P. pisum*. However, there are other two exceptions in the Mediterranean waters: the Alboran Sea where *A. monodi*, *P. pectunculi* and *P. bicristatus* are also present (Perez-Miguel *et al.*, 2019, Cuesta *et al.*, 2019), and the Catalan Sea where *P. bicristatus* was reported (Marco-Herrero *et al.*, 2020).

MATERIAL AND METHODS

In October 2024, seven specimens of *Pinnotheres bicristatus* were collected in the frame of the monitoring program for the Marine Strategy Framework Directive (MSFD - Directive 2008/56/EC), under Descriptor 2 (D2) "Non-indigenous species." Since 2017, semi-annual sampling of hard substrate macrobenthos was conducted by scraping a 33.3 × 33.3 cm square (0.1 m²), with a total of three samples at 2 m depth and three at 5 m. The monitoring station

Fig. 1: Trieste and the port area with the sampling station located in the port breakwaters.
Sl. 1: Trst in tržaško pristanišče z označeno lokaliteto vzorčenja na pristaniških valobranih.

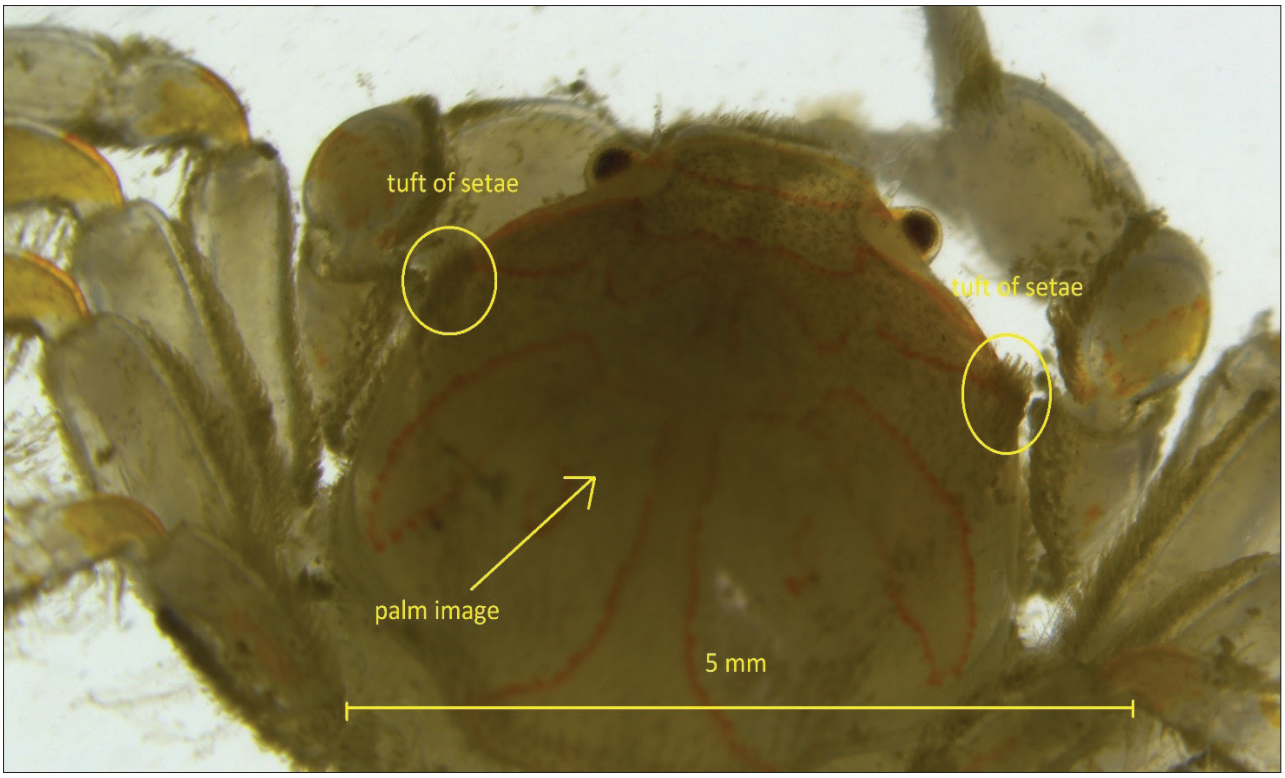

Fig. 2: Male specimen of** Pinnotheres bicristatus **sampled in the port of Trieste (photo Arpa FVG).
Sl. 2: Samček vrste** Pinnotheres bicristatus **iz vzorca v tržaškem pristanišču (slika Arpa FVG).

(45°37′28.2″ N, 013°44′13.8″ E) is located along the vertical wall of the breakwaters facing the industrial port of Trieste (Fig. 1).

Specimens were identified based on morphological features following the description in Cuesta *et al.* (2019). Total genomic DNA was extracted from 20 mg of muscle tissue from the propodus of one specimen using the PCRBIO Rapid Extract PCR Kit (PCR Biosystems, London, UK), following the manufacturer's instructions. PCR amplification was performed using PCRBIO Taq Mix Red (PCR Biosystems, London, UK) with an annealing temperature of 50 °C and 400 nM of CoxI primers LCO1490 and HCO2198, as designed by Folmer *et al.* (1994). The final sequence obtained was compared with Pinnotheridae CoxI sequences available in the GenBank database.

RESULTS AND DISCUSSION

The specimens of *Pinnotheres bicristatus* were identified by two unique carapace characteristics: a pair of dorso-anterolateral tufts of curved setae resembling tubercles and an orange-reddish "palm-tree" marking covering the dorsal surface (Fig. 2) (Cuesta *et al.*, 2019). Species identity was also confirmed using the CoxI DNA marker, which showed 100% similarity to *P. bicristatus* sequences in GenBank.

This record represents the fourth occurrence of the species in the Mediterranean Sea. The first was from the Alboran Sea (Cuesta *et al.*, 2019), the second from the Catalan Sea (NW Mediterranean) (Marco-Herrero *et al.*, 2020), and the third from the Adriatic Sea on September 5, 2021, when a specimen was photographed at a depth of approximately 9 m in Slovenian waters in the Gulf of Trieste (Mavrič & Vrezec, 2024).

During previous semi-annual samplings of hard substrates, Pinnotheridae specimens were collected in July 2021 (two individuals at -2 m), followed by two others in October 2021 at -5 m and one in October 2022 at -2 m. These specimens were 'soft' females and initially identified simply as *Pinnotheres* sp., as their morphological characteristics did not match those of *Nepinnotheres pinnotheres* and *Pinnotheres pisum,* common species in the Gulf of Trieste. Unfortunately, these specimens were not preserved, but the presence of *P. bicristatus* in the study area prior to the present record is plausible.

Despite the permanent and abundant presence of *Anomia ephippium* and *Ostrea edulis* in the breakwater of the Trieste port, sampled since 2017, the first pinnotherid specimens only appeared in 2021, coinciding with the record by Mavrič & Vrezec (2024).

The first description of Pinnotheridae in the Adriatic Sea dates to Scopoli (1763), who described *Cancer nu-*

trix as a crab living inside *Ostrea edulis* (Cuesta *et al*., 2019). De Wulfen (1791) described *Cancer minutus* from the Adriatic Sea, a species very similar to *C. nutrix* and living inside *O. edulis* and other bivalves (Ng *et al*., 2008). In this context, Cuesta *et al*. (2019) assumed that both taxa belong to *P. pisum*, because it is present in the Adriatic Sea and inhabits several different species of hosts, including *O. edulis*, and moreover there are not extant type specimens of these two species that would allow checking their identity.

Cuesta *et al*. (2019) did not rule out the possibility that *P. bicristatus* could be one of those described by Scopoli (1763) and de Wulfen (1791). Thus, either this species has remained hidden even though *A. ephippium* has always been an abundant bivalve on both soft and hard bottoms of the Adriatic Sea, or we were unable to identify it prior to the description by Cuesta *et al*. (2019). Also, the saddle oyster has no commercial interest, therefore it is not commonly sampled and examined, and for this reason the presence of *P. bicristatus* could have gone unnoticed. Besides, the pinnotherid identification is not easy due to their small sizes and changes in the morphology depending on the stage of their life cycle. Therefore, samples examined by non-specialists may have been attributed to the more common species like *P. pisum*.

Although *O. edulis* is a bivalve of commercial interest, highly consumed and extensively studied, until now only one specimen of *P. bicristatus* has been recorded inside a flat oyster (Marco-Herrero *et al*., 2020). Probably it was an accidental host, surely due to a high infestation rate of *A. ephippium* in the area with consequent problems for preferential host availability.

The real distribution of *P. bicristatus* may mirror that of its host, *A. ephippium,* that was reported from Iceland to Angola (in the Atlantic Ocean) and in the Mediterranean, Marmara and Black seas (MolluscaBase eds., 2025). Although other parameters, as temperature, could determine the pea crab distribution and therefore does not need to completely overlap with that of its host (Cuesta *et al.,* 2024). In conclusion, we can argue that *P. bicristatus* is not an exotic species in the Adriatic Sea, maybe it could be considered as unreported or confounded with *P. pisum*.

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DODATNI ZAPIS O POJAVLJANJU STRAŽNE RAKOVICE VRSTE *PINNOTHERES BICRISTATUS* (BRACHYURA: PINNOTHERIDAE) V JADRANSKEM MORJU

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POVZETEK

Stražna rakovica vrste Pinnotheres bicristatus *je manjša rakovica, ki sobiva s sedlasto ostrigo* Anomia ephippium. *Prvi zapis o pojavljanju te vrste izvira iz leta 2021, ko je bil v Tržaškem zalivu opažen en samec. Avtorji poročajo o dodatnih primerih pojavljanja te vrste iz vzorcev obrasti, pobrane v tržaškem pristanišču v okviru programa spremljanja Okvirne direktive o morski strategiji. Na podlagi morfoloških značilnosti so identificirali 7 primerkov in to potrdili še z analizo markerjev CoxI DNA, ki so pokazali 100% ujemanje s sekvencami v bazi GenBank. Ta zapis predstavlja četrti primer pojavljanja vrste v Sredozemskem morju in potrjuje njeno prisotnost v Jadranu. Zaradi težav pri določevanju stražnih rakovic, njihovih majhnih velikosti in dejstva, da sedlasta ostriga kot njihov gostitelj nima komercialne vrednosti, je bila vrsta* P. bicristatus *v preteklosti spregledana, njena realna razširjenost pa bi lahko bila večja oziroma podobna razširjenosti njenega gostitelja.*

Ključne besede: *Pinnotheres bicristatus*, *Anomia ephippium*, DNA barcoding, CoxI, Tržaški zaliv

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