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A new species of carnivorous sponge (Demospongiae: Cladorhizidae) from a Mediterranean cave

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Abstract

A cladorhizid sponge which has been shown to be carnivorous, is described as a new species of *Asbestopluma*, *A. hypogea* sp. nov. The sponge lives in a Mediterranean cave, 17 to 22 meters deep, between 15 to 60 m inside from the cave opening, in a trapped mass of cold water (13 - 14.7 °C). Preserved specimens differ significantly in shape from the living ones and varia-tions also occur according to the feeding status. Forceps spicules are present only during spermatogenesis. The nearest species is *A. hydra* Lundbeck, from the Arctic (1847 and 2394 m).
Keywords : Porifera, new species, Mediterranean, submarine cave, carnivory.

Résumé

Une nouvelle espèce d'éponge de la famille profonde Cladorhizidae, à régime alimentaire carnivore, est décrite dans le genre *Asbestopluma*, *A. hypogea* sp. nov. Elle vit dans une grotte de Méditerranée, de 17 à 22 m de profondeur, entre 15 et 60 m de distance de l'ouverture, dans une masse d'eau froide à 13 - 14,7 °C piégée par densité. La forme des spécimens *in situ* est modifiée par la fixation ou la récolte, et varie également selon l'état de nutrition. Les spicules forceps sont présents seulement durant la spermatogenèse. L'espèce la plus proche est *A. hydra* Lundbeck, de l'Arctique (1847 et 2394 m).
Mots-clés : spongiaires, nouvelle espèce, Méditerranée, grotte sous-marine, carnivorie.

Introduction

It has been recently shown that the deep-water cladorhizid sponges differ significantly from other sponges in both anatomy and feeding habits. Being devoid of an aquiferous system, the "sponges" belonging to genera *Asbestopluma* and *Cladorhiza* do not fit the conventional definition of the phylum Porifera. They have developed carnivorous feeding, by trapping small prey such as tiny crustaceans on their appendages, which have raised hook-like microsclere spicules (VACELET & BOURY-ESNAULT, 1995a). The third important genus of the family, *Chondrocladia*, has buds which are shrunken modifications of spheres normally inflated *in situ* (TENDAL *et al.*, 1993). This may be a different, and as yet unknown adaptation to deep-sea environments.

These novel characteristics of the biology of deep-sea sponges have been discovered recently in a species of Cladorhizidae which has colonized a Mediterranean littoral cave with stable low-temperature conditions (VACELET *et al.*, 1994). This habitat offers exceptional opportunities to study several deep-sea organisms, which are normally near impossible to access. We describe this sponge as a new species of *Asbestopluma*, the deepest known genus of sponges with a record depth of 8,840 m (KOLTUN, 1970), and hitherto unknown in the Mediterranean. We also give preliminary observations on the cytology and reproduction.

Material and Methods

The specimens were collected by scuba diving in a Mediterranean cave near La Ciotat (coast of Provence, France). The population density was estimated from underwater photographs. Sponges were either preserved in formalin or fixed *in situ* for cytological study. Two fixatives were used : 2.5% glutaraldehyde in a buffer composed of 0.4 M sodium cacodylate / sea water (1/1) for approximately 20 hours, followed by postfixation in 2% osmium tetroxide in sea water (BOURY-ESNAULT *et al.*, 1984) - and a mixture of osmium tetroxide and mercury chloride (6/1) (JOHNSTON & HILDEMANN, 1982). For light and electron microscopy, the specimens were embedded in Araldite. Semithin sections were stained with toluidine blue. Thin sections, contrasted with uranyl acetate and lead citrate, were observed under a Hitachi Hu 600 transmission electron microscope (TEM). For scanning electron microscopy (SEM), the specimens were fractured in liquid nitrogen, then critical-point-dried, sputter coated with gold-palladium, and observed under a Hitachi S 570.

Fig. 1. - Holotype of *Asbestopluma hypogea* (center) with entangled filaments due to turbulence and fixation in formalin. Scale bar = 2.4 mm.
Fig. 2. - Live specimens in aquarium. Scale bar = 3.5 mm.

Systematics

Order **Poecilosclerida** TOPSENT, 1928
Family **Cladorhizidae** DE LAUBENFELS, 1936
Genus ***Asbestopluma*** NORMAN, 1882
Asbestopluma hypogea sp. nov.

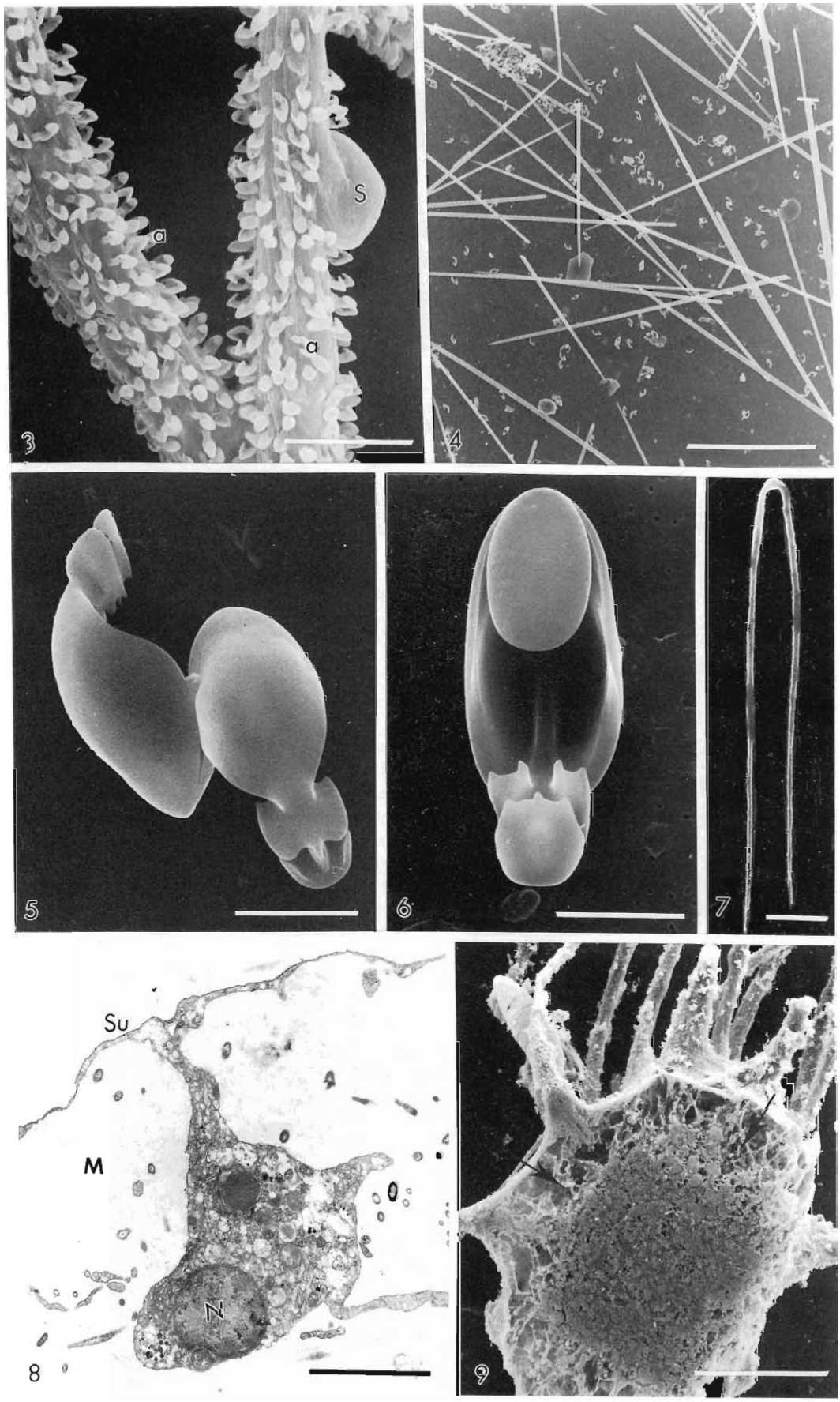
TYPE MATERIAL

Holotype : Muséum National d'Histoire Naturelle, Paris, n° MNHN DJV 49, 3PP cave, La Ciotat (France), 20 m, 24 September 1992 (Fig. 1).
Paratypes : Muséum National d'Histoire Naturelle, Paris, n° MNHN DJV 50, 3PP cave, La Ciotat (France), 20 m, 24 September 1992 (4 specimens).

LOCALITY AND HABITAT

The sponge was found in a submarine cave ("3PP cave") near "Anse Gameau", La Ciotat, Mediterranean coast of France. The environment of the cave has been described elsewhere (VACELET *et al.*, 1994). The sponge grows on rocky surfaces, being abundant at the base of the cave walls near the mud floor, between 15 to 20 m from the entrance, 17 to 22 meters deep. A density of up to 23 individuals /25 cm^2 has been observed. Deeper in the cave, up to 60 m from the opening, specimens are sparsely distributed, most often occurring in aggregates of 5 to 10 individuals in large areas devoid of any individuals. However, the sponge does not live in the furthest reaches of this 120 m long cave, contrary to another deep-sea sponge, the hexactinellid *Oopsacas minuta* TOPSENT. Most specimens hang down from the walls, although a few have been found growing vertically on small pieces of rock partially embedded in the mud floor. Sufficient light is present at the site of maximum density for the sponge to be seen without artificial light.

Fig. 3. - Filaments, densely covered with raised anisochelae. Note spermatocyst. SEM. Scale bar = 50 μm.
Fig. 4. - Spicules. SEM. Scale bar = 150 μm.
Fig. 5. - Profile and back view of anisochelae. SEM. Scale bar = 4.2 μm.
Fig. 6. - Front view of an anisochela. SEM. Scale bar = 3.8 μm.
Fig. 7. - Forceps. SEM. Scale bar = 4.3 μm.
Fig. 8. - T-shaped pinacocyte. TEM. Scale bar = 3 μm.
Fig. 9. - Transverse section through body. Note low density area near surface (arrows). SEM. Scale bar = 380 μm.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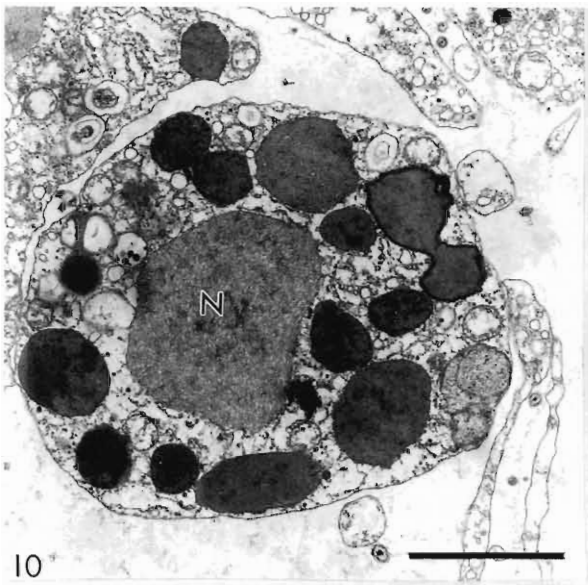

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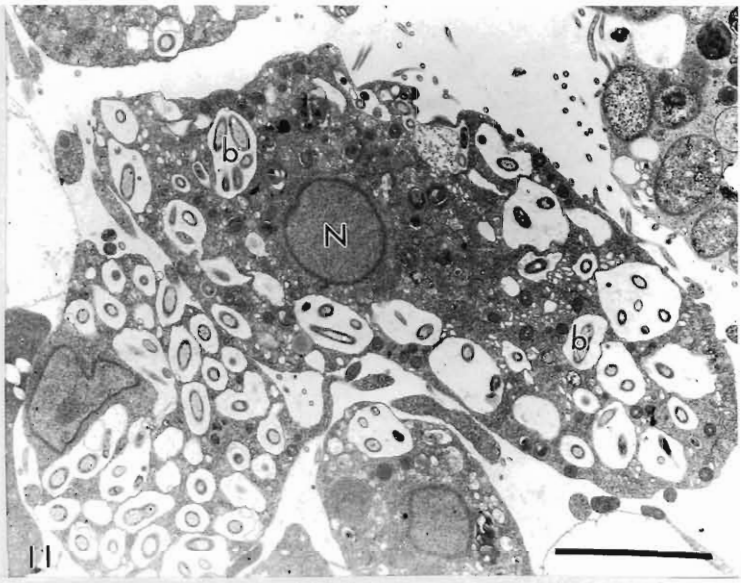

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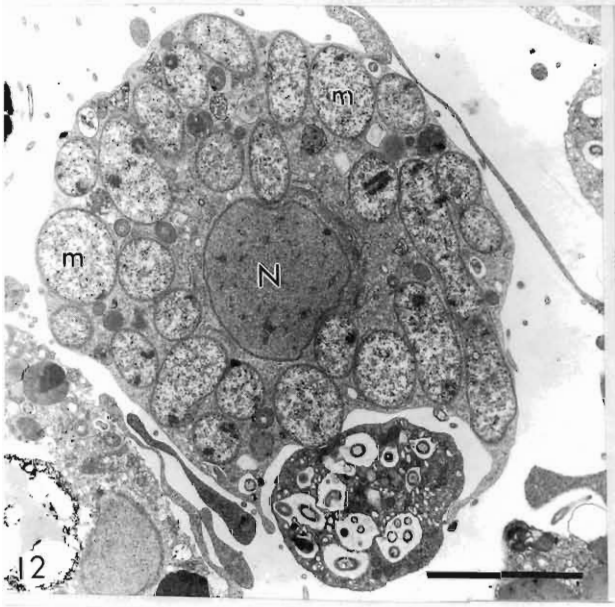

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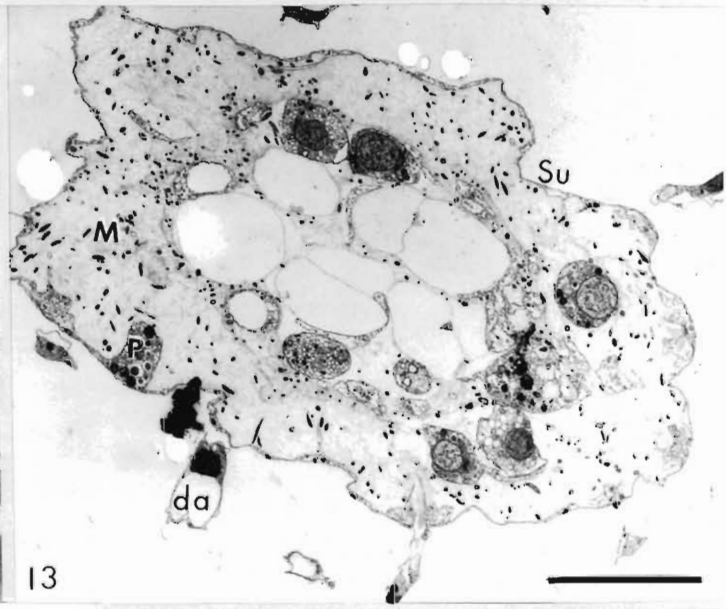

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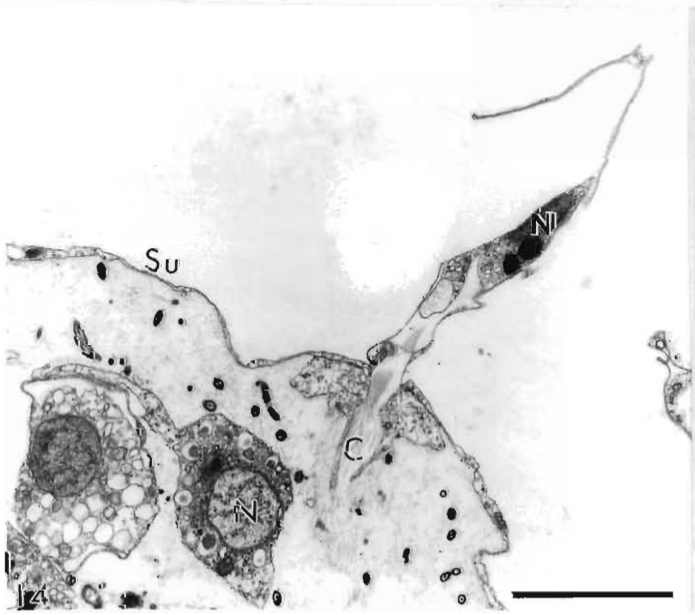

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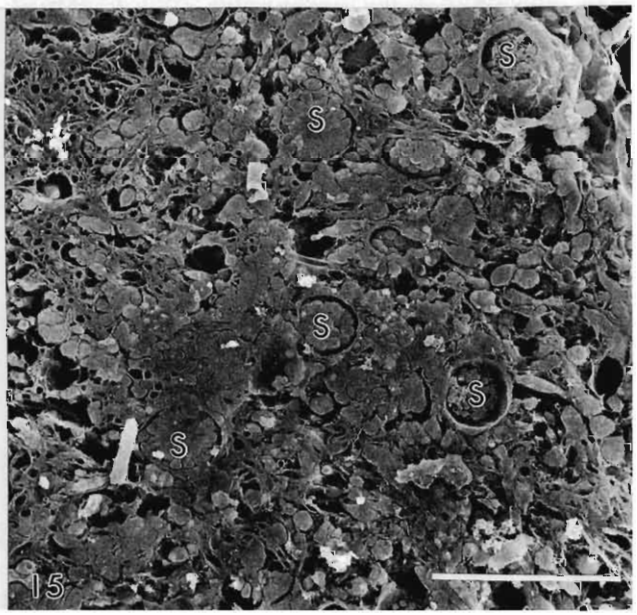

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All the specimens live in a cold body of water trapped by density during winter, and the temperature varies only from 13 to 14.7 °C year-round.

DESCRIPTION

Shape and size
Sponge stipitate, with a stalk 3.7-14 mm long, 0.1-0.18 mm in diameter, fixed on rock by an enlarged base, never branching. The ovoid body, 1.9-6.5 mm / 0.7-1.2 mm, is well distinguished from the stalk. It bears 30 to 60 lateral filaments, up to 10 mm long, 50-80 μm in diameter, arising along the body (Fig. 1, 2). The filaments become entangled when exposed to turbulence or after fixation in glutaraldehyde or formalin (Fig. 1).

Colour
White in life and in alcohol.

Surface
The surface of the stalk and the body is smooth, without any aperture in undisturbed specimens fixed *in situ*. The filaments are finely shaggy and adhesive, due to a dense cover of raised anisochelae (Fig. 3).

Skeleton
The stalk is made of an axis of 70-100 megascleres arranged in parallel and embedded in spongin. In the body, the axis diverges into secondary multispicular fibres toward the filaments, where they constitute a central 10 spicule wide fibre. This fibre is reduced to one or two spicules at the filament extremity. The palmate anisochelae microscleres are located externally at right angles on the filaments, where they protrude perpendicular to the surface with only the small end embedded in the tissue. They are rare on the body and nearly absent from the peduncle. Forceps, present only during spermatogenesis, form two diametrically opposite tufts in mature sperm cysts, localised along the filaments.

Spicules
(50 measurements in light microscopy), Fig. 4 - 7.

Megascleres
Styles or subtylostyles with a slight swelling on the head, straight or very slightly flexuous, length 385-780 μm ($\overline{x}$ = 498 μm ± 22.55), width 2-7 μm ($\overline{x}$ = 4.9 μm ± 0.26) in body and filaments, slightly larger in the stalk : length 350-920 μm ($\overline{x}$ = 674 μm ± 36.07), width 3-7 μm ($\overline{x}$ = 5.06 μm ± 0.25).

Microscleres
Palmate anisochelae (Fig. 5-6). The tooth of the large end is 4.2-6.5 μm long. The small end (foot) consists of a front denticulate tooth and two lateral denticulate teeth, welded with the front one through a visible suture line : 9-13 μm ($\overline{x}$ = 11.2 μm ± 0.21). A hole is present between the foot teeth.
Forceps (Fig. 7), absent in non-reproductive specimens, are 25-34 μm long ($\overline{x}$ = 31.4 μm ± 1.2).

Cytology
The sponge surface is covered by a pinacoderm made of T-shaped pinacocytes (Fig.8).
The sponge body (Fig. 9) is totally devoid of choanocyte chambers and canals. It is composed of low density tissue, containing collagen fibrils, intercellular symbiotic bacteria, and various types of cells. The tissue density is specially low in a 250-350 μm thick zone under the ectosome, corresponding to an area which may be inflated after starvation in an aquarium. Five different cell types are present : (i) Stellate cells with highly ramified processes, which are the main component of the tissue. (ii) Cells with dense, irregular inclusions, 1-3 μm in diameter, are rather uncommon (Fig. 10). (iii) Bacteriocytes I are large cells with a nucleolate nucleus and well developed dictyosomes. They have large vacuoles containing ovoid or rod-shaped bacteria. All the intermediate stages between bacteriocytes I, containing a few vacuoles localised on one side of the cell, and cells with a large number of vacuoles opening into the tissue, were observed (Fig. 11). (iv) Bacteriocytes II are cells containing numerous ovoid inclusions, 1-2 μm wide and 3-6 μm long, which are tentatively interpreted as intracytoplasmic micro-organisms. The inclusions are separated from the cell cytoplasm by two dense lines; they have no well defined nuclear area, and two parallel dense bodies are visible in some of them (Fig. 12).

Fig. 10. - Cell with dense inclusions. TEM. Scale bar = 2.2 μm.
Fig. 11. - Bacteriocytes I. TEM. Scale bar = 4.4 μm.
Fig. 12. - Bacteriocyte II and Bacteriocyte I (bottom). TEM. Scale bar = 4.4 μm.
Fig. 13. - Transverse section through a filament. Note longitudinal fascicle of styles which appear as holes in centre after desilicification by HF. TEM. Scale bar = 8.2 μm.
Fig. 14. - A desilicified palmate anisochela protrudes perpendicular to surface of a filament with only small end embedded in tissue. A collagen fascicle anchors spicule within mesohyl. TEM. Scale bar = 3.4 μm
Fig. 15. - Various stages of spermatocysts inside body of sponge. SEM. Scale bar = 86 μm.
Captions. - a : anisochelae; b : bacteria; C : collagen; da : desilicified anisochela; M : mesohyl; m : intracytoplasmic microorganisms; N : nucleus; P : pinacocyte; S : spermatocyst; Su : surface.

(v) Spiculocytes are abundant and display the usual characteristics of sponge spiculocytes, with numerous mitochondria and small vacuoles. The intercellular bacteria are most frequently thread-shaped, 0.15-0.23 µm in diameter, with a dense central area, although the ovoid type, found in bacteriocyte I, is also present.
The filament tissue has a structure similar to that of the sponge body (Fig. 13, 14). However, the stellate cell processes and most of the other cells are elongated along the filament axis. Bacteriocyte I cells are less numerous, bacteriocyte II cells are absent and the intercellular, thread-shaped bacteria are more abundant.
The palmate anisochelae protruding from the filaments are always enclosed in a sclerocyte, which is reduced to a thin cytoplasm lining the larger tooth (Fig. 14). The elongate nucleus, some dense inclusions and a vesicular cytoplasm, are positioned close to the inner side of the shaft. The smaller end of the spicule is anchored by the basal part of the sclerocyte through the pinacoderm. A condensation of collagen fibrils occurs through the hole between the teeth of the small end and reinforces the anchorage of the spicule to the tissue.
At the base of the filament, the anisochelae do not protrude, but lie under the pinacoderm, parallel to the surface. In this position, the sclerocytes contain a larger amount of mitochondria.

Reproduction
Embryos have been observed very rarely in May and November. Their structure cannot be described yet.
Various stages in spermatogenesis have been observed frequently in specimens collected in all seasons. Spermatocysts appear first in the body as mass of large spermatogonia surrounded by a thin envelope (Fig. 15). During the development, the envelope becomes thicker and the cysts migrate towards the filaments, where they appear as swellings, 35-45 µm in diameter (Fig. 3). The mature sperm cysts are located near the end of the filaments. The sperm cells, with thick flagella, clear vesicles, and a dense, elongated nucleus, form a central mass surrounded by two envelopes, the outer one formed by closely intertwined cells. The mature sperm cysts possess forceps, which constitute two diametrically opposed tufts.

Etymology
From *hypoge* = subterranean.

Discussion

The genus *Asbestopluma* presently includes 27 species, of which several may be synonyms (KOLTUN, 1970). LUNDBECK, 1905 has proposed three subgenera : *Asbestopluma s. str.* (penniform, with lateral filaments bi- or pluriserially arranged, with a special stalk coating of spicules, and sigma); *Cotylina* (with a caliciform head, no stalk coating and no sigma); and *Lycopodina* (with filaments arising all round the axis or from the top of the body, no stalk coating and no sigma). As pointed out initially by HENTSCHEL 1914, some of the new species described since LUNDBECK's work contain a mixture of the characters of these subgenera, which are now not used. However, the new species, which is the first representative of the genus found in the Mediterranean, belongs to the *Lycopodina* group. It differs from the previously known species by its shape, consisting of a thin peduncle supporting a clearly delimited oval body. Such a shape is found only in *A. callithrix* HENTSCHEL, 1914, from the Antarctic (which differs by having larger megascleres), and in *A. hydra* LUNDBECK, 1905, from the Arctic, 1847 m and 2394 m depth with filaments mostly grouped on the upper part of the body, thicker and shorter megascleres and slightly larger anisochelae with a larger frontal tooth (Fig. 16).

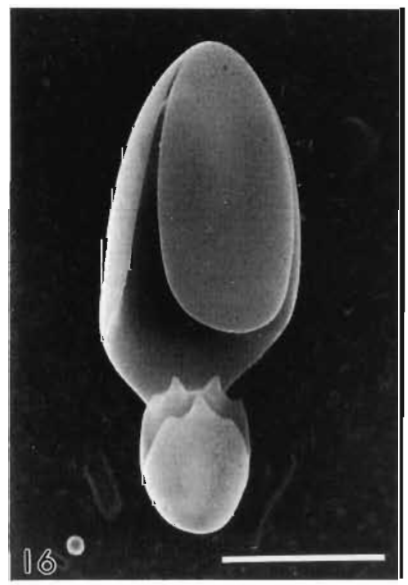

Fig. 16. - Front view of an anisochela of *Asbestopluma hydra* LUNDBECK, 1905 (paratype). SEM. Scale bar = 4.3 µm

In making such a comparison, one must be aware that the morphology of these fragile deep-sea sponges differs between that seen in natural environments and in collected specimens. The filaments of *A. hypogea* entangle and coalesce under any slight turbulence, and the morphology of the sponge is modified by formalin preservation (compare Fig. 1 and 2). The coalescence of filaments, their length and diameter appear also to vary according to the feeding stage. Long, thin filaments are characteristic of starving sponges, whereas coalescing, short filaments are observed after feeding (VACELET & BOURY-ESNAULT, 1995b). However, the morphological differences between *A. hypogea* and the nearest deep-sea species (which appears to be *A. hydra*) are always evident. Current knowledge

of the biology of this species suggests that the presence or absence of forceps is not a good diagnostic character in the genus, as their presence is related to male reproductive stages.

The new species is known only from this peculiar cave. However, it is most probably also present in deep-sea Mediterranean habitats. It has not yet been found in the Cassidaigne Canyon (100 - 3,000 m deep) located 7 km from the cave. The canyon is the likely place from which deep-sea populations living on rocky surfaces could produce the propagules which have colonised the cave. This area is the site of the strongest upwelling along the north Mediterranean coast (MILLOT, 1979). The sponge fauna of the canyon has been thoroughly studied (VACELET, 1969), but the presence of such a small organism could easily be overlooked if it occurs in caves or under ledges of the abundant cliffs in this canyon (LABOREL *et al.*, 1961). In deep-sea environments, the known Cladorhizidae mostly live on muddy bottoms, anchored by a rhizoid system or attached to small debris. However, the existence of a non-accessible fauna of small species on deep overhanging or vertical surfaces is likely. It is highly probable that *A. hypogea* is a member of the poorly known communities living on such rocky surfaces in the deep sea.

The organisation and biology of *A. hypogea* differ widely from that known in other sponges (VACELET & BOURY-ESNAULT, 1995a). However, it is likely that these features are common in the other deep-sea Cladorhizidae, and the interpretation of such bizarre structures and absence of apertures has been hampered by poor preservation. The use of microscleres to trap prey is a unique example of the use of microscleres in sponges. Microscleres are usually considered as having no clear role in sponges (DENDY, 1921), although they have been considered as "protective" against parasites or predators, without any evidence. Their role is here clearly "aggressive".

Acknowledgements

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