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## Research article

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# *Umbellula pomona* sp. nov., a new sea pen from Mar del Plata Submarine Canyon (Cnidaria: Octocorallia: Pennatulacea)

Jessica RISARO<sup>1</sup>, Gary C. WILLIAMS<sup>2</sup>, Daniela PEREYRA<sup>3</sup> & Daniel LAURETTA<sup>4</sup>

<sup>1,3,4</sup>Museo Argentino de Ciencias Naturales “Bernardino Rivadavia” – CONICET,  
Av. Ángel Gallardo 470, Buenos Aires, Argentina.

<sup>2</sup>Department of Invertebrate Zoology and Geology, California Academy of Sciences,  
Golden Gate Park, San Francisco, California 94118, USA.

\* Corresponding author: [jessicarisaro@gmail.com](mailto:jessicarisaro@gmail.com)

<sup>2</sup>Email: [gwilliams@calacademy.org](mailto:gwilliams@calacademy.org)

<sup>3</sup>Email: [mdr.pereyra@gmail.com](mailto:mdr.pereyra@gmail.com)

<sup>4</sup>Email: [dlauretta@gmail.com](mailto:dlauretta@gmail.com)

<sup>1</sup>[urn:lsid:zoobank.org:author:7FC3A3C5-D9D1-443E-AD57-D11493E4B8B7](https://zoobank.org/author:7FC3A3C5-D9D1-443E-AD57-D11493E4B8B7)

<sup>2</sup>[urn:lsid:zoobank.org:author:2C3F7EA8-C963-4514-B299-E2867CA85C98](https://zoobank.org/author:2C3F7EA8-C963-4514-B299-E2867CA85C98)

<sup>3</sup>[urn:lsid:zoobank.org:author:F96E16B1-548B-4430-B94A-1495A7C1749F](https://zoobank.org/author:F96E16B1-548B-4430-B94A-1495A7C1749F)

<sup>4</sup>[urn:lsid:zoobank.org:author:1E61C098-4037-4002-A4DA-C2118339163E](https://zoobank.org/author:1E61C098-4037-4002-A4DA-C2118339163E)

**Abstract.** Sea pens (Cnidaria: Anthozoa: Pennatulacea) constitute a distinctive group of colonial marine invertebrates. They inhabit the world’s oceans, from shallow to deep waters. Studies about this group in Argentina are scarce, and no species have been described in the area in over a decade. Based on samples collected in Mar del Plata Submarine Canyon at about 3000 m deep we describe a new species of sea pen, *Umbellula pomona* Risaro, Williams & Lauretta sp. nov. This is a spiculate *Umbellula* that differs from other species of *Umbellula* with sclerites, by the number, development and distribution of the autozooids in its terminal cluster, as well as the shape of its axis. Molecular data also distinguishes it from other known species. Of the forty-three described species approximately ten are considered valid for the genus *Umbellula*, four of them are registered for the South Atlantic Ocean and only three are described for the Antarctic region. Since sampling efforts in this area have been scarce, the number of species of sea pens from the region is likely to increase substantially in the coming years.

**Keywords.** Argentina, benthos, octocorals, taxonomy, biodiversity.

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## Introduction

Pennatulaceans, known as sea pens, are a distinctive group of octocorals, in the order Pennatulacea Verrill, 1864 (Cnidaria, Anthozoa). They are formed from a single large primary polyp with (usually)

lateral buddings of the body wall that give rise to all secondary zooids that comprise the colony. Sea pens are almost exclusively adapted for soft substrata (Williams 1992) with a few exceptions (Williams & Alderslade 2011). Hitherto, of the approximately 450 described species, at least 200 are estimated to be valid (Williams 1995), grouped in thirty-seven genera and fourteen families of living pennatulaceans (Williams 2011, 2015; García-Cárdenas *et al.* 2019). Despite the fact that only a few works deal with the phylogenetic relationships of the group, recent data using mitochondrial genes (mtMutS and ND2) confirmed the Pennatulacea as a monophyletic group (McFadden *et al.* 2006; Dolan *et al.* 2013; Kushida & Reimer 2019).

Only a few taxonomic works dealing with sea pens from the southwestern Atlantic Ocean (SAO) (from the area of Argentina) have been made so far (Kölliker 1880; Acuña & Zamponi 1992; Zamponi & Pérez 1995; Pérez & Zamponi 2004; Williams 2011). This is the first description of a new species for the area in fifteen years because of the low number of studies made in this region, so we estimate that while more expeditions can be made, the biodiversity of this group will increase considerably and nowadays its biodiversity is likely to be underestimated. *Umbellula* Cuvier, 1798 is the only genus described within the family Umbellulidae Lindahl, 1874. It is characterized by an elongate and slender rachis with autozooids restricted to the distal region of the colony, usually forming a dense umbellate cluster. Siphonozooids are present at the base of the autozooids or on bare parts of the rachis. Most of the species lack sclerites, but when present, they are rods, spindles or needles, three-flanged, and usually round in cross-section (Williams 1990).

The Mar del Plata Submarine Canyon is located at the continental margin of Argentina at about 38° S latitude. The geomorphology of the external shelf and the submarine canyon of the Argentinian continental margin are strongly influenced by the Malvinas Current, a branch of the Antarctic Circumpolar Current that runs towards the northeastern region of the Argentinian continental margin (Piola & Matano 2001). The Malvinas Current transports cold subantarctic water and collides with the Brazil Current that carries warm waters along the continental slope of South America. This collision generates one of the most energetic regions of the world ocean (Piola & Matano 2001).

Although submarine canyons are known to be hotspots of benthic biomass and are globally numerous, these environments are very poorly sampled (Del Río Iglesias *et al.* 2012), therefore it is very important to study their biodiversity. Based on the specimens collected during the expeditions to the canyon (2012 and 2013), several new records and new species of Cnidaria Verrill, 1865, Mollusca Linnaeus, 1758 and Echinodermata Bruguière, 1791 have already been published (Cerino & Lauretta 2013; Martínez *et al.* 2014; Farías *et al.* 2015; Signorelli & Pastorino 2015; Maggioni *et al.* 2016; Pastorino & Sánchez 2016; Pastorino 2016, 2019; Martínez & Penchaszadeh 2017; Pereira & Doti 2017; Bernal *et al.* 2018). Here, we describe a new species of *Umbellula* from Mar del Plata Submarine Canyon based on specimens collected between 2934 m and 3282 m deep, using both morphological and molecular data.

## Material and methods

### Taxonomic data

During the “Talud Continental III” expedition to Mar del Plata Submarine Canyon in September 2013, four specimens of *Umbellula pomona* Risaro, Williams & Lauretta sp. nov. were collected at stations N° 45 (38°1.913' S, 53°39.268' W, 2934 m depth) and N° 46 (38°5.310' S, 53°39.988' W, 3282 m depth) (Fig. 1) using trawls and preserved in ethanol 96%. All the studied specimens were deposited in the Argentinian Museum of Natural Sciences “Bernardino Rivadavia” (Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”- MACN), Buenos Aires, Argentina.

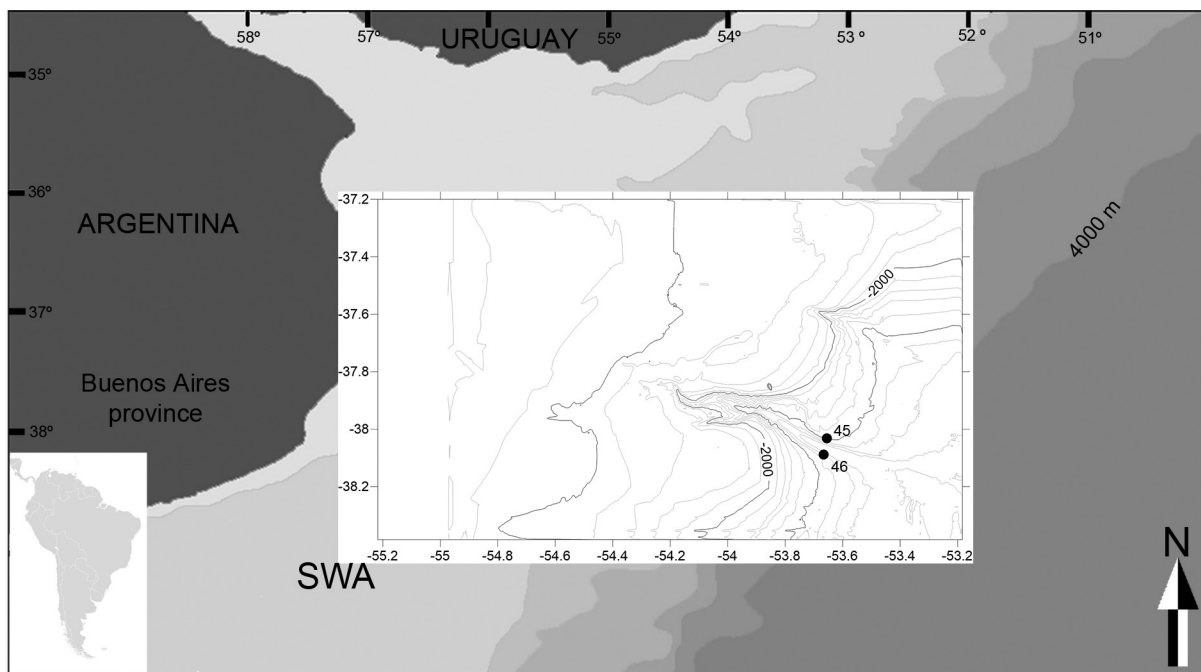
The general morphology of the specimens, the distribution of sclerites and the shape of transverse sections of the axis were studied by the naked eye and using a stereoscopic microscope. For the sclerites' shape

we followed the nomenclature of Bayer *et al.* (1983). Scanning electron microscopy (SEM) was used to examine the shape and length of the sclerites from the tentacles, polyp body and rachis of the colonies and the shape of the axis in transverse section. All measures of the peduncle (when possible), rachis, polyp's body and tentacles were done with a digital caliper and correspond to the holotype. Photographs of the colonies were taken using a digital SLR Nikon D800 camera with a Nikkor 60 mm F2.8 macro lens. To separate and prepare the sclerites of rachis, polyp's body and tentacles to examine them by SEM, a portion of each tissue was cut and treated with diluted sodium hypochlorite (commercial bleach) for fifteen minutes and then washed with distilled water. Finally, the residual of water was evaporated with ethanol 96% and the sclerites were placed on SEM tubs; for the axis sections, a portion of it was cut and all soft tissue was retired, then the same protocol of the sclerites was followed.

### Molecular data

Small tissue fragments were obtained from the holotype of *Umbellula pomona* sp. nov. and one paratype, both preserved in 96% ethanol. The samples were preliminarily washed with deionized water, allowing removal of ethanol. Total genomic DNA was extracted following the salting-out method (Miller *et al.* 1988). Following previous published works (Dolan *et al.* 2013; Kushida & Reimer 2019) we amplified two mitochondrial regions: mtMuts (France & Hoover 2002; Sánchez *et al.* 2003) and ND2 (McFadden *et al.* 2004). The primer's sequences and thermocycling profiles used to amplify each target gene are described in Table S1 (Supplementary File 1).

Amplifications were carried out in 20  $\mu$ l reaction mixtures containing 1  $\times$  PCR buffer mix (the buffer includes dNTPs and  $MgCl_2$ ), 0.2  $\mu$ M of each primer, 1 U of Taq polymerase (MyTaq DNA Polymerase, Bioline), 4  $\mu$ g of Bovine Serum Albumin (BSA), 2  $\mu$ l containing < 10 ng template DNA, and brought to final volume with  $dH_2O$ . The PCR reactions were performed in a T100TM thermocycler (Bio-Rad, USA), with negative controls included to verify the absence of contamination. The PCR products size was



**Fig. 1.** Distribution of *Umbellula pomona* Risaro, Williams & Lauretta sp. nov. in Mar del Plata Submarine Canyon.

confirmed via 1.5% agarose gel electrophoresis. PCR products were purified and sequenced in Macrogen, Korea.

### Molecular analysis

We added our sequences to the data set used in Dolan *et al.* (2013) (Table 1), since we are dealing with a deep-sea species of *Umbellula* (which is the target genus of this work) and no new data on this genus was added in Kushida & Reimer (2019). All the sequences were downloaded from GenBank, although the mtMutS sequence of *Anthoptilum* sp. (KF313832) based on a primnoid (as noted by Kushida & Reimer 2019), so we did not include this sequence in our data matrix. The new sequences were edited using the software Geneious ver. 5.6.7 and checked using BLAST (Altschul *et al.* 1990) to rule out contamination and to confirm gene identity. The concatenated data set consisted of 41 taxa and 1257 bp. Sequence alignment was performed using MAFT (Multiple Alignment using Fast Fourier Transform) ver. 7 (<https://mafft.cbrc.jp/alignment/server/>) using L-INS-i strategy and default parameters. Trees were built using Bayesian analysis (BA) and maximum likelihood (ML). To determine the evolution model (GTR+G and GTR+I+G for mtMuts and ND2, respectively), we implemented the Akaike information criterion (AIC) in PartitionFinder 2 software (Lanfear *et al.* 2016). Ellisellids were chosen as outgroup (Dolan *et al.* 2013; Kushida & Reimer 2019). The Bayesian tree was built using MrBayes (Ronquist *et al.* 2012) on Cipres science gateway (Miller *et al.* 2010): number of runs: 2, number of chains: 4, number of generations: 100 000 000, chain sample frequency: 10 000. The first 25% of each search was discarded (burninfrac = 0.25). To ensure that the Markov chains reached stationarity (effective sample size values over 200) we used the software Tracer ver. 1.7 (Rambaut *et al.* 2018). Maximum likelihood analyses were performed using PhyML ver. 3.1 (Guindon *et al.* 2010) for each individual gene and as a complete set. Bootstrap support was calculated based on 1000 rounds.

## Results

### Systematics

Phylum Cnidaria Verrill, 1865  
Class Anthozoa Ehrenberg, 1834  
Subclass Octocorallia Haeckel, 1866  
Order Pennatulacea Verrill, 1864

Family **Umbellulidae** Lindahl, 1874

### Type genus

*Umbellula* Cuvier, 1798.

**Diagnosis** (adapted from Williams 1990, modifications in bold)

Rachis long and slender. Axis quadrangular to round in cross section. Autozooids restricted to the distal terminus, usually forming an umbellulate cluster. Siphonozooids are present on the rachis at the base of the autozooids or on bare parts of the rachis. Sclerites either present in peduncle, **rachis**, and terminal cluster, or totally absent. When present, sclerites are rods, spindles or needles, three-flanged round in cross-section.

### Included genera

*Umbellula* Cuvier, 1798.

### Distribution

Cosmopolitan, from 250 m to over 6200 m deep (Williams 2011).

**Table 1** (continued on the next page). GenBank accession numbers of the sequences analyzed in this study. New specimens/sequences in bold. – = data not available; \* = data not used (see comment in text). NOCS = National Oceanography Centre, Southampton (UK); a = Natural History Museum (London, UK); b = NIWA (New Zealand); c = MACN (Argentina).

Taxon	Location	Depth	Catalogue#	mtMutS	ND2
Anthoptilidae					
<i>Anthoptilum marrayi</i>	Tasman Sea, AUS	–	–	–	DQ302938
<i>Anthoptilum</i> sp. 1	Subantarctic	–	2010.18a	KF313832*	KF313805
<i>Anthoptilum</i> sp. 2	Sumatra, Indian Ocean	1714	NOCS	–	KF313806
Funiculinidae					
<i>Funiculina armata</i>	NE Atlantic	350	2010.11a	KF313833	KF313807
<i>Funiculina quadrangularis</i>	NE Atlantic	173	NOCS	KF313834	–
Halipteridae					
<i>Halipterus finmarchica</i>	Tasman Sea, AUS	–	–	DQ302868	DQ302941
<i>Halipterus finmarchica</i>	New Zealand, W Pacific	–	28801b	KF313835	KF313808
Kophobelemnidae					
<i>Kophobelemnon macrospinosum</i>	Tasman Sea, AUS	–	–	DQ302865	DQ302937
<i>Kophobelemnon pauciflorum</i>	Crozet, S Atlantic	4189	2010.21a	KF313836	KF313809
<i>Kophobelemnon</i> sp. 1	Koster Channel, NE Atlantic	70	2010.13a	KF313837	KF313810
<i>Kophobelemnon</i> sp. 2	Monterey, E Pacific	2456	2010.10a	KF313838	KF313811
<i>Kophobelemnon</i> sp. 3	New Zealand, W Pacific	1812	28827b	KF313839	KF313812
<i>Sclerobelemnon theseus</i>	Colombia	-	-	DQ311679	DQ311678
Pennatulidae					
<i>Pennatula aculeata</i>	Monterey, E Pacific	2456	2010.15a	KF313840	KF313813
<i>Pennatula phosphorea</i>	Millport, NE Atlantic	55	NOCS	KF313841	KF313814
<i>Pennatula murray</i>	Monterey, E Pacific	3208	2010.9a	KF313842	KF313815
<i>Pennatula</i> sp.	Tasman Sea, AUS	–	–	DQ302870	DQ302943
Protoptilidae					
<i>Distichoptilum gracile</i>	Tasman Sea, AUS	–	–	DQ302866	DQ302939
<i>Distichoptilum gracile</i>	New Zealand, W Pacific	1211	28813b	KF313843	KF313816
<i>Distichoptilum gracile</i>	Monterey, E Pacific	2456	NOCS	–	KF313817
<i>Protoptilum</i> sp.	Monterey, E Pacific	3208	2010.20a	KF313844	KF313818
Pteroeididae					
<i>Gyrophyllum</i> sp.	NE Atlantic	1580	NOCS	KF313845	–
<i>Gyrophyllum</i> sp.	New Zealand, W Pacific	997	28779b	KF313846	KF313819
<i>Pteroeides</i> sp.	Tasman Sea, AUS	–	–	DQ302871	DQ302944
Renillidae					
<i>Renilla muelleri</i>	GOM, Florida, USA	–	–	DQ297432	DQ297451
Scleroptilidae					
<i>Scleroptilum grandiflorum</i>	Mid-Atlantic Ridge	2190	2010.14a	KF313847	KF313820

**Table 1** (continued). GenBank accession numbers of the sequences analyzed in this study. New specimens/sequences in bold. – = data not available; \* = data not used (see comment in text). NOCS = National Oceanography Centre, Southampton (UK); a = Natural History Museum (London, UK); b = NIWA (New Zealand); c = MACN (Argentina).

Taxon	Location	Depth	Catalogue#	mtMutS	ND2
Umbellulidae					
<i>Umbellula carpenteri</i>	Crozet, S Atlantic	4189	NOCS	KF313848	KF313821
<i>Umbellula encrinus</i>	Arctic Ocean	1400	2010.8a	KF313849	KF313822
<i>Umbellula huxleyi</i>	NE Atlantic	1512	2010.17a	KF313850	KF313823
<i>Umbellula magniflora</i>	Marguerite Bay, Antarctica	840	2010.22a	KF313851	KF313824
<i>Umbellula monocephalus</i>	Indian ocean	4229	2010.16a	KF313852	KF313825
<i>Umbellula thomsoni</i> 1	Crozet, S Atlantic	4189	2010.19a	KF313853	KF313826
<i>Umbellula thomsoni</i> 2	Cascais Canyon, NE Atlantic	3476	NOCS	KF313854	KF313827
<i>Umbellula</i> sp. 1	Whittard Canyon, NE Atlantic	4040	2009.8a	KF313855	KF313828
<i>Umbellula</i> sp. 2	Crozet, S Atlantic	4189	2009.6a	KF313856	KF313829
<b><i>Umbellula pomona</i> sp. nov. (holotype)</b>	<b>Mar del Plata Submarine Canyon, SAO</b>	<b>2934</b>	<b>42608 c</b>	<b>MT467665</b>	–
<b><i>Umbellula pomona</i> sp. nov. (paratype)</b>	<b>Mar del Plata Submarine Canyon, SAO</b>	<b>3282</b>	<b>42609 c</b>	–	<b>MT467666</b>
Virgulariidae					
<i>Virgularia mirabilis</i>	Sweden, NE Atlantic	36.5	2010.7a	KF313857	KF313830
<i>Virgularia mirabilis</i>	Portland, UK, NE Atlantic	12	2010.23a	KF313858	KF313831
Outgroup: Ellisellidae					
<i>Ctenocella barbadensis</i>	Unknown	–	–	–	AY534736
<i>Verrucella</i> sp.	Unknown	–	–	–	DQ302936

## Remarks

Since sclerites were found in the rachis of *U. pomona* sp. nov., we added this character to the family diagnosis.

Genus *Umbellula* Cuvier, 1798

## Type species

*Isis encrinus* Linnaeus, 1758.

## Diagnosis

Same characteristics of the family.

**Valid species** (based on Broch 1957; Williams 1995; López-González & Williams 2011)

*Umbellula encrinus* (Linnaeus, 1758), *Umbellula lindahli* Kölliker, 1874, *Umbellula thomsoni* Kölliker, 1874, *Umbellula carpenteri* Kölliker, 1880, *Umbellula durissima* Kölliker, 1880, *Umbellula huxleyi*



Kölliker, 1880, *Umbellula pellucida* Kükenthal, 1902, *Umbellula spicata* Kükenthal, 1902, *Umbellula hemigymna* Pasternak, 1975, *Umbellula monocephalus* Pasternak, 1964, *Umbellula pomona* sp. nov.

### Remarks

Although in his revision of the family, Broch (1958) synonymized *Umbellula dura* Thomson & Henderson, 1906 with *Umbellula durissima* Kölliker, 1880 and *Umbellula rosea* Thomson & Henderson, 1906 with *Umbellula thomsoni* Kölliker, 1880, we consider that, based on the original descriptions and images of *U. dura* and *U. rosea*, those species have important morphological characters to compare with our specimens.

*Umbellula pomona* Risaro, Williams & Laretta sp. nov.

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Figs 2–7

### Differential diagnosis

*Umbellula pomona* sp. nov. is a spiculated *Umbellula* with three autozooids in its terminal cluster, a central well-developed polyp and two lateral, symmetric and smaller ones. It presents large sclerites in all its tissues as well as siphonozooids all along the rachis. Its central axis is circular in cross section all along its extension, and does not vary throughout the colony.

### Etymology

The species is named after the birthplace of the first author (JR), Pomona (Río Negro, Argentina). The word ‘pomona’ is used as a noun in opposition.

### Material examined

#### Holotype

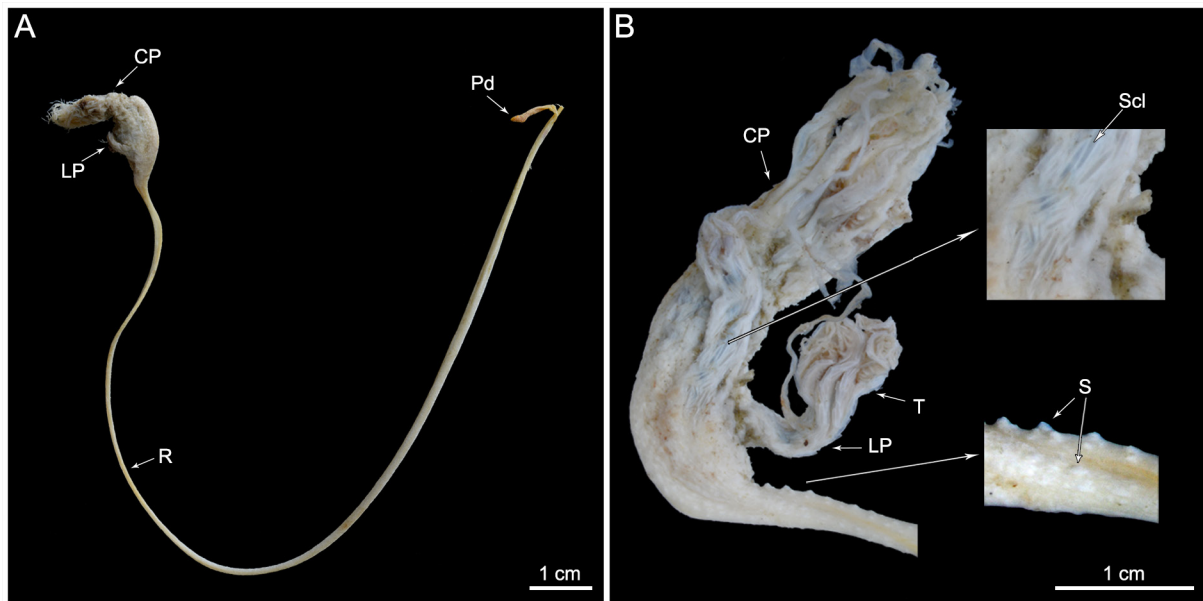
SW ATLANTIC OCEAN • one complete spec. (preserved in 96% ethanol); Mar del Plata Submarine Canyon, “Talud Continental III” exped., stn N° 45; 38°1.913' S, 53°39.268' W; 2934 m deep; Sep. 2013; Daniel Laretta leg.; MACN-IN 42608.

#### Paratypes

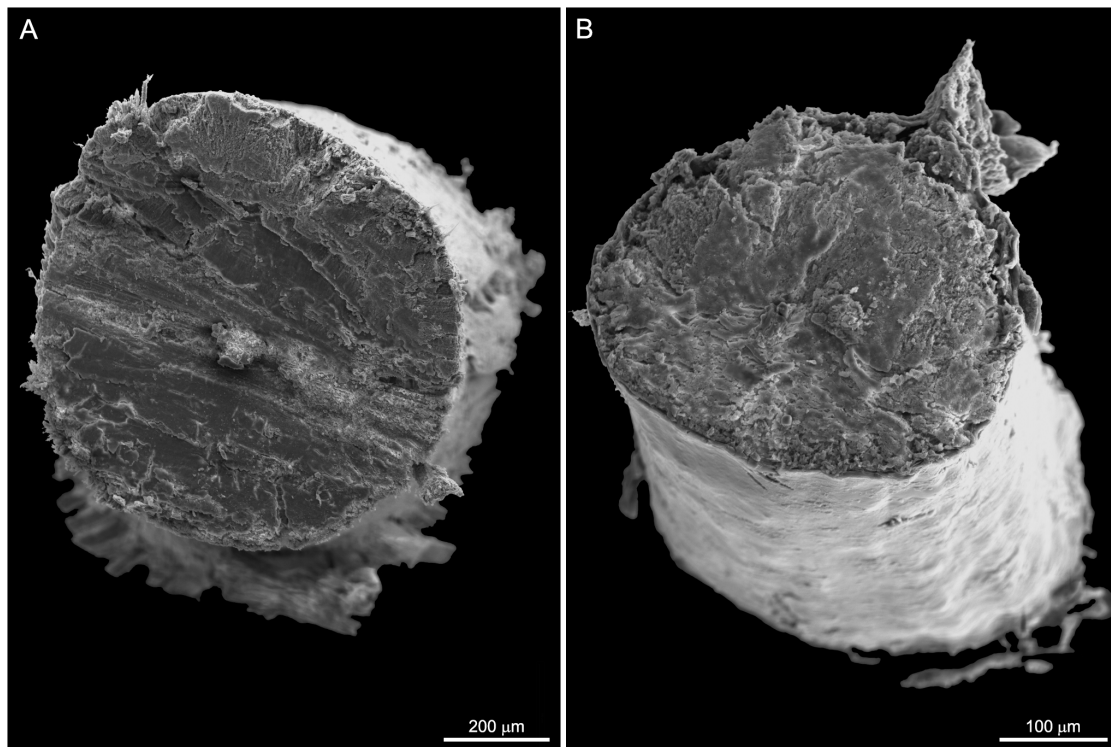
SW ATLANTIC OCEAN • 3 specs (two adult, without the peduncle: paratypes A and B; and one juvenile like, complete: paratype C; preserved in 96% ethanol); Mar del Plata Submarine Canyon, “Talud Continental III” exped., stn N° 46; 38°5.310' S, 53°39.988' W; 3282 m deep; Sep. 2013; Daniel Laretta leg.; MACN-IN 42609.

### Description (holotype MACN-IN 42608)

The colony looks rugous in all its extension, especially the terminal cluster of autozooids. The color of the polyps, rachis and the peduncle is white or light yellow (preserved). The holotype is 214 mm in length. It has a terminal cluster of three autozooids with tentacles, one central and larger polyp growing on the distal-most region of the rachis, and two smaller but well-developed lateral polyps growing at the base of the central one (Fig. 2). The central polyp is 25.3 mm in length and the two lateral polyps' lengths are 3.0 mm and 2.6 mm. The tentacles of the two types of polyps are larger than its body, the measures are 12.3 mm for the central autozooid, and 1.1 mm and 1.7 mm for the lateral ones. The axis is 178 mm in length and it is circular in cross-section, approximately 0.74 mm in diameter. The rachis is approximately 0.8 mm in diameter in the middle zone between the peduncle and the terminal cluster. The peduncle is 8.68 mm in length, and its appearance is soft and smooth. The autozooids of the cluster grow with a kind of orientation determining a ‘dorsal’ region where the axis inserts and a ‘ventral’



**Fig. 2.** A. General aspect of *Umbellula pomona* Risaro, Williams & Lauretta sp. nov. A. Holotype (MACN-IN 42608). B. Detail of the terminal cluster of paratype A (MACN-IN 42609), showing three autozooids that form the terminal cluster and amplifications of sclerites (up) and siphonozooids (down). Abbreviations: CP = central polyp; LP = lateral polyp; Pd = peduncle; R = rachis; T = tentacle; S = siphonozooids; Scl = sclerites.

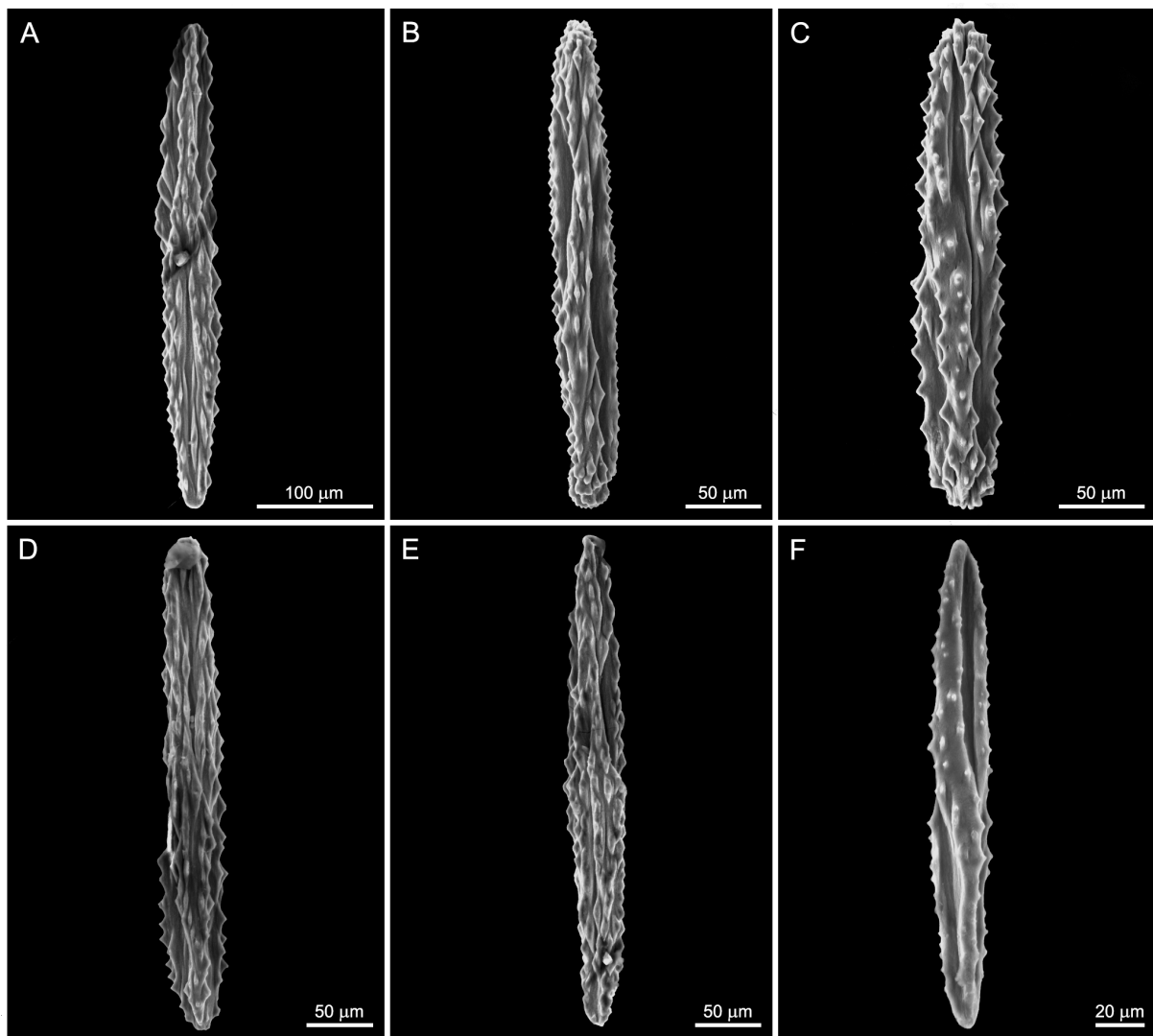


**Fig. 3.** Transversal section of the central axis of paratype B of *Umbellula pomona* Risaro, Williams & Lauretta sp. nov. (MACN-IN 42609). A. Transversal section of the central axis between the peduncle and the rachis. B. Transversal section of the central axis near the terminal cluster.



region towards which the polyps come together (Fig. 2). The siphonozooids are numerous and resemble small white dots, and are distributed all along the rachis from the base of the autozooids to the middle of the rachis. These polyps are circular and inconspicuous; and have a diameter of about  $393 \pm 93 \mu\text{m}$  (299–486  $\mu\text{m}$ , N = 36).

Sclerites are conspicuous, along much of the length of the colony (polyps, rachis, tentacles and pinnules). They are translucent and colorless, rod-shaped and spindle-shaped with spines (Figs 4–7). Those present in the rachis are spindle-shaped, have triangular protuberances all along their length (Fig. 4) and their sizes are  $333.5 \pm 71.1 \mu\text{m}$  (262.4–404.6  $\mu\text{m}$ , N = 10), the body wall of the polyps has different types of sclerites, two of them are rough and rod-shaped, but differ in their measurements, while the others are spindle-shaped with spines along their lengths and also have different sizes (Fig. 5). The largest type is rod-shaped and approximates  $1446 \pm 32.7 \mu\text{m}$  (1413.3–1478.7  $\mu\text{m}$ , N = 5) in length (Fig. 5A); another type, smaller and spine-shaped, approximates  $687 \pm 45.6 \mu\text{m}$  (641.4–732.6  $\mu\text{m}$ , N = 5) (Fig. 5B–C). The other types are much smaller, and one type is rod shaped, like the largest one (Fig. 5D), and the other

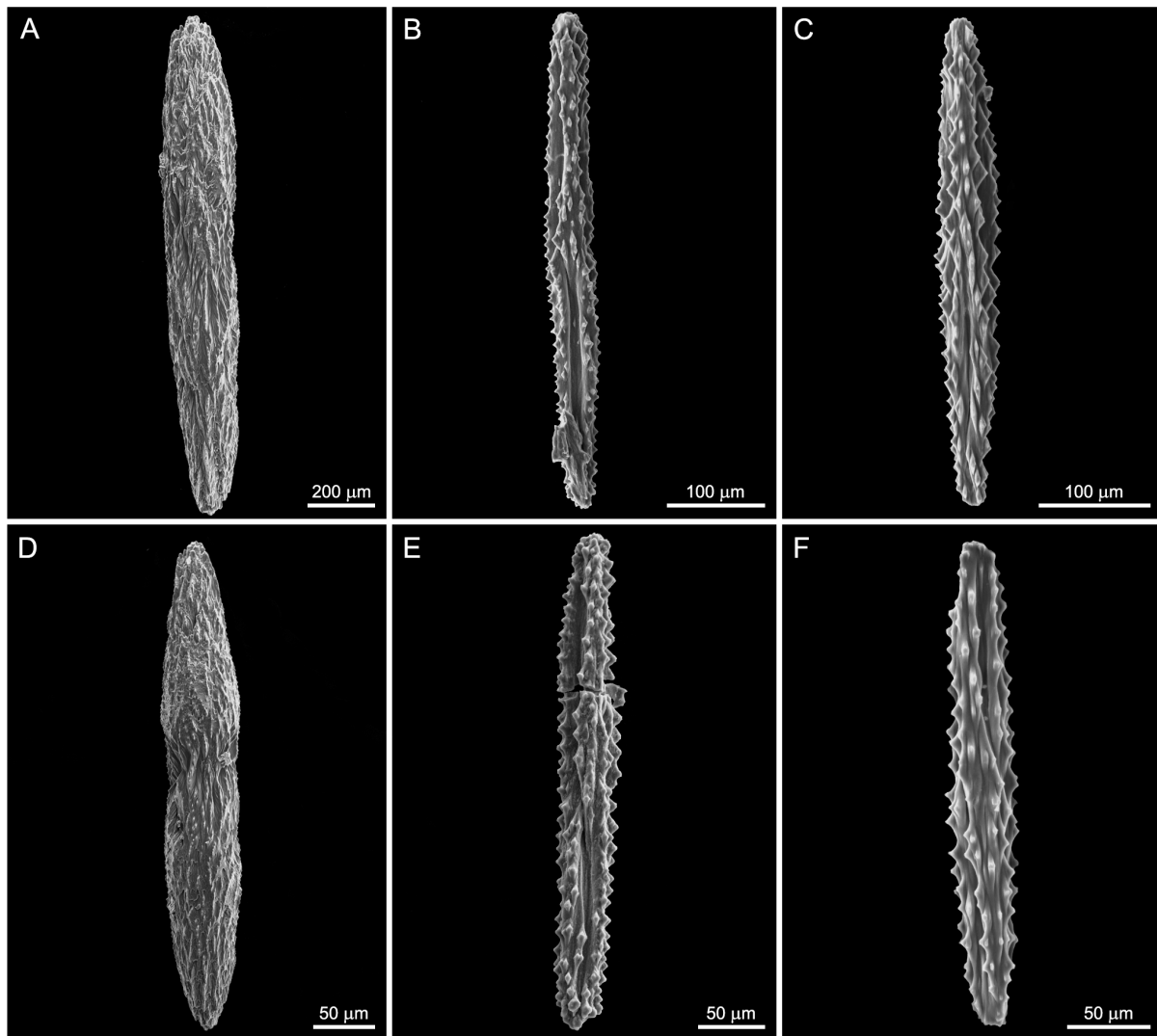


**Fig. 4.** Variability of sizes and ornamentations of the rachis' sclerites of the holotype of *Umbellula pomona* Risaro, Williams & Laurretta sp. nov. (MACN-IN 42608).

type is spine-shaped (Fig. 5E–F); these two types of sclerites have similar sizes, length approximates  $534 \pm 43.4 \mu\text{m}$  ( $490.6\text{--}577.4 \mu\text{m}$ ,  $N = 5$ ). Finally, the tentacles have rod-shaped sclerites (Fig. 6) with two sizes: the larger ones are  $1223.2 \pm 280.1 \mu\text{m}$  ( $943.1\text{--}1503.3 \mu\text{m}$ ,  $N = 5$ ) (Fig. 6A–C) and the smaller ones are  $632.8 \pm 96.2 \mu\text{m}$  ( $536.6\text{--}729 \mu\text{m}$ ,  $N = 5$ ) in length (Fig. 6D–F), and those of the pinnules are rod-shaped with some protuberances along their lengths and their sizes are approximately  $269.1 \pm 50.3 \mu\text{m}$  ( $218.8\text{--}319.4 \mu\text{m}$ ,  $N = 10$ ) (Fig. 7). In both tissues the sclerites are placed along the main axis, not transversally.

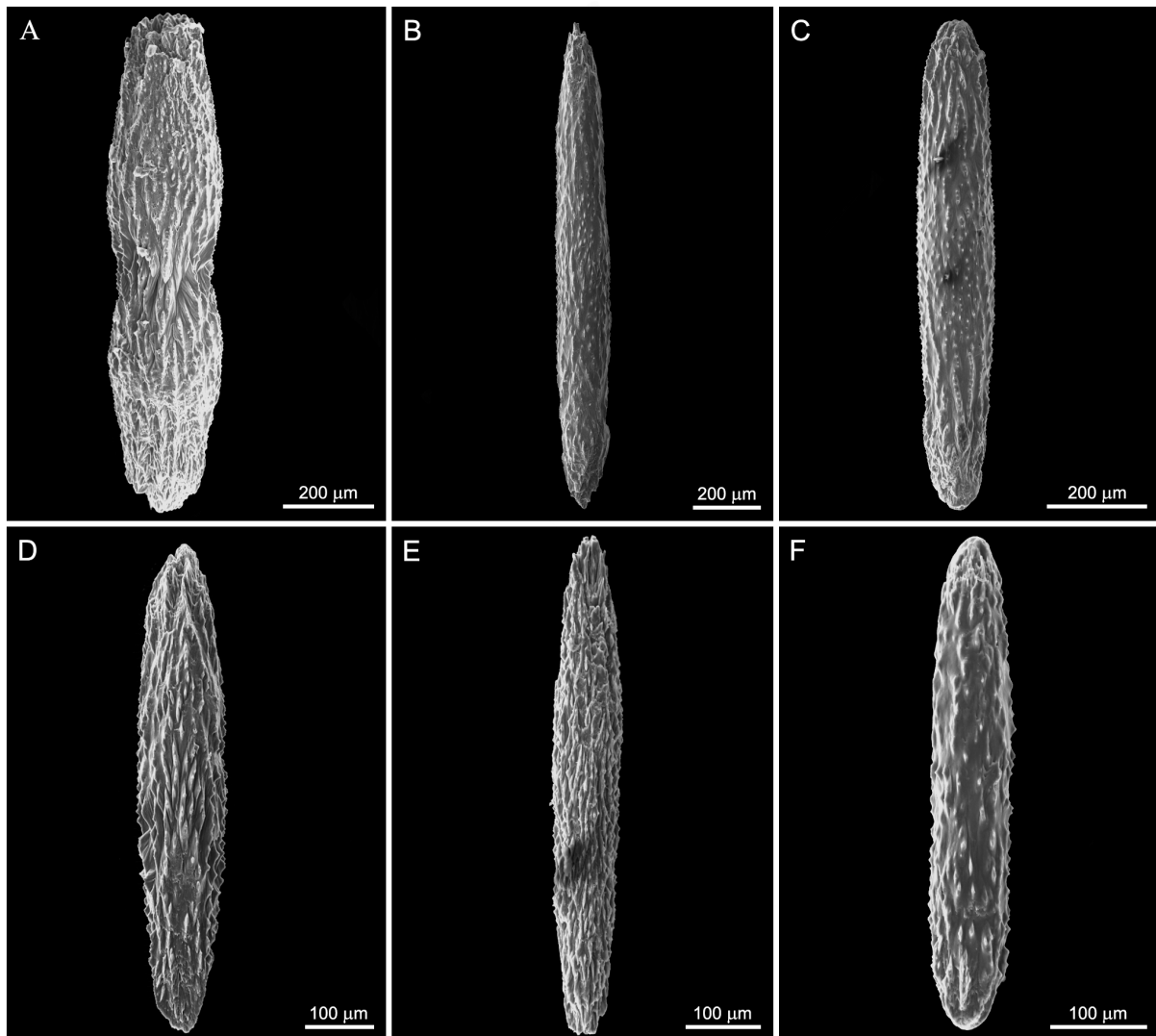
### Variability

Of the three paratypes (A, B and C), one (paratype C) is significantly smaller than the others, so we consider it could be a juvenile (Fig. 8). The color of the three colonies is white or light yellow when preserved, all of them have three autozooids with the same grade of development as the holotype (as it can be seen in Fig. 2B) and its central axis is round in all its extension (Fig. 3). The large paratypes (A and B) lack their peduncle. Their general aspect is just like the holotype, they are rugous in all their



**Fig. 5.** Variability of sizes and ornamentations of the polyps' sclerites of the holotype of *Umbellula pomona* Risaro, Williams & Laurretta sp. nov. (MACN-IN 42608).

length because of the presence of conspicuous sclerites in the autozooids and rachis (Fig. 2B). The total length of the large paratypes is 134 mm and 231 mm, their central autozooids are 36 mm and 34 mm long (length of the tentacles: 20 mm and 12 mm, length of the columns: 16 mm and 22 mm, respectively) while the dimensions of the lateral ones are 4.8 mm and 5.6 mm in one paratype and 6.4 mm and 7 mm in the other. Their rachis are 97 mm and 200 mm in length and their diameters are 0.9 mm and 2.2 mm at their widest sections. Their axis' diameter is 0.83 mm and 1.3 mm. Their siphonozooids are 313  $\mu\text{m}$  and 370  $\mu\text{m}$  (mean) in diameter and look like the siphonozooids of the holotype. Finally, the sclerites of these paratypes look alike and have similar sizes as the sclerites of the holotype. The juvenile paratype's (C) total length is 110 mm, its central autozoooid is 7.9 mm in length (column and tentacles are 4.4 mm and 3.4 mm long, respectively), and the length of the lateral ones is 2.9 mm (column and tentacles are 1.4 and 1.5 mm in length, respectively). The peduncle's length is 4.6 mm and the rachis' 95 mm, while its diameter is 0.41 mm at its widest section. Its central axis is 0.35 mm in diameter. Finally, the siphonozooids of this paratype are tiny spots with the same aspect and distribution as those on the holotype and the largest paratypes.



**Fig. 6.** Variability of sizes and ornamentations of the tentacles' sclerites of the holotype of *Umbellula pomona* Risaro, Williams & Laurretta sp. nov. (MACN-IN 42608).

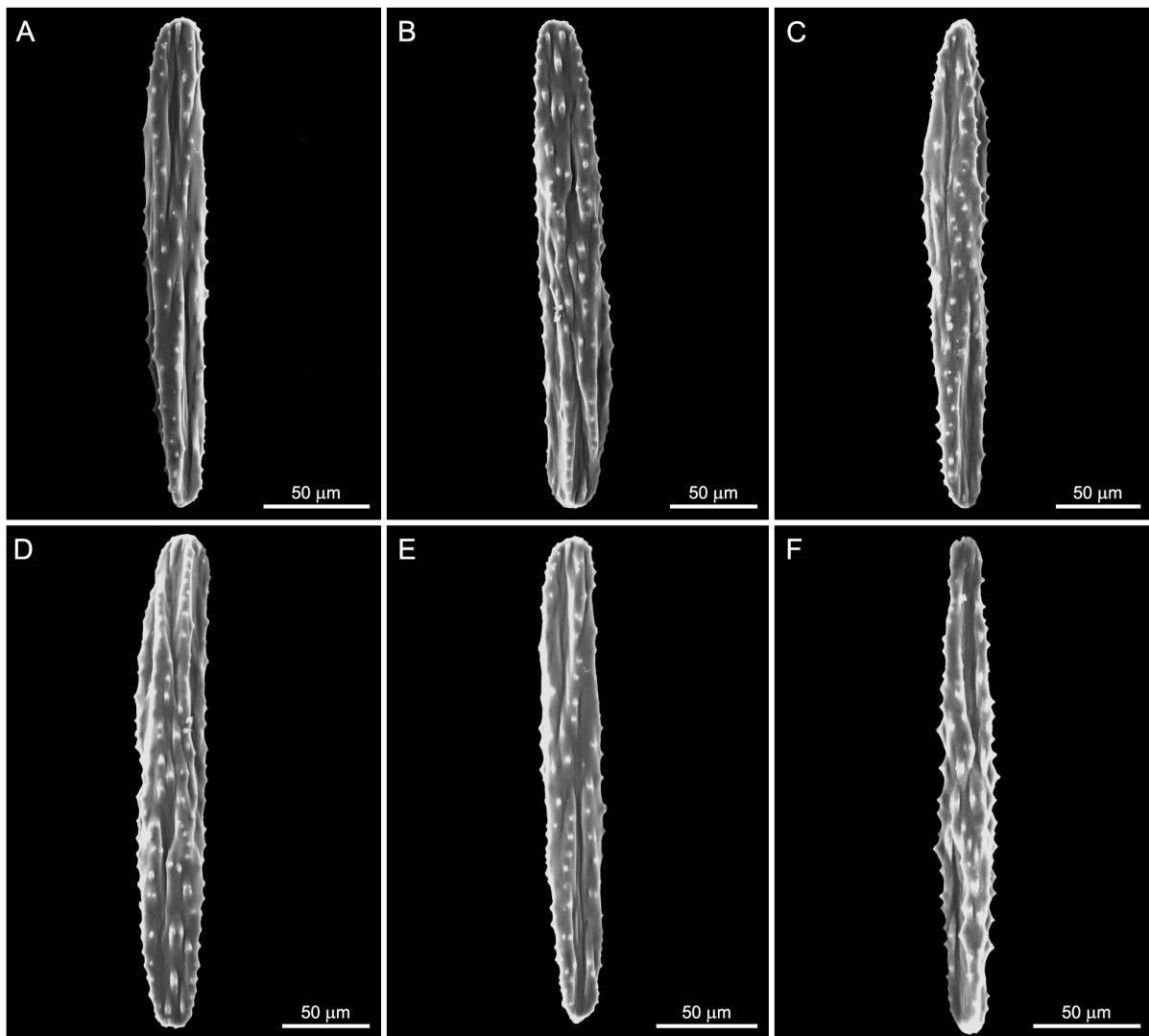
### Phylogenetic analysis

Both phylogenetics reconstructions (i.e., BA and ML) agree in the basic topology of the trees (for simplicity we only show BA). Both type specimens of *U. pomona* sp. nov. were grouped together with low support values within the same group (possibly because we only have one gene sequence for each species). *Umbellula* spp. were recovered in two clusters, *Umbellula* clade I including most of the included *Umbellula* species and a second one (*Umbellula* clade II) including only *U. monocephalus*, *Umbellula pomona* sp. nov. and *Umbellula* sp. 2 from Dolan *et al.* (2013) (Fig. 9).

### Discussion

#### Regarding *Umbellula pomona* sp. nov.

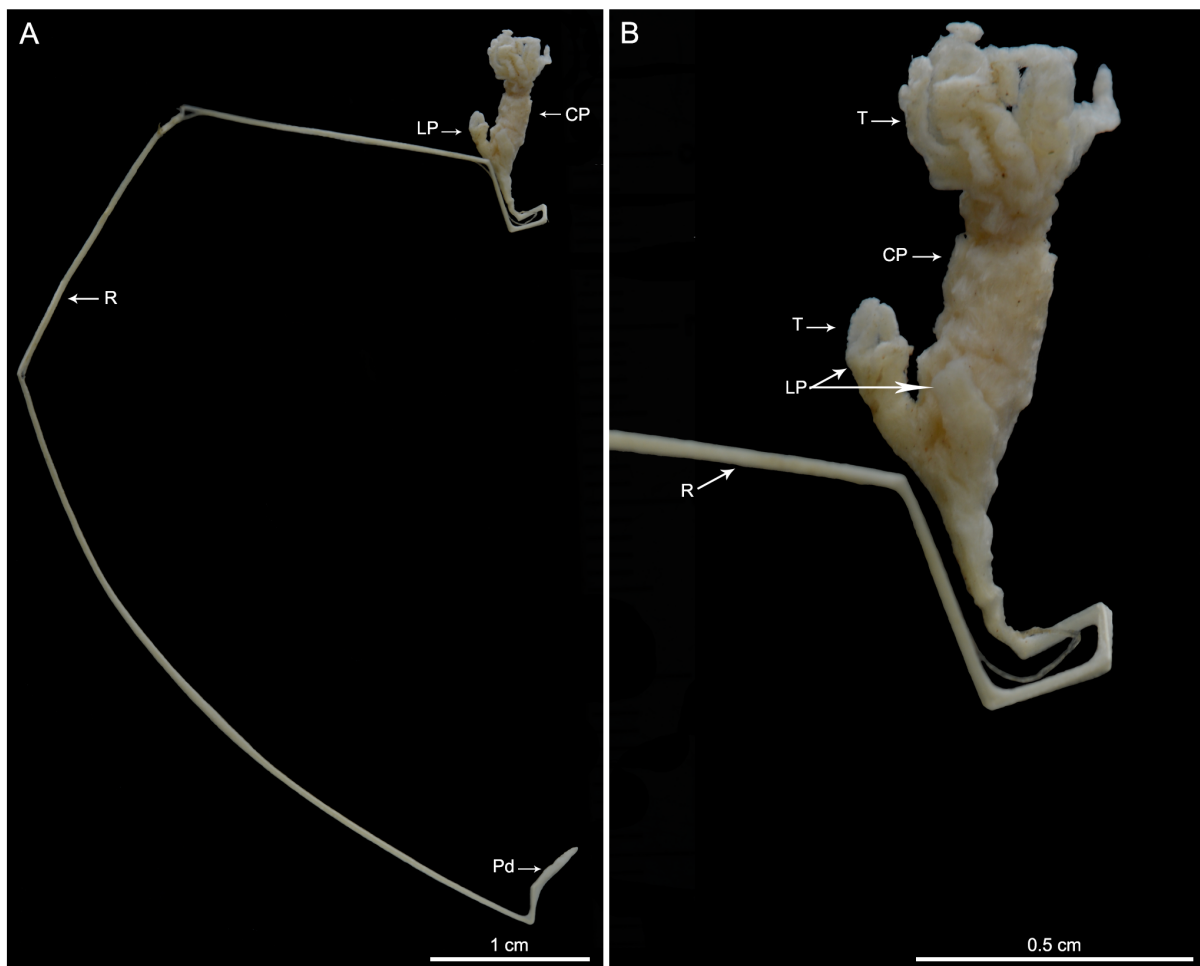
As currently defined, our specimens agree with the current set of characters assigned to *Umbellula*. They have a long, slender rachis, a cluster of autozooids on their distal-most part and numerous siphonozooids all along the rachis, in addition to conspicuous, rod- or needle-like sclerites. *Chunella* Kükenthal, 1902



**Fig. 7.** Variability of sizes and ornamentations of the pinnules' sclerites of the holotype of *Umbellula pomona* Risaro, Williams & Laretta sp. nov. (MACN-IN 42608).

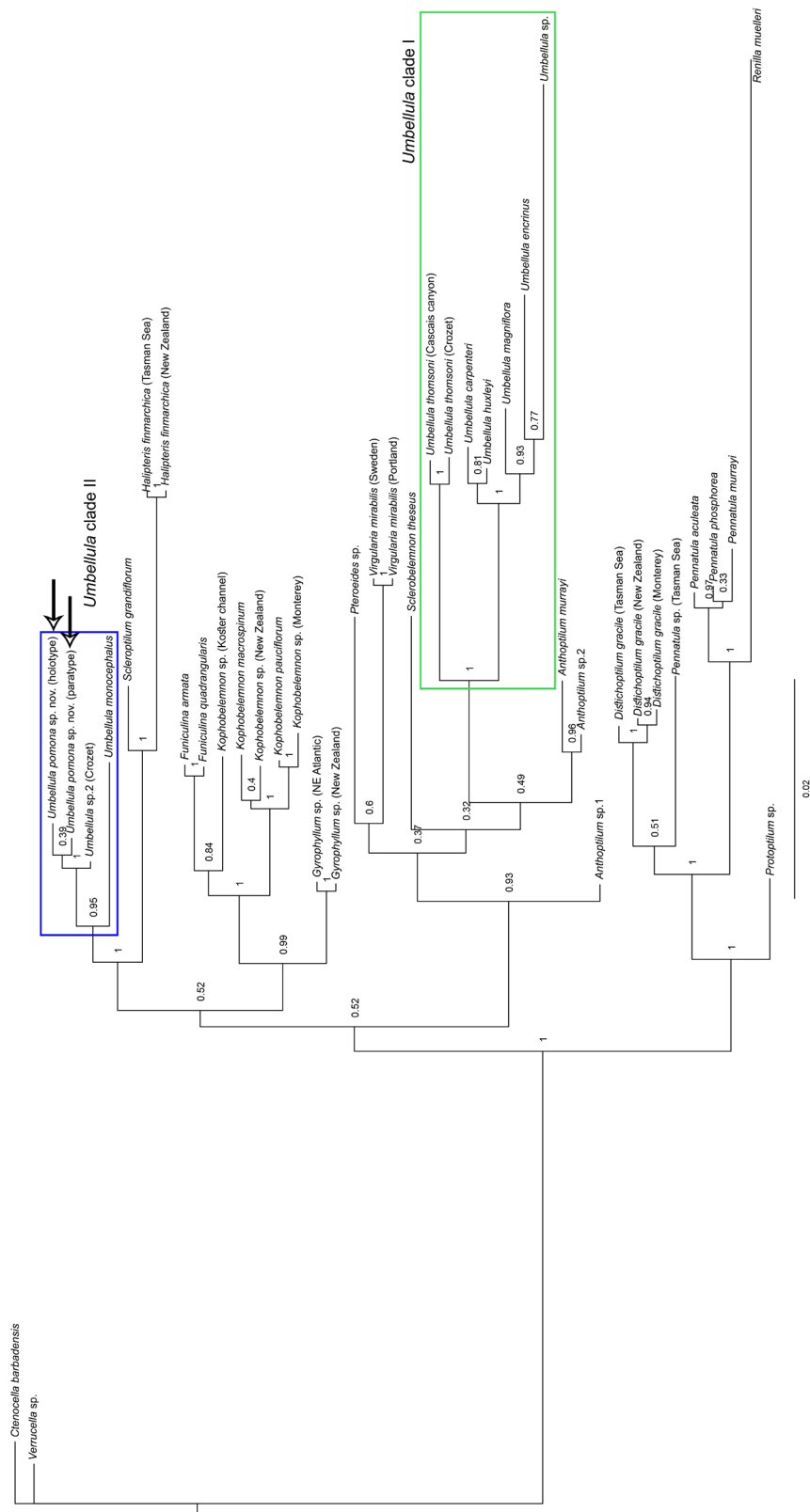
and *Amphiacme* Kükenthal, 1902 are other similar genera, but have several polyp clusters disposed in intervals along the rachis and do not have sclerites (Kükenthal 1915).

From a morphological point of view, there are two major species groups in the genus *Umbellula*, based on the presence/absence of sclerites. Up to date, of all described species, five are spiculate (of which only three are probably valid) whose morphology needs to be compared: *U. thomsoni*, *U. durissima*, *U. dura*, *U. rosea* and *U. monocephalus*. None of them share the diagnostic set of characters (arrangement of the polyp cluster, form of the central axis and sclerite sizes) of our specimens (Table 2). *Umbellula pomona* sp. nov. has a reduced number of polyps, forming a terminal cluster with one central autozoooid and two smaller, lateral ones growing from its base, a cylindrical central axis, and the sizes of their sclerites range from 1446  $\mu\text{m}$  to 269.1  $\mu\text{m}$ , depending on location in the tissues. *Umbellula thomsoni* has been reported with three to ten autozoooids in its terminal cluster growing like a flower or an umbrella, with a similar grade of development among them. The axis of this species differs in its shape in cross section, as near the cluster it is quadrangular, while it becomes cylindrical near the peduncle. Its sclerites vary from approximately 1300  $\mu\text{m}$  (pinnules and tentacles) to less than 300  $\mu\text{m}$  (rachis and body wall of the polyps) (Kükenthal 1915; Williams 1990). *Umbellula monocephalus* has only one polyp in the distal region of the rachis (Pasternak 1964; Grasshoff 1972; Tiefenbacher 2001). *Umbellula durissima* has



**Fig. 8.** General aspect of the unique specimen of a juvenile-like paratype of *Umbellula pomona* Risaro, Williams & Laretta sp. nov. (MACN-IN 42609, paratype C). Abbreviations: CP = central polyp; LP = lateral polyp; R = rachis; PD = peduncle; T = tentacles.





**Fig. 9.** Phylogenetic reconstruction of the concatenated set of the two mitochondrial genes (mtMutS and ND2) based on the Bayesian analysis. Nodes include the Bayesian posterior probabilities. Arrows point to the new specimens of *Umbellula* Cuvier, 1798 included in the analysis.

**Table 2** Comparative descriptions of the spiculated species of *Umbellula* Cuvier, 1798 and known distribution of each species.

Species	Polyp's size (mm)	Maximum number of polyps in the cluster	Cluster shape (data taken from original descriptions)	Axis form in cross section	Measurements of sclerites	Distribution / depth (m)
<i>Umbellula thomsoni</i> * Kölliker, 1874	15–20	10	Polyps distributed in a circle, forming an 'umbrella'.	Quadrangular near the terminal cluster and becoming cylindrical to the peduncle.	Rachis, polyps' column and base of the tentacles: < 300 µm. Terminal section of tentacles and pinnules: 500–1300 µm. Peduncle: 80–110 µm (ovals).	Cosmopolitan / 1300–6200
<i>Umbellula durissima</i> * Kölliker, 1880	21	5	One terminal and large polyp, two undeveloped ones. Two or three small and undeveloped polyps.	Cylindrical in all its extension.	Polyps and tentacles: 2000–2800 µm. Distal zone of rachis: 190–260 µm. Proximal zone of rachis: 76–200 µm.	North Pacific Ocean, south of Tokyo Japan / 1000
<i>Umbellula dura</i> * Thomson & Henderson, 1906	–	3–6	In juvenile, one central and smaller polyp and two lateral ones. In developed states, four large and two small autozooids.	Cylindrical in all its extension.	Polyps: 140–1500 µm. Rachis: 140–110 µm (X-shaped).	Indian Ocean / 1300
<i>Umbellula rosea</i> * Thomson & Henderson, 1906	12	5	Two pairs of lateral polyps (2 and 2) and one terminal.	Quadrangular in all its extension.	Polyps: 220–300 µm. Rachis: 90–160 µm (ellipses).	Indian Ocean / 3200
<i>Umbellula monocephalus</i> Pasternak, 1964	40	1	Formed by a unique terminal polyp.	In the original description of the species and different tabular keys don't specify the form of its internal axis	In the original description of the species and different tabular keys don't specify the dimensions of its sclerites.	Indian Ocean and Atlantic Ocean / 4635–4700
<i>Umbellula pomona</i> sp. nov.	36	3	One central terminal polyp and one pair of lateral, smaller polyps growing from the base of the central one. All of them are completely developed.	Cylindrical in all its extension.	Polyps: 490.6–1413.3 µm. Rachis: 262.4–404.6 µm. Tentacles: 536.6–1503.3 µm. Pinnules: 218.8–319.4 µm.	38°5.310' S, 53°39.988' W— Southwestern Atlantic Ocean / 3300

\*Some authors consider *U. dura* as a synonym of *U. durissima* and *U. rosea* as a synonym of *U. thomsoni* (Broch 1958)

numerous polyps forming an apical cluster of three autozooids, one terminal and well developed, two lateral and two or three less developed ones (without tentacles) below the principal cluster. Its central axis is cylindrical in all its length. The sclerites vary in length from 2800  $\mu\text{m}$  (body wall of the polyps and tentacles) to approximately 200  $\mu\text{m}$  (rachis), additionally, this species does not have siphonozooids along the rachis, but they are grouped between the autozooids in the terminal cluster (Kölliker 1880; Thomson & Henderson 1906; Kükenthal 1915). *Umbellula dura* has three autozooids growing at the same level forming a kind of circle in which the central autozoid is smaller than the other laterals in juvenile states, but in more developed states have six autozooids in total, four large and two very small, all disposed in three sets. Its central axis is cylindrical in cross section and the sclerites vary in length from 1500  $\mu\text{m}$  to 300  $\mu\text{m}$  in the autozooids and from 140  $\mu\text{m}$  to 100  $\mu\text{m}$  in the rachis, also, in these tissue has X-shaped sclerites (Thomson & Henderson 1906), that are absent in *U. pomona* sp. nov. *Umbellula rosea* is the more similar species to *U. pomona* sp. nov., but it can be differentiated because although it has a central terminal polyp and two pairs of lateral autozooids below it, the axis of *U. rosea* is quadrangular in cross section throughout (Thomson & Henderson 1906) while the axis of *U. pomona* sp. nov. is cylindrical in all its extension. In addition, Kükenthal (1915: 54), and Thomson & Henderson (1906: 5) describe the presence of small, thick, oval sclerites in the peduncle of *U. rosea*, which are absent in *U. pomona* sp. nov. Finally, some paratypes of *U. rosea* have just a few sclerites in their autozooids' tissue of around 250  $\mu\text{m}$  long (Thomson & Henderson, 1906) while *U. pomona* sp. nov. has very numerous and conspicuous sclerites in these tissues, from 1446  $\mu\text{m}$  to 543  $\mu\text{m}$  long. Some paratypes of *U. pomona* sp. nov. have oocytes, which confirms that these are adult specimens. In consequence, we describe here a new species for our specimens. A summary of all diagnostic characters is presented in Table 2.

*Umbellula pomona* sp. nov. inhabits the deep region of Mar del Plata Submarine Canyon, at a depth of about 3000 m. Sixty-four sampling stations were established during three expeditions, from 200 m to 3500 m deep, over 150 specimens of sea pens were collected but only four specimens of the new species were found. This low number of specimens is not rare because in the study area, many species report a very low abundance. Many deep-sea invertebrate species from Mar del Plata Submarine Canyon have been registered/described based on a few (and even only one) specimens (Pastorino 2016; Lauretta & Penchaszadeh 2017; Martinez *et al.* 2019). Since sampling efforts in this area have been almost non-existent until a few years ago, the number of species of sea pens (and other invertebrates) from Mar del Plata Submarine Canyon is likely to increase substantially in the coming years.

#### **Phylogenetic position of *Umbellula pomona* sp. nov.**

In all the phylogenetic reconstructions made, both specimens of *U. pomona* sp. nov. were grouped together (with low support, since we could only amplify one different gene for each specimen). They were grouped within a cluster composed by only two other species of *Umbellula*: *U. monocephalus* and *U. sp. 2* (from Dolan 2008; Dolan *et al.* 2013). Unfortunately, no molecular data of *U. rosea*, *U. durissima* or *U. dura* (the most morphologically similar species to *U. pomona* sp. nov.) were available, so it was impossible to compare the species from the molecular point of view.

There are only two molecular phylogenies available for sea pens that include several species and families (i.e., Dolan *et al.* 2013; Kushida & Reimer 2019). Both were constructed using two mitochondrial genes (mtMutS and ND2), and the latter was an extension of the former, including shallow-water species. Both papers agree that *Umbellula* is a polyphyletic group, with two clearly separate clades (here '*Umbellula* clade I' and '*Umbellula* clade II'). '*Umbellula* clade I' includes most of the species of *Umbellula* with available molecular data, including *U. encrinus*, the type species of the genus. '*Umbellula* clade II' includes only three species: *U. monocephalus* (an atypical *Umbellula* since it has only one terminal polyp), *U. pomona* sp. nov. (also an atypical species since the polyps have different sizes) and a non-identified *Umbellula* sp. (*Umbellula* sp2 from Dolan *et al.* 2013) with a typical polyp cluster (according to the figure in Dolan 2008). According to Dolan *et al.* (2013), '*Umbellula* clade II' was characterized by

**Table 3.** Distribution of the currently considered valid species of the genus *Umbellula* Cuvier, 1798.

Species	Presence of sclerites	Distribution	Main references
<i>Umbellula encrinus</i> (Linnaeus, 1758)	No	Arctic Ocean	Linnaeus 1758; Kükenthal 1911, 1915; Williams 1995
<i>Umbellula thomsoni</i> Kölliker, 1874	Yes	Cosmopolitan	Kölliker 1874; Kükenthal 1911, 1915; Williams 2011
<i>Umbellula carpenteri</i> Kölliker, 1880	No	Antarctic Ocean, Antarctic Indian Ocean	Kölliker 1880; López-González & Williams 2011
<i>Umbellula durissima</i> Kölliker, 1880	Yes	North Western Pacific Ocean, East Indian Ocean, North Atlantic, West Atlantic and South Atlantic Ocean, Antarctic Ocean	Kölliker 1880; Kükenthal 1915; Broch 1958; Williams 1995
<i>Umbellula huxleyi</i> Kölliker, 1880	No	North Pacific Ocean, East Indian Ocean, North Atlantic and South Eastern Atlantic Ocean	Kölliker 1880; Kükenthal 1915; Broch 1958; Williams 1995; López-González & Williams 2011
<i>Umbellula pellucida</i> Kükenthal, 1902	No	North Indian Ocean	Kükenthal 1902, 1915; Broch 1958; Williams 1995
<i>Umbellula spicata</i> Kükenthal, 1902	No	Indian Ocean	Kükenthal 1902, 1915; Broch 1958; Williams 1995
<i>Umbellula monocephalus</i> Pasternak, 1964	Yes	North Atlantic Ocean, Indian Ocean	Pasternak 1964; Williams 1995; López-González & Williams 2011
<i>Umbellula hemigymina</i> Pasternak, 1975	No	Caribbean Sea	Pasternak 1975; Williams 1995
<i>Umbellula pomona</i> sp. nov.	Yes	South Atlantic Ocean, Mar del Plata Submarine Canyon	This paper

species with sclerites and a round axis, characters also presents in *U. pomona* sp. nov., which supports this clade from the morphological data. If both clades were to be separated in two genera, ‘*Umbellula* clade I’ would retain the generic name *Umbellula* (since it includes the type species of the genus) and a new genus would have to be proposed for ‘*Umbellula* clade II’. Pending a complete revision of the nominal species assigned to *Umbellula*, we prefer to be conservative and include our species within *Umbellula* until a such revision is done and a decision based on molecular and morphological data can be made.

#### Valid species within *Umbellula*

Up to date, there are 43 nominal species within *Umbellula* (Williams 1995; Cordeiro *et al.* 2019; this paper). At least nine species were recognized as probably valid by Williams (1995) (*U. durissima*, *U. monocephalus*, *U. thomsoni*, *U. hemigymina*, *U. huxleyi*, *U. lindahli*, *U. pellucida*, *U. spicata* and

*U. encrinus*). Later, López-González & Williams (2011) disagreed with some specimens' identifications and shared the vision of Pasternak (1962) that *U. carpenteri* and *U. magniflora* Kölliker, 1880 are possibly the same species. On the other hand, Broch (1957) in his revision of the family considered *U. thomsoni* and *U. leptocaulis* Kölliker, 1880 as the same species. In this work, we consider the valid species according to the revision made by Broch (1957) and López-González & Williams (2011). Clearly, a complete revision of *Umbellula* spp. is needed, probably using both morphological and molecular data. Table 3 shows the valid species of *Umbellula* with their known distributions considered in this paper.

### **On the distribution of *Umbellula* spp. of the South Atlantic Ocean (SAO)**

*Umbellula* is a cosmopolitan genus (Table 3). Specimens of this group have been reported from all over the world, from the equator to the poles. Reports of *Umbellula* in the South Atlantic are limited; according to the papers made by Broch (1958) and Williams (1995), only four species were reported in the SAO: *U. durissima*, *U. monocephalus*, *U. thomsoni* and *U. lindahli*, of which only *U. thomsoni* and *U. lindahli* correspond to the southwestern Atlantic Ocean (SAO), where our specimens are located. The diversity of the group in Antarctic waters is also low, with three species reported (*U. durissima*, *U. lindahli* and *U. carpenteri*) (Kölliker 1880; Broch 1958; López-González & Williams 2011). As mentioned before, the conditions in the sampled area are strongly influenced by the Malvinas Current and the Brazil-Malvinas Confluence, which may explain the presence of Antarctic species in the SAO deep sea. Given the depths where our specimens came from (2934 m and 3282 m), it is possible that this record is (or is near to) the southern limit distribution of the species, since in the confluence area at that depth the North Atlantic Deep waters are present (see Voigt *et al.* 2013), which run southward from the northern hemisphere and in the confluence area divide the Circumpolar Deep Water vertically in two, staying at a depth of 2000–3000 m. A similar situation has also been proposed in the case of the gastropod *Theta lyronuclea* (Clarke, 1959), previously reported from the North Atlantic Ocean and recently found in the same station as *U. pomona* sp. nov. (Sánchez & Pastorino 2020).

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### **Compliance with ethical standards**

#### **Conflict of interest**

The authors declare that they have no conflict of interest.

#### **Ethical approval**

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

#### **Sampling and field studies**

All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgements.



### Data availability

The datasets generated during the current study are available from the corresponding authors on reasonable request.

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### **Supplementary material**

Supplementary file 1. Primer's sequences and PCR conditions used to amplify partial regions of the mitochondrial mtMutS and ND2 genes. <https://doi.org/ejt.2020.720.1121.2933>