Tropical Isognomonids in the Mediterranean Sea: When the West Atlantic Met the Indo-Pacific Region in the South Aegean Sea

Akis Angelidis[1] · George Polyzoulis[2] · Chrysoula Gubili[3,4]

Abstract

The introduction and establishment of the bivalves *Isognomon bicolor* and *Isognomon* aff. *legumen* in the South Aegean Sea, specifically on the islands of Chalki and Alimia, marks a significant ecological event. These species, native to different parts of the world, have now been confirmed in the Mediterranean through DNA barcoding and phylogenetic analyses. These methods are crucial for accurate identification within the genus *Isognomon*, as traditional morphological identification is challenging due to the variable and habitat-dependent shell shapes of these bivalves. *Isognomon bicolor* is originally from the West Atlantic, while *Isognomon* aff. *legumen* hails from the Indo-Pacific region. Their presence in the Mediterranean, specifically in the intertidal and shallow subtidal zones, indicates they have found suitable habitats in their new environment. Over a four-year period of field observations, researchers gathered valuable ecological data, noting the species' habitat preferences and their establishment dynamics. In July 2024, researchers observed the formation of new, extensive dense aggregations, or beds, of the invasive *I. bicolor* on the coastal rocks of Chalki and Alimia. The formation of these beds is a clear indicator of a progressing invasion, which poses a significant threat to the native ecosystem. The establishment of *I. bicolor* and *I.* aff. *legumen* could disrupt local biodiversity, outcompete native species, and alter the ecological balance in these regions. This situation underscores the importance of continuous monitoring and the use of advanced identification techniques like DNA barcoding to track and manage invasive species. Such efforts are essential to mitigate the impacts of these invasions and protect native marine ecosystems.

Keywords Isognomon bicolor · Isognomon aff. legumen · Non-indigenous mollusc species · DNA barcoding · Invasion · Greece

Introduction

The Mediterranean Sea has become increasingly accessible and suitable for the establishment of nonindigenous species (NIS) due to various environmental, ecological, and anthropogenic factors. Rising sea temperatures, driven by climate change, have made the Mediterranean more hospitable to tropical and subtropical species, which previously could not survive in cooler waters. Altered currents and circulation patterns further facilitate the transport of NIS to new areas within the Mediterranean, aided by both human activities and natural range expansions. The introduction and dispersal of tropical marine species into the Mediterranean Sea can involve multiple pathways and mechanisms, which may alternate during their journey from the donor to the recipient area. According to Essl et al. (2019), these pathways include both natural expansions and human-mediated introductions.

The construction of the Suez Canal created a significant pathway for species from the Indo Pacific province to enter the Mediterranean. This pathway supports the poleward expansion of many species through direct human agency, leading to the unintentional creation of a major dispersal corridor. According to Essl et al. (2019), species entering via the Suez Canal should not be considered neo-native due to their introduction through human intervention rather than natural range expansion.

✉ Akis Angelidis
   fortuno.akis@gmail.com

1   7, Kapetan Vangeli Str, Thessaloniki 54646, Greece

2   24, Agias Sofias Str., Thessaloniki 54622, Greece

3   Fisheries Research Institute, Hellenic Agricultural Organization-DIMITRA, Nea Pergamos, Kavala 64007, Greece

4   School of Science, Engineering & Environment, University of Salford, 43 Crescent, Salford M5 4WT, UK

Tropical Atlantic species may enter the Mediterranean via the Gibraltar Straits, following a natural poleward range expansion along the West African coast. Albano et al. (2024a) highlight the increasing significance of this biogeographical corridor, which has been enhanced by the weakening of the cold Canary barrier due to climatic shifts. Species that enter through this natural corridor may be considered neo-native (Essl et al. 2019).

Direct human actions, such as fouling on ships, untreated ballast water dumping, macrolitter rafting, aquaculture, and the aquarium trade, also introduce tropical species to the Mediterranean (Katsanevakis et al. 2013; Ulman et al. 2019). These species are classified as NIS or alien species, distinct from neo-native ones.

Natural or anthropogenic rafting on hard debris, as observed with *Isognomon bicolor*, serves as a critical dispersal mechanism (Breves et al. 2014). Rafting differs from planktonic larval dispersal, with variable dispersal distances influenced by currents and wind (Thiel and Haye 2006). This mechanism is not easily categorized as either a poleward range expansion or a direct human introduction mechanism.

Among the dominant aiding factors for the successful establishment of non-indigenous species (NIS), especially in the eastern Mediterranean basin, is the increase in sea surface temperature (SST) due to global warming. In fact, satellite observations from 1985 to 2008 revealed a significant SST increase of approximately 1 °C in the area, with the highest peak for the Aegean Sea reported regularly in August (on average 1.23 °C) (Nykjaer 2009; Raitsos et al. 2010). This process is expected to intensify in the coming decades since the Mediterranean Sea is the fastest warming sea globally, with sea surface temperatures increasing 20% faster than the global average (Givan et al. 2017; Peleg et al. 2019; Albano et al. 2021a).

The steady increase in SST has changed biotic interactions and affected temperate marine systems through a process known as "tropicalization of marine environments" (Bianchi and Morri 2003; Scuderi and Viola 2019). Through this process, thermophilic species expand their natural range to novel areas/higher latitudes (Bianchi and Morri 2003; Scuderi and Viola 2019; Albano et al. 2021a). Similarly, there is an increased occurrence of warm water biota in the Mediterranean Sea, where approximately 1,000 alien species have been recently recorded (Zenetos et al. 2022). Moreover, shallow subtidal zones are more exposed to thermal variability, and they are more affected by sea warming and tropicalization (Vergès et al. 2014). In some cases, ectothermic malaco-fauna react accordingly, and new southern thermophilic species are established, whereas indigenous species recede (Albano et al. 2021a). Interestingly, the shift from assemblages composed of Mediterranean species to those dominated by tropical NIS in shallow Israeli reefs (Albano et al. 2021a) highlights that the transformation of subtidal marine ecosystems could have

significant effects on the marine biota in the eastern Mediterranean basin. Similar thermal conditions occur in the South Aegean Islands (Greece), which are part of the fast-warming Levantine Basin. Concurrently, local water circulation patterns, such as those in the mid-Mediterranean Jet, create a complex system of cyclonic-anticyclonic eddies and fronts in the area, favouring larval transport (Valdés et al. 2013). Such conditions result in a high recruitment of non-indigenous species on the southern Dodecanese Islands, turning the area into a hot spot for their establishment (Crocetta et al. 2017).

As of 2022, 173 non-indigenous molluscs have been reported as established in the Mediterranean Sea, representing an increase of 38% during the last 20 years (Zenetos et al. 2022). The establishment of sessile bivalve NIS is further facilitated locally by both the availability of suitable substrata and increases in water temperature, especially in coastal habitats (Albano et al. 2021a). Despite the magnitude of bivalve invasions in the Levantine basin, the ecological effects on the benthic community have not been thoroughly studied (Diga et al. 2023). Six non-indigenous sessile bivalve species, including members of the genus *Isognomon* (Lightfoot 1786), have been reported in southern and warmer localities of Greece, such as the southern Dodecanese Islands (Crocetta et al. 2017). They prevail in infralittoral sessile communities and alter hard substrata assemblages (Albano et al. 2021a).

Sessile epibyssal bivalves of the genus *Isognomon* are widespread in the intertidal and infralittoral zones of the tropics and subtropics (Benthotage et al. 2020). They have pearly interior and highly irregular shapes (Reeve 1858; Printrakoon 2015). Recent molecular studies have shown that they belong to the superfamily Pterioidea [Gray, 1847 (1820)] (Tëmkin 2010). The family Isognomonidae [Woodring, 1925(1828)] is easily distinguished from the rest of the Pterioidea [Gray, 1847 (1820)] by its multivincular ligament (Stenzel 1971). There are 16 valid species belonging to the genus *Isognomon* (Benthotage et al. 2020). However, the diagnostic characteristics used to discriminate these species are particularly problematic because they are mainly based on descriptive features of the shell, such as the outline of the shell margin, obliquity, extent of the auricles, and various aspects of its shape and colour (Tëmkin 2006; Ubukata 2003). Four of them, *Isognomon australicus* (Reeve 1858), *Isognomon isognomum* (Linnaeus, 1758), *Isognomon legumen* (Gmelin 1791), and the *Isognomon nucleus* (Lamarck, 1819), were recorded in the Red Sea (Vine 1986), representing potential Lessepsian introductions due to their geographic proximity. Moreover, *Isognomon ephippium* (Linnaeus, 1875) was recorded in Israel (Mienis 2004) as a non-Indo-Pacific immigrant; however, this record was not confirmed.

Only two species, *I. legumen* and *I. australicus*, have been recorded in the Mediterranean Sea, the first from Israel, Greece, Turkey and Italy (Mienis 2016; Micali et al. 2017; Ovalis and

Zenetos in Stamouli et al. 2017; Scuderi and Viola 2019), and the latter from Astypalaia, Saronicos, and Plakias Crete in Greece (Angelidis and Polyzoulis 2018; Albano et al. 2021b; Manousis et al. 2021). Very recently Albano et al. (2024b) used for the first time, molecular analysis to identify new records of *I. legumen* in various localities in Cyprus. In the literature, *I. legumen* is acknowledged as a well-established species in the Mediterranean Sea; however, there is scepticism about the establishment of *I. australicus* (Zenetos et al. 2018; Crocetta et al. 2017; Garzia et al. 2022). A third isognomonid species, *Isognomon bicolor* (CB Adams, 1845), was recorded for the first time in the Mediterranean, in southern Italy by Garzia et al. (2022) using molecular means, while very recently Albano et al. (2024b) documented new records of. *I. bicolor* in Cyprus (16 specimens from Agia Triada), in Israel (two specimens from Skimona) and in Greece (2 specimens from Heraklion) using molecular analysis for their identification. *Isognomon bicolor* was originally described in Jamaica (Caribbean Sea, western Atlantic), and it has become a widespread invasive species across the Brazilian coast, having a negative impact on the local biota (Domaneschi and Martins 2002; Breves-Ramos et al. 2010; López et al. 2014).

Hence, our knowledge of the genus *Isognomon* in the Mediterranean Sea remains controversial. The sole use of a morphological approach for species identification has proven unreliable due to the overall similarity of morphologies and the lack of solid diagnostic characters to distinguish species of the genus (Benthotage et al. 2020). Garzia et al. (2022); Albano et al. (2024b) in their recent studies, attempt to re-evaluate previous identifications in literature, based on morphological similarities between published photos and genetically analysed specimens. In this study, we attempt to elucidate the establishment of Isognomonidae in the South Dodecanese Islands, Greece, through an integrated solid strategy based on DNA barcoding, morphology, and field observations. Through this strategy we focus on the macroscopic signs that support the presence of a large-scale invasion of *I. bicolor* and a lesser one of *I. legumen* in Halki and Alimia islands of the South Aegean Sea.

Materials and methods

Specimen Collection

Specimens were collected by snorkelling in July 2021 and July 2022 at Pontamos beach, Chalki Island (Rhodes, South Aegean Sea) and in July 2022 at Alimia Island (Rhodes, South Aegean Sea) (Fig. 1; Table 1). In total, 30 specimens attached to infralittoral rocks were hand-picked, and an additional 26 specimens were collected by turning rocks upside-down in deeper water in both localities. Sample collection was conducted during five separate "events"
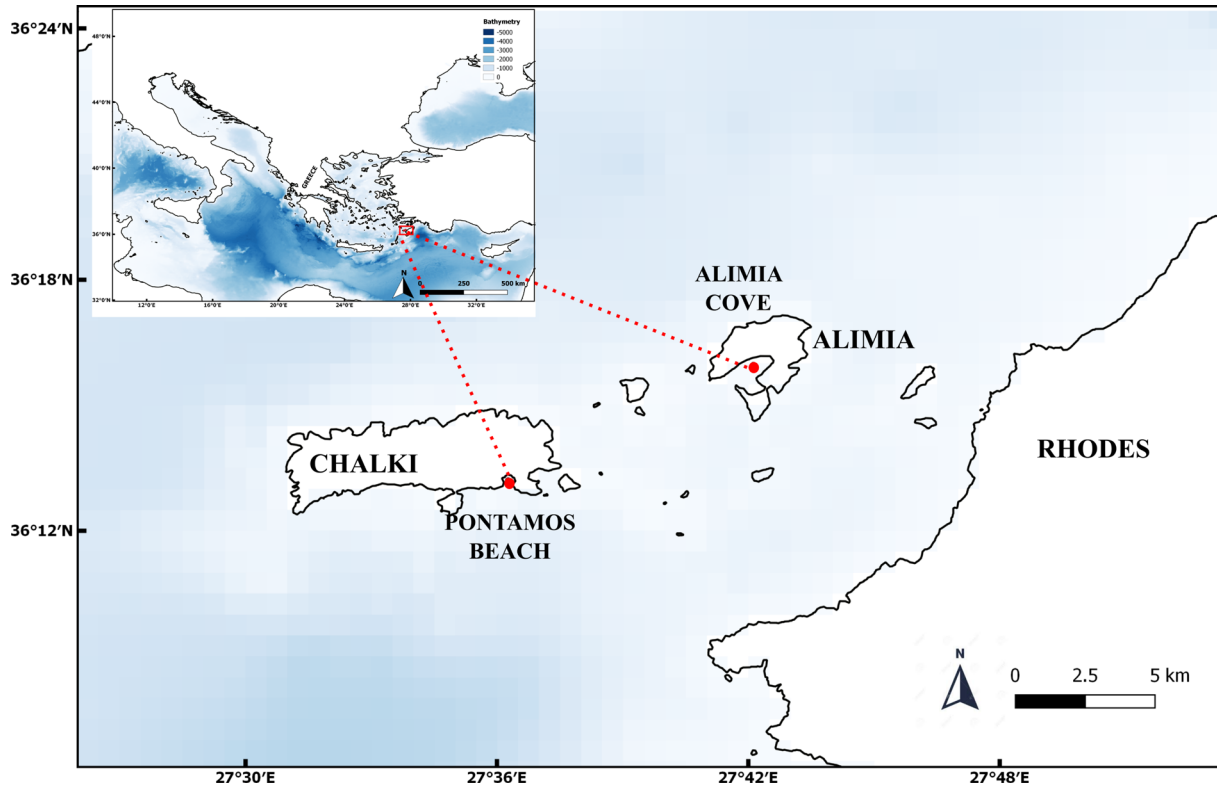

Fig. 1 *Isognomon* spp. sampling localities: Pontamos beach, Chalki Island (N 36.221666; E 27.604444) and Alimia cove, Alimia Island (N 36.266225; E 27.708494)

Table 1 Number of samples and field notes per sampling location

Sampling Event	Locality	Coordinates	Number of specimens	Depth	Habitat type	Number of sequenced specimens tags correspond to sequences in supplementary Table 2
1. July/2021	Pontamos beach, Halki Il.	(N 36.221666; E 27.604444)	8	0.30–1.5 m	Into rock crevices	2: (IB7, IB8)
2. July/2022	Pontamos beach, Halki Il.	(N36.221666; E 27.604444)	12	1–2 m	Under stones	1: (IL3)
3. July/2022	Alimia Il.	(N36.266225; E 27.708494).	22	0.10–1 m	Into and around rock crevices	6: (IB1, IB2, IB3, IB4, IB5, IB6)
4. July/2022	Alimia Il.	(N36.266225; E 27.708494).	14	1–2 m	Under stones	3: (IL1, IL2, IL4)
5. July/2022	Pontamos beach. Halki Il.	(N36.221666; E 27.604444)	2	4 m	Free standing on rocky bottom	1: (IL5)

according to their habitat at each study site (Table 1). All specimens were stored in 95% ethanol for further analyses. Additionally, photographs of sessile cluster fragments from Alimia cove were taken by removing stones from the water and then returning them to their original spot after imaging. An isolated group of four shells was observed free standing and attached vertically to a broad rock at a depth of four meters in Pontamos Beach. Two of them were collected for further examination because neither their habitat conditions nor their shell shape and colour were similar to those of any other observed or analysed specimens. One of those specimens (IL5, Table 1) was labelled and sequenced. Field observation notes were taken from both localities. A 10×10 cm quadrat was used to measure bivalve densities in both localities.

DNA Extraction, Amplification and Sequencing

Specimens were preliminarily sorted according to their habitat (under stones or within rock crevices), locality, and overall morphology (Table 1) and were kept in 95% ethanol for DNA sequencing. Thirteen specimens corresponding to all sampling events (Table 1) were labelled and sequenced. A small piece of the adductor muscle was clipped, and genomic DNA was extracted from approximately 20 mg of tissue from each specimen using the NucleoSpin Tissue Extraction Kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. The partial region of the cytochrome c oxidase subunit 1 mitochondrial gene (COI; 660 bp) was amplified by PCR using the primer set jgLCO1490: 5′-TITCIACIAAYCAYAARGAYATTGG-3′ and jgHCO2198: 5′-TAIACYTCIGGRTGICCRAARA-AYCA-3′ (Geller et al. 2013). The PCR cycling conditions included initial denaturation at 94 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 50 °C for 40 s, 72 °C for 40 s, and a final extension at 72 °C for 5 min. PCR was conducted in 25 µl volumes, and the reaction mixtures contained 1 µl of DNA, 5 µl of GoTaqx5 reaction buffer (Promega, USA), 2 µl of $MgCl_2$ (Promega, USA), 0.5 µl of deoxyribonucleotide triphosphate (dNTP, Promega, USA), 2.5 µl of each primer, 0.4 µl of GoTaq G2 Flexi polymerase (Promega, USA), and 11.1 µl of molecular grade water. PCR products were sequenced at the Macrogen Sequencing Service (Macrogen, The Netherlands). Sequences were checked and manually edited using ProSeq 3.0 (Filatov 2002). The partial COI sequences were translated and checked for stop codons and mitochondrial pseudogenes (NUMTs), and all generated sequences were compared against the GenBank database with nucleotide BLAST (blastn) (http://blast.ncbi.nlm.nih.gov/Blast.cg).

Following species identification, we amplified the D1-D3 fragment of the large ribosomal subunit sequence (28S rDNA) and the large subunit 16S ribosomal DNA (16S rRNA) of two individuals per species. Fragments for the 28S and 16S genes were amplified by polymerase chain reaction (PCR) using the primer sets D1F: 5'-GGGACTA CCCCCTGAATTTAAGCA-3' and D6R: 5'-CCAGCTAT CCTGAGGGAAACTTCG-3' (Park and Foighil 2000) following Tëmkin (2010) and 16Sar: 5'-CGCCTGTTTATCA AAAACAT-3' and 16Sbr: 5'-CCGGTCTGAACTCAGATC ACGT-3' according to Kessing et al. (1989), respectively. Amplicons were purified and sequenced by the Macrogen Sequencing Service (Macrogen, The Netherlands).

Phylogenetic Analyses

Three separate datasets, one for each gene, were created using the newly generated sequences and GenBank sequences belonging to the genus *Isognomon* (Supplementary Table 1). Each fragment was analysed independently due to sample size differences among the markers. Sequences of *Pinctada maxima* (Jameson, 1901) and *Magallana gigas* (Thunberg, 1793) were used as outgroup (Supplementary Table 1). Each dataset was aligned using ClustalX 2.1 (Larkin et al. 2007) and verified by eye. Uncorrected *p*-distances were calculated in MEGA 11.0.13 (Tamura et al., 2021) only for *Isognomon* species.

Maximum-likelihood (ML) analyses of the nonpartitioned protein-coding (COI), 28 S and 16 S gene sequences were performed on the PhyML 3.0 webserver (http://www.atgc-montpellier.fr/phyml/; Guindon et al. 2010) for 1,000

replications using automatic model selection by Smart Model Selection (Lefort et al. 2017) and the Bayesian Information Criterion (BIC). Bayesian analyses (BAs) were carried out with MrBayes v.3.2.7a (Ronquist et al. 2012) following the selection of the most appropriate model of evolution by jmodeltest 2.1.9 (Guindon and Gascuel 2003; Darriba et al. 2012) for each dataset using the BIC (COI: HKY+G; 16 S: TPM3uf+G; 28 S: TrN+G). Analyses were performed for two Markov chains of 2,000,000 generations each, with sampling every 100 generations resulting in 20,000 trees, of which the first 25% were discarded as burn-in. Figtree 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) was used to visualize both the ML and BI trees.

Morphological Analyses

Only labelled and sequenced specimens were examined under a stereomicroscope (Zeiss STEMI 508). They were also cleaned to produce empty shells. Subsequently, they were measured with the aid of a calliper to the nearest 0.01 mm. The total length (TL), length of the pearly layer (LPL), hinge length (HL), and hinge point thickness (HPT) were measured following Cavalcante and Gomes (2022): total length (TL) is the distance between the apex of the hinge and the margin of the lower part of the shell; length of the pearly layer (LPL) comprises the inner face of the valves in the region that houses the body, which is coated with a shiny nacre; and hinge point thickness (HPT) is the thickness at the hinge region of the closed shell (Supplementary Tables 3 and 4). All photos were taken using a Nikon D700 DSLR camera and processed by editing software to produce the image plates. Smartphone pictures of sessile fragments were taken in situ.

Results

Sequence Analyses, Species Identification and Phylogenies

In total, 13 individuals (22.42% of the 58 specimens collected) were successfully molecularly identified. BLAST hits for the COI gene showed high similarity (100%) with existing reference sequences in GenBank (Supplementary Table A and 2). Eight specimens were identified as *I. bicolor*, six from Alimia (IB1, IB2, IB3, IB4, IB5, and IB6) and two from Pontamos beach (IB7 and IB8) (Fig. 2a, Supplementary Table 2). Five individuals were identified as *I.* aff. *legumen*, two from Alimia (IL1, IL2) and three from Pontamos beach (IL3, IL4, IL5) (Fig. 2b, Supplementary Table 2). High similarity with GenBank reference sequences was also detected for both the 16 S and 28 S markers (100% and

99.9%, respectively; Supplementary Table 2). The intraspecific genetic distances were low for both species (*I. bicolor*: 0.01% for COI, 0.21% for 28 S, and 0.11% for 16 S; *I. aff. legumen*: 0.22% for COI, 0.14% for 28 S, and 0% for 16 S), whereas interspecific distances in the monotypic Isognomonidae family that contains only the genus *Isognomon*, ranged from 16.50 to 33.33% for COI, 1.03–4.65% for 28 S, and 4.57–26.05% for 16 S. Additionally, each of the three sequenced datasets produced topologies that confirmed our initial DNA barcoding results (Fig. 3a, b and c).

Morphological Characteristics and Identification

Specimens that were genetically identified as *Isognomon bicolor* were collected from inside and around the crevices of the rocky subtidal substrata at both localities. Their morphology exhibited the following characteristics: a rather small, solid, thin, slightly inequivalve shell with a straight hinge margin and a conspicuous byssal notch. The shape is irregular but mostly obliquely oval, pointed at the byssal edge. Interior nacreous extending to a shorter nonnacreous fringe. Hinge lacking teeth, multilingual ligament with six to seven grooves along the dorsal margin. The shell is conspicuously concentrically laminated with laminae loosely overlapping at narrow increments. The colour varies from blackish brown to yellowish brown.

The specimens, which were genetically identified as *Isognomon* aff. *legumen* (Fig. 2b), were collected by turning small bottom rocks upside down at both sampling sites. Their morphology presented the following characteristics: medium size, thin, semi-transparent, anomia-like, and highly irregularly shaped shell following the shape of its habitat. The colour is whitish in juvenile specimens and light yellow in adults. The interior of both valves is nacreous, followed by a wide fringe of irregularly developed noncancerous shell extension. The muscular scar is separate. Rudely laminated laminae compressed together to be almost obsolete. The external surfaces of both valves are similar in texture, with blister-like weak radial lines on the laminae. The ligament is multivincular with four to five pits that are relatively widely spanned. The valves are unequal to form the byssal opening. Byssus is formed by thin threads.

The specimen from the fifth sampling event, which was standing at 4 m depth, was genetically identified as *Isognomon* aff. *legumen*. Its shell was dark brown, and its shape was evenly oblong and tongue like, with TLs and LPLs equal to 4.7 and 2.4 cm, respectively. Additionally, the exterior of the valves was coarsely flaked in the first growth part, with concentric growth lines in the second half. The hinge length was 0.8 cm (Fig. 2b, No 4). The TL of *I. bicolor* varied from 1 to 2.5 cm (largest specimen), whereas that of *I.* aff. *legumen* varied from 1.6 to 6.8 cm (largest specimen). The average

Fig. 2 a) *Isognomon bicolor* sequenced specimens: 1 = IB1, 2 = IB2, 3 = IB3, 4 = IB4, 5 = IB5, 6 = IB6, 7 = IB7, and 8 = IB8 (labels correspond to sequences in Supplementary Tables A and 2); **b)** *Isognomon* aff. *legumen* sequenced specimens: 1 = IL1, 2 = IL2, 3 = IL3, 4 = IL5, and 5 = IL4 (labels correspond to sequences in Supplementary Tables A and 2). The scale bar is 2 cm
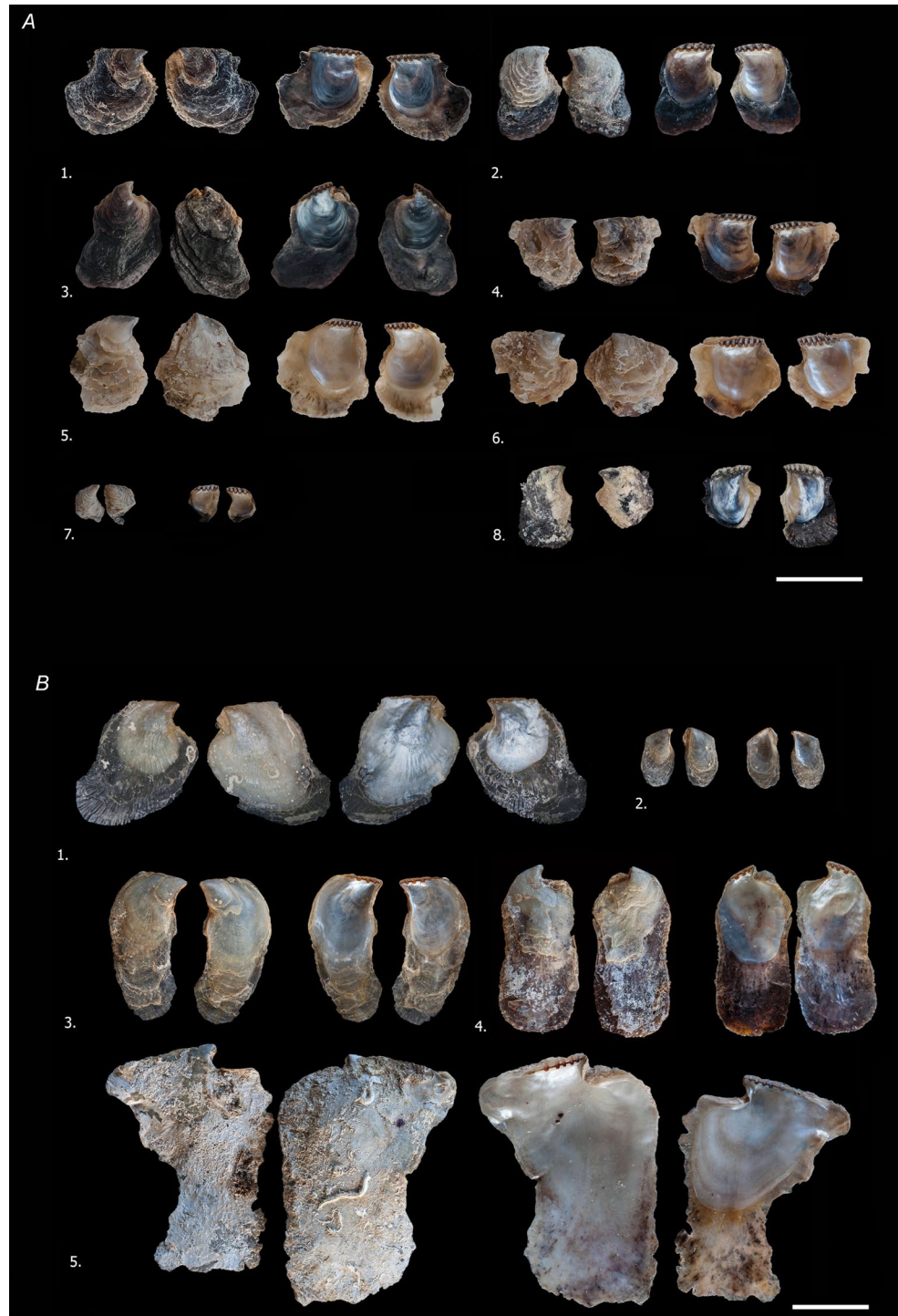
TL of the eight *I. bicolor* specimens was 2.04 cm, and the average TL for *I.* aff. *legumen* was 4.08 cm. The measured *I.* aff. *legumen* specimens were almost double the size of the corresponding *I. bicolor* shells. HPT measurements were quite similar in both species (avgHPT*bicolor* = 0.56 cm; avgHPT*legumen* = 0.56 cm); however, the TL-to-HPT ratio was considerably lower in *I. bicolor* (3.63 cm) than in *I.* aff. *legumen* (7.29 cm), suggesting that the latter has

a thinner shell. The average size difference between the TL and the length of the pearly layer (TL-LPL) was considerably greater in *I.* aff. *legumen* (avgTL-LPL = 2.12 cm) than in *I. bicolor* (avgTL-LPL = 1.28 cm). Consequently, the size difference between the two species is because *I.* aff. *legumen* develops a much larger non-nacreous fringe than does *I. bicolor* (supplementary Tables 3–4).

Fig. 3 (**a**) Maximum Likelihood phylogeny inferred from the large subunit 16 S ribosomal DNA. The tree is rooted with Pinctada maxima and Magallana gigas. Values in branches indicate support for each node based on Bayesian/ML inference. Bootstrap values under 80% are not shown. (The labels correspond to the sequences in Supplementary Table 2). (**b**) Maximum Likelihood phylogeny inferred from the large ribosomal subunit sequence 28Sr DNA sequences. The tree is rooted with Pinctada maxima and Magallana gigas. Values in branches indicate support for each node based on Bayesian/ML inference. Bootstrap values under 80% are not shown. (The labels correspond to the sequences in Supplementary Table 2). (**C**) Maximum Likelihood phylogeny inferred from the cytochrome c oxidase subunit 1 mitochondrial gene. The tree is rooted with Pinctada maxima and Magallana gigas. Values in branches indicate support for each node based on Bayesian/ML inference. Bootstrap values under 80% are not shown. (The labels correspond to the sequences in Supplementary Table 2). * Questionable species identification for I. Australicus
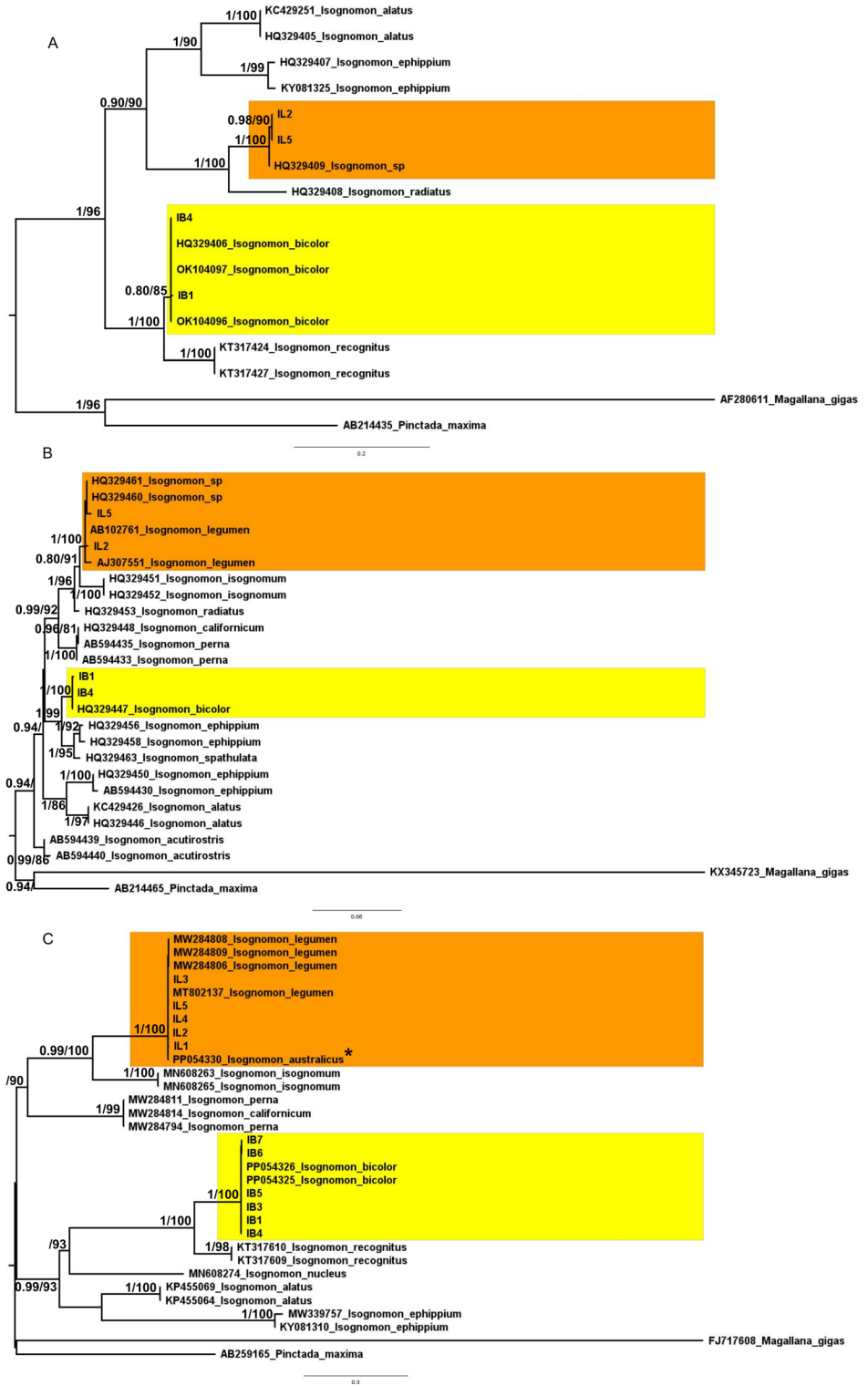
The identification of non-sequenced specimens was based on the diagnostic shell characteristics and habitat observations of the sequenced specimens. For example, I. aff. *legumen* barcoded specimens (Fig. 2b) developed an elongated non-nacreous extension around the nacreous part of the shell (a character missing in *I. bicolor*) and were all found under rocks. On the other hand, the *I. bicolor* specimens had a subquadrate shape and an indicative blackish colour (Fig. 2a), similar to those described by Cavalcante and Gomes (2022), and all samples were collected on rock surfaces. Moreover, although both species were found in the same area, they were never observed in mixed aggregations.

Thus, we could group the nonsequenced collected specimens as follows (see Fig. 4 and 5):

1. Six *I. bicolor* specimens from superficial rock crevices on Pontamos beach.
2. Eleven *I.* aff. *legumen* specimens under stones on Pontamos beach.
3. One *I.* aff. *legumen* specimen free standing on the rocky bottom of Pontamos beach.
4. Sixteen *I. bicolor* specimens in and around superficial rock crevices in Alimia.
5. Eleven *I.* aff. *legumen* specimens under stones in Alimia.

Field Observations

The subtidal and intertidal marine environments from both localities were inhabited by a rich biota dominated by non-indigenous species. Additionally, tides at both sampling sites were insignificant and did not have any effect on species assemblages (McElderry 1963). The *I. bicolor* aggregations at the Alimia cove remain generally submerged, unlike the regions of origin where they inhabit intertidal ocean zones with wide water height ranges and are regularly uncovered (Breves-Ramos, 2010). The coastal rocks at Alimia present a low relief with shallow crevices; the shore deepens smoothly, and the upper rocks are protected from waves. We also discovered a few individuals of the epibyssals *Brachidontes pharaonis* (P. Fischer, 1870) and *Septifer cumingii* (Récluz, 1849) in the same aggregations. No *I. legumen* morphotype was observed.

In July 2022 in the most populated areas in Alimia, we counted 35 *I. bicolor* individuals (four juveniles of 4–8 mm) per 100 cm^2. No empty shells were observed around the measured area. Approximately 12 *I. legumen* individuals (three juveniles) per 100 cm^2 were measured in the most populated areas of each locality (see Figs. 5).

In July 2024 we observed that in many parts of the coastal rocks in Alimia including the parts surveyed in 2022, at a shallow depth ranging from ten to sixty centimetres, *I. bicolor* had formed extensive dense clusters (beds) covering large surfaces. The density (over 150 individuals per 100 cm^2) and coverage of those beds had altered significantly the physical structure of the colonized substrate clearly indicating a progressing invasion (Figs. 6 and 7). Similar extensive *I. bicolor* beds were observed along the rocky coast east of Halki port area.

Fig. 4 *Isognomon* aff. *legumen* specimens, sorted *a posteriori* following the morphology and habitat preferences of the sequenced specimens. The scale bar is 2 cm

Fig. 5 *Isognomon bicolor* specimens sorted *a posteriori* following the morphology and habitat preferences of the sequenced specimens. The scale bar is 2 cm
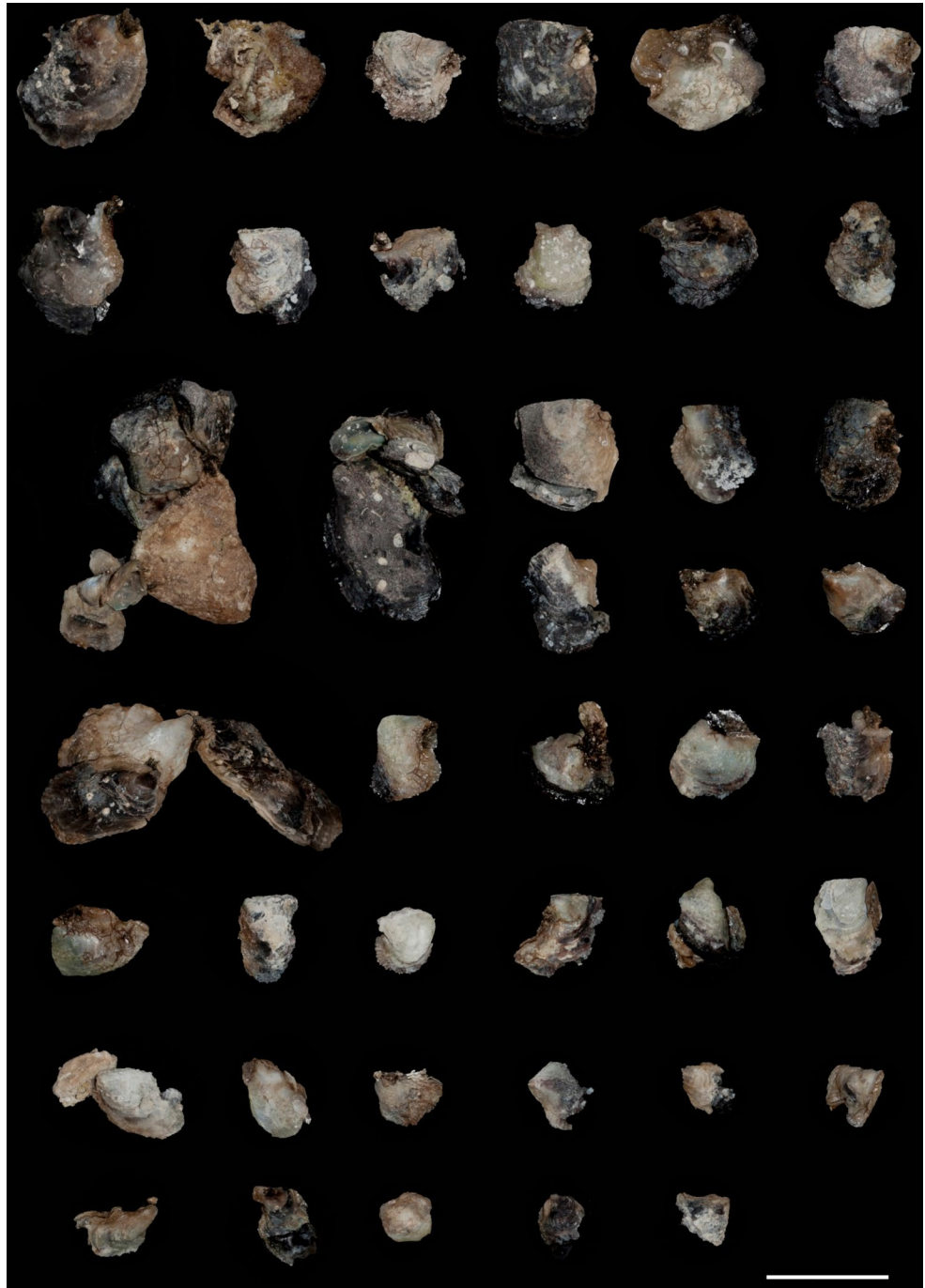
Individuals of two predatory murex species, the indigenous *Thais haemastoma* (Linnaeus, 1767) and the NIS *Ergalatax junionae* (Houart, 2008) were observed on the beds possibly feeding on *I. bicolor*.

At Pontamos beach, the shore deepens abruptly, and subtidal substrata are formed by rocks vertically disposed. The presence of *I. bicolor* is much less prominent than that in Alimia (only 3–5 individuals/100 cm²). Moreover, *Chama pacifica* (Broderip, 1835) and *Dendostrea frons* (Linnaeus, 1758) dominated the sessile assemblages, whereas *Pinctada*

radiata (Leach, 1814) was also present in small numbers in both localities. No *I.* aff. *legumen* morphotype was observed on this substratum. Bivalves macroscopically diagnosed as *I.* aff. *legumen* at both localities were found in high numbers exclusively under rocks at shallow depths (1–2 m; Fig. 4). The observed specimens were side attached to rock cavities, grew irregularly, and had a whitish colouration. No *I. bicolor* morphotype was detected under the rocks.

Fig. 6 Rock fragment detached from a large *I. bicolor* bed, showing the degree of invasion. The arrow indicates an *Ergatalax Junionae* predator found on the bed. Scale line is 2 cm

Fig. 7 Photo of an *Isognomon bicolor* bed under formation in Alimia
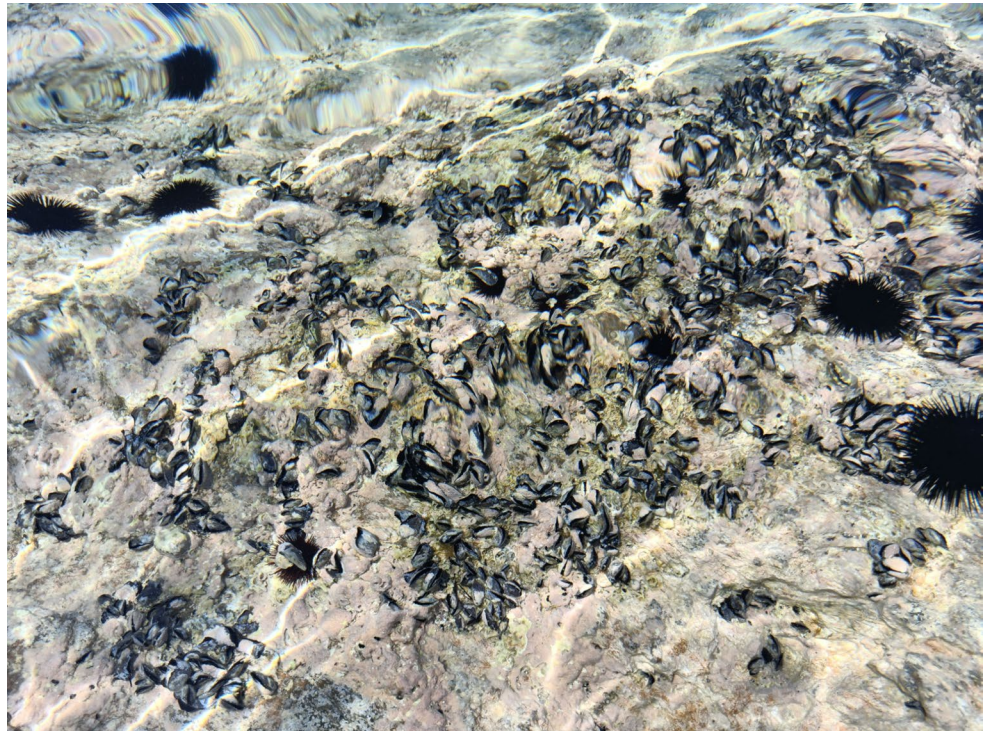
Discussion

In this study, two different non-indigenous species of the genus *Isognomon* established stable populations in two South Aegean islands, Chalki and Alimia (Fig. 1). Their identification was corroborated by DNA barcoding. The first records of *I. bicolor* in the Mediterranean Sea were recently documented and confirmed with molecular tools (Garzia et al. 2022; Albano et al. 2024b). Despite the numerous records of *I.* aff. *legumen* in the Mediterranean Sea (see above), its identification using molecular methods was confirmed for the first time in the Mediterranean Sea by Albano et al. (2024b). DNA barcoding is a particularly useful tool for species identification (Sun et al. 2016; Hamaguchi et al. 2017) and is essential for the genus *Isognomon* due to its highly irregular and habitat-dependent shell shapes (Fischer-Piette 1976; Coan et al. 2000; Wilk and Bieler 2009) and convergent interspecific morphologies (Benthotage et al. 2020), which render its identification unreliable using solely morphological features (Wilk and Bieler 2009). Nevertheless, we believe that both molecular and morphological (conchological) approaches should be applied for the identification of this genus due to the up-to-date unreliability of reference sequences in public databases (Briski et al. 2016), lack of reference sequence data (e.g., *I. australicus*), and difficulty in collecting new samples. This is particularly true for the taxa investigated here, as both species of interest (*I. bicolor* and *I. legumen*) have limited available reference data in public databases and are difficult to distinguish morphologically,

which complicates the use of barcoding as an identification tool. The limited reference sequences and the overall inconsistencies in public databases make identification within the genus using DNA barcoding difficult. For example, the first 16 S sequences for *I.* aff. *legumen* from the Mediterranean Sea were generated in this study. Additionally, taxonomic misidentifications were detected in biological databases for the *Isognomon* species *I. australicus* in this study (see Fig. 3, Supplementary Table 2), that could have increased the risk of unreliable species records with the application of a single genetic marker. Therefore, immediate revisions in taxonomic accuracy in reference databases are needed. The aforementioned issues have been the cause of concern for many studies and the reason for using multiple genetic markers to confirm the presence of both *Isognomon* species in the South Aegean Sea. Moreover, the newly generated sequences exhibited a high match rate (almost 100%) for both species, which was corroborated by further phylogenetic analyses. Therefore, morphometric characteristics (Reeve 1858; Printrakoon 2015; Angelidis and Polyzoulis 2018) and ecological observations of barcoded specimens are particularly useful for obtaining integrated identification and further distinguishing in situ populations. We are sceptical of the attempt by Albano et al. (2024b); Garzia et al. (2022) to re-evaluate previews records of *I. bicolor* and *I. legumen* based on published photographs. However if we accept those re-evaluations, the first record of *I.bicolor* in Greece is from Astypalaia, where it was misidentified as *I. legumen* by Angelidis and Polyzoulis (2018).

In fact, the habitat preference of each species could offer additional diagnostic information for species identification. For instance, *I.* aff. *legumen* was found to live under rocks at both study sites (Supplementary Fig. 3). Moreover, *I.* aff. *legumen* specimens were also observed to occupy the same habitat niche as in other Greek sites, such as Astypalaia (Kounoupa Islet), Karpathos (Amopi beach), and the Chalki port area (pers. obs. A. Angelidis). This bivalve was observed to develop a more regular elongated shell shape and darker colour when it grew in direct access to light and water, not antagonizing for space under stones/rocks (Fig. 2b, number 4). Therefore, we suggest that *I.* aff. *legumen* morphology depends on habitat conditions, which should be taken into account when considering the spread of this species in the basin.

In this study we aimed to establish a method of macroscopically distinguishing in situ populations of the two investigated species, based on morphological differences and similarities between genetically analysed specimens and ecological observation. This strategy however, requires further support trough application to a greater number of specimens accros more localities. This is indispensable for

the early detection and management of a progressing invasion, such as that of *I.bicolor*.

There is no evidence that the two Isognomonids under study were introduced to the investigated localities by their own means, such as through a natural poleward range expansion described by Essl et al. (2019). The East Atlantic corridor, the only route that could enable such a range expansion via the Gibraltar Strait (Albano et al. 2024a), must be excluded due to the lack of the necessary hard substrata for attachment, along the sandy West-African coast (see introduction) and also because these species do not occur in tropical W Africa. Consequently, the two investigated species qualify as true NIS as they were clearly introduced by human-induced factors.

The introduction of isognomonids could have been facilitated by their ability to attach to marine litter (Winston et al. 1997). However, this vector of introduction has not been evaluated in the Mediterranean Sea (Katsanevakis and Crocetta 2014). Additionally, the settlement of such species could have further eased locally due to favourable conditions, such as the availability of suitable substrate and water temperature. Similar temperatures (~ 21–$26\,°C$) were also reported in Brazil, where isognomonid bivalves have been established by occupying a wide range of the intertidal zone of rocky shores (Breves-Ramos et al. 2010; de Guimaraens and de Souza 2018). The Mediterranean dispersal of *I. bicolor* and *I.* aff. *legumen* larvae via water circulation and their selective recruitment to the South Dodecanese hotspot (Crocetta et al. 2017) is unequivocally supported by the increase in global temperature (Albano et al. 2021a). Elevated temperatures could facilitate fertilization and larval development, thus allowing species establishment (Gewing et al. 2022), which affects more sessile bivalves according to their thermal affinity as they become more exposed to SSTs as coastal inhabitants (Diga et al. 2023). This implies that global warming facilitates the establishment of non-indigenous mollusc species in the Mediterranean Sea (Givan et al. 2017; Peleg et al. 2019; Albano et al. 2021a) after their introduction.

The Mediterranean introduction and dispersal of *I.* aff. *legumen* seems to follow the known patterns of Lessepsian introduction via the Suez Canal, amplified by the tropicalization of the Levantine Basin. However, this is not the only introduction path and should not monopolize the relevant speculations for nonindigenous introductions (Garzia et al. 2022). This bivalve is also very likely to have been introduced by direct human action such as propagule spreading by ballast water discharge, rafting on anthropogenic debris and relocation of platforms (Mienis 2004). Introductions and establishment depend on the drivers of the recruitment of sessile marine organisms, which are often poorly understood due to the rapidly changing requirements experienced

during early ontogeny. The complex suite of physical, biological, and ecological interactions beginning at larval settlement involves a series of trade-offs that influence recruitment success (Doropoulos et al. 2016). The study of these mechanisms was not the focus of this study, although we offer some information that may be useful for identifying associations between differences in local substrata structure and recruitment success (see section field observations).

The classic vectors of transportation, such as ballast water, relocation of oil platforms, and rafting on hard debris (Schembri et al. 2010; Katsanevakis et al. 2013), may explain the trans-Atlantic transport of *I. bicolor*. The successful introduction of *I. bicolor* from the east and southeast coasts of the USA to the British shores using bait pots supports trans-Atlantic rafting on the macrolitter of this epibyssal bivalve (Holmes et al. 2015). Rafting as a migration vector of *I. bicolor* to the shores of Uruguay has also been established (Breves et al. 2014). The Mediterranean introduction and dispersal of *I. bicolor* may be explained by larval transport following hydrographic circulation, as suggested for other tropical Atlantic species (Valdés et al. 2013). The weakening of the cold Canary Current due to global warming, has allowed various tropical Atlantic species to enter through the Strait of Gibraltar but for sessile bivalves, this process must not be confused with a natural range expansion process.

The only possible way, as we see it, for those species to have entered the Mediterranean Sea in larval form is to be transported by rafting or other anthropogenic vectors close enough to the Gibraltar Strait to enter via the Atlantic inflow. Following their introduction to the Mediterranean Sea, *I. bicolor* subsequently dispersed via the strong Algerian Current and the Mid-Mediterranean Jet across the Mediterranean Sea (Vermeij 2012; Valdés et al. 2013), bypassing the West Mediterranean basin. Moreover, larvae of tropical species could be recruited locally under more favourable thermal conditions, such as those in the Levantine basin, and subsequently disperse westwards. Thus, the *I. bicolor* population recorded in both study areas may have acted as a donor pool for the southeastern coast of Italy, in accordance with Garzia et al. (2022). In this study, although eastwards dispersal is easily assumed for *I. bicolor*, it is likely that Ionian colonization is a result of secondary dispersal from the Aegean Sea. This hypothesis of Garzia et al. (2022), that we support, is based on lack of evidence of established populations of *I.bicolor* in the west Mediterranean basin and needs further study. Even if such populations occur in the future, we still have to investigate their eastwards or westwards origin.

The invasion of *I. bicolor* has been thoroughly studied along the tropical Brazilian coast (4,000 km), where extremely high population densities of 800-1,000 individuals per 100 cm^2 were reported from several localities (Lopez 2008; Breves-Ramos 2010). In July 2022, in Alimia cove, the population density did not exceed 35 individuals per 100 cm^2 in the most populated parts of the rocky substrates, two years after in the same localities the population densities exceeded the 150 individuals per 100 cm^2 and extensive beds were formed clearly indicating a progressing invasion. The low number of *I. bicolor* individuals observed in Pontamos suggests that the different coastal rock reliefs there do not favour the recruitment of the species as much as those in Alimia. Consequently, it is still too early to establish dense populations of *I. bicolor* in the area. Moreover, Brazilian populations of *I. bicolor* have a high number of adults and only a few recruits throughout the year (Rocha 2002; Breves-Ramos 2010). However, this type of population structure is not commonly recorded in bivalves, where high and continuous recruitment is usually expected, especially in introduced species. In contrast, *I. bicolor* is a concern for benthic communities not because of the intensity of recruitment but because of the stability of the bank, the longevity of adults, and the low, although regular, recruitment associated with low mortality and morphological plasticity (Cavalcante and Gomes 2022). Accordingly, the population structure of both investigated species in the Alimia cove involved low recruitment (four juveniles of 4–8 mm per 100 cm^2) but was sufficient to indicate healthy community growth (Cavalcante and Gomes 2022) and thus successful establishment. The *I. bicolor* individuals observed in the Alimia cove that are aggregating in rock crevices of the subtidal zone include adult individuals of larger sizes than those from Brazil (Cavalcante and Gomes 2022). The lack of empty shells in the investigated areas indicates low mortality, and the large adult sizes suggest the longevity and stability of both species' populations in situ.

Conclusions

This study confirms the recent establishment of two additional non-indigenous species (NIS) belonging to the genus *Isognomon* in the tropicalized marine ecosystem of two small islands in the southern Aegean Sea. Their identification was consolidated through an integrated strategy involving DNA barcoding, morphological analysis, and ecological observation. This approach also enabled the macroscopic recognition in situ of the significantly sized populations of both species.

These sessile bivalves, introduced by human-induced vectors from two different tropical donor regions—the West Atlantic and the Indo-Pacific—were found coexisting and expanding in the same localities but under different habitat conditions.

The first species, the Indo-Pacific *Isognomon* aff. *legumen*, which has been recorded in the eastern Mediterranean basin since 2001, was found to expand inconspicuously by attaching to the undersides of stones at shallow depths, establishing considerable populations there. The second, the West Atlantic *Isognomon bicolor*, is a highly invasive species well known for its fast and extensive colonization of the rocky coasts of Brazil. Its presence in the Mediterranean was very recently confirmed, and this study already records it forming conspicuous extensive beds in the intertidal coastal zone of the study area, altering the structure of the hard substrates it covers and impacting the local ecosystem.

With this study, updated in July 2024, we confirm the presence and progression of a significant and irreversible invasion of *I. bicolor* in the South Dodecanese marine ecosystem. The case of *I. bicolor Isognomon bicolor* underscores the need for continuous monitoring and the use of advanced identification methods to manage its invasion and mitigate its ecological impacts. Understanding these dynamics is crucial for protecting native marine ecosystems and maintaining biodiversity in the Mediterranean Sea.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s41208-024-00752-9.

Declarations

References

Albano PG, Steger J, Bošnjak M, Dunne B, Guifarro Z, Turapova E, Hua Q, Kaufman DS, Rilov G, Zuchin M (2021a) Native biodiversity collapse in the eastern Mediterranean. *Proceedings of the Royal Society B: Biological Sciences*. 288. 20202469. https://doi.org/10.1098/rspb.2020.2469

Albano PG, Steger J, Bakker PA-J, Bogi C, Bošnjak M (2021b) Numerous new records of indigenous species in the Eastern Mediterranean highlight the challenges of their recognition and identification. *ZooKeys*. 1010:1–95. https://doi.org/10.3897/zookeys.1010.58759

Albano PG, Schultz L, Wessely j, Taviani M, Dullinger S, Danise S (2024a) The dawn of the tropical Atlantic invasion into the Mediterranean Sea. Proceedings of the National Academy of Sciences.2024 April 121 (15) e2320687121. https://doi.org/10.1073/pnas.2320687121

Albano PG, Hong Y, Steger J, Yasuhara M, Bartolini S, Bogi C, Bošnjak M, Chiappi M, Fossati V, Huseyinoglu MF, Jiménez C, Lubinevsky H, Morov AR, Noè S, Papatheodoulou M, Resaikos V, Zuschin M, Guy-Haim T (2024b) New records of non-indigenous species from the eastern Mediterranean Sea (Crustacea, Mollusca), with a revision of genus Isognomon (Mollusca. Bivalvia) PeerJ 12:e17425. https://doi.org/10.7717/peerj.17425

Angelidis A, Polyzoulis G (2018) New distributional records of four Indo-Pacific species from Astypalaia Island. South Aegean Sea Xenophora Taxonomy 21:1–12

Benthotage C, Cole VJ, Schulz KG, Benkendorff K (2020) A review of the biology of the genus *Isognomon* (Bivalvia; Pteriidae) with a discussion on shellfish reef restoration potential of Isognomon ephippium. Molluscan Res. https://doi.org/10.1080/13235818.2020.1837054

Bianchi CN, Morri C (2003) Global sea warming and tropicalization of the Mediterranean Sea: biogeographic and ecological aspects. Biogeographia – The Journal of Integrative Biogeography, 24. https://doi.org/10.21426/B6110129 Retrieved from https://escholarship.org/uc/item/7bj69490

Breves A, Scarabino F, Leoni V (2014) First records of the nonnative bivalve *Isognomon bicolor* (C. B. Adams, 1845) rafting to the Uruguayan coast. Check List 10(3):684–686. https://doi.org/10.15560/10.3.684

Breves-Ramos A, Junqueira AOR, Lavrado HP, Silva SHG, Ferreira-Silva MAG (2010) Population structure of the invasive bivalve Isognomon bicolor on rocky shores of Rio De Janeiro State (Brazil). J Mar Biol Association United Kingd 90(3):453–459

Briski E, Ghabooli S, Bailey Sarah AM, & Hugh J (2016) Availability of sequences of aquatic and terrestrial taxa in genetic databases (GenBank and BOLD - the Barcode of Life Database) in 2010, 2012 and 2016 [dataset]. PANGAEA. https://doi.org/10.1594/PANGAEA.859211

Cavalcante LS, Gomes ATA (2022) Population structure of the invasive bivalve *Isognomon bicolor* (C.B. Adams, 1845) on rocky beaches in northeastern Brazil: Estrutura populacional da bivalve invasora *Isognomon bicolor* (C.B. Adams, 1845) nas praias rochosas do nordeste do Brasil. *Studies in Environmental and Animal Sciences*, 3(2), 245–257. https://doi.org/10.54020/seasv3n2-006

Coan EV, Valentich-Scott P, Bernard FR (2000) Bivalve seashells of Western North America: Marine Bivalve molluscs from Artic Alaska to Baja California. Santa Barbara Museum of Natural History. Monograph 2. Stud Biodivers, (2), 195–197 pp

Crocetta F, Gofas S, Salas C, Tringali L, Zenetos A (2017) Local ecological knowledge versus published literature: a review of non-indigenous Mollusca in Greek Marine waters. *Aquatic invasions*. 12(4). https://doi.org/10.3391/ai.2017.12.4.01

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9(8):772. https://doi.org/10.1038/nmeth.2109

de Guimaraens MA, de Souza JR (2018) Temporal and spatial variation of benthic intertidal community of a sandstone reef in Pernambuco-Brazil as a tool to evaluate bioinvasion. J Environ Sci Eng A 76. https://doi.org/10.17265/2162-5298/2018.02.003

Diga R, Gilboa M, Moskovich R, Darmon N, Amit T, Belmaker J, Yahel G (2023) Invading bivalves replaced native Mediterranean bivalves, with little effect on the local benthic community. Biol Invasions 25(5):1441–1459. https://doi.org/10.1007/s10530-022-02986-1Epub 2022 Dec 20. PMID: 36570095; PMCID: PMC9764321

Domaneschi O, Martins CM (2002) Isognomon bicolor (CB Adams) (Bivalvia, Isognomonidae): primeiro registro para o Brasil, rede-scrição Da espécie e considerações sobre a ocorrência e distri-buição de Isognomon na costa brasileira. Revista Brasileira De Zoologia 19:611–627

Doropoulos C, Roff G, Bozec YM, Zupan M, Werminghausen J, Mumby PJ (2016) Characterizing the ecological trade-offs throughout the early ontogeny of coral recruitment. Ecol Monogr 86:20–44. https://doi.org/10.1890/15-0668.1

Essl F, Dullinger S, Genovesi P, Hulme PE, Jeschke JM, Katsanevakis S, Küh I, Lenzner B, Pauchard A, Pyšek P, Rabitsch W, Richard-son DM, Seebens H, van Kleunen M, van der Putten WH, Vilà M, Bacher S (2019) A conceptual Framework for Range-Expanding species that Track Human-Induced Environmental Change. Bio-science 69(11):908–919. https://doi.org/10.1093/biosci/biz101

Fischer-Piette E (1976) Revision Des Aviculidees: 1. Crenatula. Pedalion, Foramelina. J De Conchyliologie 113(1–2):3–42

Garzia M, Furfaro G, Renda W, Rosati AM, Mariottini P, Giacobbe S (2022) Mediterranean spreading of the bicolor purse oyster, *Isog-nomon bicolor*, and the chicken trigger, *Malleus* sp., vs. the les-sepsian prejudice. Mediterranean Mar Sci 23(4):777–788. https://doi.org/10.12681/mms.29218

Geller J, Meyer C, Parker M, Hawk H (2013) Redesign of PCR prim-ers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. Mol Ecol Res 13:851–861. https://doi.org/10.1111/1755-0998.12138

Gewing MT, Goldstein E, Buba Y et al (2019) Temperature resilience facilitates invasion success of the solitary ascidian *Herdmania momus*. Biol Invasions 21:349–361. https://doi.org/10.1007/s10530-018-1827-8

Givan O, Edelist D, Sonin O, Belmaker J (2017) Thermal affinity as the dominant factor changing Mediterranean fish abundances. Glob Change Biol. https://doi.org/24

Guindon S, Gascuel O (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Syst Biol 52:696–704. https://doi.org/10.1080/10635150390235520

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML3.0. Syst Biol 59:307–321. https://doi.org/10.1093/sysbio/syq010

Hamaguchi M, Manabe M, Kajihara N et al (2017) DNA barcoding of flat oyster species reveals the presence of *Ostrea stentina* (Payraudeau, 1826) (Bivalvia: Ostreidae) in Japan. Mar Biodiv-ers Rec 10:4. https://doi.org/10.1186/s41200-016-0105-7

Holmes AM, Oliver PG, Trewhella S, Hill R, Quigley DTG (2015) Trans-Atlantic rafting of inshore Mollusca on macrolitter: Ameri-can molluscs on British and Irish shores, new records. J Conchol-ogy 42:41–49

Katsanevakis S, Crocetta F (2014) Pathways of introduction of marine alien species in European waters and the Mediterranean–A possi-ble undermined role of marine litter. In CIESM Workshop Mono-graph (pp. 61–68)

Katsanevakis S, Zenetos A, Belchior C, Cardoso AC (2013) Invad-ing European seas: assessing pathways of introduction of marine aliens. Ocean Coast Manag 76:64–74. https://doi.org/10.1016/j.ocecoaman.2013.02.024

Kessing B, Croom H, Martin A, McIntosh C, Owen Mcmillan W, Palumbi S (1989) The simple fool's guide to PCR. University of Hawaii, Honolulu, Hawaii

Larkin A, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948. https://doi.org/10.1093/bioinformatics/btm404

Lefort V, Longueville J-E, Gascuel O (2017) SMS: Smart Model Selection in PhyML. Mol Biol Evol 34(9):2422–2424, 2017. https://doi.org/10.1093/molbev/msx149

Lopez MS (2008) O bivalve invasor *Isognomon bicolor* (C.B. Adams, 1845) e seu papel nas comunidades de entremareˊs rochoso na regiaˊo de ressurgencia do Cabo Frio, RJ. PhD thesis. Departa-mento de Ecologia, Universidade Federal do Rio de Janeiro, RJ, Brazil

López M, Lavrado H, Coutinho R (2014) Structure of intertidal sessile communities before and after the invasion of Isognomon bicolor (C.B. Adams, 1845) (Bivalvia, Isognomonidae) in southeastern Brazil. Aquat Invasions 9(4):457–465. https://doi.org/10.3391/ai.2014.9.4.04

Manousis T, Zaminos G, Kontadakis C, Bazios G, Porfyris A, Galinou-Mitsoudi S (2021) New records of Mollusca for the Mediterra-nean and the Hellenic seas. Xenophora Taxonomy 31

McElderry JH (1963) Mediterranean tides and currents. Ir Astron J 6(1):12. https://ui.adsabs.harvard.edu/abs/1963IrAJ…

Micali P, Siragusa F, Agamennone F, Germanà A, Sbrana C (2017) Karpathos Island (Greece) and its Indo-Pacific alien species. Bol-lettino Malacologico 53:40–49

Mienis HK (2004) New data concerning the presence of Lesses-sian and other Indo-Pacific migrants among the molluscs in the Mediterranean Sea with emphasis on the situation in Israel. in B. Oztiirk & A. Salman (eds.) Proceedings 1st National Malacology Congress, 1–3 September 2004, Izmir. *Turkish Journal of Aquatic Life* 2(2): 117–131

Mienis HK, Rittner O, Shefer S, Feldstein T, Yahel R (2016) First record of the Indo-Pacific Isognomon legumen from the Medi-terranean coast of Israel (Mollusca, Bivalvia, Isognomonidae). Triton 33:9–11

Nykjaer L (2009) Mediterranean Sea surface warming 1985–2006. Climate Res 39(1):11–17. http://www.jstor.org/stable/24870419

Ovalis P, Zenetos A (2017) The ascent of Lessepsian Mollusca con-tinues: on the establishment of two newcomers in the eastern Mediterranean, in: Stamouli C New Mediterranean Biodiversity Records (December 2017), *Mediterranean Marine Science* 18(3) 534–556. https://doi.org/10.12681/mms.15823

Peleg O, Guy-Haim T, Yeruham E, Silverman J, Rilov G (2019) Clas-sification to traits of various taxa from three shallow rocky reef habitats in the southeastern Mediterranean. *PANGAEA*, https://doi.org/10.1594/PANGAEA.908353; In supplement to: Peleg, O. (accepted): Tropicalization may invert trophic state and carbon budget of shallow temperate rocky reefs. *Journal of Ecology*, https://doi.org/10.1111/1365-2745.13329

Printrakoon C (2015) Morphology and taxonomy of *Isognomon Spath-ulatus* (Reeve, 1858), a cryptic bivalve from the mangroves of Thailand. Plazi.org *taxonomic treatments database*. Checkl Data-set. https://doi.org/10.15468/h3xuyu

Raitsos DE, Beaugrand G, Georgopoulos D, Zenetos A, Pancucci-Papadopoulou AM, Theocharis A et al (2010) Global climate change amplifies the entry of tropical species into the eastern Mediterranean Sea. Limnol Oceanogr 55(4):1478–1484. https://doi.org/10.4319/lo.2010.55.4.1478

Reeve LA (1858) Monograph of the Genus Perna. In: Reeve LA (ed) Conchologia Iconica, or illustrations of the shells of molluscous animals, vol 11. Lovell Reeve, London, pp 1–8

Rocha FM (2002) Recruitment e sucessaˊo de uma comunidade ben-tonica de mesolitoral dominada pela especie invasora *Isogno-mon bicolor*. Bivalvia: Isognomonidae) (C.B. Adams, 1845) em dois costoes rechooses submetidos a diferentes condiçoˊes de

batimento de ondas. Dissertacao De Mestrado em Ecologia. Instituto De Biologia. Universidade Federal do Rio de Janeiro, RJ, Brazil

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard M A, & Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*(3), 539–542. https://doi.org/10.1093/sysbio/sys029

Schembri PJ, Bodilis P, Evans J, Francour P (2010) Occurence of barred knifejaw, *Oplegnathus fasciatus* (Actinopterygii: Perciformes: Oplegnathidae), in Malta (central Mediterranean) with a discussion on possible modes of entry. Acta Ichthyol Piscat 40(2):101–104. https://doi.org/10.3750/AIP2010.40.2.01

Scuderi D, Viola A (2019) The last alien reaching Sicily: Mollusca Bivalvia Isognomonidae: *Isognomon Legumen* (Gmelin 1791). Biodivers J 10(4):337–342. https://doi.org/10.31396/Biodiv.Jour.2019.10.4.337.342

Stenzel HB (1971) Oysters. In: Treatise on invertebrate palaeontology, Part N: Mollusca 6, Bivalvia, vol. 3 (R.C. Moore & C. Teichert, eds), N953–N1224. *Geological Society of America and University of Kansas Press*, Lawrence, Kansas

Sun S, Li Q, Kong L et al (2016) DNA barcoding reveal patterns of species diversity among northwestern Pacific molluscs. Sci Rep 6:33367. https://doi.org/10.1038/srep33367

Tamura K, Stecher G, Kumar S, (2021) MEGA11: Molecular evolutionary genetics analysis Version 11, Mol Bio and Evol, vol 38, Issue 7, July 2021, pp 3022–3027, https://doi.org/10.1093/molbev/msab120

Tëmkin I (2006) Morphological perspective on the classification and evolution of recent pterioidea (Mollusca: Bivalvia). Zool J Linn Soc 148:253–312. https://doi.org/10.1111/j. 1096-3642.2006.00257.x

Tëmkin I (2010) Molecular phylogeny of pearl oysters and their relatives (Mollusca, Bivalvia, Pterioidea). BMC Evol Biol 10:342. https://doi.org/10.1186/1471-2148-10-342

Thiel M, Haye P (2006) The Ecology of Rafting in the Marine Environment. Iii. Biogeographical and Evolutionary consequences. Oceanogr Mar Biology 44:323–429. https://doi.org/10.1201/9781420006391.ch7

Ubukata T (2003) A morphometric study on morphological plasticity of shell form in crevice-dwelling Pterioida (Bivalvia). Biol J Linn Soc 79:285–297. https://doi.org/10.1046/j.1095-8312.2003.00144.x

Ulman A, Ferrario J, Forcada A et al (2019) Alien species spreading on recreational vessels in the Mediterranean Sea. J Appl Ecol 56:2620–2629. https://doi.org/10.1111/1365-2664.13502

Valdés A, Alexander J, Crocetta F, Yokes MB, Giacobbe S (2013) The origin and dispersal pathway of the spotted sea hare *Aplysia dactylomela* (Mollusca: Opisthobranchia) in the Mediterranean Sea. Aquat Invasions 8(4):427–436. https://www.um.edu.mt/library/oar//handle/123456789/20993

Vergés A, Steinberg PD, Hay ME et al (2014) The tropicalization of temperate marine ecosystems: climate-mediated changes in herbivory and community phase shifts. *Proc Biol Sci*. 2014;281(1789):20140846. https://doi.org/10.1098/rspb.2014.0846

Vermeij GJ (2012) The tropical history and future of the Mediterranean Biota and the west African enigma. J Biogeogr 39:31–41. https://doi.org/10.1111/j.1365-2699.2011.02601.x

Vine P (1986) Red Sea invertebrates. Immel Publishing. Ely House, 37 Dover St. London WIX 3 RB

Wilk J, Bieler R (2009) Ecophenotypic variation in the flat Tree Oyster, *Isognomon alatus* (Bivalvia: Isognomonidae), across a tidal microhabitat gradient. Mar Biol Res 5(2):155–163. https://doi.org/10.1080/17451000802279644

Winston JE, Gregory MR, Stevens LM (1997) Encrusters, epibionts, and other biota associated with pelagic plastics: a review of biogeographical, environmental, and conservation issues. In: Coe JM, Rogers DB (eds) Marine debris. Springer, New York, pp 81–97

Zenetos A, Corsini-Foka M, Crocetta F, Gerovasileiou V, Karachle PK et al (2018) Deep cleaning of alien and cryptogenic species records in the Greek seas (2018 update. Manage Biol Invasions 9(3):209–226. https://doi.org/10.3391/mbi.2018.9.3.04

Zenetos A, Albano PG, Lopez GE, Stern N, Tsiamis K, Galanidi M (2022) Established non-indigenous species increased by 40% in 11 years in the Mediterranean Sea. Mediterranean Mar Sci 23. https://doi.org/10.12681/mms.29106