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Mediterranean record of *Eulalia ornata* (Annelida: Phyllodocidae) corroborating its fidelity link with the *Sabellaria alveolata* reef habitat

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Abstract

Among marine habitats, *Sabellaria alveolata* reefs deserve protection as they provide important ecosystem services and are advantageous for the biodiversity. Several marine species are listed among the *S. alveolata* reef-associated fauna, but characteristic species have been only seldom reported, with the exception of *Eulalia ornata* (Annelida: Phyllodocidae), which is common/abundant in the *S. alveolata* reefs of the Eastern Atlantic. The most evident geographical mismatch in the distributions of *E. ornata* and these biogenic reefs occurs in the Mediterranean Sea, where *S. alveolata* reefs are commonly found, but *E. ornata* has never been recorded. However, *E. viridis*, a non-Mediterranean species, was previously listed among the dominant reef-associated taxa. The faunal characterization of the sabellarid reefs along the Sicily Channel revealed an *Eulalia* species as the dominant taxon associated with that habitat. A taxonomical approach integrated with DNA bar-coding and comparisons with closely related species helped us determine *E. ornata* as a new species for the Mediterranean Sea. We have described the patterns of abundance and distribution and corroborated its status as a preferential species in the *S. alveolata* reef habitat. Focusing on the biology and ecology of *E. ornata* will enable us to better understand the dynamics and functioning of this valuable European shallow marine habitat.

Keywords: Bio-construction, bar-coding, new record, Mediterranean sea, *Sabellaria alveolata*, characteristic species, *Eulalia viridis*, *Eulalia clavigera*.

Introduction

Sabellaria alveolata (Linnaeus, 1767) (Annelida: Sabellariidae) is a gregarious tube-dwelling polychaete that is commonly referred to as a bio-constructor. Under particular environmental conditions (i.e. moderate hydrodynamics with high suspended sediment load, low salinity and availability of hard substrates), this species builds reefs by assembling tubes with sand, grains and shell fragments (Dubois *et al.,* 2005, 2006; La Porta & Nicoletti, 2009; Desroy *et al.,* 2011; Becker *et al.,* 2012). The resulting biogenic reefs are considered as a valuable marine habitat to be protected under the designation of Special Areas of Conservation in the EU (Annex I of the EC Habitats Directive). In fact, the *S. alveolata* reefs are structurally complex and highly dynamic; they provide important ecosystem services such as filtering of large volumes of water (Dubois *et al.*, 2006), stabilising sediments (Dias & Paula, 2001) and, particularly, increasing local biodiversity (Dubois *et al.,* 2002). *S. alveolata* reefs provide crevices and settling substrate, food and breeding grounds for an array of marine benthic organisms (Dubois *et al.,* 2002, 2006; Cocito, 2004; Pearce *et al.,* 2011; Nahuelhual *et al.*, 2012). Several taxa are associated with this peculiar habitat along the Atlantic coast of Europe; molluscs, mainly mussels (e.g. *Mytilus edulis* L., 1758) and oysters (e.g. *Crassostrea gigas* Thunberg, 1793), crustaceans (several decapod species, the tanaid *Parasinelobus chevreuxi* Dollfus, 1898 and the isopod *Campecopea hirsuta* Montagu, 1804), sipunculids (e.g. *Golfingia vulgaris* de Blainville, 1827), several sponges, bryozoans and cnidarians. However, majority of the associated fauna is usually represented by other polychaetes (Dias & Paula, 2001; Dubois *et al*., 2006). Notably, most of the *S. alveolata* reef-associated species have been commonly recorded in other benthic habitats, whereas 'exclusive' or 'preferential' species (*sensu* Pérès & Picard, 1964), that contribute to displaying of the faunal peculiarity of *S. alveolata* reefs with respect to other habitats, are exceptions. Among them, the phyllodocid polychaete *Eulalia ornata* Saint-Joseph, 1888 is an intertidal species that has been occasionally recorded in the crevices of hard substrata, but is rather common and abundant in the European Atlantic *S. alveolata* reefs, which suggests that this habitat is particularly suitable for its settlement (Pleijel, 1993; Dubois *et al.,* 2003).

Presently, the *E. ornata*'s distributional range includes the Eastern Atlantic, from the British Islands to the coast of Morocco, including the Iberian Peninsula; this species is particularly common along the English Channel and the French Atlantic coast (Fauvel, 1936; Pleijel, 1993; Dauvin *et al*., 2003; Serrano & Preciado, 2007). In addition to the

Fig. 1: Sampling locations along the southern coast of Sicily (Sicily Channel).

In order to collect live specimens of the associated benthic fauna, additional blocks were collected, stored in seawater tanks and transported to the facilities of the University of Palermo. Live specimens were photographed and stored immediately in absolute ethanol for molecular analysis. *Eulalia* specimens were identified to species level following Pleijel (1993), Bonse *et al.* (1996) and Viéitez *et al.* (2004). Additional comparative material of *E. ornata* was collected in the Bristol Channel during August 2010 for morphological and molecular analyses, at a depth of 22.5 m, on sand and gravel bottoms in association with *S. alveolata* specimens.

The morphological characterization of *E. ornata,* including morphometrics, was performed as per the method of Pleijel (1993). Mediterranean specimens were observed under the microscope and measured, and their number-of-segments/body-length ratio was compared with that of the Atlantic specimens of *E. ornata* from Brittany and Arcachon (France), as per the method of Pleijel (1993). The differences obtained were tested by analysis of variance (ANOVA).

Extraction and amplification of DNA

The morphological analysis was integrated by DNA bar-coding, which was aimed at confirming the identity of the species. *E. ornata's* DNA was extracted from two Mediterranean individuals and one individual from the Bristol Channel. The archival organic DNA extracted from the Mediterranean *E. ornata* specimens was deposited together with the voucher specimens at the Zoological Museum of the University of Palermo (MZPA; catalogue no. MZPA-ANN-0025).

Total genomic DNA extraction was performed using the Genomic DNA Extraction Kit Tissue (RBC Bioscience, Taiwan). The target mitochondrial *cytochrome oxidase subunit 1* (*COI*) gene and the D9 and D10 domains of the nuclear ribosomal large subunit (LsuRNA) 28S gene were amplified.

Almost 600 base pair (bp) fragment of the *COI* gene was amplified with primers LCO-1490 and HCO-2198 (Folmer *et al.*, 1994). PCR was performed using a 25-µL volume of 1X PCR buffer, 4 mM $MgCl_2$, 0.4 mM dNTP, 2.5 U *Taq* polymerase (Roche, Italy), 1.6 µM of each primer and 80–100 ng of DNA template. PCR with the 2-steps annealing method was performed under the thermal cycling conditions, with initial denaturation at 95°C for 5 min, 35 cycles at 95°C for 60 s, followed by annealing at 48°C for 60 s for the first 5 cycles and 52°C for 60 s for the remaining 30 cycles, followed by 72°C for 60 sec. Final extension was performed at 72°C for 8 min, followed by final cooling at 4°C.

A 324-bp fragment of the LsuRNA 28S D9/D10 region was also amplified using the primer pairs 28S_D9/D10-F (at position 2279) and 28S_D9/D10-R (at position 2661) (Zardoya *et al.*, 1995). PCR was performed using a 25-µL volume of 1× PCR buffer, 1.3 mM $MgCl_2$, 0.2 mM dNTP, 4 U *Taq* polymerase (Roche, Italy), 0.36 µM of each primer and 80–100 ng of DNA template. The thermal cycling conditions were as follows: initial denaturation at 95°C for 4 min; 35 cycles of 95°C for 60 s, 55°C

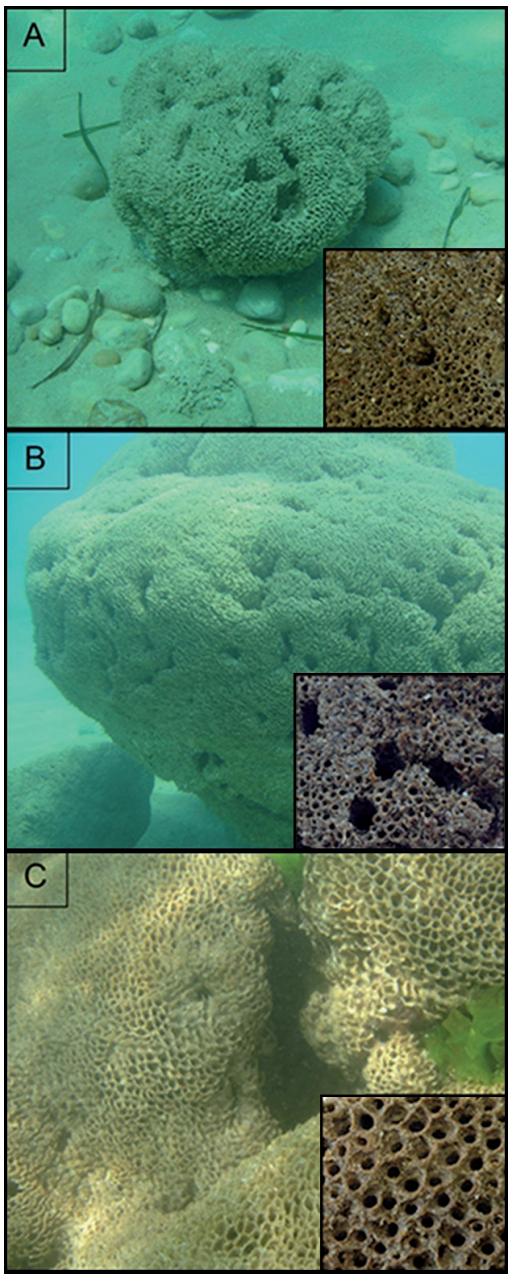

Fig. 2: *Sabellaria alveolata* reefs from the sampled Sicilian locations: (A) Triscina, a brain-like structure of about 0.5 m in diameter, (B) Eraclea Minoa, a brain-like structure of about 2 m in diameter (C), Donnalucata, a pillow-like structure of about 1 m in diameter. For each reef, a close-up picture of *S. alveolata* tubes is provided.

for 60 s and 72°C for 60 s, followed by a final extension at 72°C for 8 min and a final cooling at 4°C.

The resulting amplified DNA fragments were purified using the QIAquick PCR Purification Kit (Qiagen) and sequenced with an Applied Biosystems (ABI) 3730xl DNA analyzer. The sequences were deposited in GenBank under the accession numbers KT709565, KT709566 and KT709567.

The fragment of the LsuRNA 28S D9/D10 region was not used in the analyses, rather merely deposited in the GenBank database for further investigations.

The COI sequences of the Mediterranean *E. ornata,* plus *E. viridis* and *E. clavigera* from several European locations (Norway, Sweden, UK, Northern France, Southern France; see Results for details) were compared with sequences deposited in the Barcode of Life Data Systems (BOLD, http://www.barcodinglife.org) (Ratnasingham & Hebert, 2007) and GenBank (http://www.ncbi.nlm.nih.gov) databases (Benson *et al.*, 2000), followed by deposition in GenBank under the Accession Number KT709556/68.

Sequence alignment and successive analyses were implemented by using the MEGA 6 software (Tamura *et al.*, 2013). Measurement of the genetic differentiation was based on the Kimura-two-Parameter (K2P) model (Kimura, 1980). Unrooted Neighbour–Joining (NJ; Saitou & Nei, 1987) trees were built, and the nodes were supported by a high proportion (>90%) of replicates in the bootstrap analysis (Felsenstein, 1985). The bootstrap test, along with reciprocal monophyly, was used to determine whether a species-like cluster was well supported.

The Automatic Barcode Gap Discovery (ABGD) method, generated on the K2P pair-wise distances, was used to support the grouping of the sequences into species. Based on the barcode gap model, it identifies whenever the average divergence among sequences within species is lower than the average divergence inter-species within the genus (Puillandre *et al.,* 2012).

The ABGD web-interface as well as a command-line program are available at http://wwwabi.snv.jussieu.fr/public/abgd.

Results

In addition to *S. alveolata* (total 17210 specimens), 3952 macro-faunal specimens were collected from the three Sicilian locations (Table 1). The polychaetes represented the main component of *S. alveolata* reef-associated fauna with 2739 specimens, followed by 1169 Crustacea and 44 specimens belonging to other phyla. Among the associated polychaetes, 1368 specimens belonged to Phyllodocidae and 1371 specimens to other families (mostly Syllidae). All of the phyllodocid specimens associated with the *S. alveolata* reefs of the Sicily Channel were identified as *E. ornata.* The density of *E. ornata* per replicate ranged from 12 to 137 individuals/100 cm^2, with the minimum mean value ± standard deviation (SD) recorded at Triscina (37.25 ± 12.56) and the highest at Eraclea Minoa (85.87 ± 27.95).

Taxonomic account

Family Phyllodocidae Örsted, 1843
Subfamily Eteoninae Bergström, 1914
Genus *Eulalia* Savigny, 1822
Eulalia ornata Saint-Joseph, 1888
Eulalia ornata, Pleijel, 1993: 103-105
Material: DL1-R1, 12 ind; DL1-R2, 41 ind; DL1-R3, 21 ind; DL1-R4, 40 ind; DL2-R1, 54 ind; DL2-R2, 109 ind; DL2-R3, 51 ind; DL2-R4, 55 ind; TRI 1-1, 27 ind; TRI 1-2,

Table 1. Total abundance of the reef-associated taxa at the three sampling locations (sum of 8 replicate blocks, 10 × 10 × 10 cm/ location).

Location	*Sabellaria alveolata*	Phyllodocidae	Other polychaetes	Crustaceans	Other taxa
Triscina	10139	298	430	535	42
Eraclea Minoa	3350	687	145	363	1
Donnalucata	3721	383	796	271	1
Total	17210	1368	1371	1169	44

38 ind; TRI 1-3, 27 ind; TRI 1-4, 41 ind; TRI 2-1, 62 ind; TRI 2-2, 27 ind; TRI 2-3, 47 ind; TRI 2-4, 29 ind; EM 1-1, 76 ind; EM 1-2, 64 ind; EM 1-3, 111 ind; EM 1-4, 95 ind; EM 2-1, 56 ind; EM 2-2, 60 ind; EM 2-3, 88 ind; EM 2-4, 137 ind;

Description: Transparent, yellowish, with two pairs of transverse olive-green bands and dark brown spots medially on each segment (Fig. 3). Colour pattern progressively disappeared after preservation in alcohol, with specimens appearing homogenously yellowish to brown (in larger specimens). Body stout, tapering at both the ends. Prostomium rounded, with same length and width, and with five antennae; the median antenna slightly shorter than the lateral ones. Eyes medium-sized, rounded and reddish-brown (Fig. 4A). Proboscis long and narrow, covered with round papillae of similar size throughout the proboscis surface. Tentacular cirri of segment 1 reached segments 3 and 4. Dorsal tentacular cirri of segments 2 and 3, reaching segments 8 and 9. Ventral tentacular cirri slightly flattened, reaching segment 4 and 5. Chaetae from segment 3 to 4. The most anterior dorsal cirri rounded at the tip and twice as long as broad; becoming lanceolate-asymmetrical by segment 10; on segment 20, the dorsal cirri appearing 2–3¼-times as long as broad (Fig. 4B). Dorsal cirri of mid-body lanceolate, slightly asymmetrical, about 3–3¼-times as long as broad (Fig. 4C), on posterior segments lanceolate, asymmetrical, 3–3½-times as long as broad (Fig. 4D). All dorsal cirri with developed cirrophore. Ventral cirri oval, long as neuropodium or shorter on posterior segments. Pygidial cirri relatively long with pointed tips (Fig. 4E).

Remarks: Table 2 summarizes the relevant diagnostic features of the Sicilian *E. ornata* specimens in comparison with those of *E. viridis* and *E. clavigera* (Pleijel, 1993; Bonse *et al.,* 1996; Viéitez *et al.,* 2004). The combination of colour and colour pattern of live specimens allows easily distinguishing of *E. ornata* from the two considered congeneric species, but inter-specific colour differences are usually lost after preservation in alcohol. Moreover, in *E. viridis* and *E. clavigera*, the prostomium is cordiform or rounded-triangular, whereas it is rounded in *E. ornata*. The eyes are larger in *E. viridis* with respect to the other two species. The shape of proboscis is another difference among the three species (clavate in *E. viridis* and *E. clavigera*, narrow in *E. ornata*). The papillae are mostly rounded and similar in size throughout the proboscis length in the analyzed specimens of *E. ornata,* and they are conical and increasing in size toward the distal end of the proboscis in the other two species. Further

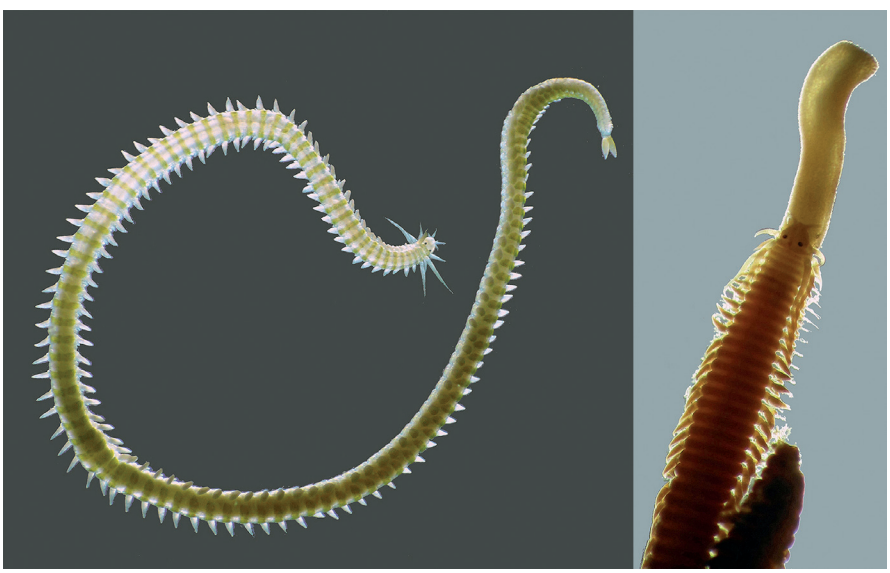

Fig. 3: *Eulalia ornata* from the Sicily Channel (Mediterranean Sea). Left, alive specimen, 45-mm long. Right, preserved specimen, 55-mm long, anterior end with everted proboscis.

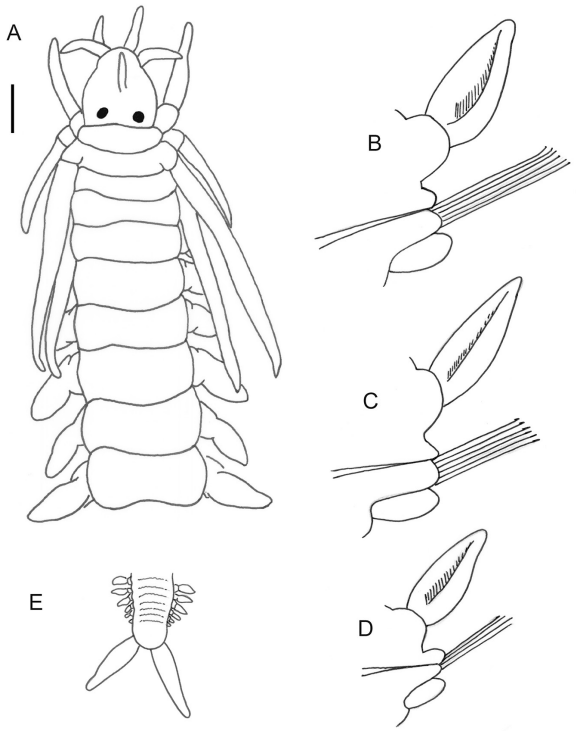

Fig. 4: *Eulalia ornata*. A: anterior end, dorsal view; B: parapodium of segment 20; C: parapodium of mid-body; D: posterior parapodium; E: pygidium. Scale bars: A–E: 200 µm; B–D: 100 µm.

difference is in the length of the median antenna, being as long as the paired ones in *E. viridis* and *E. clavigera*, but slightly shorter in *E. ornata*. In *E. viridis*, the tentacular cirri of segment 1 are short, lanceolate, reaching segments 2 and 3, and the dorsal tentacular cirri of segments 2 and 3 reaching segments 6 to 7. In *E. clavigera*, the tentacular cirri of segment 1 are short fusiform and reaching segments 2 and 3; the dorsal tentacular cirri of segments 2 and 3 are subulate and reaching segments 7 and 8. In both these two species, the cirri of the first three segments appeared shorter than in *E. ornata* (Table 2). Differences among the three species may be also seen in the shape of dorsal cirri. The length/width ratio of anterior and mid-body dorsal cirri is lower in *E. clavigera* than in the other two species; differences may be also seen in the symmetry of dorsal cirri throughout the body, with *E. clavigera* bearing strongly asymmetrical dorsal cirri on segment 20 and symmetrical cirri at the posterior end, while asymmetry of dorsal cirri is always present, but less marked in the other two species. Further differences concerning the ventral cirri length being always shorter than neuropodium in *E. clavigera*, as long as neuropodium at mid-body in *E. viridis,* but longer both on the anterior and posterior segments. Whereas, in *E. ornata,* ventral cirri are as long as neuropodium throughout the body length, except in the posterior segments, where they are shorter.

Morphometrics

The length and total number of segments in *E. ornata* were measured in a random pool of Mediterranean specimens (8 per location). The specimens from the Sicilian reefs measured 4–60 mm in body length, with the minimum average value (± SD) recorded at Triscina (22.1 ± 4.7 mm) and the highest at Eraclea Minoa (27.4 ± 5.23 mm). The number of segments was 20–190. The specimens from Triscina showed the minimum average value (± SD) (101.4 ± 42.9 segments), while those collected at Eraclea Minoa had the highest average value (111.8 ± 51.47 segments).

Figure 5 showed the number of segments plotted against the length of *E. ornata* specimens from the Sicily Channel as compared to the Atlantic specimens of the

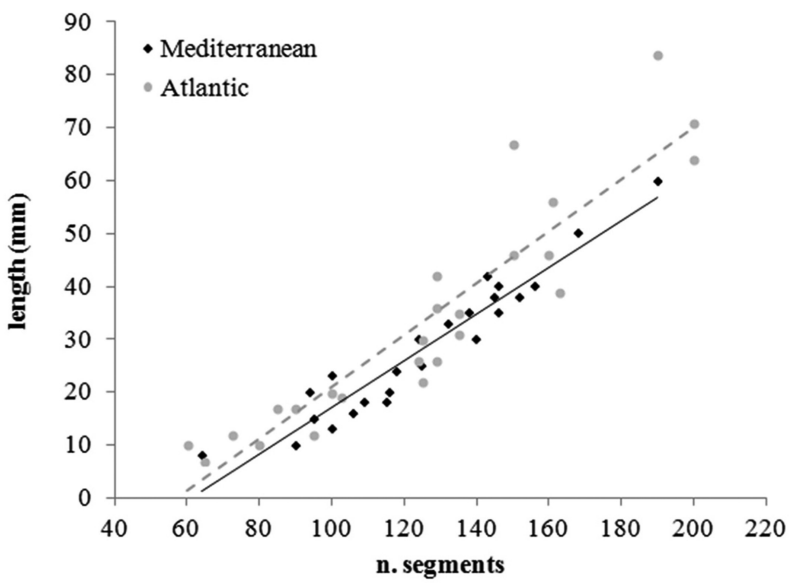

Fig. 5: *Eulalia ornata*—The relationship between the number of segments and the body length. Comparison between Mediterranean (Sicilian) specimens in the present paper versus Atlantic specimens from Pleijel (1993).

Table 2. Comparison of diagnostic features of *Eulalia ornata*, *E. viridis* and *E. clavigera.*

	E. ornata (present paper)	***E. viridis*** (Bonse *et al*., 1996; Pleijel, 1993)	***E. clavigera*** (Bonse *et al*., 1996; Viéitez *et al*., 2004)
Color: alive specimens	Faint yellow	Yellowish green	Emerald green
Color: preserved specimens	Yellowish/greenish/brown (in ethanol)	Yellow/light green/dark brown	Green/greenish brown/brown
Pigmentation pattern: alive specimens	Two pairs of transverse olive-green bands and dark brown spots medially on each segment	Sometimes reddish-brown segmental bands and dark brown spot above and below each parapodium	Sometimes dark spot below the parapodia
Pigmentation pattern: preserved specimens	Absent (in ethanol)	If present, dark brown parapodial spots may disappear in alcohol	Absent
Prostomium	Rounded	Cordiform or rounded triangular	Cordiform or rounded triangular
Median antenna	Slightly shorter than paired anterior antennae	Same size as paired antennae	Same size as paired antennae
Eyes	Medium-size	Relatively large	Medium-size
Proboscis	Narrow	Clavate	Clavate
Distribution pattern of papillae	Rounded, similar size throughout the proboscis length	Conical, increasing in size toward distal end of the proboscis	Conical, increasing in size toward distal end of the proboscis
Tentacular cirri	Cirri segm. 1 reaching segm. 3-4. Dorsal cirri segm. 2-3 reaching segm. 8-9	Cirri segm. 1 reaching segm. 2-3. Dorsal cirri segm. 2-3 reaching segm. 6-7	Cirri segm. 1 reaching segm. 3. Dorsal cirri segm. 2-3 reaching segm. 7-8
Dorsal cirri: Anterior	Rounded at tip; twice as long as broad; lanceolate, asymmetrical by segm. 10.;2 - 3 ¼ times as long as broad on segm. 20	Broadly rounded, twice as long as broad; lanceolate by segm. 8; 2 ½ - 3 ¼ times as long as broad on segm. 20	Broadly rounded, 1½ times as long as broad, elongate-triangular by segm 10; strongly asymmetrical, twice as long as broad on segm. 20
Dorsal cirri: Mid-body	Lanceolate, slightly asymmetrical, 3 - 3 ¼ times as long as broad	Lanceolate/triangular 2¼ - 3 times as long as broad	Lanceolate, asymmetrical, 1 ¾ - 2 ¼ times as long as broad
Dorsal cirri: Posterior	Lanceolate, asymmetrical, 3 - 3 ½ times as long as broad	Asymmetrical, 2½ - 3½ times as long as broad	Symmetrical, about 2 ½ times as long as broad
Ventral cirri	Oval, as long as neuropodium, shorter on posterior segm.	Oval, as long as neuropodium at mid-body, longer on anterior/posterior segm.	Oval, clearly shorter than neuropodium, shorter on posterior segments.

same species, as analyzed by Pleijel (1993). Black diamonds representing the Sicilian specimens were spread among the grey circles, representing specimens from Brittany and Arcachon (France) with the trend lines having a similar slope (0.44 and 0.49, respectively). The ANOVA test for differences in the number of segments/body length ratio between Atlantic and Mediterranean specimens revealed no significant difference (MS = 1.7684; $F_{1,47}$ = 0.60887; p = 0.45).

Molecular data

The morphological identification of species of the genus *Eulalia* was verified by genetic analysis, based on the COI sequences. The dataset used in this work is given in Table 3.

Molecular data discriminated the three common species in three monospecific clades, supported by robust nodes (bootstrap value = 100%), as shown in the NJ tree of Figure 6, and by the K2P distances pattern, supported by a 'barcode gap' (Fig. 7).

The species *E. ornata*, represented by two individuals collected from the Mediterranean Sea and by one specimen from the Bristol Channel, is clearly distinguishable from the other species as its sequences form a distinct, non-overlapping cluster. The *E. viridis* lineage, containing specimens from northern and north-eastern Atlantic Ocean (Norwegian Sea, northern North Sea and the Gulf of St. Lawrence) as well as from Arctic Ocean (White Sea). The *E. clavigera* lineage, composed of specimens from the north-eastern Atlantic (English Channel) and western Mediterranean (France), showed clades separated from each other and from the *E. ornata* clade.

Intra-specific K2P distances were 0.004–0.013, while K2P distances between the congeneric species were

Table 3. Details of the COI reference sequences of *Eulalia* species downloaded and (*) those herein sequenced shown in the NJ tree.

Accession Number (A.N.)	Sampling site	Depth/habitat	Reference
Eulalia ornata			
KT709566*	Bristol Channel (UK), NE Atlantic Ocean	22.5 m, sand/gravel in association with *S. alveolata*	Coll. Arne Nygren
KT709567*	Donnalucata (Italy), Mediterranean Sea	0.5 m, *S. alveolata* reef	Coll. Luigi Musco
KT709565*	Donnalucata (Italy), Mediterranean Sea	0.5 m, *S. alveolata* reef	Coll. Luigi Musco
Eulalia viridis			
GU672585	Kandalaksha Bay (Russia), NW White Sea, Arctic Ocean		Hardy *et al*. (2011)
HQ024018	Gulf of St. Lawrence, (Canada), N Atlantic Ocean		Carr *et al.* (2011)
GU672434	Kandalaksha Bay (Russia), NW White Sea, Arctic Ocean		Hardy *et al.* (2011)
AY996122	Koster area (Sweden), North Sea		Eklöf *et al.* (2007)
GU672477	Kandalaksha Bay (Russia), NW White Sea, Arctic Ocean		Hardy *et al*. (2011)
GU672436	Kandalaksha Bay (Russia), NW White Sea, Arctic Ocean		Hardy *et al*. (2011)
KT709560-KT709561-KT709562*	Koster area (Sweden), North Sea	10-20 m, gravel/algae	Coll. Arne Nygren
KT709563*	Gullmarsfjorden (Sweden), North Sea	33-36 m, gravel/stones	Coll. Fredrik Pleijel
KT709564*	Trondheimsfjorden (Norway), Norwegian Sea	Low tide, among mussels	Coll. Fredrik Pleijel
Eulalia clavigera			
KT709556- KT709558*	English Channel, Plymouth, (UK), NE Atlantic Ocean	Low tide, rocks	Coll. Fredrik Pleijel
KT709557*	Baie de Douarnenez, Brittany (France), NE Atlantic Ocean	Low tide, rocks	Coll. Franck Gentil
KT709559*	Baie de Lannion , Brittany (France), NE Atlantic Ocean	Low tide, rocks	Coll. Franck Gentil
KT709568*	Banyuls (France), Mediterranean Sea	0.5 m, epifauna on dock	Coll. Arne Nygren

0.19–0.22, that is, 20- fold higher. Such patterns illustrate the utility of COI sequences in providing species-level resolution. In fact, the histogram obtained from ABGD (Fig. 7) and based on the assumption that intra-specific divergence ranges should not overlap the inter-specific divergence range, a significant bar-coding gap can be seen, which indicates a high reliability in discriminating different species Our analysis revealed that the maximum

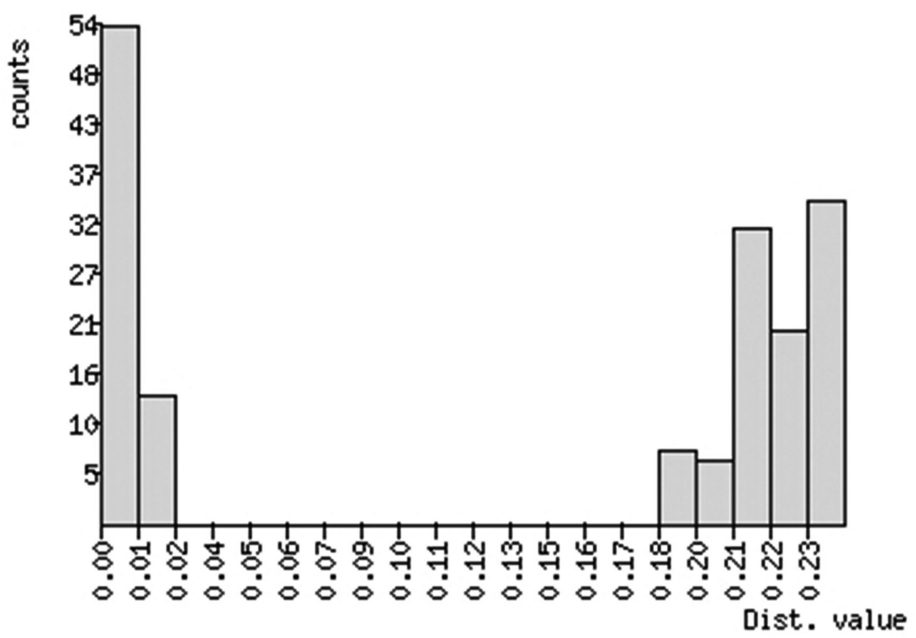

Fig. 7: Distribution of COI pair-wise K2P distance values, performed by ABGD, showing the 'barcode gap' between the putative maximum co-specific divergence (0.02) and the minimum congeneric divergence (0.18).

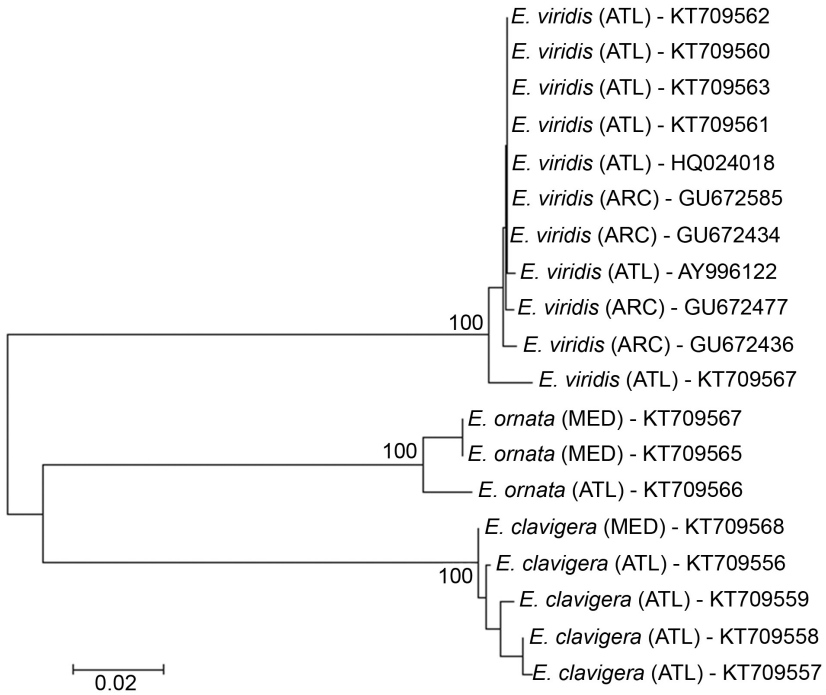

Fig. 6: Tree inferred using the Neighbor-Joining method on Kimura-2-Parameter COI distances. The tree is drawn to scale. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the nodes. *herein sequenced. (ATL) Atlantic Ocean, (ARC) Arctic Ocean, (MED) Mediterranean Sea.

intra-species values in our dataset fell into the range of 0.00–0.02, and the minimum inter-cogeneric species value was >0.18.

Discussion

The first merit of this study is the record of *E. ornata* as a new species for the Mediterranean fauna, which is supported by the results of combined molecular and morphological approaches. This study reviewed the range of distribution for this species, demonstrating that *E. ornata* should be considered as a typical Atlantic-Mediterranean species, similar to a large part of the polychaetes listed for the Mediterranean Sea (Arvanitidis *et al.*, 2002; Musco & Giangrande, 2005). However, the most important finding was the evidence for the fidelity of *E. ornata* to the *S. alveolata* reef habitat in the Mediterranean Sea. In particular, this phyllodocid always appeared as the dominant species associated with *S. alveolata* reefs along the Sicily Channel, being present and abundant in every collected reef sample along approximately 190-km stretch of coast. This finding would re-establish an almost complete match between the ranges for *E. ornata* and *S. alveolata* reefs, evidencing a preferentiality linkage between the phyllodocid and the biogenic habitat from the British Islands to the Atlantic coast of Morocco, including the Mediterranean area. The depicted bio-geographic regularity has its most apparent exceptions in the *S. alveolata* reefs from the Tyrrhenian coast of Central Italy and the Mediterranean Coast of Spain, considered to host *E. viridis* rather than *E. ornata* among the dominant taxa (Porras *et al.,* 1996; La Porta & Nicoletti, 2009; Marzialetti *et al.,* 2009). However, it should be noted that problems concerning morphological characterization could have led to errors or uncertainties in the identification of species of *Eulalia* within the Mediterranean Basin.

Prior to this study, it was generally accepted that the records of *E. viridis* in the Mediterranean area probably referred to *E. clavigera* (Bonse *et al.,* 1996; Dauvin *et al.,* 2003; Çinar & Gönlügür-Demirci, 2005; Parapar *et al*., 2009; Kurt Şahin & Çınar, 2012). *E. viridis* and *E. clavigera* are morphologically extremely similar species. They mainly differed in the shape and size of the dorsal cirri (Viéitez *et al*., 2004). The length of anterior dorsal cirri was about twice as long as broad in *E. viridis* and 1.5-times as long as broad in *E. clavigera* (Çinar & Gönlügür-Demirci, 2005). This diagnostic feature can cause confusion in species identification, as noted by Çinar & Gönlügür-Demirci (2005) after morphological comparison of the Eastern Mediterranean specimens, which was identified as *E. viridis,* and specimens of *E. clavigera* deposited at the Zoological Museum of Copenhagen. *E. clavigera* is undoubtedly present in the Western Mediterranean, as demonstrated by the molecular dataset in the present study (Fig. 6).

The bar-coding analysis confirmed the morpho-recognition of *E. ornata*, and it clearly delimits it from the congeneric species. However, problems in correct morphological identification should also be considered for *E. ornata*. In fact, this last species has been under scientific

debate since only a few years after original description. *E. ornata* was originally described by Saint-Joseph (1888) as an Atlantic species similar to *E. viridis*. Subsequently, McIntosh (1908) considered *E. ornata* as a variety of *E. viridis* due to diagnostic features not sufficiently robust to distinguish between the two species. Finally, according to Allen (1915), differences in the colour pattern and general shape between *E. viridis* and *E. ornata* actually support the validity of this last species. Such taxonomic uncertainty may have been caused by variations in the median antenna and tentacular cirri of the third segment during ontogenesis of *Eulalia* species (Pleijel, 1991) or by artefacts due to preservation, as specimens of both the species appear yellow/brown and may lose the colour pattern once stored in alcohol.

The above-mentioned taxonomic considerations indicate that the *Eulalia* specimens associated with the Mediterranean *S. alveolata* reefs from Central Italy (La Porta & Nicoletti, 2009; Marzialetti *et al.,* 2009) and Spain (Porras *et al.,* 1996) probably do not belong to *E. viridis*. At present, it is not possible to clarify their identity, although both *E. ornata* and *E. clavigera* appear to be good candidate species. The collection of live *Eulalia* specimens from the two mentioned Mediterranean locations and a combined molecular/morphological approach to species identification are needed. Regarding the morphological approach, our analysis indicates that live specimens of *E. ornata* are easily distinguishable from both *E. clavigera* and *E. viridis* due to the clear differences in colour and colour pattern, while preserved specimens should be carefully identified based on characteristics such as the proboscis shape and the length and shape of appendages, including the median antenna, dorsal and ventral tentacular cirri (Table 2). However, simple ecological considerations such as the fidelity (intended as frequency and abundance) of *E. ornata* to *S. alveolata* reefs over a wide geographical range and the lack of *E. clavigera* recorded on the same habitat suggest that the above Central Italian and Spanish Mediterranean specimens of *Eulalia* possibly belong to *E. ornata.*

From an ecological viewpoint, *E. ornata* may play an important role in the ecosystem functioning of *S. alveolata* reef-dominated areas of the Atlantic-Mediterranean coast due to its abundance and preferential association with this biogenic habitat. Jumars *et al.* (2015) suggested that the phyllodocids, including *E. viridis,* are mostly carnivorous. At present, the feeding behaviour of *E. ornata* is unknown; however, if carnivorous, the species may play the key role in structuring the reef as the huge amount of specimens observed herein would have to rely on the large amounts of animal biomass that in the biogenic reef is basically represented by the bio-constructor itself.

Focusing on the biology and ecology of this phyllodocid may provide important insights for the better understanding of the reef dynamics and the functioning of an important European shallow marine habitat.

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References

Allen, E.J., 1915. Polychaeta of Plymouth and the South Devon Coast, including a list of the Archiannelida. *Journal of the Marine Biological Association of the United Kingdom*, 10 (4), 592-646.

Arvanitidis, C., Bellan, G., Drakopoulos, P., Valavanis, V., Dounas, C. *et al*., 2002. Seascape biodiversity patterns along the Mediterranean and the Black Sea: lessons from the biogeography of benthic polychaetes. *Marine Ecology Progress Series*, 244, 139-152.

Becker, P.T., Lambert, A., Lejeune, A., Lanterbecq, D., Flammang, P., 2012. Identification, characterization, and expression levels of putative adhesive proteins from the tube-dwelling polychaete *Sabellaria alveolata*. *The Biological Bulletin,* 223, 217-225.

Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Rapp, B.A. *et al*., 2000. GenBank. *Nucleic Acids Research,* 28, 15-18.

Bonse, S., Schmidt, H., Eibye-Jacobsen, D., Westheide, W., 1996. *Eulalia viridis* (Polychaeta: Phyllodocidae) is a complex of two species in northern Europe: Results from biochemical and morphological analyses. *Cahiers de Biologie Marine,* 37, 33-48.

Cardell, M.J., Gili, J.M., 1988. Distribution of a population of annelid polychaetes in the "trottoir" of the midlittoral zone on the coast of North-East Spain, Western Mediterranean. *Marine Biology,* 99, 83-92.

Castelli, A., Bianchi, C.N., Cantone, G., Çinar, M.E., Gambi, M.C. *et al*., 2008. Annelida Polychaeta. In Relini G. (ed) *Checklist della Flora e della Fauna dei Mari Italiani (Parte I)*. *Biologia Marina Mediterranea*, 15 (S1), 323-373.

Çinar, M.E., 2005. Polychaetes from the coast of Northern Cyprus (Eastern Mediterranean Sea), with two new records for the Mediterranean Sea. *Cahiers de Biologie Marine,* 46, 143-159.

Çinar, M.E., 2013. Polychaetes (Annelida, Polychaeta) associated with *Posidonia oceanica* (L.) Delile along the coasts of Turkey and Northern Cyprus. p. 77-95. In: *1st International Workshop on* Posidonia oceanica *(L.) Delile on the coasts of Turkey.* Aktan Y. & Aysel V. (Eds*)*. Turkish Marine Research Foundation. Publication n. 39.

Çinar, M.E., Gönlügür-Demirci, G., 2005. Polychaete assemblages on shallow-water benthic habitats along the Sinop Peninsula (Black Sea, Turkey). *Cahiers de Biologie Marine,* 46, 253-263.

Çinar, M.E., Dagli, E., Açik, S., 2011. Annelids (Polychaeta and Oligochaeta) from the Sea of Marmara, with descriptions of five new species. *Journal of Natural History*, 45(33-34), 2105-2143.

Çinar, M.E., Dağli, E., Kurt Şahin, G., 2014. Checklist of Annelida from the coasts of Turkey. *Turkish Journal of Zoology,* 38, 734-764.

Cocito, S., 2004. Bioconstruction and biodiversity: their mutual influence. *Scientia Marina*, 68, 137-144.

Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Ben Rais Lasram, F. *et al*., 2010. The biodiversity of the Mediterranean Sea: estimates, patterns, and threats. *PLoS ONE,* 5 (8): e11842.

Dauvin, J.C., Dewarumez, J.M., Gentil, F., 2003. Liste actualisée des espèces d'Annélides Polychètes présentes en Manche. *Cahiers de Biologie Marine,* 44, 67-95.

Delbono, I., Bianchi, C.N., Morri, C., 2003. Le biocostruzioni di *Sabellaria alveolata* come indicatori ambientali: area costiera fra Chiavari e Sestri Levante. p. 130-140.In: *Studi per la Creazione di Strumenti di Gestione costiera: Golfo del Tigullio*. Ferretti, O. (Ed). ENEA, Centro Ricerche Ambiente Marino, La Spezia.

Desroy, N., Dubois, S.F., Fournier, J., Ricquiers, L., Mao, P.L. *et al*., 2011. The conservation status of *Sabellaria alveolata* (L.) (Polychaeta: Sabellariidae) reefs in the Bay of Mont-Saint-Michel. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 21, 462-471.

Dias, A.S., Paula, J., 2001. Associated fauna of *Sabellaria alveolata* colonies on the central coast of Portugal. *Journal of the Marine Biological Association of United Kingdom,* 80, 169-170.

Dorgham, M.M., Hamdy, R., Al-Rashidy, H.H, Atta, M.M., Musco, L., 2014. Distribution patterns of shallow water polychaetes (Annelida) along the coast of Alexandria, Egypt (Eastern Mediterranean). *Mediterranean Marine Science,* 15 (3), 635-649.

Dubois, S., Barillé, L., Cognie, B., 2009. Feeding response of the polychaete *Sabellaria alveolata* (Sabellariidae) to changes in seston concentration. *Journal of Experimental Marine Biology and Ecology,* 376, 94-101.

Dubois, S., Barille, L., Retière, C., 2003. Efficiency of particle retention and clearance rate in the polychaete *Sabellaria alveolata* L. *Comptes Rendus Biologies,* 326, 413-42.

Dubois, S., Retière, C., Olivier, F., 2002. Biodiversity associated with *Sabellaria alveolata* (Polychaeta: Sabellariidae) reefs: effects of human disturbances. *Journal of the Marine Biological Association of United Kingdom,* 82, 817–826.

Dubois, S., Barillé, L., Cognie, B., Beninger, P.G., 2005. Particle capture and processing mechanisms in *Sabellaria alveolata* (Polychaeta: Sabellariidae). *Marine Ecology Progress Series,* 301, 159-171.

Dubois, S., Commito, J.A., Olivier, F., Retière, C., 2006. Effects of epibionts on *Sabellaria alveolata* (L.) biogenic reefs and their associated fauna in the Bay of Mont Saint-Michel. *Estuarine, Coastal and Shelf Science*, 68, 635-646.

Ergen, Z., Çinar, M.E., 1997. Polychaeta of Antalya Bay (Mediterranean coast of Turkey). *Israel Journal of Zoology*, 43(3), 229-241.

Fauvel, P., 1936. Contribution a la faune des Annélides Polychètes du Maroc. *Mémoires de la Société des Sciences Naturelles du Maroc,* 43, 1-143.

Felsenstein, J., 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution,* 39, 783-791.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology,* 3 (5), 294-299.

Imajima, M., 2003. Polychaetous Annelids from Sagami Bay and Sagami Sea collected by the Emperor Showa of Japan and deposited at the Showa Memorial Institute, National Science Museum, Tokyo (II). Orders included within the Phyllodocida, Amphinomida, Spintherida and Eunicida. *National Science Museum Monographs,* 23, 1-221.

Jumars, P.A., Dorgan, K.M., Lindsay, S.M., 2015. Diet of worms emended: An update of Polychaete feeding guilds. *Annual Review of Marine Science,* 7, 497-520.

Kato, T., Pleijel, F., Mawatari, S.F., 2001. *Eulalia gemina* (Phyllodocidae: Polychaeta), a new species from Shirahama, Japan. *Proceedings of the Biological Society of Washington,* 114, 381-388.

Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution,* 16, 111-120.

Kurt Şahin, G., Çinar, M.E., 2012. A check-list of polychaete species (Annelida: Polychaeta) from the Black Sea. *Journal of the Black Sea/Mediterranean Environment,* 18 (1), 10-48.

La Porta, B., Nicoletti, L., 2009. *Sabellaria alveolata* (L.) reefs in the central Tyrrhenian Sea (Italy) and associated polychaete fauna. *Zoosymposia,* 2, 527-536.

Lo Brutto, S., Sparla, M.P., 1993. Anfipodi associati dei banchi di filtratori nel golfo di Castellammare (Sicilia N/W). *Biologia Marina* (Supplemento Notiziario SIBM), 1, 295-296.

Marzialetti, S., Nicoletti, L., Ardizzone, G.D., 2009. The polychaete community of the Fregene artificial reef (Tyrrhenian Sea, Italy): a 20-year study (1981-2001). *Zoosymposia,* 2, 551-566.

McIntosh, W.C., 1908. *A Monograph of British Annelids II. Part I. Polychaeta. Nephthydidae to Syllidae*. Ray Society of London, 232 pp.

Mikac, B, 2015. A sea of worms: polychaete checklist of the Adriatic Sea. *Zootaxa,* 3943 (1), 1-172.

Musco, L., Giangrande, A., 2005. Mediterranean Syllidae (Annelida: Polychaeta) revisited: species diversity, biogeography and faunal fidelity to environmental features. *Marine Ecology Progress Series,* 304, 143-153.

Nahuelhual, E., Carcedo, C., Fiori, S., 2012. Fauna asociada a los arrecifes de *Sabellaria nanella* (Sabellariidae) en el intermareal de Monte Hermoso, Argentina. *BioScriba*, 5, 43-48.

Parapar, J., Martínez-Ansemil, E., Caramelo, C., Collado, R., Schmelz, R., 2009. Polychaetes and oligochaetes associated with intertidal rocky shores in a semi-enclosed industrial and urban embayment, with the description of two new species. *Helgoland Marine Research*, 63, 293-308.

Pearce, B., Hill, J.M., Grubb, L., Harper, G., 2011. *Impacts of Marine Aggregate Dredging on Adjacent* Sabellaria spinulosa *Aggregations and other Benthic Fauna*. Marine Aggregates Levy Sustainability Fund MEPF 08/P39 and

the Crown Estate. Marine Ecological Surveys Limited, 3 Palace Yard Mews, BATH, BA1 2NH. 35 pp.

Pérès, J.M., Picard, J., 1964. Nouveau manuel de bionomie benthique de la Mer Mediterranée. *Recueil des Travaux de la Station Marine d'Endoume*, 31 (47), 5-137.

Pleijel, F., 1991. Phylogeny and classification of the Phyllodocidae. *Zoologica Scripta*, 20, 225-261.

Pleijel, F., 1993. Polychaeta Phyllodocidae. *Marine Invertebrates of Scandinavia* 8: 1-159.

Porras, R., Bataller, J.V., Murgui, E., Torregrosa, M.T., 1996. Trophic structure and community composition of polychaetes inhabiting some *Sabellaria alveolata* (L.) reefs along the Valencia Gulf Coast, western Mediterranean. *PSZNI Marine Ecology*, 17, 583-602.

Poulain, P.M., Zambianchi, E., 2007. Surface circulation in the central Mediterranean Sea as deduced from Lagrangian drifters in the 1990s. *Continental Shelf Research*, 27, 981-1001.

Puillandre, N., Lambert, A., Brouillet, S., Achaz, G., 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology*, 21, 1864-1877.

Ratnasingham, S., Hebert, P.D., 2007. Bold: The Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes*, 7, 355-364.

Saint-Joseph, A., 1888. Les annélides polychètes des côtes de Dinard. Seconde partie. p. 141-338. In: *Annales des Sciences Naturelles, Zoologie et Paléontologie.* Paris. Série 7, 5(2).

Saitou, N., Nei, M., 1987. The Neighbor-Joining method - a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4, 406-425.

Serrano, A., Preciado, I., 2007. Environmental factors structuring polychaete communities in shallow rocky habitats: role of physical stress versus habitat complexity. *Helgoland Marine Research*, 61, 17-29.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA 6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 30, 2725-2729.

Viéitez, J.M., Alós, C., Parapar, J., Besteiro, C., Moreira, J. *et al*., 2004. Annelida Polychaeta I. In: *Fauna Iberica.* Vol. 25. Ramos M.A. *et al.* (Eds). Museo Nacional de Ciencias Naturales, CSIC. Madrid. 530 pp.

Zardoya, R., Costas, E., López-Rodas, V., Garrido-Pertierra, A., Bautista, J.M., 1995. Revised dinoflagellate phylogeny inferred from molecular analysis of large-subunit ribosomal RNA gene sequences. *Journal of Molecular Evolution*, 41, 637-645.