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Updating the hosts and distribution range of the pea crab *Pinnotheres bicristatus* (Brachyura: Pinnotheridae)

Elena MARCO-HERRERO[1], Eve GALIMANY[1], Pere ABELLÓ[1], Jose A. CUESTA[2], Pilar DRAKE[2] and Montserrat RAMÓN[1]

[1] Institut de Ciències del Mar ICM-CSIC. Passeig Marítim de la Barceloneta, 37-49, 08003, Barcelona, Spain
[2] Instituto de Ciencias Marinas de Andalucía ICMAN-CSIC. Avda. República Saharaui, 2, 11519 Puerto Real, Cádiz, Spain

Corresponding author: elena.marco@ieo.es

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Abstract

Small crabs belonging to the family Pinnotheridae are characterized by living mainly inside bivalves. In European waters the recently described pea crab *Pinnotheres bicristatus* increased to five the known number of pinnotherid species. The known distribution of *P. bicristatus* encompassed the area between the Gulf of Cádiz (NE Atlantic) and the western Alboran Sea (SW Mediterranean). The species has so far been reported to occur in the pallial cavity of the common saddle oyster, *Anomia ephippium*, between 11 and 52 m depth. Over a period of two years, a total of 43 specimens of *P. bicristatus* were collected in two different projects/surveys along the Mediterranean coasts of the Iberian Peninsula, thus enlarging its known occurrence to the eastern Alboran Sea and the NW Mediterranean at depths of between 37 and 71 m. We also report a new bivalve host for the species, the flat oyster, *Ostrea edulis,* and provide population structure information of these new records. As a conclusion, this study considerably extends the known geographical distribution of *P. bicristatus* in latitude, longitude and depth.

Keywords: Alboran Sea; *Anomia ephippium*; Catalan Sea; Mediterranean; *Ostrea edulis.*

Introduction

Pinnotheridae De Haan, 1833 is a brachyuran family of small crabs that live in symbiosis/commensalism with other invertebrates, bivalves being their main hosts (Schmitt *et al.*, 1973). According to Udekem d'Acoz (1999), Pinnotheridae were represented in European waters by five species: *Nepinnotheres pinnotheres* (Linnaeus, 1758), *Pinnotheres ascidicola* Hesse, 1872, *Pinnotheres marioni* Gourret, 1887, *Pinnotheres pectunculi* Hesse, 1872 and *Pinnotheres pisum* (Linnaeus, 1767). Later, Becker (2010) and Becker & Türkay (2010) examined the adult morphology of European pinnotherids and resolved that *P. ascidicola* and *P. marioni* were junior synonyms of *N. pinnotheres*, thus restricting the European pinnotherid species to only three, *N. pinnotheres*, *P. pisum* and *P. pectunculi*. Subida *et al.* (2011) reported for the first time the African pea crab *Afropinnotheres monodi* Manning, 1993 in the Gulf of Cádiz (SW Iberian Peninsula). For these four pinnotherid species, Perez-Miguel *et al.* (2019) provided a review of their distribution, hosts, larval stages and DNA markers.

Recently, a new species of pea crab, *Pinnotheres bicristatus* Garcia Raso & Cuesta, 2019, was described, thus increasing to five the known number of pinnotherid species in European waters. Previously, some pinnotherid larvae identified by DNA barcodes as belonging to a yet unknown species of the genus *Pinnotheres* Bosc, 1801 had been reported from plankton samples collected along the coast of Doñana National Park (Gulf of Cádiz, SW Spain) (Marco-Herrero *et al.*, 2018). Later, these authors managed to match DNA barcodes of adult crabs they had collected with those of the above larvae. The new species, *P. bicristatus*, was then morphologically described on the basis of the adult morphology (Cuesta *et al.*, 2019). Until now, the known distribution of this pea crab encompassed the Gulf of Cádiz (NE Atlantic) and the westernmost Alboran Sea (SW Mediterranean), with the common saddle oyster, *Anomia ephippium* Linnaeus, 1758, being the only known host species. Depths of *P. bicristatus* occurrence ranged between 11 and 52 m (Cuesta *et al.*, 2019).

The study of species distribution is important for understanding ecological and evolutionary processes and for managing global change (Jetz *et al.*, 2012). However, knowledge of the spatial distribution of species is much less developed than knowledge of other environmental datasets (Jetz *et al.*, 2012). In the case of pea crabs, studying the distribution patterns and hosts is crucial because

pea crabs are symbionts of bivalves and are sometimes considered parasites, which may cause problems for bivalve aquaculture and fisheries through consumer complaints and biosecurity concerns (Hutson & Cain, 2019). In addition, the association of pea crabs with bivalves has sometimes been considered to negatively contribute to the preservation of healthy bivalve resources (Mena *et al.*, 2014). Thus, investigating the relationship between Pinnotheridae and their hosts will help to establish best management plans for bivalve populations. The aim of the present study is to report a new bivalve host for *P. bicristatus* and to expand its known geographical distribution to the eastern Alboran Sea (SW Mediterranean) and the Catalan Sea (NW Mediterranean).

Materials and Methods

Samples were obtained from two different surveys: RASTELL and MEDITS. The aim of the RASTELL project was to assess the status of the fishery targeting benthic commercial molluscs along the coasts of Catalonia (NW Mediterranean), including Vilanova i la Geltrú, a village SW of Barcelona. Samples were collected on board commercial fishing boats using a bottom-towed dredge, locally known as *rastell*, from October 2016 to September 2017. Details of the methodology may be found in Galimany *et al.* (2019). In summary, this bottom-towed dredge (6-8 m long) consists of a bag mounted on a rigid rectangular metal frame (2 m wide and 40 cm high). The upper part of the bag has netting, whereas the lower part has metal chains. Total catch was transferred to the ICM-CSIC laboratory, where all the organisms were sorted and identified to species level.

In the MEDITS_ES2018 survey, the samples were collected along the Mediterranean coasts of the Iberian Peninsula. The specifications of the MEDITS surveys may be found in Bertrand *et al.* (2002). The gear used is a bottom trawl designed for experimental fishing for scientific purposes with a 16 m width and a 2 m vertical opening (Fiorentini *et al.*, 1999). MEDITS surveys are aimed at quantitatively assessing fauna, biomass, density and population parameters of the demersal fishery target species throughout the Mediterranean Sea (Bertrand *et al.*, 2002) (Fig. 1, Table 1). For each sampling station, all bivalve specimens were sorted, identified, counted and weighed on board.

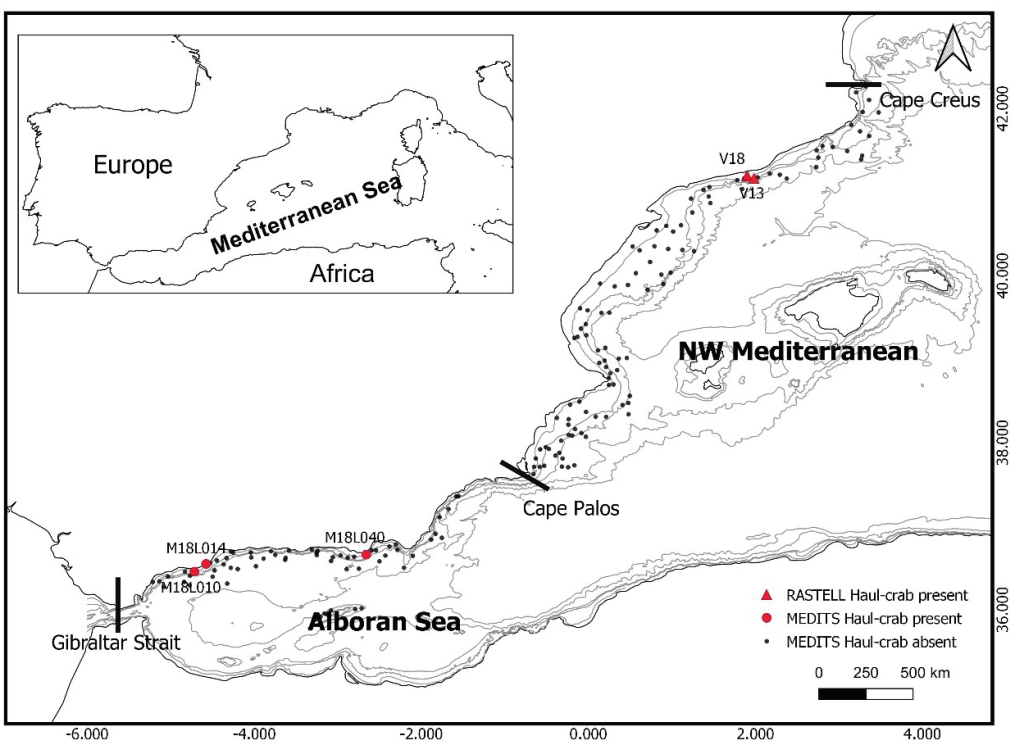

Fig. 1: Map of the study area showing the sampling points in the Alboran Sea and NW Mediterranean. The 50-100-150-200 m isobaths are shown.

Table 1. Information relative to the collection of pea crabs *Pinnotheres bicristatus*. Haul codes as in Figure 1.

Area	Haul code	Latitude	Longitude	Depth (m)	Survey	Date
NW Mediterranean	V13	41.198° N	1.8986° E	38	RASTELL	02/07/2017
NW Mediterranean	V18	41.198° N	1.9015° E	37	RASTELL	17/03/2017
Alboran Sea	M18L010	36.455° N	-4.7168° E	71	MEDITS	29/04/2018
Alboran Sea	M18L014	36.548° N	-4.5783° E	48	MEDITS	30/04/2018
Alboran Sea	M18L040	36.661° N	-2.6608° E	52	MEDITS	06/05/2018

On this cruise, all bivalve species, or a representative sample, were frozen on board and later examined in the laboratory (ICMAN-CSIC and ICM-CSIC), where they were measured and inspected for the presence of pea crabs in their pallial cavity. In order to account for any geographical differences, we divided the study area into two main sectors: the Alboran Sea, between Gibraltar Strait and Cape Palos, and the NW Mediterranean, between Cape Palos and Cape Creus, according to previous biogeographical results obtained in the area (Abelló *et al.*, 2002).

Four demographic categories were assigned to the collected pea crabs: (1) "hard" males; (2) "hard" females (both hard males and females are the swimming phase of pea crabs and have their ambulatory legs covered with natatory setae); (3) "soft" females (the sedentary phase with soft uncalcified carapace and ambulatory legs without natatory setae); and (4) ovigerous soft females (Cuesta *et al.*, 2019; Drake *et al.*, 2014). Males and hard females of *P. bicristatus* can be differentiated from those of other pinnotherid species by two singular characters on their carapace: a pair of dorso-anterolateral tufts of curved setae that resemble two tubercles and the orange-reddish 'palm tree' marking covering their dorsal surface. Soft females are characterized by the subtrapezoidal shape of their carapace and by the dactyli of the third pereiopods, which are appreciably longer than the dactyli of other pereiopods (Cuesta *et al.*, 2019).

All collected pinnotherid crabs were preserved in ethanol for further molecular identification, demographic observations and morphometric measurements. Carapace length (CL=distance from the rostrum to the posterior margin of the carapace) and width (CW=maximum carapace width) were measured (accuracy 0.1 mm) under a stereomicroscope provided with a calibrated ocular micrometer. Photographs of crabs were taken using a smartphone mounted on a stereomicroscope (Fig. 2). Muscular samples were collected for identification by molecular techniques.

For the examined bivalves, maximum shell length and width were measured along the anterio-posterior (SL) and dorso-ventral (SW) axes to the nearest 0.1 mm with a Vernier caliper.

All the specimens of *P. bicristatus* collected during the RASTELL and MEDITS surveys and one specimen of the host species *Anomia ephippium* and *Ostrea edulis* Linnaeus, 1758 were deposited in the Biological Collections of Reference of the Institut de Ciències del Mar (ICM-CSIC) in Barcelona under accession numbers ICMD002662 – ICMD002666 (*P. bicristatus,* RASTELL), ICMD002667 – ICMD002669 (*P. bicristatus*, MEDITS), ICMB000135 (*Ostrea edulis,* RASTELL) and ICMCBR000375 (*Anomia ephippium,* RASTELL).

Identification of Pinnotheres bicristatus *by DNA marker*

Total genomic DNA was extracted from pereiopod muscle tissue following a modified Chelex 10% protocol by Estoup *et al.* (1996). Partial sequences of the nuclear H3 gene were amplified. The cycling conditions of the polymerase chain reaction (PCR), the length of the sequence obtained and the primers used were the same as in Perez-Miguel *et al.* (2019). PCR products were sent to Stab-Vida laboratories to be purified and to apply bidirectional sequence. Sequences were edited using the Chromas software version 2.0. The final DNA sequences obtained were compared with those from Pinnotheridae available in the GenBank database for identification.

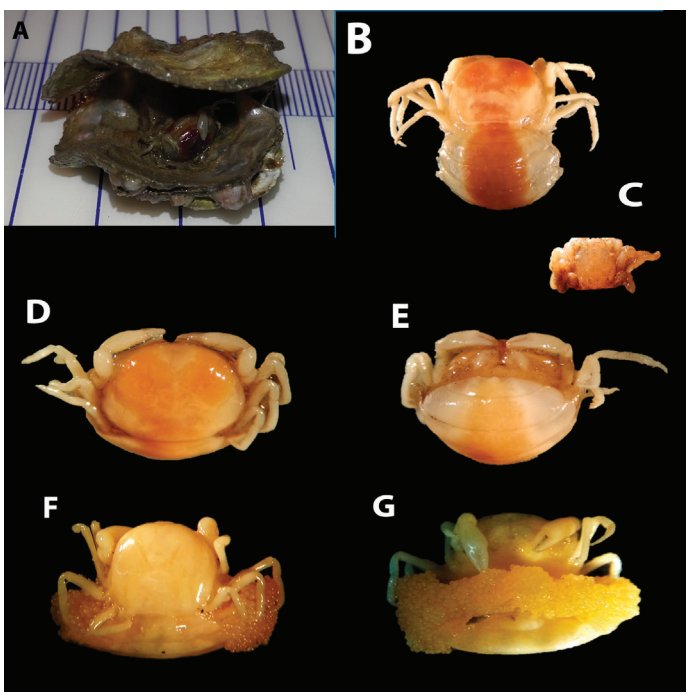

Fig. 2: *Pinnotheres bicristatus* García Raso & Cuesta, 2019. A, Soft female inside *Anomia ephippium* Linnaeus, 1758. B, Soft female found in *Ostrea edulis* Linnaeus, 1758. C, Hard female. Soft female: D, dorsal view; E, ventral view. Ovigerous female: F, dorsal view; G, ventral view.

Results

A total of 9 specimens of *Pinnotheres bicristatus* (1 ovigerous female, 7 soft females and 1 hard female) were morphologically identified in two hauls of the RASTELL survey made in the vicinity of Vilanova i la Geltrú (Table 1). Eight pea crabs were found inside seven specimens of the bivalve *Anomia ephippium* collected in haul V18 at 37 m depth, while another pea crab was found within an *Ostrea edulis* collected in haul V13 at 38 m depth (Table 2). The species identity was also confirmed by comparing the partial sequences of the DNA gene H3 obtained from the male holotype (collected in the Gulf of Cádiz) (Cuesta *et al.* 2019) and other specimens deposited in GenBank under the accession codes MK415381- MK415384 with those from three individuals from the Catalan Sea. All the sequences fitted 100% in the common 304 bp. The new H3 sequences were deposited in GenBank under the accession codes MT590759-MT590762.

Table 3 shows the number of pinnotherid crabs obtained per bivalve species in the two sectors studied in the MEDITS 2018 trawl survey and for the whole sampled area (both sectors together). Of the 14 bivalve species analysed, only 2 hosted pinnotherid crabs: *A. ephippium* and *Mytilus galloprovincialis* Lamarck, 1819.

Overall, two pinnotherid crab species were recorded in the two surveys: one individual of *Pinnotheres pisum* was collected in the Alboran Sea inside the Mediterranean mussel *M. galloprovincialis* as the host species; and 34 specimens of *P. bicristatus* (10 males, 1 hard female, 20 soft females and 3 ovigerous females) were collected in 56 specimens of *A. ephippium*.

Table 2 shows the mean sizes, sex and demographic category of the pea crabs collected per host and haul. Overall, carapace length ranged between 2.50 and 3.95 mm in males and between 3.68 and 6.66 mm in females.

Table 2. Characteristics of the pea crabs *Pinnotheres bicristatus* collected in this study, with indication of the host. Abbreviations: CL, carapace length (mm); CW, carapace width (mm); F, female; M, male; HF, hard female; SF, soft female; OF, ovigerous female.

Area	Host	Sex	Demographic category	nº of crabs	Mean CL ± SE	CL range	Mean CW ± SE	CW range
NW	*Ostrea edulis*	F	SF	1	7.06	-	9.06	-
	Anomia ephippium	F	HF	1	2.08	-	2.46	-
		F	SF	6	5.22 ± 0.41	3.68-6.66	5.95 ± 0.53	3.90-7.95
		F	OF	1	5.42	-	7.16	-
SW	*Anomia ephippium*	F	SF	20	4.99 ± 0.15	4.17-6.21	5.86 ± 0.24	4.30-7.24
		F	OF	3	6.22 ± 0.31	6.25-7.24	7.64 ± 0.48	7.08-8.62
		F	HF	1	2.67	-	2.5	-
		M		10	3.60 ± 0.09	2.50-3.95	3.46 ± 0.08	2.50-3.75

Table 3. Number of bivalves and pinnotherid crabs found by geographical sector (Alboran Sea and NW Mediterranean) and bivalve species examined in the MEDITS survey. (Pb) *Pinnotheres bicristatus*, (Pp) *Pinnotheres pisum*.

MEDITS2018						
	Alboran Sea		NW Mediterranean		Total	
Bivalve species	**Bivalve_N**	**Crab_N**	**Bivalve_N**	**Crab_N**	**Bivalve_N**	**Crab_N**
Anomia ephippium Linnaeus, 1758	54	34 (Pb)	2	0	56	34 (Pb)
Mytilus galloprovincialis Lamarck, 1819	4	1 (Pp)			4	1 (Pp)
Acanthocardia echinata (Linnaeus, 1758)	1	0	12	0	13	0
Anadara gibbosa (Reeve, 1844)			1	0	1	0
Arca tetragona Poli, 1795	52	0			52	0
Modiolus barbatus (Linnaeus, 1758)			1	0	1	0
Neopycnodonte cochlear (Poli, 1795)	338	0	152	0	490	0
Ostrea edulis Linnaeus, 1758	1	0			1	0
Pecten maximus (Linnaeus, 1758)	7	0			7	0
Pteria hirundo (Linnaeus, 1758)	11	0	12	0	23	0
Venus gallina Linnaeus, 1758	1	0			1	0
Venus nux Gmelin, 1791	90	0	53	0	143	0
Total	559	35	233	0	792	35

New host of Pinnotheres bicristatus

Of the 43 specimens of *P. bicristatus* collected in this study, a soft female was found in the pallial cavity of an individual of *Ostrea edulis* (SL: 59.7 mm; SW: 43.1 mm). The remaining 42 crabs (2 hard females, 26 soft females, 4 ovigerous females and 10 males) were all found inside *A. ephippium* (NW Mediterranean, SL mean 17.3 ± 0.4 mm and SW mean 12.3 ± 0.2 mm; Alboran Sea, SL mean 37.0 ± 1.9 mm and SW mean 32.5± 1.5 mm). Of the 7 specimens of *A. ephippium* of the NW Mediterranean hosting crabs, 1 hosted a pair of crabs (two females), whereas of the 26 specimens of *A. ephippium* of the Alboran Sea hosting *P. bicristatus*, 8 hosted a pair of crabs (one male plus one female in all cases).

Discussion

Five pinnotherid crab species had been previously reported in European waters, including *Pinnotheres bicristatus* (Cuesta *et al.*, 2019). So far, the known distribution of *P. bicristatus* had been restricted to the south of the Iberian Peninsula, but this new record supports the suggestion of Cuesta *et al.* (2019) that *P. bicristatus* most likely has a wider distribution throughout European waters in relation to its presence in other bivalve hosts. In the present study, a total of 43 specimens of *P. bicristatus* were collected along the Mediterranean coasts of the Iberian Peninsula. Moreover, nine of them were reported for the first time in the central Catalan Sea at 37-38 m depth, thus greatly expanding the distribution range of the species known so far. Understanding species distribution is important for studying species interactions and ecosystem biodiversity for better management programmes.

Until now, only two of the pinnotherid crab species reported in European waters had been cited in the NW Mediterranean: *Nepinnotheres pinnotheres* and *P. pisum* (Cuesta *et al.*, 2019). *P. bicristatus* can be differentiated from the other pinnotherid species inhabiting the same geographic area (*N. pinnotheres* and *P. pisum*) by the peculiar characteristic carapace of males and hard females, the shape and frontal region on the carapace and the finger fixed on the cheliped in males and soft females, on the first gonopod in males and on the third maxilliped and walking legs in soft females (see Table 4). In the present study, hauls of the MEDITS survey along the Mediterranean coast were examined for the presence of pinnotherids, but *P. bicristatus* was found only in the localities where *Anomia ephippium* was present, supporting a preference for this host. The distribution of *A. ephippium* encompasses the Atlantic Ocean, from Iceland and Great Britain to Guinea, Angola and the Canary Islands, as well as the Mediterranean Sea (Gofas *et al.*, 2011). Future studies on this bivalve species throughout its distribution could clarify whether the occurrence area of *P. bicristatus* completely overlaps that of its bivalve host or may be restricted due to the temperature requirements of the crab.

On finding larval stages (zoeae and megalopae) of *P. bicristatus* in November 2013 in the Gulf of Cádiz and one ovigerous female on 25 April 2017 in the Alboran Sea, Cuesta *et al.* (2019) hypothesized that the species may have a long reproductive period extending from spring to autumn. In the present study, one ovigerous female was collected on 17 March 2017 in the Catalan Sea and could represent a slightly early start of the reproductive period, perhaps related to higher temperatures in that month in this area than in the Alboran Sea.

Table 4. Morphological comparison of the adult phases of the three known pinnotherid species from the W Mediterranean.

			Nepinnotheres pinnotheres	***Pinnotheres pisum***	***Pinnotheres bicristatus***
Male & hard female	Carapace	*Anterolateral*	No noteworthy feature	No noteworthy feature	Pair of tufts of curved setae
		Colour and pattern on dorsal surface	No colour pattern	Like an orange-yellowish “palm tree” but without the “trunk”	Orange-reddish «palm tree»
	Cheliped	*Fixed finger*	With triangular tooth	Without triangular tooth	With triangular tooth
Male	Gonopod	*First*	L-shaped	Straight and wide, narrowed and curved on distal part	Curved, narrowed and curved on distal part
Soft female	Carapace	*Shape*	Subglobular (width > length)	Subglobular	Subtrapezoidal
		Frontal region	Bilobed by a median incision, a little projected	Slightly rounded	Rectilinear, not projected
	3rd Maxilliped	*Dactylus*	Reaches or exceeds the distal part of the propodus Medial insertion	Does not reach the distal part of the propodus. Basal insertion	Does not reach the distal part of the propodus. Basal insertion
	Walking legs	*Pereiopods*	Shorter and robust	Shorter	Longer and thinner
		Relative length of the dactylus	Short	Equal length on all pereiopods	Longer in third pereiopod

The reproductive cycle of pinnotherid crabs is accompanied by complex life history traits and requires shell hardness in order to mate (Mclay & Becker, 2015). The lack of small-size females observed in the present study is most likely a consequence of this complex life cycle: small hard-shell females swim searching for a bivalve host and, once inside, they become soft-shelled crabs ready for reproduction. For this reason, the probability of finding hard females inside bivalves is lower than that of finding soft females, which cannot leave the bivalve for the rest of their life.

The number of bivalve hosts for European pea crabs ranges between 2 (*N. pinnotheres*) and 28 (*P. pisum*) species (Perez-Miguel *et al.*, 2019; Triay-Portella *et al.*, 2018). Citing a new host might not be totally unexpected for a recently described species of pinnotherid, but it is necessary knowledge for understanding its biology and behavioural ecology. The large size of the reproductive female found within *Ostrea edulis* would support the hypothesis that space availability inside a host is a relevant factor in determining the final size of the crab in its sedentary phase (Cuesta *et al.*, 2019; Soong, 1997).

Some European pinnotherid species have specificity for hosts. *P. pectunculi* has been found only inside five species of Veneridae Rafinesque, 1815, while *N. pinnotheres* is mainly a symbiont of ascidians, but it has been also found in bivalve species such as *Pinna nobilis* Linnaeus, 1758 and *Atrina pectinata* (Linnaeus, 1767), both of them belonging to the family Pinnidae Leach, 1819 (Perez-Miguel *et al.*, 2019). In the case of *P. bicristatus*, it is still early to infer a host preference pattern, but though *Anomia* and *Ostrea* belong to separate families, they have a similar morphology and habitat because they are fixed to hard substrates through one of the valves.

The methodology used was not specific to determine the distribution and hosts of this pea crab in the central Catalan Sea. Therefore, the fact that one specimen was found inside one oyster cannot determine the nature of the host for *P. bicristatus*, i.e. facultative or specific. However, if oysters were a regular host of this pea crab species, it is surprising that the presence of *P. bicristatus* would have been unknown until now because this bivalve is a common commercial species. An explanation would be that *P. bicristatus* could have been misidentified as *P. pisum*, a species that has been found in *O. edulis* (Triay-Portella *et al.*, 2018). Other species of bivalves were not analysed for the presence of pea crabs in the samples of the central Catalan Sea, so further studies are necessary to understand the relationship between *P. bicristatus* and *O. edulis*, as well as their life history throughout its known distribution range, which will certainly increase in the next few years.

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