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## Distribution and phenotypic variability of the Mediterranean gorgonian *Paramuricea macrospina* (Cnidaria: Octocorallia)

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### Abstract

The genus *Paramuricea* is present in the Mediterranean Sea with two species, *P. clavata* and *P. macrospina*. These species have similar bathymetric distributions and can live in sympatry. *P. macrospina* shows morphological plasticity leading sometimes to an erroneous classification as *P. clavata*. Studying four ambiguous morphotypes of *P. macrospina* collected in different localities of the Mediterranean Sea, we provide new insights on both the taxonomy and the distribution of this species. Our analyses reveal high morphological plasticity within the sampled colonies, which in two cases were characterised by a peculiar pigmentation and a morphological pattern resembling that of *P. clavata*. After having confirmed the genetic identity of the samples, we tested the validity of traditional taxonomic characters, and found that the most reliable character to discriminate between *P. macrospina* and *P. clavata* is the number of rows of spindles in the collaret of the polyps. All other features are highly variable and therefore do not allow a correct identification. The bathymetric and geographic distribution of *P. macrospina* is here updated, showing, with the exception of the south-eastern side, broad preference in the whole basin. Moreover, further studies are needed to investigate the gene flow among and within the Mediterranean populations of *P. macrospina* and to assess whether the morphological plasticity is driven by adaptive genetic processes of populations exposed to different environmental, climatic and bathymetric conditions.

**Keywords:** Morphotypes, sclerites, taxonomy, morphology plasticity, Plexauridae

### Introduction

The taxonomy of gorgonians is mainly based on morphological characters such as the branching pattern of the colony, the shape of the anthocodia and the type and arrangement of sclerites (Bayer 1961). These characters show high morphological plasticity due to the influence of environmental factors (Sánchez et al. 2007; Prada et al. 2008; Gutiérrez-Rodríguez et al. 2009) which, sometimes, may lead to the formation of puzzling features (West et al. 1993; Wirshing & Baker 2015). Therefore, species-level identification is not a trivial process, hiding, in some cases, distinct phylogenetic lineages (Grigg 1972; Brazeau & Lasker 1988; Prada et al. 2008;

Gutiérrez-Rodríguez et al. 2009; Bilewitch et al. 2014; Wirshing & Baker 2015; Ament-Velásquez et al. 2016).

The genus *Paramuricea* (Kölliker, 1865) includes 25 valid species, distribute in tropical, temperate and polar areas (Cordeiro et al. 2018). In the Mediterranean Sea, only two species are known, *Paramuricea clavata* (Risso, 1826) and the endemic *Paramuricea macrospina* (Koch, 1882). While the interest in *P. clavata* as an ecosystem engineer is rapidly growing (Ponti et al. 2014, 2016, 2018; Valisano et al. 2016), especially after its severe involvement in widespread and frequent massive mortality events in shallow waters (Cerrano et al. 2000; Calvisi et al. 2003; Linares et al. 2005; Garrabou

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et al. 2009; Huete-Stauffer et al. 2011; Vezzulli et al. 2013), only little, scattered information, mostly in the form of records, is available for *P. macrospina* (Aguilar et al. 2015; Grinyó et al. 2017). The importance of gorgonians as an “animal forest” in the Mediterranean Sea has been documented (Cerrano et al. 2000) and is currently receiving attention (Ponti et al. 2016; Valisano et al. 2016; Gori et al. 2017) due to their structural and functional role. They increase the three-dimensional complexity of the habitat, modifying local environmental parameters such as water movement and sedimentation processes (Valisano et al. 2016), creating new ecological niches and therefore affecting the diversity of associated species (Linares et al. 2005; Ponti et al. 2014, 2018; Gori et al. 2017). Gorgonians can build wide facies, sometimes monospecific assemblages, as reported for *P. macrospina* in the Marmara Sea and Menorca Channel (Topçu & Öztürk 2015; Grinyó et al. 2016). Despite being assessed as “Data Deficient” due to little information and few records (Aguilar et al. 2015; Otero et al. 2017), *P. macrospina* was listed on the International Union for Conservation of Nature (IUCN) Red List as suspected to be in decline, owing to indiscriminate bottom trawling and trammel net fishing activities. *Paramuricea macrospina* inhabits hard, detritic or sandy bottoms, preferentially between 20 and 150 m in depth (Carpine & Grasshoff 1975; Topçu & Öztürk 2015; Grinyó et al. 2016), and except for the Aegean Sea and Marmara Sea, where it was found only between 20 and 50 m depth (Carpine & Grasshoff 1975; Vafidis et al. 1994; Bo et al. 2012; Topçu & Öztürk 2015), it usually colonises mesophotic habitats in the Mediterranean Sea below 60 m depth. *Paramuricea clavata* shows a bathymetric distribution ranging from 10 to 200 m in depth (Mokhtar-Jamai et al. 2011; Gori et al. 2017), and is more frequent on hard substrates with vertical sloping (Carpine & Grasshoff 1975). The morphological difference between the two species is an overall characterisation (Carpine & Grasshoff 1975). *Paramuricea clavata* shows usually large colonies (up to 1 m high) with thick branches, completely purple or purple to yellow in colour, polyps closely packed on the branches, thornscales of the calyx without a long point and five to eight rows of spindles in the collaret of the polyps with tubercles mainly on the convex side. In contrast, samples of *P. macrospina* are usually described as small colonies (up to ca. 10 cm) with rather thin branches and unpacked polyps, yellow to pinkish in colour, the thornscales in the calyces may have a very long point, and there

are three to four rows of spindles in the collaret, covered all around by tubercles (Stiasny 1942; Carpine & Grasshoff 1975; Grasshoff 1977). Unlike *P. clavata* which shows more stable characters, colonies of *P. macrospina* with different shapes and colours have been recorded in the Mediterranean Sea (Pax & Müller 1962; Carpine 1963; Topçu & Öztürk 2013, 2015; Grinyó et al. 2016, 2017). For instance, Carpine (1963) reported pinkish-grey colonies (up to 20 cm) from Corsica (France), while Pax and Müller (1962) recorded bright orange, translucent white and almost colourless colonies in the Adriatic Sea. In the Marmara Sea, *P. macrospina* colonies are usually yellow, but sometimes creamy to orange and brownish pink variations in colour are recorded (Topçu & Öztürk 2013, 2015). Specimens collected in the Marmara Sea and in Menorca Channel showed taller colonies (up to 22 and 56 cm high, respectively) well ramified (Topçu & Öztürk 2013; Grinyó et al. 2016, 2017). Colonies of *P. macrospina* are characterised by peculiar long thornscales points in the calyx, which were firstly described by Koch (1882) in holotype material. This character is largely variable in terms of shape and size, representing one of the main causes of erroneous identification among and within *P. clavata* and *P. macrospina* colonies (Carpine & Grasshoff 1975). Indeed, thornscales have variable sizes and, few years later, the same author (Koch 1887) considered *P. macrospina* to be a variety of *P. clavata* (*P. chamaeleon* var. *macrospina*). Afterwards, Stiasny (1942) wrote that *P. macrospina* might be clearly distinguishable from *P. clavata*, but the considered diagnostic characters are not fully accepted. For example, the long thornscales sclerites recorded in many specimens of *P. macrospina* (Koch 1882; Stiasny 1942; Pax & Müller 1962 as *P. placomus*) are considered too variable (Carpine & Grasshoff 1975). By contrast, the same authors, following Stiasny (1942), claimed that the shape and number of spindles in the collaret of the polyps might be considered the main diagnostic features to differentiate the two species. Nevertheless, these features have been overlooked, and were not taken into account by all authors. Due to its wide morphological and chromatic plasticity, *P. macrospina* has often been confused with *P. clavata* (Koch 1887; Stiasny 1942; Pax & Müller 1962; Carpine & Grasshoff 1975; Aguilar et al. 2015) even if these two species are genetically separated (Poliseno et al. 2017).

The present study was triggered by the finding of several puzzling colonies of *Paramuricea* with unusual phenotypes. However, given the lack of morphological diagnostic characters and the high phenotypic plasticity

showed by the investigated samples, two partial mitochondrial genes, *COI* and *mtMutS*, have been sequenced in order to validate the species identification of the colonies. The aim of this study is to update the taxonomic and ecological knowledge on the Mediterranean endemic *P. macrospina*, by testing the validity of traditional taxonomic characters for congeneric discrimination and assessing its bathymetric and geographic distribution by reviewing the available literatures.

## Materials and methods

### Morphological characterisation

Samples from four distinct morphotypes of *Paramuricea macrospina* and from colonies of *P. clavata* were collected by scuba diving at a depth ranging from 30 to 70 m in different Mediterranean areas (Table I).

All specimens were photographed before sampling to describe the general shape of the colonies. Two branches for each colony were collected and preserved in absolute ethanol for molecular analysis, whereas further branches preserved in 75% ethanol were considered for morphological characterisation. The morphological characterisation includes the general shape of the colony, anthocodia distribution, sclerite arrangement and shape and size of sclerites. For each sample, we took digital images using a Nikon D600 camera attached to a Nikon SMZ18 stereomicroscope. Sclerites were analysed by dissolving the different parts of the colony separately, with sodium hypochlorite: the coenenchyme, the anthostele and the anthocodia. For each part, several fragments were analysed to encompass the variability of sclerites inside one colony. Non-permanent slides were prepared following Bayer (1961) and observed under a

Nikon Eclipse Ni-U microscope and the morphology and the dimension of the sclerites were recorded. The sclerites were also cleaned for Scanning Electron Microscopy (SEM) analysis following Fabricius and Alderslade (2001). The sclerites were mounted on stubs, coated with gold–palladium in a Balzer Union evaporator and examined with a Philips XL20 SEM. The total length of the sclerites was then measured by means of ImageJ software.

### Molecular analyses

DNA extraction of ethanol-preserved samples was carried out using either a modified Cetyltrimethylammonium Bromide (CTAB) phenol-chloroform protocol (Doyle & Doyle 1987) or the Macherey-Nagel NucleoSpin® Tissue kit (M&N, Düren, Germany). Partial fragments (< 1000 bp) of *COI* and *mtMutS* were amplified following McFadden et al. (2011). PCR products were purified using a polyethylene glycol (PEG)-NaCl precipitation and were sequenced with the BigDye® Terminator v. 3.1 chemistry in an ABI PRISM® 3700 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Partial *mtMutS* and *COI* sequences of *P. macrospina* (SNSB-BSPG 2015 XXXII GW4713) and *P. clavata* (SNSB-BSPG 2015 XXXII GW4701) were extracted from the complete mitochondrial DNA sequences available in the public repositories (LT576167–LT576168). The recently published *mtMutS* sequence (LT576169) of *P. macrospina* (SNSB-BSPG 2015 XXXII GW4778) was also considered in this study. Sequences generated here were deposited at the National Center for Biotechnology Information under accession numbers MH251313–MH251317 (see Table S2). Affinity among the sequences obtained in this study and those deposited in the public repositories

Table I. Studied sites and codification of *Paramuricea* spp. sampled.

Voucher	ID	Site	Locality	Date	Lat N	Long E	Depth (m)
GW 4556	<i>P. macrospina</i>	Sicily	Sciacca Shoal	10 August 2014	37°29'0.10"	13°0'1.03"	33
GW 4778	<i>P. macrospina</i>	Pantelleria I.	Punta Spadillo	5 October 2011	36°49'28"	12°0'52"	70
GW 4781	<i>P. macrospina</i>	Skerki Bank	Sylvia Knoll	3 August 2013	38°2'48.98"	11°11'13.99"	30
GW 4713	<i>P. macrospina</i>	Corsica I.	Scandola	4 June 2014	42°23'24"	8°32'39.12"	60
GW 4702	<i>P. macrospina</i>	Corsica I.	Capo Rosso	3 June 2014	42°14'11.76"	8°31'52.98"	60
GW 4714	<i>P. macrospina</i>	Corsica I.	Scandola	4 June 2014	42°23'24"	8°32'39.12"	60
GW 4704	<i>P. macrospina</i>	Corsica I.	Capo Rosso	3 June 2014	42°14'11.76"	8°31'52.98"	60
GW 4712	<i>P. macrospina</i>	Corsica I.	Revelatta	5 June 2014	42°35'35.76"	8°43'21.3"	57
GW 4701	<i>P. clavata</i>	Ustica I.	Secca della Colombara	24 August 2014	38°43'32.53"	13°11'17.2"	50
GW 1862	<i>P. clavata</i>	Croatia	Molunat	11 September 2015	42°26'44.69"	18°26'11.32"	45
GW 1863	<i>P. clavata</i>	Croatia	Molunat	12 September 2015	42°26'44.69"	18°26'11.32"	46
GW 1864	<i>P. clavata</i>	Croatia	Molunat	13 September 2015	42°26'44.69"	18°26'11.32"	47
GW 1865	<i>P. clavata</i>	Sardinia	Secca del Papa	19 September 2015	40°54'52.98"	9°44'52.08"	40
GW 1867	<i>P. clavata</i>	Sardinia	Boa verde	14 September 2015	41°15'4.10"	9°11'42.25"	28
GW 1869	<i>P. clavata</i>	Sardinia	Corallo nero	15 September 2015	41°15'405"	9°12'227"	36

was assessed through the Basic Local Alignment Search Tool (BLAST) in National Center for Biotechnology Information (NCBI) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). All *P. macrospina* morphotypes considered in this study share identical haplotypes for both *COI* and *mtMutS*. Thus, for simplicity, the phylogenetic tree of *mtMutS* was performed considering a single specimen per morphotype. The *mtMutS* sequences generated for *P. macrospina* morphotypes were added to the dataset generated by Polisenio et al. (2017) (DOI: <http://dx.doi.org/10.5282/ubm/data.89>) and were aligned using MUSCLE (Edgar 2004) in SeaView 4.5.3 (Gouy et al. 2010). For phylogenetic reconstructions, the maximum likelihood (ML) and Bayesian trees were inferred using RAxML 7.2.8 (Stamatakis 2006) and BEAST 2.4.4 (Bouckaert et al. 2014), respectively. The ML analysis was performed with a GTR+ G substitution model, using a rapid bootstrap analysis (Stamatakis et al. 2008) and 1000 pseudo-replicates for branch support. For Bayesian analysis the best-fit substitution model (GTR+ G) was selected using the Akaike information criterion (AIC) in jModeltest 2.1.3 (Darriba et al. 2012). Metropolis-coupled Markov chain Monte Carlo (MCMCMC) ran for 10 million generations, sampling every 1000, and a quarter of the sampled trees was discarded as burn-in. MCMCMC convergence was assessed with Tracer 1.6 (Rambaut et al. 2014).

#### Statistical analysis

Among the analysed specimens, we selected four different specimens of *P. macrospina* and one specimen of *P. clavata* in order to explore whether sclerite length could be considered a valid diagnostic feature. For each type of sclerite (thornscale of the anthostele, spindle of the coenenchyme, hockey-stick spindle of the point, spindle of the collaret and rod of the tentacle) 30 sclerites were measured using a Nikon Eclipse Ni-U microscope. Sclerite lengths are reported as minimum–average  $\pm$  standard deviation–maximum size. Because the assumptions for parametric analyses were not met, the equality of the medians of the length of sclerites was tested using Kruskal–Wallis analyses (statistic: *H*). Each type of sclerite was considered. Post hoc comparison was made using Bonferroni-corrected Mann–Whitney pairwise tests.

#### Geographic and bathymetric distributions

In order to assess the bathymetric and geographic distribution of *Paramuricea macrospina*, we reviewed the information published so far in 30 papers, including the present one. Records of occurrence,

together with the location and bathymetric range, were compiled (Table S1). Information about the substrate was also included in the analysis when available. The coordinates of the records were plotted using the open-source software QGIS 2.4.0 (<http://www.qgis.org/it/site/>).

## Results

### Morphological characterisation

The specimens of *Paramuricea clavata* collected from different geographic areas (Croatia, Sardinia and Ustica Island) did not show significant variation in the morphology of sclerites. Thus, a single sample (i.e. GW4701) is here considered for morphological descriptions. Regarding *P. macrospina*, the phylogenetic tree of the partial mitochondrial *MutS* gene indicates that the studied morphotypes fall into a single group (Table I; Figure S1), despite the fact that our morphological analysis showed wide variation. Colonies of *P. macrospina* from Skerki Bank, Sciacca Shoal and Pantelleria Island are characterised by different, distinctive and peculiar shapes and morphological characters, while *P. clavata* showed a more constant morphology among the studied specimens. Below are reported the descriptions of *P. macrospina* morphotypes. However, colonies from Corsica Island (Figure 1a), identified as *P. macrospina*, encompass a heterogeneous set of morphological characters shared with other morphotypes (Pantelleria Island and Sciacca Shoal) and therefore are not described. In order to highlight the morphological divergence(s) among different *P. macrospina* morphotypes we compared them against a typical colony of *P. clavata* colonies collected from Ustica Island.

#### *Paramuricea macrospina* morphotype GW4781, Skerki Bank

The colony is dark yellow to orange in colour, up to 33 cm high (Figure 1b). The arborescent, irregularly branched colony has a more or less planar shape with polyps densely packed along the branches (Figure 2a). The anthostele is not spiny and is formed by thornscales arranged “en chevron” (Figures 2a and 3a). Thornscales of the anthostele are small with a short point and a well-developed base (Figure 4a). The coenenchyme is composed by spiny spindles, sometimes bent (Figure 4b). The retractile polyps have sclerites arranged in “collaret and point” (Figure 3a,b). The collaret is formed by 3–4 rows of spindles (Figure 3b). These latter are arched in shape with the tubercles homogeneously

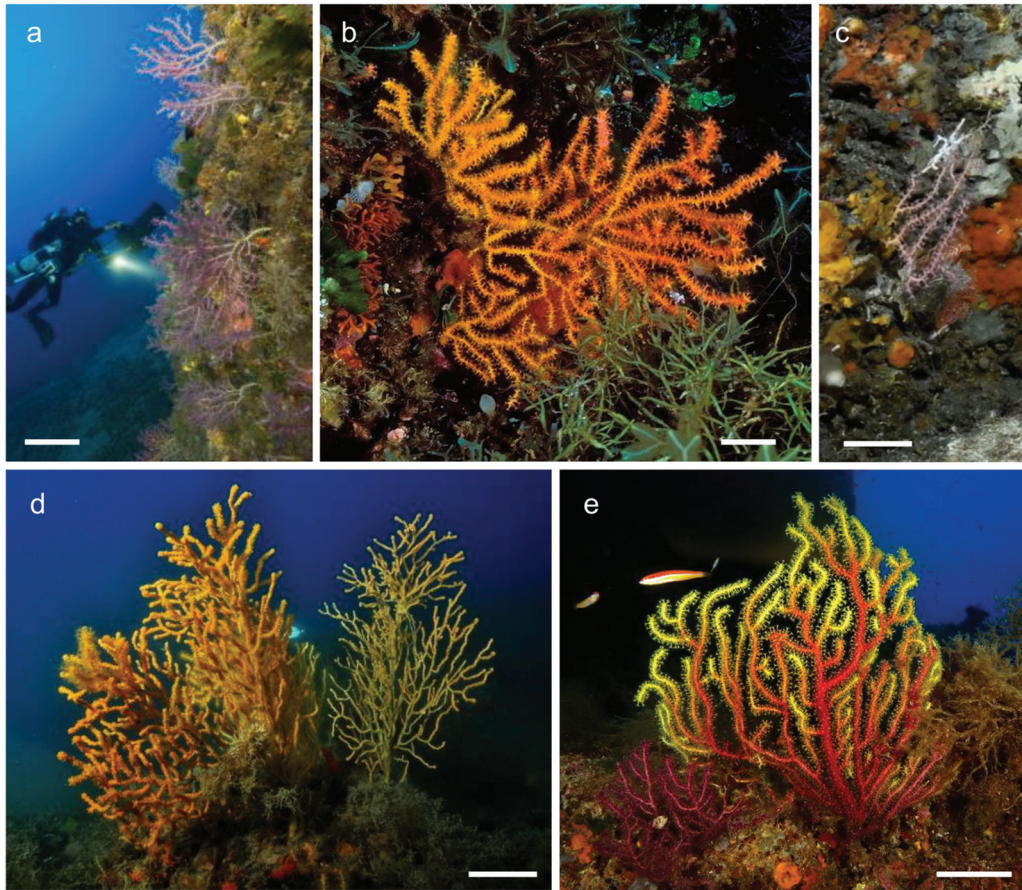


Figure 1. Morphological and chromatic variations of *Paramuricea macrospina* (a–d) and *P. clavata* (e). (a) Corsica Island (credit: Laurent Ballesta, Andromède Océanologie). (b) Skerki Bank. (c) Pantelleria Island. (d) Sciacca Shoal. Scale bars: a–d = 5 cm; e = 10 cm.

scattered along the sclerite, and sometimes are more developed on the convex side (Figure 4c). The points are made by hockey-stick spiny spindles (Figure 4d) arranged at the base of the tentacles. The tentacles contain flattened rods with small tubercles (Figure 4e). The size of sclerites is reported in Table II.

*Paramuricea macrospina morphotype GW4778, Pantelleria Island*

The colony is violet with a pale-coloured base and up to 10 cm high (Figure 1c). The colony is slight with few dichotomously ramifications arranged in one plane. The polyps are unpacked on the branches and the anthostele has very long thornscales, which give a spiny aspect to the colony (Figure 2b,e). The thornscales have long points and their base has very few digitiform processes (Figure 5a). The coenenchyme contains spindles that are often bent and branched, completely covered by tubercles (Figure 5b). The retractile polyps have sclerites

arranged in “collaret and point” (Figure 3c,d). The collarets of the polyps are formed by long bent spindles completely covered by tubercles (Figure 5c), and are organised on the polyps in 2–3 rows (Figure 3c,d). The points are made by hockey-stick spindles with the tubercles mainly in the basal part (Figure 5d). The tentacles have rods with flattened points, with few tubercles (Figure 5e). The size of the sclerites is reported in Table II.

*Paramuricea macrospina morphotype GW4556, Sciacca Shoal*

The colonies are dark yellow to pale orange in colour, up to 30 cm high (Figure 1d). They are robust, with numerous branches, which often give the colonies a bushy aspect (Figure 1d). Polyps are crowded along the branches (Figure 2c). The anthostele is a little spiny with sclerites arranged “en chevron” (Figure 3e,f). The thornscales are more or less fusiform, with short digitiform processes at the base (Figure 6a). Sometimes they

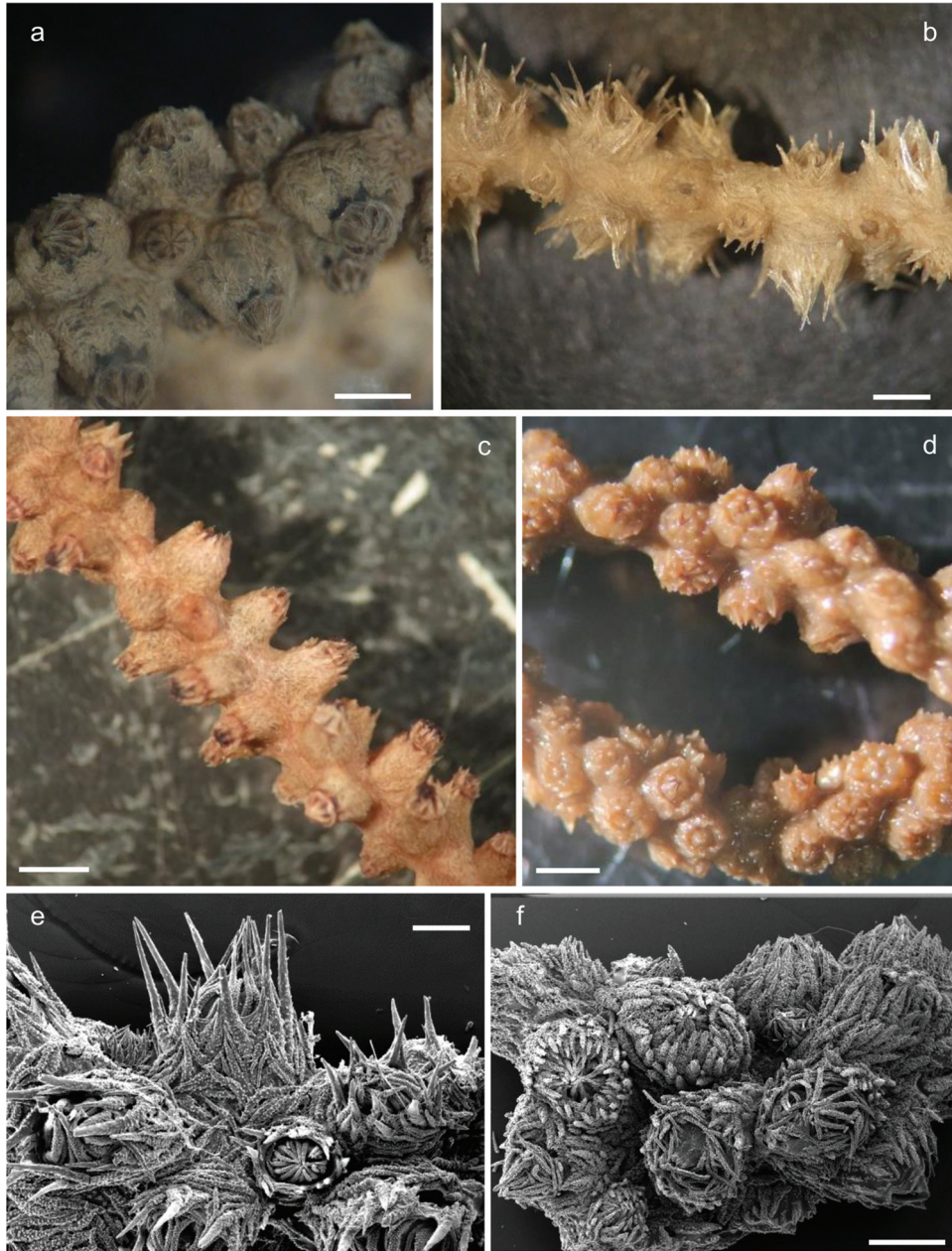


Figure 2. Morphology of the branches and arrangement of the polyps in *Paramuricea macrospina* from: (a) Skerki Bank; (b) Pantelleria Island; (c) Sciacca Shoal. (d) Branches of the colony of *Paramuricea clavata*. Scanning electron microscope pictures of sclerite shape and arrangement in: (e) *P. macrospina* from Pantelleria Island and (f) *P. clavata*. Scale bars: a–d, f = 1 mm; e = 0.5 mm.

show a spindle-like shape with two well-defined points. The coenenchyme is formed by slightly bent or branched spindles (Figure 6b). The retractile polyps have sclerites arranged in “collaret and point” (Figure 3e,f). The spindles of the collaret are curved and tubercled, but sometimes the tubercles are more developed on the convex side of the sclerite (Figure 6c). The collaret is formed by 2–3 rows of sclerites (Figure 3e,f). The hockey-

stick spindles of the point have many tubercles along the entire sclerite (Figure 6d). The tentacle has flattened spiny rods (Figure 6e). The size of the sclerites is reported in Table II.

#### *Paramuricea clavata* GW4701, Ustica Island

The colonies are usually planar in shape, but sometimes can be bushy, and may show different

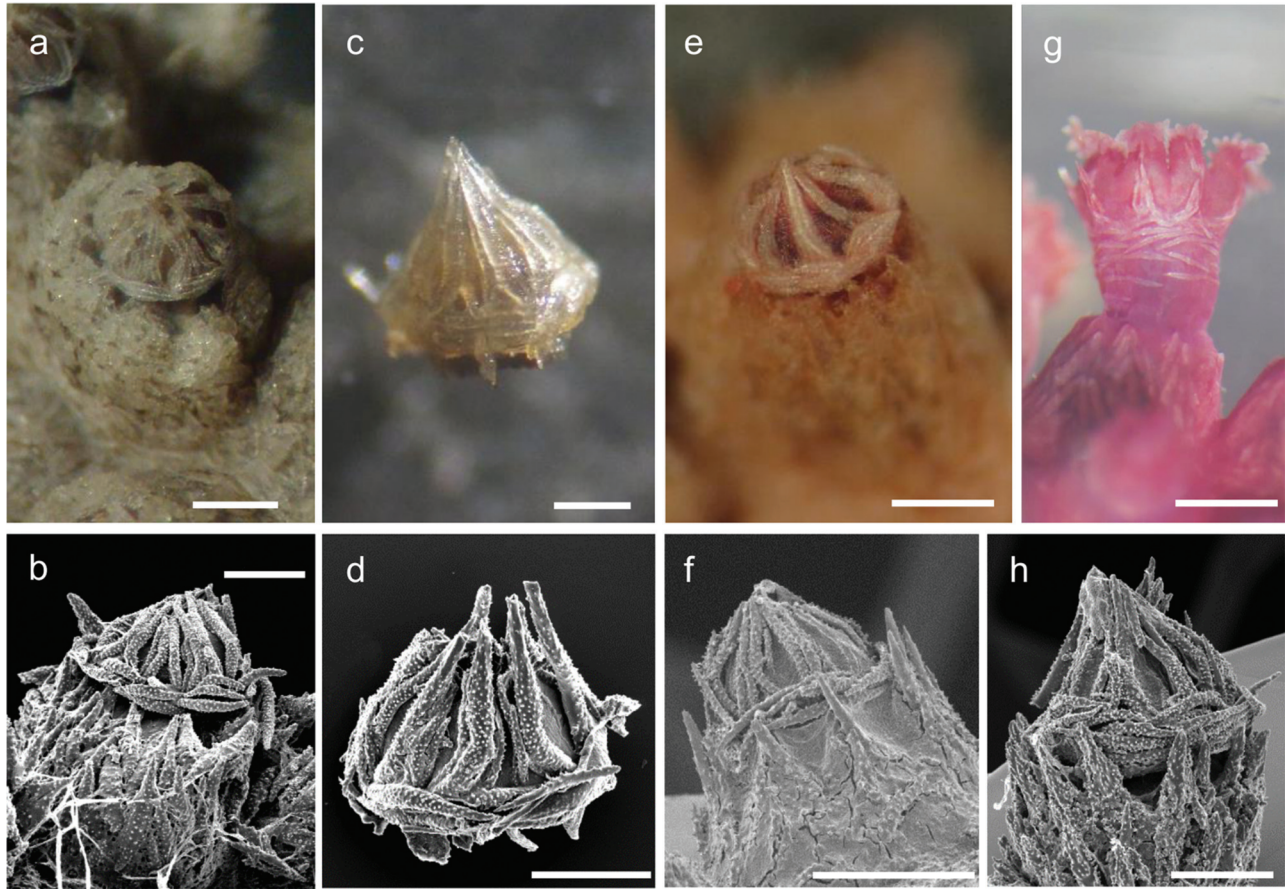


Figure 3. Arrangement of sclerites in the polyps of *Paramuricea macrospina* collected in (a,b) Skerki Bank; (c,d) Pantelleria Island; (e,f) Sciacca Shoal. (g,h) Arrangement of sclerites in the polyps of *P. clavata*. Scale bars: 0.5 mm.

colour patterns, varying from purple to yellow. The colonies are up to 70 cm high (Figure 1e). The branched colonies are usually planar in shape, but sometimes can be bushy. Polyps are closely packed along the branches (Figure 2d,f). The anthostele is not spiny (Figure 2f) and is composed by thornscales with short tubercled points and numerous short digitiform processes at the base (Figure 7a). The spindles of the coenenchyme are usually highly tubercled but also tiny and branched (Figure 7b). The retractile polyps have sclerites arranged in “collaret and point” (Figure 3g,h). The collaret of the polyps is composed by spindles with the tubercles mainly on the convex side (Figure 7c). The collaret is composed by 5–8 rows of sclerites (Figure 3g,h). The points are made by hockey-stick spindles, homogeneously covered with tubercles (Figure 7d). The rods of the tentacles are flattened, with few tubercles (Figure 7e). The size of the sclerites is reported in Table II.

#### Statistical analyses

Kruskal–Wallis analysis evidences significant differences in relation to the sclerite length of the studied colonies, except for the spindle of the collaret. In particular, Bonferroni-corrected pairwise Mann–Whitney shows significant differences according to the type of sclerite investigated. Significant differences are reported between Pantelleria Island and Sciacca Shoal for the spindles of coenenchyme and hockey-stick spindles of the point ( $p < 0.01$  and  $p < 0.05$ , respectively). The sizes of thornscales, hockey-stick spindles and rods were significantly different between Sciacca Shoal and Skerki Bank ( $p < 0.01$ ). The size of spindles of the coenenchyme was significantly different between Pantelleria Island–Skerki Bank ( $p < 0.01$ ) and Ustica Island–Skerki Bank ( $p < 0.05$ ). Rods of the tentacle of Ustica Island were significantly different from those of Sciacca Shoal and Corsica Island ( $p < 0.01$  and  $p < 0.05$ , respectively).



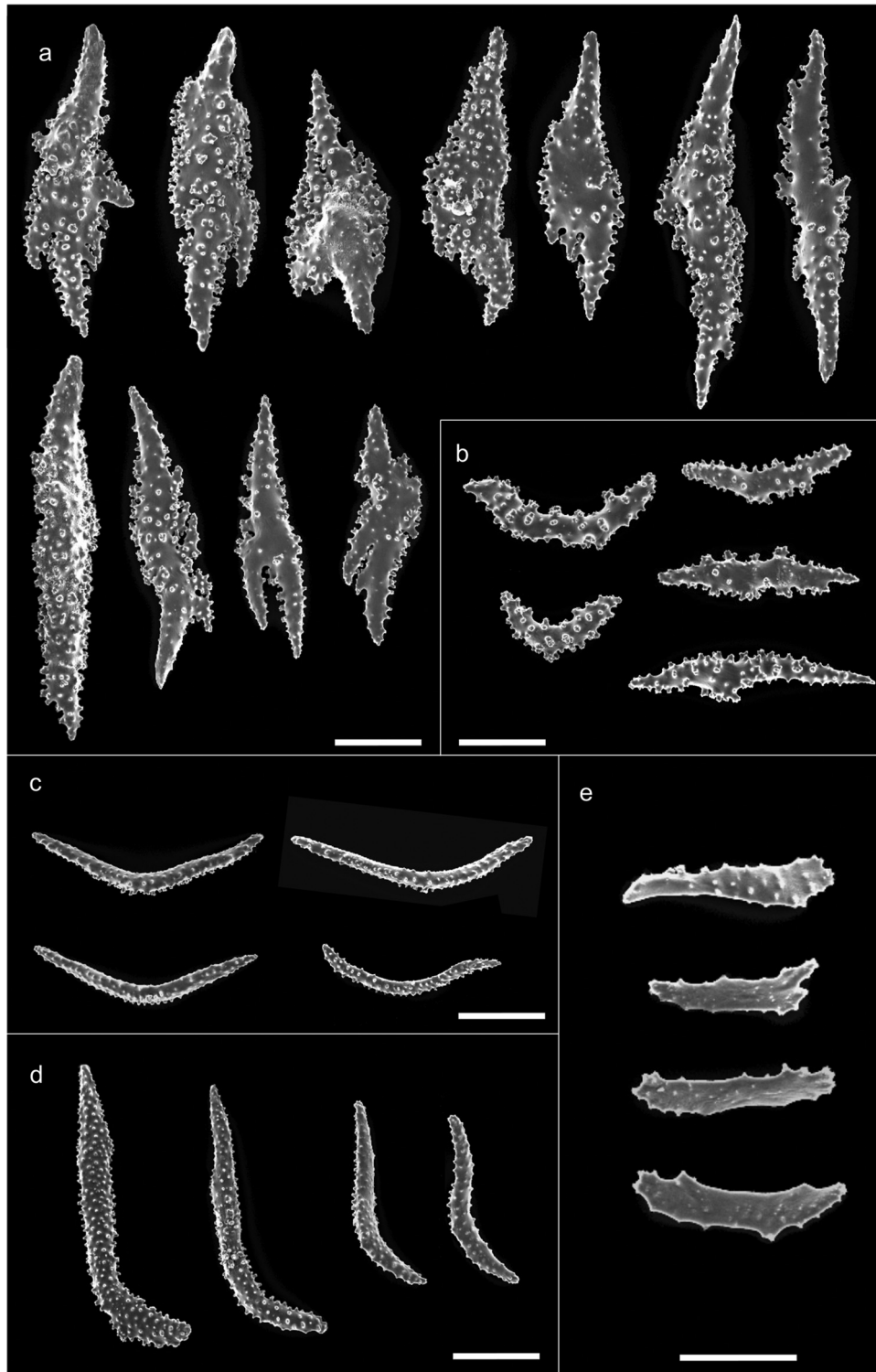


Figure 4. Scanning electron microscope pictures of sclerites of *Paramuricea macrospina* from Skerki Bank. (a) Thorn-scales from anthostele; (b) spindles of the coenenchyme; (c) spindles of the collaret; (d) hockey-stick spindles of the point; (e) rods of the tentacle. Scale bars: a–d = 200  $\mu\text{m}$ ; e = 100  $\mu\text{m}$ .

#### *Geographic and bathymetric distributions*

Among the 30 reviewed papers naming *Paramuricea macrospina*, 78 records were extracted (Table S1).

This species was mainly recorded in the western Mediterranean Sea (Figure 8a). Only a few records from the continental shelves of the eastern Mediterranean Sea Basin are reported (Figure 8a).

Table II. Length of sclerites ( $\mu\text{m}$ ), with mean and standard deviation for each morphotype analysed, of *Paramuricea macrospina* and the specimen of *P. clavata*. The distribution of the sclerites in the colony is reported in parentheses. Length is expressed in  $\mu\text{m}$  as minimum–mean  $\pm$  standard deviation–maximum.

Sclerite type	<i>Paramuricea macrospina</i>				<i>P. clavata</i>
	Skerki Bank, GW4781	Pantelleria I., GW4778	Sciacca Shoal, GW4556	Corsica I., GW4713	Ustica I., GW4701
Thornscale (anthostele)	400–669.1 $\pm$ 116.7–870	570–927.8 $\pm$ 216.3–1200	477.8–610.7 $\pm$ 107.9–870	350–672.5 $\pm$ 152.1–912	490–587.4 $\pm$ 81.0–800
Spindle (coenenchyme)	230–414.4 $\pm$ 89.9–600	220–487.0 $\pm$ 134.3–740	270–486.8 $\pm$ 91.8–660	120–486.8 $\pm$ 203.9–929	334.7–456.4 $\pm$ 64.4–610.4
Hockey-stick spindle (point)	380–550.7 $\pm$ 92.3–725.9	350–543.4 $\pm$ 149.7–1000	344.2–466.4 $\pm$ 57.6–570	430–520.8 $\pm$ 57.9–640	354.5–556.2 $\pm$ 119.4–800
Spindle (collaret)	250–376.3 $\pm$ 94.6–590	266.3–437.6 $\pm$ 105.4–698.7	180–376.7 $\pm$ 93.8–576.6	250–415.9 $\pm$ 87.6–620	210–456.4 $\pm$ 99.4–690
Rod (tentacle)	87.4–159.9 $\pm$ 32.3–232.9	90–156.2 $\pm$ 48.5–246.6	80–129.2 $\pm$ 29.6–187.6	60–129.1 $\pm$ 40.7–199	150–168.5 $\pm$ 14.6–200

These records are mostly from the Adriatic Sea and Aegean Sea. *Paramuricea macrospina* has been recorded between 30–1002 m in depth, although the deepest record is yet to be confirmed, as stated by Cartes et al. (2009) (Table S1). The shallower records ( $\leq 50$  m deep) are rare and are reported in the Marmara Sea, Aegean Sea, Gallinara Island, Corsica, Sardinia and Tunisian coasts (Figure 8b). To date, the majority of the records are distributed between 50 and 200 m in depth (Figure 8c) while few are  $\geq 200$  m depth and are limited to central and western Mediterranean (Figure 8d). In 10.3% of the records reviewed (eight out of 78), the substrate features where *P. macrospina* lived were not specified (Table S1). 56.4% of the records (44 out of 78) were found on hard substrate, in particular 25.4% (20 out of 78) identified as coralligenous habitat. In 30.8% (24 out of 78) the bottom was detritic, for example maerl bed, fragments of corals, mollusc shells, and coarse gravel and sand (Table S1). In only two records, *P. macrospina* was found on a muddy sand bottom.

## Discussion

According to the descriptions reported in the literature (Koch 1882; Stiasny 1942; Pax & Müller 1962), *Paramuricea macrospina* shows a higher morphological plasticity than its Mediterranean congener. This variability often caused misidentification of some specimens that were erroneously identified as *P. clavata* or *P. placomus* (Koch 1887; Kükenthal 1924; Pax & Müller 1962). Although some authors (Stiasny 1942; Carpine & Grasshoff 1975) recognised in the shape and the number of the spindles in the collaret of the polyps, two distinct diagnostic characters useful for species discrimination among Mediterranean *Paramuricea* until now, no one had tested the validity of these morphological characters with the support of genetic analyses.

In the present paper, genetic analyses were performed as an integrative approach with the aim to validate the species identification of the colonies based on morphology. Our morphological analyses revealed high morphological plasticity within *P. macrospina* colonies sampled from different geographic sites. In particular, the colonies of *P. macrospina* collected from Skerki Bank and Sciacca Shoal have a general shape, polyp distribution and sclerite morphology more closely resembling *P. clavata* colonies than the typical *P. macrospina* reported in the literature. However, the genetic analysis confirms the species identification of the colonies as *P. macrospina*.



Figure 5. Scanning electron microscope pictures of sclerites of *Paramuricea macrospina* from Pantelleria Island. (a) Thornscales from anthostele; (b) spindles of the coenenchyme; (c) spindles of the collaret; (d) hockey-stick spindles of the point; (e) rods of the tentacle. Scale bars: a–d = 200 µm; e = 100 µm.

The two morphotypes show large colonies, up to 33 cm, with a bright orange to yellowish colour. The size of these specimens is unusually big and to

date only Grinyó et al. (2016) described colonies of *P. macrospina* up to 55 cm high from Menorca. Regarding the colour, only Pax and Müller (1962)

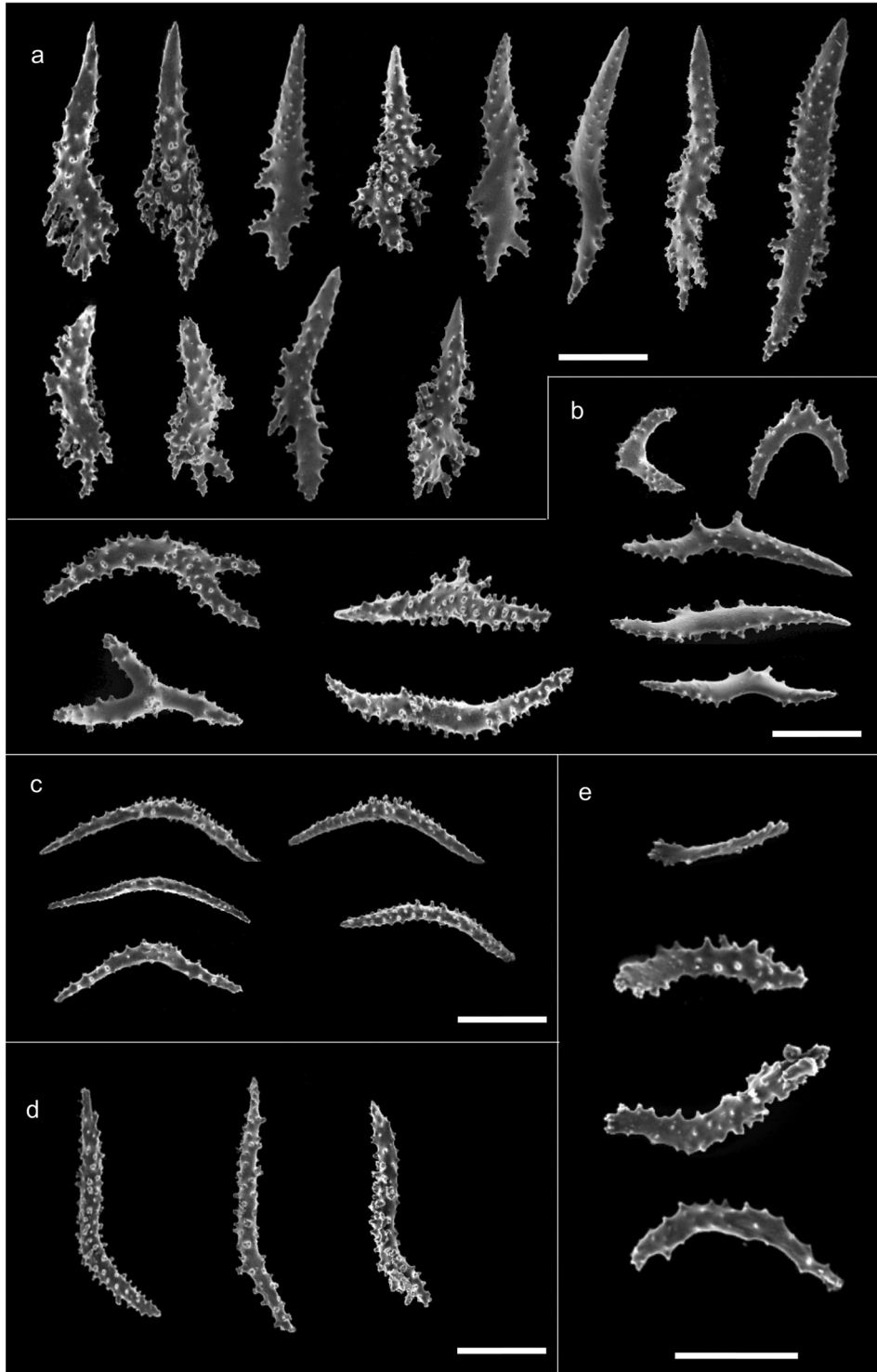


Figure 6. Scanning electron microscope pictures of sclerites of *Paramuricea macrospina* from Sciacca Shoal. (a) Thornscales from anthostele; (b) spindles of the coenenchyme; (c) spindles of the collaret; (d) hockey-stick spindles of the point; (e) rods of the tentacle. Scale bars: a–d = 200  $\mu$ m; e = 100  $\mu$ m.

and Topçu & Öztürk (2013, 2015) reported yellowish-orange colouration of the specimens. In fact, colonies of *P. macrospina* were described in the past

as whitish/yellowish to pinkish-grey, or sometimes translucent white in colour (Stiasny 1942; Carpine 1963; Carpine & Grasshoff 1975). By contrast, *P.*

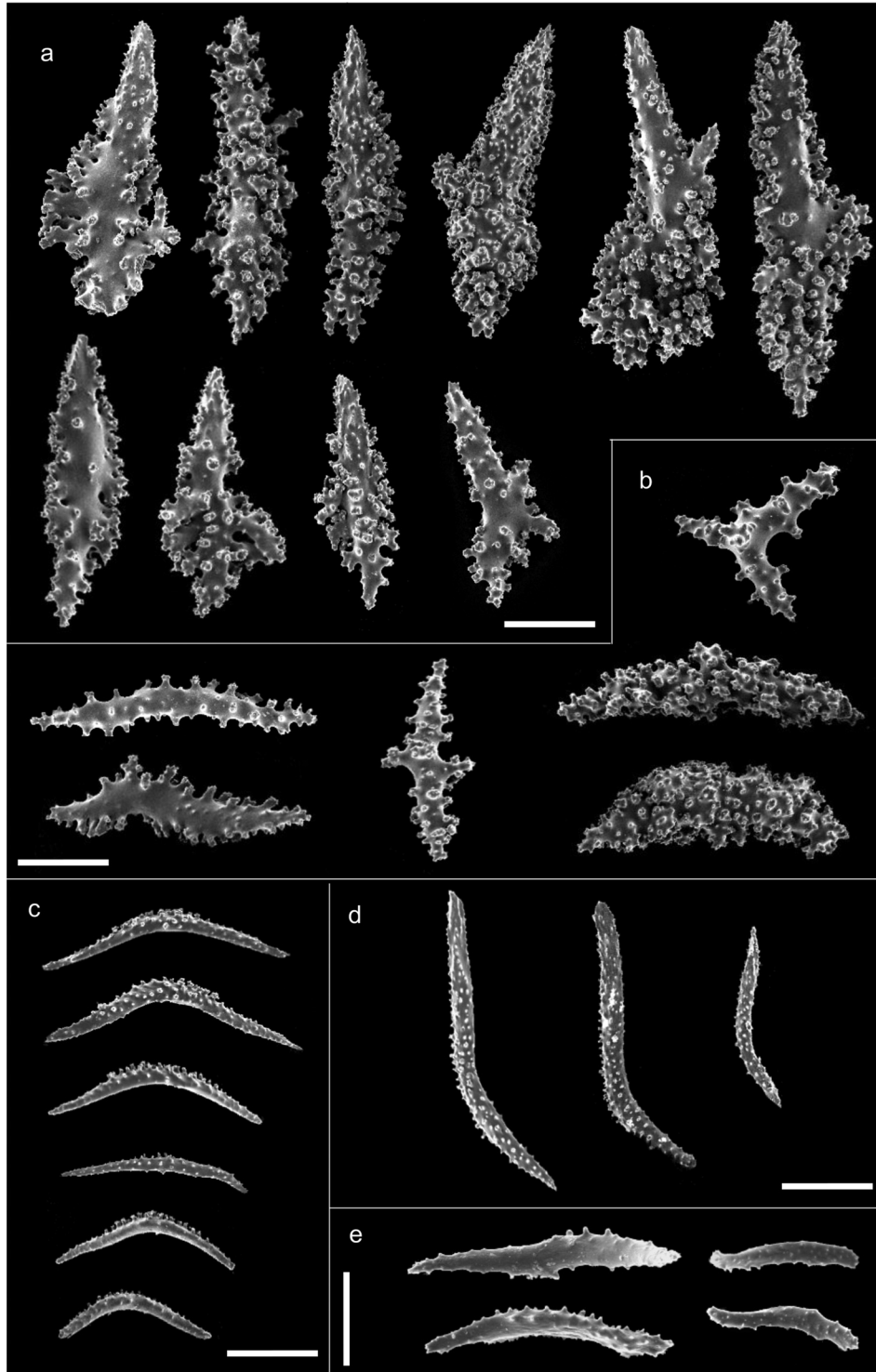


Figure 7. Scanning electron microscope pictures of sclerites of *Paramuricea clavata*. (a) Thornscales from anthostele; (b) spindles of the coenenchyme; (c) spindles of the collaret; (d) hockey-stick spindles of the point; (e) rods of the tentacle. Scale bars: a–d = 200 μm; e = 100 μm.

*clavata* was usually described as purple, purple/yellow or completely dark yellow, similarly to *P. macrospina* from Sciacca Shoal (Carpine & Grasshoff 1975). In agreement with Carpine and

Grasshoff (1975), we showed that the only taxonomic character actually reliable for species discrimination in Mediterranean *Paramuricea* is the number of rows in the collaret (5–8 rows in *P.*

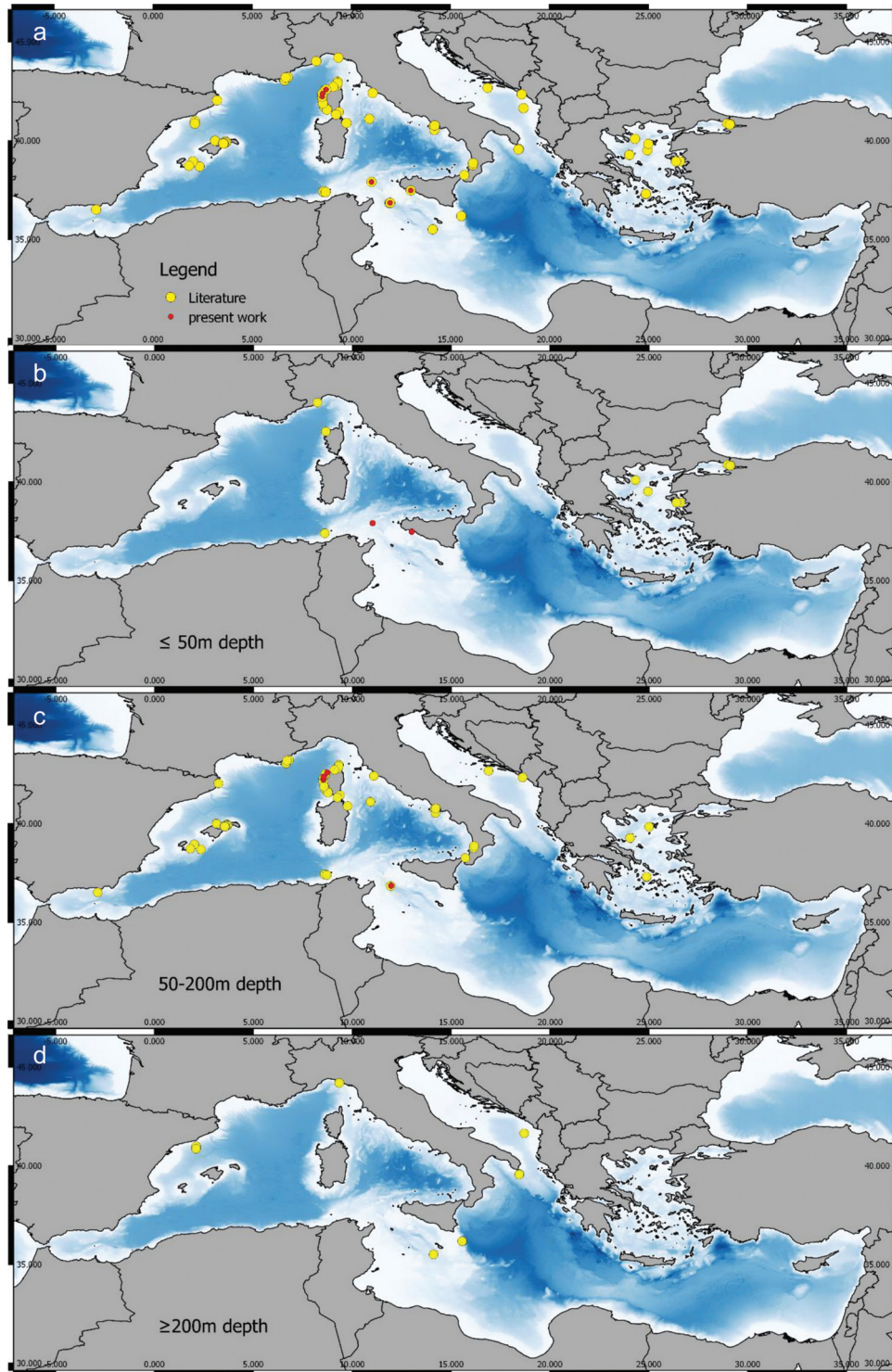


Figure 8. Distribution of *Paramuricea macrospina*. (a) All the records from the literature (yellow dots) and the present work (red dots). (b) Records up to 50 m depth. (c) Records between 50 m and 200 m depth. (d) Records below 200 m depth. (See Table S1.)

*clavata* vs 2–4 rows in *P. macrospina*). Moreover, the spindles of the collarlet in the Mediterranean *Paramuricea* species may be different: in *P. macrospina* the spindles in general have tubercles more or

less dispersed, while in *P. clavata* the tubercles are mainly located on the convex side of the spindles. This character was considered by Carpine and Grasshoff (1975) to be diagnostic in discriminating

*P. clavata* from *P. macrospina*, but we showed that it is too variable and therefore not suitable for species-level identification. In this respect, colonies of *P. macrospina* from Skerki Bank and Sciacca Shoal have many spindles of the collaret with tubercles mainly located on the convex side, as reported in *P. clavata*.

The typical, but not diagnostic, long thornscales of the anthostele were recorded only in the colony from Pantelleria. Thornscales and spindles of the coenenchyme in *P. macrospina* from Skerki Bank and Sciacca Shoal are, in terms of shape, close to those of *P. clavata*. These sclerites, in fact, are described as too variable in shape and length to be considered a valid diagnostic taxonomic character (Carpine & Grasshoff 1975).

Even though more colonies should be studied, our preliminary statistical analyses of the size of sclerites showed that in *P. macrospina* this character is variable enough that it cannot be used to discriminate between the two Mediterranean species. Sclerites show a high variability in shape and size, and constitute an important trait in octocoral taxonomy (e.g. Bayer 1961; Fabricius & Alderslade 2001; Tentori & Ofwegen 2011). Moreover, abiotic and biotic factors such as light intensity, water movement, depth and predation rate can affect their shape (Velimirov 1976; West et al. 1993; West 1998; Kim et al. 2004; Clavico et al. 2007; Prada et al. 2008), but clear links still need to be demonstrated. The sharing of partial morphological features between red gorgonians and some *P. macrospina* morphotypes highlights the role of the environment in species adaptation and plasticity. Environmental conditions may influence the branching pattern of the colonies and the size of sclerites of *Paramuricea macrospina*, as already reported for other gorgonian species (Sánchez et al. 2007; Prada et al. 2008; Gutiérrez-Rodríguez et al. 2009), leading to the formation of these morphotypes in shallow water. The “thin morph” of *P. macrospina* is likely more common where water movement is low, while the “thick morph” has been found in more exposed shallow areas.

The Mediterranean *Paramuricea* species represent two highly divergent lineages resulting from two independent vicariance events related to the Messinian and Gelasian crises, as documented by Polisenio et al. (2017). This diversity has been considered to reflect also the ecology of the endemic *Paramuricea* species, with *P. clavata* occurring at shallower depths and *P. macrospina* being restricted to deeper habitats. The present data suggest a higher morphological and ecological plasticity of *P.*

*macrospina* that, in shallow habitats, has similar characters of *P. clavata*.

This study updates and summarises the distribution of *P. macrospina* from the literature data considering the whole Mediterranean Basin (Table S1). Our results show that this species is widely distributed, with the exception of the eastern side (Marmara Sea). Within the Mediterranean Sea there is an overall lack of knowledge regarding the area between the Northern and Southern sides and the Western and Eastern parts of the basin. In particular, only a few studies concerning the distribution of gorgonians from the eastern Mediterranean are available (Di Camillo et al. 2018), so the absence of *P. macrospina* in the region could mirror this gap. Over the last two decades, new data on the distribution of several Mediterranean benthic species have been provided through the exploration of mesophotic and deep habitats (from 60 to 300 m depth; i.e. Aguilar et al. 2009; Bo et al. 2011, 2012; Giusti et al. 2015; Grinyó et al. 2016). Data from remotely operated vehicle (ROV) studies may explain in part why the majority of the records from the recent literature are reported from 50–200 m depth, confirming the preferential bathymetric distribution of *P. macrospina* (Carpine & Grasshoff 1975). Colonies of *P. macrospina* were also recorded in deeper waters; however, the deepest record, reported from 970–1002 m depth in the Catalan Basin, is considered doubtful due to the uncertainty of the species identification (Cartes et al. 2009). Further deep-sea records were reported also for the Apulian coast at 538–826 m depth (Mastrototaro et al. 2010) and for the Montenegrin slope at 430–490 m depth (Angeletti et al. 2014), confirming the scattered presence of this species in the Mediterranean deep-sea waters.

In the present work, we report two new records of *P. macrospina* from the Skerki Bank and Sciacca Shoal, widening the distribution of its upper limit. Although observations from recreational divers, including technical divers, can contribute to increase our knowledge on the actual distribution of several species in shallow waters (Cerrano et al. 2016), currently few records of *P. macrospina* have been reported (Carpine & Grasshoff 1975; Balduzzi et al. 1994; Vafidis et al. 1994; Topçu & Öztürk 2013, 2015, 2016). Given the difficulties of species discrimination among *P. clavata* and the puzzling shallow-water colonies of *P. macrospina*, we suppose that this species may have a wider bathymetric distribution. Further ecological surveys together with valid specimen identification should be carried out

to confirm the real distribution of *P. macrospina* in the Mediterranean Sea.

*P. macrospina* colonises a wide range of sea bottoms such as rocks, coralligenous habitat, maerl, detritic and sandy-mud bottoms (Carpine & Grasshoff 1975; Bo et al. 2012; Topçu & Öztürk 2015). The literature reviewed in this study shows that this species is usually found in the coralligenous outcrops together with other gorgonians, such as *P. clavata*, and anti-patharians (Gori et al. 2017). Differently from *P. clavata*, *P. macrospina* is able to form dominant assemblages on more unstable bottoms, such as coastal detritic and maerl (Topçu & Öztürk 2015; Grinyó et al. 2016). The ability to colonise this habitat is probably related to its greater adaptability in deep waters and its fast growth rate with respect to *P. clavata* (Bo et al. 2012; Topçu & Öztürk 2015).

More specimens should be collected to investigate in depth the intraspecific variation of *P. macrospina* species in the Mediterranean Basin. Population genetics and connectivity studies could be useful to better understand the gene flow among and within the Mediterranean populations of *P. macrospina*. Furthermore, the use of new molecular technologies such as next-generation sequencing (NGS) could be also exploited to assess whether the morphological plasticity is driven by adaptive genetic processes of populations exposed to different environmental, climatic and bathymetric conditions. We need to increase the number of samples of these peculiar colonies of *P. macrospina*, and a tailored citizen science project for volunteer divers (Cerrano et al. 2016) could be launched in order to increase the chance of finding new colonies.

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### Supplemental data

Supplemental data for this article can be accessed [here](#)

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