A new species of carnivorous deep-sea sponge
(Demospongiae: Cladorhizidae)
associated with methanotrophic bacteria

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Abstract: A new species of Cladorhiza, C. methanophila sp. nov., is described from mud volcano fields and active sites of methane expulsion in the Barbados Trench, from 4700 to 4900 m depth. This species, the first reliable record of the genus in the Central Eastern Atlantic, is both carnivorous and associated with methanotrophic bacteria.

Résumé : Une nouvelle espèce d'éponge abyssale carnivore (Demospongiae : Cladorhizidae) associée à des bactéries méthanotrophes. Une nouvelle espèce de Spongiaires du genre Cladorhiza, C. methanophila sp. nov., est décrite, provenant de volcans de boue et de sources de méthane du prisme de la Barbade, situé à 4700-4900 m de profondeur. Cette espèce, la première du genre connue du centre de l’Atlantique occidental, est à la fois carnivore et associée à des bactéries méthanotrophes.

Keywords: Porifera; Cladorhizidae; Cladorhiza; deep sea; new species; methanotrophy.

Introduction

The cladorhizids are strange deep-sea sponges with a plant-like or hydroid-like shape highly unusual in the phylum Porifera. Their symmetrical body is provided with lateral filaments or expansions, and is supported by a stalk anchored by a basal root system adapted to life in soft sediments. Thanks to a representative living in a littoral cave, these deep-sea sponges have been recently shown to have developed a carnivorous feeding habit (Vacelet & Boury-Esnault, 1995, 1996).

The family Cladorhizidae, Dendy 1922 includes to date 88 species classified in three genera (Hajdu & Vacelet, in press). It includes the deepest known sponge, a species of the genus Ashbestopluma Topsent, 1901 found at 8840 m depth in the Pacific (Koltun, 1970). They probably all have the same unusual feeding habit, with a body plan different from that of the other Porifera, including the disappearance of the aquiferous system and the development of branching processes covered with microsclere spicules allowing the capture of small prey. However, the anatomy and organization of the deep-sea species are poorly known, especially in the case of the genus Chondrocladia Thomson, 1873, in which a carnivorous feeding habit occurs together with a modified aquiferous system (Kübler & Barthel,
1999). The monophyly of the family in the order Poecilosclerida may be questioned.

Like many deep-sea organisms, most of the cladorhizid sponges are known only from a few records, and their taxonomy is confused, especially at species level. Although they are widely distributed in the world oceans, only two poorly described species, Crinorhiza amphactis Schmidt, 1880 and Cladorhiza concrescens Schmidt, 1880 are known from the West-Central Atlantic. These species are at present classified in the genus Chondrocladia due to their microscleres, and considered as unrecognizable (Hajdu & Vacelet, in press; Soest, 1984; Soest & Stentoft, 1988). Recently, it has been found that an undescribed species of Cladorhiza Sars, 1872 thrives near methane sources of the Barbados Trench and derives part of its carbon from symbiosis with methanotrophic bacteria (Vacelet et al., 1995, 1996). We give here the taxonomic description of this new species.

**Material and methods**

**Sampling**
Specimens were collected from mud volcano fields in the Barbados Trench during the BARESNAUT (1987) and MANON (1992) cruises (Henry et al., 1990; Le Pichon et al., 1990; Olu et al., 1997). Field observations and collections were made by means of the submersible ‘Nautil’ on the diatreme Atalante and on the diapir Mount Manon.

Small samples of the specimens from MANON were either preserved for cytology or frozen. The remaining specimens, approximately one hundred individuals, and those of BARESNAUT were preserved in alcohol.

For the study of spicules, the tissue was digested by boiling in nitric acid. The dissociated spicules were either separated by filtration on a 0.1 µm Cyclopore membrane (Reiswig & Browman, 1987) or deposited after sedimentation on a glass coverslip, sputter-coated with gold-palladium, then observed under a Hitachi S570 scanning electron microscope (SEM). The skeletal architecture, anatomy and gross structure of the tissue were studied in light microscopy, on thick polished sections obtained by sawing specimens embedded in Araldite with a low speed saw, using a diamond wafering blade, and wet-ground with abrasive paper.

For cytology and transmission electron microscopy (TEM), the specimens were fixed in 3% glutaraldehyde in 0.4 M cacodylate buffer and postfixed in 1% osmium tetroxide in the same buffer, then embedded in Araldite. Semi-thin sections for light microscopy were stained with toluidine blue. Thin sections for TEM were cut after local desilicification in 5% hydrofluoric acid applied on the free surface of the trimmed block (Borojevic & Lévi, 1967). They were contrasted with uranyl acetate and lead citrate, and observed under a Hitachi Hu 600 or Zeiss EM 912 transmission electron microscope.

**Systematics**

Order Poecilosclerida Topsent, 1928
Family Cladorhizidae Dendy, 1922
Genus Cladorhiza Sars, 1872

Type species: Cladorhiza abyssicola Sars, 1872
Definition: Cladorhizidae with anchorate unguiferate anisochelae.

Cladorhiza methanophila sp. nov.

**Etymology:**
From gas “methane” and “phil”, friend, referring to the association of the new species with methanotrophic bacteria.

**Type material**
Holotype: Muséum national d’Histoire naturelle, Paris, n° MNHN. D JV 57. From the diapir Mount Manon, dive MANON 1992 MA06, Barbados Trench, 4718 m depth.
Some specimens from MANON MA06 (dry), MA03 and BARESNAUT PL 94/3 4935 m depth are preserved in the authors’ collection in the Centre d’Océanologie de Marseille, Station Marine d’Endoume.

**Locality**
The specimens were living in mud volcano fields in the Barbados Trench on the diatreme Atalante and on the diapir Mount Manon. They were collected during the dives MANON MA01 (13°49.36N, 57°39.58W, 4943 m depth), MANON MA03 (13°49.36N, 57°39.58W, 4930 m depth), MANON MA06 (13°46.74N, 57°32. 51W, 4718 m depth) and BARESNAUT PL 94/3 (13°49N, 59°37W, 4935 m depth).

**Description**

**Shape and size** (Figs 1-2)
The holotype from the diapir Mount Manon (MA 06) is a complete branching specimen, 44 mm high. It is attached to a thin plate, 6 mm in largest dimension, made of sediment trapped in a feltwork of megascleres. The root system that usually anchors the cladorhizid sponges in sediment is absent. Stem and branches, smoothly curved, are covered
with lateral expansions, 2 to 3 mm long and 0.12 to 0.25 mm in diameter, approximately 0.5 mm apart, mostly perpendicular to the axis. The lateral expansions arise from all round the stem. A similar complete specimen was collected on the same diapir during the dive MA 03. The photographs taken from the submersible indicate that the sponge forms small, dispersed bushes in this site.

The numerous specimens of *Cladorhiza methanophila* sp. nov. collected on the diatreme Atalante are larger and often display the shape of a bottle brush. The maximum length of the collected specimens is approximately 20 cm, but observations and photographs from the submarine indicate that the sponge clumps may be more than 40 cm high. The specimens consist of a long, bare stem, up to 1 mm in diameter, without soft tissue and appendage, which ramifies irregularly several times. The branching is usually at near right angles to the main axis, but oblique or dichotomous branching and anastomoses are also observed. The branches are attached to the main stem by an enlarged base. Anastomoses between twigs also result in a flattened widening of the axis. The soft tissue and lateral expansions occur only at the end of the branches of the collected specimens, rarely on more than 5 cm. However, the photographs from the submarine indicate that this is due to poor preservation and degradation of the specimens after collection, as the axis appears in situ to be wholly covered by living tissue. The lateral expansions are thicker and shorter than in the better preserved specimens from Mount Manon. In a few cases, their disposition is biserial. The base of these specimens was not collected.

All specimens are white or light grey. They are devoid of aperture, ostia or osculum.

**Skeleton**

The skeleton is made of styles longitudinally arranged along the axis of stem and lateral expansions, with a spirally twisted disposition in the larger axis. The main axis is up to 1 mm in diameter. The lateral expansions are supported by an axis of a few tens of megascleres, tapering toward the end. The living tissue contains a large number of microscleres, mostly anisochelae, usually arranged haphazardly, roughly perpendicular to the surface of the expansions in well preserved areas. **Spicules** (Figs 3-6)

**Megascleres**

Styles (Figs 3, 4), straight, fusiform, often with a slightly enlarged head. Size (180 measurements in 6 specimens): length 310-680 µm (\(\bar{x} = 486 \mu m \pm 11.3\)), width 5-20 µm (\(\bar{x} = 13.18 \mu m \pm 0.48\)) in body and lateral expansions, slightly thicker in the peduncle: length 320-680 µm (\(\bar{x} = 515 \mu m \pm 10.53\)), width 10-25 µm (\(\bar{x} = 16.87 \mu m \pm 0.48\)). Holotype (30 measurements): length 320-670 µm (\(\bar{x} = 534 \mu m \pm 31.48\)), width 10-25 µm (\(\bar{x} = 16.75 \pm 1.34\)).

**Microscleres**

Anchorate unguiferous anisochelae (Figs 3, 4, 6), absent in the bare stem. The shaft is curved and bears two fimbriae near the larger end, which has five large teeth, 9-10 µm in length, with a sharp, abrupt point. The five teeth of the small end are 4-4.5 µm in length. Developing spicules, with the same length as the fully developed ones have smaller teeth and a thinner shaft. The two ends are similar in the youngest spicules observed, which bear small swellings in place of recognizable teeth. Total length (180 measurements in 6 specimens): 20-25 µm (\(\bar{x} = 22.44 \mu m \pm 0.17\)). Holotype (30 measurements): 20-27.5 µm (\(\bar{x} = 22.5 \pm 0.47\)).

Sigmans (Fig. 3), with equal ends in the same plane, rare in some specimens. Size (150 measurements in 6 specimens): 95-145 µm (\(\bar{x} = 110 \mu m \pm 5.02\)). Holotype (30 measurements): 100-140 µm (\(\bar{x} = 124 \mu m \pm 2.82\)). Thickness approximately 5 µm.

Sigmancistras (Fig. 5), with ends in different planes and less strongly bent than in the sigma, without notch on the shaft. They are rare in two specimens from Atalante, abundant in the others. Size (125 measurements in 6 specimens): 40-55 µm (\(\bar{x} = 44.69 \mu m \pm 0.57\)). Holotype (30 measurements): 35-47.5 µm (\(\bar{x} = 42.6 \mu m \pm 1.21\)).

**Living tissue** (Figs 7-11)

Canals and choanocyte chambers are absent in *C. methanophila*. The tissue, of low general density, is composed of various types of rounded or stellate cells. The most abundant are cells with long pseudopodia extending in the intercellular matrix, and with vacuoles containing symbiotic bacteria, most often in the process of degradation, which could be isolated or grouped in large inclusions, already described (Vacelet et al., 1996). A few cells with a nucleolate nucleus contain dense, homogeneous granules (Fig. 7), up to 1.8 µm in diameter, which have a characteristic shape: spherical with a thick, conical expansion with a striated content. Scleroocytes are abundant in the mesohyl. Collagen fibrils (Fig. 8), 15-18 nm in diameter, with a transverse banding of approximately 10 nm period, are dispersed or grouped in small fascicles in the intercellular matrix. The tissue contains two, possibly three, morphological types of inter- and intracellular bacteria, whose distribution and morphology have been described in a previous work (Vacelet et al., 1996). The most abundant are coccoid bacteria (Fig. 7), 1-1.7 µm in diameter, containing stacks of flattened vesicles, whose morphology is typical of methanotrophic bacteria. Healthy bacterial cells are found in the extracellular matrix, whereas cells in various stages of degradation are found inside sponge cells. **Reproduction** (Fig. 9-11)

Embryos, 250 to 300 µm in maximum diameter, have been observed near the axis in several specimens of *C. methanophila*. They were never found in the lateral expansions. An early developmental stage, 250 µm in
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diameter, has been observed in TEM (Fig. 10). At this stage, the embryos, devoid of spicules, consist of a mass of blastomeres containing numerous yolk and lipid inclusions. These cells, which are devoid of symbiont, are irregular in shape, size, and density, most often with a polygonal outline. They contain a large number of lipid inclusions and clear vesicles. The nucleus is round, up to 10 µm in diameter, often with several nucleoli. The smallest cells, which are usually denser, are frequently encircled and apparently phagocytized by the larger cells. The blastomeres are surrounded by an outer layer of flattened follicular cells, 3 to 6 µm in thickness, generally displaying an outer area with homogeneous cytoplasm, and an inner area with numerous clear vesicles and larger vacuoles which contain undigested symbiotic bacteria, apparently healthy (Vacelet et al., 1996). Homogeneous lipid inclusions, 0.5 to 1 µm in diameter, are present locally in the inner area. The embryo is surrounded by a layer of collagen fibrils more densely packed than in the normal tissue, 2 µm thick.

Other stages in embryogenesis have been observed under light microscopy on poorly preserved specimens (Fig. 11). The maximum size of embryos is 300 µm and their cellular structure could not be clearly resolved. A few of them, formed of large embryonic cells similar to those described in TEM, contain a few fully-grown or developing anisochelae. The most advanced stage observed is an ovoid embryo, 300 µm / 130 µm, in which a mass of very small cells (approximately 4-5 µm with a nucleus of 2 µm) is surrounded by an acellular envelope. Its central part contains a longitudinal fascicle of thin styles (180 µm / 4 µm) and numerous fully grown anisochelae of normal size. Sigmas and sigmancistras are absent.

Both the body and the lateral expansions of several specimens locally contain round bodies, 30 to 60 µm in diameter, which are interpreted from light microscopy observations as spermatic cysts (Fig. 9). We could not observe the ultrastructure of these bodies. The largest contain small cells, 4 µm in diameter. The smallest contain an array of filaments with dark spots, probably sperm cells. The cysts are surrounded by a thin cellular envelope, 0.5 µm in thickness, which is more visible around the small cysts, the fibrillar content of which is frequently depressed on one side by a cell with a nucleolate nucleus. The sigmancistra microscleres are more abundant in the areas where the cysts are observed, although there is no direct relation between cysts and microscleres. These round bodies resemble the spermatic cysts observed in another cladorhizid, Asbestopluma hypogea Vacelet & Boury-Esnault, 1996.

Distribution and Ecology

Fields of Cladorhiza methanophila have been observed in a diapiric field, 12 km seaward of the Barbados accretionary complex deformation front, around a flat mud volcano, the diatreme “Atalante”, and on the top of cone-shaped diapiric mounds, “Mount Manon” and “Volcano A”. The characters of the diapiric field and the precise distribution of the “methanotrophic” sponges versus the “sulphur-oxidizing” clams Calyptogena have already been described (Le Pichon et al., 1990; Olu et al., 1997) and will be only summarized here. In the diatreme Atalante, an active site of methane expulsion, the central depression (eye) of the flat volcano is filled with warm mud (21 ° C at 1.8 m) covered by a carbonate crust. The sponges occurred here in a concentric zonation. They were most abundant at the immediate periphery (0-100 m) of the eye, in “léopard facies” (Le Pichon et al., 1990), mostly on the east side, forming dense clumps up to 2 m in diameter and 40 cm high, of several hundred individuals (Fig. 1). They occurred as smaller bushes (0.2-0.5 m in diameter) between 100 m and 250 m from the edge of the eye, in “yellow facies”. In the central mud lake of the eye where no animal life has been seen, several sponge clumps were observed buried by a very recent mud flow (Le Pichon et al., 1990), indicating that C. methanophila is able to temporarily thrive near the mud vent. On Mount Manon, the sponge bushes, at the top of the volcano, were smaller (20 cm in diameter) and less numerous. Some bushes were mixed with dense aggregations of vesicomyid clams (Calyptogena sp.). One large bush was observed on the top of another volcano.
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(Volcano A) of the same field. No sponges were seen on the diatreme Cyclope, in which the estimated methane flux (60 kg day\(^{-1}\)) is less than 1% of the diatreme Atalante (8000 kg day\(^{-1}\)) (Olu et al., 1997). In all locations, most of the sponge bushes were attached to the carbonate crusts.

Several amphipod and copepod debris, 0.5-3 mm in length, have been observed trapped in the appendages of the studied specimens. Some bunches of *C. methanophila* collected during the BARESNAUT cruise have provided several species of annelids and of crustaceans (Bellan-Santini, 1990; Ségonzac, pers. comm.). They were considered as an associated fauna, but they are more likely prey in the process of capture or digestion.

### Discussion

These specimens of *Cladorhiza methanophila* sp. nov. from the Barbados Trench belong to a group of *Cladorhiza* from the North-East Atlantic possessing relatively small styles, less than 700 to 800 µm in length: *C. abyssicola* Sars, 1872, *C. corticocancellata* Carter, 1876, *C. gelida* Lundbeck, 1905, and *C. iniquidentata* Lundbeck, 1905. In this group, the specimens of *C. methanophila* are close to *C. abyssicola*, the type-species of the genus. They differ from this species by slight differences in spiculation, and by their shape and size. We thus propose to consider them as a new species, taking also into account the disjunct geographic distribution and their special ecological localization.

The spiculation of the new species differs from that of *C. abyssicola* by thicker styles (maximum width 25 µm instead of 19 µm), absence of notch in the shaft of the sigmancistra, and absence of the special sigma (ancistras). Ancistras were not reported in the original description of *C. abyssicola*, but were figured by Lundbeck (1905) in specimens from the Norwegian Sea and were described by Topsent (1909) in Madeira and Canary islands.

The new species also has a larger size than all the specimens identified as *C. abyssicola*, and forms large bushes. Large specimens of *Cladorhiza* sp. forming bushes or shrubs up to 50-80 cm high have been reported from the Faroe Channel (Thomson, 1873), but the species was unidentified. This large size may also be linked to the association with methanotrophic bacteria near methane source, allowing the sponge to thrive in a favorable environment.

A further difference is the absence of root in all the collected specimens of *C. methanophila*. This character could be related to life on a relatively hard substrate consisting of carbonate crusts near the mud volcano. However, all the known specimens of *C. abyssicola* were provided with a richly branched root, the only exception being one specimen from Tenerife, identified as *C. abyssicola* by Topsent (1909).

*Cladorhiza abyssicola* appears to be the most common species of the genus. It is known from the North Atlantic and Norwegian Sea, and is also recorded from Madeira, the Canary Islands (Topsent, 1909) and the Mediterranean. The specific identity of the northern and southern specimens needs to be checked, but even considering that they actually belong to the same species, there is a wide distribution gap between this North-East Atlantic *C. abyssicola* and the new species from the Barbados Trench. *Cladorhiza methanophila* is the first record of the genus *Cladorhiza* in the West-Central Atlantic, the poorly described *Cladorhiza concrescens* Schmidt, 1880 being more probably an unrecognizable representative of *Chondrocladia* (Lundbeck, 1905; Soest, 1984).

The poor state of preservation of most specimens unfortunately does not allow elucidation of the embryology and larva structure of the Cladorhizidae, which is still problematic. Our study provides some data on the ultrastructure of the early embryos, indicating a direct transmission of the symbionts included in follicular cells, but the following stages remain poorly known. We confirm that, as shown mostly by Lundbeck (1905), the embryos have, at a late stage, microscleres and a central axis of megascleres, similar to what is known in some other

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**Figure 7.** Cell with inclusions and, in the extracellular space, two methanotrophic microsymbionts. Scale bar = 0.85 µm.

**Figure 8.** Intercellular collagen fibrils. Scale bar = 0.1 µm.

**Figure 9.** Light microscopy, polished section, showing spermatic cysts *(arrows)*. Scale bar = 80 µm.

**Figure 10.** Early stage of embryogenesis. *(B)* blastomere; *(FC)* follicular cell; *(arrows)* symbiotic bacteria in the follicular cell. Scale bar = 1.9 µm.

**Figure 11.** Light microscopy, polished section through an advanced embryo. Scale bar = 26 µm.
Poecilosclerida, especially in the genera Desmacidon Bowerbank, 1861 and Mycale Gray, 1867 (Lévi, 1956). But there is still no precise data on the apparently special envelope of these embryos, which suggested to Topsent (1909) that they were gemmules.

The presence of polychaetes and amphipods trapped in the appendages of C. methanophila, which is devoid of aquiferous system and filtration device, confirms that this sponge is carnivorous. Carnivory, for which direct evidence has been given in the genera Asbestopluma (Vacelet & Boury-Esnault, 1995) and Chondrocladia (Kübler & Barthel, 1999) is now directly documented in the third genus of cladorhizid sponges, Cladorhiza. This feeding mode is thus very likely to occur in all the deep-sea Cladorhizidae.

The morphology of the associated bacteria, which is typical of methanotrophs, their relationships with the sponge tissue, the very negative d13C values of the sponge tissue, the occurrence of methanol deshydrogenase activity, all indicate that the sponge utilizes methanotrophy in addition to a carnivorous feeding habit. This is confirmed by the unusually large size and the great abundance of Cladorhiza methanophila, which is clearly correlated with the methane flow near the most active vents (Olu et al., 1992). However, large bushes of Cladorhiza sp. have been reported from the Faroe Channel (Thomson, 1873). It would be particularly interesting to check whether this abundance could be indicative of a local fluid expulsion.

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