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NOTE

First record of a trophonema in black corals (Cnidaria: Antipatharia)

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Abstract The gametes of many groups of anthozoans contain a funnel-shaped cluster of specialized cells that facilitate the transport of nutritive substances, known as the trophonema. This structure has been found within all orders of anthozoans, with the exception of pennatulaceans, scleractinians, and antipatharians, the latter of which are commonly known as black corals. Based on the histological study of eight specimens of the black coral *Dendrobathypathes grandis* Opresko (Zool Med Leiden 76(22):411–442, 2002) from the southwestern Atlantic, we report for the first time the presence of a trophonema within the order Antipatharia. A trophonema was found only in oocytes from one of the seven female specimens studied. Since *D. grandis* contains the largest oocytes among antipatharians that have been studied to date, it remains unknown whether the presence of a trophonema is an exceptional case, or whether these structures have simply been overlooked in other black corals.

Keywords Argentina · Histology · Oocyte · Reproduction · Southwestern Atlantic

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Introduction

The cnidarian class Anthozoa is characterized by an exclusively polypoid lifestyle, as well as by possessing polyps that are radially divided by sheets of tissue, known as mesenteries. Some of these mesenteries contain the reproductive structures or gonads, which are gametogenic regions that contain aggregations of oocytes or sperm cysts (reviewed by Fautin and Mariscal 1991). Funnel-shaped clusters of specialized cells, known as the trophonema (Fig. 1a), are found in some groups of anthozoans and are thought to facilitate the transport of nutritive substances from the mesentery to gametes undergoing vitellogenesis (Schmidt and Zissler 1979; Schmidt and Schaefer 1980; Larkman and Carter 1982; Fautin and Mariscal 1991). The trophonema was first described by Hertwig and Hertwig (1879) in the oocytes of the sea anemone *Calliactis parasitica* (Couch, 1842) under the German word "Fadenapparates," which translates into filamental apparatus. Hertwig (1882) later described the same structure in the corallimorpharian *Corallimorphus rigidus* Moseley, 1877. Nyholm (1943) found this structure in a ceriantharian and termed it trophonema, a name still in use for this structure to this day (e.g., Dunn 1975; Fautin and Mariscal 1991; Shikina and Chang 2016; and references therein). Trophonemas are associated mostly with oocytes, although such structures have also been reported in association with sperm cysts (Larkman and Carter 1982).

The name of trophonema given by Nyholm (1943) implied that this structure was involved in nutrition; however, this suspected function was based only on circumstantial evidence (Fautin and Mariscal 1991). The nutritive function of the trophonema was only demonstrated close to 150 yr later, when Larkman and Carter (1982) studied this structure in the sea anemone *Actinia fragacea* Tugwell,

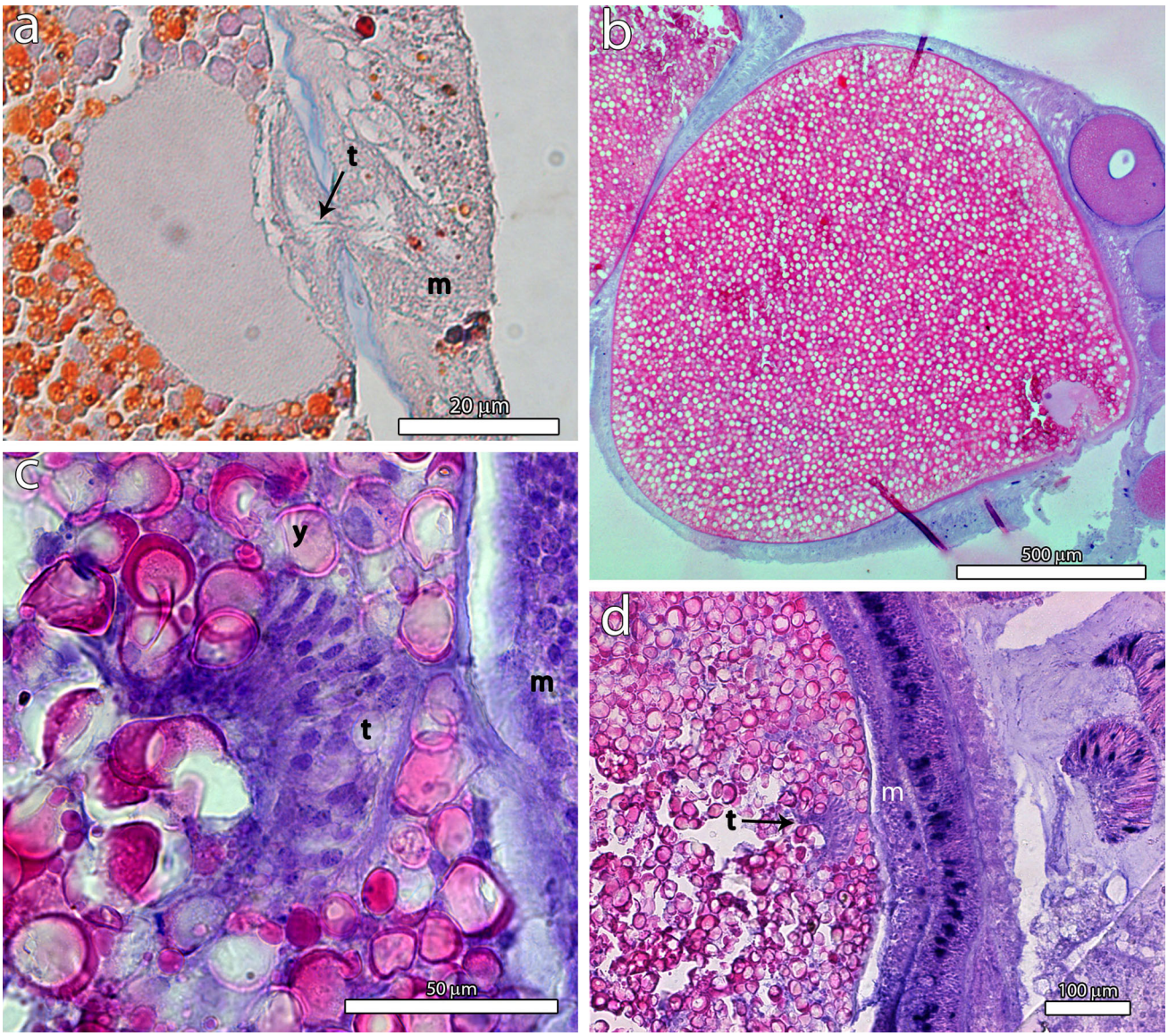

Fig. 1 **a** Trophonema in the sea anemone *Choriactis* sp. (stained with azocarmine triple stain); note the peripheral position of the nucleus. **b–d** *Dendrobathypathes grandis* oocytes. **b** Large oocyte with peripheral nucleus. **c** Trophonema close-up. **d** Oocyte with trophonema in general view (*m* mesentery, *t* trophonema, *y* yolk granule)

1856. Using electron microscopy and autoradiography, they found that the cells of the trophonema are in direct contact with oocytes, and that those cells are more active than the rest of endodermal cells in incorporating small molecules (Larkman and Carter 1982). To date, trophonemas have been found within all orders of anthozoans with the exception of pennatulaceans (Eckelbarger et al. 1998), scleractinians (Shikina and Chang 2016), and antipatharians (Schmidt and Schaefer 1980). The latter order, commonly known as the black corals, encompasses close to 250 described species, over 75% of which are restricted to depths below 50 m (Cairns 2007). Little is known about the sexual reproduction of antipatharians. This is largely because antipatharians are primarily found in deep (> 50 m) environments that are difficult to access, and consequently reproduction has only been studied for very few antipatharian species in detail (reviewed by Wagner et al. 2011, 2012). While information on the reproductive anatomy of antipatharians exists for 56 species, to date no study has found evidence for trophonemas within black corals (reviewed in Schmidt and Schaefer 1980; Wagner et al. 2012). Here, we provide the first report of a trophonema within the order Antipatharia, based on histological examinations of specimens of *Dendrobathypathes grandis* Opresko, 2002 collected from deep waters off Argentina in the southwestern Atlantic.

Materials and methods

During 2012 and 2013, three expeditions aboard the O/V *Puerto Deseado* (Talud Continental I, II and III) surveyed the Mar del Plata submarine canyon and surrounding area. A total of 11 specimens of *D. grandis* were collected during these expeditions at depths between 819 and 2204 m using fishing nets or trawls. Of these, three were dried, and the rest were fixed in 70% ethanol, 96% ethanol, or 4% formalin in sea water, the latter of which were transferred to 70% ethanol for long-term storage. The antipatharian specimens were all deposited in the Bernardino Rivadavia Argentinian Museum of Natural History (MACN) under collection numbers MACN-IN 41148 to 41153 (see Lauretta and Penchaszadeh 2017 for more information about these specimens). For histological analyses, several polyps from all of the ethanol/formalin-preserved specimens ($n = 8$) were dehydrated, embedded in paraffin or Leica HistoResin®, sectioned at 5–10 µm, stained with Azocarmin triple stain or hematoxylin–eosin stain (Humason 1967; Suvarna et al. 2013) and photographed with a Zeiss AxioCam HRc camera. Oocyte diameters were measured from photographs of histological sections as described by Lauretta and Penchaszadeh (2017).

Results and discussion

Of the eight specimens that were analyzed, one was a male and seven were females. The male specimen contained several spermatic cysts in different developmental stages, but none were full of sperm, because it is possible that it was not completely mature (Lauretta and Penchaszadeh 2017). No trophonema was observed in this male colony. The seven female specimens all contained oocytes in different stages of maturation, including specimens with large oocytes up to 1.5 mm in diameter (Fig. 1b) (Lauretta and Penchaszadeh 2017), which represent the largest oocytes found in black corals to date (see Wagner et al. 2012). Despite the fact that histological sections were made of several polyps with oocytes, trophonemas were only found in oocytes from one specimen (lot MACN-IN 41149; labeled 0236). The complete or partial structure of the trophonema was observed in six oocytes from five different polyps.

Many of the observed oocytes did not have the nucleus at a centered location, but rather shifted to the border of the oocyte. This was particularly noticeable in larger oocytes (Fig. 1b), but also seen in some smaller ones (i.e., $\sim$ 200 µm in diameter). The peripheral position of the nucleus is consistent with previous trophonema reports in other anthozoans (Schmidt and Schaefer 1980; Fautin and Mariscal 1991; Eckelbarger et al. 2008), but it was not possible to see the nucleus and the trophonema in the same oocyte, so no statement about the relationship between the trophonema and the nucleus can be made. The failure to see both structures in the same oocyte is not surprising, since it was very difficult to obtain complete sections of large oocytes with the intact yolk granules and nucleus (see Lauretta and Penchaszadeh 2017).

The observed trophonemas were all small (< 70 µm in diameter), shaped like an inverted cup, and clearly separate from the rest of the oocyte (Fig. 1c, d). Its origin seems to be in the mesenterial tissue and appears to be an invagination of the surrounding tissue inside the oocyte (Fig. 1c). Trophonemas were always found close to the surface of the oocyte with a thin (but clearly distinct) layer of tissue that kept it connected to the mesenterial tissue, which is consistent with a nutritive function (Larkman and Carter 1982). In the hematoxylin–eosin-stained sections, trophonema cells were colored strong violet (like the tissue of the mesentery), which differentiated them from the light red or pink color of the yolk granules. The general shape and size of the trophonema structures observed in *D. grandis* here, were similar to those described for the anemone *Calliactis parasitica* (Hertwig and Hertwig 1879). While this is the first report of a trophonema within the order Antipatharia, it remains unknown whether this is an exceptional case, or whether these structures have simply not yet been recorded in black corals due to the lack of studies focusing on this group. The particularly large oocytes of *D. grandis* ($\leq$ 1.5 mm in diameter) compared to other antipatharians might have facilitated the visualization of trophonemas in this study. It is also possible that the unusually large oocytes of *D. grandis* are a consequence of the presence of the trophonema. Since the trophonema facilitates the incorporation of molecules from the endoderm to the oocyte, this may be the reason why oocytes of this species can grow up to 1.5 mm, when oocytes in the order are usually under 0.2 mm (see Wagner et al. 2012 for a summary of known oocyte sizes in the antipatharian order). Future histological studies will need to be undertaken to determine whether trophonemas are more widespread within the order Antipatharia.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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