

First in situ record of the medusa stage of *Cladonema radiatum* (Cnidaria: Anthoathecata) in the South Atlantic Ocean

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Hydromedusae are important predators in marine habitats and information on their distribution is essential for understanding biogeographic patterns and marine ecosystem functioning (Colin et al., 2003; Tewksbury et al., 2014). Contrary to most hydromedusae, species of the family Cladonematidae Gegenbaur, 1857 (Anthoathecata) are characterized by a mostly or exclusively benthic mode of life with specialized adhesive structures on the tentacles used to adhere and move over the substrate (Schuchert, 2006).

The medusae of the genus *Cladonema* Dujardin, 1843 are distinguished from other Cladonematidae by their tentacles being branched more than once, and by having two types of tentacle branches: some ending in an adhesive structure, and others having nematocyst clusters; and the presence of simple oral knobs of nematocysts. *Cladonema* species have undergone several groupings and splittings since they were first described. Currently, six valid species are recognized but species distinction is still somewhat controversial (Schuchert et al., 2006; Gershwin and Zeidler, 2008; Cedeño-Posso, 2014; Schuchert, 2020).

To date, in the Southwestern Atlantic the genus has been recorded in subtropical waters (23°S) based on hydroids and laboratory-reared medusae (Migotto, 1996). In the present work, we report the first *in situ*

observation of the medusa stage of *Cladonema radiatum* in the South Atlantic Ocean associated with two different substrates, collected with emergence traps. This record extends the known geographic occurrence of the species to northeastern Brazil. We additionally provide insights on methodologies that can improve the collection efficiency of organisms with demersal habits.

The specimens were collected in the Abrolhos Archipelago, which is part of the Abrolhos Bank. The Abrolhos Bank is an extension of the Brazilian continental shelf, off Bahia state. It occupies an area of approximately 46,000 km², between 16°40'-19°40'S and 37°20'-39°10'W, composed of coral reefs, rhodolith beds, volcanic islands, shallow banks and channels (Leão and Kikuchi, 2001; Moura et al., 2013; Francini-Filho et al., 2013). The Abrolhos Archipelago is formed by five volcanic islands surrounded by fringing reefs and the sediment among the islands is mainly sandy and calcareous with the presence of rhodolith beds. The islands are surrounded by scleractinian coral colonies that dominate the substrate and a diverse seaweed community (Leão and Kikuchi, 2001; Torrano and Oliveira, 2013). The area is surrounded by unique mushroom-shaped structures formed by corals and calcareous algae known as "chapeirões" (Leão et al., 2019).

The samplings were conducted in April 2016 (austral fall) during 3 consecutive days in triplicate. Emergence traps were set up 1m from the bottom at two different sites. One site was located on a rocky bottom, dominated by turf, calcareous algae and scleractinian coral. The second site was located on a

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coarse sand bottom. Both sites were approximately 60 km from the coast with an average depth of 6 m (Table 1). The samplings were performed using emergence traps, consisting of a conical net with 1 m diameter mouth and 1.5 m long (200 μ m mesh size), the traps ended in a catch chamber with a funnel to prevent organisms from escaping (Figure 1) (Melo et al., 2010). In each site, three traps were installed at dusk and removed at sunrise during three days, totalizing 9 samples for each substrate. After withdrawals, the samples were transferred to 500 mL flasks and fixed with 4% formaldehyde buffered with sodium tetraborate (Harris et al., 2000).

Water temperature and salinity were measured with a Seabird SBE 19 plus CTD (Table 1). Six specimens were deposited in the *Museu de Oceanografia Professor Petrônio Alves Coelho* from *Universidade Federal de Pernambuco* (COL202002-02). The abundance and environmental parameters were tested for normality (Kolmogorov-Smirnov) and homoscedasticity (Levene). Subsequently, these parameters were tested between substrates using *t*-test. The tests were carried out on the software PAST 3.20, values of $p < 0.05$ were considered significant.

A total of thirty-five medusae specimens were identified as *Cladonema radiatum* (Table 1). Twenty-two were found in the sand traps and thirteen in the reef traps. Despite the greater abundance over the sand bottom no statistical significance could be found between the two substrates ($p = 0.49$); temperature and salinity were similar between the two stations with no statistical difference between them ($p = 0.46$; $p = 0.41$ respectively) (Table 1).

The specimens ranged from 0.32 to 0.57 mm in bell diameter and 0.4 to 0.48 mm in bell height, usually slightly wider than higher. Umbrella bell-shaped, almost spherical with short rounded apical projection (Figure 2a, b). Mouth with six bulbous nematocyst clusters (Figure 2a, b). The specimens presented a narrow manubrium, spindle shaped and never projected beyond the bell opening. Some specimens were young (Figure 2a) and others presented gonads in the beginning of their development, forming six rounded pouches around the middle portion of the manubrium (Figure 2b). All specimens had nine radial canals reaching bell margin, with one to three of them emerging from a bifurcation. Marginal tentacles branched; the proximal 1-2 branches with

Table 1. *Cladonema radiatum* occurrences on the Abrolhos Archipelago, Brazil. F.O. = Frequency of occurrence; T = temperature; S = salinity.

| Substrate | Location | No. of Specimens | F.O. (%) | T (°C) | S | Bottom depth (m) |
|-------------|----------------------|------------------|----------|--------|-------|------------------|
| Sand bottom | 17.9525 S, 38.7006 W | 22 | 55.55 | 28.57 | 37.27 | 5~6 |
| Coral reef | 17.9526 S, 38.6857 W | 13 | 44.44 | 28.53 | 37.13 | 5~6 |

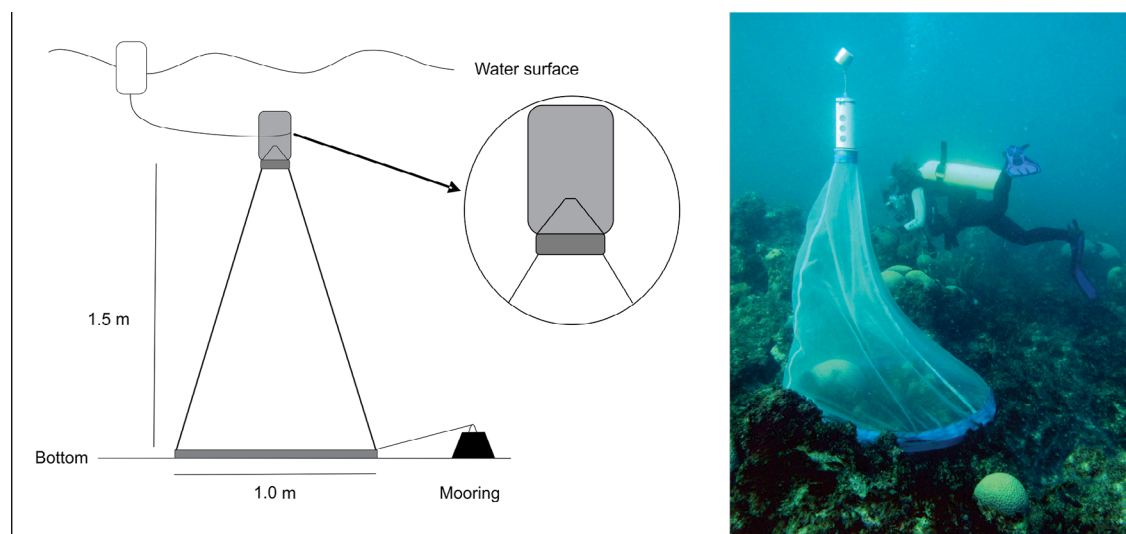


Figure 1. Schematic view (left) and photo (right) of the trap used for collecting the demersal fauna (scheme adapted from Melo et al., 2010 and photo from Fernando Moraes - Rede Abrolhos).

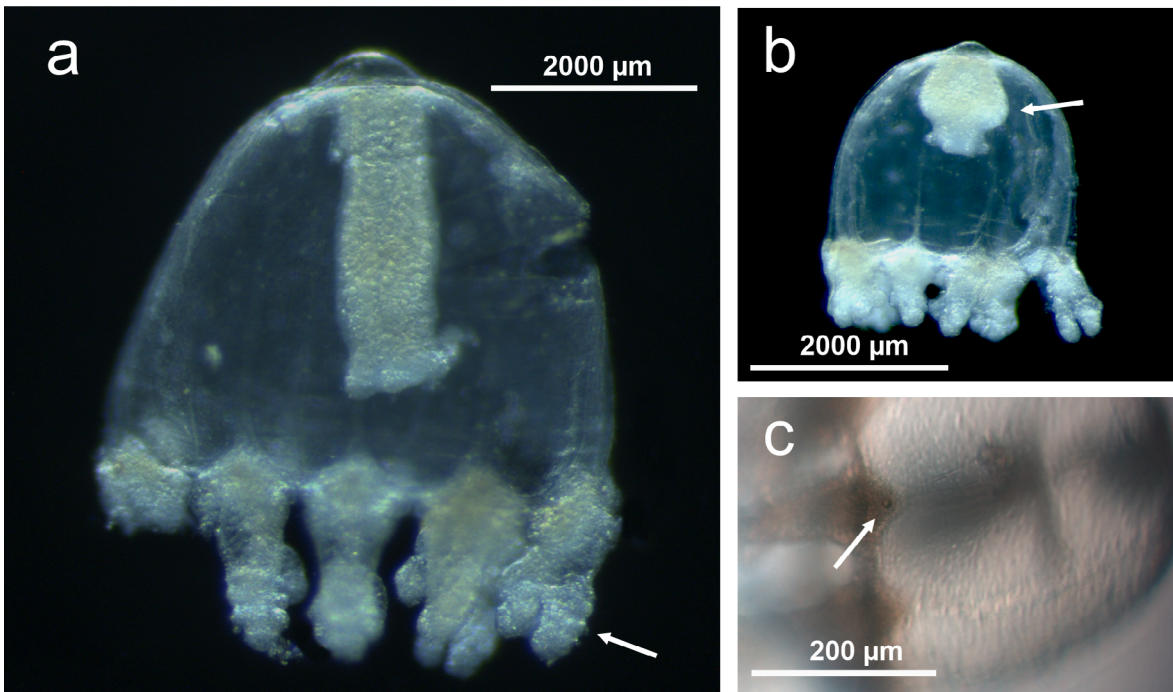


Figure 2. Fixed medusa of *Cladonema radiatum* Dujardin, 1843 from Abrolhos Bank, Brazil. Lateral view showing nematocysts clusters on tentacle (a; arrow) and the gonads in the manubrium (b; arrow). Detail of the tentacular bulbs showing an abaxial ocelli (c; arrow).

adhesive organs, distal ones bearing nematocyst clusters (Figure 2a, b). Number of tentacular bulbs corresponded to the number of radial canals, each with an abaxial ocellus (Figure 2c).

Cladonema radiatum medusae are highly variable in the number and branching pattern of radial canals and number of oral knobs, and many described species based on these meristic characters have been posteriorly proposed as synonymies of *C. radiatum* (Schuchert, 1996). Among the currently valid species (Table 2), *Cladonema californicum* Hyman, 1947, *Cladonema myersi* Rees, 1950, and *Cladonema timmsii* Gershwin & Zeidler, 2008 medusae differ from the present specimens in having only unbranched radial canals (Hyman, 1947; Rees, 1950; Gershwin and Zeidler, 2008). *Cladonema californicum* also differs from all other species in having only one adhesive tentacle branch, and one or two nematocyst tentacle branches. *Cladonema pacificum* Naumov, 1955 medusae differ from other species by their gonads covering almost the whole manubrium without any pouch. *Cladonema novaezelandiae* Ralph, 1953 differs from *C. radiatum* only in the higher number of tentacular branches, although currently *C. novaezelandiae* is considered valid (Schuchert, 2020), both species were considered

as synonymies in the past (Schuchert, 1996). This distinction should be better characterized since the number of branches in *C. radiatum* is highly variable and the polyp stage of *C. novaezelandiae* remains unknown.

Medusa stages of the genus *Cladonema* are not commonly reported, and most records refer to the hydroid stage with medusae reared in the laboratory (Migotto, 1996; Schuchert, 2006; Cedeño-Posso, 2014). First described from the Mediterranean Sea (Dujardin, 1843), *Cladonema radiatum* was found in temperate coastal areas in the Mediterranean Sea (Boero and Fresi, 1986; Bouillon et al., 2004), Black Sea (Revkov et al., 2002), Northeastern Atlantic (Teissier, 1965; López-González and Medel, 1996), North Sea (Russel, 1953) and eastern Pacific off China, Korea and Japan (Uchida, 1958, Chow and Huang, 1958; Hirohito, 1988; Park, 1996), the few reports off the west coast of USA and Canada considered it as an invasive species (Mills et al, 2007). In tropical and subtropical latitudes, the species was recurrently found in the Northwestern Atlantic around the Caribbean Sea (Wedler & Larson, 1986; Calder, 1991), however occasional catches occurred in coastal waters from all ocean basins. The largest number of records in temperate latitudes is expected since those areas historically received more

Table 2. Comparison of main medusae characters of the valid species of the genus *Cladonema*.

| Species | <i>C. californicum</i> | <i>C. myersi</i> | <i>C. novaezelandiae</i> | <i>C. pacificum</i> | <i>C. radiatum</i> | <i>C. timmsii</i> |
|--------------------|------------------------------------|--------------------------------|---------------------------------|---|--|---|
| Bell size | Up to 3 mm wide | Up to 0.8 mm wide | Up to 3 mm wide | Up to 3.5 high | Up to 4 mm high and 3 mm wide | NI |
| Radial Canals (RC) | 9 (rarely 11); unbranched | 7 (rarely 5 or 6); unbranched | Usually 9, some may be branched | 9; sometimes six, with every other one branching dichotomously so that nine radial canals reach the bell margin | Sometimes bifurcated, forming 8-10 canals | 9; unbranched |
| Tentacles | =RC | =RC | Usually 9 | 9 | =RC | =RC |
| Stinging branches | 1 or 2 | Much branched | 05 to 06 | Much branched | Usually 4-6 | 7 |
| Adhesive branches | 1 | up to 3 | 03 to 04 | 01 to 03 | Usually 1-4 | 6 |
| Manubrium | Beyond bell margin | Half the height of bell cavity | NI | Without pouches | Not extending beyond bell margin | Not extending beyond bell margin |
| Oral knobs | 6 | 6 | 6 | 6 | 0-4 | 6 |
| Gonads | On 6 to 7 pouches around manubrium | NI | On 6 pouches around manubrium | Around manubrium | Upper 2/3 of manubrium and on 4-6 pouches around manubrium | Female gonad completely encircling stomach in upper half, lacking pouches, male gonad on 6 pouches around manubrium |
| Ocelli | Present | Present | NI | NI | Present | Present |
| Reference | Hyman (1947) | Rees, (1950) | Ralph (1953) | Rees (1982) | Bouillon et al (2004); present study | Gershwin & Zeidler (2008) |

NI - Not Informed .

attention. However, proportionally the number of occurrences of *C. radiatum* in tropical areas was lower when compared to other species with temperate and tropical distributions (e.g. Nogueira-Junior et al., 2015; Tosetto et al., 2018), which could indicate a preference of the species for temperate latitudes.

Previous records of the species and genus in the South Atlantic were based on hydroid samplings with medusae reared in the laboratory from subtropical Brazil (around 23.8°S; Migotto, 1996; Oliveira & Marques, 2011), thus this study represents the first *in situ* occurrence of the medusa stage in South Atlantic waters. The new finding extends in more than 500 km the known distribution of the species in the Southwestern Atlantic Ocean, reaching tropical areas up to 17.3°S.

The specimens of this study were observed near sand and reef substrates in a shallow tropical high saline coastal environment (Table 1). Although slightly more abundant over sandy

bottom, that was without statistical significance. Other environmental variables were similar among samples (Table 1), with no clear indicative of habitat preference. Despite the wide distribution of *C. radiatum* in temperate waters, the ecological requirements of the species are still poorly known. Further detailed studies on *C. radiatum* ecology are needed to fill this gap. Furthermore, *C. radiatum* medusae were found in half of the traps, suggesting that this may be an appropriate methodology for its capture. Due to its benthic behavior, adhering to substrate with the adhesive branches of tentacles (Schuchert, 2006), traditional pelagic trawls used to sample hydromedusae may be inadequate to catch the species (Nogueira-Junior et al., 2015; Tosetto et al., 2019). Several studies showed differences when using trawl nets or traps for collecting organisms with demersal behavior (Emery, 1968; Sale et al., 1976; Figueiredo et al., 2018), emphasizing the traps capacity to include the entire migration variation

existent in this community (Porter and Porter, 1977; Smith et al., 1979). Thus, the application of more appropriate methodologies for its lifestyle, as emergency traps (used here) may help to understand the biogeography and ecology of *C. radiatum* and other demersal species.

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AUTHOR CONTRIBUTIONS

G.B.F.: Writing - original draft, Conceptualization, Methodology, Validation, Formal Analysis, Writing-Reviewing and Editing. S.N.L.: Writing-Reviewing and Editing. P.A.M.C.M.: Writing-Reviewing and Editing. M.N.J.: Conceptualization, Validation, Writing-Reviewing and Editing. E.G.T.: Writing - original draft, Conceptualization, Methodology, Validation.

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