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New kid in town: *Pinna rudis* spreads in the eastern Mediterranean

Maria ZOTOU[1], Orestis PAPADAKIS[1], Gaetano CATANESE[2,3], Yolanda STRANGA[1], Michail RAGKOUSIS[1], Thodoros E. KAMPOURIS[1], Roxani NAASAN AGA-SPYRIDOPOULOU[4], Evangelos PAPADIMITRIOU[1], Drosos KOUTSOUBAS[1] and Stelios KATSANEVAKIS[1]

[1] Department of Marine Sciences, University of the Aegean, Lofos Panepistimiou, 81100 Mytilene, Greece
[2] IRFAP-LIMIA, Institut de Recerca i Formació Agroalimentària i Pesquera de les Illes Balears, Laboratori d'Investigacions Marines i Aqüicultura, Govern de les Illes Balears, 07157 Port d'Andratx, Spain
[3] INAGEA-UIB, Agro-Environmental and Water Economics Research Institute, University of the Balearic Islands, 07122 Palma, Spain
[4] iSea, Environmental Organisation for the Preservation of the Aquatic Ecosystems, Kritis 12, 54645 Thessaloniki, Greece

Corresponding author: Maria ZOTOU; m.maryzotou@gmail.com

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Abstract

In the Mediterranean Sea, the genus *Pinna* encompasses two large fan-shaped bivalve species, *Pinna nobilis* and *Pinna rudis*. Historically, both species coexisted in the western Mediterranean until a mass mortality event (MME) brought *P. nobilis* to the brink of extinction. Notably, *P. rudis* remained unaffected by the MME, and its recent successful recruitment and further spread have been hypothesized to be linked to the local extinction of *P. nobilis*. Although *P. rudis* has been sparsely recorded in the eastern Mediterranean Sea (with some of these records being doubtful), reports emerging in the summer of 2023 from researchers and citizens have unveiled its sudden spread in the region. This study documents the recent presence of *P. rudis* within Greek waters through a dedicated survey and molecularly confirmation through two distinct molecular methods. Information derived from a citizen science initiative, following photo-identification of the species was also included. Furthermore, an updated review of the distribution of *P. rudis* in the Mediterranean Sea, integrating data from literature and online repositories is provided. This research confirms the recent spread of *P. rudis* in Crete, the Greek Ionian Sea, and Cyprus (first verified records of the species in the regions), in marine areas where *P. nobilis* has become extinct, further strengthening the hypothesis that *P. rudis* has benefited from the collapse of *P. nobilis* populations.

Keywords: Pinnidae; *Pinna rudis*; Distribution; Mediterranean Sea; Molecular identification.

Introduction

The family Pinnidae comprises large bivalve mollusks with a distinctive fan-like shape, anchored in the substrate by byssus threads (Gvozdenović *et al.*, 2019; Deidun *et al.*, 2022) and consists of two genera, *Atrina* and *Pinna* (Lemer *et al.*, 2014). The genus *Pinna* encompasses the two largest bivalve species of the Mediterranean Sea, *Pinna nobilis* (Linnaeus, 1758) and *Pinna rudis* (Linnaeus, 1758) (Zavodnik *et al.*, 1991; Galinou-Mitsoudi *et al.*, 2006). These congeneric species, while sharing certain characteristics, also exhibit notable differences. *Pinna nobilis* can attain an impressive shell length of up to 120 cm (Huber, 2010), and its maximum recorded lifespan exceeds 45 years (Rouanet *et al.*, 2015). In contrast, *P. rudis* reaches a maximum shell length of only 50 cm (García-March & Kersting, 2006) and a maximum age of 31 years (Nebot-Colomer *et al.*, 2016).

Although both species inhabit the Mediterranean Sea, *P. nobilis* is confined solely to this region while *P. rudis* has a broader distribution, occurring also along the African Atlantic coasts and Macaronesian islands (Barreiros *et al.*, 2016; Gvozdenović *et al.*, 2019; Kersting & Ballesteros, 2021). The habitat preferences and depth distributions of the two species differ: *Pinna nobilis* is closely associated with *Posidonia oceanica* meadows and soft substrates (Addis *et al.*, 2009; Vázquez-Luis *et al.*, 2014; Deudero *et al.*, 2015) and is found at depths ranging from 0.5 m to 60 m (Garcia-March & Kersting, 2006; Katsanevakis, 2007; Vázquez-Luis *et al.*, 2017). *Pinna rudis* can also inhabit similar habitats (such as seagrass meadows, maerl, detritus and boulder beds, as described in Kersting & García-March, 2017), but shows a preference for gravel bottoms and hard substrates (Poppe & Goto, 1993) to depths down to 40 m (Sempere *et al.*, 2006). Their populations overlap in areas where both species coexist, particularly in the western Mediterranean (Kersting & García-March, 2017). This overlap occasionally leads to the misidentification of *P. rudis* as *P. nobilis* (e.g., Gvozdenović *et al.*, 2019; Kersting & Ballesteros, 2021). Morphologically, the most distinct feature of *P. rudis* lies in its shell, which appears more robust and triangular, with fewer but larger protruding scales compared to that of *P. nobilis* (Cosentino & Giacobbe, 2006). The external color of *P. rudis* valves is reddish brown, and valves possess a symmetrical, triangular shape with transparent ends, notably delicate, featuring 5–10 radiating ribs, neatly arranged in rows. Adult *P. nobilis* tends to be larger and lacks the protuberances present on the surface of the shell in *P. rudis*. Additionally, *P. rudis* typically displays a white and iridescent mantle border, while *P. nobilis* exhibits a pink one. Both species have similar-sized nacre lobes (García-March & Vicente, 2006; Gvozdenović *et al.*, 2019).

Pinna spp. play a unique and crucial role in the ecosystem. *Pinna nobilis* acts as a host to many marine species, including invasive species, on and within its shell, providing a hard substrate amidst the soft bottom surrounding, functioning as an ecosystem engineer (Giacobbe, 2002; Rabaoui *et al.*, 2009; Katsanevakis *et al.*, 2016; Kersting & García-March, 2017; Çinar *et al.*, 2021), as does *P. rudis* shells (Cosentino & Giacobbe, 2007; Lopes *et al.*, 2020). Furthermore, *Pinna* spp., being filter-feeding organisms, can decrease water turbidity and retain organic material (Trigos *et al.*, 2014; Basso *et al.*, 2015). From a cultural ecosystem services perspective, they are considered attractions for SCUBA divers (Morrocco *et al.*, 2018; Deidun *et al.*, 2022) since the species are emblematic, adding complexity and dimension to the marine environment. Both species are considered endangered due to various anthropogenic activities threatening their populations, such as poaching, anchoring, trawling, and habitat degradation (Katsanevakis, 2007; Barea-Azcón *et al.*, 2008; Deudero *et al.*, 2015; Kersting *et al.*, 2019). Consequently, they have been included in Annex II of the Bern and the Barcelona Conventions.

Since 2016, *P. nobilis* populations have experienced a substantial decline due to mass mortality events (MMEs) that emerged across the Mediterranean Basin (Vázquez-Luis *et al.*, 2017; Kersting *et al.*, 2019; Zotou *et al.*, 2020; Özalp & Kersting, 2020; Katsanevakis *et al.*, 2021). The MMEs have been linked to the presence of the parasite *Haplosporidium pinnae* (Catanese *et al.*, 2018; Grau *et al.*, 2021), although *Mycobacterium* sp. and *Vibrio mediterranei* were also detected in dead or moribund individuals (Prado *et al.*, 2020; Carella *et al.*, 2020; 2023). The mortality rates reached 100% in most Mediterranean locations (Katsanevakis *et al.*, 2021). As a result, in 2019, the species' conservation status was evaluated as "critically endangered" on the IUCN Red List (Kersting *et al.*, 2019), and the attention of marine scientists focused on actions to prevent the global extinction of *P. nobilis*. Remarkably, *P. rudis* remained unaffected by the pathogen responsible for the high mortality observed in its congeneric species (Catanese *et al.*, 2018). Recently, in regions where the two species co-existed, an increase in *P. rudis* recruitment has been reported (Kersting & Ballesteros, 2021) and small populations of *P. rudis* have been observed in areas where the species had not been recorded previously (Donato *et al.*, 2021; present study).

Unfortunately, *P. rudis* is understudied compared to *P. nobilis*, with scarce publications assessing the population dynamics or the distribution of the species in the Mediterranean (Nebot-Colomer *et al.*, 2016; Kersting & García-March, 2017; Gvozdenović *et al.*, 2019). The first mapping of *P. rudis* occurrences in the Mediterranean, based on the existing literature, was published in 2019 (Gvozdenović *et al.*, 2019). Most of these observations were concentrated in the western Mediterranean, with the majority of studies being conducted in Spain. Notably, in Greece, only two research articles have mentioned the presence of *P. rudis* in the Aegean and the Ionian Sea (Salomidi *et al.*, 2016; Galinou-Mitsoudi *et al.*, 2023).

Citizen science represents a collaborative and inclusive approach that engages both professional scientists and citizens (Kelly *et al.*, 2021). The integration of citizen science into monitoring programs offers valuable information while reducing the time, effort, and costs associated with scientific fieldwork. Simultaneously, this integration is viewed as a pivotal process for nurturing a new generation of citizens with a profound comprehension and appreciation of the natural environment (Mokos *et al.*, 2020). In recent years, an increasing number of research papers have incorporated citizen science (Cabanellas-Reboredo *et al.*, 2019; Deidun *et al.*, 2022; Kampouris *et al.*, 2022), and networks of environmentally conscious citizens willingly share their valuable observations with scientists.

The primary aim of this study was to assess if the distribution of *P. rudis* is changing in the Mediterranean Sea after the MME of *P. nobilis*. To this aim, an assessment of the distribution of *P. rudis* in Greek waters, particularly southern Crete, combined with opportunistic records from other areas, was conducted. To describe the changes in the range of the species, the study also includes a comprehensive review of its past distribution in the Mediterranean Sea. The key objectives of this research were: i) to document the recent expansion of *P. rudis* in the eastern Mediterranean, with a focus in Crete; ii) to provide molecular confirmation of the correct identification of the species; iii) to evaluate information arising from our citizen science network about *Pinna* populations in Greece and Cyprus; and iv) to map the present distribution of *P. rudis* in the Mediterranean Sea utilizing existing literature and including supplementary data sourced from online databases (GBIF, WoRMS).

Materials and Methods

Main study area

Crete, the largest island in Greece and the fifth largest in the Mediterranean Sea, boasts a coastline that spans more than 1046 km (Alexandrakis, 2014). Positioned approximately 160 km south of the Greek mainland, it occupies a strategic location within the eastern portion of the Mediterranean basin. The island has an elongated shape, stretching approximately 260 km from its eastern to western extents, with a width fluctuating between 12 and 60 km from north to south. The southern Cretan coastal waters are relatively unaffected by significant river flows; consequently, this region stands out as one of the most oligotrophic regions within the Mediterranean. This is manifested by notably low levels of primary productivity and chlorophyll concentrations (Karakasis & Eleftheriou 1998; Psarra *et al.*, 2000; Bosc *et al.*, 2004). Sea surface temperature along the southern Cretan coastal waters spans from 16.5°C during winter to a peak of 27.5°C in summer (2010-2023 E.U. Copernicus Marine Service Data). Salinity levels within this region span from 39.2 to 39.5 psu (2021-2023 E.U. Copernicus Marine Service Data). The circulation patterns in the region are influenced by multiple factors, including wind patterns, water temperature, and the larger-scale Mediterranean circulation. It is a complex oceanographic circulation system where water masses from the Western Mediterranean, the Ionian, and the Levantine Seas interact (Zodiatis 1993; Akpinar *et al.*, 2016). The local circulation structures of south Crete exhibit a series of permanent and recurring gyres and jets, including the Cretan Cyclone, the Mid Mediterranean Jet, and the Ierapetra Anticyclone (Robinson *et al.*, 1992; Fusco *et al.*, 2003).

Sampling and documentation

Samples for molecular identification were collected through SCUBA diving at a site with high *Pinna* population densities, pinpointed by a citizen scientist (Site #6, Kalypso, S. Crete; Table 1) in July 2023. The sampling methodology adhered to the non-lethal technique proposed by Sanna *et al.* (2013), to reduce any damage to the shell and soft tissues of the specimens (Scarpa *et al.*, 2020). The collected tissue samples were stored in 10-ml tubes and preserved in 99.8% ethanol. In total, eight mantle samples were acquired from living individuals. All sampled individuals were photographed *in situ* with a digital camera (Sony RX100 III).

Furthermore, tens of individuals were documented at several other surveyed sites in southern Crete during the same period. To visually depict the distribution of the documented populations, a map was generated using QGIS software (version 3.24.1). Additional observations contributed by both researchers and citizen scientists from other locations in Greek waters, confirmed through photographic evidence, were also mapped.

Molecular confirmation

Total genomic DNA was extracted from the eight mantle biopsies using the DNA NucleoSpin® Tissue extraction kit following the instructions provided by the manufacturer. Two distinct methods were carried out for species identification. A first analysis was performed following the method described by Catanese *et al.* (2022a) but applied directly to the extracted DNAs from the bivalve samples (approximately 30 ng). The method is based on a multiplex-PCR which selectively amplifies amplicons of different lengths for each *P. nobilis* (a fragment of 175 bp using the primers Pnob12SF/Pnob12SR) and *P. rudis* species (a fragment of 123 bp using the primer pair Prud16SF/ Prud16SR). The method employs species-specific primers designed on the mitochondrial genes 12S rDNA and 16S rDNA, which have been previously characterized for their inter-specific differences (Catanese *et al.*, 2022b). In addition, a 28S rDNA fragment (205 bp using the primers Pinna28SF/ Pinna28SR) is included in the multiplex-PCR as a PCR positive control of amplification for *Pinna* sp. and *Atrina* sp.

For the second method, DNA samples underwent PCR amplification and sequencing targeting a segment of 1150 base pairs (bp) within the 28S rDNA nuclear gene. Two distinct regions were amplified using the primers 28S_Pinna_F/ 28S_Pinna_R to target a first portion (fragments A and B in Vázquez-Luis *et al.*, 2021) and the primers 28Sa/ 28srd5b (fragment C in Vázquez-Luis *et al.*, 2021) to target a second portion. The PCR reactions were conducted in a total volume of 20 µL. The reaction mixture consisted of 10 µL of KAPA Taq Ready Mix PCR kit, 0.4 µL of each primer (stock 20 µM), 1 µL of DNA (approximately 200 ng/µL), and the remaining volume was made up with water.

Table 1. Locations with coordinates in decimal degrees indicating the start of each dive (and maximum distance of approximately 200 m from reported individuals), and dates when each location was visited. Mantle samples for molecular confirmation were collected from Kalypso (Station #6) and are marked with an asterisk.

Station	Location	Latitude (N)	Longitude (E)	Sampling date
1	Ag. Antonios	35.245514°	23.836447°	5/7/2023
2	Domata	35.229138°	23.905206°	5/7/2023
3	Glika nera	35.200247°	24.106286°	6/7/2023
4	Plakias	35.191408°	24.373022°	8/7/2023
5	Myrtos	34.996291°	25.572163°	10/7/2023
6	Kalypso*	35.172628°	24.398562°	8/7/2023

The temperature profile for the PCR reaction was as follows: an initial denaturation step at 94°C for 2 minutes, succeeded by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 20 seconds, and extension at 72°C for 90 seconds. The PCR products were separated on 2% agarose gels in TAE 1x buffer (w/v). The gels were stained with GelRed and included a High-Ranger DNA ladder size standard 50 bp (for multiplex PCR) or 1kb (for nuclear PCR amplification). The amplified DNA fragments were visualized using a UV transilluminator and excised from the agarose gel. Subsequently, they were purified using the Metabion International mi-Gel Extraction Kit, following the manufacturer's instructions. The two obtained PCR fragments underwent bi-directional sequencing using the Applied Biosystems 3130xl DNA automated sequencer. The sequences were edited and aligned using the BioEdit version 7.2.5 software (Hall, 1999) and MEGA X (Kumar *et al.*, 2018).

The citizen science network

For several years, a network of dedicated SCUBA divers has actively collaborated with the 'Marine Biodiversity and Ecosystems Management' lab of the University of the Aegean, sharing valuable information regarding marine biodiversity. Additionally, within the framework of another research project with a specific focus on sharing information regarding the endangered *P. nobilis* populations in Greece, the pre-existing citizen-science Facebook group Red Fish Project (https://www.facebook.com/groups/481084575908558/), that monitors threatened aquatic species was utilized, following the protocols and principles described by Kampouris *et al.* (2022). Presence data collected from the environmental organization iSea, through their Facebook group (https://www.facebook.com/groups/104915386661854) and their field actions were also incorporated in the dataset. As of June 2023, there have been notable citizen observations of live *Pinna* individuals in locations where *P. nobilis* has faced local extinction due to recent MMEs; however, upon careful examination of the photographs submitted alongside the reports, it became evident that while the genus was accurately identified, the species in question was not *P. nobilis*, which is typically found in Greece, but rather *P. rudis*. To investigate this further and validate the observations, we communicated with the citizens who had made the "uncommon" pinnid observations, and subsequently, a survey was organized in southern Crete to collect samples for molecular species identification.

Review of the past distribution of **Pinna rudis** *in the Mediterranean*

A systematic literature review was undertaken to retrieve information on the past distribution of *P. rudis* in the Mediterranean, employing Scopus and Google Scholar as the primary search platforms. For the Scopus and Google Scholar search, the keywords "*Pinna rudis*" and "Mediterranean Sea" were used to query titles and abstracts, together and apart. The Google Scholar search was restricted to the initial 100 documents (sorted by relevance), as subsequent results did not yield any additional relevant information. Studies conducted beyond the Mediterranean Sea were omitted from the analysis. To augment the dataset, additional records were obtained by backward searching a systematic review article by Gvozdenović *et al.* (2019). Additionally, *P. rudis* georeferenced data from GBIF and WoRMS were included in the review. Subsequent to the elimination of duplicates, all available records from literature and online databases until 31 July 2023 were assimilated. The spatial data was visualized on a map using the QGIS software (version 3.24.1).

Results

Documentation of the population in south Crete

During the visual observations conducted in July 2023 in southern Crete (Fig. 1), *Pinna rudis* individuals were found at six locations (Table 1, Table 2). A total tally of 31 individuals was recorded, of which 28 were alive whilst three were dead. Of the total population in southern Crete, 24 individuals were classified as adults, constituting 77.4% of all documented *P. rudis* individuals, while seven individuals were characterized as presumed juveniles, based on their small size (less than 6 cm shell height; Fig. 2). Notably, all individuals were found on rocky or pebbly substrates at depths ranging between 5 and 20 meters.

Among these locations, Kalypso (Site #6) hosted the largest population, with 19 individuals. This site was the only sampling site where dead (presumed juvenile) individuals were encountered; however, the reason for their mortality was unclear. Samples for molecular analyses were also collected from Site #6. In the three locations (Sites #2, #3, #5) where fewer individuals were observed (1 or 2 individuals/site), the presence of presumed juvenile individuals was consistently noted. These findings provide evidence for the establishment of a *P. rudis* population in southern Crete, characterized by the successful recruitment of juveniles and the presence of adult individuals.

Additional locations

During the summer months of 2023, *P. rudis* individuals were occasionally recorded across various locations in Greece. A total of 29 individuals was documented at 17 sites from three locations (Crete and Antikythera, Ionian Sea, Cyprus) (Fig. 3). Specifically, one individual was observed and photographed by one of the authors (SK) on the island of Antikythera at 5 m depth on a rocky substrate in August 2023. In October 2023, another individual was reported by a researcher (D. Poursanidis, pers, comm,) on the northern coast of Crete at 15 m depth. An additional ten individuals were recorded by citizen

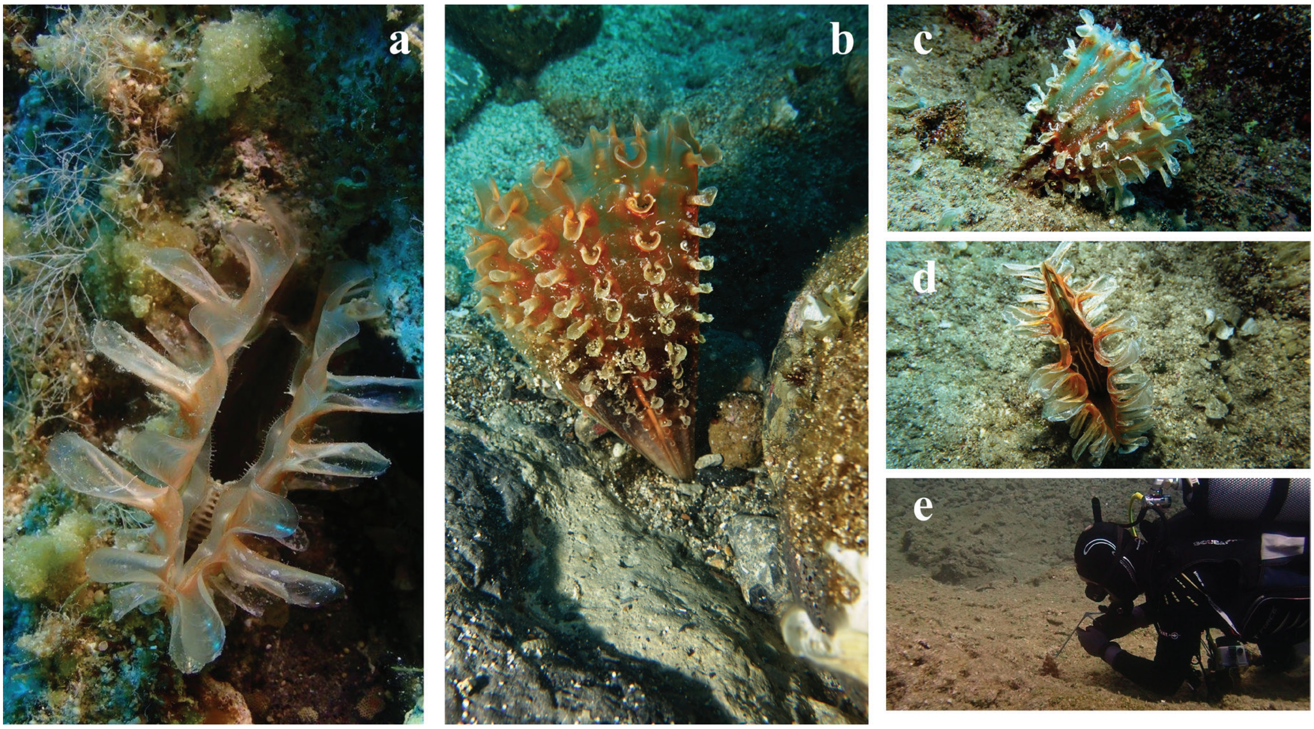

Fig. 1: Photographs taken during sampling in southern Crete, Greece: a, d: View from above of a *Pinna rudis* individual with visible mantle. b, c: *P. rudis* individuals with distinctive shell ornamentation. e: Non-lethal mantle sampling for molecular confirmation of the species.

Table 2. Number of individuals found in sampled locations, categorized by age (adult/presumed juvenile) and status (alive/dead).

Station	Location	Living individuals	Dead individuals	Juveniles	Adults
1	Ag. Antonios	3	-	1	2
2	Domata	2	-	1	1
3	Glika nera	1	-	1	-
4	Plakias	4	-	-	4
5	Myrtos	2	-	1	1
6	Kalypso	16	3	3	16

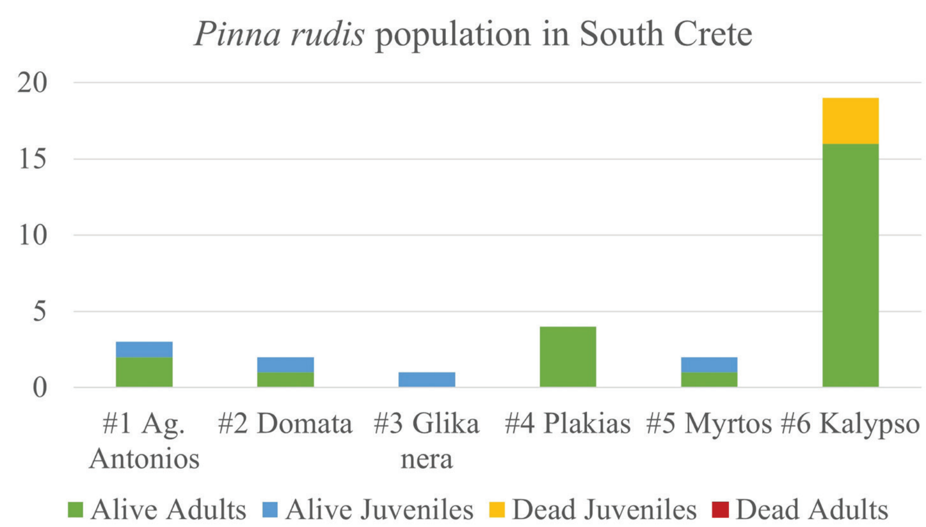

Fig. 2: Population characteristics (adult/presumed juvenile individuals) and their status (alive/dead) in visited locations of South Crete.

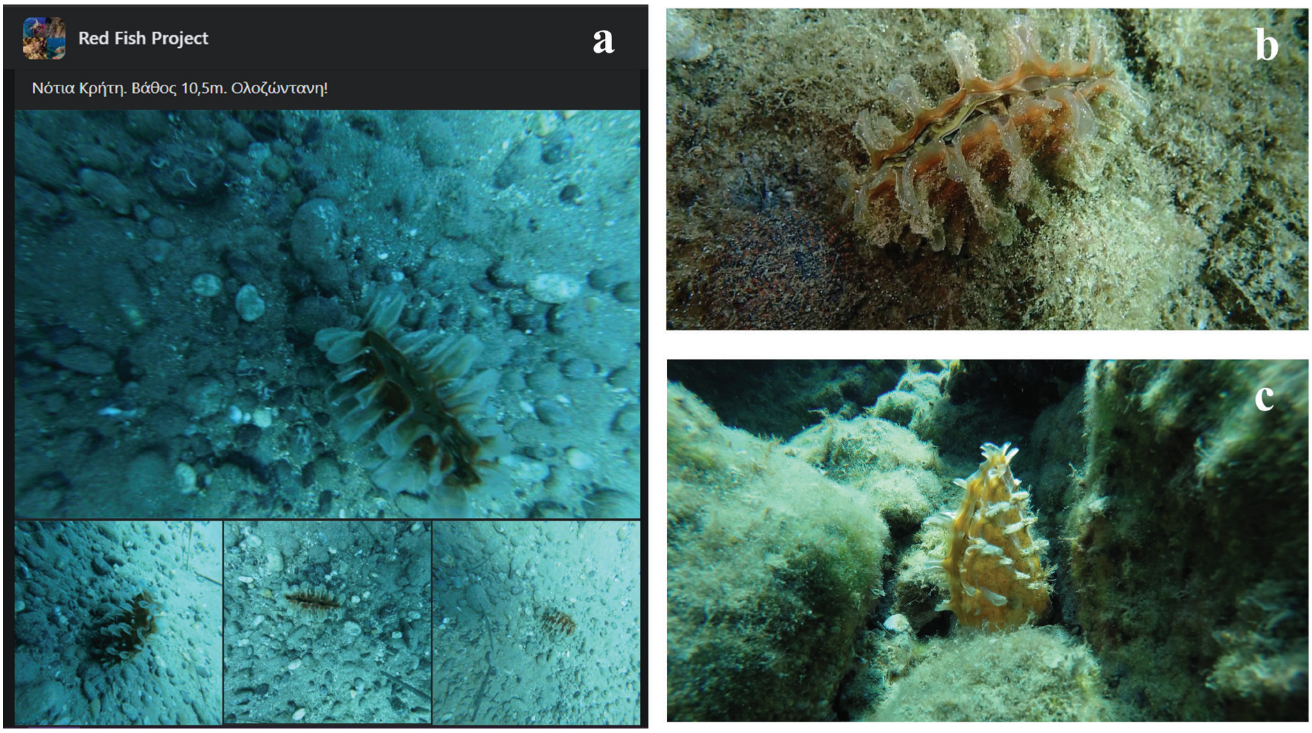

Fig. 3: Photograph identification of opportunistic records made by researchers or citizen scientists. a: Print screen of a citizen scientist's post on Red Fish Project with a living individual found in southern Crete. b: Photograph taken by a citizen scientist at Parga (Ionian Sea). c: Photograph of a living specimen taken by S. Katsanevakis at Antikythera Island.

scientists participating in the Red Fish Project in south and east Crete and western Cyprus during the summer of 2023. These individuals were found across five dive sites at depths between 4 and 35 m. The environmental organization iSea documented seven individuals in three islands of the Ionian Sea (Paxos, Corfu, and Ithaca).

Furthermore, reports from citizens, supported by photo-identification of the individuals, indicated the presence of *P. rudis* in other areas of the Ionian Sea. Near Parga, ten individuals were spotted across three dive sites, between 2 and 17 m deep. There was also information on *P. rudis* presence at Kefalonia Island (Ionian Sea), but these individuals were not photographed; thus the information was not included in the distribution map of the species' presence in the Greek seas (Fig. 4). Data about locations and the recorded individuals by site can be found in Table 3.

Molecular confirmation

All 205 bp fragments from the 28S rDNA gene of the examined samples were successfully amplified during the multiplex-PCR tests, serving as a positive control for the two *Pinna* species and *Atrina* spp. Moreover, only the 123 bp PCR product, specific for the 16S rDNA gene of *P. rudis*, was obtained from the eight samples. No cross or simultaneous amplification of the two species-specific fragments were observed in any PCR.

In the PCR targeting the 28S rDNA nuclear gene, two amplicons of differing lengths, approximately 900 bp and 350 bp, were consistently generated in all samples. Upon sequence analysis, the same haplotype was identified across both fragments for all examined individuals.

BLAST analysis revealed that the obtained sequences exhibited a 100% identity similarity with a sequence previously deposited in GenBank under accession number LC634517, obtained from *P. rudis*. Analyzing the chromatograms of the 28S rDNA sequences, the nine diagnostic nucleotide sites for the identification of *Pinna* species or putative hybrids as described by Vázquez-Luis *et al.* (2021), showed the typical pattern of *P. rudis* species. Additionally, no insertion/deletion events were detected in the obtained sequences.

Review of the past distribution of **Pinna rudis** *in the Mediterranean*

A comprehensive total of 399 records containing georeferenced information on *P. rudis* distribution in the Mediterranean were compiled. Among these records, 118 (30%) were derived from the literature review and forward searches of scientific articles, while most data (281 records, 70%) were acquired from online databases (Fig. 5).

Among the contributing countries, Spain stood out with 226 records, followed by France (75), Italy (49), and Algeria (24). Conversely, countries with limited records were Croatia (1 record), Lebanon (1), Libya (1), Montenegro (2), Tunisia (2), Morocco (2), Greece (6), and Malta (10) (Fig. 6).

In terms of ecoregions (as classified in Spalding *et al.*, 2007), the Western Mediterranean Sea exhibited the most substantial presence of *P. rudis* records, encompassing 70.4% of all records. The Alborán Sea accounted for 18.6% of the records, while the remaining ecoregions collectively contributed 11.0% (Ionian Sea 7.3%, Adriat-

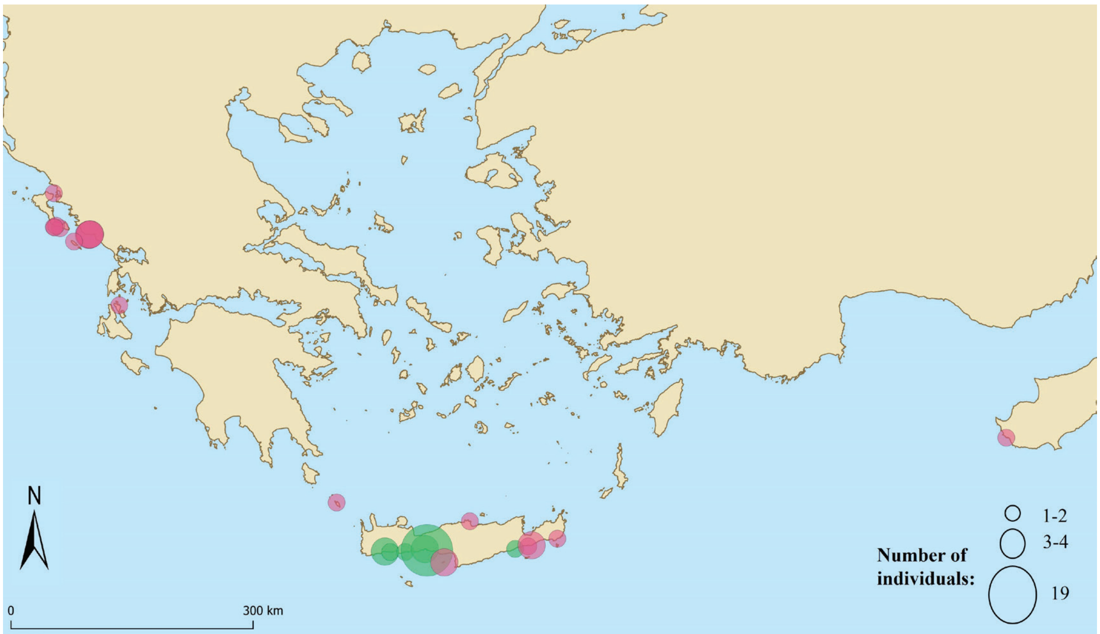

Fig. 4: The map displays the herein documented records of *Pinna rudis* in Greece and Cyprus, with circle size representing the number of individuals found in each location. The green color represents the documented population of southern Crete by our dedicated survey, while the pink color indicates opportunistic records made by researchers or citizen scientists. The opacity of symbols is set to 60% to highlight overlapping observations.

Table 3. Additional locations where opportunistic records of *Pinna rudis* individuals occurred. Locations with coordinates in decimal degrees indicating the start of each dive (and maximum distance of approximately 200 m from reported individuals), depth range, and number of individuals found in each site.

Location	Latitude (N)	Longitude (E)	Depth range	Number of individuals
Antikythera Island	35.883338°	23.292293°	5 m	1
Parga (Ionian Sea)	39.278193°	20.386764°	2-17 m	4
Parga (Ionian Sea)	39.282090°	20.404204°	2-17 m	3
Parga (Ionian Sea)	39.279557°	20.400047°	2-17 m	3
Ierapetra (S. Crete)	34.994694°	25.750889°	25 m	1
Ierapetra (S. Crete)	34.999222°	25.798389°	4-11m	3
Sitia (S. Crete)	35.011583°	26.154472°	10 m	1
Heraklion (N. Crete)	35.401219°	25.03381°	15 m	1
Paksimadia Isles (S. Crete)	35.003915°	24.601525°	12-35 m	4
Pegia (W. Cyprus)	34.856784°	32.348314°	10 m	1
Peristeres Island (North Straits Corfu)	39.792189°	19.95975°	4 m	1
Paxos Island	39.225982°	20.164533°	2 m	1
SW. Corfu Island	39.417611°	19.903028°	6-15 m	1
SW. Corfu Island	39.393917°	19.989389°	6-15 m	1
SW. Corfu Island	39.427598°	19.9374°	2.5-5 m	1
SW. Corfu Island	39.410789°	19.912179°	2.5-5 m	1
Ithaca Island (Kioni port)	38.44959°	20.690681°	3 m	1

ic Sea 1.8%, Aegean Sea 1.3%, Tunisian Plateau/Gulf of Sidra 0.5%, Levantine Sea 0.3%; Fig. 7).

Overall, the presence of *P. rudis* in the western Mediterranean Sea has been thoroughly documented and studied, while information about the eastern part of the basin is scarce. This highlights the current knowledge that the distribution range of the species is primarily concentrated in the western Mediterranean Basin (Fig. 8). Regarding the six past records within the Greek seas, five records corresponded to a single article (Salomidi

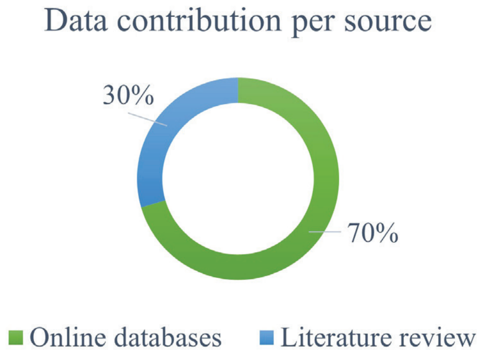

Fig. 5: Contribution of sources used for the distribution review of *Pinna rudis* in the Mediterranean. The green color represents records retrieved from online databases (GBIF, WoRMS), while the blue color corresponds to spatial information obtained from the literature.

et al., 2016) and were situated within Santorini's islands complex (South Aegean Sea), and one record occurred from a specimen recently caught on fish farming nets in Thesprotia region (Ionian Sea) (Galinou-Mitsoudi *et al.*, 2023). There was one additional record retrieved from the GBIF database, originating from a citizen scientist's observation in Lesvos Island (North Aegean Sea), but it was a clear misidentification of *P. nobilis*, as judged by observing the accompanying photos, and was discarded.

Discussion

The findings of the present study have illuminated a broader distribution range for *P. rudis* in the Mediterranean Sea than previously known. The integration of data sourced from online databases has significantly augmented the records indicating the species' presence. However, the unexpected discovery of a verified population in southern Crete, alongside reported occurrences from other locations in Greece and Cyprus, suggests that the species either has been present but undocumented or that its distribution range has recently expanded into the eastern Mediterranean. While the former appears implausible given that Crete and the regions of the Ionian Sea (islands and mainland) have been subject to thorough investigations and *P. rudis* had not been previously identified there, the latter hypothesis gains traction. The Marine Biodiversity and Ecosystems Management lab (Dept. Marine Sciences) diving team has extensively surveyed several sites in southern Crete and the Ionian Sea during the past five years, and *P. rudis* had never been detected. This underscores the novelty of the species' presence in the region. A previous study that focused on mapping endangered species in the Aegean Sea had reported the presence of *P. nobilis* in southern Crete (Sini *et al.*, 2017). However, the subsequent outbreak of MMEs led to the collapse of all known *P. nobilis* populations on the island (Zotou *et al.*, 2020). The emergence of *P. rudis* populations in areas affected by *P. nobilis'* MMEs has been increasingly reported throughout the Mediterranean by scientist and citizen observations uploaded to citizen science platforms and social media. In locations where the two species coexisted, the successful recruitment of *P. rudis* has been linked to the decline of *P. nobilis* (Kersting & Ballesteros, 2021). It is also possible that in locations where mixed populations occurred, misidentifications might have taken place (not only in juvenile individuals but also in adults), thereby overlooking the presence of *P. rudis* (D. Kersting, pers. comm.). After the MME, *P. rudis* individuals present in those mixed populations have become more "visible" and easier to correctly identify. Notably, the findings of the present study mirror similar observations made by Donato *et al.* (2021), who documented *P. rudis* populations in locations where the species had not previously been observed in Italy. This growing body of evidence underscores the potential interaction between the two species and the ecological dynamics at play in the wake of significant disturbances, as initially hypothesized by Kersting & Ballesteros (2021).

The review of the past distribution in the Mediterranean Sea coincides with the results of Gvozdenović *et al.* (2019), considering that the majority of sites with confirmed presence of *P. rudis* are concentrated in the western Mediterranean Sea, while data originated from the eastern, central, and northern Mediterranean Sea are scarce. The low population density, the cryptic habitat (Gvozdenović *et al.*, 2019), and the potential misidentification of *P. rudis* for *P. nobilis* may have impeded monitoring efforts and underestimated the distribution range of the species.

The distribution range of marine species depends on a complex interplay of factors, including temperature,

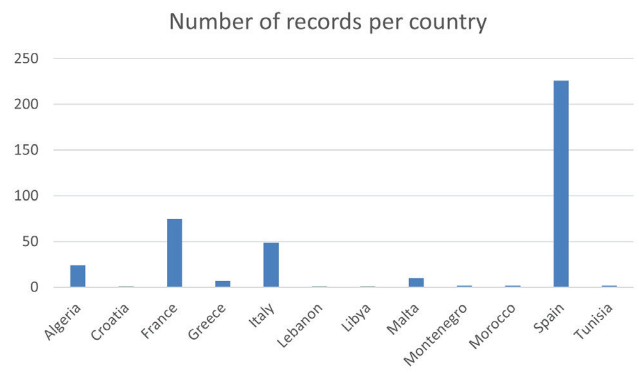

Fig. 6: Number of records per country collated for the distribution review of *Pinna rudis* in the Mediterranean.

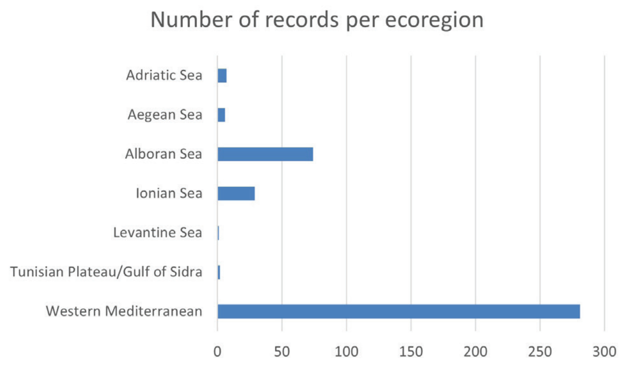

Fig. 7: Number of records per ecoregion collated for the distribution review of *Pinna rudis* in the Mediterranean.

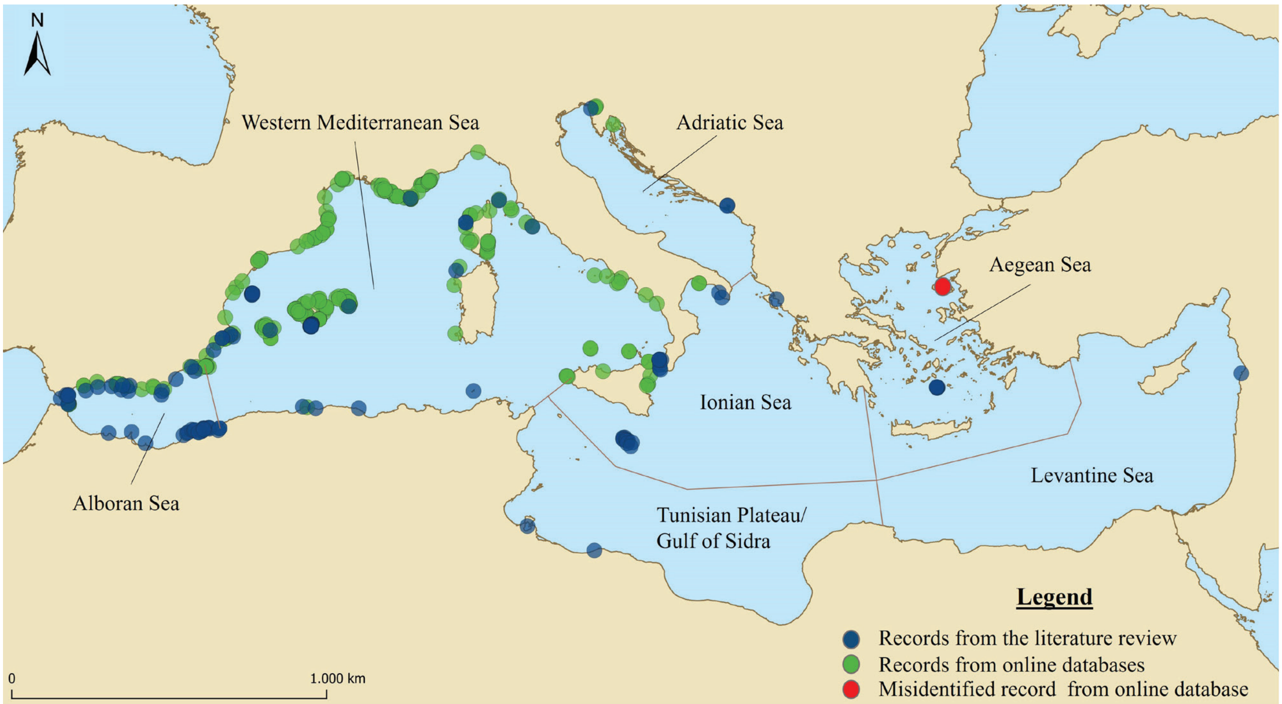

Fig. 8: Distribution map of *Pinna rudis* in the Mediterranean Sea. Different colored circles correspond to different data sources. The higher opacity of symbols indicates the occurrence of multiple records. The Mediterranean Basin is divided into ecoregions based on the classification proposed by Spalding *et al.* (2007).

currents, interspecific interactions, and habitat availability (Dunstan & Bax, 2007). Species with pelagic larval stages generally exhibit higher dispersal potential, facilitated by ocean currents (Donelson *et al.*, 2019). The recent colonization success and range shift of *P. rudis* in the Mediterranean has yet to be fully understood, with several hypotheses proposed to explain it. One hypothesis posits that interspecific competition between the two *Pinna* species, when coexisting, may have constrained *P. rudis.* Another hypothesis proposes the consumption of *P. rudis* larvae by the larger-sized *P. nobilis* as the main reason for restriction of the population density of *P. rudis*. With the subsequent collapse of most *P. nobilis* populations, the survival and establishment of *P. rudis* larvae may have been enhanced (Kersing & Ballesteros, 2021). Another possible explanation lies in the thermophilic nature of *P. rudis* and the ongoing warming trends in the Mediterranean (Kersing & Ballesteros, 2021; Donato *et al.*, 2021). Climate change has triggered local extinctions, substantial changes in food webs, and shifts in species distribution in other groups (Bianchi, 2007; Nikolaou & Katsanevakis, 2023), which may have been beneficial for the thermophilic *P. rudis* to flourish (Kersting & Ballesteros, 2021; Donato *et al.*, 2021).

Although previous research has hinted at the presence of *P. rudis* in the Aegean Sea (Salomidi *et al.*, 2016) in a study about gathering ecological information for the establishment of a potential Marine Protected Area on Santorini Island, no image documentation was provided. Furthermore, none of the experienced scientific divers of our group, with a collective log of more than 4,000 dives in Greek waters and > 25 publications on *Pinna*, has previously observed *P. rudis* in the region. In a more recent article (Galinou-Mitsoudi *et al.*, 2023), a single juvenile *P. rudis* individual was collected from fish farming nets in the Thesprotia region of the Ionian Sea in February 2022. This individual was subsequently photographed on three separate occasions over a nine-month period. This rarity of documented sightings underscores the significance of our study's findings in shedding light on the noteworthy shift in the species distribution within the Greek Seas.

The integration of data from online repositories (GBIF, WoRMS) was instrumental in enriching the spatial information available for the distribution mapping of *P. rudis* in the Mediterranea. However, the reliability of such data can be compromised by misidentifications and inadequate documentation. A good example of this pertains to a record from GBIF, which indicated the presence of five dead *P. rudis* individuals in the Kalloni Gulf, Lesvos Island (Greece), as reported by a citizen. The accompanying photographs clearly depicted *P. nobilis* individuals. Considering that the area held a large, well-documented population of *P. nobilis* (Katsanevakis *et al.*, 2019; Zotou *et al.*, 2020), the specific unverified citizen science records from GBIF are a clear case of species misidentification, underscoring the need for cautious consideration of unverified citizen science records.

The studied individuals observed during the field survey exhibited the distinct shell morphology (large protuberances on the surface of their shells) and the iridescent mantle border of *P. rudis* and did not show any indications of the putative hybrids that might manifest traits of both *P. nobilis* and *P. rudis*. Likewise, molecular analyses confirmed the absence of cross-species or introgression. In fact, the molecular results undoubtedly identified all the analyzed individuals to be *P. rudis,* as confirmed both at the mitochondrial (multiplex-PCR) and nuclear level (ribosomal gene sequences).

The contribution of local ecological knowledge was pivotal in documenting the *P. rudis* population in southern Crete. Similarly, citizen scientists across various regions of the country are actively sharing more information about areas of interest and live pinnids through social media platforms. Networks of environmentally aware citizens, guided by members of the scientific community to validate and oversee shared information, not only promote ocean literacy but also strive to maximize data reliability by training the observers. It is worth noting that since resilient *P. nobilis* populations still survive in a few areas in Greece (Papadakis *et al.*, 2022; 2023) and putative *P. rudis-P. nobilis* hybrids have been documented in sites where both species coexisted (Vázquez-Luis *et al.*, 2021; Kersting & Ballesteros, 2021), accurate species identification becomes challenging without molecular confirmation.

Following the mass mortality of *P. nobilis* in the eastern Mediterranean Sea, the ecological niche it once occupied has remained vacant, and its ecological role has not been replaced; it is premature to assert that *P. rudis* is fulfilling this ecological role, especially considering the difference in densities of *Pinna* spp. before and after the MME. The knowledge about the ecology and biology of *P. rudis* remains limited (Nebot-Colomer *et al.*, 2016; Gvozdenović *et al.*, 2019; Lopes *et al.*, 2020; Kersting & Ballesteros, 2021), let alone about putative hybrids. Hence, further research is essential to gain a deeper understanding of the life cycle, reproduction, recruitment, growth, population dynamics, and ecosystem functioning of *P. rudis*. Extensive monitoring in regions affected by MMEs might reveal further unknown cases of the species' establishment.

In conclusion, given the ecological similarities between *P. rudis* and *P. nobilis*, coupled with the threats both species face from human activities, it is crucial for Greek national and international legislation to encompass *P. rudis* within existing protective laws established for *P. nobilis*. Rigorous enforcement of these protection measures is crucial to prevent poaching and exploitation of *P. rudis*, particularly in the wake of the dramatic decline of its congeneric species, which has historically suffered from illegal activities in the Greek Seas (Katsanevakis, 2007; 2016).

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Supplementary Data

The following supplementary information is available online for the article: Zotou_Pinna_rudis_Supplementary_File.xlsx.