

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

above-mentioned area, *E. ornata* was recorded along the eastern coast of Scotland (Pleijel, 1993) and in Japan (Imajima, 2003); however, the last record requires further confirmation by comparison with type specimens of *E. gemina* Kato, Pleijel & Mawatari, 2001, a morphologically similar species endemic for Japanese waters (Kato *et al.*, 2001). *E. ornata* does not list among the 8 species of *Eulalia* reported from the Mediterranean Sea (Coll *et al.*, 2010).

Notably, the present distribution of *S. alveolata* reefs along the European coast (Dubois *et al.*, 2009) mostly overlaps the *E. ornata*'s range of distribution, and the specimens of *E. ornata* found along the Atlantic Moroccan coast by Fauvel (1936) coexist with considerable masses of *S. alveolata* tubes. Presently, the most evident mismatch in the distributions of *E. ornata* and *S. alveolata* reefs occur in the Mediterranean area.

S. alveolata reefs are actually commonly reported along the Western Mediterranean basin although they are usually less broad as compared to their Atlantic counterparts (La Porta & Nicoletti, 2009). The limited extension of *S. alveolata* reefs in comparison to the other important Mediterranean habitats may explain the surprisingly scant number of faunal studies on sabellarid reefs in the area (Cardell & Gili, 1988; Lo Brutto & Sparla, 1993; Porras *et al.*, 1996; Delbono *et al.*, 2003; La Porta & Nicoletti, 2009). The limited available studies suggest that the Mediterranean *S. alveolata* bio-constructions host-diversified faunas include polychaetes as the dominant group, with Phyllodocidae being among the most abundant taxa (La Porta & Nicoletti, 2009). In particular, *Eulalia viridis* L., 1767 is a dominant taxon associated with *S. alveolata* reefs along the Tyrrhenian coast of Central Italy and the Gulf of Valencia along the Mediterranean coast of Spain (Porras *et al.*, 1996; La Porta & Nicoletti, 2009; Marzialetti *et al.*, 2009). This species has been reported several times in the shallow hard-bottom environments of the Mediterranean and Black Seas (Pleijel, 1993; Ergen & Çinar, 1997; Çinar & Gönlügür-Demirci, 2005; Castelli *et al.*, 2008). However, Bonse *et al.* (1996) argue that the Eastern Atlantic and Mediterranean records of *E. viridis* could refer to the congeneric species *Eulalia clavigera* (Audouin & Milne-Edwards, 1833), since *E. viridis* is probably limited to the North Sea and the Scandinavian coast. In fact, recent faunal papers do not mention *E. viridis* among the Mediterranean polychaetes, and an accurate revision of the previous records of this species outside the Scandinavian area is needed (Çinar & Gönlügür-Demirci, 2005; Kurt Şahin & Çinar, 2012; Çinar *et al.*, 2011, 2014; Çinar, 2013; Dorgham *et al.*, 2014; Mikac, 2015). *E. clavigera* has been usually recorded on rocky reefs in crevices, among algae, mussel beds, *Balanus* blocks, *Dendropoma* reefs, *Posidonia oceanica* meadows, and coralligenous formations (Bonse *et al.*, 1996; Viéitez *et al.*, 2004; Çinar, 2005). To the best of our knowledge, *E. clavigera* has never been listed among the *S. alveolata*-reef associated species.

In the framework of a study performed to characterize the fauna of the *S. alveolata*-reef habitat in the Central Mediterranean area, we collected large numbers of phyllodocid specimens belonging to the genus *Eulalia*. Based on a taxonomic approach integrated by DNA barcoding, and after comparisons with closely related *Eulalia* species reported from the Mediterranean area, we hereby report *E. ornata* as a new species for the Mediterranean Sea and describe its patterns of abundance and distribution along the Sicily Channel.

Materials and Methods

Study area and habitat description

The Sicily Channel (Central Mediterranean Sea) connects the Western and Eastern Mediterranean sub-basins, and it is directly influenced by the Atlantic Ionian Stream (AIS) originating from the Atlantic waters entering through the Gibraltar Strait and moving eastward. Temperature and salinity fields in the Sicily Channel are thus greatly influenced by the AIS dynamics (Poulain & Zambianchi, 2007), with sea surface temperature ranging from 15°C in February to 22–24°C in June–July. Along the Sicilian coast of the Channel, several rivers flow into the sea and the coast is shallow and characterized by sandy bottoms locally interspersed with *P. oceanica* meadows and rocky shores. The tide excursion is limited to a few centimetres in the area.

S. alveolata reefs were sampled in June 2013 in the Sicily Channel along an approximately 190-km stretch of coast of southern Sicily at three locations: Triscina–TR (37.58°N, 12.80°E), Eraclea Minoa–ER (37.39°N, 13.28°E) and Donnalucata–DO (36.75°N, 14.64°E) (Fig. 1).

The sampled reefs appeared as pillow-like structures of different sizes, with crevices and holes and a brain-like appearance (Fig. 2). At Triscina, the reefs were about 0.5 m in diameter and about 30-cm high, lying at 3-m depth on sandy bottom interspersed with pebbles, close to a *P. oceanica* meadow; the diameter of *S. alveolata* tubes appeared homogeneously small in the study area. At Eraclea Minoa, the reefs were considerably bigger, measuring up to 2 m in diameter and 1.5 m in height, lying at 3-m depth on sandy bottom; tubes of different diameters (both big and small) characterized the reefs. At Donnalucata, the reefs were about 0.5-m high, laying at 1.5-m depth on sandy bottom at the base of breakwater rocky barriers and were up to 1 m in diameter; tubes of the sampled reefs appeared homogeneously large (Fig. 2).

Sampling design and material collection

At each location, 8 replicates blocks (10 × 10 × 10 cm) of *S. alveolata* reef were randomly collected from biogenic structures laying few meters apart from each other using a hammer and a putty knife and then stored in 70% ethanol. *S. alveolata* specimens and the associated fauna were sorted under a stereo microscope and counted.



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 Zoologi-

cal 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- μ L volume of 1X PCR buffer, 4 mM MgCl₂, 0.4 mM dNTP, 2.5 U *Taq* polymerase (Roche, Italy), 1.6 μ M 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- μ L volume of 1 \times PCR buffer, 1.3 mM MgCl₂, 0.2 mM dNTP, 4 U *Taq* polymerase (Roche, Italy), 0.36 μ M 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

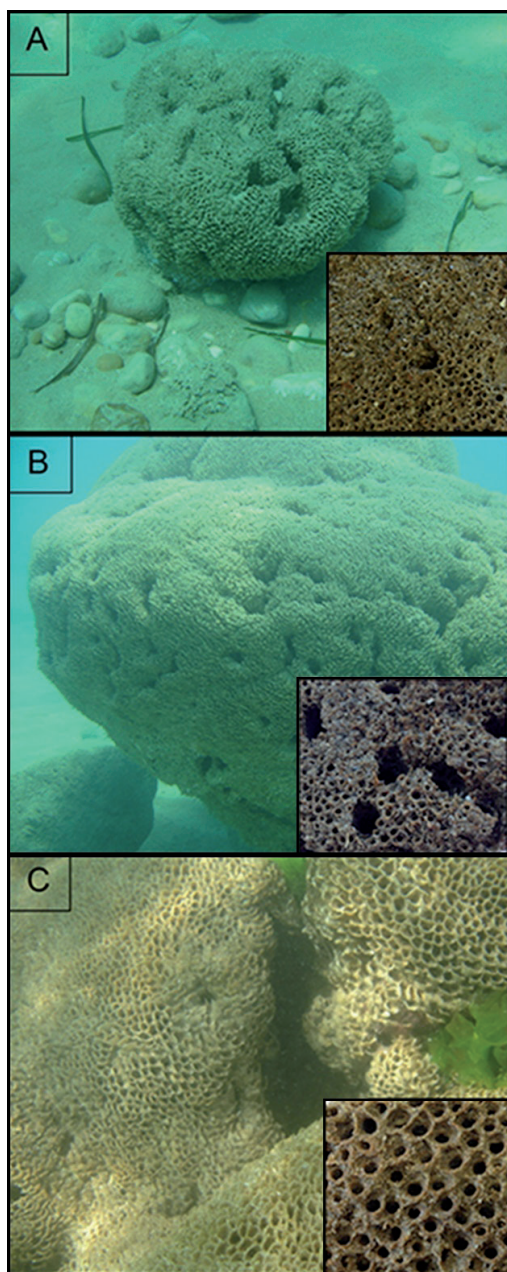


Fig. 2: *Sabellaria alveolata* reefs from the sampled Sicilian locations: (A) Triscina, a brain-like structure of about 0.5 m in diameter, (B) Eraclea Minoa, a brain-like structure of about 2 m in diameter (C), Donnalucata, a pillow-like structure of about 1 m in diameter. For each reef, a close-up picture 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://www.wabi.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 cm², with the minimum mean value \pm 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	<i>Sabellaria alveolata</i>	Phyllodoceidae	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 homogeneously 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 lan-

ceolate, 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

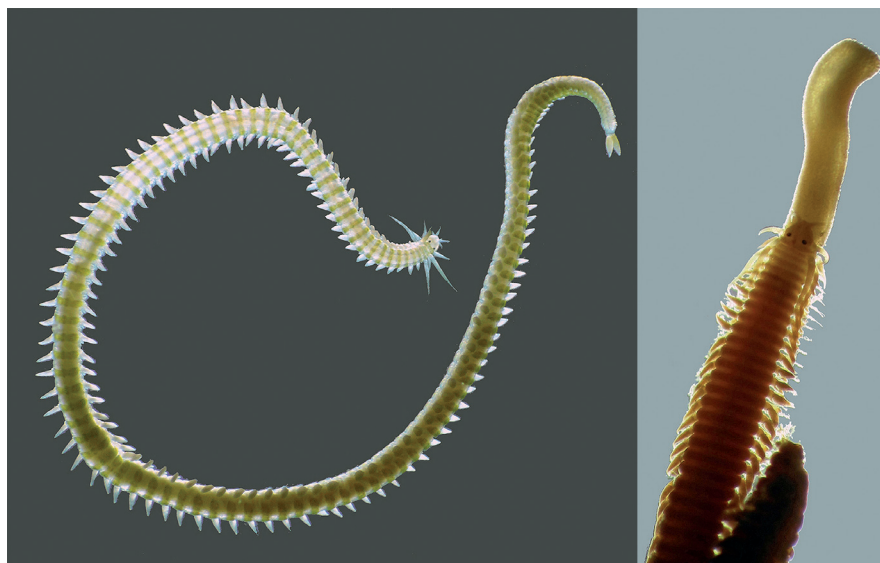


Fig. 3: *Eulalia ornata* from the Sicily Channel (Mediterranean Sea). Left, alive specimen, 45-mm long. Right, preserved specimen, 55-mm long, anterior end with everted proboscis.

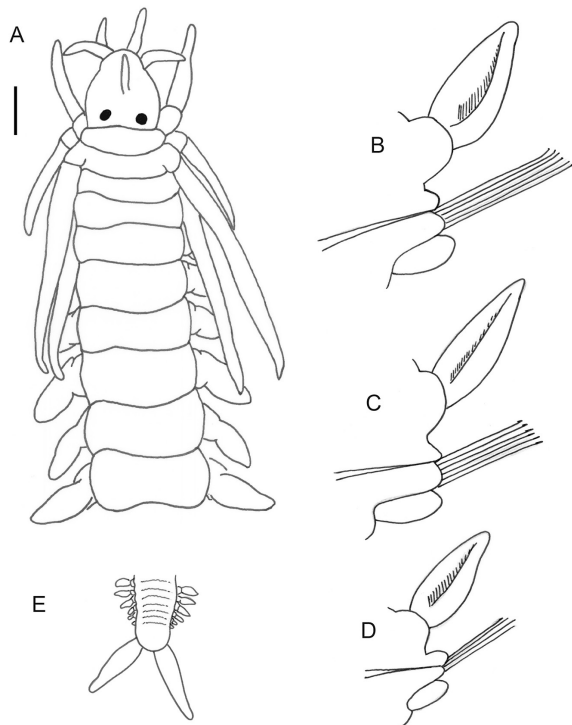


Fig. 4: *Eulalia ornata*. A: anterior end, dorsal view; B: parapodium of segment 20; C: parapodium of mid-body; D: posterior parapodium; E: pygidium. Scale bars: A–E: 200 μm ; B–D: 100 μm .

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 (\pm 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 (\pm 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

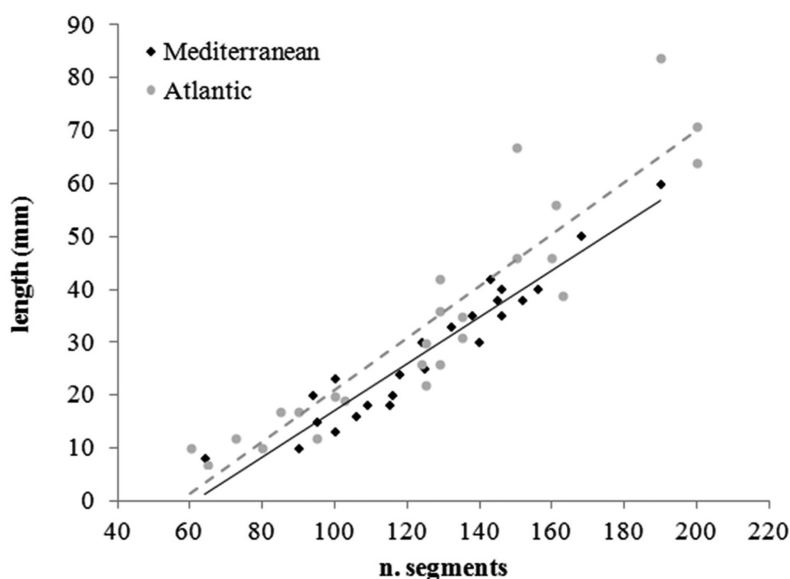


Fig. 5: *Eulalia ornata*—The relationship between the number of segments and the body length. Comparison between Mediterranean (Sicilian) specimens in the present paper versus Atlantic specimens from Pleijel (1993).

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

	<i>E. ornata</i> (present paper)	<i>E. viridis</i> (Bonse <i>et al.</i> , 1996; Pleijel, 1993)	<i>E. clavigera</i> (Bonse <i>et al.</i> , 1996; Viéitez <i>et al.</i> , 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 (A.N.)	Number	Sampling site	Depth/habitat	Reference
<i>Eulalia ornata</i>				
KT709566*		Bristol Channel (UK), NE Atlantic Ocean	22.5 m, sand/gravel in association with <i>S. alveolata</i>	Coll. Arne Nygren
KT709567*		Donnalucata (Italy), Mediterranean Sea	0.5 m, <i>S. alveolata</i> reef	Coll. Luigi Musco
KT709565*		Donnalucata (Italy), Mediterranean Sea	0.5 m, <i>S. alveolata</i> reef	Coll. Luigi Musco
<i>Eulalia viridis</i>				
GU672585		Kandalaksha Bay (Russia), NW White Sea, Arctic Ocean		Hardy <i>et al.</i> (2011)
HQ024018		Gulf of St. Lawrence, (Canada), N Atlantic Ocean		Carr <i>et al.</i> (2011)
GU672434		Kandalaksha Bay (Russia), NW White Sea, Arctic Ocean		Hardy <i>et al.</i> (2011)
AY996122		Koster area (Sweden), North Sea		Eklöf <i>et al.</i> (2007)
GU672477		Kandalaksha Bay (Russia), NW White Sea, Arctic Ocean		Hardy <i>et al.</i> (2011)
GU672436		Kandalaksha Bay (Russia), NW White Sea, Arctic Ocean		Hardy <i>et al.</i> (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
<i>Eulalia clavigera</i>				
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

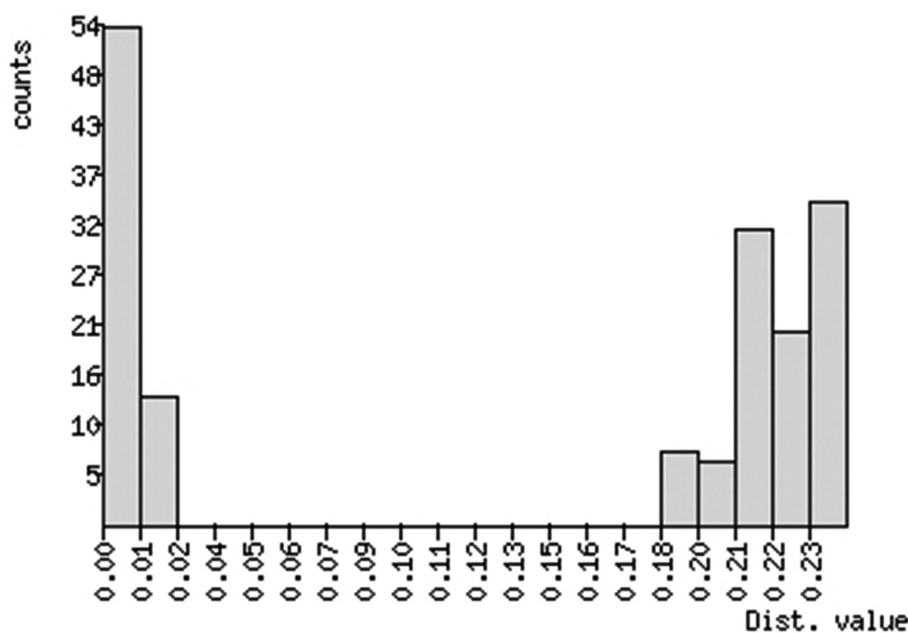


Fig. 7: Distribution of COI pair-wise K2P distance values, performed by ABGD, showing the ‘barcode gap’ between the putative maximum co-specific divergence (0.02) and the minimum congeneric divergence (0.18).

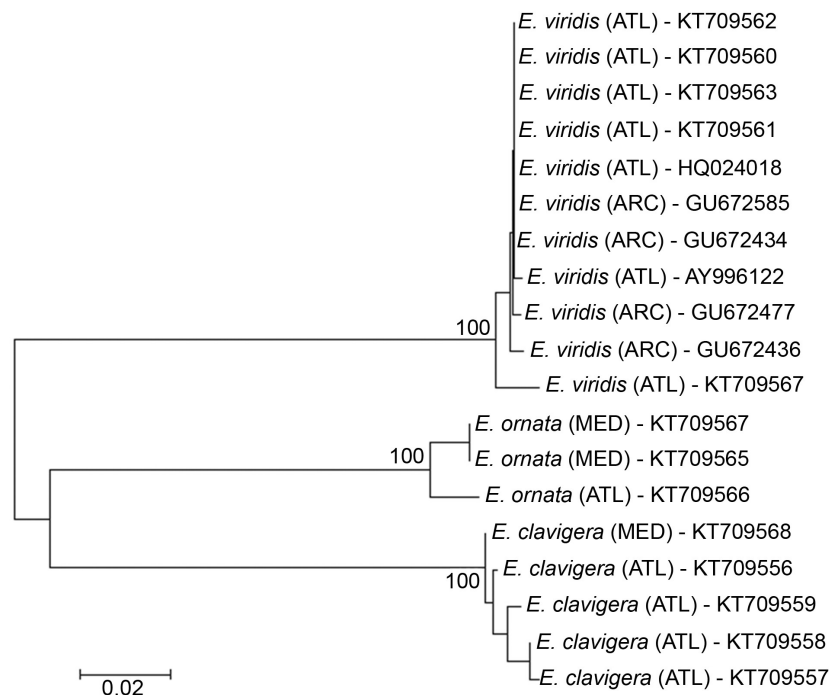


Fig. 6: Tree inferred using the Neighbor-Joining method on Kimura-2-Parameter COI distances. The tree is drawn to scale. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the nodes. *herein sequenced. (ATL) Atlantic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

dis rather than *E. ornata* among the dominant taxa (Porras *et al.*, 1996; La Porta & Nicoletti, 2009; Marzialelli *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 & Gönlgür-Demirci, 2005; Parapar *et al.*, 2009; Kurt Şahin & Çinar, 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 & Gönlgür-Demirci, 2005). This diagnostic feature can cause confusion in species identification, as noted by Çinar & Gönlgür-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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