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ON AN EARLY RECORD OF THE ALIEN CLAM *MYA ARENARIA* IN THE IBERIAN PENINSULA AND ITS LIKELY CONFUSION WITH *SCROBICULARIA PLANA* (BIVALVIA)

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MYA ARENARIA
SCROBICULARIA PLANA
POPULATION PARAMETERS
LIMA ESTUARY
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ABSTRACT. – The first record of the invasive clam *Mya arenaria* in the Iberian Peninsula was reported by Guimarães & Galhano in the "*Mya* zone", Lima estuary. However, we hypothesize that these authors taxonomically confused *Mya arenaria* with the native clam *Scrobicularia plana*. Our results point in this direction from a species and a bionomical approach. Population parameters such as density or asymptotic shell length and the analysis of the expected composition of the macrobenthic assemblages support our findings. Nonetheless, *Mya arenaria* is actually present in the Lima estuary: apart from empty shells of the invasive clam lying on the sediment surface, at least a low density population was observed in a lower intertidal level off the "*Mya* zone". Shell length comparisons strongly suggest that the invasion happened earlier in northern Portugal than in the Tagus estuary, where the invasive clam was recently reported to be established.

INTRODUCTION

The soft-shell clam *Mya arenaria* (Linnaeus, 1758) is a native species from the east coast of North America. This clam is considered one of the oldest invasive marine species in European waters (Reise *et al*. 1999). Nowadays, *Mya arenaria* is distributed from the White Sea in northern Europe (Maximovich & Guerassimova 2003) to the Tagus estuary in Portugal (Conde *et al*. 2010), southern Europe. The invasive clam is also established in the Mediterranean Sea (Crocetta & Turolla 2011 and literature therein), namely in the Gulf of Lions, France (Zenetos *et al*. 2004), Sacca di Goro Lagoon, Italy (Crocetta & Turolla 2011) and in the eastern Mediterranean Sea (Saronikos Gulf in Greece and Tenedos, Turkey; Zenetos *et al*. 2004, 2009, Zenetos pers. comm.). *Mya arenaria* has also been described in the Black Sea as an invasive species (Gomoiu & Petran 1973).

In contrast to the smaller and softer-shell clam *Scrobicularia plana* (da Costa 1778), *Mya arenaria* has an oval less rounded shell with a posterior explicit gap, the inhalant and exhalant siphon are fused together and the chondrophore, a specific spoon-like structure, is located in the hinge of the left valve. An important feature to highlight is the longevity of *Mya arenaria*: Brousseau (1978) reports a lifespan of almost 13 years for a population from Gloucester, Massachusetts. This long lifespan allows the clam to attain up to 100 mm long shell length, or longer (Brousseau 1979; Palacios *et al*. 2000).

The first record of *Mya arenaria* in the Iberian Peninsula is attributable to Guimarães & Galhano 1988. However, this early record has been referred to by other authors with certain doubts. Strasser (1999), when describing the worldwide distribution of *Mya arenaria*, stated that "The southern limit on the Atlantic east coast appears to be Portugal (Guimarães & Galhano 1988)". Moreover, the last publications in relation to the macrofaunal assemblages of the Lima estuary do not include *Mya arenaria* among the observed species (Sousa *et al*. 2006, Sousa *et al*. 2007, Costa-Dias *et al*. 2010). The absence of *Mya arenaria* in these latter studies, led Conde *et al*. (2010) to also show skepticism about the early record of the species in the Iberian Peninsula.

Viéitez (1976), in a study concerning the polychaetes and mollusc assemblages in a beach located in the inner region of the Ria of Vigo, barely 50 km north from Lima estuary, stated that "*Mya arenaria* do not reach the southern Cantabric Sea" when describing a *Cardium* (= *Cerastoderma*) *edule-Scrobicularia* community (Thorson 1957). Accordingly, Guimarães & Galhano (1988) reported the presence of *Cerastoderma edule* (Linnaeus, 1758) and *Hediste diversicolor* (O.F. Müller, 1776), both of them characteristic species of the *Cerastoderma edule-Scrobicularia* community. However it is strange that there was no reference to *Scrobicularia plana* in their survey, a dominant species of this community.

The purpose of this study was to provide a reassessment of the early record of the invasive clam *Mya arenaria*. We intend to clarify a potential taxonomical confusion in the early report of *Mya arenaria* in the Lima estuary (Guimarães & Galhano 1988) with the native clam *Scrobicularia plana*. In order to find a satisfactory answer to this question, we considered population parameters and proceeded to an ecological analysis. Additionally, we investigated on the current occurrence of the invasive clam in the Lima estuary.

MATERIALS AND METHODS

The Lima estuary is located on the northern Portuguese coast. It belongs to the river Lima hydrological basin that is mainly composed of a granitic basement. The river Lima originates in Galicia and extends for 108 km in a NE-SE direction. In the lower part of the estuary some industries have remarkable importance such as the Shipyards of Viana do Castelo as well as the port activity.

Sampling was carried out during the ebb tide (the lowest low tide limit was approximately 1.2 m above the datum) at the beginning of May 2010. Three stations were considered in the same estuarine region as in Guimarães & Galhano (1988) (Fig. 1), corresponding to the south bank of the river Lima, near the Eiffel bridge. One sampling station was situated in the "*Mya* zone" (a salt marsh environment), another station was located in the "*Cardium* zone". Guimarães & Galhano (1988) defined these two "zones". One last sampling station was located seawards from the former in a place where we observed signs of *Mya arenaria* in the sediment surface (key-like holes). Hereafter, our sampling stations (Fig. 1) are named as MZ (in the "*Mya* zone"), CZ (in the "*Cardium* zone") and AS (meaning "Additional Station"). They were respectively located at 60 mm, 195 mm and 135 mm under the mean high water tidal level.

Temperature, salinity, oxygen, redox potential and pH were measured *in situ* (in the interstitial water) using a field standard probe (WTW 340i). The top 2 cm of the sediment were used for sediment analysis determined by the sieving method.

Sampling for infaunal organisms was conducted with a corer of 0.095 m of inner diameter (five replicates per sampling station) inserted to a depth of 25 cm, covering a 0.035 m^2 of total surface area for each sampling site. Every sample was washed through a 1 mm sieve. Benthic animals were sorted under the dissecting microscope, counted and identified to the lowest possible taxonomic level.

In order to obtain information from dead individuals of *Mya arenaria*, a collection of empty shells lying on the sediment surface was conducted in two different places: (1) empty shells of *Mya arenaria* were collected in the same location of the Tagus estuary where Conde *et al*. (2011) studied some aspects of the reproductive success of *Mya arenaria* (see box on Fig. 1) and (2) empty shells of the invasive clam were also searched for in the Lima estuary. The searching area (approximately 2000 m^2) and searching effort (of about 60 min) were similar in both cases. Shell length was only measured in the left valve for articulated and single valves to the nearest 1 mm with a vernier caliper. Strasser *et al*. (1999) and Palacios *et al*. (2000) previously used empty shells of *Mya arenaria* for interpreting the historical evolution of populations of this clam.

Hypothesis testing and data analysis: Guimarães & Galhano (1988) described *Mya arenaria* as a key species of a particular macrobenthic assemblage of their study site, so characterized in terms of abundance that this region of the Lima estuary was designated as "*Mya* zone". In contrast to what would be expected, they did not observe the estuarine bivalve *Scrobicularia plana* among the malacofaunal species. Therefore, we hypothesise that there was a taxonomic confusion of *Mya arenaria* with the native clam *Scrobicularia plana* in the study of Guimarães & Galhano (1988). In accordance to this hypothesis, the dominance of *Scrobicularia plana* should obscure *Mya arenaria* in the so called "*Mya* zone" (Fig. 1) showing additionally higher abundance than in the remaining sampling stations. Thus, the number of individuals of *Scrobicularia plana* in each sampling station was tested by fitting Generalized Linear Models (GLM) with a log link function and a Poisson distribution for the error term (McCullagh & Nelder 1989).

We applied a two-tailed t-test to compare means of abun-

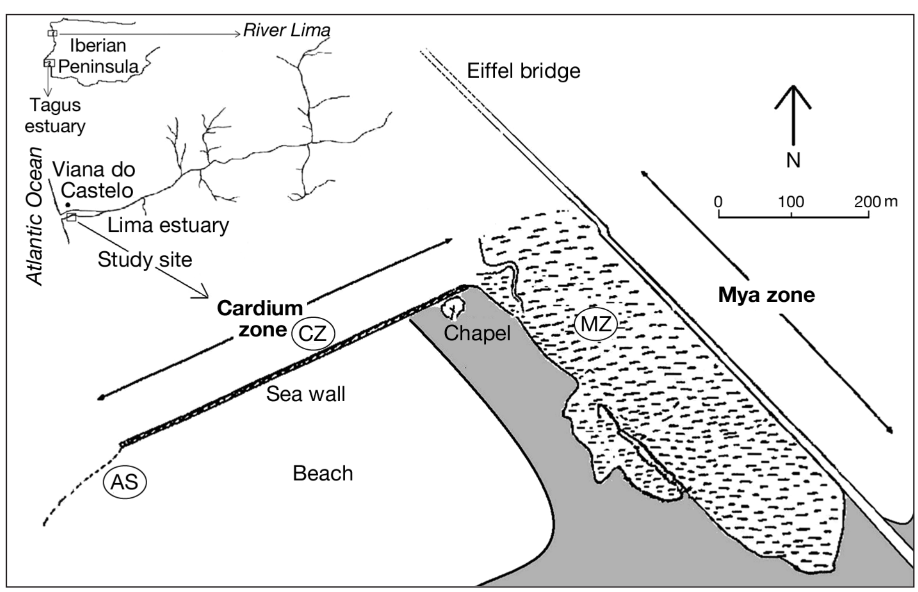

Fig. 1. – Map of the Lima estuary with the location of the sampling stations MZ, CZ and AS. The "*Mya* zone" and the "*Cardium* zone" are shown as defined by Guimarães & Galhano (1988). The stripped area highlighted the "*Mya* zone", a salt marsh. The location of the Tagus estuary is given at the top left corner of the figure. Inland areas are in grey.

Table I. – Density of the species in each site (mean number 0.007 m^{-2} ± SD). The total abundance of the species is shown in the last column. The values of the ecological parameters (mean ± SD) are also shown at the bottom of the table.

		Sampling	Stations		
Species	Taxa	MZ	CZ	AS	Totals
Hediste diversicolor (O.F. Müller, 1776)	Polychaete	11.4 ± 2.9	2.4 ± 3.7	3 ± 1.6	84
Nematode ind.	Nematoda	0	9.6 ± 9.7	0.2 ± 0.4	49
Hydrobia ulvae (Pennant, 1777)	Gastropoda	3.6 ± 2.7	2.4 ± 2.7	3.4 ± 1.8	47
Scrobicularia plana (da Costa, 1778)	Bivalvia	5.2 ± 1.6	0	0.6 ± 0.5	29
Tubificidae ind.	Oligochaeta	0	2.6 ± 3.3	1.8 ± 3.5	22
Cyathura carinata (Krøyer, 1847)	Isopoda	1.4 ± 2.2	0	0.6 ± 0.9	10
Pygospio elegans Claparède, 1863	Polychaete	0.2 ± 0.4	1 ± 1.2	0.8 ± 1.3	10
Nemertean ind.	Nemertean	0	1.2 ± 1.3	0.4 ± 0.9	8
Carcinus maenas (Linnaeus, 1758)	Decapoda	1.4 ± 1.1	0	0	7
Capitella capitata (Fabricius, 1780)	Polychaete	0	0.2 ± 0.4	0.4 ± 0.9	3
Scolelepis squamata (Müller, 1806)	Polychaete	0	0.6 ± 0.5	0	3
Alkmaria romijni Horst,1919	Polychaete	0.6 ± 0.9	0	0	3
Cerastoderma edule (Linnaeus, 1758)	Bivalvia	0	0.4 ± 0.5	0	2
Crangon crangon (Linnaeus, 1758)	Decapoda	0	0.4 ± 0.5	0	2
Glycera convoluta Keferstein, 1862	Polychaete	0	0.4 ± 0.9	0	2
Ecological parameters		MZ	CZ	AS	
Abundance		23.8 ± 6.1	21.2 ± 13.5	11.2 ± 7.4	
Species Richness		4.8 ± 1.6	6 ± 1.2	4.4 ± 1.7	
Diversity		1.8 ± 0.3	2.1 ± 0.6	1.8 ± 0.5	
Evenness		0.8 ± 0.05	0.8 ± 0.2	0.9 ± 0.03	

dance and shell length between populations of bivalves when data were normally distributed (Shapiro-Wilk test); otherwise a Wilcoxon's signed-rank test was used. Going beyond a two-species approach, an ecosystem level approach was considered to provide data from another perspective. A one-way ANOVA was carried out in the ecological parameters abundance, species richness, species diversity (H', Shannon index, Log_2) and evenness (J', Pielou's index) in order to characterize the sampling stations and find any difference between the "*Mya* zone" and the remaining sampling sites. Before conducting the test, data were checked for normality and heterocedasticity. Evenness violated normality (Shapiro-Wilk statistic = 0.785, $P = 0.002$) but the results were considered valid because ANOVA is quite robust to non-normality when independence of the samples and homocedasticity are met in a balanced design (Underwood 1997). Additionally, the multivariate PERMANOVA test (Anderson 2001) was used in order to find any significant difference among the considered assemblages in each sampling station (Bray-Curtis dissimilarity was used), followed by a post-hoc analysis in the case of finding any significant difference (Anderson 2001). The degree of matching of the biotic and abiotic similarity matrices among the sampling stations, based on Spearman correlation, was estimated by using the function BIOENV in Vegan package (Oksanen *et al*. 2010, Clarke & Ainsworth 1993). In order to look for a general pattern in the study site and a higher degree of coherence of the results, a multivariate regression tree (MRT) analysis was applied (De'Ath 2002) to the nine most abundant species plus the bivalve *Cerastoderma edule*, along with the matrix with the considered environmental parameters. The last statistical technique explores and describes the relationship between the species matrix and the accompanying environmental characteristics.

Mean values are reported ± one standard deviation (± SD). All statistics were considered significant at $P < 0.05$ and were calculated by use of an R package (R Development Core Team 2009). The PERMANOVA test was run using the free computer program provided by Dr Anderson (Anderson 2005).

RESULTS

We found high numbers of *Scrobicularia plana* in the "*Mya* zone" (Table I). The GLM model applied to the abundance of *Scrobicularia plana* showed clear significant differences in its distribution (χ^2 statistic = 44.429; $P < 0.0001$). The comparison between sites showed that the abundance of *Scrobicularia plana* in the sampling station located in the "*Mya* zone" (equivalent to a density of 742.8 ind.m^{-2}) was significantly higher than in the AS sampling station ($W = 25$, $P = 0.010$). In accordance to Guimarães & Galhano (1988) the mean density (in fact the grand mean over six mean density values provided in their study) of the described population of bivalves in the "*Mya* zone" was of 708.2 ind.m^{-2}, statistically equal to our estimated population density of *Scrobicularia plana* in the MZ sampling station (t-test = -0.461, $P = 0.664$).

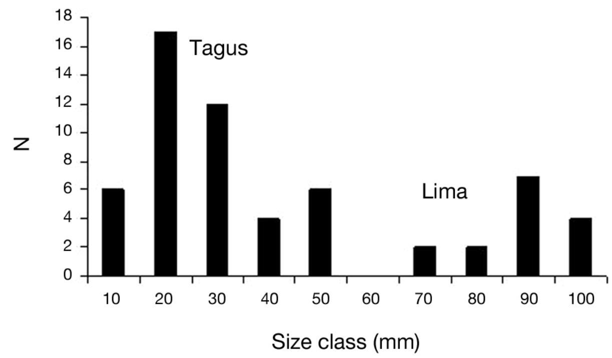

Fig. 2. – Size class distribution of empty shells *of Mya arenaria* collected in the Tagus and Lima estuaries.

The mean shell length of the population of bivalves observed by Guimarães & Galhano (1988) in the "*Mya* zone" during their spring campaign was of 19.8 ± 6 mm, significantly different (W = 149, P = 0.0001) than the mean shell length of 27.7 ± 4.9 mm measured in this study (n = 17) for *Scrobicularia plana*.

The frequency of the empty shells of *Mya arenaria* collected in the Tagus and Lima estuary (n = 44 and 16 respectively) clearly showed significant differences (W = 0, P < 0.0001) in the size classes distribution (Fig. 2). While the empty shells of *Mya arenaria* from the Tagus had a mean value of 31.2 ± 10.7 mm (maximum of 56.2 mm), the Lima estuary empty shells had a mean value of 91.6 ± 12.1 mm, with a maximum of 104.2 mm.

Regarding the assemblages composition, the polychaete *Hediste diversicolor* was the most abundant observed species in the study site. It dominated the MZ sampling station where it reached a maximum in abundance along with *Scrobicularia plana* (Table I, Fig. 3). The last species was absent in the CZ sampling station where a nematode species dominated the assemblage. The AS sampling station presented common species in relation to the remaining sampling sites, with the gastropod *Hydrobia ulvae* (Pennant, 1777) as the most abundant species. *Mya arenaria* was not observed among the fauna collected in any replicate sample.

Still, after collecting all the samples, we were able to find four individuals of the invasive clam near the AS sampling station after an active search in approximately 100 m^2. In consequence, this population was characterized by very low density. These specimens had either a significant lower mean shell length (75.3 ± 14.3 mm) than the empty shells collected in this estuary (91.6 ± 12.1 mm; W = 130, P = 0.0015) and a significant higher mean length in relation to the empty shells of *Mya arenaria* from the Tagus estuary (W = 0, P < 0.0001). Inside the "*Mya* zone" neither empty shells of *Mya arenaria* on the surface of the sediment nor its remains in any sample were observed.

The ecological parameter abundance, species richness, diversity and evenness showed some fluctuation in their values (Table I) although there were evidences of no significant differences among the sampling stations when each of the parameters was separately tested in an ANOVA

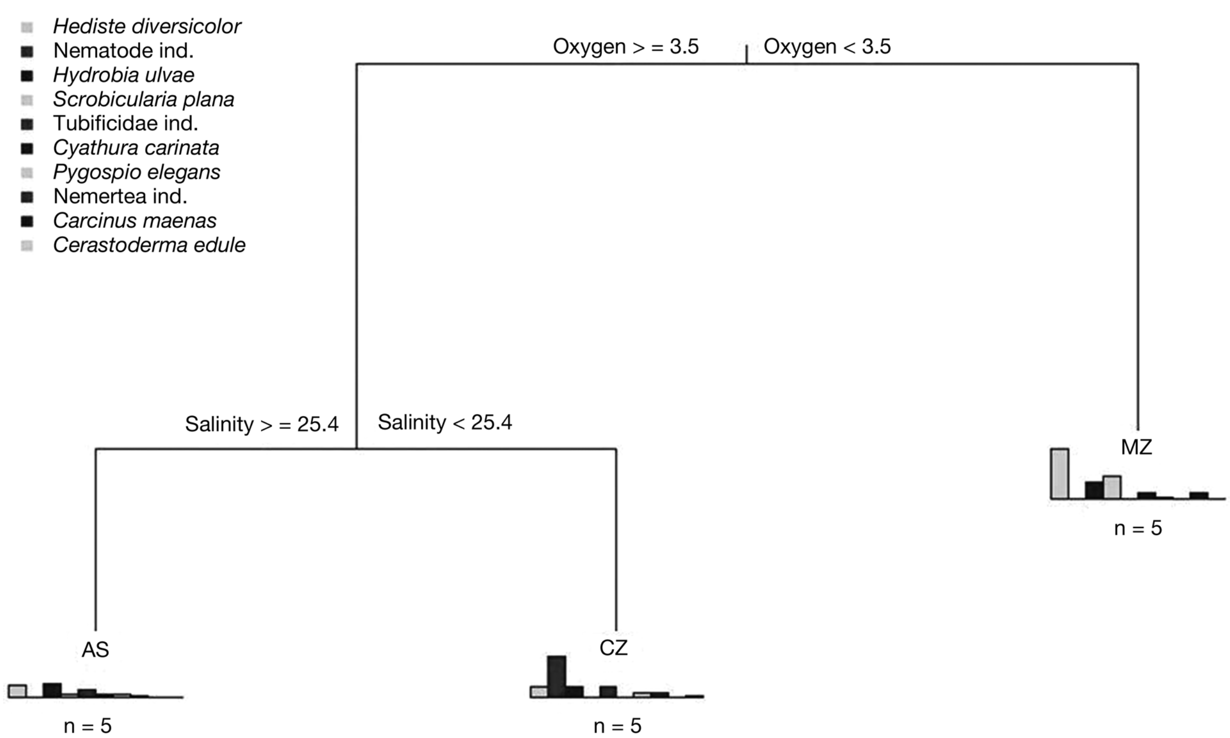

Fig. 3. – MRT analysis relating the species composition of each sampling station (MZ, CZ and AS) with the explanatory environmental variables oxygen and salinity. The number of observations (n) is displayed under the barplots.

Table II. – Summary of the one-way ANOVA to test for differences of the ecological parameters between sites. The outcome of the PERMANOVA test is shown at the bottom of the table. Significant values are in bold.

Test	Response variable	Source of variation	df	MS	F	P
Anova	Abundance	Sites	2	221.27	2.426	0.130
		Residual	12	91.20		
	Species richness	Sites	2	3,467	1.486	0.265
		Residual	12	2.333		
	Diversity	Sites	2	0.143	0.515	0.610
		Residual	12	0.279		
	Evenness	Sites	2	0.011	0.857	0.449
		Residual	12	0.013		
Permanova	Multivarate (spp.)	Sites	2	6662.48	5.401	**0.0002**
		Residual	12	1233.43		

($P > 0.12$, Table II). However, despite this homogeneity in the ecological descriptors, the composition of the assemblages did reveal to be significantly different in accordance to the PERMANOVA test ($P = 0.0002$; Table II). In addition, pair-wise comparison showed significant differences among the assemblages of the sampling stations with a $P < 0.01$ in all cases except between the assemblages of the CZ and AS sampling stations ($P = 0.03$).

The MRT analysis (Fig. 3) allows explaining the distribution of the observed assemblages in relation to the environmental variables oxygen (first split) and salinity (second split). Thus, the assemblage described for the MZ sampling station was found to be different in relation to the other two assemblages (oxygen values ≥ 3.55 mg.l^{-1}, Fig. 3). On the other hand, the observed assemblages of the CZ and AS sampling stations were linked together. Salinity (reference value 25.4, Fig. 3) was the environmental constraint that conditioned the composition of the latter assemblages previously split by oxygen. The result of the BIOENV analysis was coincident in the selection of the best set of environmental parameters (Spearman rho = 0.64 for both "oxygen-salinity" and "oxygen-salinity-tidal height") that better explained the dissimilarities in species composition among the assemblages.

DISCUSSION

The absence of *Mya arenaria* in the MZ sampling station ("*Mya* zone"), corroborated our main hypothesis by, in turn, confirming the existence of an abundant population of *Scrobicularia plana*. The GLM model showed that *Scrobicularia plana* was a characteristic species in the MZ sampling station, as would be expected for a key faunal component of the Iberian estuarine systems (Viéitez 1976, Moreira *et al*. 1993, Sola 1997, Calvário 2001, Silva *et al*. 2006, Conde *et al*. 2010). In accordance, this species was previously reported in the Lima estuary (Sousa *et al*. 2006, 2007). Additionally, *Scrobicularia plana* showed a 100 % chance of capture at any sample, indicating a homogeneous distribution and high density in the "*Mya* zone". In fact, the estimated mean density for this species is in accordance with the mean density value reported by Guimarães & Galhano (1988), suggesting that in both cases the population parameter density belong to the same species, i.e., *Scrobicularia plana*.

Guimarães & Galhano (1988) reported an asymptotic shell length of about 40 mm, very close to the 36.8 mm asymptotic shell length attributed to *Scrobicularia plana* by Sola (1997). Thus, if describing a population of the invasive clam, at least a low pool of reproductive individuals with a higher shell length above the asymptotic shell length of 40 mm would be expected in the report of Guimarães & Galhano (1988). For instance, see the size class of 50 mm in Fig. 2 for the case of *Mya arenaria* in the Tagus estuary. In fact, it is common to observe populations of *Mya arenaria* dominated by young individuals in which exists a reproductive pool of adults with a significant larger shell length (Strasser *et al*. 1999, Maximovich & Guerassimova 2003), even in the case of an early stage of the invasion (Gomoiu & Petran 1973). The absence of a reproductive pool of individuals makes difficult to explain a high density population for *Mya arenaria* as described in Guimarães & Galhano (1988). In addition, the average 19.8 ± 6 mm shell length provided by Guimarães & Galhano (1988) for their spring campaign fits within the 21.8 mm average shell length of the first age class (that passed one winter) described by Sola (1997) in the Bidasoa estuary. Furthermore, the 29.9 mm mean shell length of *Scrobicularia plana* in the second age class (that survived for two winters) reported by Sola (1997) is similar with the average shell length of 27.7 ± 4.9 mm found in this study.

The refuge depth in which *Mya arenaria* is almost safe from predators is about 15 cm, corresponding to individuals of 45-50 mm in shell lengths (Zwarts & Wanink 1989). Accordingly, the longer empty shells from the Lima estuary may correspond to individuals that died of natural causes. In turn, the shells from the Tagus might correspond to younger individuals that were mainly killed by predation or displacement (Strasser *et al*. 1999), among other possible causes, such as summer overheating on shallower buried young clams (see for thermal tolerance in Strasser 1999). The differences in the mean of empty shells between both estuaries may reflect different stages of the invasive process. The shells from dead and live individuals of *Mya arenaria* in the Lima estuary were in both cases significantly higher than the observed shell length for the empty shells of *Mya arenaria* from the Tagus estuary. These findings led us to the conclusion that the invasion of the soft-shell clam happened earlier in northern Portugal than in the Tagus estuary, the only confirmed locations where the invasion of *Mya arenaria* took place in the Iberian Peninsula.

Although we found significant differences in relation to the assemblages of the sampling stations (PERMANOVA test, Table II), the similar values of the ecological parameters suggest that the assemblages were in equilibrium with the environmental conditions in the study site. As such, Sousa *et al*. (2007) described significant differences among the assemblages of the lower Lima estuary but these assemblages were quite stable in composition on a seasonal basis. Moreover, in agreement with our results, these authors found that salinity and oxygen were among the important set of environmental variables that better explain the structure of the estuarine community in the Lima estuary. These data suggest that the "*Mya* zone" and "*Cardium* zone" regions defined by Guimarães & Galhano (1988) have maintained their identity through time, and thus also the dissimilarities in the composition of their assemblages.

The invasion of *Mya arenaria* may outcompete native species, as it was the case for *Corbula mediterranea* Da Costa, on the Romanian coast, Black Sea (Gomoiu 1981). However, the coexistence of populations of *Mya arenaria* and *Scrobicularia plana* is commonly observed on the European Atlantic coast (Bocher *et al*. 2007). In this sense, more than the presence of *Mya arenaria* it is the absence of *Scrobicularia plana* which likely explains the doubts expressed by other authors (Strasser 1999, Conde *et al*. 2010) about the report of *Mya arenaria* by Guimarães & Galhano (1988). The misidentification of *Scrobicularia plana* may be explained by the use of scarce and inappropriate identification keys for bivalves, the book of McLusky (1981) on estuarine ecosystems being the only well-known reference on macrofaunal assemblages quoted by Guimarães & Galhano (1988).

Thus, our findings indicate that the population of bivalves of the "*Mya* zone" described by Guimarães & Galhano (1988) was most likely a population of *Scrobicularia plana*. However, the invasive species *Mya arenaria* has established populations in the Lima estuary and quite probably it was already part of the estuarine assemblages at the time of the study of Guimarães & Galhano (1988), although distributed in a lower intertidal level than that defined by the "*Mya* zone". Therefore, northern Portugal should be considered the first known location in which *Mya arenaria* was first recorded in the Iberian Peninsula. This conclusion is also important in order to identify the potential donor region for explaining the invasion of *Mya arenaria* in the Tagus estuary.

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