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Gigantic oocytes in the deep sea black coral *Dendrobathypathes grandis* (Antipatharia) from the Mar del Plata submarine canyon area (southwestern Atlantic)

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ABSTRACT

The black coral *Dendrobathypathes grandis* (Cnidaria: Antipatharia) is studied for the first time in the southwestern Atlantic off Argentina. This is the only antipatharian reported from the Atlantic between 35°S and 54°S. Eleven specimens were collected at depths of 819–2204 m during three expeditions to the Mar del Plata submarine canyon (2012–2013); seven were females. The species is gonochoric, and the polyps in female colonies contain up to nine oocytes per polyp, which can reach 1500 µm in diameter. In contrast, the largest oocyte currently reported for antipatharians is 500 µm, and usual diameters do not exceed 200 µm. These large oocytes have over 20 times more volume than the biggest oocyte reported, but over 800 times more volume when compared with the common oocyte size of the group. Sperm size and morphology is similar to previous data from other species. As in previous studies, neither embryos nor larvae were found in any specimens. This species was previously only reported from waters off South Georgia Island, and so these specimens expand the known distribution north by 1800 km.

1. Introduction

Antipatharians (commonly known as black corals) form a relatively small order of anthozoan cnidarians that includes about 250 species in 41 genera and 7 families (Brugler et al., 2013). Although they are present from pole to pole and from 4 m to 8900 m, they are most common in tropical and subtropical waters (including shallow water tropical reefs), and predominantly in the deep sea, since about 75% of the species occur from 50 m deep (Brugler et al., 2013; Cairns, 2007). Since they are (or were) collected in order to make jewelry, medicines and for food (Wagner et al., 2012), the entire group is listed in the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES), appendix II.

Numerous species have abeen found in the Atlantic Ocean (especially in the north), but there is a gap in the southwestern Atlantic (SWA) between Uruguay (35°S) and the South Georgia Island (54°S). No studies of antipatharians have been reported in this region.

Antipatharians are mostly a deep-water species with over 75% found at depth below 50 m (Cairns, 2007). Only little is known about their reproduction (Wagner et al., 2011): most species studied so far are gonochoric (only two are hermaphrodites, Wagner et al., 2011); fertilization occurs externally and oocytes are usually under 200 µm in diameter, with a single report of a particularly large size oocytes up to 500 µm in specimens of *Schizopathes affinis* (Brook, 1889) from about 1600 m in the northern Indian Ocean (Cooper, 1909).

The genus *Dendrobathypathes* Opresko (2002) was created to accommodate two new species: *Dendrobathypathes grandis* Opresko (2002) from South Georgia Island (417–686 m) and *D. isocrada* Opresko (2002) from Australia and New Zealand (659–1110 m). Later, two new species were added: *D. boutillieri* Opresko (2005) from Alaska (1707–2109 m) and *D. fragilis* Opresko (2005) from Japan (587 m).

Here we study 11 specimens of *D. grandis*, providing additional descriptive characters, extending the species distribution about 1800 km to the north into the SWA reporting the presence of the largest oocytes to date in the order Antipatharia worldwide.

2. Materials and methods

The specimens studied are deposited in the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN), Argentina (Table 1). During 2012 and 2013 three expeditions were made to the Mar del Plata submarine canyon and surrounding area onboard of the R/V *Puerto Deseado* (Fig. 1). Samples were taken from 201 m to 3447 m depths at 64 stations using fishing nets or trawls. The antipatharians

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Table 1
Distribution of the collected specimens during "Talud Continental" expeditions on board of the O/V *Puerto Deseado*.

Station	Lat. (°S)	Long. (°W)	Depth (m)	Number of specimens	Date	Museum collection number
15	38° 0500´	54° 25,069´	1200	1	August 2012	MACN-IN 41148
16	37° 57,288´	54° 23,456´	1308	4	August 2012	MACN-IN 41149
31	38° 1499´	54° 44,171´	819	1	August 2012	MACN-IN 41150
35	37° 54,045'	54° 24,091'	1245	1	May 2013	MACN-IN 41151
36	37° 57,508'	54° 23,989'	1289	2	May 2013	MACN-IN 41152
42	37° 59,110'	54° 41,136'	877	1	May 2013	MACN-IN 41153
56	37° 54.840'	54° 2.470'	2204	1	September 2013	MACN-IN 41154

were fixed in 70% or 96% ethyl alcohol, 4% formalin in seawater or dried. To gather the taxonomic information from the skeleton the soft tissue was removed manually from small fragments of the colony using a fine tip tweezer, and the skeletal samples were mounted on a scanning electron microscope (SEM) stub for examination of the spines (specimen MACN-IN 41148). The samples were coated with 60% gold 40% palladium, and SEM images were taken using a Phillips XL30 microscope. The separation between spines was measured between the center of the bases of two adjacent spines, and the height from the base to the apex. All measurements correspond to the specimen MACN-IN 41151, except when otherwise stated. Interior and distal angle measured on specimen MACN-IN 41149. For the reproductive information, the size of the largest oocytes was measured with micrometer digital calypter directly from oocytes separated from the polyp (20 oocytes from four specimens) with tweezers under stereoscopic microscope or using an ocular micrometer (seven oocytes from one specimens). To estimate the fecundity of the colony, a complete primary pinnule of the middle zone of one colony was cut and weighted. The total number of polyps present was registered. All the polyps in the fragment were dissected under stereoscopic microscope and the proportion of polyps with oocytes was calculated. The total number of fertile polyps in the colony was calculated by multiplying the number of fertile polyps in the sectioned pinnule by the total weight of the colony and then divided by the weight of the sectioned pinnule. The maximum number of oocytes in the colony was calculated by multiplying the total number of fertile polyps in the colony by the maximum number of oocytes found in a single polyp. The maximum number of oocytes in one polyp was determined in the histological serial section of fourteen polyps from three specimens. For histological studies several polyps from all the colonies (except the dried ones) were dehydrated, embedded in paraffin or Leica historesin®, sectioned at 5–10 µm and stained with Azocarmin triple stain or eosin-hematoxylin stain (Humason, 1967, Suvarna et al., 2013). Digital images were taken using an AxioCam HRc camera on an Axio Imager Z1 Zeiss microscope. Feret diameter of oocytes and spermatic cysts were calculated from digital images of the oocytes using the software Image J (Schneider et al., 2012). Oocyte diameter was measured for 18 oocytes in sections where the nucleus and nucleolus was visible.

3. Results

3.1. Species identification

Order Antipatharia
Family Schizopathidae Brook (1889)
Dendrobathypathes Opresko (2002)

Diagnosis (after Opresko, 2005*, modifications in bold)*

Corallum monopodial to extensively branched, and pinnulate. **Basal disc not modified, attached to hard substrate**. Stem, branches and primary pinnules generally in one plane. Primary pinnules arranged alternately in two anterolateral to lateral rows. Secondary pinnules, when present, arranged uniserially and/or biserially on anterior (polypar) or anterolateral sides of primaries. Tertiary and higher order subpinnules, when present, also arranged uniserially and/or biserially, on the anterior (polypar) or anterolateral side of the lower order subpinnules. Spines triangular to subconical in lateral view, smooth, acute,

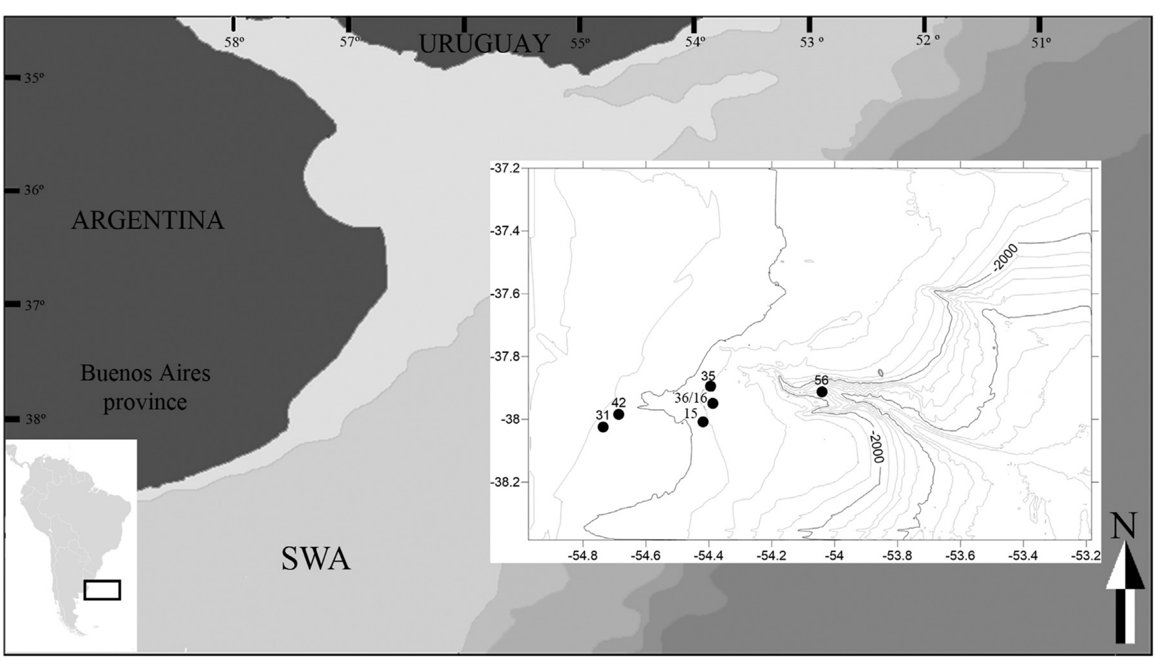

Fig. 1. *Dendrobathypathes grandis* in the southwestern Atlantic (SWA) off Argentina.

Table 2
Morphological traits of *Dendrobathypathes grandis*. n, number of measurements; N, number of specimens.

Morphological traits	Min.	Max.	Mean	Standard deviation	n	N
Polyp diameter (mm)	2.5	4.5	3.5	0.6	18	3
# polyp cm^{-1}	2.5	4.0	3.3	0.5	10	4
Distance between rows of spines (µm)	95.2	190.5	135.1	31.0	8	1
Separation between spines in the same row (µm)	187.3	454.5	345.0	82.6	11	1
Height of spines (µm)	39.7	107.6	63.7	21.6	8	1
Wide of spines (µm)	100.9	241.1	157.41	51.3	8	1

simple or rarely bifurcate at apex; up to about 0.2 mm tall; subequal or with polypar spines larger than abpolypar spines. Maximum size of polyps 2.5–5 mm in transverse diameter.

Type species: *Dendrobathypathes grandis* Opresko (2002)
Included species: *D. grandis*; *D. isocrada* Opresko (2002); *D. boutillieri* Opresko (2005) and *D. fragilis* Opresko (2005).

Distribution of the Genus: South Georgia Island, Australia, New Zealand, Alaska and Japan. New record from the southwestern Atlantic off Mar del Plata city.

3.1.1. Description of specimens of D. grandis collected in the SWA

Of several hundred anthozoan cnidarians collected, only 11 specimens of *D. grandis* were collected at seven stations (Table 1, Fig. 1). Live polyps, bright orange in color, are up to 4.5 mm in diameter (Table 2) and situated on the front side of the corallum (Fig. 2a). There are about three polyps per cm (rarely four) (Table 2), with a separation between 0.35–0.7 mm. The biggest specimen (MACN-IN 41151) is 38 cm tall, including the base, 9.4 cm wide, and 6.15 cm thick. The holdfast is dome shaped, 12.9 mm high and 15.4 mm in diameter (max.), however, it is less developed in other specimens (Fig. 2e-g). There are up to four order pinnules; the diameter of the stem is 4.3 mm close to the holdfast and 0.9 mm at the distal end. The first primary pinnule occurs 2.4 mm above the base (only a small fragment remains). These pinnules are arranged anterolaterally on each side of the stem in an alternate fashion with a distal angle at about 60°–70°. One of the specimens (MACN-IN 41149) presents a distal lateral ramification of about 60 mm. The separation between primary pinnules is about 5.0–24.0 mm and they are 40.0–55.0 mm long (25.0 and 50.0 in MACN-IN 41150). The angle formed by the lateral pinnules (interior angle) is about 120°. The pinnules are curved backward, forming an incomplete channel in the abpolypar region of the specimen. The secondary pinnules arise uniserially from the primaries, up to eight (usually six), more or less perpendicular to the primary; about 0.65 mm in diameter and up to 40 mm long. From these arise tertiary pinnules in the same plane, up to four (usually one or two) about 0.42 mm in diameter and up to 20 mm long. Some quaternaries pinnules may be present in large specimens, especially in the distal part of the specimen. One quaternary pinnule presents a small ramification.

The pinnular spines are conical, acute and compressed (Fig. 3d). They are arranged in regular rows (Fig. 3a, b), with a row separation between 95.2 µm and 190.5 µm (Table 2). The distance between the spines in each row is from 187.3 µm to 454.5 µm (Table 2). The spines are from 39.7 to 107.6 µm tall (Table 2); the larger spines tend to occur on the polypar side (Fig. 3c). Many of the spines tend to be directed toward the distal end of the pinnule.

3.2. Reproduction of Dendrobathypathes grandis from the SWA

Seven of the specimens collected were female and one was male.

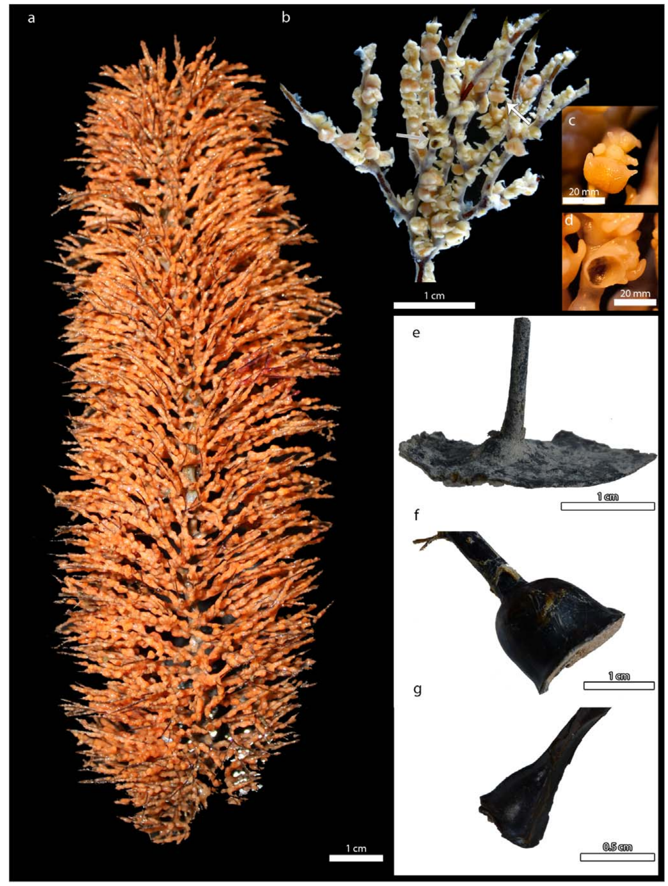

Fig. 2. *Dendrobathypathes grandis.* a, recently collected specimen (MACN-IN 41150); b, section of specimen MACN IN-41149 showing preserved polyps with large oocytes, c, close-up of a swollen polyp, indicated by the white arrow in b; d, close-up of a polyp after the removal of a large oocyte, indicated by the gray arrow in b; e-g, bases of specimens MACN-IN 41154, MACN-IN 41151 and MACN-IN 41149, respectively.

Other three were dried and no information about sex could be obtained from them. Small and large oocytes were found in samples from May and August. Five specimens presented oocytes clearly visible with the naked eye or using a stereoscopic microscope. They were big orange spheres seen through the polyp tissues once fixed (Fig. 2b–d). The specimens presented oocytes in different developmental stages (Fig. 4e), up to nine per polyp and up to 11844 oocytes per colony (Table 3). In the same polyp it was possible to find previtelogenic oocytes (up to 196.6 µm, Fig. 4a), intermediate vitelogenic oocytes (Fig. 4b–d)) and one or two large oocytes, over 900 µm (Fig. 4f). Largest oocyte found measured under stereoscopic microscope was 1575 µm (Table 3). Average size measured with digital caliper of the largest fresh oocytes found was 1381 µm (1421.4 µm with ocular micrometer) (Table 3); largest vitelogenic oocyte measured in histological section with nucleus and nucleolus visible was 1407.8 µm. Maximum diameter of yolk granules in a large oocyte was 23 µm. No evidence of embryos or larvae was found in any of the females polyps analyzed. Polyps with large oocytes maintain their usual structure (i.e. no loss of tentacles were detected), except for being swollen. Spermatic cysts were found in different developmental stages in the same polyp (Fig. 4g), up to mature sperm (Fig. 4h), (Table 3). Anterior portion of the spermatozoa more or less round, about 2.5 µm. Largest cysts 351.7 µm (Table 3). All eight polyps analyzed from MACN-IN 41149 had spermatic cysts.

4. Discussion

Based on general morphology, our specimens clearly belong to the genus *Dendrobathypathes*. The specimens found (n = 11) are very

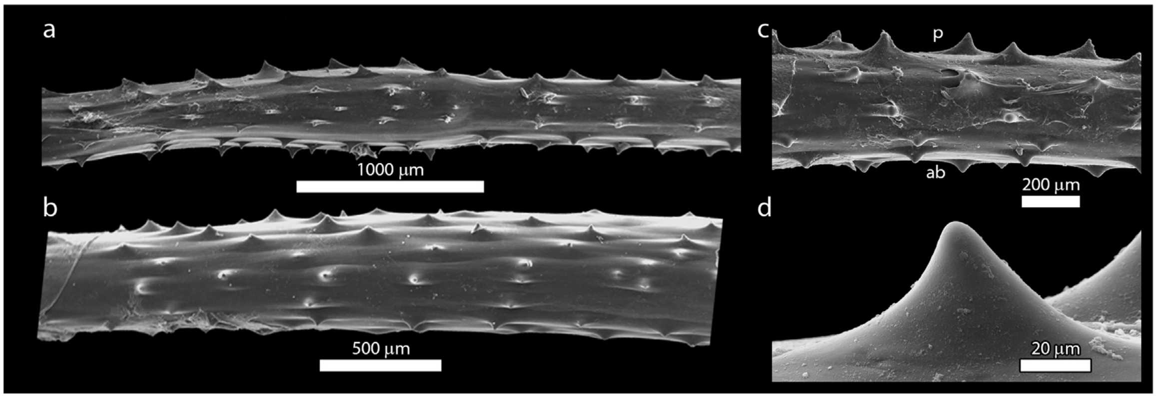

Fig. 3. Spines of *Dendrobathypathes grandis* MACN-IN 41148. a, on a primary pinnule; b, on a secondary pinnule; c, pinnule showing polypar (p) and abpolypar (ab) spines; d, close up of a spine.

similar to the type specimens of *D. grandis*, although some differences were found in size and disposition of the pinnular spines. Our specimens present spines clearly arranged more or less evenly spaced in longitudinal rows (see Fig. 3), and the spines are not as large as in the type specimens. Most of our specimens lack the lateral branch present in the holotype and paratype, and the stem is thicker (although this may be a result of the lack of the holdfast in type specimens). Nevertheless, the differences are not enough to describe a new species; they may represent variability between different populations.

The lack of information of antipatharians in the southern part of the

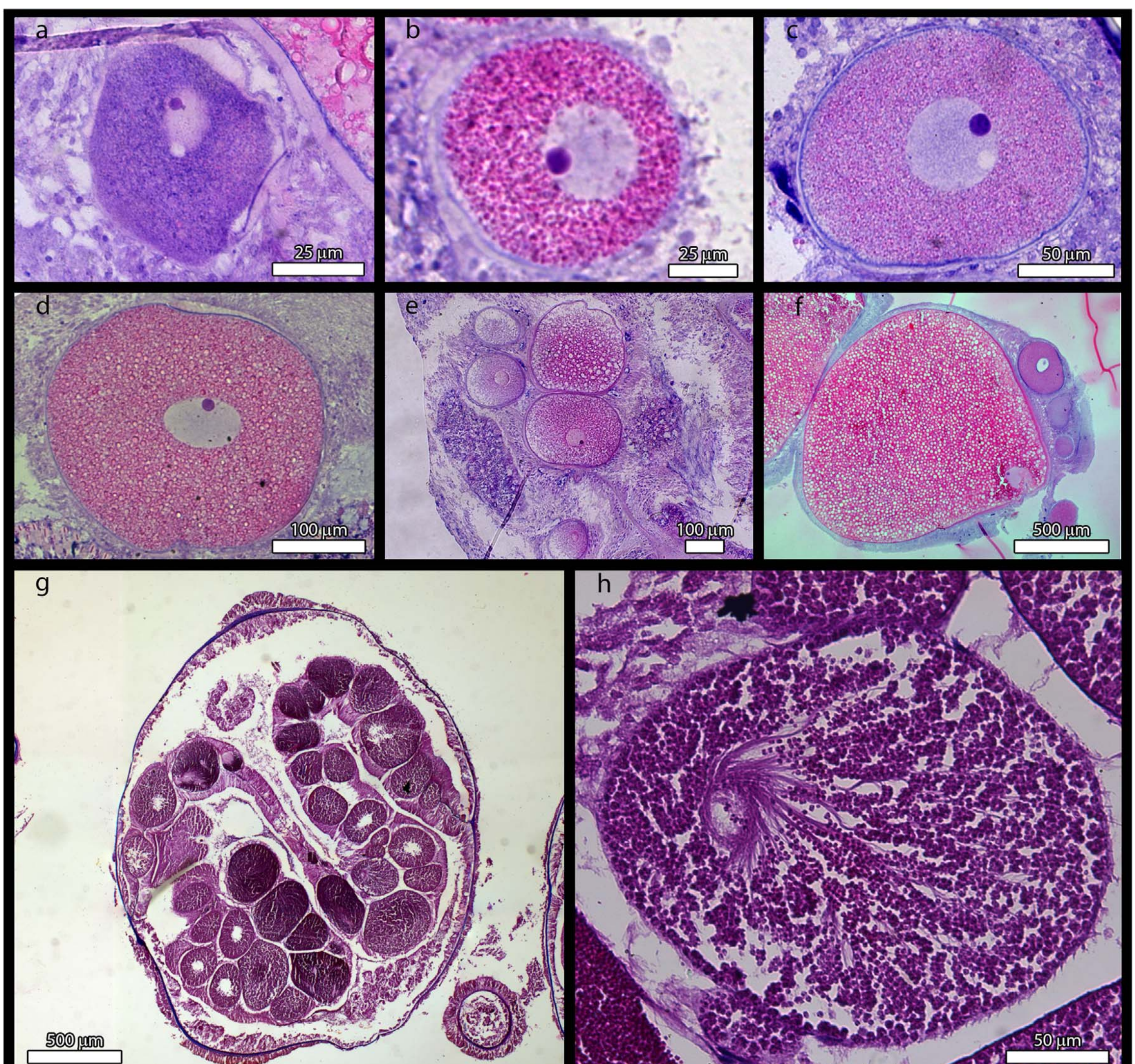

Fig. 4. Histological sections of *Dendrobathypathes grandis*. a, previtelogenic oocyte; b–d, small vitelogenic oocytes; e, oocytes in different developmental stages; f, large vitelogenic oocytes; g, spermatic cysts in different developmental stages; h, spermatic cysts with mature sperm.

Table 3
Reproductive traits of *Dendrobathypathes grandis*. n, number of measurements; N, number of specimens.

Reproductive trait	Min.	Max.	Mean	Standard deviation	n	N
# polyps in colony	–	2800	–	–	–	1
# fertile polyps in the colony	–	1316	–	–	–	1
# oocytes per fertile polyp	3	9	5.8	2.0	14	3
# of oocytes in the colony	–	11844	–	–	–	1
Oocytes Feret diameter (µm)	89.0	1407.8	284.1	366.1	18	3
Previtelogenic oocytes Feret diameter (µm)	59.2	196.6	89.0	29.8	14	3
Vitelogenic oocytes Feret diameter (µm)	308.1	1407.8	849.7	451.4	4	3
Diameter of largest fresh oocytes with calypter (µm)	920.0	1575.0	1381.3	159.8	20	4
Diameter of largest fresh oocytes with ocular micrometer (µm)	1200.0	1500.0	1421.4	122.0	7	1
Spermatic cyst Feret diameter (µm)	125.2	351.7	263.5	57.5	16	1
Sperm "head" size (µm)	2.2	2.6	2.5	0.18	4	1

SWA reflects the lack of study of the deep sea invertebrates in the region, not the absence/low abundance of the group. The closest records to this area are from the South Georgia Island (*D. grandis*) and east of Montevideo, Uruguay, 35°39'S, 50°47′W, 3474 m (Brook, 1889) (*Schizopathes crassa*). These two locations are separated from each other by about 2200 km. Portela et al. (2012) mentioned *Dendrobathypathes grandis* in a book chapter. No description, photographs, reference to deposited specimens, precise localities, depths, related information, or citation sources were provided. As far as we know, the present paper represents the first study of an antipatharian from this region in the scientific literature.

The order appears not to be abundant (or diverse) in this region. We found specimens during the three expeditions made, so this may be due to the lack of studies in the deep sea region of the southern part of the SWA. The location where the specimens were found was included in an area of high habitat suitability (Yesson et al., 2015), which is as long as the continental slope of the Argentinian Sea, and this species likely occurs all along the slope to the south.

The specimens of *Dendrobathypathes grandis* from the SWA present extraordinary large oocytes for the order Antipatharia. The presence of oocytes of such a large size is responsible for most of the bright orange color of the polyps. The biggest oocyte size recorded up to date in the order is about 500 µm, but usual sizes are under 150 µm (see Wagner et al., 2011). Our maximum oocyte diameter is 1407.8 µm. In consequence these oocytes have over 20 times more volume than the biggest oocyte reported in antipatharians (i.e. 500 µm), but over 800 times more volume when compared with the common oocyte size of the group (i.e. 150 µm).

Large oocytes are nevertheless not unusual among other hexacorals. Large eggs have been reported in sea anemones, like in *Urticina lofotensis* (800 µm, Wedi and Fautin Dunn, 1983), *Urticina crassicornis* (700 µm, Chia and Spaulding, 1972), *Actinostola spetsbergensis* (750 µm, Riemann-Zürneck, 1976) and *Aulactinia vladimiri* (2200 µm, Sanamyan et al., 2015). Also in scleractinian corals: 1200 µm in *Flabellum angulare* (Mercier et al., 2011), 925 µm in *Flabellum alabastrum* (Waller and Tyler, 2011), and even over 5000 µm in *Flabellum curvatum* and *Flabellum impensum* (Waller et al., 2008). Nevertheless, such large oocytes have not been found before in the order Antipatharia, despite the fact that studies about reproduction in this group have included over 55 species from all over the world. More information on the group would increase our knowledge on possible ways of dispersal and geographical distribution of the species in the region.

The number of oocytes per polyp in our specimens (up to 9, 1407.8 µm max. diameter) is rather low compared to other black corals. Fecundity in other species can reach up to 173 oocytes (to 140 µm each) per polyp in *Antipathes fiordensis* (Parker et al., 1997), and to 309 oocytes (to 349 µm each) in *Antipathella wallastomi* (Rakka et al., 2016). Since oocyte size influences the number of offspring that can be produced (as the energy expended on individual offspring is increased, the number of offspring that parents can produce is decreased) along with the fitness of the offspring (Smith and Fretwell, 1974; Levitan, 2006), the size and number of oocytes in our specimens are in line with the different reproductive strategies adopted by different species (many small oocytes vs. few large oocytes). *A. fiordensis* and *A. wallastomi* present a large number of small oocytes, whereas *D. grandis* from the SWA exhibits a small number of large oocytes. Based on the yolk content of these oocytes, it is likely that this species produces a non-feeding lecitotrophic larvae, which has also been suggested by Goenaga (1977) for *Stichopathes* sp. Such a larva may be able to disperse using the ocean currents, which may explain the great distribution of the species. As in other species of antipatharians, no evidence of internal fertilization, and no evidence of hermaphroditism were found.

Only one male specimen was found. It presents polyps with several spermatic cysts, but only a few cysts contain mature sperm, and none was filled entirely. The spermatic cysts size and mature sperm form and size are similar to that reported by other authors (Gaino et al., 2008; Gaino and Scossia, 2009, 2010; Wagner et al., 2011). Since it was not possible to find any fully matured sperm cysts, it is probable that the specimen was not completely sexually mature, although females collected during the same expedition had large vitelogenic oocytes indicating maturity.

We found a large proportion of female over male colonies (7:1). Deviation from equal sex proportion in antipatharians has been reported in Hawaiian species, both in favor of female or male colonies (Wagner et al., 2011). Since we collected few specimens, it is not possible to rule out a bias in the sex proportion due to the low number of specimens found.

The finding of oocytes in different developmental stages in the same polyp and in samples from May and August may indicate a continuous reproductive strategy, although it would be necessary to analyze the development of the oocytes over a longer period of time to definitively establish the reproduction strategy and periodicity of the species. For the first time this study highlights the largest oocytes observed in the order of antipatharians. Additionally, our findings increase the knowledge of the distribution and reproductive biology of deep sea antipatharians, which is overall scarce for these deep sea species.

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