

Research Article

First records of non-indigenous *Timarete caribous* (Grube, 1859) (Polychaeta; Cirratulidae) in the Western Mediterranean, and its ecology in the Mar Menor (Murcia, SE Spain)

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Abstract

The knowledge and control of invasive species are essential in the management of marine ecosystems. The Mediterranean Sea is one of the marine areas with the highest number of invasive species detected, most of them invertebrates. On a smaller scale, colonization by non-native species in coastal lagoons depends directly on their connectivity with the adjacent sea and on the changes in their environmental conditions induced mainly by human interventions. In this study, the cirratulid species *Timarete caribous* Grube, 1859, native from the Western and Central Atlantic Ocean, was detected for the first time in a Western Mediterranean coastal lagoon, the Mar Menor. It has been found in dense aggregates on both artificial and natural rocky substrates, mainly in the most important communication channel with the Mediterranean Sea. Following the evolution of this NIS will be important for understanding the dynamic and spread of the species and its possible effects on the indigenous biological communities.

Key words: allochthonous species, invasive species, non-indigenous species, alien species, transitional waters, coastal lagoon, Annelida

Introduction

One of the greatest current challenges in the management and conservation of marine ecosystems is the knowledge and control of invasive species, mainly related to their impact on the extinction of other species and, in general, on the functioning and loss of services of ecosystems (Çınar 2013; Katsanevakis et al. 2014; Giakoumi et al. 2019). According to the European Environment Agency, 1223 marine non-indigenous species (NIS) were present in the European seas in 2017, of which 1039 (nearly 81%) were first recorded in the period 1949–2017, and mostly consisted of invertebrates (63%, approximately). The same source of information indicated that their number was highest in the Mediterranean Sea, where 838 (69%) of all these NIS have been recorded and their number increased with a rate of 8 species per year for the 2017–2019 period (EEA 2010; Zenetos and Galanidi 2020).

The opening of seaways is one of the main species transfer vector, being the Suez Canal the most important one in the case of the Mediterranean Sea. Its continuous dredging and maintenance activities reduce the existing biogeographical barriers for the so-called Lessepsian species. Other important, and sometimes synergistic, routes of entry for invasive marine species include shipping (through fouling or ballast water) and aquaculture (Çinar 2013; Katsanevakis et al. 2013). Moreover, the effect of climate change on the expansion and distribution of non-indigenous species must not be omitted, as well as its overall effect on the functioning and composition of ecosystems concerning their native species (Occhipinti-Ambrogi 2007). With regards to the abundance of macrozoobenthos groups, polychaetes stand out with an estimation of 154 alien species in the Mediterranean basin, just behind molluscs and crustaceans (Zenetos et al. 2010, 2012, 2022). A factor that obscures the real pattern of NIS distribution is the variable degree of taxonomic expertise regarding specific groups at local or national scale, which is considered an “extrinsic factor” by Giangrande and Licciano (2004), as well as the partial information in most studies focusing on a specific group rather than on the total assemblages (Tempesti et al. 2020).

Coastal lagoons often represent stepping-stones in the colonization process of many NIS, offering the opportunistic species the ideal conditions, such as abundant food resources and a general absence of predators or diseases, which are more common in the coastal open sea (Pérez-Ruzafa et al. 2019a). Coastal lagoons are transitional ecosystems between land and sea with a relative isolation from the latter. They are dynamic systems with strong physical, chemical, and biological gradients, and a great spatio-temporal variability characterized by frequent environmental disturbances and fluctuations, both natural and human induced (Barnes 1980; UNESCO 1981; Kjerfve 1994; Pérez-Ruzafa et al. 2019a). Furthermore, coastal lagoons are among the most productive and valuable ecosystems in the world as a consequence of their physiographic, hydrological, and biological characteristics (Anthony et al. 2009; Russi et al. 2013; Pérez-Ruzafa et al. 2019a). They harbour very prominent biodiversity values (EEA 2010; De Wit 2011), sheltering a collection of habitat types for many species and offering conditions for the maintenance of their lifecycles, while functioning as refugia, nursery areas and feeding grounds for many marine migratory organisms (Sabetta et al. 2007; Vasconcelos et al. 2011; Pérez-Ruzafa et al. 2019a). Among the ecological services they provide, coastal lagoons can act as genetic or species biodiversity reservoirs which favour the selection of locally adapted populations, to more extreme and restricted conditions and, thus, more likely to face future climatic or other changes and pressures (Pérez-Ruzafa et al. 2019a). Moreover, they may sometimes offer extreme environmental conditions that favour the exclusion of parasites and predators, allowing the conservation of species

threatened by them, as in the case of the Mediterranean endangered species *Pinna nobilis* Linnaeus, 1758 in Mar Menor (López-Núñez et al. 2022; Nebot-Colomer et al. 2022). However, some of these features (high productivity, extreme conditions, exclusion of many parasites and predators) can favour some NIS and opportunistic species.

The colonization of lagoons by NIS generates changes in their assemblages, originating new ecological states associated with future implications for their functioning, provision of services, or management (Secco et al. 2005). This colonization and subsequent settlement processes are highly dependent on the connectivity with the adjacent sea, and their success and rate are the main factors determining the species composition and their spatio-temporal dynamics at a lagoon basin scale (Perez-Ruzafa and Marcos 1992, 1993; Ghezzi et al. 2015; Pérez-Ruzafa et al. 2019b). The restricted connectivity produced by the inlets results in low probability and randomness in the colonization processes leading to a high spatio-temporal variability both between lagoons and within the same lagoon (Pérez-Ruzafa et al. 2019a). For example, approximately 40% of the macrofaunal benthic invertebrates in Venice Lagoon change from one year to another (Sigovini 2011), and a similar percentage has been observed in the ichthyoplankton interannual composition in the Mar Menor (Quispe 2014; Pérez-Ruzafa et al. 2019a). On the other hand, and in the same sense of ecological change, the presence and expansion of NIS sometimes lead to the creation of new habitats in the ecosystem or to an increase in its diversity and heterogeneity (Zaiko et al. 2009; Arias et al. 2013). Magni et al. (2019) showed that, given that these ecosystems are under so strong anthropic pressure, including dystrophic crises and episodes of hypoxia or anoxia, colonisation processes by opportunistic NIS could result in an increase in the number of species in highly altered ecosystems, always considering a careful monitoring process.

Within polychaetes, cirratulids inhabiting the Mediterranean Sea have rarely been subject of special interest and have normally been reported in general fauna or ecological works (Çinar 2007). The Family Cirratulidae comprises 10 genera and 21 species in the Mediterranean Sea, being present in a wide variety of habitats (Çinar 2007). Despite some recent increases in the documentation of this family (Çinar and Dagli 2021), its knowledge in the Mediterranean area is still poor. Notable exceptions are represented by the works of Gravina et al. (1989), Çinar and Ergen (2007), Çinar (2009), Simboura et al. (2010), Chambers et al. (2011), Lezzi et al. (2016), Lezzi (2017), Grossi et al. (2017), Munari et al. (2017) or Langeneck et al. (2020), some of them expressly making reference to NIS polychaetes in this sea.

Worldwide, the family Cirratulidae includes 16 genera (WORMS 2022). These are poorly defined, and some authors group them into multi-tentaculate and bi-tentaculate forms (Blake 1996). The multi-tentaculate cirratulids form a group of five genera: *Cirratulus* Lamarck, 1801, *Timarete*

Kinberg, 1866, *Protocirrinervis* Czerniavsky, 1881, *Cirriformia* Hartman, 1936, and *Fauvelicirratulus* Çinar & Petersen, 2011. All of them possess more than a single pair of tentacles attached on the dorsal part of anterior segments and organized in longitudinal rows or in transverse or oblique groups (Magalhães et al. 2014). The number, segmental origin, and distribution of branchial filaments are also of taxonomic interest at genus level.

Blake (1996), Çinar (2007), Magalhães and Bailey-Brock (2010) and Magalhães et al. (2014) conducted taxonomic revisions for the genus *Timarete*. Magalhães et al. (2014) provided a systematic review of diverse multi-tentaculate cirratulids from Brazil, including the species *Timarete caribous* Grube, 1859, *Timarete oculata* Treadwell, 1932 and the description of *Timarete ceciliae* Magalhães, Seixas, Paiva and Elias, 2014.

In the Mediterranean area, a number of *Timarete* species have been reported, of which *Timarete filigera* Delle Chiaje, 1828 is considered as the only native one and has a wide distribution within this sea. The presence of the non-native *Timarete anchylochaeta* Schmarda, 1861 and *Timarete dasylophius* Marenzeller, 1879 has been recorded from the Sea of Marmara (Rullier 1963) and Lebanon (Laubier 1966). However, the original report, which is the basis for subsequent ones, contains a very brief description and no figures, so its accuracy is dubious; thus, these two species are mentioned as questionable reports by Çinar et al. (2005) and Zenetos et al. (2010), and have been removed from subsequent inventories of species introduced to the Mediterranean Sea. Afterwards, *Timarete punctata* Grube, 1859, another alien species, was reported from the Levantine coast of Turkey (Çinar 2007). In this case, the description is detailed and accompanied by adequate figures and with a comparison between the type and Turkish material, so identification is reliable, and the species should be treated as an established alien (Zenetos et al. 2010). Finally, Çinar (2009) reported the presence of *Timarete caribous* Grube, 1859 in SE Turkey. As in the previous report, a detailed description is provided and comparison with type specimens has been made. In spite of small differences with the type material, Zenetos et al. (2010) treated the species as a casual record, and Zenetos and Galanidi (2020) as an established alien. All of the non-native *Timarete* species have been reported from the eastern Mediterranean, and the scarce information on their populations, stresses the need of an effort in the identification and revision of these species and their distribution throughout the Mediterranean basin.

In this context, the aim of this work is to describe and study the presence and population dynamics of the non-indigenous *Timarete caribous* in an Iberian Peninsula coastal lagoon (Mar Menor, Murcia – SE coast of Spain), to increase the knowledge of its actual distribution and to update the polychaetes NIS list in Mediterranean marine habitats.

Materials and methods

Study area

The Mar Menor is a hypersaline coastal lagoon located in the Region of Murcia, in the southeast of Spain (Western Mediterranean). With a surface area of 136 km² and a perimeter of 59.5 km, it is one of the largest lagoons in the Mediterranean. It is separated from the open sea by a sandy bar 22 km long and 100–900 m wide, crossed by three main channels that allow the communication between the two water bodies. The width of the Mar Menor inlets is approximately 645 m, leading to a restriction ratio of 0.015 (Pérez-Ruzafa et al. 2005a), a water renewal rate of 318 days, with net flow from Mediterranean water into the lagoon, and a classification of this as a restricted lagoon (Ghezzi et al. 2015). The lagoon has an average depth of 3.6 m, a maximum depth of 6.1 m, and a total volume of water of 598.8 hm³ (Ghezzi et al. 2015; García-Oliva et al. 2018; Pérez-Ruzafa et al. 2020). Due to human actions, its salinity has been decreasing in the last years from an annual average of 50.60 ± 0.27 in the 1970s to range between 43.21 ± 0.09 to 45.70 ± 0.09 in the 1980–1990s, after the dredging of the Estacio inlet. Since 2019, it ranges between 40.54 ± 0.09 and 42.49 ± 0.09 due a rising water table after changes in human uses and inadequate management actions on the watershed, which resulted in continuous flows of fresh water. Its temperature ranges between 11.3 °C and 31.3 °C (García-Oliva et al. 2018; Pérez-Ruzafa et al. 2019c). The water circulation within the lagoon is primarily driven by winds, and the system of currents follows a counter-clockwise model, with 3 main gyres (Pérez-Ruzafa and Marcos 1993) located in the north, centre, and south, determining three differentiated basins with different degrees of confinement. Finally, the predominant bed sediments are muddy in the central areas of the lagoon and sandy in shallow exposed areas (Pérez-Ruzafa 1989). Hard bottoms are located around the five volcanic islands that exist within the lagoon as well as in artificial surfaces corresponding to ports and piers along its coastline.

The lagoon has suffered various changes throughout its history that have had consequences on its environmental characteristics and the functioning of its ecosystem. The intense tourism development on its coast since 1950, the changes in the channels communicating with the Mediterranean Sea (opening or dredging of them), the change in the agricultural model in its drainage basin (from rainfed to irrigated agriculture), and the coastal works for the improvement and maintenance of its beaches stand out among the most recent and important actions (Pérez-Ruzafa et al. 1987, 1991, 2005a, 2019c). Two of the most important based on the ecological impacts they caused were the enlargement of El Estacio Channel with the purpose of making it navigable and the inputs of nutrients as a result of the intensive agricultural activity surrounding the coastal lagoon.

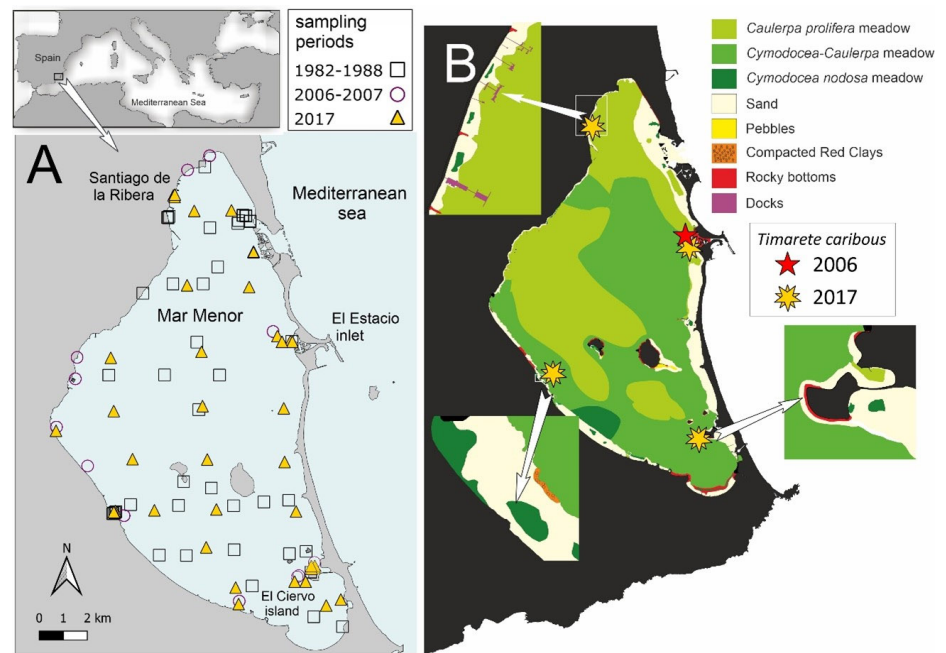


Figure 1. (A) Mar Menor and studied localities in the three periods (1982–1988; 2006; 2017); (B) Composition map of different communities found in the Mar Menor and localities where *T. caribous* has been found.

The dredging of the main communication channel originated a properly called “Mediterranization” process in the sense of a decrease in salinity values, a softening of extreme temperatures, and a progressive colonization by Mediterranean species (Perez-Ruzafa et al. 1991). One of these was *Caulerpa prolifera* (Forsskal) J.V.Lamouroux, 1809, which caused a significant effect on the nature of the sediments. Its expansion increased the organic matter content and led to anoxic conditions affecting also the pre-existing *Cymodocea nodosa* (Ucria) Ascherson, 1870 meadows (Pérez-Ruzafa 1989; Pérez-Ruzafa et al. 2008, 2012), as well as to changes in the associated benthic communities (Perez-Ruzafa et al. 1991; 2006). Moreover, in the last three decades the intensive agricultural activity surrounding of the coastal lagoon has increased the inputs of nutrients (Pérez-Ruzafa et al. 2005b) and has caused a severe eutrophication process, leading to extreme dystrophic crises since 2016 (Pérez-Ruzafa et al. 2019c; Fernández-Alías et al. 2022).

Sampling

Since the 1980s, periodic sampling of the Mar Menor benthic communities has been carried out in the context of different research projects, maintaining a series of basic sampling locations (Figure 1A). One of the last sampling efforts was carried out in 2017, in the context of a monitoring plan following the dystrophic crisis event that occurred in the summer of 2016 because of the eutrophication process that the lagoon had been undergoing (Pérez-Ruzafa 1989; Pérez-Ruzafa et al. 2005b). In 2017, the samples were collected following a tri-monthly schedule to include variability within the year and to cover all the seasons (winter, spring, summer, and autumn). In this

study, 26 locations in the deeper areas with soft bottoms were sampled from boat using a van Veen grab (400 cm²). Three different locations in shallow areas were sampled by diving, scraping a surface of 400 cm² using a shovel in rocky bottoms: El Estacio inlet (Es), El Ciervo Island (Ci) and Santiago de la Ribera piers (Ri). Vertical zonation was defined in these study locations as midlittoral (ML_ROCK) when depth was < 0.5 m, and infralittoral (WI-IL-ROCK) when depth was 0.5–1 m. In the case of Ri, samples only corresponded to midlittoral zone (Eleftheriou 2013). One location (Los Urrutias) was sampled by using a core of 11.4 cm internal diameter in *Cymodocea nodosa* meadows on sand (Eleftheriou 2013; Hummel et al. 2017). Two replicates were collected at each site. All samples were sieved in situ using 500 µm mesh bags; the fauna was stored in jars for identification and subsequent quantification in the laboratory. The retained material was stored in marine water for its transport to the laboratory. The separation, counting, and first classification of polychaete were carried out with the aid of an Olympus SZX7 stereomicroscope, and all the specimens were preserved in 70% ethanol. The posterior and detailed identification to species level was carried out at the Autonomous University of Madrid, using a Nikon XN stereomicroscope and a Nikon microscope. Pictures were made using a MOTICAM 1080 camera adapted to the stereomicroscope. Some specimens were selected for scanning electron microscope (SEM) in the University of Murcia. All the specimens were deposited at the laboratory of the research group “Ecology and management of coastal marine ecosystems” of the University of Murcia. After identifying the abundant presence of *Timarete caribous* in some of the localities, the samples taken in previous projects and stored in the collections of the research group were also revised.

Results

Systematic

Family Cirratulidae

Genus *Timarete* Kinberg, 1866

***Timarete caribous* (Grube, 1859)**

Type locality

St. Croix, Caribbean Sea, Western Atlantic Ocean.

Material examined

A total of 4434 specimens of *T. caribous* were collected in the Mar Menor coastal lagoon, divided in 329 individuals in 2006 (all from El Estacio inlet) and 4105 in 2017 (from several localities). Material examined was deposited in the laboratory of the research group “Ecology and Management of Coastal

Table 1. Species list of Cirratulids (Polychaeta: Terebellida) cited in Mar Menor and their cumulative mean abundance (indivs./400 cm² ± se) in the studied periods for all bottoms. No standard error value involves that the species was recorded in only one occasion and site.

Family	Specie	1982–1988	2006	2017
Cirratulidae	<i>Caulleriella alata</i> (Southern, 1914)	20	5.2 ± 1.8	
	<i>Caulleriella bioculata</i> (Keferstein, 1862)	29.8 ± 17.4	8.5 ± 3.3	
	<i>Chaetozone</i> sp. (Malmgren, 1867)	1		
	<i>Cirriiformia tentaculata</i> (Montagu, 1808)	5 ± 4	6.4 ± 1.14	3.8 ± 1.9
	<i>Dodecaceria concharum</i> (Örsted, 1843)	6.2 ± 2.9	2.9 ± 0.5	2
	<i>Timarete caribous</i> (Grube, 1859)		11.7 ± 2.46	132.3 ± 32.6

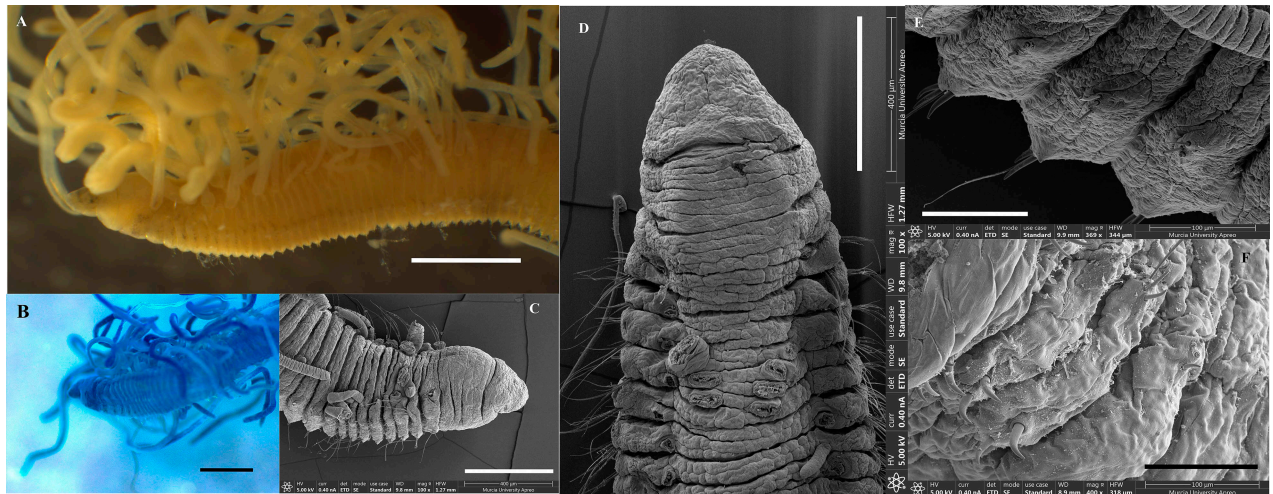


Figure 2. *Timarete caribous*. (A) Anterior end lateral views showing the dorsal disposition of branchiae. (B) Anterior end ventral view Methyl green pattern. (C) SEM micrograph anterior dorsal view. (D) SEM micrograph anterior dorsal view detail of prostomium, peristomium and tentacular filaments. (E) SEM micrograph thoracic notopodia and neuropodia lateral view detail. (F) SEM micrograph abdominal chaetigers details. Scale bars – A, B: 1 mm; C, D: 400 µm; E: 100 µm. Photos by Antonio Sala-Mirete.

Marine Ecosystems” of the University of Murcia. Record information, access code and abundance of each sample are presented in Supplementary material Table S1. Mean abundances (individuals/400 cm²) for each period and stations are presented in Table 1.

Description

Specimens 15–36 mm long, with 110–157 segments. Body ventrally flattened in the anterior region, without ventral groove. Body of living specimens dark orange, light yellow and pale in preserved ones; with some dark pigmentation located on ventral anterior, clearly visible after preservation (Figure 2A, B). Prostomium short and rounded; as long as wide. Peristomium with three rings, longer than prostomium (Figures 2C, 3A). Palps absent. First pair of branchiae arising on the margin of third peristomial lobe, in the intercept with the first chaetiger (Figures 2C, 3A, B). Branchial filaments located dorsally and close to notopodia up to chaetigers 9–10; shifting gradually to mid-dorsum thereafter, distance between left and right branchiae of the same segment equal or slightly longer than distance to corresponding notopodia (Figures 2A, C, 3B). Tentacular cirri above two or three chaetigers, e.g. 4–5, 3–4, loosely grouped by 2–3 tentacular filaments

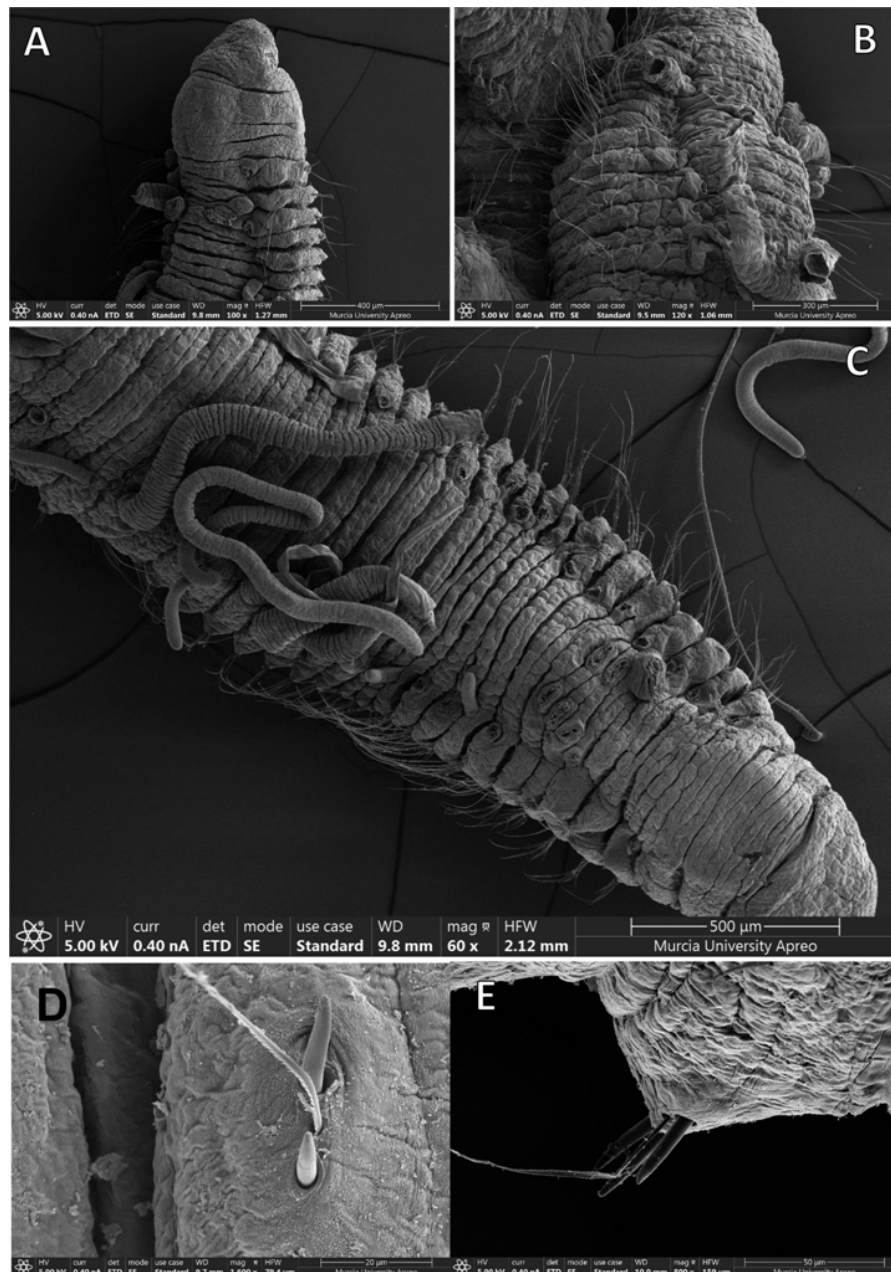


Figure 3. *Timarete caribous*. SEM micrographs. (A) Anterior end dorso-lateral view, showing detail of prostomium, peristomium and branchiae insertion. (B) Anterior end in lateral view showing detail of the branchial gradual shift. (C) Dorsal view with detail of tentacular cirri location. (D) Abdominal notopodial spines and capillary chaeta. (E) Abdominal neuropodial spines. Scale bars – A: 400 µm; B: 300 µm; C: 500 µm; D: 20 µm; E: 50 µm. Photos by Antonio Sala-Mirete.

on each side of corresponding chaetiger. Intraspecific variability was found regarding to the number of cirri and the chaetiger where present. (Figures 2C, D, 3C). Notopodia and neuropodia widely separated, poorly developed. Anterior notopodia bearing 2–3 spines with 1–2 capillaries from chaetiger 24 (Figures 2E, 3D). Anterior neuropodia bearing 3–4 spines combined with 1 capillary chaetae from chaetiger 11–14. Posterior neuropodia alternating groups of 2 spines similar in length and shape to single ones (Figures 2F, 3E). Spines slightly sigmoid with pointed tips; dark brownish coloured; about one third of the length of capillaries (Figures 2F, 3E).

Taxonomic remarks

Timarete species usually have branchiae that originate from the posterior end of the peristomium, anteriorly to the dorsal tentacles. These branchiae shift posteriorly to a more dorsal location, as in the herein described specimens, whereas the branchial filaments in *Cirriformia* species remain close to the notopodial lobes throughout the body. Branchiae in species of *Cirratulus* begin on the same segment as the tentacular filaments, usually chaetiger 1. The recently described genus *Fauvelicirratulus* has a similar arrangement of branchiae and tentacles as in *Cirratulus* but differs by the presence of more than a single pair of branchiae per segment and by different types of acicular spines.

Within the genus *Timarete* there are characters shared among the species *T. punctata*, *T. caribous*, *T. ceciliae* and *T. filigera* and absent in other species, such as the shape of the prostomium, absence of eyes, origin of branchiae, origin and form of notopodial spines, or the presence of thicker neuropodial spines. *Timarete punctata* differs from the other species of this group by the presence of a conspicuous pigmentation both along the body and on the tentacular cirri; by the number of acicular spines on parapodia (3–4) and their anterior appearance at chaetigers 8–25, and by the number and position of chaetigers bearing feeding tentacles (3 and 4 or 4 and 5) (Çinar 2007; Magalhães et al. 2014). In the species *T. filigera*, native from the Mediterranean Sea, the branchiae shift abruptly on chaetigers 15–20 from notopodia to body dorsum, a characteristic also present in *T. caribous*. However, neuropodial spines in *T. filigera* appear on chaetigers 15–21 and form groups of 4 or 5 (up to 6) throughout the entire body length, whereas in *T. caribous* neuropodial spines form groups of 3 or 4 from chaetiger 10–15 and change to a single one from chaetigers 34–38. For *T. ceciliae*, the shifting of the branchiae from notopodium to dorsal area is not abrupt but gradual. Another difference very important to Magalhães et al. (2014), is the number of neuropodial spines. *T. ceciliae* shares with *T. caribous* the anterior origin of these spines, but in *T. ceciliae* neuropodial spines, which are only slightly larger than the notopodial ones, form groups of at least 2 or 3 throughout the entire body.

Çinar (2009) reported subtle differences between Mediterranean specimens and the syntypes of *T. caribous*. The notopodial acicular spines appeared in chaetiger 30 in the Mediterranean specimens and in chaetiger 40 in the syntypes, while neuropodial spines did so in chaetiger 50 in the Mediterranean material and in chaetiger 100 in the type material. The author considered these differences to be size dependent, as eastern Mediterranean specimens were clearly smaller. Regarding those found in the Mar Menor, the largest specimen is 36 mm long, which is more similar to the Caribbean syntype. The neuropodial hooks are first present on chaetiger 11 to 14 for specimens reported in Mar Menor, which is consistent with the other two specimens

Table 2. *T. caribous* mean density and standard error (SE) (indiv/400cm²) in studied periods (2006; 2017), seasons and locations where present. El Ciervo island (Ci); El Estacio inlet (Es); Santiago de la Ribera (Ri).

Values (indiv/400cm ²)	2006						2017					
	Ci	SE	Es	SE	Ri	SE	Ci	SE	Es	SE	Ri	SE
Winter	0	0	13.66	2.37	0	0	0	0	391.25	36.05	8.5	7.5
Spring	0	0	8.98	2	0	0	3	1	196.5	91.10	2	1
Summer	0	0	2	1	0	0	9	8	114.75	52.17	78	0
Autumn	0	0	7.37	2.92	0	0	1.5	0.5	285.5	121.74	8.66	2.60

from the Mediterranean and Caribbean Seas (Çinar 2009). In this respect, our specimens are more similar to those from Levantine Sea than to those from native range, even though size in the largest individuals from Mar Menor is similar to those of type series. The only remarkable difference is the much smaller number of tentacular cirri forming each bundle, which in western Mediterranean specimens ranges from two to four and in those from Turkey is more than 30. The importance of this character is very hard to assess, as it is omitted in many case descriptions (see Magalhães et al. 2014), making it difficult to know the intraspecific variation. Considering this, we consider the most reasonable option to assign our specimens to *T. caribous*.

Cirratulid assemblages in the Mar Menor

Cirratulids in the Mar Menor are represented by a total of 5 genera and 6 species (Table 1). However, the cirratulid fauna in the lagoon has changed in composition and number of species throughout the studied periods and some species, like *Chaetozone* sp., have been collected in only one locality and occasion (Table 1). While the genera *Caulleriella* spp. Chamberlin, 1919 decreased in abundance since 1980s and was not found in 2017, *Cirriiformia* and *Dodecaceria* Örsted, 1843 were present in all studied periods and in similar population densities (Table 1). *Timarete caribous* appeared for the first time in 2006 in both mid- and infralittoral communities on rocky substrates in El Estacio inlet (Es). It spread its distribution and greatly increased its abundance in 2017, with an average abundance in the lagoon of 263.67 ± 83.87 indiv./400cm² and maximum values of 566 indiv./400 cm² in the midlittoral rocky bottoms in Es during the winter (Table 2). Other Cirratulids occurring on hard bottoms were *Caulleriella* spp., for 1982–1989 period, but were not identified in 2017 in the same community. In 2006, midlittoral and infralittoral rocky bottoms presented a higher number of species than in the other two periods (Figure 4).

In El Ciervo island (Ci), *T. caribous* highest abundance was 17 indiv./400 cm², reached in summer 2017 in midlittoral rocky bottoms. This sampling site is a shallow natural rocky bottom, characterized by the presence of photophilic algae dominated by *Cystoseira compressa* (Esper) Gerloff & Nizamuddin, 1975, *Palisada tenerrima* (Cremades) D. Serio, M. Cormaci, G. Furnari & F. Boisset, 2010 at mid- and infralittoral rocky bottoms (Pérez-Ruzafa et al. 2008; García-Sánchez et al. 2012) and *Caulerpa prolifera* at the transition to

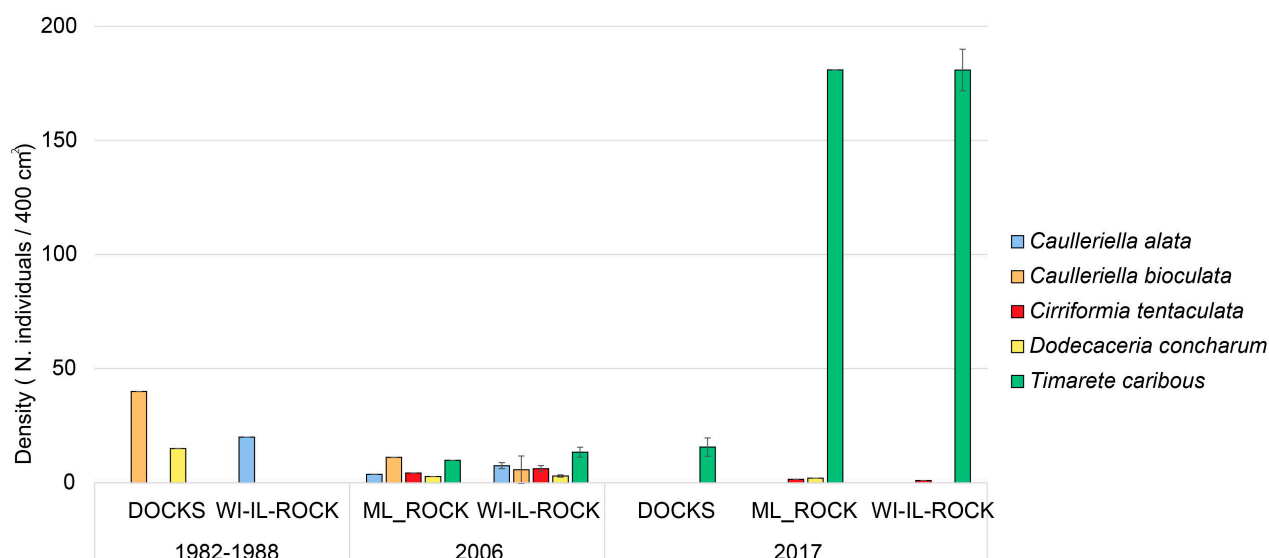


Figure 4. Mean density of cirratulid faunal composition in hard bottoms within the Mar Menor lagoon during the study periods (1982–1988; 2006; 2017) in hard bottoms: Santiago de la Ribera piers with low illumination (DOCKS); well illuminated mid-litoral communities (ML_ROCK); and well illuminated infra-litoral communities (WI-IL-ROCK).

soft bottom in the infralittoral zone. In Santiago de la Ribera (Ri), population density of *T. caribous* reached 78 indiv./400 cm² in the infralittoral sciaphilous community on rock in pillars under pier-spas in summer (Table 2).

Discussion

Mediterranean marine region presents the highest number of polychaete alien species (154), 71 of which were already considered as established (Zenetos et al. 2010, 2022; Zenetos and Galanidi 2020). However, after a revision made by Zenetos et al. (2022), 22 species of polychaete were excluded from the list, 24 were considered as questionable, three as failed introductions, and 16 as casual species. Therefore, it is necessary to correctly consider the definitions when dealing with invasive species, as well as to perform monitoring studies to define them as occasional or as naturalized or established in the ecosystem under study. In this sense, an alien species may be considered on a scale of colonization as unestablished, established, invasive and noxious species, following population expansion and impact on the ecosystem (Occhipinti-Ambrogi and Galil 2004). With that in mind, an “established alien” is considered an introduced species settled in the wild in at least one free-living, self-sustaining population by reproduction outside its natural range and independent of humans. For practical reasons, species with at least two records in the area over time and space are so considered (Occhipinti-Ambrogi and Galil 2004; Zenetos et al. 2010). Thus, truly established aliens need to be separated from species with seemingly isolated records and from those that have naturally spread to regions beyond their usual range (Zenetos et al. 2010).

In coastal lagoons in particular, the degree of isolation or confinement becomes one of the most determining factors of the colonization processes,

and therefore of the structure of the assemblages and communities in these environments (Guelorget and Perthuisot 1983; Pérez-Ruzafa and Marcos 1992, 1993; Pérez-Ruzafa et al. 2019a). Consequently, in coastal lagoons, the first records of the species frequently occur in or near channels communicating with the open sea, and only after a few years do they expand throughout the lagoon, constituting a population adapted to the local environmental conditions (Pérez-Ruzafa et al. 2011a, b).

The physical, chiefly geomorphological and hydrographic, barriers found in transitional ecosystems are one of the main factors that determine their species composition (Pérez-Ruzafa 2007a, b) and control the colonization probability. The larval colonization probability is very low, even in coastal lagoons with higher water renewal rates (Ghezzi et al. 2015). Thus, colonization becomes a largely random process that explains the low number of non-migrant species shared by different lagoons and the high spatio-temporal and interannual variability of assemblages in a given basin, contributing to shape the heterogeneity and homeostatic capacity of these ecosystems (Pérez-Ruzafa et al. 2008, 2019a). It is worth mentioning that a dystrophic crisis occurred after 25 years of eutrophication process of the Mar Menor lagoon. It started in 1990s, but the ecosystem showed a high homeostatic regulation capability until it collapsed in 2016 (Pérez-Ruzafa et al. 2002; Pérez-Ruzafa 2005b, 2019c). The increasing abundance of *T. caribous* could be part of the system response. As mentioned above, coastal lagoons are considered as naturally stressed ecosystems. They present an inherent heterogeneity in their faunistic composition that is reflected in both spatial and temporal variability at different scales. Thus, many species that appear in a given period could be naturally absent in the following one (Sigovini 2011; Quispe 2014). Therefore, Cirratulid assemblages of the Mar Menor and their shift from being dominated by *Caulleriella* spp. to *Cirriiformia tentaculata* and *T. caribous* can be explained by this interannual variability. Furthermore, it should not be underestimated that the degradation caused by the dystrophic crisis in 2016 was an additional selection factor for species more tolerant to altered conditions. However, data presented in this study would not be considered sufficient to determine a correlation between *T. caribous* and the other Cirratulids, as it does not determine whether this is an outcome of interspecific competition, a selection of more tolerant species, or part of the natural dynamic of this heterogeneous ecosystem.

With respect to the different hard substrate communities, *T. caribous* showed a clear dominance on hard bottoms of anthropogenic origin, such as the photophilous mid- and infralittoral biocenoses in El Estacio inlet (Figure 5). This dominance with respect to the rest of the communities may be result of the influence of the Mediterranean Sea. In such inlet areas, the influence of the open sea has two components that can operate in opposite directions. On one hand, according to the confinement theory

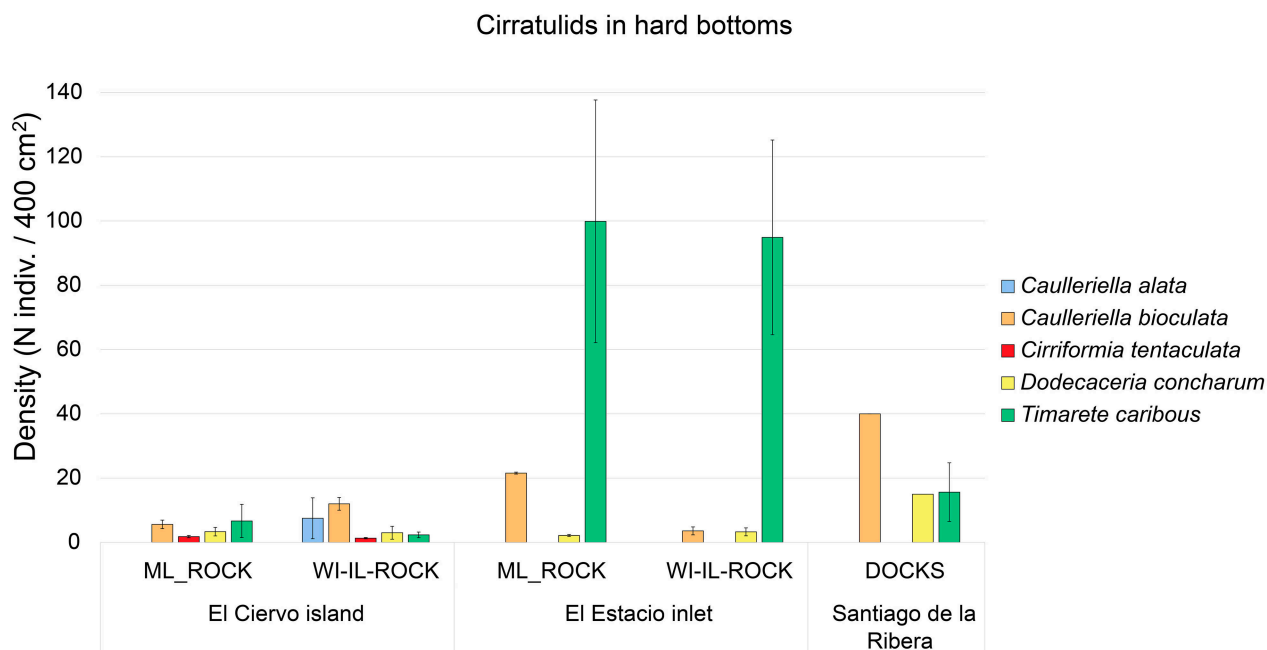


Figure 5. Cumulative density of the species of cirratulids (colour scale) found in the Mar Menor for the three periods (1982–1988; 2006; 2007) in hard bottoms: Santiago de la Ribera piers with low illumination (DOCKS); well illuminated mid-litoral communities (ML_ROCK); and well illuminated infra-litoral communities (WI-IL-ROCK).

(Guelorget and Perthuisot 1983; Pérez-Ruzafa and Marcos 1992, 1993), the settlement of non-native species, not necessarily adapted to the lagoonal conditions, in the inlet area, is favoured both due to the greater probability of colonization and by the fact that the salinity and temperature conditions are less extreme than those inside the lagoon. But, on the other hand, they also present a high frequency in the fluctuation of these environmental conditions due to the changes in the direction of the incoming and outgoing currents, which in the Mar Menor take place approximately every six hours (Arévalo 1988). Frequently, as has also been observed in algal populations, this oscillation rhythm tends to be a more important factor of stress for the communities than extreme values that remain more stable over time following seasonal patterns in the inner parts of the lagoon (Pérez-Ruzafa et al. 2008). Such conditions in the inlets would therefore be more favourable for opportunistic species, allowing their greater demographic growth. Over time, new colonizing species can develop populations whose individuals have been selected to withstand extreme conditions and, in this way, also settle inside the lagoon where they can coexist with native species. In this way, it is explained that in the rocky substrates of El Ciervo Island, the number of species was the highest of all the communities on hard substrates studied (Figure 5). Furthermore, at this location, the mid- and infralittoral rocky communities are colonised to varying degrees by *C. prolifera* meadows, which favours a more heterogeneous environment with patches of higher organic matter content and thus promoting higher diversity. In the biocenosis developed on the pillars under piers, in a poorly illuminated environment, the Cirratulid community is composed, as in El

Estacio inlet, only of encrusting species (Figure 5). The density of individuals in this community in Santiago de la Ribera does not show as much dominance as in El Estacio inlet. This may be due both to the lower light exposition and to a greater isolation from the communication inlet.

Therefore, the application of monitoring studies would permit to analyse the dynamic and trophic role of *T. caribous* in the basin, and to determine if this has only been an eventual occurrence of the species or if it has successfully colonized the lagoon, likewise previous cases of established alien polychaetes such *Branchiomma boholense* Grube, 1878 (Román et al. 2009) or *Perinereis linea* Tradwell, 1936 (Arias et al. 2013). These monitoring studies are critical to understand and determine if the competition with native species and the resulting community rearrangement can affect the ecosystem or, rather the contrary, the colonization of new established or occasional species could help the natural heterogeneity and the homeostatic responses and thus the equilibrium of this system.

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Authors' contribution

APR, CM, and EL conceived the study. ASM and OSF performed the field work and species collection. ASM and EL carried out the identification of species and revision of taxonomy. ASM carried out the statistics and wrote the first draft of the manuscript. All the authors contributed to the manuscript revision, read and approved the submitted version.

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Supplementary material

The following supplementary material is available for this article:

Table S1. *Timarete caribous* access codes.

This material is available as part of online article from:

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