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Rapid Communication

The brown shrimp, *Penaeus aztecus* Ives, 1891, reaches the Iberian Peninsula Mediterranean coasts

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Abstract

The penaeid shrimp *Penaeus aztecus* Ives, 1891 has been collected off the Catalan coast for the first time. This represents the first report of the species for the Iberian Peninsula waters. Its morphological description, as well as the DNA sequenced fragment, agree with the descriptions assigned to *Penaeus aztecus*. This constitutes the first record of the species in the Spanish Mediterranean coast and the confirmation the species westward expansion along the Mediterranean Sea probably been drifted from the later invasion in the Gulf of Lions.

Key words: Western Mediterranean, allochthonous species, non-indigenous species, Catalan coast, sequencing

Introduction

The Mediterranean Sea has historically been one of the busiest seas in the world, and is currently one of the most sensitive areas for the appearance of non-indigenous (NIS) species (Zenetos et al. 2010). This generates great concern about the loss of biodiversity (Boudouresque 2004). More than 500 alien species have been reported in the Mediterranean Sea (Galil 2007, 2009; Zenetos et al. 2010). Their monitoring plays an important role in controlling the expansion of the species and contributes to the adoption of management measures (Zenetos and Galanidi 2020).

In the western Mediterranean, different species of allochtonous decapod crustaceans have been recently reported, such as *Callinectes sapidus* Rathbun, 1896 (Castejón and Guerao 2013) and *Charybdis* (*Charybdis*) *feriata* (Linnaeus, 1758) (Abelló and Hispano 2006; Colmenero et al. 2019). The acquisition of knowledge on the occurrence, dispersal and ecological role of non-native species is very important to try to know and control the ecological balance among the species and ecological systems, especially so concerning species with high fecundity and previous evidence of causing relevant damage in the ecosystems they settle in. Depending on the life history characteristics of the incomers, their trophic role, and the effects they can generate on invaded

ecosystems (Hänfling et al. 2011), trouble and damage may take place if they negatively affect sensitive habitats and/or fisheries (Clavero et al. 2022). On the other hand, in the case of invasive species with putative fisheries interest, they may become a new source of income for local fisheries and become not only an ecological problem but also a sociological and socioeconomical issue (Mancinelli et al. 2017).

Penaeus aztecus Ives, 1891, is a penaeid shrimp, native to the Western Central Atlantic. Its original present distribution is limited to the East coast of the United States and the Gulf of Mexico (Cook and Lindner 1970). The habitat of this species is estuarine and coastal waters, with greater presence in muddy bottoms, and higher densities between 27–55 m depth (Williams 1984). As in adults, in larval stages, the optimal temperature ranges around 28 degrees Celsius and as with other members of the Penaeidae family, *P. aztecus* presents variations in the salinity conditions of the environment according to its stage of development (Uludağ and Aktaş 2021). The first record of *P.aztecus* in the Mediterranean Sea was obtained off Antalya, Turkey, in December 2009 (Deval et al. 2010). It was later reported along the Mediterranean coast of Turkey, Aegean Sea, Ionian Sea, Adriatic Sea, Tyrrhenian Sea, Gulf of Lions, Black Sea and Libya (Galil et al. 2017; Özcan et al. 2019; Gönülal and Türetken 2019; Abdulrraziq et al. 2021) (Figure 1). The present study confirms the expansion of the species through the western Mediterranean, previously detected in the Gulf of Lions (Galil et al. 2017).

Materials and methods

The first specimen we recorded, was a pale, large, unmarked penaeid shrimp, clearly attributed to the genus *Penaeus*. It was collected on February 21, 2022, on muddy substrates by the trawling vessel "Maireta IV" off Barcelona (41.33°N; 2.37°E), at a depth of 51 m (Figure 1). Taxonomic identification was carried out according to the descriptions and keys in Perez-Farfante (1969, 1988). All measurements were taken with a digital caliper with a 0.01 mm accuracy. The weight was taken with a balance, with an accuracy of 0.001 g. The specimen (Figure 2) was deposited at the Marine Biological Reference Collections (CBMR) of the Institut de Ciències del Mar (ICM-CSIC, Barcelona, Spain) under the accession code ICMD 13020101 (Guerrero et al. 2023).

We dissected one pleopod of this specimen to determine species identification using molecular tools. We stored the sample in 90° high quality ethanol (Supelco 1.11727) at −80 °C until analyses were performed. From the extracted pleopod of this individual, we obtained 29.6 ng μL^{-1} of DNA. DNA isolation was carried out using the E.Z.N.A Tissue DNA kit (Omega Bio-tek), strictly following the manufacturer's instructions. DNA was resuspended in a final volume of 100 µL. A negative control that contained the sample was included in the DNA isolation procedure to check for contamination. DNA was quantified using the Qubit High Sensitivity dsDNA Assay (Thermo FisherScientific). The mitochondrial COI (mitochondrial

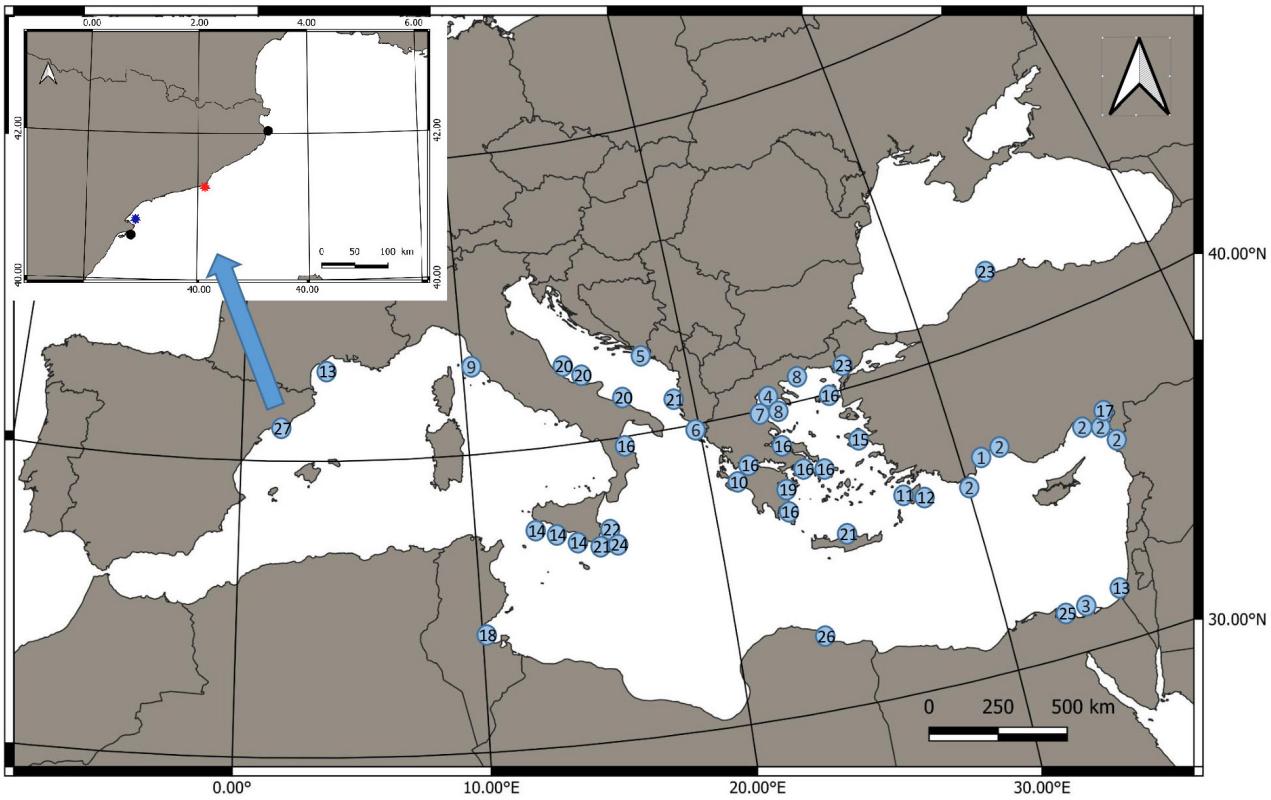

Figure 1. Location of the presence of the *Penaeus aztecus* Ives, 1891 in the Mediterranean Sea, in chronological order: 1 – Deval et al. 2010; 2 – Bilecenoglu et al. 2013; 3 – Sadek et al. 2018; 4 – Nikolopoulou et al. 2013; 5 – Marković et al. 2013; 6 – Kapiris et al. 2014; 7 – Kevrekidis 2014; 8 – Minos et al. 2015; 9 – Cruscanti et al. 2015; 10 – Crocetta et al. 2015; 11 – Kondylatos and Corsini-Foka 2015; 12 – Kondylatos et al. 2020; 13 – Galil et al. 2017; 14 – Scannella et al. 2017; 15 – Bakır and Aydin 2016; 16 – Mytilineou et al. 2016; 17 – Özcan et al. 2019; 18 – Ben Jarray et al. 2019; 19 – Kapiris and Minos 2017; 20 – Zava et al. 2018; 21 – Kampouris et al. 2018; 22 – Stern et al. 2019; 23 – Gönülal and Türetken 2019; 24 – Katsanevakis et al. 2020; 25 – El-Deeb et al. 2020; 26 – Abdulrraziq et al. 2021; 27 – Present study (In red the specimen collected in Barcelona. In blue the specimens collected in L'Ametlla de Mar. In black circles the presences detected by fishermen), for details see Supplementary material Table S1.

Figure 2. Original photograph of the recently captured specimen (by David Albiol).

cytochrome c oxidase subunit I) region was amplified by polymerase chain reaction (PCR). A 664-base pair (bp) fragment of the COI gene was amplified using the primers COIAL2o_mod (5' ACG CAA CGATGA TTA TTY TCT AC 3') and COIAH2m_mod (5' GAC CRA AAA ATC ARAATA ART GTT G 3'), modified from Mantelatto et al. (2016). The PCR reaction

was carried out in a final volume of 12.5 µL, containing 3.13 µL of Supreme NZYTaq Green PCR Master Mix (NZYTech), 0.5 µM of each primer, 1.25 µL of the template DNA solution, and PCR-grade water up to 12.5 µL. The thermal cycling conditions were as follows: an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 47 °C for 45 s, extension at 72 °C for 45 s; and a final extension step at 72 °C for 7 min. A negative control that contained no DNA was included in every PCR round to check for cross-contamination. The PCR products were run on a 2% agarose gel stained with GreenSafe (NZYTech), and imaged under UV light to verify the amplicon size. The PCR products were bi-directionally sequenced on a ABI 3730xl DNA Analyzer (Thermo Fisher Scientific), using the PCR primers.

Electropherogram analysis was conducted using Geneious 11.1.5 program. During the analysis, the primer annealing regions and the low quality regions at both ends of eachelectropherogram were trimmed (error probability limit of 0.03). Sequence reads were manually checked for sequencing errors or ambiguous base calls. Ambiguities and heterozygous positions were coded using the IUPAC ambiguity code (e.g., K:G or T). The final edited COI sequence was translated into amino acids (genetic code = invertebrate mitochondrial) to detect stop codons, which might indicate the amplification of nuclear mitochondrial pseudogenes (NUMTS). The sample was identified to the lowest possible taxonomic level by comparing the sequences obtained against the NCBI's (National Center for Biotechnology Information) Nucleotide database using the BLASTn server, and following the best close match criteria, *sensu* Meier et al. (2006). In addition, the COI sequence was also compared to the COI records available in the Barcode of Life Data System (BOLD) database, using the BOLD Identification Engine. When the query sequence shared similarly high identity values with reference sequences belonging to different species, the sample was only identified to the lowest common taxonomic level (e.g., genus).

Some months later, on October 19th, 2022 we collected, within the frame of a fisheries monitoring programe (ICATMAR), four additional individuals from the south of Catalonia, in the fishing area of the town of L'Ametlla de Mar (40.81°N; 0.82°E), at a depth of 29 m, on muddy substrates (Figure 1). These specimens were collected by the trawling vessel "Peret i Paquita". Sex, carapace length (distance between the ocular cavity and the distal end of the cephalothorax) and weight measurements were taken for all samples.

Results

The first individual, collected off Barcelona, was an adult male, weighed 23.13 g and had a carapace length of 31.74 mm. All measurements obtained in the identification are shown in Table 1.

The sequencing results showed that the COI 624 bp fragment sequenced presented 100% nucleotide identity with the accession number KU958164.1

Table 1. Measurements (in mm) taken from the *P. aztecus* collected in Barcelona

Carapace length	31.74
Gastrofrontal sulcus broad	2.30
Gastrofrontal sulcus length	6.36
Cervical sulcus	6.86
Hepatic carina	11.92
Length of antennular flagellum	9.50
Length of antennular pedunculum	18.90
Scaphocerite length	23.36
Scaphocerite maximun width	8.74
Length of sixth abdominal segment	17.59

in the reference databases, and was therefore assigned to the species *Penaeus aztecus* Ives, 1891.

Four more individuals were later collected further southwest off L'Ametlla de Mar. They were four adult females, had a carapace length of 43–47 mm, and weights of 61–77 g. All these female specimens showed advanced stages of maturity, with a greenish coloration in the gonads.

Discussion

The presence of *P. aztecus* was confirmed along the Catalan coasts with both morphological and molecular methods. All morphological characteristics coincided with those reported in Perez-Farfante (1969, 1988). Concerning molecular analyses, *P. aztecus* samples in the Mediterranean Sea were previously reported by Galil et al. (2017) and El-Deeb et al. (2020), showing the COI fragment with the accession number KU958164 as in the present study.

The presence of *P. aztecus* in the Catalan coast represents the first record of the species along the Iberian Peninsula Mediterranean coasts, and the confirmation of its westward expansion along the whole Mediterranean Sea (Özcan et al. 2019; Abdulrraziq et al. 2021). According to fishermen, scattered individuals of this species are irregularly captured along the Catalan coast since 2021 (Figure 1), along their usual trawling paths, together with other captures of the autochtonous congeneric species *Penaeus kerathurus* (Forskål, 1775).

Occurrences of *P. aztecus* in the Mediterranean Sea were previously reported in other areas, such as the eastern and central Mediterranean (Deval et al. 2010; Galil et al. 2017). As happened in other areas, its presence and expansion may be underestimated (Ugarković and Crocetta 2021). Kampouris et al. (2018) reported, for this species, wide bathymetric ranges and a rapid expansion. The effects of this expansion, especially on the local fauna are yet unknown, but they could act as a vector of the ectoparasitic bopyrid isopod *Epipenaeon ingens* (Deval and Koçanci 2021). This expansion could have a negative effect on the congeneric, native species *P. kerathurus*, but so far, no effects have yet been reported. However, the important fishing pressure to which *P. kerathurus* is subjected may facilitate the establishment of *P. aztecus* (Kevrekidis 2014).

The authors of the first report of *P. aztecus* in the Mediterranean Sea (Deval et al. 2010; Nikolopoulou et al. 2013) reported ballast water as the main entry putative vector in the Mediterranean, and indicated that oceanic transport of larvae influenced its expansion. Other authors report that the occurrence of contemporaneous citations in different areas (Figure 1) cannot only be explained by this route of entry and suggest that some reports may be due to illegal farming (Cruscanti et al. 2015; Galil et al. 2017; Abdulrraziq et al. 2021). In relation with the individuals captured in the Catalan sea (present study), they probably originated from settled individuals from the Gulf of Lions through the Northern Current (Clavel-Henry et al. 2020).

Many of the fishermen near the influence of the Ebro Delta have confirmed to the authors that they accidentally catch *P. aztecus* mixed with *P. kerathurus*. These catches have taken place since 2021. Since, as can be seen in the specimens we presently report, adult females have been found with advanced stages of gonad maturation, this would indicate that the species thrives in the area, thus showing a full population establishment in the area. The environmental conditions present in the Ebro Delta with high temperatures and relatively low salinity may favor the establishment of *P. aztecus* (Haas et al. 2001), as already shown for other recently settled non-indigenous species, such as the American Blue Crab *C. sapidus* (Clavero et al. 2022).

The biological and fishery study of the population, together with the monitoring of fisheries catches should provide a more solid understanding of the population evolution and the effects on the local fauna, especially so in relation with *P. kerathurus*.

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Authors' contribution

RS designed the study, collected the samples, performed the laboratory work, led the writing of the manuscript with contributions from all authors. GR contributed to design the study, led genetic analysis, performed the laboratory work, contributed to the writing of the manuscript. PA contributed to design the study, data interpretation, and writing.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Specimens of *Penaues aztecus* collected at Western Mediterranean.

This material is available as part of online article from:
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