

## First record of *Protoptilum carpenteri* (Cnidaria: Octocorallia: Pennatulacea) in the Mediterranean Sea

F. MASTROTOTARO<sup>1\*</sup>, G. CHIMIENTI<sup>1</sup>, F. CAPEZZUTO<sup>1</sup>, R. CARLUCCI<sup>1</sup>, & G. WILLIAMS<sup>2</sup>

<sup>1</sup>Department of Biology, Local Research Unit CoNISMA, University of Bari, Bari, Italy, and <sup>2</sup>Department of Invertebrate Zoology and Geology, California Academy of Sciences, San Francisco, CA, USA

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### Abstract

This paper reports the first finding of the sea pen *Protoptilum carpenteri* Kölliker, 1872 in the Mediterranean Sea. A total of three colonies were collected in 2010 with an epibenthic sledge, and one colony in 2013 with a bottom-trawl net, in the Santa Maria di Leuca (SML) coral province in the Ionian Sea. The main anatomical features and taxonomic characteristics are reported and discussed in order to update the knowledge of this species. A description of morphologies and dimensions of the sclerites, taken from different parts of the colony, is reported. A comparison with Atlantic records is given and discussed.

**Keywords:** *Protoptilum carpenteri*, *Pennatulacea*, *Mediterranean Sea*, *biometry*, *cold-water coral community*

### Introduction

*Protoptilum carpenteri* Kölliker, 1872 is a sea pen belonging to the family Protoptilidae, which currently includes six accepted species of the genus *Protoptilum* and one of the genus *Distichoptilum*. This family is characterized by long slender colonies, with bilateral symmetry and with a prominent axis, rounded in cross-section or sometimes slightly flattened and present throughout the length of the colony (Kükenthal 1915; Williams 1990). The rachis presents solitary polyps that arise laterally and sometimes ventrally on the colony. Autozooids are arranged in one to three more or less distinct longitudinal series, which can simultaneously form diagonally ascending rows (Kükenthal 1915). Polyps are retractile into spiculate calyces, which appear asymmetrically developed, and adhere to the rachis along their inner proximal portions and face upward. Between the autozooids there are several to numerous siphonozooids. The sclerites are numerous, three-flanged and mostly spindles (Williams 1995). *Protoptilum carpenteri* is characterized by a narrow, thin rachis, ca. 1–2 mm wide, with a narrow stripe bare of zooids on the dorsal side and throughout the length of the rachis (Jungersen 1904;

Kükenthal 1915). The colony is supported by a calcareous axis, cylindrical in cross section, about 0.4 mm in diameter, and located in the whitish coenenchyme throughout the whole colony. Autozooids of *P. carpenteri* are arranged in two longitudinal rows along opposite sides of the rachis: a ventral and a dorsal row on either side (Jungersen 1904; Kükenthal 1915). Autozooid calyces are formed like a horn or straight cornucopia, without evident teeth, and are 2–3 mm long and 1–1.1 mm wide (Jungersen 1904; Kükenthal 1915; Grasshoff 1981). Polyps are reinforced by sclerites arranged obliquely and only located along the aboral side of the tentacle-stem (Jungersen 1904; Kükenthal 1915). Siphonozooids have a calyx of similar shape and appearance to that of the autozooids, but much smaller. Along the colony of *P. carpenteri*, there are more than 2–3 siphonozooids per autozooid, often numerous and congested around the autozooids. The siphonozooids are arranged in 2–3 rows along each side of the dorsal midline. However, on the ventral side, there are fewer siphonozooids, approximately half the number of the dorsal ones (Jungersen 1904; Kükenthal 1915).

\*Correspondence: F. Mastrototaro, Department of Biology, Local Research Unit CoNISMA, University of Bari, Via E. Orabona 4, 70125 Bari, Italy. Tel: +39 080 5443344. Fax: +39 080 5442871. Email: [francesco.mastrototaro@uniba.it](mailto:francesco.mastrototaro@uniba.it)

The sclerites of the polyps, calyces, rachis and peduncle are three-flanged spindles, needles or rods, completely smooth on their surface (Jungersen 1904; Kükenthal 1915). The sclerites of calyces and rachis are bright red in color and give this color to the rachis too, with the exception of the bare streak on the dorsal side which is yellowish-white. The sclerites of calyces and rachis are about 0.240–0.480 mm long and 0.016–0.032 mm wide (Jungersen 1904; Kükenthal 1915), whereas polyp sclerites are smaller, measuring about 0.064 mm in length and up to 0.0112 mm in breadth (Jungersen 1904). These sclerites form a red streak on the tentacle-stem, which is clearly visible due to the contrast with the whitish polyps.

The sclerites on the peduncle are yellowish to white, as is the whole peduncle. Their size is not reported in the literature.

The presence of a terminal bulb at the base of the colony is not a specific character of the species (Jungersen 1904).

This paper reports the first record of *Protoptilum carpenteri* in the Mediterranean Sea. Furthermore, a detailed description of the collected colonies is provided in order to increase the knowledge of this species.

#### *Distribution of Protoptilum carpenteri*

*Protoptilum carpenteri* shows a North Atlantic distribution and it is considered a deep-sea species (Kükenthal 1915). It has been found in several areas of the North Atlantic Ocean, from Canada and Northern Europe to West Africa. In particular, *P. carpenteri* has been found from Massachusetts to North Carolina, in West Africa and Northern Europe, between 1334 and 2194 m in depth (samples in the US National Museum of Natural History collection, Ocean Biogeographic Information System); south of New York by the Challenger Expedition (indicated as *P. aberrans*), at depths of 2268, 2469 and 3109 m (Kölliker 1880); in the North Atlantic Ocean by the Porcupine Expedition, at 1262 and 1326 m depth (Marshall & Fowler 1888); south of Iceland by the Ingolf Expedition, at 1545 and 2150 m in depth (Jungersen 1904); from the North Atlantic to the Azores, between depths of 650 and 4270 m (Grasshoff 1981); from Baffin Bay to Newfoundland (Canada), between depths of about 600 and 1000 m (Gilkinson & Edinger 2009); in Newfoundland, off the Grand Banks, between about 400 and 2300 m in depth (Baker et al. 2012). Therefore, this species can live in a wide bathymetric range, since it is certainly present between depths of 400 and 4270 m in the Atlantic Ocean.

Although *Protoptilum carpenteri* is considered to be present in the North Atlantic and European waters

(Williams & van der Land 2001), there are no previous documented records for the Mediterranean Sea.

#### Material and methods

Sampling was carried out in April 2010, in the Santa Maria di Leuca (SML) coral province (Ionian Sea; Central Mediterranean) (Figure 1) (Tursi et al. 2004; Taviani et al. 2005; Corselli 2010), during a research cruise on board the R/V (Research Vessel) *Minerva I*, within the EU 7FP CoralFISH project. The first findings (Stations BT47 and BT64; Table I) were carried out by means of an epibenthic sledge with a fixed iron opening of 3.8 m by 0.46 m and a mesh size of 0.5 cm. Sampling was carried out between 412 and 451 m in depth on soft bottom habitats (SB *sensu* Vertino et al. 2010) characterized by a gently sloping sea floor and a smooth surface, without mounds or any other seafloor irregularity (Mastrototaro et al. 2013). Each tow was carried out for about 30 min at a speed of about 3 knots.

A second benthic survey took place in June 2013, in the same area, during an experimental bottom trawl fishing sampling within the MEDITS (International bottom trawl survey in the Mediterranean) project. This second sampling event was carried out using an experimental trawl net on a muddy bottom area (Station ST69; Table I), close to the SML coral province, in a depth range of 240–244 m. The trawl net was equipped with a SCANMAR (sonar system for monitoring fishing gears) net sensor to monitor the net opening and a MINILOG submersible temperature logger.

The sampled colonies of *Protoptilum carpenteri* were anaesthetized with a saturated solution of menthol in seawater for 2–4 hours according to the size of the colonies and the contractions of the polyps. After this procedure, the colonies were preserved in 5% seawater–formalin solution.

All the collected colonies were measured. The total length of colonies and the lengths of rachis and peduncle were measured, as well as the width of the colonies (measured at the middle area of each colony, without including autozoid or siphonozoid calyces). The colonies were identified considering the main taxonomic characters of the species, such as the distribution of polyps on the rachis, the morphology of autozooids and siphonozoids, and the shape and the distribution of carbonatic sclerites in both the rachis and peduncle. The length and width of calyces of autozooids and siphonozoids and the distance between autozoid calyces along the axis for the whole length of rachis were also measured.

Microscope slides of sclerites were prepared by collecting small portions of tissue from the peduncle and rachis. From the rachis in particular, separated

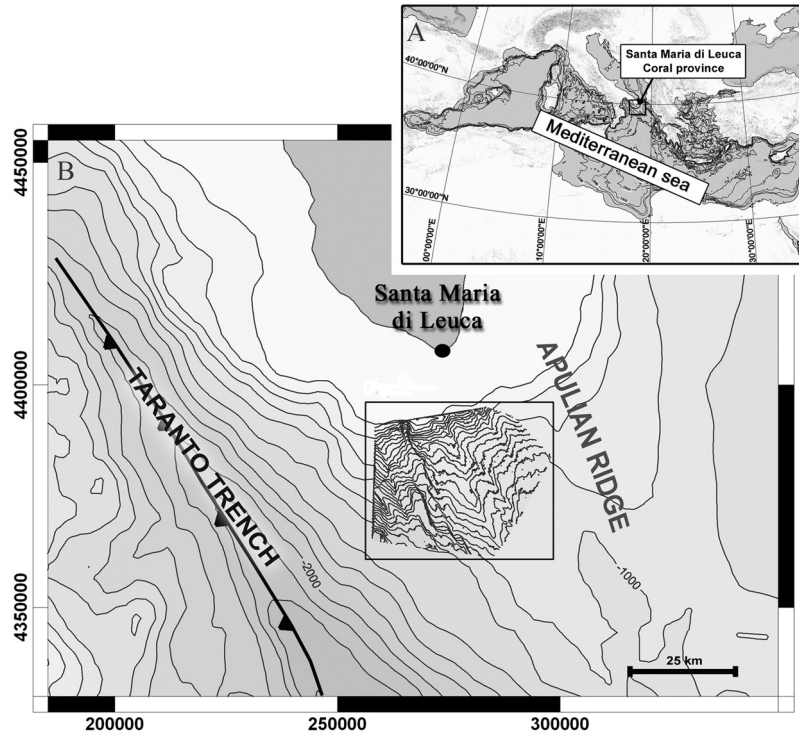


Figure 1. Study area: (a) location within the Mediterranean Sea; (b) bathymetric map of the area with detail of the Santa Maria di Leuca (SML) coral province.

Table I. Number of colonies of *Protoptilum carpenteri* collected at each sampling station, relative period, depth range, geographic coordinates and sampling tool.

Station	N	Period	Depth (m)	Latitude N	Longitude E	Sampling tool
BT47	2	April 2010	429–451	S: 39°36.59' E: 39°36.78'	S: 18°22.42' E: 18°20.40'	Epibenthic sledge
BT64	1	April 2010	412–446	S: 39°36.58' E: 39°36.73'	S: 18°21.86' E: 18°19.89'	Epibenthic sledge
ST69	1	June 2013	240–244	S: 39°39.95' E: 39°38.77'	S: 18°25.20' E: 18°21.80'	Bottom trawl net

S, start sampling; E, end sampling.

samples were taken from the polyps, autozooid calyces, siphonozooid calyces and body portions without zooids, in order to isolate and distinguish sclerites from different parts of the colony. Each portion of tissue was digested in sodium hypochlorite; then the sclerites were washed in distilled water and dehydrated with a sequence of alcohol solutions (70%, 80%, 95% and absolute ethanol). A portion of the sclerites was transferred to slides, observed and measured using an optical microscope. The length and width of a total of 750 sclerites were measured: 150 for each portion of the colony (peduncle, polyps, autozooids calyces, siphonozooid calyces and rachis without zooids). The Student's t-test was also applied for samples with similar variance to compare the size

of sclerites from the autozooid calyces, siphonozooid calyces and body parts without any calyces.

## Results

During the sampling that occurred in April 2010 with the epibenthic sledge, a total of three colonies of *Protoptilum carpenteri* were collected: one colony between 412 and 446 m in depth and two colonies between 429 and 451 m in depth. One of these two colonies was incomplete so it was not possible to take biometric measurements. Another colony of *P. carpenteri* was collected during the bottom trawl fish sampling in June 2013, between 240 and 244 m in depth (Table I). The bottom temperature registered was



14.00°C. Two complete colonies of *P. carpenteri* collected in April 2010 in the SML coral province are now part of the collection of the Zoological Museum of the University of Bari (Code MUZAC-6277 for the BT47 colony and MUZAC-6278 for the BT64 one).

*Morphology and biometry of the colonies*

The colonies are long and thin, with a red–orange rachis and a yellowish-white peduncle. The colonies

are supported by a central calcareous axis, whitish and rounded in cross section, of about 0.5 mm in diameter. The axis extends for the whole length of the colony within the coenenchyme. There is no evident terminal bulb in any of the colonies sampled (Figure 2a).

Along the entire length of the rachis there are solitary autozooids and siphonozooids, with the exception of a thin white dorsal streak devoid of zooids. This stripe becomes wider towards the peduncle.



Figure 2. *Protopitulum carpenteri* K lliker, 1872 collected in the Santa Maria di Leuca (SML) coral province (Mediterranean Sea): A, whole colony 451 mm long; B, rachis with autozooids (Aut) and siphonozooids (Siph); C, detail of the middle zone of rachis with autozooids and siphonozooids; D, polyp of autozooids; E, sclerites of autozooid polyp; F, autozooid calyx with sclerites; G, detail of polyp tentacle. Scale bars: A, 20 mm; B, C, 2 mm; D, E, 1 mm; F, G, 0.40 mm.

On the ventral side of the neck area of the colony (between the rachis and peduncle) where the bare streak widens, there is a bare white area which, going down towards the peduncle, joins with the opposite dorsal bare area. The two bare areas, the dorsal one and the ventral one, widen to meet each other and to open up the peduncle. However, going up towards the rachis, the ventral bare area soon disappears while the polyps on the two lateral sides join, and the only bare area that remains is the dorsal streak.

The solitary autozooids arise laterally and ventrally on the rachis, arranged in one to three more or less distinct longitudinal series along opposite sides (Figures 2b and c). The calyces are spiculated, horn-like in shape, adherent to the rachis along their ventral side and facing upward, without evident teeth (Figure 2f). The polyps are retractile into the calyces and have obliquely located sclerites along the aboral side of the tentacles (Figures 2d, e and g). Several siphonozooids are present along the whole rachis, between and around autozooids, sometimes in groups and sometimes forming longitudinal series.

The three complete colonies collected measure between 350 and 451 mm in length and between 2.0 and 2.5 mm in width, with a rachis between 275 and 331 mm long that represent 73–81% of the total length of the colony (Table II). The peduncles are between 74 and 120 mm long.

The autozooid calyces have a mean length of  $2.44 \pm 0.92$  mm, with a mean width of  $1.05 \pm 0.33$  mm. The mean length of the siphonozooid calyces is  $0.54 \pm 0.12$  mm, and their mean width is  $0.20 \pm 0.07$  mm. Moreover, the mean distance between autozooid calyces along the same line of the colonies is  $4.96 \pm 2.38$  mm (Table II).

#### Description and biometry of sclerites

Sclerites of rachis are fusiform macrosclerites, up to 1.05 mm long and 0.06 mm wide, with a three-

flanged spindle shape and with an evident orange colour that also persists in the preserved colonies (both in 5% seawater–formalin solution and in 99% ethanol solution). The fusiform shape of the macrosclerites can be more or less convex in the central part or, sometimes, in the distal part of the sclerite (Figure 3a). Fusiform macrosclerites are distributed throughout the rachis, covering it completely.

In contrast, the peduncle is covered by elongate round-ended microsclerites, up to 0.17 mm long and 0.02 mm wide, with a three-flanged rod shape and a pale yellowish color. Some of these microsclerite rods can be more or less clavate (Figure 3c).

The sclerites of the polyps are three-flanged rods, like the peduncle sclerites but more robust and bright orange colored (Figure 3b), up to 0.56 mm long and 0.05 mm wide. However, it is also possible to find some clavate rod-like sclerites and some fusiform spindle-like ones. The measurements of sclerites from the different parts of the colony are reported in Table III, expressed as the minimum, maximum and mean with standard deviation.

The difference in length and width between the sclerites of the autozooids and those of the siphonozooids is statistically significant ( $\alpha = 0.01$ ;  $p = 0.00001688$  for length;  $p = 0.00165768$  for width) as is the difference between the sclerites of siphonozooids and the ones taken from the parts of the body without polyps ( $\alpha = 0.01$ ;  $p = 0.000000077$  for length;  $p = 0.00007549$  for width), while the difference in size between the autozooid and body sclerites is not statistically significant ( $\alpha = 0.01$ ;  $p = 0.0633$  for length;  $p = 0.1814$  for width).

#### Discussion

The finding of *Protoptilum carpenteri* in the SML coral province represents the only documented record of the species in the Mediterranean Sea and

Table II. Minimum, maximum, mean and standard deviation of the biometric measurements (mm) and the % of the rachis of *Protoptilum carpenteri* in relation to the total length of the colony in the four colonies collected close to the Santa Maria di Leuca (SML) coral province.

	Min	Max	Mean	Standard deviation
Total length (mm)	350	451	383.7	58.3
Width (mm)	2	2.5	2.2	0.3
Rachis length (mm)	275	331	296.3	30.3
Rachis %	73.4	80.9	77.6	3.8
Peduncle length (mm)	74	120	102.2	19.8
Distance between autozooid calyces (mm)	1.62	8.91	4.96	2.38
Autozooid calyces				
Length (mm)	1.13	3.86	2.44	0.92
Width (mm)	0.48	1.61	1.05	0.33
Siphonozooid calyces				
Length (mm)	0.34	0.47	0.54	0.12
Width (mm)	0.13	0.26	0.20	0.07

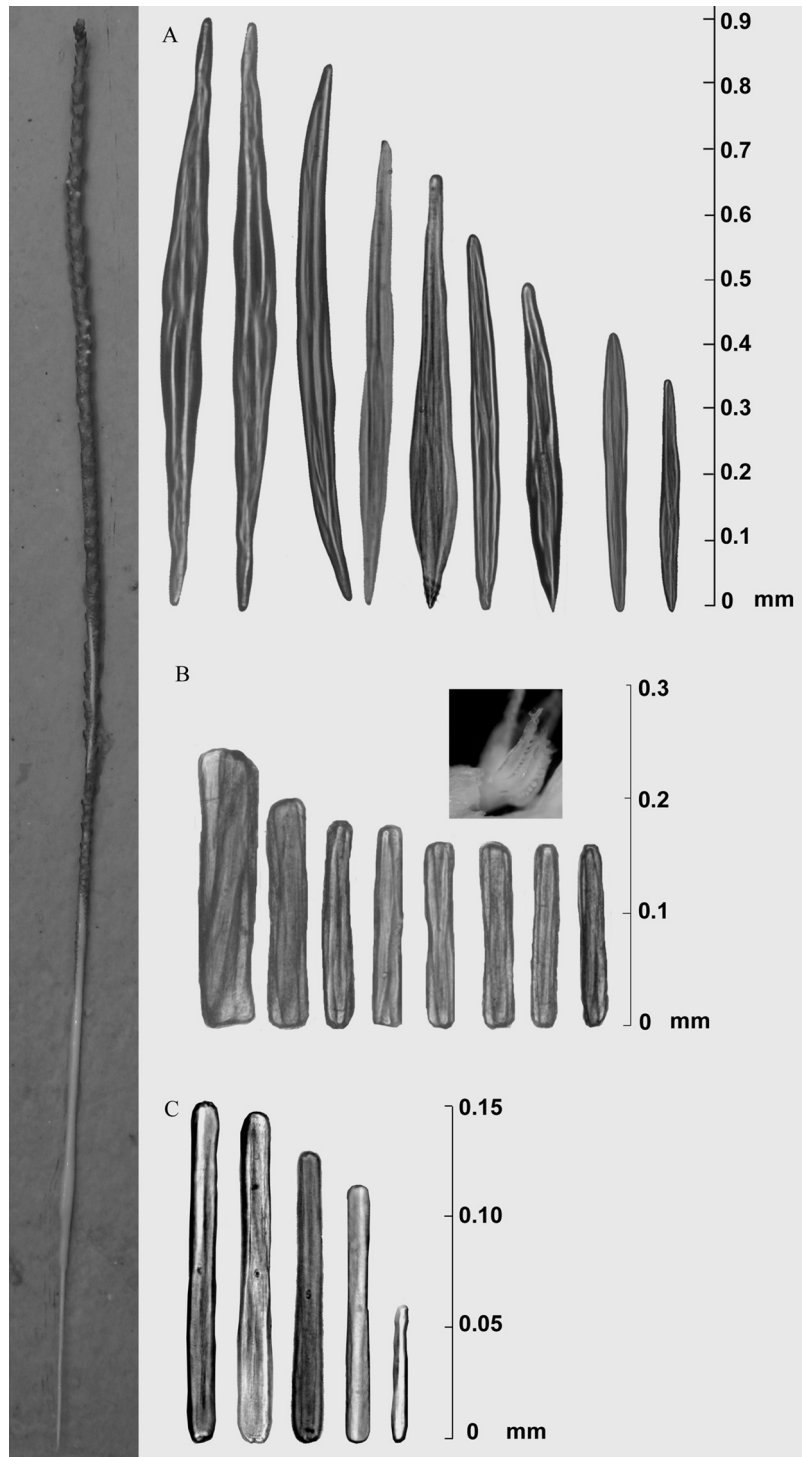


Figure 3. Different types of sclerites of *Protophilum carpenteri*: from A, rachis, B, polyps, C, peduncle.

increases the knowledge of the biodiversity of this area (Carlier et al. 2009; Mastrototaro et al. 2010; Vertino et al. 2010).

The three colonies sampled in April 2010 were found within the facies of *Kophobelemnon stelliferum*

described in the same area by Mastrototaro et al. (2013) and wrongly reported as *Distichoptilum* sp.

The depth of the first finding, 412–451 m, is in agreement with the lower limit of distribution of the species reported in the literature for the Atlantic

Table III. Sclerite size (mm) of one colony of *Protoptilum carpenteri* expressed as minimum, maximum, mean and standard deviation (SD).

	Length (mm)				Width (mm)			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Body sclerites from rachis	0.108	1.047	0.553	0.207	0.0105	0.0595	0.0370	0.0109
Peduncle sclerites	0.059	0.175	0.131	0.026	0.0045	0.0162	0.0110	0.0019
Autozooid sclerites	0.105	0.928	0.519	0.175	0.0070	0.0596	0.0358	0.0108
Siphonozooid sclerites	0.119	0.854	0.433	0.176	0.0070	0.0630	0.0321	0.0111
Polyp sclerites	0.052	0.564	0.168	0.052	0.0035	0.0500	0.0229	0.0077

Ocean (Baker et al. 2012). By contrast, the depth of the second finding, about 242 m, lowers the minimum bathymetrical limit known for this species.

The water temperature of 14°C, recorded during the second finding (June 2013), widens the temperature range of the species previously recorded in Atlantic waters at 3.3°C (2150 m depth in South of Iceland) (Jungersen 1904).

The presence of *P. carpenteri* in the bathyal mud near the SML coral province is most probably due to the protection from the trawl fishing which occurs around this area offered by hard substrates and coral mounds. Indeed, hard substrates interspersed with coral mounds and muddy bottoms are less accessible to trawl fishing activities, and therefore they can provide a natural refuge not only for mobile fauna, as reported by D'Onghia et al. (2011), but also for sessile species such as *P. carpenteri* and other sea pens (Mastrototaro et al. 2013). It is well known that the Mediterranean bathyal mud was covered by octocoral facies, such as the gorgonian *Isidella elongata* on compact mud or the sea pen *Funiculina quadrangularis* on viscous mud (Pérès & Picard 1964). These facies constitute an essential habitat for some commercial crustaceans, such as *Aristeus antennatus* and *Aristeomorpha foliacea* in the first case, and *Parapenaeus longirostris* and *Nephrops norvegicus* in the second one. Both *I. elongata* and *F. quadrangularis* facies have almost completely disappeared due to trawl fishing activity in many Mediterranean areas (D'Onghia et al. 2003; Sardà et al. 2004). This could also be true for sea pens such as *P. carpenteri*, although the actual distribution and abundance of this species in the Mediterranean is not known. Indeed, the low number of colonies found with both the epibenthic sledge and the trawl net could also be related to the highly patchy distribution of the species, which usually characterizes sea pens and other sessile organisms in deep-sea habitats (Carpine & Grasshoff 1975; Marshall 1979; Gage & Tyler 1991; Williams 1992; Morris 2011; Mastrototaro et al. 2013).

Concerning the biometry of *P. carpenteri*, the colonies described here (between 350 and 451 mm in

length) are much longer than the ones found by Jungersen (1904) (from 75 to 190 mm in length). Moreover, in the colonies described by Jungersen (1904), the rachis represents a lower percentage of the colony (about 56%) than the values found in the present study (about 78%). On the contrary, the width of the colonies here measured, from 2 to 2.5 mm, is in accordance to the values reported in the literature (Jungersen 1904; Kükenthal 1915). The recorded autozooid calyx sizes expand the known interval from 2–3 mm long and 1–1.1 mm wide (Jungersen 1904; Kükenthal 1915; Grasshoff 1981) to 1.1–3.9 mm long and 0.5–1.6 mm wide.

The length and width of the siphonozooid calyces, as well as the distance between the autozooid calyces, can be informative of the biometry of the species, even though never previously recorded.

The analysis of sclerite size and morphology also increases knowledge about this species. The range of known rachis sclerite dimensions has been expanded to 0.10–1.05 mm in length and to 0.007–0.063 mm in width, amplifying the previously known range of about 0.240–0.480 mm in length and 0.016–0.032 mm in width (Jungersen 1904; Kükenthal 1915). In addition, it has been possible to distinguish between the dimensions of rachis sclerites from autozooids and those from siphonozooids (Table III). In fact, autozooid calyx sclerites seem to be about 20% longer and 10% wider than those from siphonozooid calyces ( $p \ll 0.01$ ), while the difference between siphonozooid sclerites and those taken from the parts of the body without polyps is not statistically significant ( $p > 0.01$ ).

The size of polyp sclerites has been reconsidered too, varying from 0.05 to 0.56 mm in length and from 0.003 to 0.063 mm in width.

The present work also provides peduncle sclerite sizes that seem to be about 25% shorter and about 30% narrower than those of the rachis.

Considering the small number of findings, it is difficult to hypothesize whether there are true biometric differences between the Atlantic and Mediterranean populations of *Protoptilum carpenteri*



or if these biometric differences are due to the intrinsic variability of the species.

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