

The sea anemone *Bunodactis octoradiata* (Anthozoa: Actiniaria) from southern Patagonia: morphological study and new records

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ABSTRACT. *Bunodactis* Verrill, 1899 comprises at present 19 nominal species of sea anemones. The validity of the genus is under discussion. The description of the species, *Bunodactis octoradiata* Carlgren, 1899, is insufficient for reliable identification, and although subsequent works have provided additional information on the species, its description still needs to be complemented. Herein we describe *B. octoradiata* based on histological sections of the internal anatomy, and give a complete and detailed description of the external anatomy. The cnidom is composed of spirocysts, basitrichs and microbasic p-mastigophores; their distribution in the organism, sizes and relative abundances are provided. The presence of zooxanthellae is reported for the first time. *Bunodactis octoradiata* is distributed in groups that vary from 5.6 to 45 ind./m² in the field. Additionally, our data extends the distribution of the species along the coast of southern Patagonia (47°44'36"-49°15'13"S and 65°50'26"-67°39'45"W).

KEY WORDS. Actiniidae; cnidom; spatial distribution; taxonomy.

Bunodactis Verrill, 1899 (Anthozoa: Actiniidae) comprises many nominal species whose validity and generic assignment are not known with certainty and need confirmation. The validity of the genus has also been questioned. CARLGREN (1949) synonymized *Bunodactis* with *Aulactinia* Verrill, 1864, as he placed the type species, *Aulactinia capitata* Agassiz in Verrill, 1864, in *Bunodactis*. Subsequently, DUNN *et al.* (1980) indicated that *Aulactinia* has priority over *Bunodactis* and, accordingly, should be the valid generic name. Recently, SPANO *et al.* (2013), in the re-description of *Bunodactis hemaphroditica* (Carlgren, 1959), discussed the nomenclature of these genera. They pointed out that the synonymization of the two genera only occurred because CARLGREN (1949) placed the type species of *Aulactinia* in *Bunodactis*. Thus, they still consider *Bunodactis* as valid until *Aulactinia* is revised, including its type material. In this study, we follow SPANO *et al.* (2013) and consider *Bunodactis* as valid.

Two species of *Bunodactis* have previously been described from Patagonia; *B. patagoniensis* (Carlgren, 1899) from Puerto Madryn (42°46'S, 65°02'W, Chubut Province, Argentina) and *B. octoradiata* (Carlgren, 1899), which is distributed in the southern most regions of Argentina and Chile (Tierra del Fuego and Straits of Magellan) and around the Malvinas/Falklands Island. The original description of *B. octoradiata* is incomplete because it does not depict characteristics of the internal anatomy and the cnidae. Although subsequent descriptions have provided some missing information (CARLGREN 1927, 1959), a detailed

study of the cnidom with images, biometry and abundance of cnidocysts, histological section of its internal anatomy, characteristics of male gametogenic tissue and ecological data of spatial distribution are still lacking. The aims of this work were to study the morphology of *B. octoradiata*, to provide new distribution records for it and to add information on its spatial distribution and density in the intertidal zone.

MATERIAL AND METHODS

During December 2009 and March 2010, a total of 114 specimens of *Bunodactis octoradiata* were collected from the following localities (Fig. 1) of Santa Cruz Province (southern Patagonia, Argentina): Península Foca (47°44'36"S, 65°50'26"W), Playa La Mina (49°09'23"S, 67°37'46"W), Cabo Curioso (49°12'11"S, 67°39'45"W), Punta Cueva (49°13'10"S, 67°40'20"W), and Península San Julián (49°15'13"S, 67°38'20"W). Sea water temperature from these areas oscillates between 5°C (winter) and 14°C (summer) and the region is considered to have cold waters of sub-Antarctic origin. All specimens were collected from the intertidal zone, anesthetized with MgCl₂ and then fixed in 5% seawater formalin and preserved in 70% ethanol. For histology, four specimens of *B. octoradiata* were embedded in paraffin, sectioned at 5 µm and stained with hematoxylin-eosin or Masson trichrome. Cnidae from 4 specimens were measured using a microscope with a micrometric

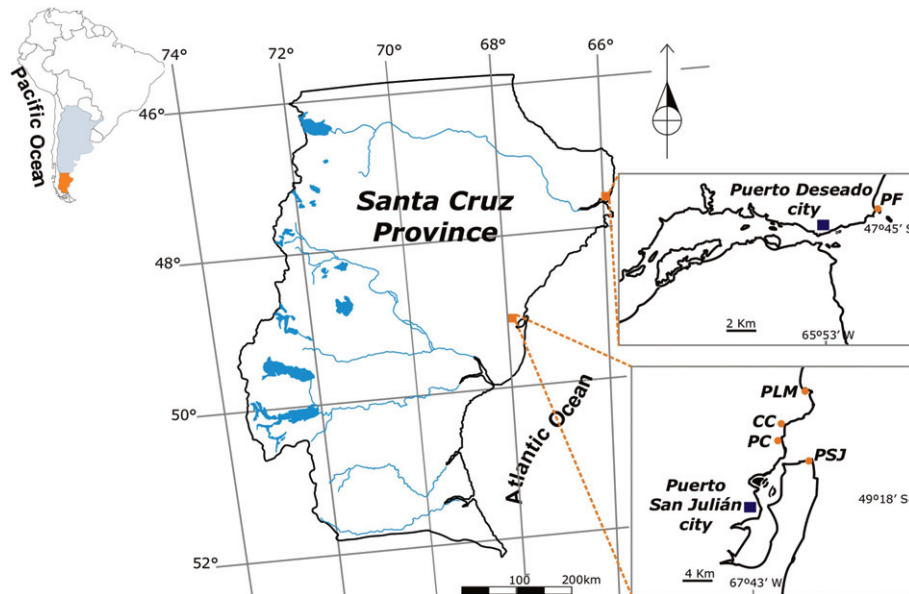


Figure 1. Sampling sites along the coast of Santa Cruz Province (Southern Patagonia, Argentina). Península Foca (PF), Punta Cueva (PC), Cabo Curioso (CC), Playa La Mina (PLM), Península San Julián (PSJ).

eyepiece at 1000X (oil immersion). From each individual, 40 unfired capsules were measured per type and tissue. Nematocysts nomenclature follows ENGLAND (1991).

Five specimens are deposited at the Actinarian Collection of the Departamento de Ciencias Marinas (Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata), with reference number C.A. 29.

The density of individuals of the species was estimated on the intertidal zone of Cabo Curioso. The sampling was carried out in transects at four stations, separated from each other by 50 m, from the middle intertidal to the lower intertidal zones. At each station, ten samples were taken using a quadrant (size: 40 x 40 cm), noting the number of specimens found to calculate density. Then, to establish the spatial distribution in the field we calculated the patchiness index as the relationship between variance and media (s^2/\bar{x}). If the values of this relationship close to 1, the distribution was considered aleatory; values of zero or close to zero indicated a uniform distribution, and values greater than 1 suggested that the distribution of the organism was grouped (SOUTHWOOD 1978).

TAXONOMY

Bunodactis octoradiata (Carlgren, 1899)

Bunodes octoradiatus Carlgren, 1899: 20-21.

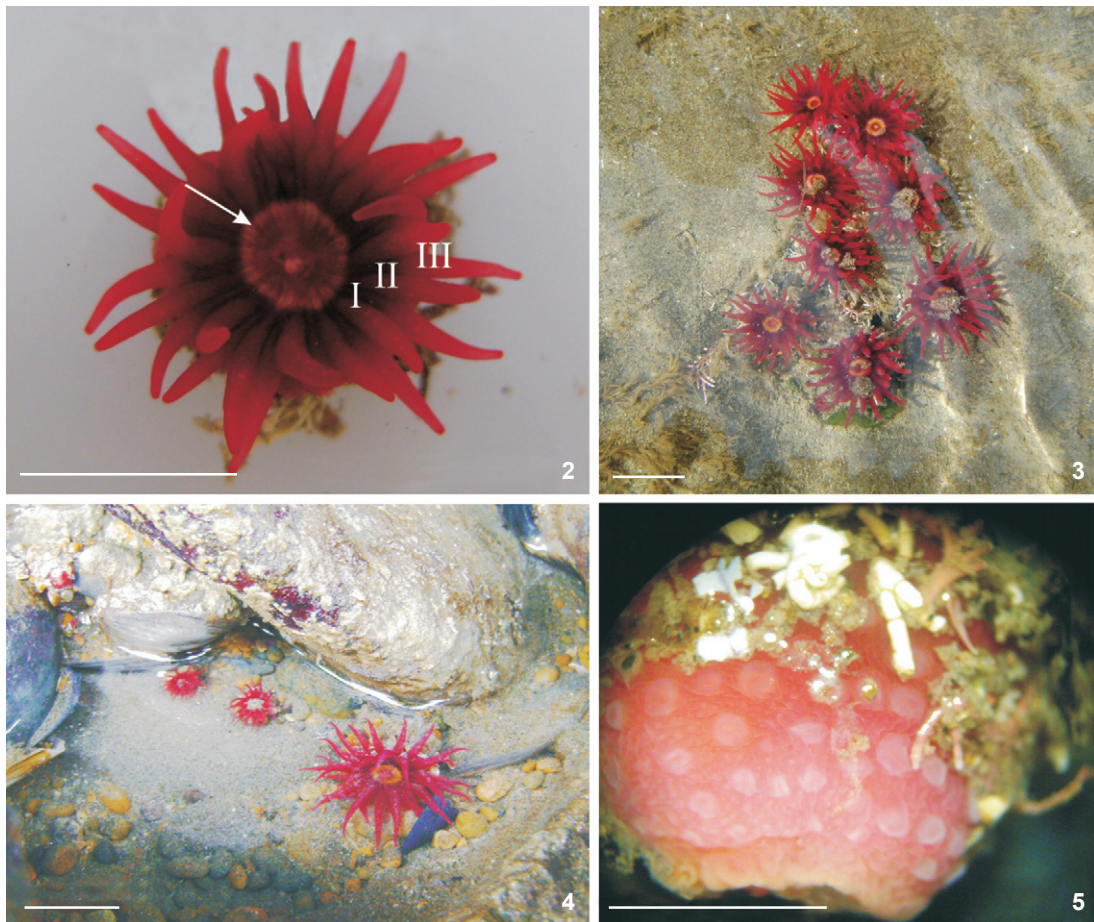
Cibrina octoradiata: Clubb, 1908: 7-8.

Bunodactis octoradiata: Pax, 1923: 26; Carlgren, 1927: 30-31; 1959: 22-23.

Aulactinia octoradiata: Rodríguez et al., 2007: 1879, 1890, 1899.

External anatomy. In preserved specimens, height of column is usually 12 mm (up to 19 mm), diameter of pedal disc is broadly 12 mm (up to 14 mm), diameter of oral disc up to 10 mm. The oral disc is dark red to black with visible mesenterial insertions, with a yellow annulus around the mouth, and 28 to 32 red tentacles arranged in three cycles (Fig. 2). The column is red, sometimes purple-colored distally. Whitish to pinkish verrucae, all about the same size, sparser in the nearest zone to pedal disc, irregularly arranged in approximately 30 vertical rows. They have foreign material such as shell remains, sand, gravel and algae attached (Fig. 5). On the margin of the column there are non-adhesive blister-shaped protuberances, clearly visible in preserved specimens, called here marginal projections. The color is very stable and very distinctive in this species, allowing easy field identification. Pedal disc well developed, more or less circular, with lightly scalloped edge in fixed material.

Internal anatomy. Actinopharynx deeply furrowed, with two well-developed siphonoglyphs. 16 pairs of perfect mesenteries arranged in two cycles (8 + 8) (Fig. 6); this arrangement is constant throughout the body with the same number of mesenteries distally and proximally. Retractor muscles diffuse, restricted and strong; parietobasilar muscles very strong with short free flap (Fig. 6); basilar muscles strong. Marginal sphincter muscle endodermal, circumscribed and palmate (Fig. 7). Longitudinal muscles of tentacles ectodermal (Fig. 8). Specimens with reproductive tissues were all males with all mesenteries fertile, in which spermatogenic follicles were observed (Figs 9 and 10). In mesenterial filaments the cnidoglandular, ciliated and reticular tracts are easily distinguishable (Fig. 11). Simple verrucae at



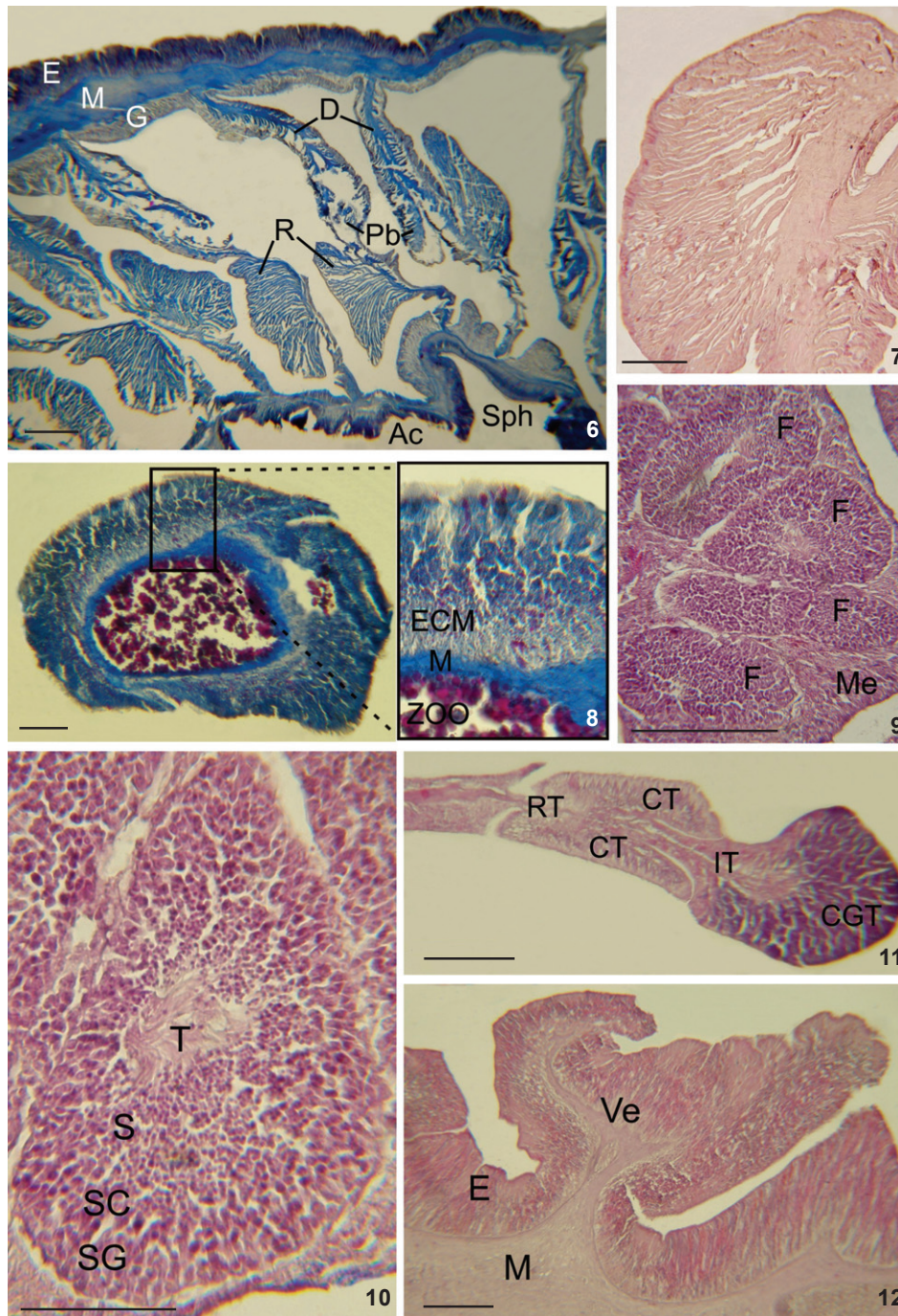
Figures 2-5. *Bunodactis octoradiata*: (2) oral view (arrow: yellow annulus; I, II and III: 1st to 3rd cycle of tentacles); (3) aggregation of individuals in situ; (4) individuals in a tide pool; (5) lateral view of column showing the verrucae and attached material. Scale bars: 10 mm.

column, both endocoelic and exocoelic (Fig. 12). Zooxanthellae with mean diameter of approximately 5 μm were observed in the endoderm of tentacles, but they were not found in other structures of specimens. This is the first time that their presence has been reported in this species (Fig. 8).

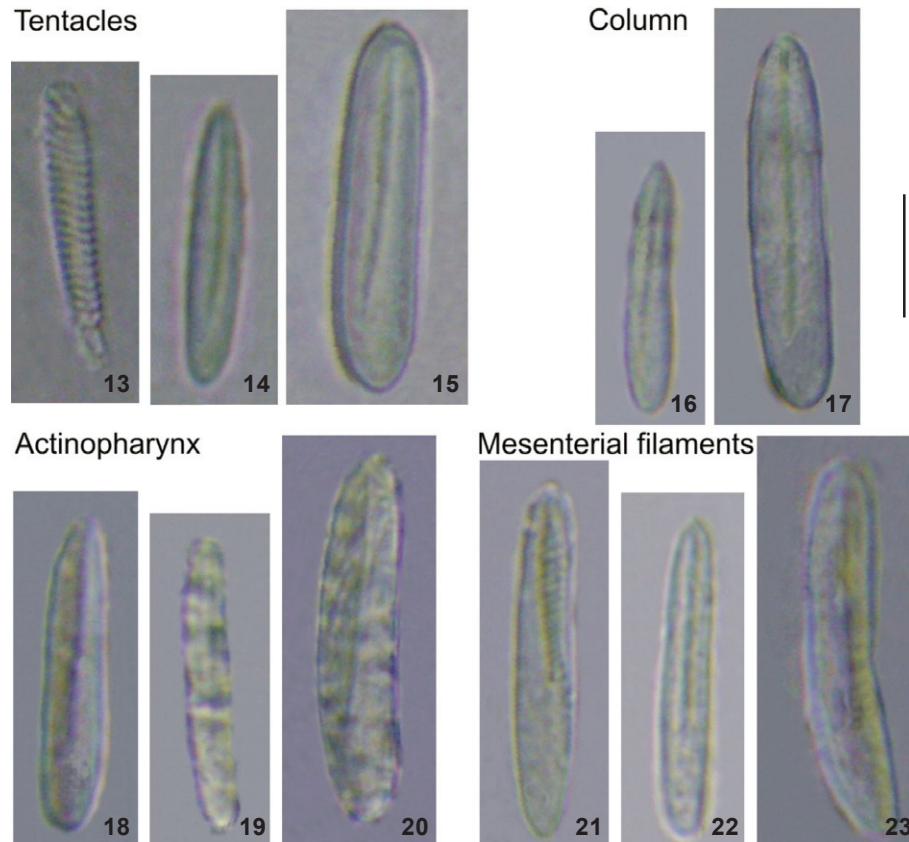
Cnidom. Spirocysts (Fig. 13), basitrichs (Figs 14-17, 19-20 and 22-23) and microbasic p-mastigophores (Figs 18 and 21) comprise the cnidom of *B. octoradiata*. See Table I for distribution, size and abundance of cnidae. All types have representative abundances in respective structures; the cnidom is qualitatively very similar between structures with the exception of spirocysts that are found only in tentacles. We also analyzed the cnidom in marginal projections. They had the same types of cnidocysts with similar sizes and abundances as in the rest of the column.

Ecological aspects. In the present study, *B. octoradiata* was found on rocky shores of the cities of Puerto Deseado and Puerto San Julián (Fig. 1). It is a common species in the inter-

tidal zones, inhabiting both exposed and protected areas, and shares habitat with other common sea anemones such as *Antholoba achates* (Drayton in Dana, 1846) and *Metridium senile* (Carlgren, 1899). Individuals of *B. octoradiata* were frequently found aggregated (Fig. 3), and usually in particular microhabitats such as crevices and tide pools (Fig. 4). Column is cover with remains of shells, sand, gravel and algae (Fig. 5). The abundance of *B. octoradiata* in Cabo Curioso was high from the middle intertidal to the initiation of lower intertidal (stations 1 to 3) and declined drastically to the lowest zone of the lower intertidal (station 4) on the border with the subtidal. The mean density was: 45 individuals per m^2 (sd = 45.5) at station 1; 35.6 individuals per m^2 (sd = 26) at station 2; 43.75 individuals per m^2 (sd = 21.87) at station 3, and 5.6 individuals per m^2 (sd = 7.43) at station 4. The values of the relationship between variance and media were 46 for station 1; 19 for station 2; 10.9 for station 3, and 9.8 for station 4; all cases indicated a grouped distribution of the species in the field.



Figures 6-12. *Bunodactis octoradiata* internal anatomy: (6) cross section through the column proximal to the actinopharynx (Ac) (D: directive mesenteries, E: ectoderm, G: gastrodermis, M: mesoglea, Pb: parietobasilar muscles, R: retractor muscles, Sph: siphonoglyph); (7) circumscribed palmate sphincter in longitudinal section, (8) tentacle cross section showing ectodermic muscles (ECM) and zooxanthellae (ZOO); (9) fertile mesentery with spermatogenic follicles (F) (Me: mesentery); (10) Spermatogenic follicles detail (S: spermatozooids, SC: spermatocytes, SG: spermatogonia, T: tails of spermatozooids); (11) mesenterial filament (CGT: cnidoglandular tract, CT: ciliar tract, IT: intermediate tract, RT: reticular tract); (12) longitudinal section of the column showing a verrucae (Ve) (E: ectoderm, M: mesoglea). Scale bars: 6 = 200 μ m, 7 = 100 μ m, 8 = 100 μ m, 9 = 50 μ m, 10 = 50 μ m, 11 = 100 μ m, 12 = 200 μ m.



Figures 13-23. Cnidom of *Bunodactis octoradiata*: (13) spirocyst; (14, 16, 19, 22) basitrich I; (15, 17, 20, 23) basitrich II; (18, 21) microbasic p-mastigophore. Scale bar: 10 μ m.

Table I. Distribution and size of cnidae of *Bunodactis octoradiata*. Range, length and width (sd = standard deviation). N: total number of measured cnidocysts. Ratio: proportion of number of specimens in which each cnida was found over the number of specimens examined. Abundance: (+) rare, (++++) very common.

Tissue and Cnida type	Length (μ m) (min-max) mean \pm sd	Width (μ m) (min-max) mean \pm sd	N	Ratio	Abundance	Range mentioned by CARLGRÉN (1927*, 1959) length x width (μ m)
Tentacles						
Spirocyst	(18-30) 24.40 \pm 3.32	(3-4) 3.10 \pm 0.30	40	4/4	++++	(14-26) x (1-2.5)
Basitrich I	(17-27) 19.82 \pm 1.66	(2-4) 3.05 \pm 0.31	40	4/4	+++	(14-17) x (3.5)
Basitrich II	(25-32) 28.62 \pm 1.77	(5-7) 5.37 \pm 0.54	40	4/4	++	(21-26) x (5-6.3)
Column						
Basitrich I	(11-20) 15.57 \pm 1.72	(2-3) 2.95 \pm 0.38	40	4/4	++++	(13.4-18) x (-)
Basitrich II	(23-34) 28.70 \pm 2.67	(4-8) 5.67 \pm 0.76	40	4/4	+++	(21-32.4) x (5-8.5)
Actinopharynx						
Microbasic p-mastigophore	(21-27) 24.37 \pm 1.46	(3-5) 4.15 \pm 0.42	40	4/4	++++	(18.3-21.8) x (3.5-4.2)
Basitrich I	(17-26) 20.30 \pm 2.13	(2-5) 3.12 \pm 0.51	40	4/4	+++	-
Basitrich II	(26-40) 32.20 \pm 3.33	(4-9) 4.95 \pm 1.06	40	4/4	+++	(24-31) x (4.2-7)
Filament						
Microbasic p-mastigophore	(21-30) 26.00 \pm 2.03	(3-5) 4.12 \pm 0.51	40	4/4	++++	(22.6-25.4) x (4.2)
Basitrich I	(18-23) 20.62 \pm 1.61	(3-4) 3.02 \pm 0.15	40	4/4	++++	(17-19.7) x (2.8-3)
Basitrich II	(26-39) 29.50 \pm 2.70	(3-6) 4.62 \pm 0.62	40	4/4	++++	(21-27.5) x (4.2-6.5)

* Only spirocysts.

DISCUSSION

Bunodactis octoradiata has been previously recorded from several localities from the southernmost end of South America (Chile and Argentina). We extend its geographical distribution range northwards, over the coast of Santa Cruz Province in the region of Puerto San Julián City and Puerto Deseado City, Argentina. Thus, *B. octoradiata* is distributed between 47°44'36"-59°15'29"S and 60°42'20"-71°55'19"W. In Table II all localities and coordinates are detailed.

There is controversy about the validity of *Aulactinia* and *Bunodactis*. CARLGREN (1949) placed the type species of *Aulactinia*, *A. capitata* Agassiz in Verrill, 1864 in *Bunodactis*, stating that *Bunodactis* was valid. However, members of *Bunodactis* never have holotrichs in the marginal structures (CARLGREN 1949) or in the column (ENGLAND 1987), as seen in the analyzed specimens in the current study. Afterwards, DUNN *et al.* (1980) pointed out that the character that distinguishes *Aulactinia* from *Bunodactis*, as defined by CARLGREN (1949), is only the presence of holotrichs (mentioned as atrichous) in the outer tentacles. DUNN *et al.* (1980) broadened the generic definition to include both previous taxa, instead of creating a new taxon, and used *Aulactinia* as the senior synonym.

On the other hand, SPANO *et al.* (2013) noted that the controversy between these genera was caused by CARLGREN (1949), who placed the type species of *Aulactinia* in *Bunodactis*, and also that the replacement of DUNN *et al.* (1980) was carried out without examining the type-material of *Aulactinia*. Thus,

they consider *Bunodactis* as still valid until *Aulactinia* is revised by including its type material. We agree with SPANO *et al.* (2013) and thus use the generic name *Bunodactis* in this study, and we also consider that the synonymization with *Aulactinia* is an unsolved problem that requires further comprehensive studies, which should include morphological and molecular characters obtained from the greatest possible number of species of the two genera in order to solve this controversy.

The presence of zooxanthellae in *B. octoradiata* had not been previously reported, and can be considered unusual for a relatively cold-water species such as this sea anemone. Regarding the cnidae, CARLGREN (1927) measured the size of the nematocysts from all tissues of *B. octoradiata*, with the exception of the mesenterial filaments, pointing out two types in the column, one type in the tentacles and two types in the actinopharynx, but he did not discriminate these into different morphological types. Additionally, CARLGREN (1927) also identified and measured spirocysts in the tentacles. Subsequently, CARLGREN (1959) identified the types of nematocysts from the different structures and provided their size ranges; these are compared in Table I with the cnidom described in the current study. The size ranges (length and width) of all types of cnidocysts in this study are similar but broader when compared to those found by CARLGREN (1927, 1959). Furthermore, with regard to the composition of cnidae, the current results are similar except that we were able to identify two types of basitrichs from the actinopharynx whereas CARLGREN (1959) mentioned only one.

Table II. Distribution of *Bunodactis octoradiata*.

Locality	Coordinates	Source
Península Foca	47°44'36"S, 65°50'26"W	This work
Playa La Mina	49°09'23"S, 67°37'46"W	This work
Cabo Curioso	49°12'11"S, 67°39'45"W	This work
Punta Cueva	49°13'10"S, 67°40'20"W	This work
Península San Julián	49°15'13"S, 67°38'20"W	This work
Malvinas Island	51°21'36"S, 60°42'20"W	CLUBB (1908), CARLGREN (1927, 1939)
Caubunco	53°01'40"S, 70°49'12"W	Sebens & Paine (1978)
Punta Arenas	53°10'48"S, 70°55'18"W	Carlgren (1899, 1959)
Punta Santa María	53°22'36"S, 70°57'06"W	Carlgren (1959)
Smyth Channel Isthmus Bay	53°28'38"S, 70°43'12"W	Carlgren (1899)
Strait of Magellan	53°30'06"S, 70°42'10"W	Carlgren (1899)
Port Bulnes	53°37'44"S, 70°55'10"W	Sebens & Paine (1978)
Port Hambre	53°37'13"S, 70°55'48"W	Sebens & Paine (1978)
Port Gallant	53°44'30"S, 71°55'19"W	Carlgren (1899)
New Year Island	54°43'33"S, 64°07'22"W	Carlgren (1927)
Staten Island	54°46'15"S, 64°16'22"W	Carlgren (1899)
Beagle Channel, Lapataia Nueva	54°49'42"S, 68°34'10"W	(Carlgren, 1899)
Navarino Island, Puerto Toro	55°04'27"S, 67°04'15"W	(Carlgren, 1899)
Saddle Island	55°31'19"S, 68°04'38"W	(Dana, 1846)
Lennox Island	59°15'29"S, 67°04'12"W	(Carlgren, 1899)

The presence of marginal projections was previously mentioned by CARLGREN (1899, 1927) as pseudo-marginal-spherules. Firstly (1899) he stated that nematocysts were more numerous in these structures than in the rest of the column; however, after carrying out new examinations he (1927) found that the cnidom of the spherules and other parts of the column were the same, in agreement with our results. The marginal projections were described by DALY (2003) as permanent, fingerlike projections, with histology and cnidom identical to those of the column, with function unknown. All examined specimens in this study were males, but CARLGREN (1927) mentioned the presence of large ova rich in yolk, and cited CLUBB (1908), who found embryos developing in external brood-chambers. Undoubtedly the reproductive biology of this sea anemone is a very interesting topic to be studied in the future, along with other aspects such as the fauna and flora attached to its column and its trophic ecology.

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