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Litophellia enoplosa sp. nov., a new species of sea anemone (Actiniaria, Metridioidea) from cold-water coral environments in the North-western Mediterranean Sea

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Abstract

A new species of sea anemone (Actiniaria, Metridioidea) is described and illustrated from cold-water coral environments at 600 m depth at the Blanes Canyon in the north-western Mediterranean Sea. *Litophellia enoplosa* sp. nov. is characterized by a column divisible into scapus and scapulus, the former with cuticle forming tenaculi, a mesogleal marginal sphincter muscle, more mesenteries proximally than distally arranged octomerously, and a single pair of directive mesenteries; it has strong retractor and parietobasilar muscles, and acontia with basitrichs and *p*-mastigophores B2a. *Litophellia enoplosa* sp. nov. is the second species described in the genus and it can be distinguished from the other species by the cuticle in the column, size ranges of the cnidae, and geographic and bathymetric distribution.

Keywords: Cnidaria; Andvakiidae; Acontiate; Blanes Canyon.

Introduction

The sea anemone fauna from the Mediterranean Sea has been studied since the 1800s by different authors (e.g., Andres, 1883; Pax & Müller, 1962; Schmidt, 1972a; Doumenc *et al.*, 1985, Vafidis *et al.*, 1997); it is considered that there are 52 species of actiniarians in the Mediterranean Sea (Badreddine *et al.*, 2022). Most of these species are distributed in the coastal and shallow waters, with only nine species of sea anemones (i.e., *Actinauge richardi* (Marion, 1906), *Amphianthus dorhnii* Koch (1878), *Calliactis palliata* (Fabricius, 1779) (listed as *Adamsia palliata*), *Calliactis parasitica* (Couch, 1842), *Hormathia alba* (Andres, 1881), *H. coronata* (Gosse, 1858), *Kadophellia bathyalis* Tur, 1989, *Sagartia undata* (Müller, 1778) (listed as *Sagartiogeton undulatus*), and *Segonzactis hartogi* Vafidis & Chintiroglou, 2002) distributed in waters deeper than 100 m (Vafidis & Chintiroglou, 2002). Recent studies of Mediterranean deep-sea and cold-water corals environments (e.g., Mastrototaro *et al.*, 2017; Taviani *et al.*, 2019; Gerovasileiou *et al.*, 2019; Angeletti *et al.*, 2020; Cartes *et al.*, 2022) have cited mainly two species of Mediterranean deep-sea anemones (*A. dorhnii* and *Protanthea simplex* Carlgren, 1891), adding another species of deep-sea anemone for the region. Nevertheless, the identity of some of the deep-sea Mediterranean actiniarian records has been contested [e.g., *H. alba* was mistaken and described as *Paracalliactis mediterranea* Ross & Zamponi, 1982 according to Tur (1993)], others are deemed *nomen dubium* (e.g., *K. bathyalis*, see Rodríguez *et al.*, 2023), and in other cases, the identity of the records must be corroborated because the studies did not have a taxonomic focus (e.g., *P. simplex* in Angeletti *et al.*, 2020). Thus, further attention to the group is needed in Mediterranean deep waters.

The Blanes Canyon is sited about 5 km off the coast of Catalunya (Spain), in the north-western Mediterranean Sea (Fig. 1). It is 60 km long and half a kilometer wide, with vertical and vaulted walls, descending up to 2,300 m in depth (Lastras *et al.*, 2011). In 2017, during

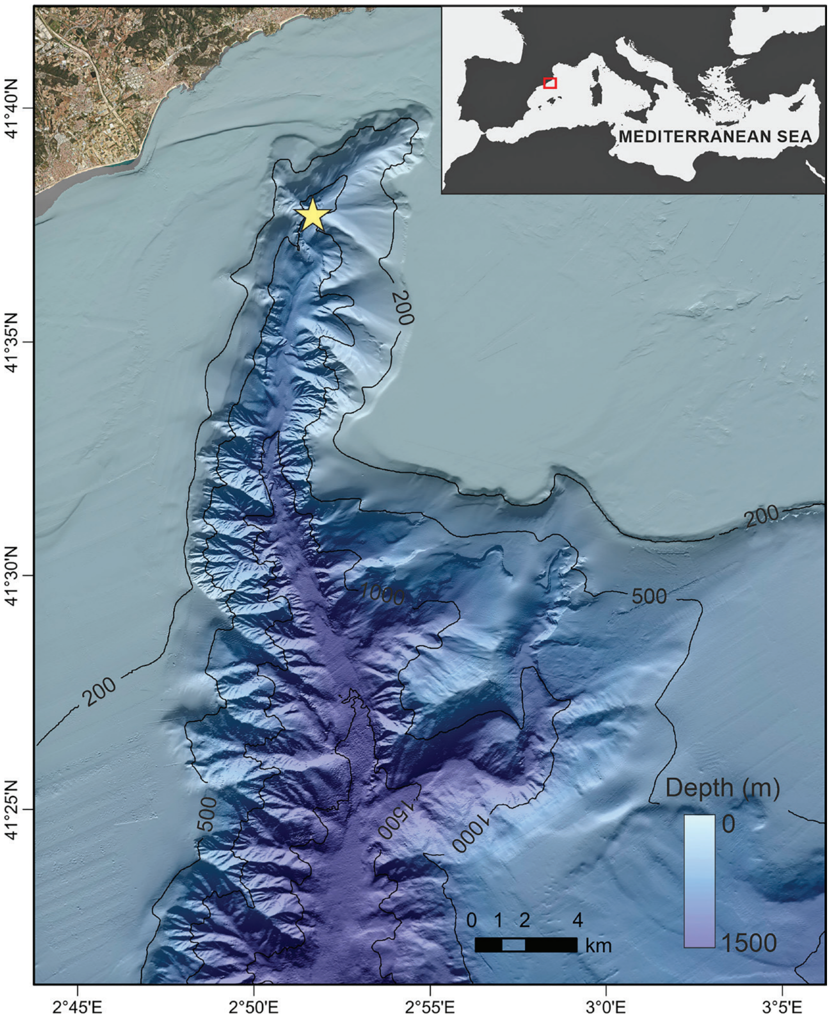

Fig. 1: Bathymetric map of the Blanes Canyon (north-western Mediterranean Sea) showing the type locality (yellow star) of *Litophellia enoplosa* sp. nov.

the project ABIDES (Assessment of Bottom-trawling Impacts in Deep-sea Sediments), which focused on the study of bottom trawling impacts on deep marine sediments on submarine canyons and flanks of the Catalan continental margin, extensive and dense colonies of deep cold-water corals were discovered in its walls (Santín *et al.*, 2020). The morphology of the Blanes Canyon (many faults and fractures) generates environments with vertical walls, terraces and rocky outcrops, promoting the formation of very well developed and structured benthic assemblages, mainly dominated by cold-water corals. Monospecific coral gardens composed by different gorgonian species, such as *Callogorgia verticillata* (Pallas, 1766) and *Muriceides lepida* Carpine & Grasshoff, 1975, and the antipatharian *Leiopathes glaberrima* (Esper, 1791), were mainly found on terraces and outcrops. The vertical canyon walls and overhangs were dominated by the solitary scleractinian *Desmophyllum dianthus* (Esper, 1794) and the colonial scleractinians *Desmophyllum pertusum* (Linnaeus, 1758) and *Madrepora oculata* Linnaeus, 1758 that achieved high densities. The Blanes canyons are classified alongside the Gulf of Lions as an Ecologically or Biologically Significant Area (EBSA) by the Regional Activity Center for Specially Protected Areas (Gabrie *et al.*, 2012).

Here we describe the sea anemone *Litophellia enoplosa* sp. nov. from two specimens collected in the Blanes Canyon head, at 623 m depth. *Litophellia enoplosa* sp. nov. is characterised by having a regionate column with cuticle forming tenaculi, tentacles and mesenteries arranged octomerously, a single pair of directive mesenteries, and acontia with two size ranges of basitrichs and one of *p*-mastigophores B2a. *Litophellia enoplosa* sp. nov. is the second species described within the genus and represents the second Mediterranean endemic actiniarian species from deep waters described to date.

Material and Methods

This contribution is part of the more detailed research project ABRIC (Assessment of Bottom-trawling Resuspension Impacts in deep benthic Communities) aimed to explore the effects of trawling sediment resuspension on the Blanes Canyon cold-water corals and to characterize the canyon walls environments and their associated benthic communities The material studied was collected in cold-water corals habitats in the Blanes Canyon (Fig. 1). The oceanographic cruise was carried out on board the R/V *Sarmiento de Gamboa* during 14/02/2020-

29/02/2020. Specimens were collected at 623 m depth on coral rubble of *D. pertusum* using the Remote Operate vehicle (ROV) *Liropus*, from the Spanish Institute of Oceanography (IEO by its acronym in Spanish).

Two sea anemones were fixed in 10% formalin in seawater and subsequently transferred to ethanol 70%. Preserved specimens were examined whole, in dissection and as serial sections. Fragments from dissected specimens were temporally stained by submerging them in methyl blue dye for one minute. Fragments of both specimens were dehydrated in graded ethanol series and then embedded in paraffin. Histological sections 10 µm thick were stained with Heidenhain's Azan Triple Stain (Presnell & Schreibman, 1997).

Cnidae measurements were taken from preserved material smeared on slides using Differential Interface Contrast (DIC) microscopy at 1000X magnification. We scanned through the slides and randomly measured 20 capsules of each type (when possible) to generate a range: frequencies given are subjective impressions based on slides. For each type, a mean and standard deviation has been provided to give an idea of the distribution of sizes; these will be not statistically significant when based on fewer than 40 capsules, or if the underlying data are not normally distributed (Williams 1998, 2000). We follow a nematocyst terminology that combines the classification of Weill (1934) modified by Carlgren (1940) thus differentiating 'basitrichs' from '*b*-mastigophores' with that of Schmidt (1969, 1972b) which captures the underlying variation seen in 'rhabdoids' (see Gusmão *et al.*, 2018 for more details). We include photographs of each type of nematocyst for reliable comparison across terminologies and taxa (see Fautin, 1988). Higher-level classification for Actiniaria follows Rodríguez *et al.* (2014). Material studied has been deposited at the American Museum of Natural History (AMNH) in New York City, USA. This article is registered in Zoobank under urn: lsid:zoobank.org:pub:9789903C-EED3-4D1E-9991-EA60B870099C.

Results

Order Actiniaria Hertwig, 1882
Suborder Enthemonae Rodríguez & Daly, 2014 in Rodríguez *et al.*, 2014
Superfamily Metridioidea Carlgren, 1893
Family Andvakiidae Danielssen, 1890

Diagnosis (After Daly & Goodwill, 2009; Rodríguez *et al.*, 2012; modifications in bold): Metridioidea with weak or absent basilar muscles and long mesogleal marginal sphincter. Elongate, cylindrical body divisible into **aboral end**, scapus, and capitulum. **Aboral end** physa-like and rounded or flattened. Scapus with cuticle and usually with tenaculi; it may have cinclides. Mesenteries not divisible into macro- and micro-cnemes but **usually dimorphic**. Perfect mesenteries fertile, with developed filaments, strong retractors, and acontia. Imperfect mesenteries small, sterile, without retractor muscles, at least some extend length of column, may have filaments and acontia. Acontia with basitrichs and *p*-mastigophores B2a. Cnidom: Gracile spirocysts, basitrichs, and ***p*-mastigophores A, B1 and B2a.**

Included genera. *Andvakia* Danielssen, 1890; *Epiphellia* Carlgren, 1950; *Euphellia* Pax, 1908; *Flosmaris* Stephenson, 1920; *Gymnophellia* England, 1992; *Isophellia* Carlgren, 1900; *Ilyactis* Andres, 1881; *Litophellia* Carlgren, 1938; *Octophellia* Andres, 1883; *Telmatactis* Gravier, 1916. *Synandwakia* Carlgren, 1947.

Remarks. We modified the familial and generic diagnoses to reflect the cnidae terminology used in this study. Similarly, we use the term dimorphic to describe mesenteries with different morphology of pairs among cycles, but that do not fit the definition of macro- and micro-cneme mesenteries –the later defined as imperfect and poorly developed, not having retractor muscles, mesenterial filaments, gametogenic tissue, or acontia (Stephenson, 1928; revised in Gusmão *et al.*, 2019). We added the possibility to have an aboral end that is not a physa or physa-like in Andvakiidae because some species within the family have an aboral end forming a flat disc (e.g., *Litophellia*, *Flosmaris*). Finally, we added the genus *Octophellia* to the family following Carlgren's (1949) comments that this genus probably belongs within Andvakiidae and provide an updated diagnosis for the genus.

Genus *Octophellia* Andres, 1883

Diagnosis (after Andres, 1883)*:* Andvakiidae with flat, circular, weakly adherent base, sometimes physa-like. Column cylindrical, divisible into scapus and scapulus; scapulus smooth and delicate, scapus with cuticle and tenacula (?). Margin tentaculate; tentacles few, up to 16, in two cycles, all similar sizes, octomerously arranged; at least 12 perfect (?) mesenteries. Marginal sphincter muscle mesogleal or not developed (?). Acontia. Cnidom: unknown.

Type species. *Phellia timida* Andres, 1881 by subsequent designation (Fautin, 2016).
Remarks. Because Andres (1883) mostly described external characters of *O. timida*, we indicated questionable characters in the diagnosis with a question mark [i.e., (?)].

Genus *Litophellia* Carlgren, 1938

Diagnosis (after Carlgren, 1949; modifications in bold)*:* Andvakiidae with **broad disc-like** base. Column elongate, not regionate and smooth, or **divisible into scapus and scapulus**; **scapus with cuticle and tenaculi.** Cinclides present. Margin tentaculate. Sphincter rather strong, mesogleal. Tentacles and mesenteries octomerously arranged. A single siphonoglyph and one pair of directives. Mesenteries similar in number proximally and distally **or more mesenteries proximally**, distinctly **dimorphic** among cycles: eight pairs of **perfect mesenteries**; **at least** two cycles of weak **imperfect mesenteries**. Retractors of **perfect mesenteries** strongly restricted, reniform. Parietobasilar muscles weak. Basilar muscles **well developed**. Acontia with basitrichs and

***p*-mastigophores B2a**. Cnidom**: spirocysts, basitrichs, and *p*-mastigophores A, B1, and B2a**.

Type species. *Litophellia octoradiata* Carlgren, 1938 by monotypy.
Remarks. Carlgren (1938) described *Litophellia* with a distinct basal disc in his original diagnosis of the genus, but he modified the diagnosis later on to include the possibility of the base being small (Carlgren, 1949). Because the only two species so far included in this genus have a distinct broad disc-like base, we have modified the diagnosis to reflect the presence of a wide flat base and the presence of well-developed basilar muscles. We also added the possibility of the column to be divided into scapus and scapulus, the former bearing a strong cuticle forming tenaculi to accommodate *L. enoplosa* sp. nov. We modified the language for the mesenteries to reflect the distinction among pairs of different cycles that do not confirm the definition of macro- and micro-cnemes (see family diagnosis remarks).

Litophellia enoplosa sp. nov.
Rodríguez & Collins
(Figs. 1-4, Table 1)
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Table 1. Size ranges of the cnidae of *Litophellia enoplosa* sp. nov. Data from *L. octoradiata* (in parenthesis) from Carlgren (1938, 1945); ranges in bold font are from Carlgren (1938). S: Sample, ratio indicates number of specimens in which each cnidae was found compared to number of specimens examined; N: indicates total number of capsules measured. F: Frequency, +++ = very common, ++ = common, + = rather common, --- = sporadic. (*), capsules present but broken; ND, No data.

Tissue/Categories	**Range of length and width of capsules (in µm)**	**Average and SD**	**S**	**N**	**F**	*L. octoradiata* **Data from Carlgren (1938) / Carlgren (1945)**
SCAPUS						
Basitrichs 1	11.2-14.4 x 2.0-3.2	12.7±1.2 x 2.5±0.5	2/2	5	---	**(14-16) x (2.5)** / (14-15.2) x (2.8)
p-mastigophores B1	12.4-19.6 x 3.7-6.8	15.9±1.6 x 5.1±0.8	2/2	18	+	**(12 -17) x (3-3.5)** / (12.7-17) x (3-4)
p-mastigophores B2a	23.4-28.5 x 4.8-5.9	26.0±2.1 x 5.4±0.4	2/2	7	---	---
SCAPULUS						
Basitrichs 1	7.6-11.0 x 1.8-2.5	9.1±1.2 x 2.1±0.3	1/1	7	+	ND
Basitrichs 2	14.5-16.2 x 2.2-3.0	15.5±0.9 x 2.7±0.4	1/1	3	---	ND
p-mastigophores B2a	24.6-29.1 x 4.9-6.8	27.4±1.4 x 5.6±0.6	1/1	8	+	ND
TENTACLES						
Basitrichs 1	12.5-14.5 x 2.2-3.4	13.5±1.4 x 2.8±0.8	1/2	2	---	**(14-17) x (2)** / ND
Basitrichs 2	18.0-29.3 x 1.9-4.4	24.3±3.9 x 3.3±0.5	2/2	20	++	**(14.0) x (3.0)** / ND
p-mastigophores B2a	22.2-31.9 x 4.1-6.2	28.3±2.1 x 5.3±0.4	2/2	38	++	---
Spirocysts(*)	16.1-34.1 x 3.4-6.6	25.0±5.4 x 4.5±0.9	2/2	20	++	**(12-24) x (1.5-3.5)** / ND
ACTINOPHARYNX						
Basitrichs 1	9.8-14.7 x 1.3-2.7	12.5±1.2 x 2.2±0.4	2/2	31	++	---
Basitrichs 2	19.0-24.8 x 2.3-3.5	21.6±1.3 x 2.8±0.3	2/2	44	++	**(24-29) x (2-2.5)** / ND
p-mastigophores A	16.9-23.2 x 4.7-6.3	20.4±1.6 x 5.6±0.5	2/2	24	++	---
p-mastigophores B2a	36.8-43.5 x 5.1-7.1	40.6±1.7 x 5.8±0.5	1/2	18	+	---
FILAMENT						
Basitrichs 1	9.3-13.9 x 1.6-2.9	11.7±1.1 x 2.3±0.3	2/2	32	++	---
Basitrichs 2	27.1-33.1 x 3.0-3.9	30.9±1.7 x 3.4±0.3	2/2	21	++	---
p-mastigophores B1	8.1-12.5 x 2.9-4.7	10.6±1.2 x 3.5±0.4	2/2	22	++	(10-14) x (4.2-5)
p-mastigophores A	15.9-21.4 x 4.7-6.1	19.3±1.5 x 5.5±0.5	2/2	12	+	(19.7-22.6) x (3.5)
ACONTIA						
Basitrichs 1	12.2-16.2 x 1.8-2.8	13.9±1.4 x 2.5±0.3	2/2	11	+	---
Basitrichs 2	26.0-34.2 x 2.9-4.2	31.1±1.4 x 3.5±0.3	2/2	39	+++	**(26-31) x (3)** / (26.8-29.6) x (3)
p-mastigophores B2a	49.6-58.4 x 4.9-8.7	54.0±2.5 x 6.0±0.7	2/2	34	+++	**(31-36) x (3.5-4)** / (31-36.7) x (4.2-4.5)

Type material. Holotype: AMNH_IZC_00361521, 1 specimen, dissected; AMNH_IZC_00361521.1-8: histological and cnidae slides; Blanes Canyon, Mediterranean Sea (Spain), 41.629º N, 2.860º E, 623 m depth, 17 February 2020. Paratype: AMNH_IZC_00361522, 1 specimen, dissected; AMNH_IZC_00361522.1-8: histological and cnidae slides; same data as the holotype.

Description

External anatomy (Fig. 2): Aboral end well developed, flat and disc-like, wider than oral disc, to 21 mm diameter. Column divisible into scapus and scapulus. Scapus with a brownish cuticle, forming tenaculi, to 10 mm height and 9 mm in diameter in preserved and contracted specimens; with cinclides scattered along column distally and proximally. Scapulus smooth, whitish, up to 2 mm height.

Oral disc narrower in diameter than proximal end in preserved and contracted specimens, not observed; most distal part of column to 9 mm in diameter. Tentacles 32 and 76 in number (in paratype and holotype, respectively), arranged in four cycles, to 2 mm in contracted and preserved specimens; outer tentacles longer than inner ones; translucent with reddish pigmented gastrodermis. Mouth not observed.

Internal anatomy (Fig. 3): More mesenteries proximally than distally. Mesenteries to 38 pairs at actinopharynx level, octomerously arranged in four cycles with forth cycle incomplete distally (8+8+16+16); cycles of mesenteries dimorphic: first cycle, eight pairs of perfect mesenteries, rest of mesenteries imperfect but some with small retractors and acontia; third and fourth cycle poorly developed; mesenteries of forth cycle only developed in exocoels adjacent to perfect mesenteries. Only one pair of directives connected with one well developed siphono-

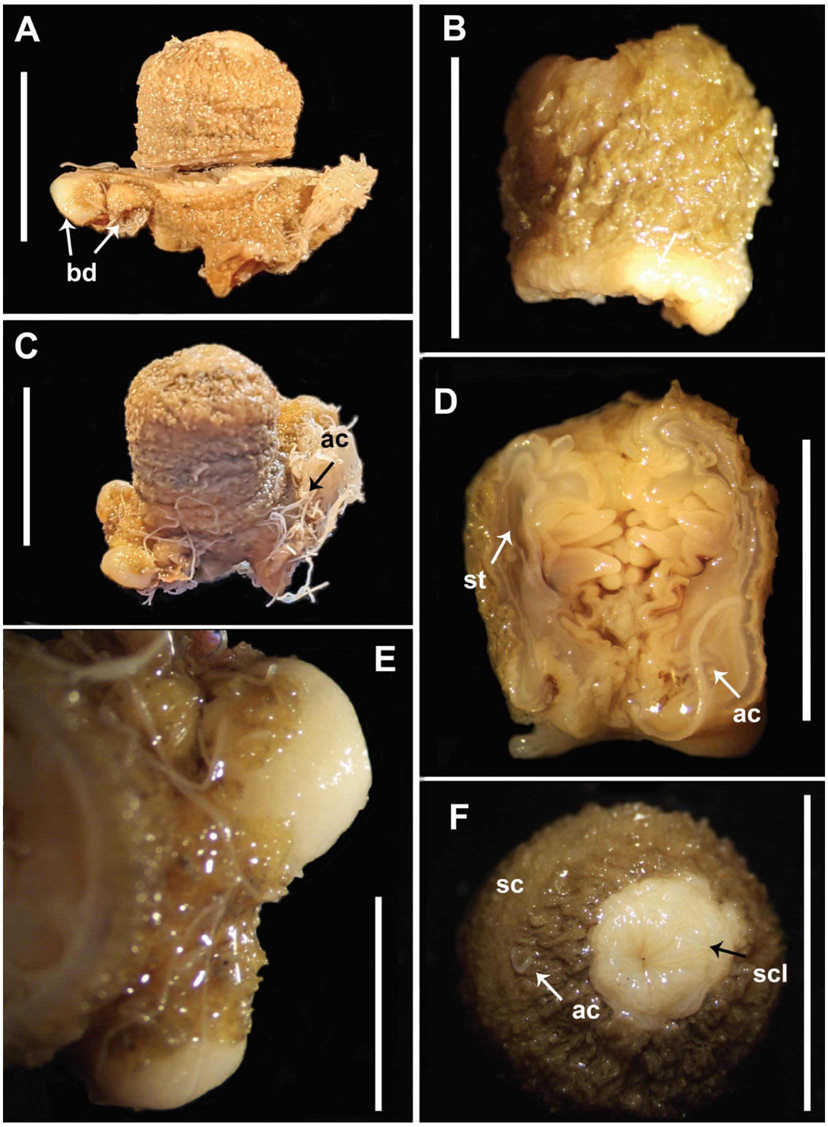

Fig. 2: External and internal anatomy of *Litophellia enoplosa* sp. nov. A) lateral view of contracted and preserved holotype AMNH_IZC_00361521; B) lateral view of contracted and preserved paratype AMNH_IZC_00361522; C) different view of whole preserved holotype; D) longitudinal section of paratype; arrow points to oral stoma; E) detail of the proximal column of the holotype showing budding; F) oral view detail of the distal column of the holotype showing an acontium expelled through a cinclide (arrow). Abbreviations: ac, acontia; bd, budding juveniles; sc, scapus; scl, scapulus; st, stomata. Scale bars: A-D, F, 5 mm; E, 3 mm.

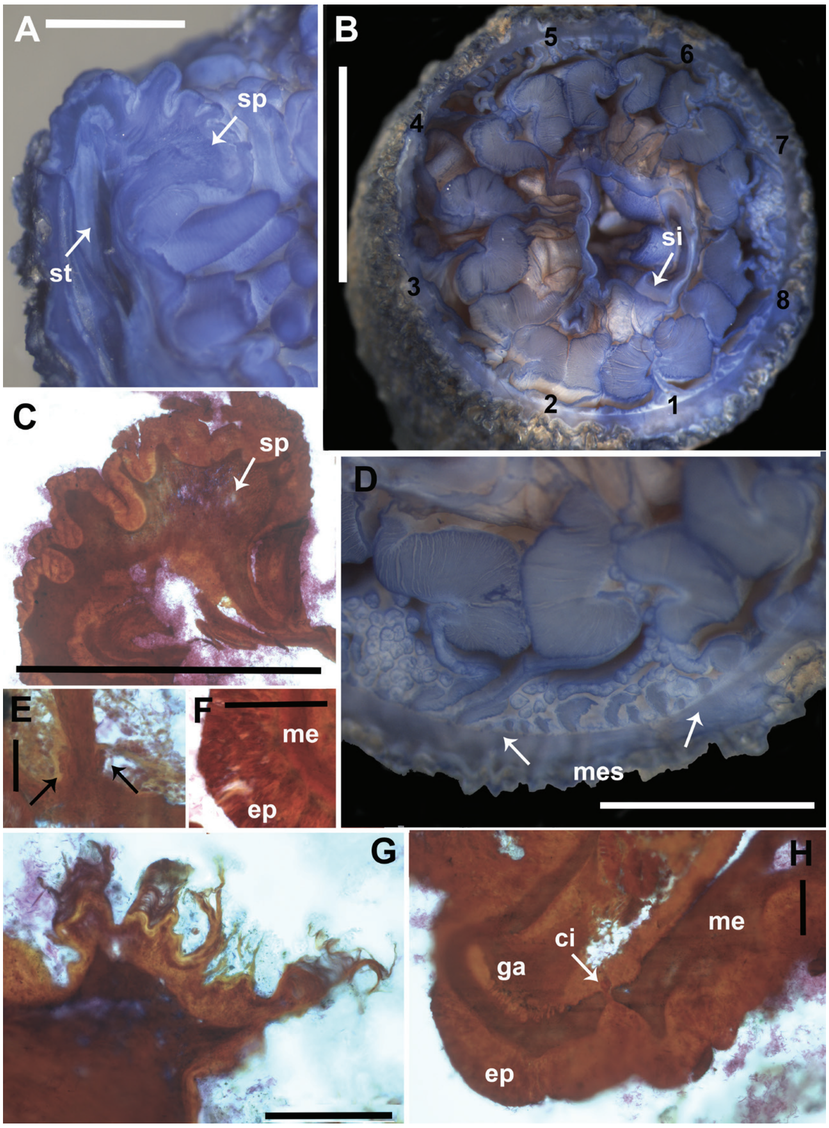

Fig. 3: Internal anatomy of *Litophellia enoplosa* sp. nov. A) longitudinal section of distal column of the paratype showing the marginal sphincter muscle and the marginal stomata; B) cross section of the column through the mesenteries at the actinopharynx level showing the eight perfect pairs of mesenteries; note single pair of directives attached to the single siphonoglyph (arrow); numbers indicate pairs of perfect mesenteries, number 1 indicates the directive pair; C) longitudinal histological section of distal column showing the mesogleal marginal sphincter muscle; D) detail of imperfect cycles of mesenteries and non-directive pair; note pairs of mesenteries of the fourth cycle (arrows); E) longitudinal section of proximal column showing the basilar muscles (arrows); F) cross section showing the ectodermal longitudinal muscles of the tentacles; G) detail of a tenacula showing the stratified cuticle; H) detail of the proximal column showing a cinclide. Abbreviations: ac, acontia; ci, cinclide; ep, epidermis; ga, gastrodermis; me, mesoglea; mes, mesenteries; si, siphonoglyph; sp, marginal sphincter muscle; st, stomata. Scale bars: A, C, 1 mm; B, 25 mm; D, 2 mm; E, 0.075 mm; F, H, 0.065 mm; G, 0.2 mm.

glyph. Oral stomata ovoid, long axis to 3 mm in diameter (Fig. 2D, 3A). Retractor muscles of perfect mesenteries strong, restricted, reniform (Fig. 3B, D). No gametogenic tissue in specimens collected in February; pedal laceration in larger specimen, three buddings (Fig. 2A, C, E). Parietobasilar muscles well differentiated; muscles fibres on broad and branched mesogleal base in mesenteries of first and second cycles (Fig. 3D). Basilar muscles developed, with short thick mesogleal pennons (Fig. 3E). Acontia in both specimens, numerous, white, not coiled; with two size ranges of basitrichs and one of *p*-mastigophores B2a.

Mesogleal marginal sphincter muscle strong and moderately long; distal part broad, of alveolar nature (Fig. 3A, C). Muscles fibres in mesoglea closer to epidermis. Tentacles and oral disc with ectodermal longitudinal muscles (Fig. 3F). Column wall of similar thickness entire length: epidermis 0.06-0.11 mm; mesoglea 0.09-0.14 mm thick, and gastrodermis 0.09-0.10 mm thick at actinopharynx level.

Cnidom (Fig. 4). Spirocysts (in tentacles), basitrichs (in scapus, scapulus, tentacles, actinopharynx, filaments, and acontia), *p*-mastigophores A (in actinopharynx and filaments), *p*-mastigophores B1 (in filaments), and *p*-mastigophores B2a (in scapus, scapulus, tentacles, actinopharynx, and acontia). A survey of cnidae size ranges is provided in Table 1.

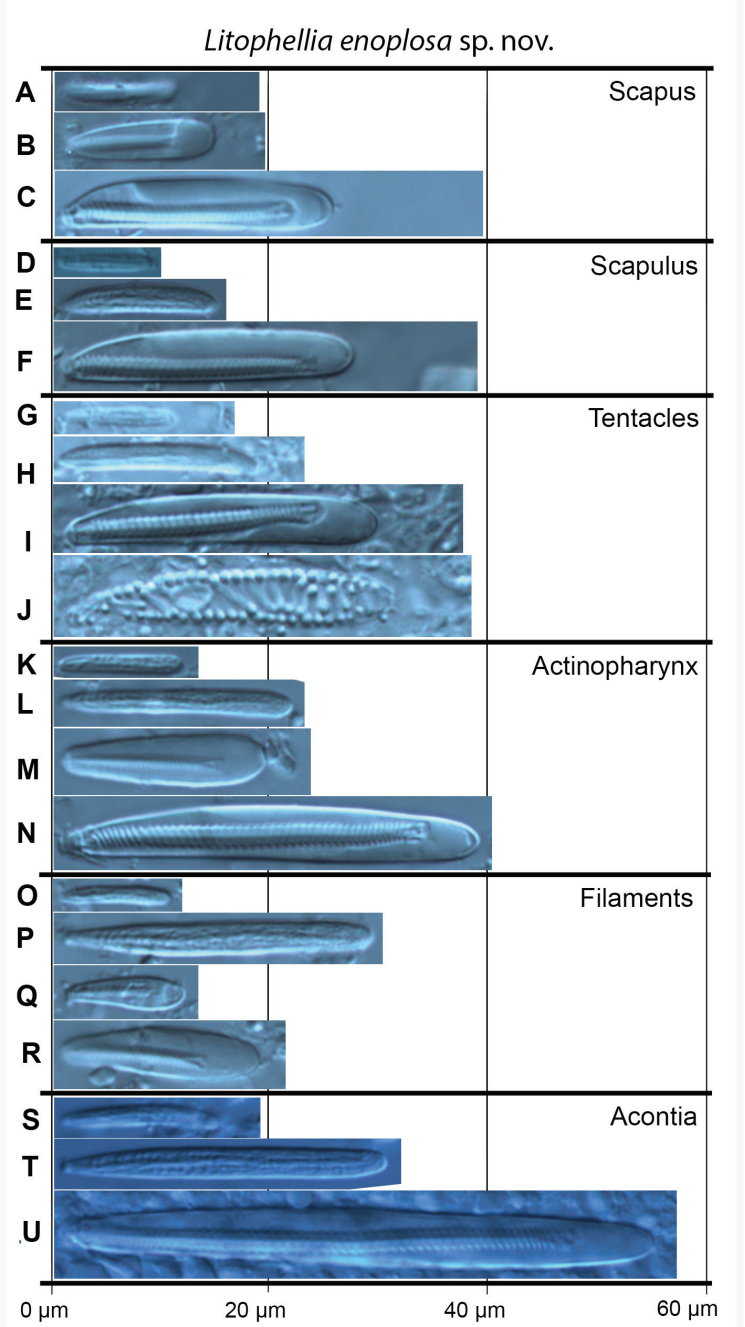

Fig. 4: Cnidae of *Litophellia enoplosa* sp. nov. A, D, E, G, H, K, L, O, P, S, T) Basitrichs; B, Q) *p*-mastigophores B1; C, F, I, N, U) *p*-mastigophores B2a; M, R) *p*-mastigophores A; J) Spirocyst.

Etymology

The specific name *enoplosa* refers to the cuticle covering the scapus of this species, a differential diagnostic feature of *L. enoplosa* sp. nov. from the Greek ἔνοπλος (énoplos) = armed.

Geographic distribution and natural history

Litophellia enoplosa sp. nov. has been collected from deep-sea waters (623 m depth) in the Blanes Canyon, in the north-western Mediterranean Sea (Fig. 1). It was collected on *D. pertusum* dead colony sections and associate with two other actiniarian species, *Protanthea* sp. and *Amphianthus* sp. and the carnivorous sponge *Lycopodina hypogea* (Vacelet & Boury-Esnault, 1996).

Discussion

Familial and generic placement

Litophellia enoplosa sp. nov. is characterized by a mesogleal marginal sphincter and acontia, characters that place these specimens within the superfamily Metridioidea (Rodríguez *et al.*, 2014). A regionate column with cuticle forming tenaculi, dimorphic cycles of mesenteries and acontia with basitrichs and *p*-mastigophores B2a further place our specimens within the family Andvakiidae.

Nevertheless, the most distinct anatomical characters of *L. enoplosa* sp. nov. are a clear octomerous arrangement of mesenteries and tentacles, and a single pair of directive mesenteries attached to a single developed siphonoglyph.

Although more common than documented, only a few genera within Actiniaria have a clear octomerous arrangement of mesenteries and tentacles (Barragán *et al.*, 2019). Examples of regular octamerous symmetry in actiniarians are found in Edwardsiidae Andres, 1881 and among Actinostoloidea Carlgren, 1932 (*Stomphia* (Gosse, 1859), *Sicyonis* Hertwig, 1882), Phymanthidae Andres, 1883 (some species of *Phymanthus* Milne Edwards & Haime, 1851), Andvakiidae (*L. octoradiata* Carlgren, 1938), Aiptasiidae Carlgren, 1924a (some species of *Bartholomaea* Duchassaing de Fombressin & Michelotti, 1864; *Aiptasiogeton* Schmidt, 1972a), Actiniidae Rafinesque, 1815 (*Bunodactis octoradiata* (Carlgren, 1898) [authorship date traditionally attributed to 1899 although original description was published in 1898, see Rodríguez *et al.*, 2023] and Peachiidae Hamilton, Daly & Rodríguez, 2022 in Hamilton *et al.*, 2022 (*Metapeachia tropica* (Panikkar, 1938)]; in addition, there are some species within some genera with octomerous symmetry (e.g., species within *Sagartiogeton* Carlgren, 1924b) (reviewed in Grebelny, 1982).

The genus *Octophellia* was described by Andres to accommodate the acontiate *O. timida* (Andres, 1881) found in the northern Gulf of Naples at 65 m depth (Andres, 1883, 1884). Because its description provided mostly external characters [i.e., cylindrical, regionate column with cuticle, 16 tentacles in two cycles, 12 mesenteries and acontia, see Andres (1883: 120-121)], the type material is not available and no additional specimens have been ever described, several authors have considered the familial position of the genus doubtful (e.g., Stephenson, 1920; Carlgren, 1921) and the species to be a *nomen nudum* (Daly & Goodwill, 2009). However, Fautin (2016) argued that because the description of *O. timida* contains features purported to differentiate the species it does not meet the definition of a *nomen nudum* according to the International Code of Zoological Nomenclature (ICZN, 2000).

We checked the available descriptions of *O. timida* (Andres, 1881, 1883) and agree with Fautin (2016) that based on the characters provided *O. timida* cannot be considered a *nomen nudum*. However, available characters are not enough to recognize without uncertainty *O. timida* under modern standards: Andres (1883) did not provide the nature of the marginal sphincter muscle (mesogleal, endodermal or not developed), details of the mesenterial arrangement or cnidae composition. Thus, we consider *O. timida* to be a *nomen dubium.* Nevertheless, based on the brief discussion provided by Andres (1883), we consider *O. timida* (and thus the genus *Octophellia*) to differ from *L. enoplosa* sp. nov. Andres (1883) commented on the similarity of *O. timida* and edwardsiid species, particularly in the physa-like proximal end and the number of tentacles (octomerous symmetry), but he distinguished *O. timida* by having acontia and 12 mesenteries ("septus") and thus, resembling more members of the family Halcampidae Andres, 1883. Although Andres (1883) did not specify whether the 12 mesenteries in *O. timida* were perfect (i.e., attached to the actinopharynx), we assume based on the number of mesenteries attributed to other taxa described in the same work that they were perfect (e.g., *Halcampella* Andres 1883, described as having 12 mesenteries but known to have a higher number because of the presence of microcnemes, Rodríguez & López-González, 2002). Andres (1883) did not provide any comments about the marginal sphincter muscle in *O. timida*, but because it has acontia, this musculature would probably be mesogleal or not developed (Rodríguez *et al.*, 2012). We agreed with previous authors (Stephenson, 1920; Carlgren, 1949) considering *Octophellia* to better align with members of Andvakiidae than those in Sagartiidae Gosse, 1858 (family in which *Octophellia* was placed up to now (Rodríguez *et al.*, 2023).

Because *L. enoplosa* sp. nov. has acontia, octomerous symmetry and a single pair of directives it strongly resembles the monotypic genus *Lithophellia*. Only four species within three families (e.g., Andvakiidae, Halcampidae, Peachiidae) have been described to have a single pair of directives without asexual reproduction documented (Barragán *et al.*, 2019). The other three species with a single pair of directives attached to one siphonoglyph other that *L. octoradiata* are *Actinothoë carlgreni* Haddon & Duerden, 1896; *Gymnophellia hutchingsae* England, 1992; and *Tenactis riosmenai* Barragán *et al.*, 2019. Previous reports lacked further discussion about the fact (e.g., Carlgren, 1938; 1949; England, 1992). Presence of a single pair of directives is the common pattern within the subclass Octocorallia, however, in octocorals the directives are not attached to the single siphonoglyph but to the opposite side of the actinopharynx (Gonçalves, 2016).

Species discussion

Litophellia enoplosa sp. nov. and *L. octoradiata* resemble each other in gross morphology and cnidom; however, both species are clearly distinguishable by external and internal anatomy, cnidae and geographic distribution.

The original description of *L. octoradiata* is based on a sole specimen described with a smooth column without cuticle or tenacula (Carlgren, 1938). However, in *L. enoplosa* sp. nov. the column is clearly regionate, divisible into a scapus and a smooth scapulus, the former with a strong cuticle forming tenacula (Fig. 3G). Carlgren (1938) found cinclides connected with some of the secondary endocoeles in *L. octoradiata* but he did not specify if these were proximally or distally in the column. We found cinclides distributed proximally and distally (Fig. 2C, D, F; Fig. 3H).

Litophellia octoradiata and *L. enoplosa* sp. nov. share having mesenteries and tentacles arranged octomerously. However, *L. enoplosa* sp. nov. has up to a fourth cycles of mesenteries (8+8+16+16=48 pairs, last cycle incomplete, at least distally) whereas *L. octoradiata* only has three cycles of mesenteries (8+8+16=32 pairs) with similar body sizes. Carlgren (1938) described *L. octoradi-*

ata with similar number of mesenteries proximally and distally, but in *L. enoplosa* sp. nov. more mesenteries are developed proximally than distally. Similarly to the holotype of *L. octoradiata* being sterile, we did not find gametogenic tissue developed in specimens of *L. enoplosa* sp. nov. collected in February. We found in the holotype of *L. enoplosa* sp. nov. three buds developing proximally and still connected to the specimen (Fig. 2A, C, E), probably produced via pedal laceration; the three buds were of different sizes and already had developed mesenteries. Although it is not possible to infer whether *L. octoradiata* might also present asexual reproduction because only a single specimen is known, the differences in reproductive strategies of the two species might be explained because *L. octoradiata* was collected during November (corresponding to the austral spring) whereas *L. enoplosa* sp. nov. was collected during winter (February).

The sizes and distribution of the cnidae also differ between both litophellid species with *L. enoplosa* having additional cnida types (e.g., *p*-mastigophores B2a in the scapus and tentacles, basitrichs in the filaments) and larger sizes that do not overlap with those of *L. octoradiata* (e.g., *p*-mastigophores B2a in the acontia). Although the type material of *L. octoradiata* was not examined here, Carlgren (1938, 1945) provided measurements that allow for comparison between both species (Table 1).

The geographical and bathymetric distribution of both species of *Litophellia* also differentiates them. *Litophellia enoplosa* sp. nov. was found among cold-water coral rubble in the north-western of the Mediterranean Sea, at 623 m depth. However, *L. octoradiata* was found in the intertidal of Isipingo (Durban, South Africa). Not only the localities are geographically separate, but the two environments where the species have been found are very different ecologically, supporting the differentiation of both species.

Similarly to other invertebrate groups of deep sea in the Mediterranean benthos (Bouchet & Taviani, 1992), deep-sea anemones show low degree of diversity and endemism in the Mediterranean compared to the Northeast Atlantic deep-sea fauna (Vafidis & Chintiroglou, 2002). *Litophellia enoplosa* sp. nov. represents the second deep-sea Mediterranean endemic actiniarian species described to date and the sixth family of the order with deep-sea representatives in the region. The only other endemic deep-sea Mediterranean species is *Segonzactis hartogi* distributed in the northern and central Aegean Sea (Vafidis & Chintiroglou, 2002) between 80 and 800 m depth on silty bottoms.

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