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Research Article

***Leodamas australiensis* (Hartmann-Schröder, 1979) (Polychaeta, Orbiniidae), a new alien species in the Mediterranean, and its ecology in the Mar Menor coastal lagoon (SE Spain)**

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Citation: Sala-Mirete A, López E, Fernández-Alías A, Sánchez-Fernández O, Marcos C, Pérez-Ruzafa Á (2023) *Leodamas australiensis* (Hartmann-Schröder, 1979) (Polychaeta, Orbiniidae), a new alien species in the Mediterranean, and its ecology in the Mar Menor coastal lagoon (SE Spain). *BioInvasions Records* 12 (in press)

Received: 18 August 2022

Accepted: 8 August 2023

Published: 1 November 2023

Handling editor: David Wong

Thematic editor: April Blakeslee

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Abstract

Invasive species, including many polychaetes, are one of the major factors affecting the diversity of the Mediterranean ecosystems. Coastal lagoons, as transitional ecosystems, present a large spatial and temporal variability that makes them suitable for opportunistic species colonization. In this work, the species *Leodamas australiensis* (Hartmann-Schröder, 1979) has been identified in the Mar Menor coastal lagoon (Spain) and this is its first report from the Mediterranean. It is characterized by the presence of large aciculae with protruding and distinctly curved tips in abdominal neuropodia and by the onset of dorsal branchiae on chaetiger 6. Molecular analyses were carried out using mitochondrial 16S rDNA gene sequences. The spatio-temporal distribution of *L. australiensis* and its relationship with other species belonging to the family Orbiniidae in the lagoon was investigated in different periods from 1982 to 2018. This species expanded from sites more connected to the Mediterranean Sea towards more confined areas of the basin. However, this expansion did not affect in an appreciable manner to native orbiniids. Follow-up studies at longer time scales are needed to determine if *L. australiensis* is a casual presence or maintains a well-established population.

Key words: coastal lagoons, invasive species, 16S rDNA, transitional ecosystems, Mediterranean Sea, non-indigenous species

Introduction

Although the Mediterranean Sea is among the richest areas in terms of biodiversity and is considered a marine biodiversity hot-spot, the invasion by non-indigenous species (NIS), together with habitat loss, fishing impact, climate change and eutrophication, is crucial in changing the biodiversity of this region, mostly in coastal areas and continental shelves (Coll et al. 2010; Occhipinti-Ambrogi and Galil 2010; Katsanevakis et al. 2020). Impacts of alien species on biodiversity and communities in coastal and transitional ecosystems affect both ecosystem functioning (changing biogeochemical cycles) and biotic relations (changes in the food web) (Katsanevakis et al. 2014; Occhipinti-Ambrogi 2021). Among the main

human activities that have been reported as NIS introduction vectors, maritime traffic, the opening of artificial channels, and aquaculture activities are the most important in terms of changes in marine biodiversity in the Mediterranean area (Galil et al. 2017, 2018).

As mentioned above, changes in biodiversity of the Mediterranean are greater in coastal zones and shelves (Coll et al. 2010). In the Mediterranean Sea, most invasion-susceptible biotopes are confined areas because of the number of potential NIS found on ship hull fouling and ballast water, which make ports and coastal lagoons NIS hot-spot environments for bioinvasion processes (Çinar 2013). In particular, coastal lagoons, as transitional ecosystems, present a large spatial and temporal variability and naturally stressful conditions that makes them suitable for opportunistic species colonization (Occhipinti-Ambrogi 2001; Gamito et al. 2005; Elliott and Quintino 2007; Munari et al. 2009; Pérez-Ruzafa et al. 2011; Ponti et al. 2011), including allochthonous taxa. Furthermore, their proximity to sources of human pressures facilitates the NIS settlement (Paavola et al. 2005; Crooks et al. 2011; Boudouresque and Verlasque 2012). For instance, marinas and aquaculture facilities are often built within these ecosystems and are among the most important vectors for NIS introduction (Cecere et al. 2016a; Cabidu et al. 2020). Thus, habitat degradation is a promoter of biological invasions, which correlate negatively with native species richness (Ardura and Planes 2017). In the Mediterranean Sea, different regions containing coastal lagoon systems are considered hot spots of introduction of marine alien species, such as Southern France (Verlasque 2001), the Northern Adriatic Sea (Marchini et al. 2015) and the Northern Ionian Sea (Cecere et al. 2016b), among others. Because of that, transitional ecosystems and, particularly, semi-enclosed coastal inlets are interesting places for the study of non-native species due to the ecotone effect and the mixed presence of marine, brackish, and freshwater native and non-indigenous species (Olenin and Leppäkoski 1999).

Regarding NIS taxonomic composition in the Mediterranean Sea, the groups containing the greatest number of alien species are molluscs, crustaceans, and polychaetes (Katsanevakis et al. 2020). Among polychaetes, the families with the highest number of NIS are Spionidae, Serpulidae, Nereididae and Sabellidae. Zenetos et al. (2022) compiled a total of 154 polychaete alien species in the Mediterranean basin. Literature points out a relation between the main introduction pathways and groups. Annelids were the most common NIS introduced through shipping, molluscs and chordates by aquaculture, and molluscs, chordates and arthropods through the Suez Canal (Nunes et al. 2014; Çinar et al. 2021).

Family Orbiniidae includes more than 240 species ranging in size from a few millimetres to 30 cm, living in a wide variety of soft bottoms and common dwellers of nearshore benthic communities along continental margins (Blake 2017). Orbiniids occur worldwide, being Asia (Asian Pacific

realm and Indo-Pacific biogeographic region) the most diverse area in terms of reported species, followed by Pacific North America, Atlantic North America and Atlantic South America. The species having the widest distributions are *Scoloplos armiger* (Müller, 1776) and *Naineris laevigata* (Grube, 1855) (Costello et al. 2017; Meca et al. 2021). Orbiniids are considered quite tolerant to the organic matter content in sediments and to ample ranges of salinity. Members of this family can be found in mud, sand, shell fragments content or mixed bottoms, showing, in some cases (*S. armiger*) a moderate resistance to anoxia (Grieshaber 1988; Meca et al. 2021). Because of their burrowing lifestyle, orbiniids can resuspend deeper sediment layers to the surface or even in the water column, contributing to the continuous mixing of sediment (Giangrande et al. 2002).

In the Mediterranean Sea, the Orbiniidae family comprises 13 native species, with a single representative of the genus *Leodamas* Kinberg, 1866: a subspecies of *Leodamas chevalieri* (Fauvel, 1902), *Leodamas chevalieri candiensis* (Harmelin, 1969). In this area, *Naineris setosa* (Verrill, 1900) is considered an established non-indigenous orbiniid species. It was first recorded out of its native geographic range (Western Atlantic Ocean) by Blake and Giangrande (2011) from an aquaculture farm in Brindisi (Adriatic Sea, Italy). Subsequently, it was reported from some Mediterranean coastal lagoons, such as Boughrara, in Tunis (Khedhri et al. 2014), Santa Gilla, in Italy (Atzori et al. 2016), or Livorno and Taranto (Langeneck et al. 2020).

The aim of this study is to report the first record of *Leodamas australiensis* (Hartmann-Schröder, 1979) in the Mediterranean Sea, specifically in a Western Mediterranean transitional ecosystem (Mar Menor coastal lagoon, Spain), and to review long-term species data from previous studies in the location in order to describe its temporal dynamic, its spatial distribution within the lagoon and its interaction with native species of orbiniids.

Materials and methods

Study area

The Mar Menor is a hypersaline coastal lagoon located in the Western Mediterranean. It is classified as choked lagoon following Umgiersser et al. (2014) and is isolated from the sea by a sandy bar 22 km long called La Manga, which is crossed by three main inlets maintaining communication between the lagoon and the open sea. In contrast with other Mediterranean coastal lagoons, the Mar Menor presents a higher diversity of environments and biological communities due mostly to the different types of sedimentary bottoms and the presence of hard substrata, maintaining high productivity (Pérez-Ruzafa 1989; Pérez-Ruzafa et al. 1989, 2005, 2012). Central areas of the Mar Menor lagoon are dominated by muddy sediments with high contents of organic matter. It is mainly covered by monospecific *Caulerpa prolifera* (Forsskål) J.V. Lamouroux, 1809 or mixed *C. prolifera* – *Cymodocea*

nodosa (Ucria) Ascherson, 1870 meadows, but some areas of muddy sediments without vegetation can be found. Sandy bottoms are restricted to the margins of the basin and surroundings of the islands and are mostly covered by more or less extensive monospecific meadows of *C. nodosa*. Regarding natural rocky bottoms, five volcanic islands occur in the Mar Menor, showing a vertical zonation similar to that in open sea communities (Pérez-Ruzafa et al. 1988; Pérez-Ruzafa 1989). Artificial hard bottoms are also present in the Mar Menor, mainly as small piers, harbour docks and beach breakwaters.

The Mar Menor has been targeted by human pressures since ancient times. Mining, urban development, enlargement of the channels communicating with the Mediterranean Sea, and changes in agricultural practices are the main activities that affect the Mar Menor functioning (Pérez-Ruzafa et al. 1987, 1991, 2002, 2005, 2019a). One of the most important changes regarding the ecology of the Mar Menor was caused by the widening of the connection channels and was characterized by the decrease of salinity values, the amelioration of extreme water temperatures, and the colonization by Mediterranean species such as *C. prolifera*, which caused changes in the sediment organic matter composition and affected the bottoms previously characterized by *C. nodosa* (Pérez-Ruzafa 1989; Pérez-Ruzafa et al. 1991, 2008, 2012). The other important and recent change in the lagoon is the eutrophication crises suffered by the ecosystem as a response to decades of inputs of nutrients resulting from the intensive agricultural activity in the surrounding fields (Pérez-Ruzafa et al. 2005, 2019a; Fernández-Álías et al. 2022).

Sampling design

Samples of the different benthic communities found in the lagoon ecosystem have been repeatedly taken from a net of fixed stations in different projects from the 1980s to 2018 (Figure 1). Samples were collected following a seasonal pattern (winter, spring, summer, and autumn) aiming to monitor the ecological state of the lagoon before and after a dystrophic crisis suffered during the summer of 2016 (Pérez-Ruzafa et al. 2019a). These samples were collected from the different bottom types of the Mar Menor to characterise and survey as many benthic biological communities as possible, covering all the spatial and temporal heterogeneity of the basin (Pérez-Ruzafa et al. 2007). Twenty-six sampling stations corresponding to soft bottoms were located throughout the lagoon basin and surveyed by means of a van Veen grab (400 cm² surface area) from boat. In addition, two coastal localities with *C. nodosa* meadows on sandy-muddy bottoms were sampled by means of a 11.4 cm internal diameter sedimentological core (Eleftheriou 2013). Different soft-bottom communities were classified according to substrate type, following the main biotopes studied in Pérez-Ruzafa et al. (2020) (Table 1). In the case of rocky substrate communities,

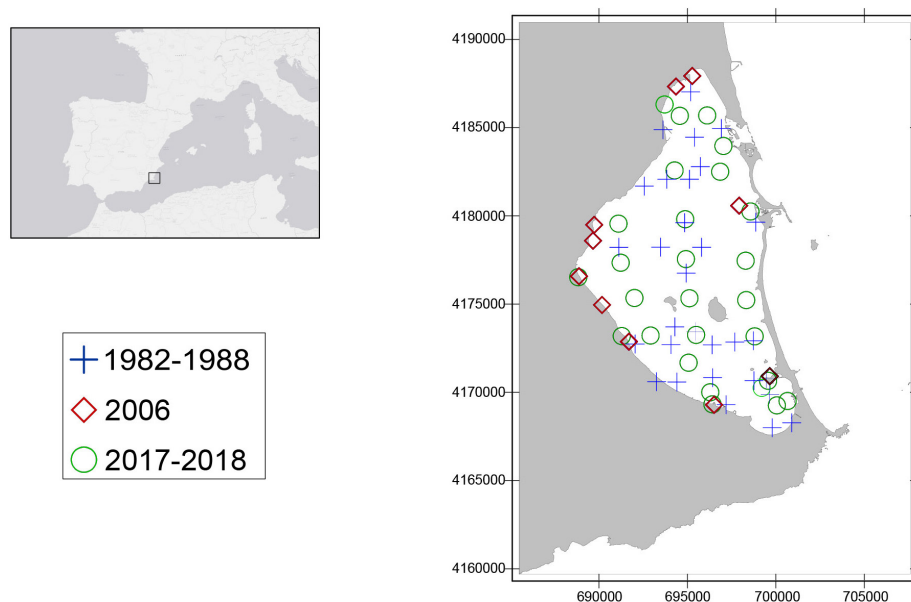


Figure 1. Mar Menor lagoon location and sampling designs in all periods studied.

Table 1. Main biotopes and biocenoses considered found in the Mar Menor. Adapted from Pérez-Ruzafa et al. (2020).

Biotopes/Biocenoses	Abbreviaton
Muddy-sand lagoon bottoms without meadows	MUD
Muddy-sand lagoon bottoms with <i>Caulerpa prolifera</i>	CA
Muddy-sand lagoon bottoms with <i>Cymodocea nodosa</i>	CY
<i>Cymodocea nodosa</i> – <i>Caulerpa prolifera</i> mixed Meadows	CACY
Sandy bottoms of coastal lagoons with <i>Acetabularia calcyculus</i> and/or <i>Acetabularia acetabulum</i>	SAND
Rocky bottoms	ROCK
Mid-litoral rocks of coastal lagoons	ML_ROCK
Well illuminated infralittoral rocks of coastal lagoons	WI-IL-ROCK
Protected shallow infralittoral rocks, poorly illuminated on pillars under docks	DOCKS

vertical zonation was taken into consideration, distinguishing between midlittoral and infralittoral biocenoses. These samples were taken from natural rocky bottoms on Ciervo Island (Ci) and from artificial rocky bottoms in Estacio Channel (Es). In addition, the specific community of the pillars under the piers in Santiago de la Ribera location (Ri), which is characterised by a relatively shadowed environment, was also studied. The methodology used for sample collection from the hard substrates was the scraping of a 400 cm² surface. In each sampling, two replicates were taken from each rocky or soft bottom locality. Samples were sieved with a 500 µm mesh size, and retained material was stored and preserved within a 70% ethanol solution. The separation and identification of faunal groups was carried out at the University of Murcia using an Olympus SZX stereomicroscope. In addition, taxonomic identification of polychaete annelids to species level was carried out at the Universidad Autónoma de Madrid, using a Nikon XN model stereomicroscope. Some specimens were selected for scanning electron microscope (SEM) at the University of Murcia. In addition, a taxonomic review of the individuals belonging to the family Orbiniidae found in previous years was carried out, obtaining the number of individuals, period, station code and community where they were found. Due to the

Table 2. PCR primers used for amplification and sequencing.

Gene fragment	Primer	Sequence (5'-3')	Reference
16S	AnnF	GCGGTATCCTGACCGTRCTAAGGTA	Sjölin et al. 2005
	AnnR	TCCTAAGCCAACATCGAGGTGCCAA	Sjölin et al. 2005

differences in sampling surface found in the review, the density of individuals was referred to as individuals/m². The specimens were deposited in the laboratory of the research group “Ecology and Management of Coastal Marine Ecosystems” at the University of Murcia.

Molecular analysis

One individual (3.1 mg), taxonomically identified as *L. australiensis*, was selected for DNA extraction, amplification, and sequencing. The DNA was extracted with QIAamp DNA Mini Kit by QIAGEN following the manufacturer’s indications. DNA concentration was measured using a NanoDrop2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The PCR of the mitochondrial 16S rDNA gene fragment was accomplished with the primer pair 16SAnnF and 16SAnnR (Table 2). PCR mixture (10 µl) consisted of 3µl of DNA template (initial concentration 5.6 ng/µl), 1x reaction buffer, 0.2 mM dNTPs, 0.5 µM forward and reverse primers, 0.4U Taq polymerase (MyTaq DNA polymerase by Biorline). The PCR profile was adapted from Zhadan et al. (2015) with an initial heat activation at 94 °C for 5’, followed by 35 cycles of denaturation at 94 °C for 30”, annealing at 60 °C for 30” and extension at 72 °C for 50”. The reaction was terminated with a final extension at 72 °C for 7’. The PCR product size was checked in an agarose gel (0.8%) and both chains were sequenced by the molecular biology section of the Research Support Service of the University of Murcia (Spain). The electropherogram was edited with SnapGene software (www.snapgene.com) and manually inspected. To genetically determine the identity of the individual we have launched a Basic Local Alignment Search Tool (BLAST) against the nucleotide database (GenBank) of the National Center for Biotechnology Information (NCBI 2022). This analysis revealed, at least, more than 9.5% genetic dissimilarity between our specimen and any other of the family Orbiniidae. Thus, we have selected all the 16S rDNA sequences from the *Leodamas* genus, including *Leodamas acutissimus* (Hartmann-Schröder, 1991), considered as *Scoloplos acutissimus* Hartmann-Schröder, 1991 (sensu Zhadan et al. 2015). Sequences KR349348 and KR349347, referred to *Leodamas dubius* (Tebble, 1955) in GenBank, are herein referred to *L. australiensis* since the specimens from which molecular material was extracted were newly identified as such (Zhadan 2020). As an outgroup of the Orbiniidae family, we have selected *N. laevigata*. Additionally, we have selected one 16S rDNA sequence from *Lumbricus terrestris* Linnaeus, 1758, to root the tree. Accession numbers are provided in Table 3.

Table 3. Taxa included in phylogenetic analyses, and GenBank accession numbers. (*) Species marked with an asterisk are revised following WORMS Editorial Board (2022); *Leodamas rubrus* and *Leodamas acutissimus* are considered as *Leodamas rubra* (Webster, 1879) and *Scoloplos acutissimus* Hartmann-Schröder (1979), respectively, in GenBank (sensu Zhadan et al. 2015). (‡) Sequences KR349348 and KR349347, referred to *Leodamas dubius* (Tebble, 1955) in GenBank, are herein referred to *Leodamas australiensis* (Australia) since the specimens from which molecular material was extracted were newly identified as such (Zhadan 2020).

Taxa	Authority	Accession no.
		16S
<i>Leodamas australiensis</i> (Australia) ‡	(Tebble, 1955)	KR349348.1 KR349347.1
<i>Leodamas johnstonei</i>	(Day, 1879)	AY532332.1
<i>Leodamas rubrus</i> *	(Webster, 1879)	FJ612460.1
<i>Leodamas tribulosus</i>	(Ehlers, 1897)	FJ612467.1 FJ612459.1 FJ612458.1
<i>Leodamas acutissimus</i> *	(Hartmann-Schröder, 1991)	KR920027.1 KR920028.1
<i>Leodamas australiensis</i> (Spain)	(Hartmann-Schröder, 1979)	Supplementary material S1
<i>Naineris laevigata</i>	(Grube, 1855)	FJ612463.1
<i>Lumbricus terrestris</i>	Linnaeus, 1758	FJ612462.1

Prior to the phylogenetic analysis, multiple alignments of the sequences were performed using ClustalW algorithm, and the sequences were trimmed to the length of the shortest sequence. Then, following Zhadan et al. (2015), a Neighbor-Joining tree was elaborated to infer the evolutionary history (Saitou and Nei 1987). We have used using Kimura-2-parameters method as the substitution method (Kimura 1980), and the bootstrap method (1000 replicates) as the test of phylogeny (Felsenstein 1985). Evolutionary analysis was performed in Mega 7 (Kumar et al. 2016).

Results

Systematics

Family Orbiniidae

Genus *Leodamas* Kinberg, 1866

Leodamas australiensis (Hartmann-Schröder, 1979)

Figs. 2, 3

Scoloplos (Leodamas) rubra australiensis Hartmann-Schröder, 1979: 131–132, figs. 276–282.

Leodamas dubia (non Tebble, 1955).- Zhadan et al. (2015): 789–792, figs. 7A–K, 8A–K.

Leodamas australiensis (Hartmann-Schröder, 1979).- Zhadan (2020): 471–473, fig. 11.

Material examined

160 specimens of *L. australiensis* were examined. 1 individual in 2006 in El Ciervo Island location (sample code, iccy2_5/2006); 1 specimen in winter 2017 (sample CiCy1_I/17); 2 individuals in summer same year (sample E14_V/17), and 71 in autumn (samples and (indiv.): CiCy2_O/17 (3), E12B_O/17 (18), E13_O/17 (1), E15_O/17 (1), E16_O/17 (48)). A total of 61 individuals were found in samples from winter 2018 (samples and (indiv.): CiCy1_I/18 (5), CiCy2_I/18 (3), E12B_I/18 (35), E13_I/18 (1), E16_I/18 (17));

19 specimens in spring 2018 (samples and (indiv.): CiCy1_P/18 (17), CiCy2_P/18 (1), E07_P/18 (1); and 5 in summer same year (samples and (indiv.): E12B_V/18 (2), E16_V/18 (3)).

Description

Small worms; largest specimen 45 mm long for 282 chaetigers (27 thoracic, 205 abdominal), 1 mm wide at mid-thorax level. Body long and narrow, thorax dorso-ventrally flattened (Figure 2A) and formed of 19–27 chaetigers, abdomen cylindrical. Prostomium conical and divided in two regions, pointed and bearing a short apical organ; eyes absent; with only one achaetous peristomial ring similar in length to first chaetiger; peristomium dorsally fused to prostomium (Figure 2A). Branchiae first appearing on chaetiger 6 (Figure 2A); triangular, with wide base and conical tips in thorax; ciliated on both sides, ciliation not reaching the tip (Figure 2B); becoming longer in abdomen, slightly longer than corresponding notopodial lobe. Thoracic notopodia bearing only crenulated capillary chaetae, with a small, rounded postchaetal lobe from chaetiger 3, better developed from chaetiger 5 (Figure 2C). Thoracic neuropodia with postchaetal lobes as low ridges, with a digitiform central projection in the last segments of the thorax (Figure 2C, E); bearing uncini with slightly curved distal end; arranged in 5 vertical rows, most anterior row extending only from dorsal portion to middle of the bundle, most posterior one doing so from ventral zone; uncini from anterior and uppermost side thicker than the those in the middle (Figure 2E). Abdominal notopodia low, supported by two thin aciculae similar in thickness to capillary notochoetae, with slightly curved and not projecting tips (Figure 2D, H); bearing foliaceous postchaetal lobes, being longer up to the posterior end; with 6–11 crenulated capillary chaetae and 1–2 forked chaetae, with smooth shaft and subequal tapering tines (Figure 2G). Abdominal neuropodia unilobed and conical, supported by a much thicker, single acicula with a conspicuously projecting tip curved to different degrees, from nearly straight to 180°, depending on the chaetiger (Figure 2F, H); bearing a digitiform postchaetal lobe attached ventrally to chaetal bundle and half as long as corresponding notopodial postchaetal lobe (Figure 2D, H); all chaetae as crenulated capillaries, numbering 3–5. Pygidium unknown.

Taxonomic remarks

Orbiniidae can be distinguished from other annelids by a conical or rounded prostomium without appendages, body subdivided into thorax and abdomen, dorsally disposed branchiae and parapodial lobes in the abdomen, and a unique type of crenulated capillary chaetae (Blake 2017). A great revision of the literature on orbiniids was made by Blake (2000, 2017, 2021), with the review of the definition of known genera or the erection of new ones from

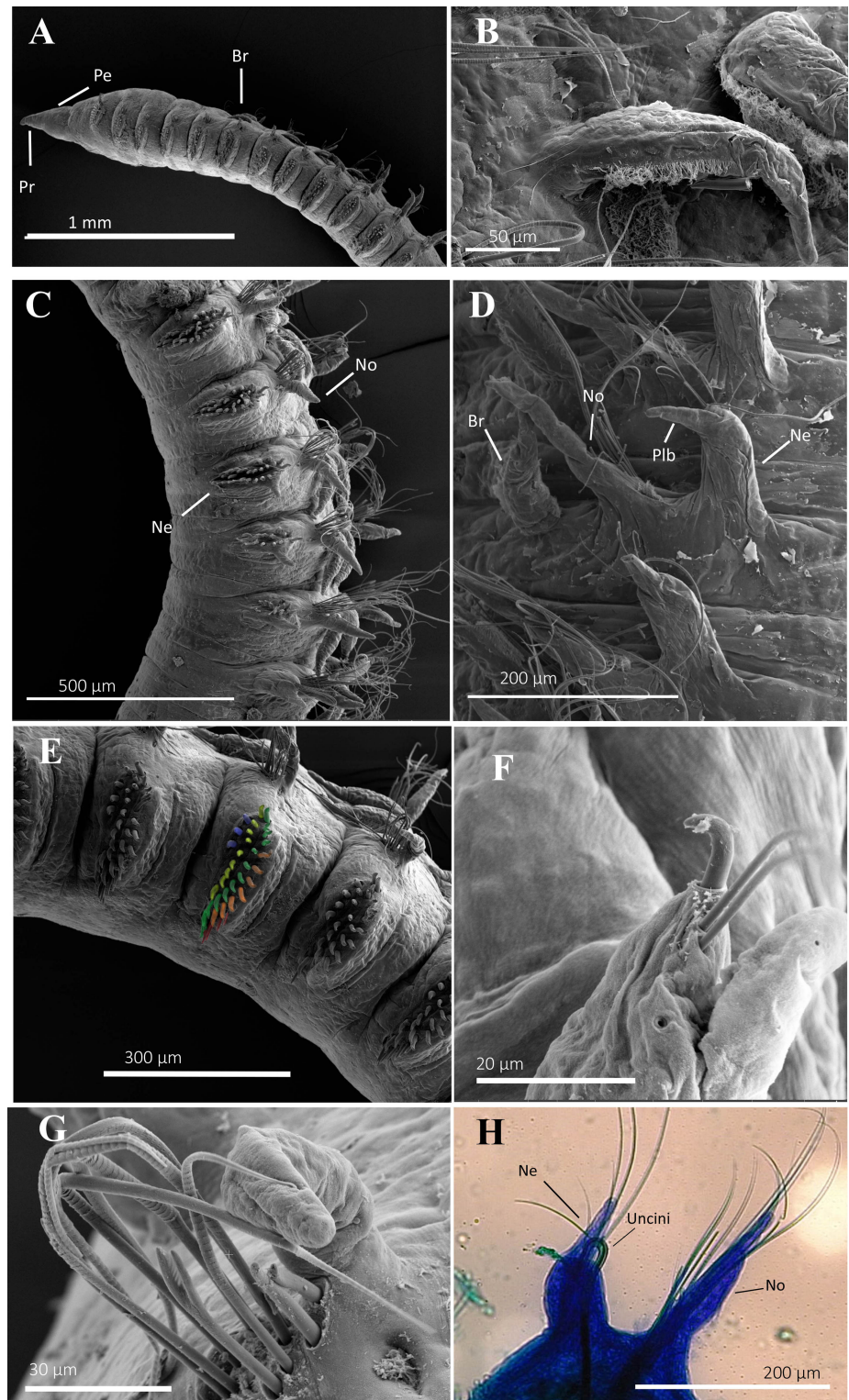


Figure 2. *Leodamas australiensis* micrographs. A Anterior end lateral view. B Detail of thoracic ciliated branchia. Chaetiger 14. C Thorax-abdomen transitional chaetigers with developed neuropodial postchaetal lobe. D Abdominal parapodia. E Thoracic neuropodia with uncini arranged in vertical rows. F Abdominal neuropodia with aciculae. G Detail of abdominal notopodia; forked chaeta present. H Methylene blue staining glycerol mount of abdominal parapodia with curved uncini. Abbreviations: Br: branchia; Ne: Neuropodium; No: Notopodium; Pe: peristomium; Pr: prostomium; Plb: postchaetal lobe. Photos by Antonio Sala-Mirete.

subgeneric status. At a regional scale, the family has been thoroughly revised by López (2012).

Hartmann-Schröder (1979) referred to *Leodamas* Kingberg, 1866 as a subgenus of *Scoloplos* Blainville, 1828. However, Blake (2000) restructured the orbiniid genera and *Leodamas* came to be considered as a valid genus. Traditionally, *Leodamas* and *Scoloplos* were chiefly distinguished by the appearance of the branchiae (on thorathic chaetigers 4–7 in the former, on chaetigers 8 or posterior in the latter), and secondarily by the presence of rows of numerous and thick neuropodial uncini in the thorax of *Leodamas* instead of a few uncini interspersed among crenulated capillaries in *Scoloplos*. Blake (2017) revised the diagnosis of the genera and dismissed the position of the first pair of branchiae as important taxonomic character. According to this, a total of 33 species of *Leodamas* are known worldwide (Blake 2017, 2020, 2021; Sun et al. 2018), with *Leodamas chevalieri candiensis* (Harmelin, 1969) as the only previously known Mediterranean species of the genus. However, the revaluation of the characters will rise the number as several species referred to *Scoloplos* might fall within the new diagnosis. For instance, two Mediterranean species *Scoloplos haasi* (Monro, 1937) and *Scoloplos typicus* (Eisig, 1914) bear thick neuropodial uncini in the thorax. The herein described specimens do not belong to any of these three species because of the presence of large and strongly curved neuropodial aciculae on abdominal chaetigers. Additionally, these specimens can be distinguished from *S. haasi* and *S. typicus* by the anterior onset of branchiae (chaetiger 6 instead of 13–14 in *S. typicus* or 22–25 in *S. haasi*) and by having unilobed instead of bilobed abdominal neuropodia (López 2012). *Leodamas chevalieri candiensis* is a more similar species having branchiae from chaetiger 6 and abdominal neuropodia with large and protruding aciculae, but in this species abdominal neuroaciculae possess straight tips, thoracic uncini have a strongly defined sculpture and thoracic neuropodial lobes completely lack postchaetal projection (Harmelin 1969). It must be noted that Harmelin reported juvenile specimens with smoother sculpture in thoracic uncini and curved tips in abdominal neuropodia uncini, but the extent of these variations was neither figured nor quantified.

Within *Leodamas*, the presence of large and protruding neuropodial aciculae in the abdominal chaetigers is not an uncommon trait, and apart of *L. chevalieri candiensis*, *Leodamas cochleatus* (Ehlers, 1900), *Leodamas dendrocirris* (Day, 1967), *Leodamas gracilis* (Pillai, 1961) and *Leodamas hamatus* Dean & Blake, 2015 (Gallardo 1968; Dean and Blake 2015; Blake 2017; Zhadan 2020) have this kind of acicula. However, only two species have them with very distinctly curved tips as in the herein described specimens: *Leodamas dubius* (Tebble, 1955) and *L. australiensis*. *L. dubius* was originally described from Ghana in Gulf of Guinea (Tebble 1955) and it has subsequently been reported from a number of Indo-Pacific localities such as Vietnam (Gallardo 1968), Thailand (Eibye-Jacobsen 2002) and Australia (Zhadan et al. 2015). However, in the specimens reported from the last two localities branchiae start on chaetiger 6 and abdominal notochaetae

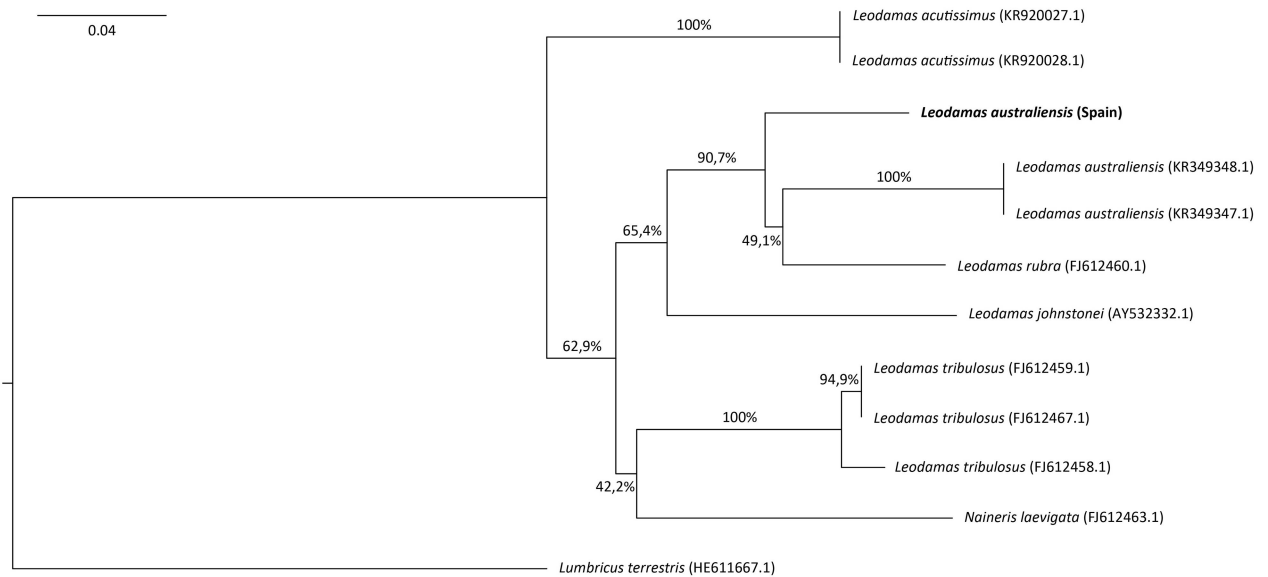


Figure 3. Neighbor-Joining phylogenetic tree based on 16S gene sequence (Table 3). The tree is drawn to scale, with *Lumbricus terrestris* as root sequence, and branch lengths in the same units as those of the evolutionary distances used for the phylogenetic tree inference. The indicated percentages indicate the statistical support using bootstrap analysis with 1000 replicates.

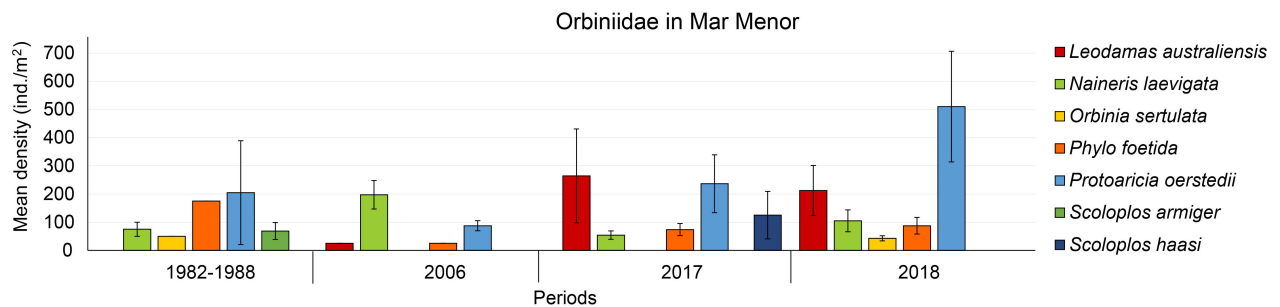
include forked ones, so they were referred to *L. australiensis*, a species from Western Australia (Hartmann-Schröder 1979), by Zhadan (2020), who compared Australian material with the type series of *L. dubius* and probed that in the later forked chaetae were absent and branchiae started on chaetiger 7. The real identity of material from Vietnam is difficult to assess, as Gallardo (1968) reported only abdominal fragments, which due to their geographical situation are likely to belong also to *L. australiensis*. In spite of type locality being farther away, our specimens fit better to the diagnosis of *L. australiensis*, so this is the first report of this species out of the Indo-Pacific area.

Molecular analysis

The molecular analysis revealed that the specimens herein identified as *L. australiensis* have a dissimilarity of, at least, 9.5% with all the other members of the Orbiniidae family that account for a 16S rDNA sequence in GenBank. The Neighbor-Joining tree supported three different clades in the *Leodamas* genus (Figure 3). A first one included material of *L. australiensis* collected from Spain, *Leodamas australiensis* from Australia, and *Leodamas rubrus* (Webster, 1879); a second one included *Leodamas tribulosus* (Ehlers, 1897), and a third one included only two sequences of *L. acutissimus*. *Leodamas johnstonei* (Day, 1879) can be included, in a more dubious clade, with *L. australiensis* and *L. rubrus* (referred as *L. rubra*, in the cladogram). Within the first clade, specific branch supporting *L. australiensis* from Spain is registered (Figure 3), and the genetic distances with *L. australiensis* from Australia and *L. rubrus* are 10.4% and 11.6%, respectively. Thus, the genetic analysis supports the taxonomical one and indicates that our individual differs from *L. australiensis* from the type location. Although the

Table 4. Mean density (indiv. / m²) ± standard deviation of orbiniids found in Mar Menor for the studied periods.

Family	Genus	Species	1982–1989	2006	2017	2018
Orbiniidae	<i>Leodamas</i>	<i>L. australiensis</i> (Hartmann-Schröder, 1979)		25 ± 0	264.3 ± 440.4	212.5 ± 280.2
	<i>Naineris</i>	<i>N. laevigata</i> (Grube, 1855)	75 ± 35.4	197.2 ± 302.9	54.2 ± 36.8	105 ± 122.4
	<i>Orbinia</i>	<i>O. sertulata</i> (Savigny, 1822)	50 ± 0			42.8 ± 23.8
	<i>Phylo</i>	<i>P. foetida</i> (Claparède, 1868)	175 ± 0	25 ± 0	73.7 ± 96.1	87.5 ± 131.9
	<i>Protoaricia</i>	<i>P. oerstedii</i> (Claparède, 1864)	204.7 ± 368.3	87.5 ± 79.3	236.4 ± 339.9	510 ± 620.7
	<i>Scoloplos</i>	<i>S. armiger</i> (Müller, 1776)	68.7 ± 59.9			
		<i>S. haasi</i> (Monro, 1937)				125 ± 168.3


Figure 4. Mean density (number of individuals/m²) of different species of orbiniids found in the different study periods. Error bars represents standard error values.

very low number of sequences considered make difficult to establish sound conclusions, the existence of a complex of Indo-Pacific species around *L. australiensis* can be suspected.

Ecology and distribution of family Orbiniidae in the lagoon

A total of 1197 individuals of Orbiniidae were identified in this study, corresponding to six genera (Table 4). The most abundant species was *Protoaricia oerstedii* (Claparède, 1868) (503 indiv.), followed by *N. laevigata* (345 indiv.), *L. australiensis* (160 indiv.), *Phylo foetida* (Claparède, 1868) (138 indiv.), *S. haasi* (20 indiv.), *S. armiger* (17 indiv.), and *Orbinia sertulata* (Savigny, 1822) (14 indiv.). Of all the study periods, 2006 is the less diverse, with only four species. In the rest of periods, the species richness was the same (five species), but with different composition (Table 4).

Naineris laevigata, *P. foetida*, and *P. oerstedii* were present in all study periods (1982–1988; 2006; 2017; 2018) (Figure 4, Table 4). *Naineris laevigata* was dominant in 2006, with a mean density of 197.2 ind./m², whereas *P. oerstedii*, showed the highest mean density value and dominance in 2018 (510 ind./m²). *Phylo foetida* showed its greatest mean density in 1982–1989 (175 ind./m²) but was not the dominant orbiniid in any study period. Other species and genera that were collected in 1982–1988, were not found in other periods. For instance, *O. sertulata*, which showed relatively lower densities respect to the other species, was collected in 1982–1988 period (50 ind./ m²) and in 2018 (42.8 ind./m²), but not in the central periods; and *S. armiger* was only found in 1982–1988 (68.5 ind./m²). *Scoloplos haasi* was identified only in 2017 period (125 ind./m²). In the case of *L. australiensis*, it was first identified in 2006 with a low density (25 ind./ m²).

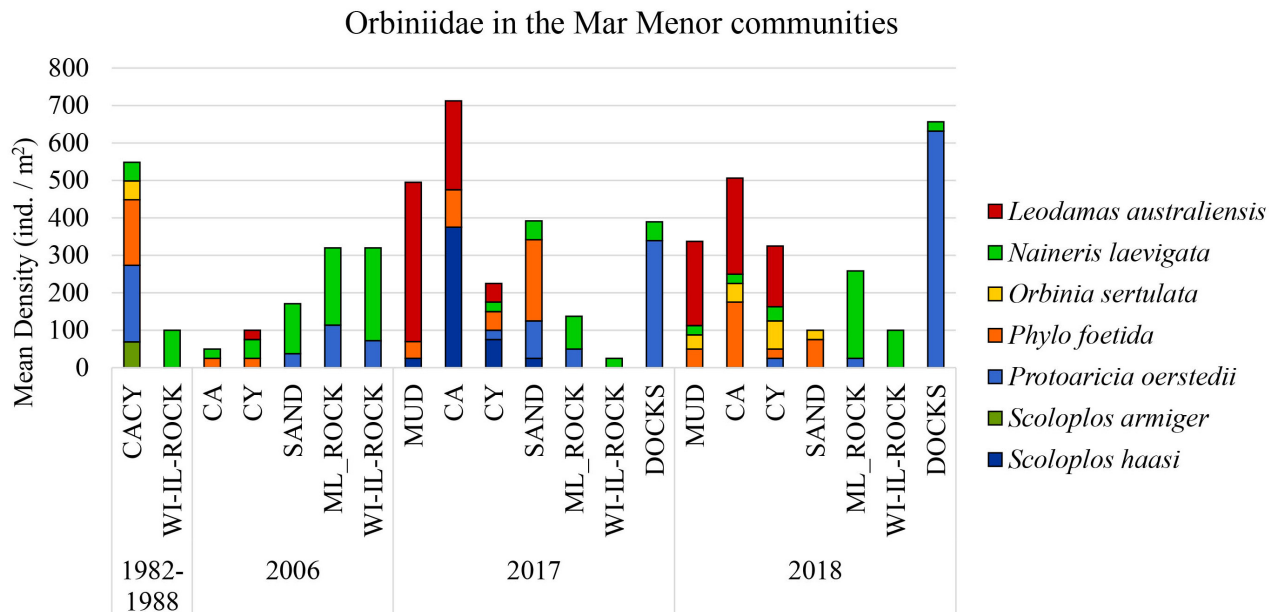


Figure 5. Cumulative mean density of orbiniids in the Mar Menor in different communities for all period studied. Mud bottoms without seagrass (MUD); monospecific *C. prolifera* meadows (CA); *C. prolifera*–*C. nodosa* mixed meadows; monospecific *C. nodosa* meadows (CY); sandy bottoms without seagrass (SAND); midlittoral rocks (ML_ROCKS); well-illuminated infralittoral rocks (WI-IL-ROCKS); protected shallow infralittoral rocks, poorly illuminated on pillars under docks (DOCKS).

Regarding to distribution of orbiniids in the communities studied, rocky bottoms (ML_ROCK, WI-IL-ROCK and DOCKS) only presented individuals of *N. laevigata* and *P. oerstedii*. The first species also occurred in soft bottoms, either in *C. prolifera* monospecific meadows (CA) or in mud bottoms without meadows (MUD) (Figure 5). All the other species occurred only in soft bottoms. *Orbinia sertulata*, *P. foetida*, *S. armiger* and *S. haasi* were found in all the biocenoses of this type (MUD, CA, CACY, CY, and SAND). Nevertheless, *L. australiensis* was absent from sandy bottoms without meadows (SAND) and from *C. prolifera*–*C. nodosa* mixed meadows (CACY). On the contrary, this species was frequent in mud bottoms without meadows (MUD), in *C. prolifera* monospecific meadows (CA), and in *C. nodosa* monospecific meadows (CY) (Figure 5).

Focusing on *L. australiensis* time and seasonal dynamics in the three periods when it was found, it is notable that it was first collected during the spring of 2006 in el Ciervo island station, showing a population density of 25 ind./m² (Figures 6, 7). During the winter of 2017, the same density than in 2006 was found in the same station (Figure 7). However, in 2017 the species expanded to a station with mud bottoms without vegetation in zone 4, where it showed a clear increase of mean density that reached a maximum in autumn of 1200 ind./m² (Figures 6, 7, Table 5). For the next period (2018), there was a clear decrease of this species mean density, from mean values around 305 ind./m² during the winter to values of 62.5 ind./m² in summer from the same year, ending with no individuals found in autumn (Figure 7). Nevertheless, *L. australiensis* was found to expand to zone 3 in spring of the same year (Figure 6, Table 5).

Leodamas australiensis

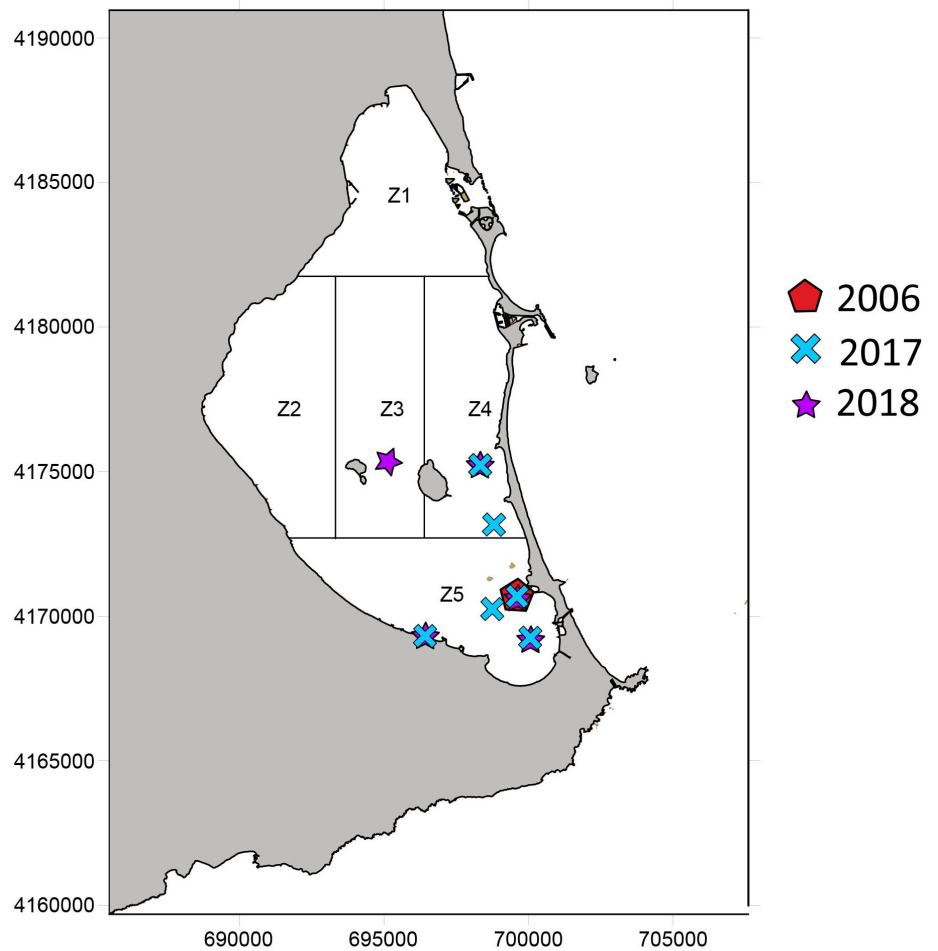


Figure 6. Presence of *Leodamas australiensis* in different zones within the Mar Menor during the studied periods.

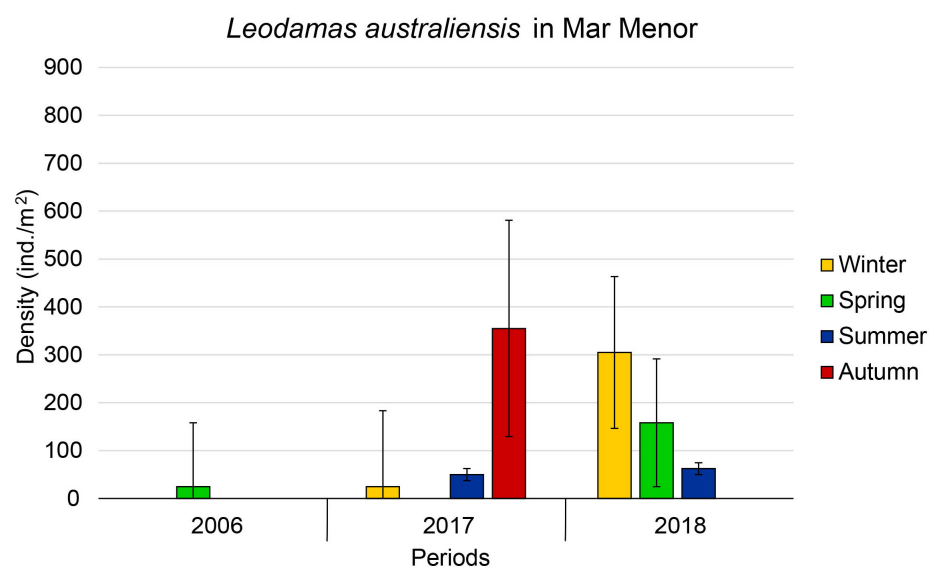


Figure 7. Seasonal mean density of *Leodamas australiensis* in the Mar Menor during in 2006, 2017, and 2018.

Table 5. Mean density of *Leodamas australiensis* (ind. / m² ± standard error (SE)) in different periods studied (2006, 2017, 2018) and zones (Z) within the Mar Menor lagoon.

Year	Zone		
	Z3	Z4	Z5
2006			25 ± 0
2017		830.9 ± 587.5	125 ± 81.7
2018	25 ± 0	247.5 ± 175	317.7 ± 120.1

Discussion

The Mediterranean region is one of those with the highest number of alien polychaete species identified, most of them translocated through shipping (Nunes et al. 2014). Not only the number of species reported is high (154), but 71 of them were already considered as established at the time of the present study (Zenetos et al. 2010; 2012; 2022; Zenetos and Galanidi 2020). However, a great number of species observed are still treated as possible casual observations or questionable invaders, hindering the study of the implications over the ecosystem they were found (Langeneck et al. 2020; Zenetos and Galanidi 2020; Zenetos et al. 2022). Furthermore, properly established monitoring programs able to detect the different scales at which this species develops are necessary to know these species population dynamics.

After the present study on the population dynamics of *L. australiensis*, it can be considered as “established species” in the sense of Occhipinti-Ambrogi and Galil (2004) and Zenetos et al. (2010), as a species with the ability to establish in an environment foreign to its natural range of distribution and to maintain this population over the time. However, in the last period in this study, *L. australiensis* population decreased and no individuals were collected in autumn 2018 campaigns. This result does not undoubtedly mean that the species had disappeared, as a number of native species could be found in some periods and not in others causing the species composition of the assemblages to change from one year to the next by 40–50% (Sigovini 2011; Quispe 2014). Therefore, long term dynamics studies of the benthic assemblages of the lagoon could help in the future to consider this species as properly established in the sense of Occhipinti-Ambrogi and Galil (2004) and of Zenetos et al. (2010).

Coastal lagoons are transitional ecosystems in which, due its restricted connectivity with the adjacent sea, colonization from the sea plays an important factor on species composition (Pérez-Ruzafa and Marcos 1992, 1993). The low probability in the colonization rates typical of most lagoons (Ghezze et al. 2015; Pérez-Ruzafa et al. 2019b) introduces a random component in the species composition of the lagoon assemblage and determines the spatial gradients and interannual variability within the same basin and with respect to other coastal lagoons (Guelorget and Perthuisot 1983; Pérez-Ruzafa et al. 2019c; Sigovini 2011). For this reason, first observations of invasive species often happen near the inlets, and they subsequently expand within the lagoon. This work corroborates this idea.

L. australiensis first appearance was in El Ciervo island, in spring 2006, near to the Marchamalo inlet in the southern basin of the lagoon. Despite Mar Menor zone 5 is the most isolated in terms of main flows (Pérez-Ruzafa and Marcos 1993) it has the characteristic of being the area with the highest salinity and temperature, followed by the zones 3 and 4 (Pérez-Ruzafa et al. 2005). After the first record, the species spread to these adjacent areas and increased its population density, resulting in an expansion to the southern region of the lagoon in the final periods of this study. Despite that, the vector of transport of this species remains obscure, the opening of the Suez Canal has allowed for the introduction of many Indo-Pacific species into the Mediterranean (Galil et al. 2017). Although shipping cannot be discarded, it does not seem likely. Orbiniid larvae tend to spend short time in planktonic stage (López 2012) and are poor candidates to be translocated in ballast water, while adults are typically soft-bottom dwellers and do not occur in typical fouling communities on ship hulls. An alternative hypothesis is unaided range expansion related to progressive warming of Mediterranean waters, as described for other marine groups as crustaceans (García Raso et al. 2013) or fishes (Azzurro et al. 2019), although distance from type and new locality is far enough to make this pathway questionable. The finding of new localities would aid to explain the process.

Despite its consideration as non-native species, *L. australiensis* has not proved to displace or affect by competition native species of Orbiniidae in the Mar Menor. Common species such as *N. laevigata*, *P. foetida* or *P. oerstedii* did not change their interannual dynamics after the appearance of *L. australiensis* in 2006. Analysing the communities in which these three species occurred, as well as their importance in them, there is no evidence of competitive exclusion by *L. australiensis* towards any of these two, as in the years in which they appeared they coexisted in communities of soft bottoms with vegetation (CA and CY), as well as in muddy areas without meadows (MUD). Therefore, temporal ranges included in this study are such different that it is difficult to demonstrate a direct relationship between the presence of *L. australiensis* and other orbiniids in the Mar Menor.

It is known that in some coastal lagoons the interannual dynamics of the assemblages cause their specific composition to change by nearly a half from one year to the next (Sigovini 2011; Quispe 2014). The results of this study corroborate this idea, since whereas some orbiniid species maintain apparently stable dynamics, *S. armiger*, *S. haasi* and *O. sertulata* emerge and disappear alternatively. *L. australiensis* also seems to show such dynamics, as no individual was collected during the 2018 period. This highlights the need to implement monitoring program lasting for a minimum period of time in these naturally stressed ecosystems, which would better describe their interannual dynamics. This kind of research will be able to establish if this or any other invasive species detected in Mar

Menor (Román et al. 2009; Arias et al. 2013) represent a casual observation or, rather the contrary, an established population with a great temporal range of variability.

Acknowledgements

We express our gratitude to all the researchers participating in the different years of the monitoring project, both in the collection and separation of samples and species and to the anonymous reviewers for their comments and suggestions during the revision process of this manuscript.

Funding declaration

This work has been financed by the General Directorate of the Mar Menor of the Autonomous Community of the Region of Murcia. AFA contribution was supported by 21449/FPI/20, Fundación Séneca, Región de Murcia (Spain). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author's contribution

ASM, APR, CM and EL conceived the study. APR and CM, conceptualization, sampling design, and provision of historical samples. ASM and OSF performed the field work and species collection. ASM and EL carried out the identification of species and revision of taxonomy. AFA and ASM carried out the molecular analysis. ASM carried out the statistics and wrote the first draft of the manuscript. All the authors contributed to interpretation of the results and discussion, the manuscript revision, read, and approved the submitted version.

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