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Sponge Burrowing: Fine Structure of *Cliona lampa* Penetrating Calcareous Substrata*

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Abstract

Cliona lampa Laubenfels (Porifera: Hadromerida), a common burrowing organism in Bermuda, has been used to study the method of sponge penetration into calcareous objects. To progress beyond the results obtained by previous authors, electron-microscope techniques, in addition to light-microscope observations, were employed. Burrowing patterns, fine structure of the tissue-substratum interface and of calcareous fragments removed by sponge activity are described on the basis of scanning electron microscopy. Cell types and cytological features have been identified by light and transmission electron microscope. One cell type of archeocyte origin is demonstrated; it carves out chips of calcium carbonate (and conchiolin) by means of filopodial extensions and etching secretions. The cells undergo plasmolysis during this process; the substratum chips are expelled through the exhalent canal system. It is calculated that only 2 to 3 % of the eroded material is removed in solution.

Introduction

Numerous representatives of 12 major systematic groups of marine plants and invertebrate animals are known to excavate hard calcareous substrata by either chemical or mechanical means or by a combination of both. During the past century, a great number of papers has been written (Clapp and Kenk, 1963) dealing with the systematics, distribution, ecology and physiology of these organisms, and with the geological, chemical, and biological effects they cause by contributing to coastal erosion, by influencing the calcium balance in the sea, and by controlling the structure of marine communities where calcium-carbonate-producing organisms are dominant. An important summary of the latest research results on calcium-carbonate penetration was presented at a recent symposium (Carriker *et al.*, 1969).

The first discovery of a sponge, *Cliona celata* Grant (1826), penetrating oyster shells started a long-lasting discussion; some workers advocated chemical burrowing, others mechanical penetration. This argument continued long after Nassonov (1883), in his fine experimental study, had shown that *Clione* (sic) *stationis* (*Cliona vastifica*) etches elliptical lines into oyster shell lamellae and, by means of cellular processes, is able to free from the substratum hemispherical fragments which are extruded from the sponge. He assumed that chemical as well as mechanical forces are employed for the process. Topsent (1887) figured and described similar calcareous chips, but also comparable conchiolin fragments from sponge-riddled oyster shells. He concluded that the mechanism of penetration had to be mechanical, and believed that it could be performed by contractile cells which he described, among other cell types, for *C. celata* and *C. vastifica*.

Cotte (1902) believed, like Nassonov (1883), in chemical freeing of the chips. He suggested that an enzyme might be responsible for attacking the conchiolin. As he could not determine the presence of an acid for etching out calcareous fragments, he speculated that its secretion might take place only intermittently. Cotte also confirmed the significance of pseudopodial cell expansions for the excavation of chips.

Until quite recently, little experimental work followed these important early contributions. The latest comprehensive review on clionid burrowing mechanisms is by Goreau and Hartman (1963). These authors also point to the fact that direct production of calcareous fine sediments by burrowing sponges has not been appreciated in the modern sedimentological literature. Finally, Neumann (1966) studied not only the rate of bioerosion caused by *Cliona lampa*, an abundant sponge in Bermuda, but also estimated production of fine sediment due to the activity of the clionid.

Warburton (1958) revived the interest in the burrowing mechanism of *Cliona*. Instead of waiting for larval stages, he let tissue fragments of adult *Cliona celata* reconstitute on transparent calcite crystals and other substrata. The small sponges obtained this way would readily penetrate calcareous materials. The author substantiated the observations by Nassonov (1883) and by Topsent (1887) on etching patterns and form of calcium carbonate and conchiolin chips

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He observed the pathway of chips from the substratum through the exhalant canals and through the oscula, around which they accumulated. He studied living and fixed stained cells suspected to be engaged in etching and removing chips. He was unsuccessful in demonstrating acidic substance or increase of dissolved calcium carbonate in dishes containing actively burrowing sponges. He suggested that the solvent is secreted in minute quantities by threads of cytoplasm, and that the amount of calcium dissolved is less than 10% of the substratum removed and could, therefore, not have been detected by his experimental method.

Cobb (1969), using the same method of culturing as Warburton, concentrated his study on the cell-substratum relationship during the burrowing process of *Cliona celata*. He confirmed the observation of earlier authors on etching patterns by using calcitic (Iceland spar, *Crassostrea* shell) and aragonitic (*Mercenaria* shell) substrata, and described these and the shape of the dislodged fragments in detail. He could also observe penetration into pieces of periostracum obtained from decalcified *Mytilus* shells, thus giving further support to the view that an enzyme could participate in the etching process.

Cobb (1969) identified one cell type, with prominent nucleus and nucleolus and basophilic granules surrounding the nucleus, as responsible for the etching process. According to his view, the etching agent is released precisely at the cell edge, which insinuates itself into the substratum as a slit-like crevice is dissolved ("chemical coring"). A noose-like constriction of the cell edge finally frees the chip. No pseudopodial extensions, as described by Nassonov (1883), Cotte (1902), and Warburton (1958), were seen participating in the process.

The results of the fine studies mentioned here show clearly that previous observations were limited by the resolving power of the light microscope. We are, therefore, attempting to introduce new evidence, based on observations with scanning and transmission electron microscopy, by describing the fine architecture of the substratum, the nature and structure of cells, and the cell-substratum relationship of *Cliona lampa* during the burrowing process.

We have followed the terminology of Carriker and Smith (1969), who suggest to consider sponge penetration, as hitherto described, as burrowing. This term is convenient also because the term "boring sponges" is generally associated with the family Clionidae: however, we now know members of other sponge families (Adociidae: Rützler, 1971; Spirastrellidae: Pang, in press; Rützler, unpublished) which efficiently excavate limestone substrata.

Material and Methods

The following method was used to initiate growth of *Cliona lampa* in Iceland spar crystals. Infested shells of the bivalve *Chama macerophylla* Gmelin and fragments (50 to 75 cm^3 volume) of coral and calcarenite containing the sponge were each wired to one crystal which, again, was attached to a wooden frame. The whole procedure was performed under water, and at the place of natural occurrence of the sponge, at Castle Harbour, Ferry Reach North Entrance and Whalebone Bay, Bermuda. Care had to be taken to leave as little crystal surface as possible uncovered to avoid fouling and boring by other organisms. Several frames were nailed to solid structures (rocks, concrete pillars) at 1 to 2 m below low tide level. The experiments were made during July, 1971 and January, 1972, and left undisturbed for periods of 1, 2 and 5 months.

Infested crystals were detached from the "mother" sponge, and kept under observation in the laboratory to assure their active state of burrowing during the moment of fixation. Activity was indicated by the continuous discharge of calcareous fragments through one or several oscula.

Hexamethylenetetramine — buffered 4% formalin-seawater was used as fixative for most light-microscope observations. Two fixatives were employed for electron microscopy: (a) 2% glutaraldehyde in 0.1 M sodium cacodylate in seawater (pH 7.4), with 1.5 g sucrose per 100 ml added to the solution; postfixation in 2% osmium tetroxide in distilled water; fixation times 1 h in each solution at 4 °C. (b) 4% glutaraldehyde in 0,1 M phosphate buffer (pH 7.4), with 6.8 g sucrose per 100 ml added to the solution; postfixation in 0.5% osmium tetroxide in 0.1 M phosphate buffer; fixation times 1 h in glutaraldehyde, 2 h in osmium tetroxide, both at 4 °C. Calcite crystals containing the sponges were broken up while they were immersed in the glutaraldehyde solution, to assure complete penetration. Spurr low-viscosity embedding medium (Polysciences, Inc., Warrington, Pennsylvania, USA) was used for embedding. After curing, one surface of each crystal was exposed by grinding off the epoxy, and the crystal was dissolved in dilute hydrochloric acid. After rinsing and drying, the specimens were re-embedded in epoxy to fill the spaces previously occupied by the crystal.

The following microscopical techniques were used for studying the material:

(a) Light microscopy. Epoxy embedded, undecalcified and decalified specimens were glued onto microscope slides, wet-ground, and polished to 50—10 μm thickness. They were either viewed unstained with phase contrast or interference contrast, or were stained in 1% aqueous toluidin or in 1% aqueous safranin and 1% aqueous crystal violet. At 60 °C on a hot-plate, the stains penetrate a few micrometers into the epoxy surface, without staining the resin itself.

(b) Scanning electron microscope (SEM). Observations were made on epoxy-embedded tissue after dissolution of the crystal. As the epoxy also infiltrates spaces between tissue and substratum, the actual tissue was not seen this way but rather a cast of the excavated

tunnels. To overcome this problem, paraffin was also used for embedding during decalcification The paraffin was then removed with xylene, the tissue washed in 100% alcohol, transferred into amyl acetate, and dried in a critical point dryer, using liquid carbon dioxyde. Fragments of the burrowed substratum were cleaned in commercial sodium-hypochloride, rinsed, cleaned in an ultrasonic cleaner, and oven dried. Crystal chips which had been removed by the sponge were pipetted from the oscular area, cleaned in an ultrasonic cleaner, and oven dried. Contact with distilled water was avoided. SEM specimens were coated with a 20 nm layer of gold, and viewed by a Cambridge Stereoscan Mark II A at 50 ×—14500 × primary magnification.

(c) Transmission electron microscopy (TEM). Specimens were sectioned, and stained with uranyl acetate in 100% ethonol. A Zeiss 9 S-2 with primary magnifications 1860 ×, 4570 × and 9140 × was employed.

Results

Morphology and Distribution

Cliona lampa Laubenfels (Porifera: Hadromerida) is a very common sponge in shallow water (intertidal to 3 m depth) of Bermuda. It is conspicuous in its typical appearance, vermilion membraneous incrustations on a variety of calcareous substrata (limestone, coral skeletons, mollusk shells) (Fig. 1:*1*). However, it is also abundant in a more cryptic form: small circular papillae, which bear either ostia or oscula, and protrude slightly above the substratum surface (Fig. 1:*2*). The orange-colored endosome of the sponge infiltrates porous calcarenite and coral rock, or it fills distinct excavated galleries in well-cemented limestone and mollusk shells (Fig. 1:*3, 4*) (Laubenfels, 1950; Neumann, 1966).

The coating of most encrusting specimens is so thin that the nature of the underlying structure can easily be recognized. Irregularly distributed oscula are circular, seldom elongate, 2 to 4 mm in diameter. They have a slightly raised rim if viewed *in situ* (Fig. 1:*1*). Specimens in protected locations can frequently be covered by fine sediment, only the oscular openings showing. The papillae of the cryptic form of *Cliona lampa* measure 0.5 to 1.5 mm in diameter. Penetration varies from 0.5 to 80 mm from the substratum surface. The excavated chambers, if well-developed, are more or less spherical, closely spaced and 0.5 to 1.5 mm in diameter.

A more detailed taxonomic description of this and other burrowing sponges in Bermuda is presented elsewhere (Rützler, in press). No skeletal or histological differences between the two growth forms of the sponge could be detected. There seems to be no correlation between substratum structure and cryptic or encrusting habit. *Cliona vastifica* Hancock is a closely related species with world-wide distribution, not reported from Bermuda but mentioned by various authors from the West Indies, Florida and Gulf of Mexico. *C. stationis* Nassonov, subject of Nassonov's study of the burrowing habit (1883), is a synonym of *C. vastifica* (fide: Vosmaer, 1933).

For the present study, *Cliona lampa* has been collected in abundance at the following locations in Bermuda: Coot Pond (0.5 to 1 m), Whalebone Bay (0 to 3 m), Three Hill Shoals (3 m) (North Shore); Ferry Reach (0.3 m); Castle Harbour (0 to 3.5 m); Harrington Sound (0.2 m); Devonshire Bay, Church Bay (0 to 1.5 m) (South Shore).

Outside of Bermuda, the species is known from the Gulf of Mexico (Laubenfels, 1953; Little, 1963), Bahamas (Rützler, unpublished) and Jamaica (Pang, in press).

Penetration into New Substrata

Iceland spar crystals containing new growth of *Cliona lampa* were carefully detached from the "mother" organism in the field and brought to the laboratory, without exposing them to air. Before fixation, they were cleaned from fouling organisms and kept for observation in an aquarium with circulating sand-filtered seawater for 12 to 60 h. Depending on the closeness of previous contact between the smooth crystal surface and the substratum of the mother sponge, a varying amount of soft tissue had remained on the crystal after separation. Although this wound healed within 2 days (Fig. 1:*5*), the surplus tissue was usually cut off with a razor blade. This exposed a number of circular perforations at the crystal surface, filled with living tissue, where the mother sponge had penetrated (200 to 400 μm in diameter) or, where burrowing processes from newly formed chambers had pierced the surface from inside the crystal (40 to 150 μm). An average of 150 perforations per cm^2 was counted, 2 to 4 (per cm^2) of the larger ones take over the function of oscula (Fig. 1:*6*). These, without doubt, had been major exhalant canals leading back to the mother sponge.

The burrow at this stage has roughly the shape of a truncated cone that extends into the crystal, perpendicular to the attached surface (Fig. 1:*7*). The direction of burrowing is not influenced by cleavage planes of the Iceland spar. The burrow is composed of a three-dimensional network of galleries, formed by spherical to ovoid chambers which are interconnected by short tunnels. In the distal portion of the burrow, the chambers are comparatively small, well-spaced, with one to a few connecting tunnels. From each chamber radiate numerous tapering tunnels, which contain the most actively burrowing tissue processes (Figs. 2:*1—3*; 3:*2*). These are spear-heading the penetration process. Formation of new chambers usually occurs where radiating burrowing processes from adjacent chambers meet. However, isolated processes may also begin forming chambers and radiating