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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

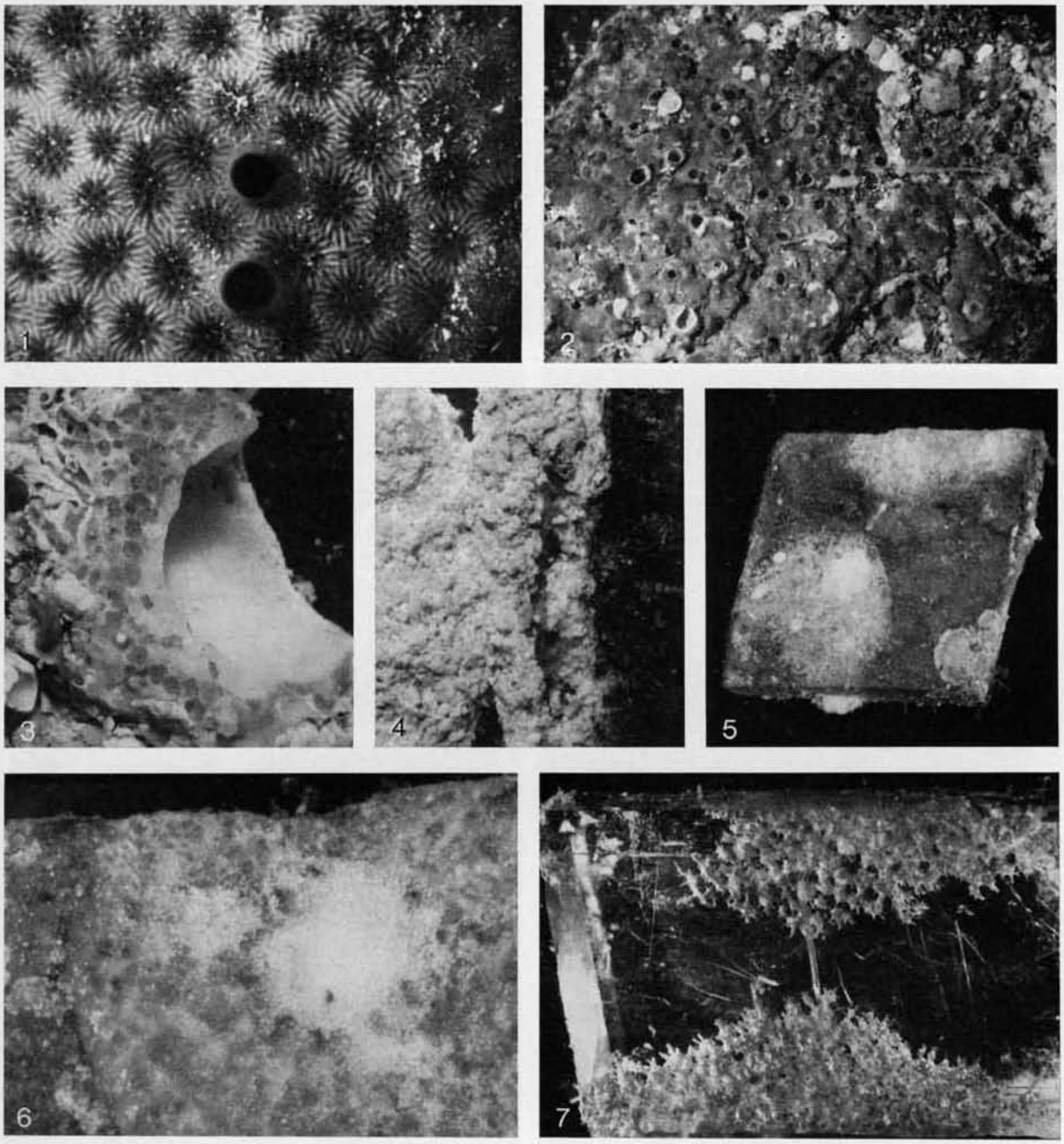

Fig. 1. *Cliona lampa*, morphology. 1: Encrusting coral *Favia fragum* (Esper); note undisturbed appearance of coral, although covered by a thin red layer of sponge tissue; 2 oscula are showing (2.4 ×). 2: Circular papillae of the other growth form, protruding through coralline alga covering rock (2.4 ×). 3: Distinct galleries formed by the sponge inside the bivalve *Chama macerophylla* Gmelin (2.4 ×). 4: Indistinct infiltration of poorly cemented calcarenite (2.4 ×). 5: Iceland spar crystal, detached from "mother" sponge after 3 months; the two large bright areas consist of healed tissue; several white spots (lower left) are areas of calcium carbonate chips accumulated around newly formed oscula (2.4 ×). 6: Enlarged view of encrusting tissue and osculum, almost buried by accumulation of chips (10 ×). 7: Side view of a crystal that had been burrowed from opposite sides; newly formed chambers and tunnels can be seen through the transparent material (2.4 ×)

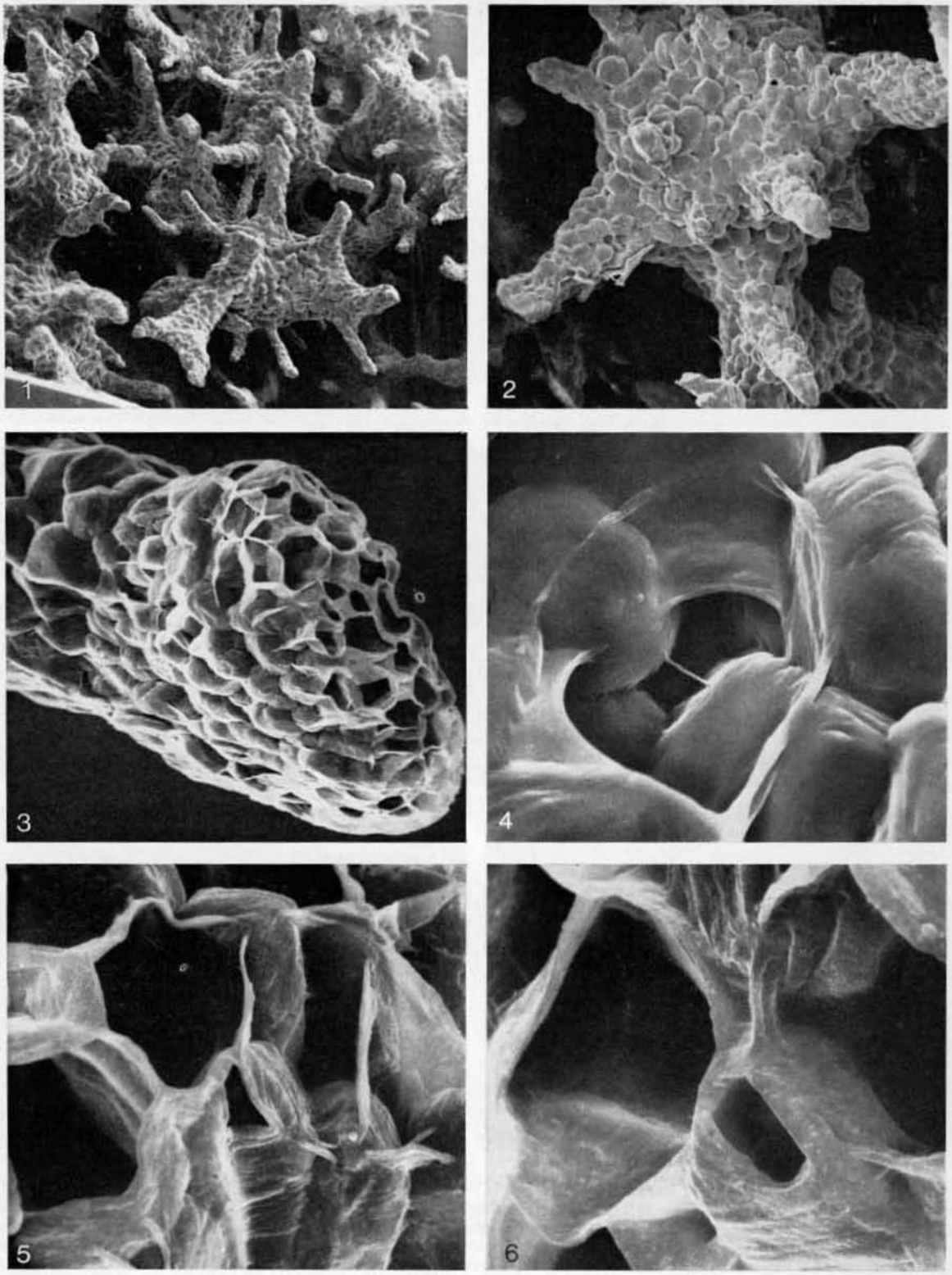

Fig. 2. *Cliona lampa*. SEM views of epoxy casts from burrowed chambers and tunnels. 1: Chambers and tunnels, showing pitted (in negative) surface where chips had been removed (40 ×). 2: One chamber with tunnels, enlarged (90 ×). 3: Apical end of tunnel cast; note membraneous structures representing casts of etched crevices (300 ×). 4: Molds of completed pits and of initial etchings (900 ×). 5: Enlarged view of molds from etched crevices (1500 ×). 6: Same, further enlarged; note constriction of crevice mold where chip is being cut off. Most crevice casts appear thicker than they actually are because of perspective distortion (3000 ×)

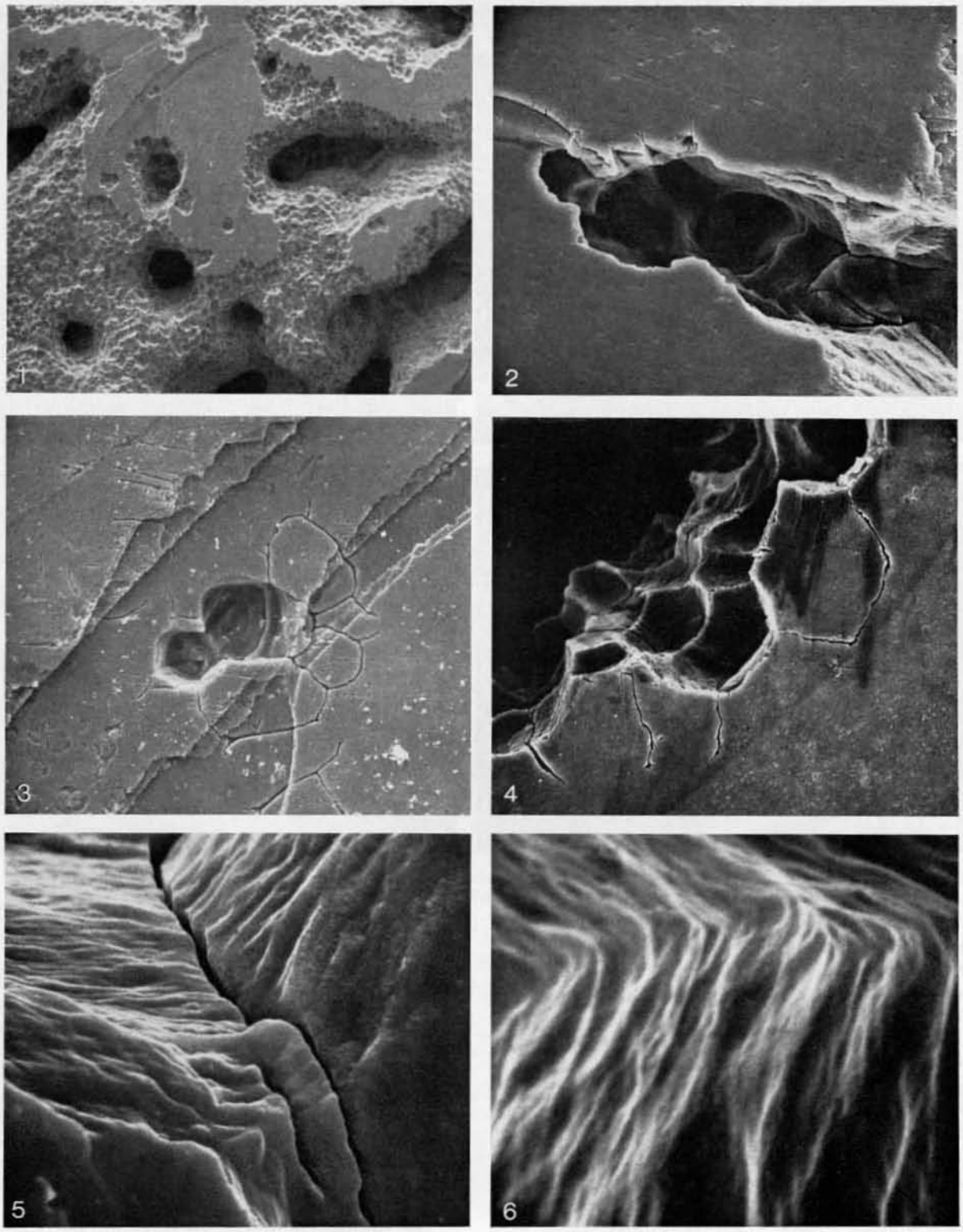

Fig. 3. *Cliona lampa*. SEM views of burrowed Iceland spar crystals. 1: Partly etched crystal surface where "mother" sponge had been attached. Note tunnels penetrating crystal (50 ×). 2: Cleaved crystal showing one of foremost tunnels (900 ×). 3: Initial etching patterns on crystal surface; two chips are already removed (500 ×). 4: Similar view at tunnel entrance (700 ×). 5: New etching on surface where chips had previously been removed (400 ×). 6: Fine structure of etching pattern produced by cellular processes (800 ×)

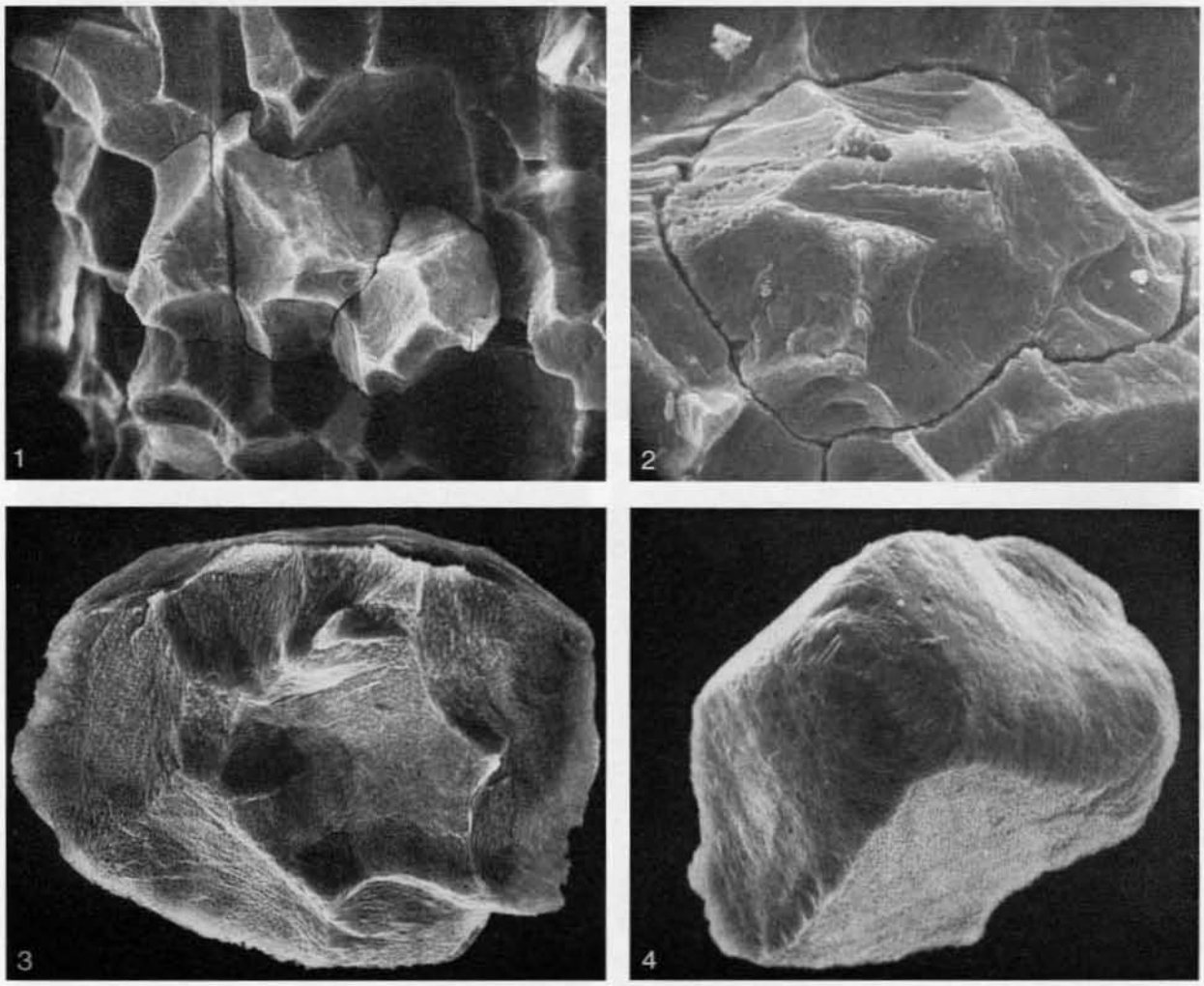

Fig. 4. *Cliona lampa*. SEM views of chips cored by sponge action. 1: Group of chips etched from substratum but still in place (600 ×). 2: Similar view, enlarged (1600 ×). 3, 4: Isolated chips discharged through sponge osculum (1500 ×)

tunnels after about 1 mm penetration. Exceptionally, these processes pierce the surface of the substratum and form inhalant or exhalant papillae.

The first chamber formation, as seen from the attached crystal surface, takes place after 0.2 to 0.5 mm. The chambers measure 0.4 to 1.3 mm, without radiating tunnels. Interconnecting passages are 0.2 to 0.4 mm wide; radiating processes attain a length of about 1 mm and taper from approximately 200 to 40 μm diameter.

Due to the transparency of the crystals, it can be clearly seen, even by low-power stereo-microscope, that the walls of the galleries are distinctly pitted. These are the places where substratum chips were freed by cellular action. The chips fill the tissue in all active areas, and move with the water current through the exhalant system. They are expelled through the oscula and, in quiet water, pile up around these openings (Fig. 1:*6*).

Fine Structure of the Substratum

A scanning electron microscope view of the attached crystal surface (after removal of the sponge tissue) shows the perforations of the first tunnelling steps and also a pitted pattern, evidence of surface erosion (Fig. 3:*1*). This surface erosion does not proceed further once the sponge has established its first galleries inside the new substratum. The same situation can be observed under the surface tissue layer on naturally occurring substrata. Complete breakdown of the limestone support, resulting in a free (gamma-) stage of *Cliona lampa*, never occurs.

The outlines of initial etchings on the smooth Iceland spar surface are shown in Fig. 3:*3, 4*. They are roughly circular in outline, join one another, and never overlap, indicating that they have been simultaneously produced. Erratic incomplete and branching lines can also be seen. Where the first chips are al-

ready removed, the crevices can be followed perpendicularly into the substratum (Fig. 3:*4*).

An epoxy mold, after dissolving the substratum, gives an informative view in negative of a chamber with burrowing processes and pits (Fig. 2:*2*). Subsequent etching on already burrowed surfaces produces etchings and almost dislodged chips, as shown in Fig. 4:*1, 2*. Epoxy molds of a distal tunnel ending give a negative, but three-dimensional, impression of the complex system of crevices produced by an actively burrowing process (Fig. 2:*3—6*). The bulbous structures (Fig. 2:*4*) are molds of completed pits. Constriction of the crevices, where the chip is to be cut off, can be noted on Fig. 2:*6*. The fine structure of burrowed surfaces is irregular but smooth, i.e., without sharp edges or corners (Fig. 3:*5, 6*). A system of shallow grooves and ridges becomes apparent at high magnification. These can be randomly oriented; more frequently, however, they appear as parallel structures on the wall of the pits, approximately concentric with the outline of the initial etching.

Initial etchings, completed around their periphery, measure 18 to 64 × 13 to 48 μm (maximum lengths of perpendicular axes), with mean dimensions of 39 × 27 μm ($n = 17$). The depth of their straight portion (before they change direction to undercut a chip) could be followed on some photographic stereo-pairs: 12 to 17 μm. The width of the crevices is fairly constant (0.15 to 0.25 μm), except at the mouth, where it is greater (0.3 to 0.8 μm).

Freshly removed chips were collected for study from the piles outside the oscula. They are circular to ellipsoid in outline, and have a surface composed of mostly concave facets. These concave facets are parts of pits left by previously removed adjacent chips (Fig. 4:*3*). One part of the surface is convex (Fig. 4:*4*), and represents the portion of the chip that had been freed from untouched substratum areas. Concentric grooves and ridges like the ones mentioned above are also apparent there.

The dimensions of the chips (length × width × height) range between 30 to 88 × 30 to 75 × 16 to 57 μm; mean values are 56 × 47 × 32 μm ($n = 50$). The means deviate from the much smaller dimensions given above for the outline etchings. This is explained by the fact that most incomplete etchings were seen near the distal ends of the tapering tunnels. Much smaller and more spheroid chips are mined there than further back in the tunnels and chambers. The measured chips, however, represent the full range of a long-term burrowing activity.

Histology and Cytology

Polished sections of epoxy-embedded specimens of *Cliona lampa* show that the ectosomal portion of the sponge is either restricted to the incurrent or excurrent papillae or to a 0.1 to 0.3 mm membrane overlaying the substratum. Densely packed spiny micro-

Table 1. *Cliona lampa. Measurements of cells, organelles and cytoplasmatic inclusions taken from transmission electron photomicrographs (μm)*

Cell type	Cell body	Nucleus	Nucleolus	Mitochondria	Inclusion droplets		Phagosomes	Glycogen granules	Small vesicles	Vacuoles
					Medium dense	Dense				
Pinacocytes	4—41 × 2—4	3—4 × 2—3	—	0.3—0.6	0.5—0.7	0.2—0.3	0.8—1.0	0.05—0.10	0.17—0.50	2.9—5.9
Collencytes	7—29 × 1—5	2—6 × 2—3	—	0.1—0.8	0.3—0.8	0.2—1.0	0.4—2.3	0.03—0.14	0.07—0.80	—
Grey cells	8—19 × 3—8	2—4 × 2—3	—	0.3—0.5	0.3—1.5	0.3—1.9	0.4—1.2	0.05—0.15	0.14—1.20	—
Sclerocytes	4—7 × 2—5	1—3 × 1—2	—	0.2—0.3	—	—	1.0—1.5	0.03—0.07	0.17—0.34	1.0—5.4
Choanocytes	6—8 × 2—5	2—3 × 2—3	—	0.3—0.5	—	0.3—1.5	0.6—2.0	0.05—0.17	0.20—0.90	1.9—4.1
Archeocytes	4—19 × 3—9	2—7 × 1—4	0.5—1.5	0.1—1.0	0.3—0.5	0.3—0.8	0.3—4.1	0.07—0.10	0.07—0.69	2.1—6.2
Etching cells	7—24 × 4—15	3—7 × 1—5	0.6—1.6	0.2—1.0	0.4—0.8	—	0.6—1.6	0.03—0.13	0.14—0.45	1.0—2.0

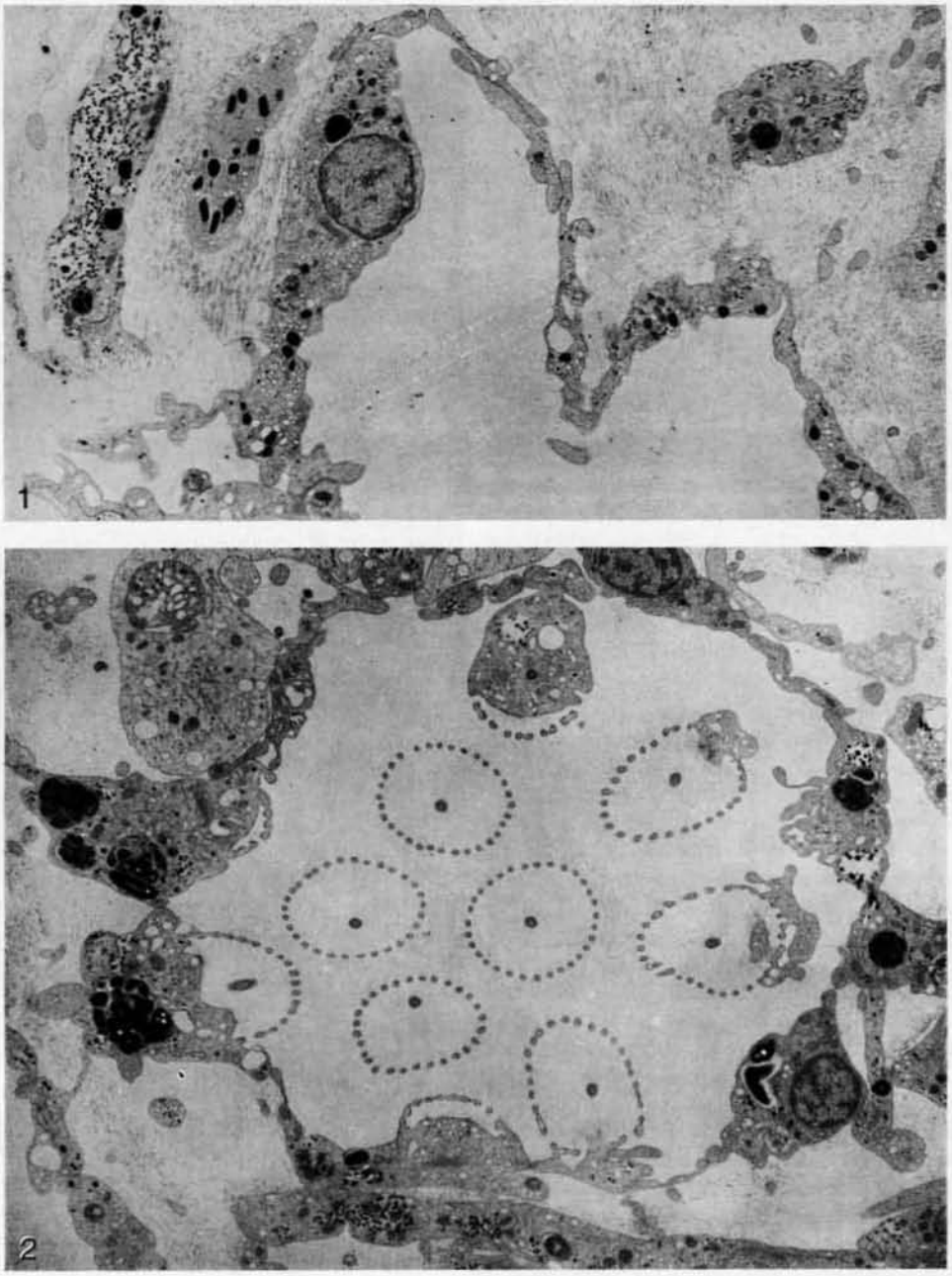

Fig. 5. *Cliona lampa*. TEM photomicrographs of cell types present. 1: Pinacocyte lining aquiferous canal (4000 ×). 2: Section through choanocyte chamber; note cross-section of collars and flagella (4000 ×)

rhabds form a delicate cortex, 20 to 40 µm thick. Tylostyles are embedded perpendicularly to the surface from which their tips frequently protrude.

The choanosome extends throughout the galleries. It is characterized by spherical choanocyte-chambers, and by numerous micro-spined oxea.

The following cell types could be localized, and are described from light-microscope observations and transmission-electron photomicrographs. Dimensions of whole cells given below are taken from polished thick-sections. Measurements of cell thin-sections, cell organelles and cytoplasmatic inclusions are summarized in Table 1.

Pinacocytes (Fig. 5:*1*) form the exopinacoderm, and line the aquiferous canals and the galleries in the substratum. The cell body around the nucleus is only

3 to 4 μm wide, and tapers in opposite directions to produce a cytoplasmic sheet, up to 40 μm long, and usually less than 0.5 μm thick. The anucleolate nucleus is spheroid to ovoid, with a Golgi complex developed at one side. Subspheroid mitochondria and loops, and strands of rough endoplasmic reticulum are evenly distributed throughout the cytoplasm. Knob-like junctions between endopinacocytes resemble those described for exopinacocytes of *Microciona* (Bagby, 1970). There is no other differentiation in the area of contact. Phagosomes occur in the cell-body and in the thicker parts of the sheets. Small vesicles are abundant, and occasional large vacuoles inflate the sheet. Small, electron-dense inclusions and glycogen granules are rare.

Collencytes are stelliform cells with long and frequently ramified filopodia located in abundance between the tylostyles in the ectosome. More mobile fusiform collencytes occur in the choanosome (Fig. 6: *1, 4*). The cell body without pseudopodial processes attains about 11 × 5 μm, the longest spindle measured was 29 μm. The nucleus is spheroid or ellipsoid, and no nucleolus was observed with certainty. Dictyosomes and associated vesicles are sometimes well developed; spheroid mitochondria and ergastoplasm are abundant and dispersed. Phagosomes and accumulations of glycogen granules are frequently seen, but other inclusions are very rare. Collencytes are always associated with collagen. The fibrils are approximately 13 nm in diameter, even in thickness, and appear in strands. They are never ramified and are never found intracytoplasmic (Pavans de Ceccatty and Thiney, 1963). We confirm the observation that parallel collagen fibrils form, the instant a secretory product leaves the cell, at certain zones where the cell membrane is not distinct (Lévi, 1964).

Gray cells appear at the transition from ectosome to choanosome, and become very abundant in the choanosome (Fig. 6:*2, 3*). Their name, coined by Wilson and Penney (1930) from their appearance through the light microscope, is now well established also in the electron optical sponge literature. Gray cells are packed with highly refractile granules, that show a strong basophilia after staining. They are irregular in outline, some are quite elongated, and twisted in three dimensions. The cells measure 13 to 40 × 3 to 13 μm (24 × 8 μm, on average). The small anucleolate nucleus is spheroid. A Golgi complex was never clearly seen, endoplasmic reticulum is little developed. Mitochondria are dispersed and moderately abundant. Phagosomes are rare, but clusters of glycogen granules are frequently amassed. The large inclusions that are characteristic for this cell type occur in an abundance of 20 to 35 per cell section. They are usually ovoid, and approximately 1 μm in diameter. The inclusions are surrounded by membranes, and vary in electron density. Some appear black, others dark gray, with or without a darker core. No structure can be resolved inside. The nature of the droplets is not yet clearly understood, but histochemical tests indicate that they are of a lipoid substance (Borojević and Lévi, 1964; Pavans de Ceccaty, 1966).

Sclerocytes are encountered everywhere in the choanosome (Figs. 6:*5*; 8:*1*). Their size depends on the development of the vacuole containing the spicule, 12 to 17 × 3 to 6 μm for fairly advanced stages with spiny microrhabds. Because of the size of the vacuole, the cell-body is reduced to a thin sheet, comparable to pinacocytes. The subspherical anucleolate nucleus causes an inflation of the cell. Golgi membranes were never observed, endoplasmic reticulum, mitochondria and inclusions, other than the spicule are very rare.

Choanocytes compose spherical chambers of 25 to 30 μm diameter (Fig. 5:*2*). There are 15 to 20 cells per cross section of the chambers. The cells measure 4 to 6 × 3 to 5 μm. Their collars are approximately 9 μm high and 4 μm in diameter. Nuclei are small, spheroid and without nucleolus. Golgi apparatus and rough endoplasmic reticulum are well developed and mitochondria are abundant. Most choanocytes contain prominent phagosomes and vacuoles filled with fibrillous material, possibly traces of bacterial digestion (Borojević and Lévi, 1964).

Archeocytes (Fig. 6:*4, 6*) occur throughout the choanosome, but they are especially abundant in the burrowing processes. They are distinguished by their rounded shape, large nucleus with prominent nucleolus, and abundance of phagosomes. Even if elongate they never assume spindle shape. Lobopodial processes are rare. The cells measure 18 to 24 × 8 to 12 μm. The nucleus is spheroid or ovoid, and contains a spherical nucleolus. Stacks of Golgi membranes with associated vesicles are always present, rough endoplasmic reticulum and large, ovoid cristae-type mitochondria are abundant throughout the cytoplasm. There are numerous small, some large vacuoles, and some multivesicular bodies. Up to 16 phagosomes per cell section can be counted. They are in different stages of development, including those containing the fibrillous material mentioned above. Many phagosomes are conspicuously surrounded by rosettes of glycogen granules. Other inclusions such as lipoid droplets are rare.

Cellular Structures Related to Burrowing

The burrowing activity of *Cliona lampa*, as judged from the presence of etching cells, is most intense at the tips of the burrowing processes, but it is not restricted to these regions. Groups of active etching cells can be observed along the sides of the processes, and almost everywhere in the peripheral parts of the galleries (Fig. 7:*1*).

The choanosome, characterized by the presence of choanocyte chambers, fills the galleries in the substratum, but does not extend into the tapering burrowing processes. These contain, in their proximal por-

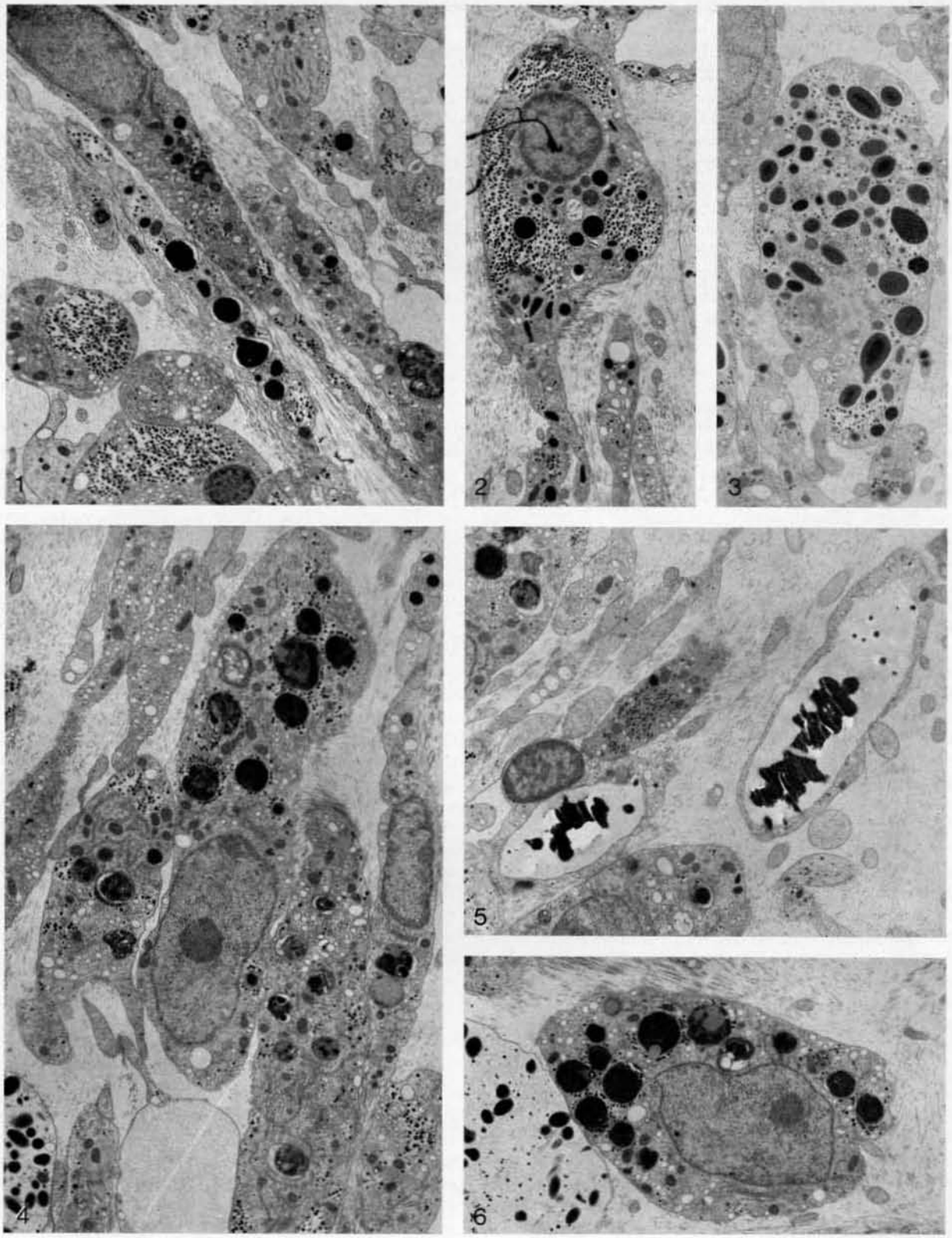

Fig. 6. *Cliona lampa*. TEM photomicrographs of cell types present. 1: Fusiform collencyte surrounded by fibrils of collagen (5100 ×). 2, 3: Gray cells with glycogen granules and lipoid droplets (5100 ×). 4: Archeocyte with nucleolate nucleus and prominent phagosomes surrounded by glycogen granules; note discharge of collagen from collencyte to the right (5100 ×). 5: Sclerocytes containing spicules (5100 ×). 6: Archeocyte containing numerous phagosomes (5100 ×)

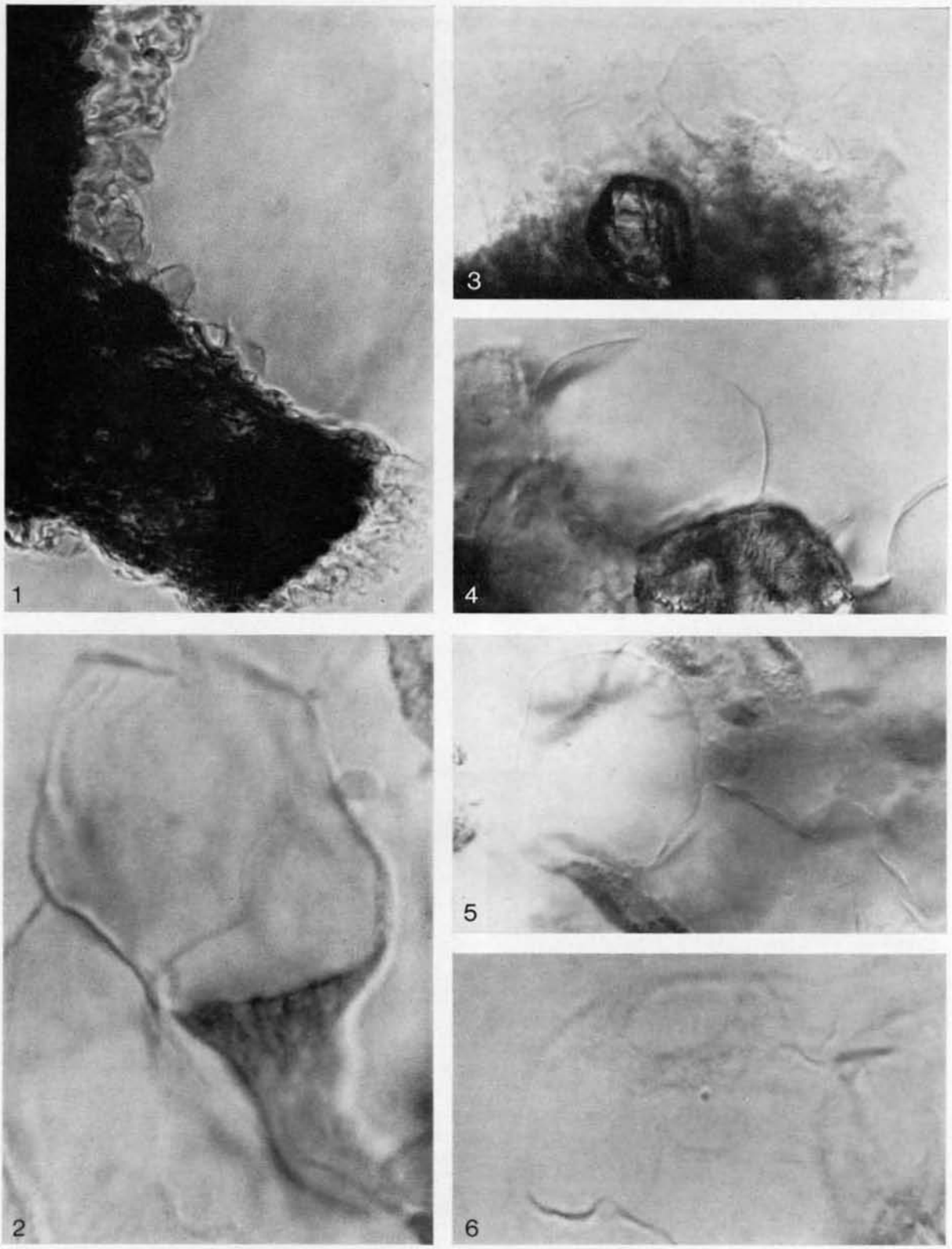

Fig. 7. *Cliona lampa*. Light photomicrographs (interference contrast) of tissue structures during penetration of substratum (epoxy embedded, decalcified). 1: Burrowing tissue process lined with active etching cells (90 ×). 2: Etching cell with filopodial extensions penetrating the substratum (1800 ×). 3: Similar view, showing several etching cells and 1 chip that had already been removed; the chip had been enveloped by epoxy during decalcification (700 ×). 4: Optical section through filopodial basket embracing a chip (dissolved), and displaced (undissolved) chip (700 ×). 5: Head-on view of filopodial baskets during the first etching steps (700 ×). 6: Filopodial basket with iris-like constriction where chip is (dissolved) being cut off (compare with Fig. 2: *6*) (1800 ×)

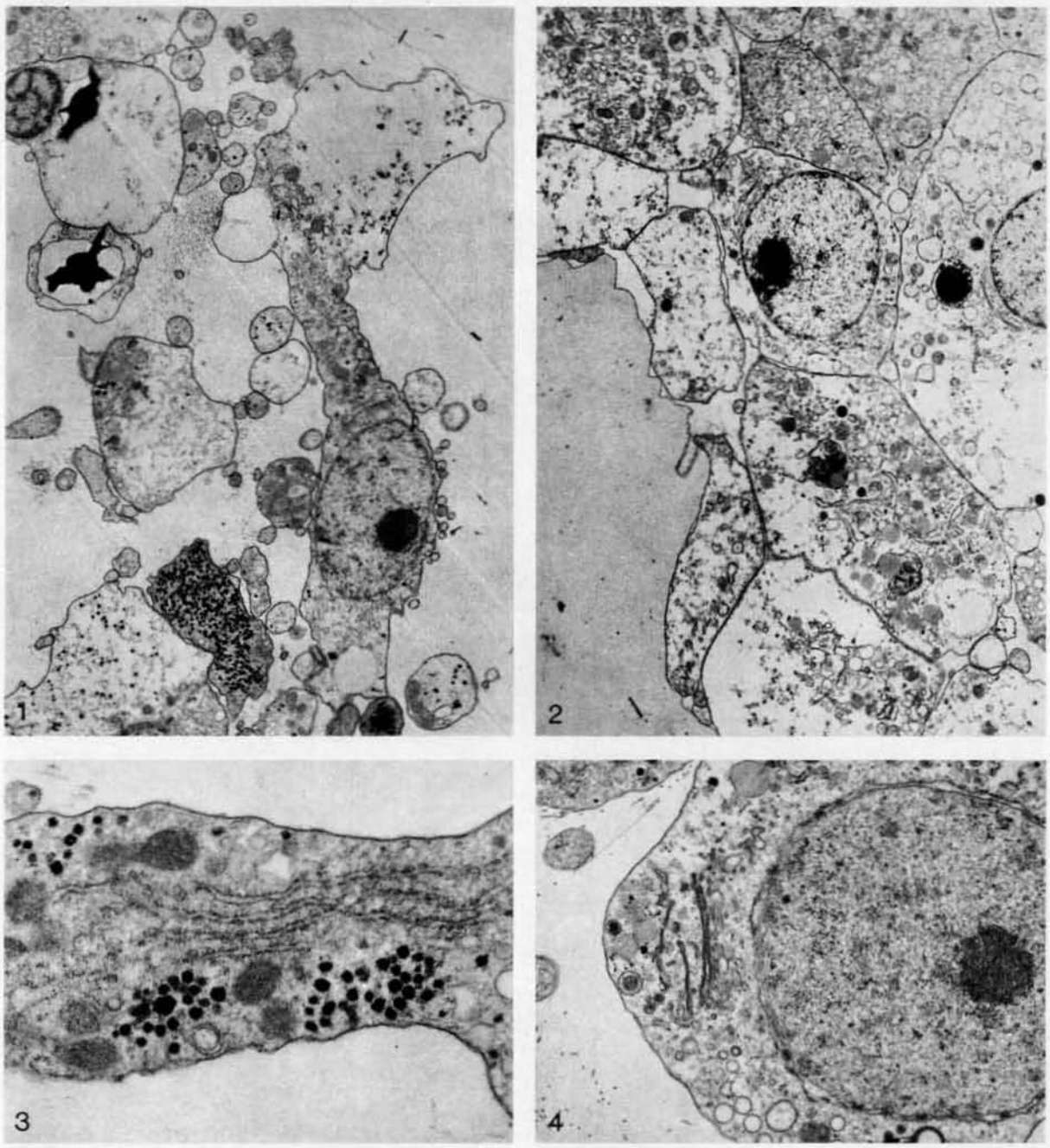

Fig. 8. *Cliona lampa*. TEM photomicrographs of etching cells. 1: Etching cell with nucleolate nucleus, showing first steps of plasmolysis and formation of flocculent secretory product; the fine line across upper right corner is an artifact, indicating position of calcareous substratum; there, after solution of the crystal, new epoxy joined the old resin during re-embedding (5200 ×). 2: Various stages of plasmolysis in etching cells at tissue-substratum interface (epoxy-replaced substratum to left) (5200 ×). 3: Development of rough endoplasmic reticulum, mitochondria, and glycogen at early stage of penetration (28000 ×). 4: Golgi complex, nucleus and nucleolus (10000 ×)

tion, sclerocytes, endopinacocytes, amoebocytes and large "bubble" cells (vesicles containing mainly a fuzzy, mucoid, basophilic substance but also a varying amount of cell remains). Occasionally seen nuclei are nucleolate, large, and oval, indicating that the vesicles derived from archeocytes. In the distal areas of actively burrowing processes, there are endopinacocytes forming canals, occasional sclerocytes, large intercellular spaces,

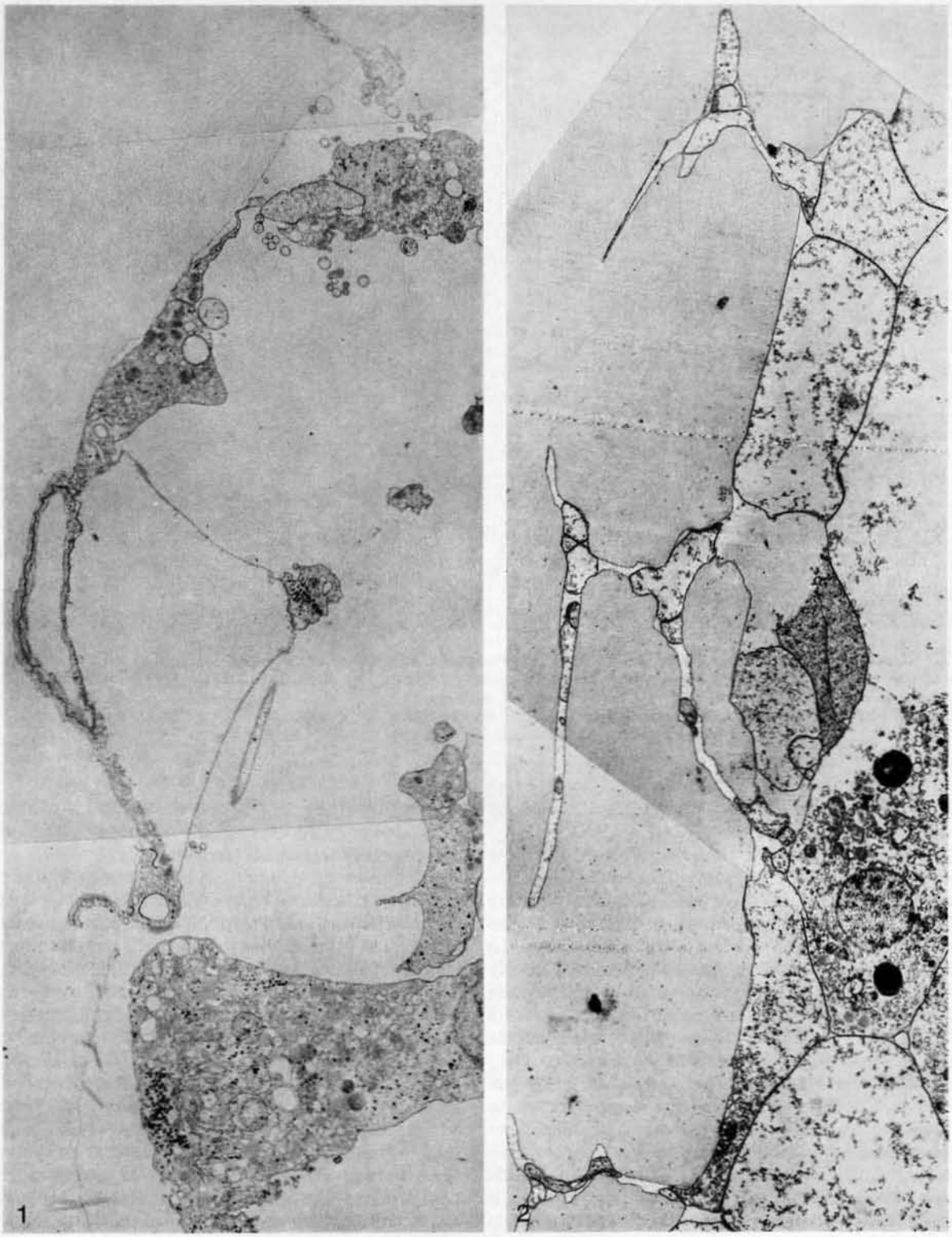

Fig. 9. *Cliona lampa*. TEM photomicrograph composite of cell-substratum relationships. 1: Early stage of filopodial penetration; cell showing part of nucleus at bottom; substratum (replaced by epoxy) lies to left, boundary is indicated by thin line (see explanation for Fig. 8:1) (4000 ×). 2: More advanced stage, two adjacent chips (dissolved) are being freed by etching filopodia (4000 ×)

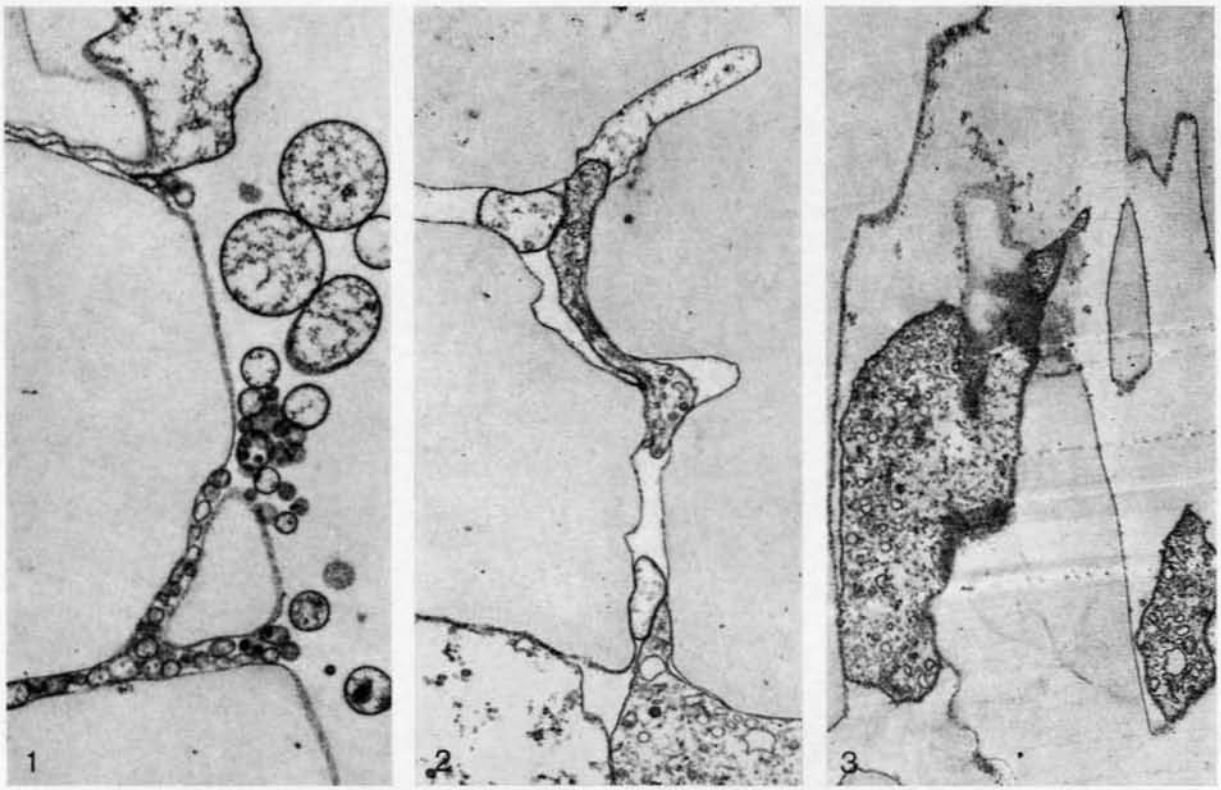

Fig. 10. *Cliona lampa*. TEM photomicrographs of etching cytoplasmic structures. 1: One longitudinal and numerous cross-sections of filopodia inside substratum crevice they produce by etching; substratum (left) is replaced by epoxy; note flocculent secretion containing isolated ribosomes (14000 ×). 2: Similar view, substratum (replaced by epoxy) occupies most of upper portion of photograph (20000 ×). 3: Tangential section through etched crevice containing cytoplasmic sheet; secretory product and rudimentary rough endoplasmic reticulum are distinct (20000 ×)

and cells which are clearly engaged in the etching activity.

The etching cells are elongate, twisted in three dimensions, and oriented perpendicular to the substratum. They are widest at the distal end, tapering towards the base. Several filopodia originate at the wide distal part of the cell body, and extend into the etched crevices of the substratum. The filopodia ramify along their way, and partly fuse again to cytoplasmic sheets. They form a delicate meshwork shaped like a basket (Figs. 7:*2—6*; 11:*1*). The cells are difficult to measure because of their twisted appearance. They reach about 30 μm in length, 5 μm at the base and 15μm at the top. The filopodial baskets correspond in size to the substratum chips they embrace. The nuclei are large, circular or oval in longitudinal sections, and provided with a distinct nucleolus. A Golgi apparatus is always present. The rough endoplasmic reticulum is prominent and extends throughout the distal portion of the cells. Large ovoid mitochondria are abundant. There are small vacuoles, multivesicular bodies, and a few phagosomes. Medium dense (gray) droplets without surrounding membranes (lipoid?) are loosely dispersed. Glycogen granule accumulations occur at the distal portions of the cells, where various stages of cytoplasmolysis can be observed (Fig. 8:*1—4*).

The disintegration of the cell contents is a process that is correlated with the penetration of the filopodial basket into the substratum. Plasmolysis starts at the distal end of the cell body, as soon as the first filopodia insinuate the substratum (Figs. 8:*1, 2*; 9:*1, 2*; 10). First the cell contents loosen, irregular loops and circular cross-sections of rough endoplasmic reticulum become dominant, and mitochondria become very abundant. Increased activity of the Golgi complex is indicated by the increasing number and size of the associated vesicles. A flocculent secretory product, which is not confined to a vacuole, appears and extends into the filopodia. The cell-body inflates as the secretion takes over and the various organelles start to decay. First the nucleolus disappears, then phagosomes, mitochondria, and nuclear membrane disintegrate. The secretory flocks contained in cells, filopodia, and cytoplasmic sheets are interspersed with occasional mitochondria, phagosomes, small loops of endoplasmic reticulum, vesicles, and rosettes of glycogen granules.

A second smaller kind of black granules are probably isolated ribosomes.

Discussion

In addition to *Cliona lampa*, investigated in this report, only two other sponge species (*Cliona celata* and *C. vastifica*) have been closely investigated for their burrowing habit. Preliminary surveys by scanning electron microscope indicate, however, that sponges have generally adopted the same mechanism for excavating calcareous materials (Rützler, unpublished). Substrata are always penetrated by removing small, approximately hemispherical fragments, leaving a characteristic pitted surface (Figs. 3:*1—4*; 4:*1—4*). There is a considerable size range in the expelled chips, but the variation is more dependent on the point of origin of the chip and on the micro-topography of the substratum than on the species and the nature of the substratum. In the distal parts of a new tunnel (Fig. 3:*2*), few cells, mining small chips, make the fastest progress. Larger fragments are removed during the widening of tunnels and chambers. From an engineering point of view, this is a most efficient method. Removal of rims between existing pits, of course, results in small chips. Crevices present in the substratum (e.g. in coral) will not only aid penetration, but also influence the size of the chips. Table 2 summarizes our information on the size range of pits and chips produced in different substrata by several species of clionids and by members of the families Spirastrellidae (*Anthosigmella*, *Spheciospongia*) and Adociidae (*Siphonodictyon*).

Sediment particles produced by sponges in the described manner measure, before they are further degraded, predominately 40 to 60 µm. Therefore, in the Wentworth scale, they would be considered part of the silt fraction, at the transition to the very fine sand fraction. Shape of the particles is so characteristic that they can be identified in freshly settled sediments, thus making it possible to calculate participation of sponge communities in coastal erosion (Rützler, in preparation; S. V. Smith, personal communication). The characterictically pitted substratum surface, on the other hand, makes positive attribution of fossil and recent sponge-burrows possible.

Table 2. *Range of maximum diameters (µm) of pits and chips produced by different sponge species in various substrata*

Pits	Chips	Species	Substratum	Source
45—70		*Cliona celata*	*Crassostrea* shell	Hartman (1958)
	30—50[a]	*C. celata*	*Crassostrea* shell	Warburton (1958)
75—82		*C. celata*	*Mercenaria* shell	Hartman (1958)
	20—60[a]	*C. celata*	*Venus* shell	Warburton (1958)
65—85		*C. celata*	Calcarenite	Rützler (unpublished)
35—45[a]	25—45[a]	*C. celata*	Calcite crystal	Warburton (1958)
	85 (average)	*C. celata*	Calcite crystal	Cobb (1969)
	15—30[a]	*C. celata*	Conchiolin	Warburton (1958)
	19—26[a]	*C. celata*	Conchiolin	Cobb (1969)
50—75	31—71	*C. lampa*	*Chama* shell	Rützler (unpublished)
18—45		*C. lampa*	Calcarenite	Rützler (unpublished)
18—64	30—28	*C. lampa*	Calcite crystal	Rützler and Rieger (this paper)
	22—40	*C. lampa*	Conchiolin	Rützler (unpublished)
24—62		*Cliona* sp. (undescribed)	Calcarenite	Rützler (unpublished)
45—65		Clionid (Pliocene)	Coral	Rützler (unpublished)
49—94		*Cliothosa hancocki*	Calcarenite	Rützler (unpublished)
20—35		*Anthosigmella varians*	Calcarenite	Rützler (unpublished)
45—60		*Spheciospongia othella*	Calcarenite	Rützler (unpublished)
30—50		*Siphonodictyon coralliphagum*	Coral	Rützler (unpublished)

[a] Measured from figures.

The kind and function of tissue components which are responsible for the excavation of chips has been little understood in the past, and it is quite possible that the cell types, organelles, and secretory products involved vary among species. Our study shows that the width of crevices produced by etching cell processes varies between 0.8 and 0.15 µm; for the most part it is 0.2 µm. It follows that these processes lie in this order of magnitude and, therefore, at the limit of resolution by light microscopy. This accounts for the fact that previous authors were unsuccessful in demonstrating pseudopodia or organelles present inside the crevices.

Nassonov (1883) described "protoplasmic extensions" penetrating into thin calcareous plates, without discussing their nature. He also mentioned and figured large pigmented club-like cells with pseudopodial processes at one end, which are comparable to etching cells described in this report (Fig. 7:*2, 3*). Further-

more, he observed mesenchymal cells with long pseudopodial expansions. Cotte (1902) confirmed this observation, and ascribed to these processes the burrowing function. Warburton (1958), after wiping off small sponges which had settled on cover glasses, found remaining cells showing "a remarkable network of thread-like interconnections and pseudopodia, often 50 µ or more long". These surrounded areas resemble figures etched into calcite. The cytoplasm is described as clear and transparent. From our own observations on thin outgrowth regions of *Cliona lampa* on coverslips and calcite crystals, it appears that these cells are collencytes with connective function, and not associated with the burrowing activity. Cobb (1969) has made the most detailed light-microscopical study on cell-substratum relationships. His observations were certainly limited by resolution and depth of field, but it is obvious that there are two major differences in our results for the two species of *Cliona*: the nature of the etching cells and the way they excavate chips.

Cobb (1969) did not describe the various cell types present in his specimens, nor did he discuss the origin of the etching cells. They correspond to granulous amoeboid cells described by Topsent (1900). Basophilia and size of the granules resemble those of gray cells, but there is a prominent nucleolate nucleus, lacking in the latter. There is no information on the fate of the granules during cell penetration. Only very occasionally could similar granules be found in the archeocytes of *Cliona lampa*, never during etching activity. Cells in *C. celata* are reported to flatten themselves on the substratum, and to commence etching activity around the periphery, and are very localized at the cell edge. Archeocytes in *C. lampa* position themselves perpendicular to the substratum, and insinuate a filopodial basket (Figs. 7:*2*, *3*, *6*; 9).

Adjacent cells maintain close contact during the etching activity. Frequently, pseudopodia of neighboring cells can be found inserted in the same crevice (Fig. 9:*1*), and usually several adjacent chips are removed simultaneously (Figs. 3:*3*; 4:*1*). It is difficult to imagine how a high degree of coordination, as demonstrated by the regularity of chambers, tunnels and pits, can be maintained. However, many erratic, incomplete etchings can also be observed (Fig. 3:*3*, *5*).

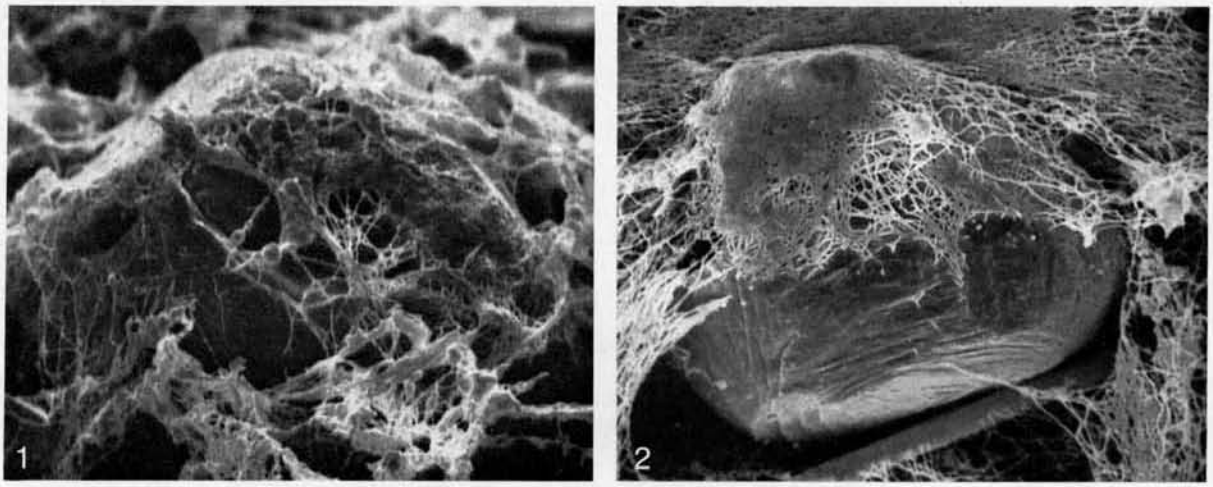

Fig. 11. *Cliona lampa*. SEM views of tissue components and chips. 1: Freshly dislodged chip, with adhering filopodial remains and secretory product (1400 ×). 2: Dislodged chip inside opening of exhalant canal; part of a spicule (tylostyle) is visible at lower right (1700 ×)

The loose tissue composition in active burrowing tunnels, and the three-dimensional, twisted appearance of etching cells, indicate great mobility of the cells during the etching process. It is assumed that the filopods are moved continuously during secretion to assure complete separation of the chips and to prevent accumulation of interstitial water with excessive calcium ion concentration. Removal of the freed chips from their position is accomplished by simple displacement by new etching cells and other tissue components moving into the newly created spaces (Fig. 11:*2*). They are forced into the exhalant canal system and discarded through the oscula. Exhausted etching cells either go the same way or are phagocytized.

In this study we have not attempted to identify the secretory product which causes the dissolution of the calcium-carbonate substratum. The flocculent material observed by us in sections through etching cells (Figs. 9:*2*; 10:*3*) is presumably responsible. The presence of a prominent nucleolus, abundance of ribo-

somes, even in advanced stages of plasmolysis, and great activity of the Golgi complex (Fig. 8:*3, 4*) suggest synthesis of both proteins and carbohydrates (Fawcett, 1966). Sensitive analytical micro-methods would be required to identify the substance and to present experimental proof for its acidic, chelating, or enzymatic effects. Judging from the fine-structure of the substratum, the etching activity is localized at the immediate area of contact between cellular processes and limestone (Fig. 10:*1, 2*). No subsequent etching of the pitted substratum surface or of chips during transport to the oscula could be observed. An analogy has been suggested with tunnelling through limestone "by carving out blocks with acid dispensed from a pipette" (Warburton, 1958). Several authors (see Cobb, 1969) have proposed an acid combined with an enzyme to account for destruction of both calcium carbonate and conchiolin present in oyster shells. Also *Cliona lampa* is able to penetrate conchiolin layers in *Chama*.

Warburton (1958) tried to demonstrate an increased content of calcium in dishes containing burrowing *Cliona celata*, but did not succeed. He estimated, taking into account the sensitivity of his experimental method, that the amount of calcium carbonate dissolved must be less than 10% of the amount of material removed. We have used our avearge measurements for chips and etched crevices (taken from scanning electron photomicrographs of substrata and epoxy molds), and approximated the shape of chips to spherical segments. As a result, we calculate that only 2 to 3% of the total calcium carbonate is removed in dissolved form.

Summary

1. Many valuable contributions to the problem of the sponge burrowing technique are found in the literature of the past 90 years. Two species, *Cliona vastifica* and *C. celata* had been studied in detail, but important observations on cell-substratum interrelationship had been missed because of the limited resolution of the light microscope.

2. *C. lampa* Laubenfels (Porifera: Hadromerida) is an abundant burrowing sponge in shallow water of Bermuda. Actively burrowing stages, penetrating homogeneous calcite crystals, were obtained and fixed for light and electron microscopy.

3. A distinctive pattern of tunnels and chambers is formed during penetration. Fine crevices (approximately 0.2 µm wide), etched by cellular activity, result in the freeing of characteristic substratum chips ($56 \times 47 \times 32$ µm mean dimensions). The remaining substratum surface has a pitted structure that seems typical for all sponge excavations, at least for the 9 species (belonging to 5 genera and 3 families) comparatively investigated for this report. Scanning electron microscopy permits interpretation of many structural details as never before.

4. Six cell types, common to many sponges, can be identified by light and transmission electron microscope: pinacocytes, collencytes, gray cells, sclerocytes, choanocytes, and archeocytes. An additional cell type, characterized by the presence of apical filopodia, interconnected to form a basket-like structure, and by progressive stages of plasmolysis, is responsible for the etching of chips from the substratum. These etching cells, judging from cytological features, are of archeocyte origin.

5. The filopodial basket penetrates the substratum by localized dissolution, caused by a secretory product that is apparently synthesized in the regions of the Golgi complex and of the endoplasmic reticulum. Thus, chips are chemically cored from calcium carbonate as well as from conchiolin layers that are present in mollusk shells. An enzymatic component of the secretion can, therefore, be assumed in addition to the calcium-carbonate solvent, as has been suggested by previous authors. Etching cells are not regenerated, but disposed. Detached chips are expelled through the exhalant system of the sponge.

6. It is calculated that not more than 2 to 3% of the substratum that is broken down by burrowing sponges go into solution.

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Literature Cited

Bagby, R. M.: The fine structure of pinacocytes in the marine sponge *Microciona prolifera* (Ellis and Solander). Z. Zellforsch. Abt. Histochem. **105**, 579—594 (1970).

Borojevic, R. et C. Lévi: Etude au microscope électronique des cellules de l'éponge: *Ophlitaspongia seriata* (Grant), au cours de la réorganisation après dissociation. Z. Zellforsch. Abt Histochem. **64**, 708—725 (1964).

Carriker, M. R. and E. H. Smith: Comparative calcibiocavitology: summary and conclusions. Am. Zool. **9**, 1011—1020 (1969).

— — and R. T. Wilce (Eds.): Penetration of calcium carbonate substrates by lower plants and invertebrates. Am. Zool. **9**, 629—1020 (1969).

Clapp, W. F. and R. Kenk: Marine borers, an annotated bibliography, 1136 pp. Washington, D.C.: Office of Naval Research, Department of the Navy 1963.

Cobb, W. R.: Penetration of calcium carbonate substrates by the boring sponge, *Cliona*. Am. Zool. **9**, 783—790 (1969).

Cotte, J.: Note sur le mode de perforation des cliones. C.r. Séanc. Soc. Biol. **54**, 636—637 (1902).

Fawcett, D. W.: The cell, its organelles and inclusions. An atlas of fine structure, 448 pp. Philadelphia: W. B. Saunders Co. 1966.

Goreau, T. F. and W. D. Hartman: Boring sponges as controlling factors in the formation and maintenance of coral reefs. *In:* Mechanisms of hard tissue destruction, Ed. by R. F. Sognnaes. Washington, D.C.: Publs Am. Ass. Advmt Sci. **75**, 25—54 (1963).

Grant, R. E.: Notice of a new zoophyte (*Cliona celata* Gr.) from the Firth of Forth. Edinb. New Phil. J. **1**, 78—81 (1826).
Hartmann, W. D.: Natural history of the marine sponges of southern New England. Bull. Peabody Mus. nat. Hist. **12**, 1—155 (1958).
Laubenfels, M. W. de: The Porifera of the Bermuda Archipelago. Trans. zool. Soc. Lond. **27**, 1—154 (1950).
— Sponges from the Gulf of Mexico. Bull. mar. Sci. Gulf Caribb. **2**, 511—557 (1953).
Lévi, C.: Ultrastructure de la larve parenchymella de démosponge. 1. *Mycale contarenii* (Martens). Cah. Biol. mar. **5**, 97—104 (1964).
Little, F. J., Jr.: The sponge fauna of the St. George's Sound, Apalachee Bay and Panama City regions of the Florida Gulf Coast. Tulane Stud. Zool. **11**, 31—71 (1963).
Nassonov, N.: Zur Biologie und Anatomie der Clione. Z. wiss. Zool. **39**, 295—208 (1883).
Neumann, A. C.: Observations on coastal erosion in Bermuda and measurements of the boring rate of the sponge, *Cliona lampa*. Limnol. Oceanogr. **11**, 92—108 (1966).
Pang, R. K.: The systematics of some Jamaican excavating sponges. Postilla (In press).
Pavans de Ceccatty, M.: Ultrastructures et rapports des cellules mésenchymateuses de type nerveux de l'eponge *Tethya lyncurium* Lmk. Annls Sci. nat. (Zool. Biol. Animale) (sér. 12) **8**, 577—614 (1966).
— et Y. Thiney: Microscopie électronique de la fibrogenèse cellulaire du collagène, chez l'éponge siliceuse *Tethya lyncurium* Lmk. C.r. hebd. Séanc. Acad. Sci., Paris **256**, 5406—5408 (1963).
Rützler, K.: Bredin-Archbold-Smithsonian biological survey of Dominica: Burrowing sponges, genus *Siphonodictyon* Bergquist, from the Caribbean. Smithson. Contr. Zool. **77**, 1—37 (1971).
— The burrowing sponges of Bermuda. Smithson. Contr. Zool. (In press).
— Participation of burrowing sponges in erosion and sediment production on the Bermuda platform. (In preparation).
Topsent, E.: Contribution à l'étude des clionides. Archs Zool. exp. gén. (sér. 2) **5**, 1—165 (1887).
— Etude monographique des spongiaries de France. III. Monaxonida (Hadromerina). Archs Zool. exp. gén. (sér. 3) **8**, 1—331 (1900).
Vosmaer, G. C. J.: The sponges of the Bay of Naples. Porifera Incalcaria. With analyses of genera and studies in the variations of species, Part V. pp 321—456. The Hague: Martinus Nijhoff 1933.
Warburton, F. E.: The manner in which the sponge *Cliona* bores in calcareous objects. Can. J. Zool. **36**, 555—562 (1958).
Wilson, H. V. and J. T. Penney: The regeneration of sponges (*Microciona*) from dissociated cells. J. exp. Zool. **56**, 73—147 (1930).

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