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Bulletin 25

The Structure and Function of Sponge Cells:

New Criteria for the Taxonomy of Poecilosclerid Sponges (Demospongiae)

by
Tracy L. Simpson

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THE STRUCTURE AND FUNCTION OF SPONGE CELLS:

New Criteria for the Taxonomy of Poecilosclerid Sponges (Demospongiae)

By Tracy L. Simpson

ABSTRACT

The skeletal morphology, histology, cytology, and cytochemistry of fifteen species of marine poecilosclerid sponges have been investigated. The following sponges were studied: *Microciona prolifera, Microciona atrasanguinea, Microciona spinosa, Microciona seriata, Microciona pennata, Plocamilla illgi, Thalysias juniperina, Thalysias schoenus, Axocielita hartmani, Clathria* sp., *Rhaphidophlus cervicornis, Tedania ignis, Tedania suctoria, Lissodendoryx isodictyalis, Lissodendoryx carolinensis.* A comparison of the skeletal morphology of these sponges with histological and cytological characteristics has resulted in the conclusion that the employment of only skeletal characteristics for species, genus, and family placement is misleading in determining the taxonomic relationship of these sponges, and that genera must be defined on the basis of cytological characters. The cytological characteristics which have been found to be of importance for this purpose comprise what have been termed here, Special Cell Types. These lack detectable RNA and mitosis and at least one of them in any particular species contains large amounts of acid mucopolysaccharide.

The clathriid sponges studied have shown that there are at least two taxonomic lines within the family Clathriidae. One of these, the *Microciona* line, appears to be a highly specialized one which has arisen from within the family. The *Thalysias* line shows a greater similarity to two genera (*Tedania* and *Lissodendoryx*) in the family Tedaniidae than does the *Microciona* line.

СТРУКТУРА И ФУНКЦИЯ КЛЕТОК ГУБКОВЫХ: НОВЫЕ КРИТЕРИИ ДЛЯ ТАКСОНОМИИ ГУБОК POECILOSCLERIDAE (DEMOSPONGIAE)

ТРЕЙСИ Л. СИМПСОН

РЕЗЮМЕ

Были исследованы скелетная морфология, гистология, цитология и цитохимия следующих 15 видов морских губок Poecilosclеridae: Microciona prolifera, Microciona atrasanguinea, Microciona spinosa, Microciona seriata, Microciona pennata, Plocamilla illgi, Thalysias juniperina, Thalysias schoenus, Axocielita hartmani, Clathria sp., Rhaphidophlus cervicornis, Tedania ignis, Tedania suctoria, Lissodendoryx isodictyalis, Lissodendoryx carolinensis. Сравнение скелетной морфологии этих губок с их гистологическими и цитологическими характерными признаками привело к заключению, что применение одних только скелетных особенностей видов, родов и семейств может привести к ошибочным выводам при определении таксономического родства этих губок, так что роды их должны определяться на основе цитологических особенностей. Цитологические особенности, имеющие значение в этом отношении, охватывают то, что здесь названо "специальные типы клеток". В них отсутствуют РНК и митоз, и по крайней мере один из них в любом из видов содержит большое количество кислого муко-полисахарида.

Изученные клатриидные губки показали, что в семействе Clathriidae есть по крайней мере две таксономические линии. Кажется, что одна из них, Microciona, в высшей степени специализована и возникла внутри семейства. Линия Thalysias показывает больше сходства к двум родам (Tedania и Lissodendoryx) в семействе Tedaniidae чем линия Microciona.

LA STRUCTURE ET FONCTION DES CELLULES DES SPONGIAIRES:

Nouveaux Critère pour la Taxonomie des Spongiaires Poecilosclérides (Demospongiae)

par Tracy L. Simpson

Résumé

La morphologie du squelette, l'histologie, la cytologie et la cytochimie de quinze espèces de spongiaires poecilosclérides ont été l'objet d'investigation. Ces spongiaires fûrent étudiés: *Microciona prolifera, Microciona atrasanguinea, Microciona spinosa, Microciona seriata, Microciona pennata, Plocamilla illgi, Thalysias juniperina, Thalysias schoenus, Axocielita hartmani, Clathria* sp., *Rhaphidophlus cervicornis, Tedania ignis, Tedania suctoria, Lissodendoryx isodictyalis, Lissodendoryx carolinensis.* Une comparaison entre la morphologie du squelette de ces spongiaires et les caractères histologiques et cytologiques a porté à la conclusion que l'emploi des caractères du squelette seulement pour la définition de l'espèce, genre et famille peut induire en erreur dans la détermination des relations taxonomiques de ces spongiaires, et que les genres doivent être definies sur la base des caractères cytologiques. Les caractères cytologiques qui ont été trouvés être important pour ce propos comprendent ceux qui ont été només ici: Types des Cellules Spéciales. Celles-ci n'ont pas de RNA et mitose détectables et, au moins une d'elles dans chaque espèce particulière, contient une grande quantité d'acide mucopolysaccharide.

Les spongiaires clathriidés étudiés ont montré qu'il y a au moins deux lignes taxonomiques dans la famille Clathriidae; une, la ligne *Microciona,* semble être celle qui, extrêmement specializée, provient de l'interne de la famille. L'autre, la ligne *Thalysias,* est plus semblable aux deux genres (*Tedania* et *Lissodendoryx*) dans la famille Tedaniidae qu'aux genres de la ligne *Microciona.*

To the memory of Professor Alexander Petrunkevitch

"The value of any classification rests on the soundness of the principles underlying it. A conspicuous character may entice the keenest observer to attribute to it greater importance than it possesses and to make use of it for a division of a natural group into two branches, one of which is distinguished from the other by the lack of that character. Rare, indeed, are the cases where such divisions are natural! More commonly they cut across the lines of evolution and confuse true relationships. The course of nature is devious and complex." (Alexander Petrunkevitch, 1933. An Inquiry into the Natural Classification of Spiders, Based on a Study of Their Internal Anatomy. Conn. Acad. Arts and Sci., Trans., 31:303.)

THE STRUCTURE AND FUNCTION OF SPONGE CELLS:
New Criteria for the Taxonomy of Poecilosclerid Sponges (Demospongiae)*

by TRACY L. SIMPSON

INTRODUCTION

Presently, skeletal morphology and external characteristics form the basis for establishing taxonomic categories among the Porifera. It is my purpose in this study to evaluate the significance of histological and cytological characteristics for defining taxonomic categories and for establishing relationships among species of sponges.

In many cases the delimiting of sponge genera and species has proven to be exceedingly difficult. This has led several workers (Vosmaer, 1935; Burton, 1963) to lump large numbers of species within a single species. Burton (1963) has carried this point of view to its extreme by placing some 500 previously described species of calcareous sponges into 47 species. In doing so, Burton (1963) has established 47 categories which are broadly defined in terms of variability. This implies that the calcareous sponges are different from all other animals in that they are characterized by a much greater degree of variability within species rather than by species which are more or less distinct units (see Hartman, 1964, for a critique of Burton's work). Furthermore, the point of view of Burton (1963) and Vosmaer (1935) leads to the assumption that members within these highly variable species are interfertile. Sarà (1956) has uncovered evidence to support this by finding that two species (i.e. species as they are normally conceived, not according to Burton, 1963) of the genus *Leucosolenia* hybridize and produce poorly viable hybrids. This finding is based upon morphological studies and not upon experimental breeding studies. Thus, even though in this case evidence of hybridization has been found, it is clear that one can morphologically distinguish the two parent species, as well as the hybrids. Burton (1963) has placed these two "parent" species of *Leucosolenia* along with 41 other species into *L. botryoides*. If one can recognize sponge species morphologically, which in essence Sarà (1956) did, why have some systematists overlooked this and emphasized the intergradations of taxonomic characteristics?

Part of the answer to this question lies in the fact that in many instances delimitation of taxonomic categories in sponges is based upon very few characteristics (as in the genera *Haliclona, Halichondria, Verongia,* etc.). Using only these few characteristics, it is often easy to find a complete series of intergradations between genera, families, and even orders. For example, Connes (1963) has found that the same sponge colony may produce two types of gemmules; one type lacks amphidiscs and the other contains them. The lack of amphidiscs within gemmules is characteristic of the genus *Spongilla* and their presence is character-

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istic of the genus *Trochospongilla*. The simplest solution out of this paradox is to merge these two genera and consider the presence of amphidiscs as a variable character. This is the type of decision which is inherent in the point of view of Burton (1963) and Vosmaer (1935). Additional support for the lumping of previously described species in a single species is also derived from the finding that even in cases where a large number of characteristics is involved, intergradations can be found. Thus, the unidentified specimen of *Clathria* described in this study possesses skeletal characteristics intermediate between *C. compressa* and *C. coralloides* (see Table 49). In this instance even though we are dealing with a large number of skeletal attributes it is not possible to make a definitive decision as to the proper species placement of this specimen. For some taxonomists this finding might lead to the conclusion that the basis for species separation between *C. compressa* and *C. coralloides* is not valid and that these two species should be considered conspecific. Without the knowledge of additional non-skeletal characteristics the decision to merge or separate species or higher taxa has to be made arbitrarily. This is precisely the type of situation which has led Vosmaer (1935) and Burton (1963) arbitrarily to place a large number of previously described species in a single species designation.

It is obvious from even this very brief discussion of the characteristics which have been used for sponge systematics that in order to limit taxonomic categories with more precision and to be able to establish more meaningful taxonomic relationships, additional characteristics are needed. Additional morphological characteristics are, of necessity, histological and cytological in nature.

The elucidation of additional characteristics permits a critical evaluation of the employment of skeletal characteristics and growth form for taxonomic purposes. For example, although Hallman (1920) enlarged the scope of the genus *Ophlitaspongia* by placing within it a species which contains palmate isochelas, de Laubenfels (1936) rejected this decision and limited *Ophlitaspongia* to sponges whose microsclere content includes only toxas. For sponges which contain the same spicule complement as *Ophlitaspongia* but which also contain chelas, de Laubenfels (1936) utilized two additional genera, *Axociella* and *Axocielita*. These two genera differ in that the species of the former produce upright branches; those of the latter are incrusting. In terms of establishing a natural classification of sponges, what is the value of the presence or absence of chelas? What is the significance of an incrusting mode of growth as compared to the production of upright branches? The family Ophlitaspongiidae as defined by de Laubenfels (1936, p. 112) contains sponges in which ". . . the fibers are echinated by smooth rather than by spiny styles." Other systematists have not emphasized this difference in megasclere content but have utilized microscleres in assigning genera in de Laubenfels' Ophlitaspongiidae to other families (e.g., Topsent, 1928). Taxonomically, what is the value of differences in the morphology of megascleres as compared to differences in microsclere content? The present work has been undertaken to study additional characteristics (cytochemical and histological) which then can serve as a basis for answering these and related questions. The results of the present study have shown that the classical taxonomic characteristics (skeletal morphology and growth form) have a limited value and are frequently misleading for establishing taxa and taxonomic relationships unless they are correlated with histological and cytological characteristics.

MATERIALS AND METHODS

In all of the sponges studied (see Table 1), parafin embedding, sectioning, and histological and cytochemical staining were carried out on adult specimens. In addition, the outgrowth region of explants was studied both live and following fixation and staining in the following species: *Microciona prolifera, Microciona spinosa, Thalysias juniperina, Thalysias schoenus,* and *Tedania ignis.* The methods of explanation have been fully described in an earlier publication (Simpson, 1963). The study of living material and the growing of explants of *M. prolifera* were carried out at the U. S. Bureau of Commerical Fisheries, Biological Laboratory, Milford, Connecticut. Specimens of *M. prolifera* were collected intertidally at West Haven, Branford, and Milford, Connecticut. *Microciona spinosa, Thalysias juniperina, Thalysias schoenus,* and *Tedania ignis* were collected and worked on at the Lerner Marine Laboratory of the American Museum of Natural History, North Bimini Island, Bahamas. Explants of *M. spinosa* and *Tedania ignis* were prepared in the same manner as *M. prolifera* (see Simpson, 1963). Explants of *Thalysias juniperina* and *Thalysias schoenus* were made by bisecting a branch longitudinally. The central cut surface was then applied to the glass substratum with the original outer surface of the sponge uppermost. For histological and cytochemical analysis, explants were tied to glass slides which were then placed in a running sea water system in the laboratory or alternatively were placed in modified slide boxes which were then hung off the laboratory dock. The sponges studied at the Lerner Laboratory were all grown by the latter method.

Microciona spinosa was obtained by dredging in 30 to 50 feet of water approximately 1/4 to 1/2 mile west of North Bimini Island. *Tedania ignis* was collected from mangrove roots in the lagoon east of the Lerner Laboratory. *Thalysias juniperina* and *Thalysias schoenus* were collected along the beach of Key Biscayne on Bear Cut southeast of Miami, Florida. These sponges had been uprooted from deeper water following strong northeasterly winds and were washed inshore where they were collected by hand. Specimens of *Microciona (= Ophlitaspongia) seriata* and *Microciona astrasanguinea* were supplied by the Marine Biological Laboratory at Plymouth, England. The remaining species were obtained from museum collections.

In several cases the fixative employed for museum specimens was unknown. However, whatever the fixative was, these specimens (which are presently preserved in alcohol) were in acceptable condition for cytological analysis. Thus, older material which is preserved in alcohol for long periods of time is still useful for the type of analysis carried out here. Iron hematoxylin, PAS, and toluidin blue staining can be carried out on this material, even in the absence of knowledge of the original fixative employed. These stains allow a minimal cytological analysis which can be utilized for comparative purposes.

The following fixatives and staining methods were employed: (1) Carnoy (3 parts 95% ethanol, 1 part glacial acetic acid) followed by the azure b bromide (Flax and Himes, 1952)—ribonuclease (McDonald, 1948) method for determination of ribonucleic acid (RNA); (2) Carnoy followed by Feulgen (Leuchtenberger, 1958) for deoxyribonucleic acid (DNA) determination; (3) Carnoy or

TABLE 1: FIELD DATA ON THE SPECIES STUDIED

Species	Origin	Habitat	Fixative	Color	Number of Specimens Analyzed
Microciona prolifera	Branford, West Haven, and Milford, Connecticut	Littoral	Bouin and Carnoy	Red	Numerous
Microciona spinosa	North Bimini Island, Bahamas	Benthic	Bouin and Carnoy	Red	Ten
Microciona atrasanguinea	Plymouth, England	Littoral	Bouin and Carnoy	Red	One
Microciona seriata	Plymouth, England	Littoral	Bouin and Carnoy	Red	One
Microciona pennata	San Juan Island, Washington	Littoral	Formalin	Red	One
Plocamilla illgi	San Juan Island, Washington	Littoral	Formalin	Red	One
Thalysias juniperina	Key Biscayne, Florida	Benthic	Bouin and Carnoy	Red	Four
Thalysias schoenus	Key Biscayne, Florida	Benthic	Bouin and Carnoy	Greenish Orange	Three
Axocielita hartmani	San Juan Island, Washington	Littoral	Formalin	Red	One
Clathria sp.	Mediterranean Sea	Probably Benthic[1]	Unknown[1]	Unknown[1]	One
Rhaphidophlus cervicornis	Palau Islands, Micronesia	Probably Benthic[1]	Unknown[1]	Unknown[1]	One
Tedania ignis	North Bimini Island, Bahamas	Shallow Water (Lagoon)	Bouin and Carnoy	Red	Ten
Tedania suctoria	Vineyard Island Sound, Martha's Vineyard, Massachusetts	Benthic	Formalin	Drab	One
Lissodendoryx isodictyalis	North Bimini Island, Bahamas	Shallow Water (Lagoon)	Bouin and Carnoy	Drab	Two
Lissodendoryx carolinensis	Woods Hole, Massachusetts; Branford, Connecticut	Dock pilings and Littoral	Bouin and Carnoy	Drab	Two

[1] The specimens of *Clathria* sp. and *Rhaphidophlus cervicornis* used in this study are museum specimens and data on habitat, color, and fixative are not available.

formalin followed by alcian blue (Pearse, 1960) for the localization of acid mucopolysaccharide; (4) Carnoy or formalin followed by Hale's dialysed iron (Pearse, 1960) for the localization of acid mucopolysaccharide; (5) Carnoy or formalin followed by the PAS—diastase or ptyalin method (Pearse, 1960) for glycogen and carbohydrate localization; (6) Kahle, Bouin, or formalin followed by 0.5% aqueous toluidin blue (Pearse, 1960) for cytological characteristics and acid mucopolysaccharide; (7) Bouin, Kahle, or formalin followed by iron hematoxylin, Mallory solution II or eosin for histological and cytological features. For the species studied by explanation (see p. 15) material was fixed in Carnoy for a period of eight to twelve hours and in Bouin for twelve to eighteen hours. Living cells in the outgrowth region were studied by phase contrast microscopy (see Simpson, 1963, for full details).

For the study of skeletal morphology, hand sections were made with a razor blade on material which had been fixed in Bouin or formalin and which was stored in 95% ethanol. These hand sections were stained in a saturated solution of basic fuchsin in 95% ethanol, dehydrated and mounted. Spicule preparations of pieces of adult sponges were made by digesting the tissue in concentrated nitric acid and then washing by repeated centrifugation and resuspension. The spicules were then resuspended in 95% ethanol, placed on a slide to dry and subsequently mounted. Field data on the specimens employed in this study are presented in Table 1.

RESULTS

FAMILY CLATHRIIDAE

Genus *MICROCIONA* Bowerbank, 1862, p. 1109

Microciona prolifera (Ellis and Solander, 1786, p. 189) Verrill, 1873, p. 741
(Pls. 1–5)

A. Skeletal Morphology (Measurements given in Table 2)

Microciona prolifera occurs quite commonly in the intertidal region as a thin incrustation (Pl. 1, figs. 1, 2). Many incrusting colonies in this environment show various stages in the formation of upright branches. Rarely, colonies which are primarily branching (Pl. 1, figs. 3, 4) are found in the intertidal region; however, this growth form is quite common in deeper water.

TABLE 2: MEASUREMENTS OF SPICULES AND SPONGIN IN MICROCIONA PROLIFERA[1]

Spicules	
Thick (= coring) styles	99.8-189.7-372.3 x 6.9-9.7-14.3
Thin styles	95.7-201.4-327.2 x 2.0-3.2- 5.8
Acanthostyles	57.1- 83.3-131.4 x 4.3-6.7- 9.0
Toxas	5.2- 15.9- 73.1
Palmate isochelas	11.2- 17.6- 22.6
Spongin	
Fiber width	30 to 100
Meshes	90.0 x 90.0 to 200.0 x 60.0

[1]Means (underlined) and extremes of 300 spicules in each category; measurements in microns.

The basal portion of incrusting colonies consists of a sheet of spongin. Arising from the basal layer of spongin are separate, upright spongin fibers in which are embedded, either partially or wholly, thick styles (referred to as coring styles), some with microspined heads (Pl. 1, fig. 5). In addition, there are shorter acanthostyles (referred to as echinating spicules) having only their heads embedded in the spongin; acanthostyles are not numerous and are widely spaced in

the spongin fibers. Thick styles are for the most part only partially embedded with their points protruding. These upright spongin fibers end at the surface of the sponge with a cluster of styles protruding, points directed outward. In the tissue of the sponge there are two types of microscleres which are randomly distributed —palmate isochelas and toxas (chelas show a tendency to be more numerous in the dermis). In the dermis and to a lesser degree in the mesenchyme are thin styles. In the mesenchyme these spicules are distributed at random without any particular orientation, whereas in the dermis they tend to lie parallel to the surface. Thin styles with the latter orientation are referred to as dermal spicules. In thicker, incrusting colonies in which the surface of the sponge has become lumpy there is a tendency in some areas for the upright spongin fibers to form bridges and thus produce an irregular system of anastomosing fibers. Upright branches contain a system of anastomosing spongin fibers (Pl. 1, figs. 6, 7). Those fibers which are closest to the dermis give off branches which end at the surface in a manner comparable to the individual fibers present in thin, incrusting colonies.

B. Adult Histology

The surface of the sponge is covered by a dermis which is composed of a thin epidermis of anucleolate cells on the outer surface; below the epidermis are nucleolate cells, rhabdiferous cells, globoferous cells and fiber cells. Since these cells occur in several layers, they give the dermis thickness. A characteristic feature of the dermis is the presence of strands of fibrous material. These strands occur in layers parallel to the surface. Beneath the dermis are subdermal spaces and below them is the mesenchyme of the sponge which extends down to the basal layer of spongin. Within the mesenchyme are all of the cell types which are present in the mesenchyme of the outgrowth region of explants (see next section). Flagellated chambers are evident and the spaces in the mesenchyme lined by thin epithelial cells are exhalant canals (Pl. 2, fig. 1). Gray cells show a tendency to be localized near the basal layer of spongin; rhabdiferous cells permeate the tissue and are also abundant in the dermis, as are globoferous cells. Scattered throughout the subdermal space are partitions of mesenchyme which run up to the dermis, thus obliterating the subdermal space in that area. Most of these partitions are partially made up of fiber cells (Pl. 2, fig. 2). These fiber cells lie parallel to one another and form short tracts (hereafter referred to as fiber cell tracts) containing thin styles. Fiber cells (see Wilson and Penney, 1930) contain elongate, anucleolate nuclei; they are thin, long cells in which the cytoplasm occurs in a long strand surrounding the nucleus and extending outward on both sides of it. Fiber cells are morphologically similar to mesenchymal, motile cells referred to as collencytes (see, for example, Borojevic, 1966). Fiber cells differ from collencytes in that they are present only in fiber cell tracts and in the dermis; they are not found throughout the mesenchyme as are collencytes. The histology of branching colonies displays the same characteristics as incrusting colonies; gray cells, however, are randomly distributed.

Annual collections of intertidal colonies of *M. prolifera* reveal that all of the special cell types (see below) present in this sponge persist throughout the year, even though canal systems, flagellated chambers, dermis, and subdermal spaces are absent from mid-November until the end of April.

Mature eggs occur free in the mesenchyme and contain nucleolate nuclei and cytoplasmic inclusions (Pl. 2, fig. 3). Sperm masses have also been found in the mesenchyme where they are surrounded by an epithelium (Pl. 2, fig. 4). Larvae are present in the mesenchyme from June through August. Immature larvae consist of a homogeneous mass of blastomeres (nucleolate cells) some of which are dividing; the mature larva (Pl. 2, fig. 1) has an outer flagellated epithelium that completely covers it. In the center of the mature larva are nucleolate cells, rhabdiferous cells, gray cells, and globoferous cells. Both megascleres and microscleres are also present within the center of the larva.

C. Morphological and Cytochemical Study of the Outgrowth Region[1]

1. Gross aspects of the outgrowth region

When explants of *M. prolifera* are tied to glass substrata they produce, as a result of the migration of cells from the explant and the subsequent mitosis of some cell types, an area of outgrowth on all sides of the explant (Pl. 2, fig. 5). As the growing edge of the explant advances, particulate matter (bacteria, algae, detritus, etc.) adhering to the coverslip is ingested by the epidermis which is in contact with the coverslip. At times during this advance the growing edge regresses temporarily. Regression involves the pulling back of the edge some 50μ or so. During temporary regression the thickness of the adjacent growth area increases. Also, as the growing edge recedes, it leaves behind it on the coverslip an area which is free of particulate matter.

The initial area of growth around the explant lacks canal systems, flagellated chambers, spongin, and spicules. A lower epidermis in contact with the coverslip is present. Early stages of spicules and newly formed flagellated chambers are the first structures to appear at this initially undifferentiated stage. Later, after the outgrowth region has increased, the innermost area becomes fully differentiated and contains masses of flagellated chambers located between the exhalant canals, numerous spicles (both megascleres and microscleres), small amounts of spongin surrounding the rounded portion of some megascleres, and an upper as well as a lower epidermis (Pl. 2, fig. 6). Many thick styles occur in clusters. The canals of the exhalant system in the differentiated areas become progressively larger in diameter as they are followed from the edge of the outgrowth region to the explant. Since the diameter of an exhalant canal increases on its course from the flagellated chambers to the explant, water in the exhalant canals flows toward the explant. At this more advanced stage, the areas which lack the above-mentioned structures and which are undifferentiated are now confined to the edge of the growth region (Pl. 2, fig. 6).

2. Cell types present in the outgrowth region (terminology according to Wilson and Penney, 1930) (Measurements of cells, nuclei and other components are given in Table 3.)

The epidermis and ostial openings

When the lower epidermis is viewed in the living state it appears as a thin

[1] See also Simpson (1963).

TABLE 3: MEASUREMENTS OF CELLS AND OTHER COMPONENTS IN MICROCIONA PROLIFERA[1]

Component	Number Measured	Nuclear Diameter	Nucleolar Diameter	Cell Size	Other Features
Eggs	20	7.2-9.0-11.7	2.7-3.7-5.2	15.0x15.0 to 44.0x44.0	
Sperm masses	Numerous				Diameter: 70.0
Larval size	Numerous				200.0 x 250.0
Ostial openings	Numerous				10.0 to 42.0
Epidermal cells	25	3.0-4.0-5.0	0.5[2]		
Flagellated chambers					15.0 to 40.0
Choanocytes	75	1.2-1.9-2.6	absent	8.0	Flagellum: 2.0
Nucleolate cells	30	3.2-3.7-4.2	0.5-1.0-1.5	19.0 x 5.0 11.0 x 12.0	
Gray cells	30	2.5-3.0-3.5	absent	25.0 x 8.0 12.0 x 9.0 15.0 x 5.0	Cytoplasmic granules: 0.2-0.4
Rhabdiferous cells	25	2.2-2.7-3.2	absent	When elongate: 88.0x4.0 to 18.0x7.0	Cytoplasmic inclusions: 1.0-2.0
Globoferous cells	20	2.1-2.5-2.9	absent	40.0 x 30.0	Small cytoplasmic inclusions: 0.5-1.0 Large cytoplasmic inclusion: 3.5
Cheloblast	Numerous	2.0 to 2.5	absent		
Fiber cells	Few	3.0 to 4.0	absent	20.0-40.0 x 2.0-5.0	

[1]Measurements in microns; means underlined. Measurements made on cells, etc. in the outgrowth region of explants except fiber cells which are only present in adult sections; Bouin fixed material.

[2]Nucleoli absent in adult sections.

cytoplasmic sheet containing nuclei, around which are a variable number of inclusions (Pl. 4, fig. 1). Cell boundaries are not evident but irregular thickenings are usually present. In some preparations the growing edge gives the appearance of being cellular, but the apparent cell boundaries cannot be traced owing to the thickness of the tissue. Many epidermal nuclei contain a single nucleolus (Pl. 4, fig. 1); a few contain two nucleoli. In stained material epidermal nuclei are surrounded by a limited area of cytoplasm; distinct cell boundaries are wanting.

Ostia are present in the upper epidermis of differentiated areas of the outgrowth region (Pl. 3, fig. 1). It is not clear whether there is a single cell which forms each ostial opening because cell bounderies are not discernible. In some ostia one epidermal nucleus appears to be more closely associated with the opening than any other, whereas in other ostia two or three nuclei are equally associated with the opening.

The upper epidermis is a composite structure containing ostia, globoferous cells, nucleolate cells, epidermal cells and rhabdiferous cells. The basal epidermis, on the other hand, is a simple squamous epithelium. No evidence of cell division has been found in the epidermis in living or stained material after exhaustive searching.

Choanoctyes and flagellated chambers

Study of the differentiated areas of the outgrowth region has shown the flagellated chambers to be located primarily in elongated areas between the exhalant canals (Pl. 2, fig. 6). Flagellated chambers are also present in areas which are in the process of differentiating; in these cases, the number of choanocytes present in a single chamber is small, four or more (Pl. 4, fig. 2). The presence of flagellar activity, however, demonstrates these young chambers to be fully functional. In the undifferentiated areas flagellated chambers are completely absent. Each choanocyte possesses a flagellum which extends into the lumen of a flagellated chamber (Pl. 4, fig. 2). Choanocyte nuclei are anucleolate.

Flagellated chambers have two types of openings. One type is produced by small spaces between the choanocytes on one side of the chamber (Pl. 3, fig. 2). The other is formed by a single cell on the opposite side of the chamber (Pl. 3, fig. 3). This cell forms a single opening similar to an ostial opening but much smaller; a single nucleus is present to one side of the opening. The latter type is presumed to be an apopyle and the former, prosopyles. Numerous mitotic figures have been found in the flagellated chambers.

Nucleolate cells

Nucleolate cells occur in all areas of the outgrowth region. When viewed in living state these cells are extremely active in the mesenchyme, displaying ameboid movement by means of short, thin pseudopodia (Pl. 4, fig. 3). The cytoplasm of these cells contains a number of inclusions, many of which are present within vacuoles. In addition, a number of minute granules are present in the cytoplasm (Pl. 4, fig. 3) and these show streaming movements as the cell moves. They are a constant feature of the nucleolate cells while the number of larger inclusions present may vary greatly. Nucleolate cells have been followed at the growing edge of the outgrowth region and in several instances the number of

large inclusions present within a cell was observed to increase. This increase occurred over a period of five to ten minutes.

In fixed and stained material nucleolate cells have been found secreting styles (Pl. 3, fig. 4). Moreover, in the outgrowth region spongin is present clothing the rounded ends of some megascleres. A ring of nucleolate cells always occurs around this spongin and these cells are responsible for spongin secretion (Pl. 3, fig. 5); these nucleolate cells are exceptionally rich in cytoplasmic RNA. The presence of spongin is revealed by staining with Mallory solution II; spongin stains a clear blue color (Gross *et al.,* 1956). Spongin has not been found in any other areas of the outgrowth region.

Most nucleolate cell nuclei contain a single nucleolus; however, a few contain two nucleoli. Many mitotic figures are present in these cells. A dividing cell in the mesenchyme can be identified as a nucleolate cell because of the presence of inclusions and cytoplasmic basophilia (see Cytochemical results, p. 24 and Table 4).

Special cell types

Gray cells

These cells are found in all areas of the outgrowth region but are less abundant in the marginal zones. In the area just under the edge of the original explant gray cells are extremely numerous. When viewed in living state they move slowly in the mesenchyme but lack well-defined pseudopodia. Although the cytoplasm is packed with numerous small granules (Pl. 4, fig. 4) there is a homogeneous cytoplasmic area at one end of the cell which lacks granules. These granules stain basophilically in iron hematoxylin. Gray cell nuclei lack nucleoli. No mitotic activity has been detected in these cells after extensive observations.

Rhabdiferous cells

Rhabdiferous cells are mainly associated with the epidermis but also occur in the thin growing edge of the outgrowth region. The cytoplasm of these cells contains inclusions which are rod-shaped, spherical and oval (Pl. 5, fig. 1). Some of the rhabdiferous cells contain long fibrous and irregular inclusions which in stained material can be seen to be erupted or secreted from the cell (Pl. 3, fig. 6). These fibrous inclusions are morphologically similar to the coiled material which is associated with toxa secretion (see p. 24). Invariably, erupting cells are present in the older areas of the outgrowth region and have never been observed in the marginal zones. The material which is released from these cells becomes associated with the epidermis and with the mesenchyme in the form of fine granular material, homogeneous deposits and irregular fibers. Rhabdiferous cells have not been observed to move although they do change shape over a period of hours. The inclusions within these cells are basophilic when stained with iron hematoxylin but this basophilia is easily removed by longer periods of differentiation of the stain. Rhabdiferous cells are elongate or irregular in shape and contain anucleolate nuclei. No evidence of cell division has been found in them.

Globoferous cells

Globoferous cells are found in highest concentration in the outer edges of the outgrowth region and associated with the upper epidermis. They are very rarely found immediately under the edge of the explant where the highest concentration of gray cells occurs. When viewed in living condition these cells show a gliding movement without the formation of pseudopodia. Their cytoplasm contains a number of spherical inclusions and, in addition, each cell contains a larger spherical inclusion (Pl. 5, fig. 2). The smaller inclusions are basophilic and the larger inclusion stains light blue in Mallory solution II. Some globoferous cells have two large inclusions rather than a single one. The larger inclusion usually occurs at one end of the cell and contains a homogeneous substance.

Globoferous cells contain anucleolate nuclei and are usually elongate but occasionally are spherical. In these cells also, no mitotic activity has been found.

3. Spiculogenesis in the outgrowth region

Megasclere secretion

In fixed and stained material nucleolate cells in the outgrowth region have been found which contain styles in their cytoplasm (31 found) (Pl. 3, fig. 4). The conclusion that these spicules are actually *within* the cytoplasm is derived from the occurrence of a thin, finely granular layer of cytoplasm surrounding the spicule and a conspicuous bulge containing a nucleolate nucleus. These styles never have spiny processes. The size of many of them is well below the size of adult styles (39.1-156.6μ $\times$ 0.5-4.1μ). Those styles which do fall within the adult size range are always within the lower limits of the size of adult styles. Larger, more mature styles and acanthostyles in the outgrowth region have two, three or four nucleolate cells associated with them along their length.

Chela secretion

Chelas are secreted for the most part in the upper epidermis where they are found within small cells (= cheloblasts) having finely granular cytoplasm and anucleolate nuclei. Chelas are also secreted in the thinner areas of the outgrowth region (Pl. 5, fig. 3).

Toxa secretion

In the earliest observed stages of toxa secretion, toxas are associated with coiled material which is curled around them and which extends into the mesenchyme (Pl. 5, fig. 4) (see p. 100). This coiled material is visible only in living material examined under phase contrast microscopy and in fixed material which is stained with PAS. Coiled material appears to be non-cellular; no nucleus is associated with it and a limiting membrane is not evident. However, it could conceivably be connected to a cell by a cytoplasmic bridge which is inconspicuous. Coiled material is morphologically similar to the fibrous inclusions within rhabdiferous cells.

4. Cytochemistry of the cells in the outgrowth region (see Table 4)

Nuclear DNA has been found in all of the cells in the outgrowth region

TABLE 4: CYTOCHEMICAL STAINING RESULTS ON THE CELLS IN THE OUTGROWTH REGION OF MICROCIONA PROLIFERA

	Feulgen staining	Beta metachromatic staining in azure b bromide	Beta metachromatic staining in azure b bromide following ribonuclease	PAS	PAS following diastase or ptyalin	Hale's dialysed iron	Alcian blue	Toluidin blue gamma metachromasia
Interpretation of staining results								
DNA	+							
No detectable DNA	-							
RNA		+	-					
No detectable RNA		-	-					
Glycogen				+	-			
Non-glycogen carbohydrate				+	+			
Acid Mucopoly-saccharide						+	+	+
Gray cells	N	-	-	+	+,-[1]	+	+	-
Rhabdiferous cells	N	-	-	+	+	+	+	+[2]
Globoferous cells	N	-	-	+	+	-	+[3]	-
Nucleolate cells	N&Ci	NL&C	-			-	-	-
Epidermal cells	N	NL&C[4]	-			-	-	-
Choanocytes	N	C	-			-	-	-
Coiled toxa material	-	-	-	+	+	-	-	-

N = nuclear; NL = nucleolar; Ci = cytoplasmic inclusions; C = cytoplasmic.

[1] Homogeneous deposit is PAS-negative. [2] Gamma metachromasia displayed both in water mounts directly following staining and in permanent preparations which were dehydrated and mounted.

[3] Large spherical inclusion fails to stain.

[4] Nucleoli are not present in all epidermal nuclei; nucleoli absent in adult sections.

(Feulgen staining). In addition, extranuclear Feulgen-positive material has been found in the nucleolate cells. This occurs in the form of cytoplasmic inclusions, many of which are present in vacuoles (see p. 22). Control slides which remained unhydrolyzed showed no staining in Schiff's reagent.

Cytoplasmic and nuclear RNA is present in nucleolate cells and epidermal cells. It is present in the nucleus within a nucleolus and in the cytoplasm as a fine granular material. Some of the inclusions in the nucleolate cells contain RNA also. Choanocytes contain only cytoplasmic RNA; gray cells, rhabdiferous cells and globoferous cells do not contain any detectable amounts of either nuclear or cytoplasmic RNA.

Gray cells, rhabdiferous cells and globoferous cells contain carbohydrate which is not glycogen (that is, PAS-positive material which is not attacked by diastase or ptyalin). This occurs in the cytoplasm of the gray cells, in the smaller inclusions and large inclusion of globoferous cells (the larger inclusion stains much more strongly than the smaller inclusions), and in both the cytoplasm and inclusions of the rhabdiferous cells. In the latter, it is difficult to determine whether the staining is solely cytoplasmic or not because the intensity of staining is very weak. The homogeneous cytoplasmic area in the gray cells (Pl. 4, fig. 4) contains glycogen (diastase and ptyalin digestible material).

The inclusions within the rhabdiferous cells contain acid mucopolysaccharide, as borne out by positive staining in Hale's dialysed iron and alcian blue, and by gamma metachromasia in toluidin blue (Pearse, 1960).

Microciona spinosa Wilson, 1902, p. 396
(Pls. 6-8)

A. Skeletal Morphology (Measurements given in Table 5)

The specimens of *Microciona spinosa* used in this study are incrusting (Pl. 6, fig. 1). The surface of the sponge contains numerous upright, hair-like processes which consist of spongin fibers covered by epidermis and mesenchyme (these processes are referred to as conules). Palmate isochelas are numerous in the epidermis and a very few thin styles occur here without any particular orientation. These thin styles also occur sparsely in the mesenchyme.

At the ends of some of the spongin fibers is a small plush of thick styles. Also, at the fiber ends, the mesenchyme is reduced and usually only the dermis is present. At the base of the spongin fibers branches arise which form a very irregular network anastomosing with branches from other fibers; basally there is a thin layer of spongin (Pl. 6, fig. 2). Within the spongin fibers and sometimes protruding from them are smooth, thick (= coring) styles which are thicker than those occurring in the dermis and mesenchyme; however, a few thin styles also occur within the spongin fibers.

There is a great deal of variation in the width of the spongin fibers. The upright fibers on the average are 50μ in width at their tip and broaden out to 500μ basally; they may be from 2.0 to 4.0 mm in height. Some fibers, however, are shorter and thinner (1.5 mm $\times$ 60μ), and some colonies have a more dominant pattern of anastomosing fibers basally; the upright fibers in these latter colonies are shorter since they originate from the upper portion of the network. In these

cases, the fiber width is 200μ or so and their height is only in the range of 500μ. In the mesenchyme long thin toxas occur quite commonly in tracts; single toxas are also present individually in the tissue.

TABLE 5: MEASUREMENTS OF SPICULES IN MICROCIONA SPINOSA[1]

Spicules		
Thick (= coring) styles		141.4-247.0-364.0 x 9.4-12.0-15.4
Thin styles		192.4-266.8-384.8 x 1.9- 3.1- 5.9
Toxas	Slight bend:	275.6-294.3-319.3
	Deep arch:	57.6- 60.2- 67.2
	Moderate arch:	12.0- 21.2- 27.6
Palmate isochelas		14.0- 15.6- 17.0
Spongin		See text

[1]Means (underlined) and extremes of twenty-five spicules in each category; measurements in microns.

B. Adult Histology

The tissue of *M. spinosa* is loosely packed in comparison to *M. prolifera* (Pl. 6, fig. 3). It consists of flagellated chambers, exhalant canals, a subdermal space and mesenchyme. The mesenchyme contains all of the cell types described in the next section; however, the cells referred to as microgranular cells are extremely rare. The dermis is a composite structure containing strands of fibrous material, anucleolate epidermal cells, nucleolate cells, rhabdiferous cells and globoferous cells; fiber cells, if present, must be very rare since none were observed. In particular, globoferous cells are very numerous in the dermis. Spongin fibers are present and are peculiar in that they contain separate, but closely associated, bands of material. These bands often form concentric circles within the fibers. In the mesenchyme, coiled material is abundant and forms tracts (see next section); many toxas are present within this material. Rhabdiferous cells are present in the dermis and also permeate the mesenchyme.

Mature larvae have been found in the mesenchyme of adult colonies (Pl. 6, fig. 3). These larvae have a uniformly flagellated epithelium. Within the larvae are nucleolate cells, rhabdiferous cells, gray cells and globoferous cells. Undifferentiated larvae consist of a spherical mass of blastomeres, as in *M. prolifera,* without a special epithelial covering. Sperm and mature eggs have also been found in the mesenchyme; the former occur in spherical masses (Pl. 6, fig. 3) surrounded by an epithelium.

C. Morphological and Cytochemical Study of the Outgrowth Region

1. Gross aspects of the outgrowth region

The outgrowth region of explants of *M. spinosa* is very thin and well suited to observations on both living, and fixed and stained material. A very characteristic feature of the outgrowth region is the great abundance of long tracts of coiled material. Flagellated chambers are present in the mesenchyme as are exhalant canals. Spicules also occur in the differentiated areas and are usually the first skeletal elements to appear in the undifferentiated regions. The growing edge displays periodic regression as in *M. prolifera* (see p. 20).

2. Cell types present in the outgrowth region (Measurements of cells, nuclei and other components are given in Table 6.)

The epidermis and ostial openings

A lower epidermis is present in the undifferentiated areas of the outgrowth region in contact with the substratum. In the differentiated areas, or the areas undergoing differentiation, an upper epidermis is present as well. The lower epidermis consists of cells with distinct boundaries as seen under phase contrast (Pl. 7, fig. 1). Many epidermal nuclei contain a small nucleolus; nucleoli are more prevalent in the lower epidermis. Surrounding the nucleus are usually a few cytoplasmic inclusions (Pl. 7, fig. 1). No cell division has been found in the epidermis.

At the growing edge of the outgrowth region particulate matter is ingested by the lower epidermis; algae have been observed being engulfed by the epidermal cells.

The upper epidermis is a composite structure containing in addition to epidermal cells, ostial openings, chelas, globoferous cells and rhabdiferous cells. Ostia may be associated with a single nucleus or with two or three nuclei as in *M. prolifera* (see p. 22); cell boundaries are indistinct. Nucleolate cells are also associated with the upper epidermis.

Choanocytes and flagellated chambers

Flagellated chambers are present in the differentiated areas of the outgrowth region and in areas undergoing differentiation; they contain the same type of opening as described in *M. prolifera* (see p. 22). The choanocytes which make up the flagellated chambers contain anucleolate nuclei. Numerous mitotic figures are present in the flagellated chambers.

Nucleolate cells

These cells are similar to the nucleolate cells in *M. prolifera*. They contain a variable number of cytoplasmic inclusions some of which are present within vacuoles. They show active movement by means of short, thin pseudopodia. Many nucleolate cells have been found dividing in the mesenchyme. Nucleolate cells vary in shape and contain nucleolate nuclei. No nucleolate cells have been found which contain more than a single nucleolus.

Spongin is present clothing the rounded ends of some megascleres in the dif-

TABLE 6: MEASUREMENTS OF CELLS AND OTHER COMPONENTS IN MICROCIONA SPINOSA[1]

Component	Number Measured	Nuclear Diameter	Nucleolar Diameter	Cell Size	Other Features
Sperm masses	Numerous				35.0-70.0
Eggs	Numerous	9.5-14.5	3.1-4.8	47.0x24.0 to 52.0x24.0	
Larval size					300.0 x 200.0
Epidermal cells	25	3.5-4.0-4.8	0.7[2]	30.0 x 30.0	
Ostial openings	25				11.0x13.0 to 48.0x39.0
Flagellated chambers	25				20.0x20.0 to 45.0x25.0
Choanocytes	25	1.8-2.2-3.0	absent	7.0	
Nucleolate cells	25	3.3-4.0-5.1	0.7 to 1.4	15.0 x 8.0 27.0 x 6.0 50.0 x 2.0	
Gray cells	25	2.0-2.3-2.7	absent	13.0 x 4.0 30.0 x 4.5	Cytoplasmic granules: 0.7-1.1
Rhabdiferous cells	25	2.3-2.8-3.6	absent	40.0 to 50.0 x 10.0	Cytoplasmic inclusions: 1.0-3.0
Globoferous cells	25	2.0-2.1-2.7	absent	17.0x5.0 to 40.0x3.0	Small cytoplasmic inclusions: 0.8-2.0 Large cytoplasmic inclusion: 3.7-5.0
Microgranular cells	Few	approx. 2.0-2.5	absent	13.0 x 2.5 40.0 x 1.5	Cytoplasmic granules: 0.3-0.8
Collencytes	Few	approx. 3.5-4.0	absent	25.0 x 1.5	
Cheloblast	Few	approx. 2.0-2.5	absent		

[1]Measurements in microns; means underlined. Measurements made on cells, etc. in the outgrowth region of explants; Bouin fixed material. [2]Nucleoli absent in adult sections.

ferentiated areas of the outgrowth region. This spongin is always encircled by a group of nucleolate cells which secrete the spongin as in *M. prolifera* (see Pl. 3, fig. 5).

Collencytes

Collencytes are characterized by anucleolate nuclei and basophilic cytoplasm. They may also contain cytoplasmic inclusions, although many lack them. The cell body is usually elongate. These cells are found throughout the mesenchyme.

Special cell types

Gray cells

Gray cells are like those described in *M. prolifera* with two minor differences: the cytoplasmic granules are larger than in *M. prolifera,* and there is a smaller area (or areas) which lacks granules (see Cytochemical results). Gray cells occur throughout the mesenchyme and move actively without the formation of well-defined pseudopodia (Pl. 7, fig. 2). No mitotic activity has been found in these cells, which contain anucleolate nuclei.

Rhabdiferous cells

Rhabdiferous cells in this sponge are similar to those in *M. prolifera.* Their cytoplasm contains numerous inclusions (Pl. 7, fig. 3) which are rod-shaped, spherical, or oval. Many rhabdiferous cells also contain coiled material which is morphologically similar to the coiled material associated with toxas (see p. 31). These cells do not show active movement in the mesenchyme and no mitotic figures have been observed. In the differentiated areas of the outgrowth region rhabdiferous cells show disruption with the release of some of their contents. These cells occur both in undifferentiated areas, and in the epidermis and mesenchyme of differentiated areas. However, many rhabdiferous cells also occur organized into tracts which run from the explant almost to the growing edge (see p. 31). These tracts also contain coiled material and globoferous cells (Pl. 8, fig. 1). Rhabdiferous cells are usually irregular in shape and contain anucleolate nuclei which are elongate.

Globoferous cells

These cells are similar to the globoferous cells in *M. prolifera* (Pl. 8, fig. 2). However, in addition to small basophilic inclusions and a single large inclusion which stains light blue in Mallory solution II, they contain a number of smaller Mallory-positive inclusions. These additional inclusions may number from two to five or may be absent; their size is similar to that of the basophilic inclusions.

Globoferous cells are exceedingly numerous in the upper epidermis and in the marginal areas of the outgrowth region. They also occur in large numbers in tracts which contain coiled material and rhabdiferous cells (see p. 31). Globoferous cells move actively in the mesenchyme without the formation of pseudopodia. They contain anucleolate nuclei. No mitotic activity has been found in these cells.

Microgranular cells

These cells occur almost exclusively in the upper epidermis. Their measurements and characteristics are similar to the cells which secrete chelas. They are also similar to gray cells except that their cytoplasm is completely filled with smaller granules and they do not contain glycogen. These cells possess anucleolate nuclei.

3. Spiculogenesis in the outgrowth region

Megasclere secretion

Nucleolate cells secrete styles in the mesenchyme of the outgrowth region; these styles are smaller than the styles present in the adult. Very early stages of style secretion have been found; these stages are elongate nucleolate cells which contain a long thin axial thread which stains basophilically. Silica has not yet been deposited along the length of these axial threads.

Chela secretion

Chelas are secreted almost exclusively in the upper epidermis by cells (= cheloblasts) with small anucleolate nuclei and with minute granules in the cytoplasm.

Toxa secretion

Toxas are secreted both in the undifferentiated and differentiated areas of the outgrowth region. In the former they occur singly in the mesenchyme (Pl. 8, fig. 3), but in the differentiated areas, they occur both singly in the mesenchyme and in tracts. In all cases, toxa secretion occurs in association with non-cellular, coiled material as in *M. prolifera* (see p. 24). The coiled material present in tracts (Pl. 8, fig. 1) is much thicker than that present around toxas which occur singly in the mesenchyme (Pl. 8, fig. 3). The presence of tracts of coiled material (Pl. 8, fig. 1) is a prominent feature of *M. spinosa*. These tracts occur throughout the mesenchyme and stain deep blue in Mallory solution II. They have associated with them rhabdiferous cells, globoferous cells and toxas. Some rhabdiferous cells contain cytoplasmic strands of material which form loops similar to those formed by coiled material around toxas which occur singly in the mesenchyme. These cells appear to be forming coiled material. Only the two longer categories of toxas are present in these tracts.

4. Cytochemistry of the cells in the outgrowth region (see Table 7)

The cells in the outgrowth region contain nuclear DNA (Feulgen staining) and some of the cytoplasmic inclusions in the nucleolate cells contain Feulgen-positive material. RNA is present in the cytoplasm of collencytes, nucleolate cells, epidermal cells and choanocytes. In addition, the nucleoli in epidermal cells and nucleolate cells contain RNA. No detectable amounts of this substance have been found in the other cell types.

Gray cells, globoferous cells and rhabdiferous cells contain carbohydrate which is not glycogen (i.e. not removed by ptyalin). This occurs in the granules

TABLE 7: CYTOCHEMICAL STAINING RESULTS ON THE CELLS IN THE OUTGROWTH REGION OF MICROCIONA SPINOSA

	Feulgen staining	Beta metachromatic staining in azure b bromide	Beta metachromatic staining in azure b bromide following ribonuclease	PAS	PAS following ptyalin	Hale's dialysed iron	Alcian blue	Toluidin blue gamma metachromasia
Interpretation of staining results	- - - - - - - - - - - - -		See Table 4		- -			
Gray cells	N	-	-	+	+,-[1]	+	±	-
Rhabdiferous cells	N	-	-	+[2]	+[2]	+	+	+[3]
Globoferous cells	N	-	-	+[4]	+[4]	+[5]	+[5]	+[3,5]
Microgranular cells	N	-	-	±	±[6]	-	-	-
Collencytes	N	C	-			-	-	-
Nucleolate cells	N&Ci	NL&C	-			-	-	-
Epidermal cells	N	NL&C	-			-	-	-
Choanocytes	N	C	-			-	-	-
Coiled material in tracts	-	-	-	+	+	-	-	-
Coiled material around toxas which occur singly	-	-	-	+	+	+	+	+[3,7]

N = nuclear; NL = nucleolar; Ci = cytoplasmic inclusions; C = cytoplasmic.

[1] Small homogeneous deposits are negative. [2] Very weak staining.

[3] Gamma metachromasia displayed in water mounts directly following staining and in permanent preparations which were dehydrated and mounted. [4] Small inclusions moderately stained, large inclusion (or inclusions) strongly stained. [5] Large inclusion (or inclusions) fails to stain. [6] No change in PAS staining. [7] Very weak gamma metachromasia.

and cytoplasm of the gray cells. Some of the small clear areas present in the gray cells, however, do contain glycogen. This is present in small irregular deposits which tend to be localized at one end of the cell (Pl. 7, fig. 2); there are usually two to five of these deposits. In globoferous cells non-glycogen carbohydrate is found in the small inclusions, which stain moderately with PAS, and in the larger spherical inclusion (or inclusions) which stains very deeply with PAS. The rhabdiferous cells stain only weakly with PAS and this staining occurs both in the cytoplasm and in the inclusions.

Rhabdiferous cells and globoferous cells both contain acid mucopolysaccharide as borne out by positive staining in Hale's dialysed iron and alcian blue and by gamma metachromasia in toluidin blue (Pearse, 1960). This occurs in the small inclusions in the globoferous cells and in the cytoplasm in small areas joining the inclusions. These narrow "links" which connect the inclusions give to the globoferous cells an appearance very similar to that of the rhabdiferous cells. In preparations stained with Hale's dialysed iron or alcian blue it is difficult to distinguish between these two cell types. The one characteristic which does separate them, however, is the presence of the large spherical inclusion in the globoferous cells, which is negative in these stains. The acid mucopolysaccharide in rhabdiferous cells is present throughout the cytoplasm of the cell and in the inclusions within the cytoplasm. The coiled material around toxas which occur singly in the mesenchyme contains acid mucopolysaccharide, although the gamma metachromasia displayed in toluidin blue is weak. The coiled material present in tracts throughout the mesenchyme stains differently from the coiled material present around toxas which occur singly; it contains non-glycogen carbohydrate but is negative in all of the stains for acid mucopolysaccharide. It is not clear whether microgranular cells contain non-glycogen carbohydrate; PAS staining is very weak but is unchanged by ptyalin treatment. Glycogen is thus absent as is RNA.

Microciona atrasanguinea Bowerbank, 1864, p. 188, type species of *Microciona*
(Text- fig. 1)

A. Skeletal Morphology (Measurements given in Table 8)

Microciona atrasanguinea is similar in skeletal morphology and growth form to *M. prolifera* except that it does not produce upright branches. It grows as an incrustation possessing a basal layer of spongin from which arise separate, upright spongin fibers. These fibers contain thick (= coring) styles, some with subtylote and/or microspined heads, and acanthostyles. Most thick styles and all acanthostyles have only the head of the spicule embedded within the spongin. At the surface of the sponge, the upright spongin fibers end with a plush of thick styles which protrude from the sponge. Thin styles, some with microspined heads, occur sparsely at the surface, most lying parallel to it; they also occur in the mesenchyme of the sponge. Palmate isochelas are present at the surface and within the tissue, as are toxas (Text-fig. 1, C, F). Most toxas, however, occur in tracts in the tissue rather than singly.

B. Adult Histology

The histology of *M. atrasanguinea* is like that of *M. prolifera*. A dermis con-

TABLE 8: MEASUREMENT OF SPICULES AND SPONGIN IN MICROCIONA ATRASANGUINEA[1]

Spicules		
Thick (= coring) styles	124.8-249.6-384.8	x 7.6-13.1-17.4
Thin styles	135.2-257.9-324.5	x 1.2- 2.8- 4.8
Acanthostyles	72.4- 89.7-126.1	x 4.8- 6.2- 7.6
Toxas	13.1- 61.9-142.8	
Palmate isochelas	12.1- 16.4- 20.2	
Spongin		
Fiber width	50.0 to 120.0	

[1]Means (underlined) and extremes of twenty-five spicules in each category; measurements in microns.

taining strands of fibrous material and other cells is present; also present are exhalant canals, subdermal spaces, flagellated chambers, nucleolate cells and fiber cell tracts (see p. 19). Gray cells and globoferous cells occur throughout the mesenchyme with gray cells being more numerous near the basal layer of spongin and globoferous cells more numerous in the dermis. Rhabdiferous cells also occur throughout the mesenchyme but are more numerous in areas which contain tracts of toxas. In addition, rhabdiferous cells are very abundant in the dermis and release acid mucopolysaccharide in both the dermis and mesenchyme. Epidermal cells in the adult lack nucleoli. Collencytes have not been found in *M. atrasanguinea.*

C. Special Cell Types, Cytochemistry and Coiled Material Associated with Toxas (see Tables 9 and 10)

The special cell types present in *M. atrasanguinea* are morphologically the same as those in *M. prolifera.* All of them lack detectable RNA but contain non-glycogen carbohydrate and anucleolate nuclei. Gray cells contain glycogen in the form of irregular cytoplasmic deposits; both rhabdiferous cells and globoferous cells contain acid mucopolysaccharide (see Table 9). This acid mucopolysaccharide occurs in the cytoplasm and inclusions of rhabdiferous cells and in the smaller cytoplasmic inclusions of the globoferous cells.

Coiled material similar to that in *M. prolifera* (see p. 24) contains non-glycogen carbohydrate (strongly PAS positive) but lacks acid mucopolysaccharide. It is present around toxas and extends into the mesenchyme. Coiled material is non-cellular; a nucleus is not associated with it and a limiting membrane is not apparent. In those areas in which toxa tracts are present, coiled material forms short tracts which are similar to, but much less abundant than, those in *M. spinosa.*

TABLE 9: CYTOCHEMICAL STAINING RESULTS ON THE CELLS IN ADULT SECTIONS OF MICROCIONA ATRASANGUINEA

	Feulgen staining	Beta metachromatic staining in azure b bromide	Beta metachromatic staining in azure b bromide following ribonuclease	PAS	PAS following ptyalin	Hale's dialysed iron	Alcian blue	Toluidin blue gamma metachromasia
Interpretation of staining results	See Table 4							
Gray cells	N	-	-	+	+,-[1]	±	±	-
Rhabdiferous cells	N	-	-	+	+	+	+	+[2]
Globoferous cells	N	-	-	+	+	+[3]	+[3]	+[2,3]
Nucleolate cells	N&Ci	NL&C	-			-	-	-
Epidermal cells	N	C	-			-	-	-
Choanocytes	N	C	-			-	-	-
Coiled toxa material	-	-	-	+	+	-	-	-

N = nuclear; NL = nucleolar; Ci = cytoplasmic inclusions; C = cytoplasmic.

[1] Homogeneous deposit is negative.

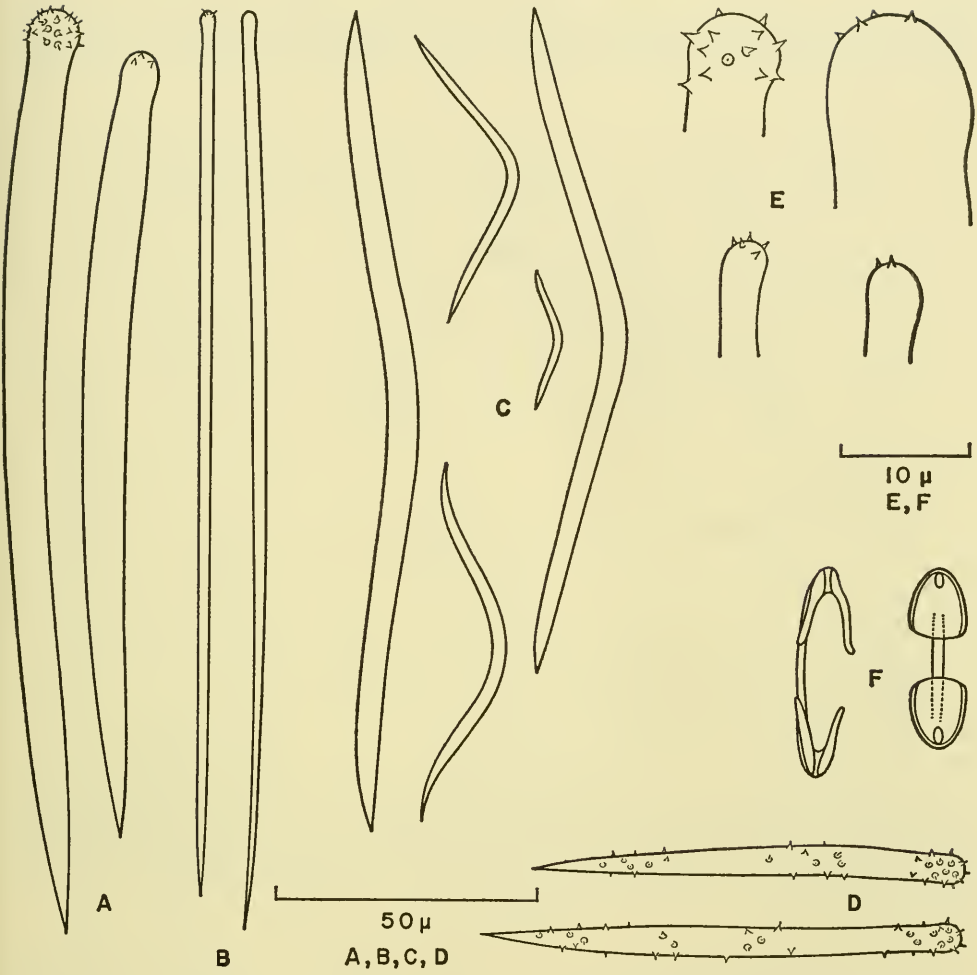
[2] Gamma metachromasia displayed both in water mounts directly following staining and in permanent preparations which were dehydrated and mounted.

[3] Larger cytoplasmic inclusion (or inclusions) fails to stain.

TABLE 10: MEASUREMENTS OF CELLS AND OTHER COMPONENTS IN MICROCIONA ATRASANGUINEA[1]

Component	Number Measured	Nuclear Diameter	Nucleolar Diameter	Cell Size	Other Features
Nucleolate cells	10	3.4-3.9-4.5	0.5-1.1-1.5	11.0 x 5.0	
Epidermal cells		same size range as nucleolate cells	absent	?	
Flagellated chambers	10				15.0x15.0 to 22.0x18.0
Choanocytes	10	1.5-1.8-2.1	absent	3.5 x 3.5 to 5.0 x 5.0	
Gray cells	10	2.2-2.6-3.0	absent	10.0 x 5.0	Cytoplasmic granules: 0.3-0.5
Rhabdiferous cells	10	2.1-2.6-3.0	absent	30.0 x 4.0	Cytoplasmic inclusions: 1.0-2.0
Globoferous cells	10	1.9-2.2-2.8	absent	19.0 x 3.0	Small cytoplasmic inclusions: 1.0-1.5 Large inclusions: 3.5

[1] Measurements in microns; means underlined. Measurements made on adult sections fixed in Bouin.



Text-fig. 1. Spicule types present in *Microciona atrasanguinea*. A. Thick (= coring) styles. B. Thin styles. C. Toxas. D. Acanthostyles. E. Spination on the heads of thick styles (above) and thin styles (below). F. Palmate isochelas.

Nucleolate cells, epidermal cells, and choanocytes contain RNA. This occurs in the cytoplasm of these cells and in the nucleoli of nucleolate cells (see Table 9).

Microciona seriata (Grant, 1826, p. 116) new combination
(see p. 93 for generic placement of this species), type species of *Ophlitaspongia*
(Pls. 9, 10, Text-fig. 2)

A. Skeletal Morphology (Measurements given in Table 11)

Microciona seriata is a red, incrusting sponge containing distinct and regularly arranged oscular openings on the surface (Pl. 9, fig. 1). Basally, there is a thin layer of spongin from which arises a prominent and very regular reticulation of spongin fibers (Pl. 9, figs. 2, 3). At the surface of the sponge this reticulation ends abruptly without the production of separate upright spongin fibers.

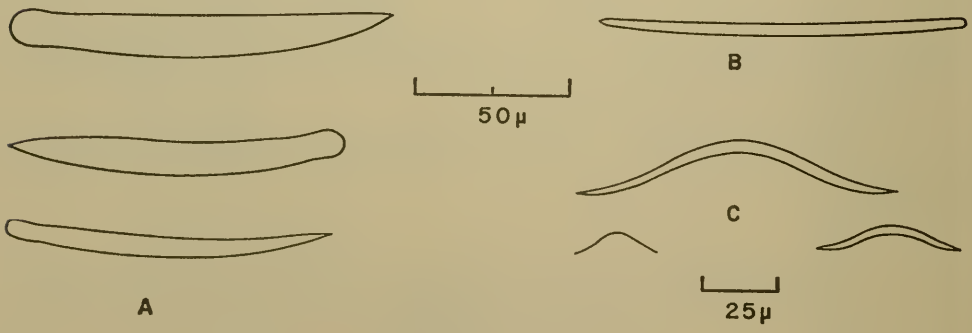
Embedded within the spongin are thick (= coring) styles, some with subtylote heads. In addition, thin styles may also be present embedded within the spongin.

TABLE 11: MEASUREMENTS OF SPICULES AND SPONGIN IN *MICROCIONA SERIATA*[1]

Spicules	
Thick (= coring) styles	78.0-113.4-132.1 x 7.1-10.5-14.8
Thin styles	83.2-111.3-130.0 x 1.2- 2.1- 3.1
Toxas	19.0- 67.8-130.9
Spongin	
Fiber width	10.0 to 50.0
Meshes	70.0 x 45.0 to 140.0 x 130.0

[1] Means (underlined) and extremes of twenty-five spicules in each category; measurements in microns.

These thin styles are also sparsely present in the surrounding mesenchyme. Toxas, none of which occur in tracts, are present in the mesenchyme and are very numerous. The spicule types present in this sponge are pictured in Text-figure 2.



Text-fig. 2. Spicule types present in *Microciona seriata*. A. Thick (= coring) styles. B. Thin style. C. Toxas.

B. Adult Histology (see Table 12)

Sections of adult colonies contain a dermis possessing anucleolate epidermal cells, strands of fibrous material, nucleolate cells, globoferous cells and rhabdiferous cells. Subdermal spaces, exhalant canals and flagellated chambers are also

TABLE 12: MEASUREMENTS OF CELLS AND OTHER COMPONENTS IN MICROCIONA SERIATA[1]

Component	Number Measured	Nuclear Diameter	Nucleolar Diameter	Cell Size	Other Features
Nucleolate cells	10	2.5-4.0-5.0	1.0-1.1-1.3	10.0 x 4.0	
Epidermal cells		same size range as nucleolate cells	absent	?	
Flagellated chambers	10				20.0x13.0 to 30.0x20.0
Choanocytes	10	1.2-1.6-2.0	absent	3.5 x 3.5	
Gray cells	10	2.0-2.3-2.9	absent	10.0 x 5.0	Cytoplasmic granules: 1.0
Rhabdiferous cells	10	1.6-2.2-2.8	absent	34.0 x 9.0	Cytoplasmic inclusions: 1.0-2.0, irregular
Globoferous cells	10	1.7-2.0-2.3	absent	15.0 x 4.0	Small cytoplasmic inclusions: 1.0-2.5[2] Large inclusion: 3.0-4.5-6.0
Sperm masses	Numerous				50.0x50.0 to 150.0x70.0
Microgranular cells	These cells are reported by Borojevic and Levi (1964) in reaggregation masses. They are like those in M. spinosa.				

[1] Measurements in microns; means underlined. Measurements made on adult sections fixed in Bouin.

[2] Small cytoplasmic inclusions are irregular in shape or are absent in globoferous cells which are present in groups in the mesenchyme.

present in this sponge. In sections which pass through oscular openings (Pl. 9, fig. 4) large exhalant canals can be seen leading from the mesenchyme to the oscular openings. Gray cells are present throughout the mesenchyme as are rhabdiferous cells which are also present in large numbers in the dermis. Globoferous cells occur throughout the mesenchyme, in the dermis, and in isolated groups in the mesenchyme (Pl. 10, figs. 1, 2). Those which occur in groups generally lack smaller cytoplasmic inclusions (see next section). Collencytes have not been found in adult sections and epidermal nuclei are anucleolate.

Sperm masses and eggs are present in the tissue; these are similar to those in *M. prolifera* (see p. 20).

C. Special Cell Types, Cytochemistry and Coiled Material Associated with Toxas (see Tables 12 and 13 and Plate 17)

The morphology and cytochemistry of gray cells, rhabdiferous cells, globoferous cells and coiled material are the same as in *M. spinosa* (see Table 13) with the exception that coiled material does not form tracts and is only PAS positive (= non-glycogen carbohydrate). It is apparently non-cellular as in *M. prolifera* (see p. 24). Those globoferous cells which are present in groups in the mesenchyme (see p. 94) are round rather than elongate and the smaller cytoplasmic inclusions are irregular rather than spherical in shape or are lacking altogether (see Pl. 10, figs. 1, 2). Rhabdiferous cells release acid mucopolysaccharide into the mesenchyme and dermis. RNA is present in choanocytes, epidermal cells, and nucleolate cells (see Table 13). Rhabdiferous cells and globoferous cells both contain acid mucopolysaccharide as in *M. spinosa.* Borojevic and Lévi (1964) have found microgranular cells in reaggregation masses of *M.(= Ophlitaspongia) seriata.* Although these cells have not been found in the present study, it is assumed from their work that they are present in adult sections. In *M. spinosa* microgranular cells are very rare in adult sections but are relatively abundant in the outgrowth region of explants.

Microciona pennata (Lambe, 1894, p. 129) new combination
(see p. 95 ff. for generic placement of this speices)
(Pl. 15)

A. Skeletal Morphology (Measurements given in Table 14)

Microciona pennata is a red, incrusting sponge which contains a thin basal layer of spongin in contact with the substratum (Pl. 15, fig. 1). Arising from this basal spongin are upright tracts of spicules containing small amounts of spongin. These tracts are connected by thin bridges consisting of one or two spicules and spongin; the bridges are approximately the length of one spicule. The spicules within this framework are thick (= coring) styles with subtylote heads some of which are spined. Most of these styles are only partially embedded in spongin. In the surrounding mesenchyme thinner styles, also with subtylote heads some of which are spined, are present as well as toxas. There is no evidence of the presence of toxa tracts; only single toxas occur in the mesenchyme.

The surface of the sponge contains plushes of thick styles where the tracts of spongin and spicules terminate; these plushes protrude from the surface with

TABLE 13: CYTOCHEMICAL STAINING RESULTS ON THE CELLS IN ADULT SECTIONS OF MICROCIONA SERIATA

	Feulgen staining	Beta metachromatic staining in azure b bromide	Beta metachromatic staining in azure b bromide following ribonuclease	PAS	PAS following ptyalin	Hale's dialysed iron	Alcian blue	Toluidin blue gamma metachromasia
Interpretation of staining results	See Table 4							
Gray cells	N	-	-	+	+,-[1]	±	±	-
Rhabdiferous cells	N	-	-	+	+	+	+	+[2]
Globoferous cells	N	-	-	+	+	+	+	+[2,3]
Microgranular cells	?[4]	(Probably negative[4])		?[4]	?[4]	?[4]	?[4]	?[4]
Nucleolate cells	N&Ci	NL&C	-			-	-	-
Epidermal cells	N	C	-			-	-	-
Choanocytes	N	C	-			-	-	-
Coiled toxa material	-	-	-	+	+	-	-	-

N = nuclear; NL = nucleolar; Ci = cytoplasmic inclusions; C = cytoplasmic.

1 Homogeneous deposit does not stain.

2 Gamma metachromasia displayed both in water mounts directly following staining and in permanent preparations which were dehydrated and mounted.

3 Larger cytoplasmic inclusion fails to stain.

4 Borojevic and Levi (1964) have described microgranular cells in this sponge. They apparently lack endoplasmic reticulum and nucleoli (=RNA). Cytochemical staining results are not recorded by the authors.

the points of the styles outermost. In addition, thin styles are present at the surface, most lying parallel to it, but in some areas groups of upright thin styles are present and these also protrude beyond the surface of the sponge. In some cases these groups of thin, upright styles are intermixed with a plush of thick styles.

TABLE 14: MEASUREMENTS OF SPICULES AND SPONGIN IN MICROCIONA PENNATA[1]

Spicules	
Thick (= coring) styles	156.0-236.1-379.6 x 12.4-18.8-23.8
Thin styles	161.2-195.5-244.4 x 2.6- 4.8- 7.4
Toxas	16.3- 27.6- 53.6
Spongin	
Width of upright fibers	30.0 to 100.0
Width of connecting fibers	10.0 to 25.0
Meshes	irregular

[1] Means (underlined) and extremes of twenty-five spicules in each category; measurements in microns.

B. Adult Histology

The histology of adult colonies is like that of *M. prolifera* (see p. 19). Fiber cell tracts containing thin styles are present. However, these are more numerous than in *M. prolifera* and in some cases they are perpendicular to the dermis. In this respect they show similarity to the dermal columns to be described later in *Thalysias*. Nucleolate cells, flagellated chambers, exhalant canals, subdermal spaces, and a dermis containing strands of fibrous material and other cell types are present in *M. pennata* as in *M. prolifera*. Gray cells are most abundant near the basal layer of spongin and near the spongin fibers; globoferous cells occur throughout the mesenchyme but tend to be more numerous in the dermis. Rhabdiferous cells permeate the mesenchyme and are numerous in the dermis. Collencytes have not been found, and epidermal nuclei are anucleolate.

C. Special Cell Types, Cytochemistry and Coiled Material Associated with Toxas (see Table 15 and Plate 17)

The following special cell types are present in *M. pennata:* gray cells, rhabdiferous cells and globoferous cells. The morphology and cytochemistry of these cells are similar to the same cell types in *M. spinosa*. The size of nuclei and cytoplasmic organelles is approximately the same as in *M. spinosa* (see Tables 6 and 7). All contain small, anucleolate nuclei. However, coiled material is like

TABLE 15: CHARACTERISTICS OF CELLS IN ADULT SECTIONS OF MICROCIONA PENNATA[1]

	Nuclei	Cytoplasm
Gray cells	anucleolate; approximately 2.5-3.0 μ in diameter	numerous small granules (1.0 μ); glycogen deposit; cytoplasm weakly PAS positive
Rhabdiferous cells	anucleolate; approximately 2.5-3.0 μ in diameter	irregular inclusions contain mucopolysaccharide; cytoplasm weakly PAS positive
Globoferous cells	anucleolate; approximately 2.5-3.0 μ in diameter	smaller inclusions (1.5 μ) are PAS positive and contain acid mucopolysaccharide; larger inclusion (3-5 μ) strongly PAS positive but lacks acid mucopolysaccharide
Nucleolate cells	nucleolate; approximately 3.5-4.0 μ in diameter	some cytoplasmic inclusions present in vacuoles
Epidermal cells	anucleolate; approximately 3.0-3.5 μ in diameter	no distinctive organelles
Choanocytes	anucleolate; approximately 2.0 μ in diameter	no distinctive organelles in addition to flagella
Coiled toxa material	none	strongly PAS positive

[1]Cytochemical staining restricted to PAS, Hale's dialysed iron, Alcian blue, and toluidin blue.

that present in *M. prolifera* (see p. 24). It is present around individual toxas, is strongly PAS positive (= non-glycogen carbohydrate), but does not stain for acid mucopolysaccharide. Toxa tracts and tracts of coiled material are not present in this sponge.

Rhabdiferous cells and globoferous cells contain acid mucopolysaccharide (see Table 15) and this is released into the mesenchyme by rhabdiferous cells. Data on RNA distribution has not been recorded, because of the lack of appropriately fixed material.

Genus *PLOCAMILLA* Topsent, 1928, p. 63[2]

Plocamilla illgi Bakus, 1966, p. 440
(Text-fig. 3)

A. Skeletal Morphology (Measurements given in Table 16)

This species is a red, incrusting sponge containing a basal reticulation of

[2] See also Burton (1935, p. 402). *Plocamilla* is synonymous with the genus *Holoplocamia* which was established by de Laubenfels (1936, p. 75).

megascleres which are mostly acanthostrongyles; an occasional acanthostyle is present in the basal region. This reticulation is characterized by anastomosing spongin fibers which form a rectangular or triangular pattern and which contain no more than two or three spicules on each side. This type of skeleton is referred to as an isodictyal reticulation. At intervals along this basal reticulation

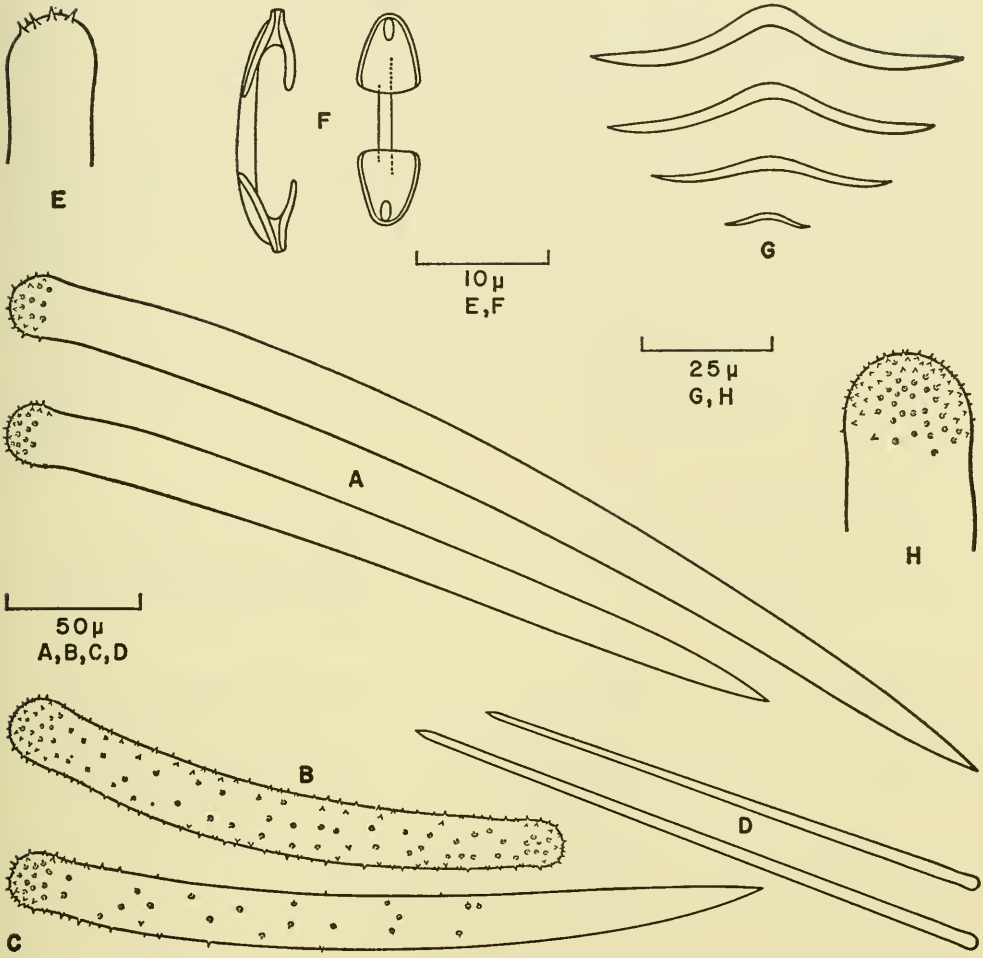
TABLE 16: MEASUREMENTS OF SPICULES AND SPONGIN IN PLOCAMILLA ILLGI[1]

Spicules	
Acanthostrongyles	192.4-251.7-282.9 x 23.1-25.9-29.8
Acanthostyles	242.3-292.2-358.0 x 17.8-23.3-28.6
Thick (= coring) styles	384.8-619.8-884.0 x 26.2-29.8-36.9
Thin styles	218.4-362.9-733.2 x 4.3- 6.7-16.7
Palmate isochelas	16.7- 18.8- 21.4
Toxas	10.7- 44.5- 72.6
Spongin	
Width of basal reticulated fibers and cross bridges	30.0
Width of upright columns	100.0
Meshes of basal reticulation	100.0 x 200.0

[1] Means (underlined) and extremes of twenty-five spicules in each category; measurements in microns.

there arise upright columns of spongin which end at the surface with a plush of thick styles protruding. These columns contain, partly embedded within them, thick (= coring) styles with microspined heads and shorter acanthostyles. At irregular intervals the columns are connected by means of cross bridges usually consisting of a single acanthostrongyle and a small quantity of spongin. In the mesenchyme thin styles, also with microspined heads, occur randomly, as do palmate isochelas. Toxas are also present in the tissue and many are present in tracts.

At the surface of the sponge, plushes of thick styles protrude and may be joined by groups of erect thin styles. The latter are also present at the surface, lying parallel to it or protruding at various angles. The spicule types present in this sponge are pictured in Text-figure 3.



Text-fig. 3. Spicule types present in *Plocamilla illgi*. A. Thick styles. B. Acanthostrongyle. C. Acanthostyle. D. Thin styles. E. Spination on the head of a thin style. F. Palmate isochelas. G. Toxas. H. Spination on the head of a thick style.

B. Adult Histology

Histological sections reveal the presence of nucleolate cells, flagellated chambers, exhalant canals, subdermal spaces, and a dermis containing fibrous strands of material and other cells. Fiber cell tracts (see p. 19) containing thin styles are present and join the dermis. These tracts are occasionally perpendicular to the dermis, producing tufts of thin styles at the surface. As in *M. pennata,* these tracts are not as well developed or as numerous as in *Thalysias.* Rhabdiferous cells occur throughout the sponge and are very abundant in the dermis. In the mesenchyme they form a network rather than being randomly distributed as in *M. prolifera.* Globoferous cells are present in the tissue and in the dermis; many of them occur in groups. These cells usually lack smaller cytoplasmic inclusions as in *M. seriata* (Pl. 10, fig. 2; Pl. 17). Collencytes are lacking, as are nucleoli in epidermal nuclei.

Sperm masses are present in the mesenchyme and are similar in size and constitution to those in *M. prolifera* (see p. 20).

C. Special Cell Types, Cytochemistry and Coiled Material Associated with Toxas (see Table 17 and Plate 17)

Plocamilla illgi contains rhabdiferous cells which stain like those in the previously described species of *Microciona* and which have the same morphology. These cells release acid mucopolysaccharide and, in addition, they abut one

TABLE 17: CHARACTERISTICS OF CELLS IN ADULT SECTIONS OF PLOCAMILLA ILLGI[1]

	Nuclei	Cytoplasm
Gray cells	anucleolate; approximately 2.5-3.0 μ in diameter	glycogen deposit present; numerous small (0.5 μ) granules or numerous small granules and a network of material or network of material only present; cytoplasm weakly PAS positive
Rhabdiferous cells	anucleolate; approximately 2.5-3.0 μ in diameter	irregular inclusions contain acid mucopolysaccharide; cytoplasm weakly PAS positive
Globoferous cells	anucleolate; approximately 2.5-3.0 μ in diameter	smaller inclusions (1.0 μ) PAS positive; larger inclusion (4-5 μ) strongly PAS positive[2]
Nucleolate cells	nucleolate; approximately 3.5-4.0 μ in diameter	some inclusions present in vacuoles
Epidermal cells	anucleolate; approximately 3.0-3.5 μ in diameter	no distinctive organelles
Choanocytes	anucleolate; approximately 2.0 μ in diameter	no distinctive organelles in addition to flagella
Coiled toxa material	none	strongly PAS positive

[1]Cytochemical staining results are restricted to PAS, Hale's dialysed iron, Alcian blue and toluidin blue.

[2]Some globoferous cells are present in groups in the mesenchyme and most of these lack smaller inclusions.

another forming a continuous network within the mesenchyme. Globoferous cells are morphologically like those in *M. seriata* except that the larger PAS positive inclusion tends to be elongate in many cells rather than spherical. Although the smaller cytoplasmic inclusions stain gamma metachromatically in

toluidin blue and positively in Hale's dialysed iron, they are negative in alcian blue and thus it cannot be concluded that they contain acid mucopolysaccharide. They do, however, contain non-glycogen carbohydrate (= PAS positive). Gray cells contain glycogen in small irregular deposits throughout the cytoplasm and have small cytoplasmic granules as do gray cells in the previously described species of *Microciona*. Some gray cells, however, contain a network composed of thin strands of material as well as cytoplasmic granules; others lack cytoplasmic granules and contain only a network (Pl. 17). Glycogen deposits, however, are present in all of the variants of gray cells. The granules, network and, to a lesser extent, cytoplasm are all PAS positive (= non-glycogen carbohydrate). Toxas are surrounded by coiled material which is similar to that in *M. prolifera* (see p. 24) and which is strongly PAS positive. Many toxas are present in tracts and have tracts of coiled material associated with them as in *M. atrasanguinea*. RNA distribution has not been recorded, due to the lack of appropriately fixed material.

Genus *THALYSIAS* Duchassaing and Michelotti, 1864, p. 86

Thalysias juniperina (Lamarck, 1813, p. 444) de Laubenfels, 1936, p. 105, type species of *Thalysias* (see Hartman, 1955, p. 171-177)
(Pls. 11, 12, Text-fig. 4)

A. Skeletal Morphology (Measurements given in Table 18)

Thalysias juniperina is a red, upright, branching sponge (Pl. 11, fig. 3). The branches fuse with one another, giving the sponge a solid appearance. A char-

TABLE 18: MEASUREMENTS OF SPICULES AND SPONGIN IN THALYSIAS JUNIPERINA[1]

Spicules		
Thick (= coring) styles	146.6-222.0-306.8 x 7.1-10.7-14.3	
Thin styles	104.0-236.7-327.6 x 2.4- 4.2- 6.4	
Acanthostyles	49.7- 57.6- 63.5 x 4.8- 5.9- 7.4	
Toxas Slight bend:	119.6-251.7-353.6	
Deep arch:	27.4- 55.5- 92.8	
Palmate isochelas	11.9- 14.5- 19.0	
Spongin		
Fiber width	25.0 to 95.0	
Meshes	80.0 x 80.0 to 300.0 x 370.0	

[1]Means (underlined) and extremes of twenty-five spicules in each category; measurements in microns.

acteristic feature of the branches is the presence of pointed processes (hereafter referred to as conule-like processes; see next paragraph) which arise from the surface. The interior of the sponge contains a network of spongin fibers (Pl. 11, fig. 4). Enclosed within the spongin fibers are smooth, thick (= coring) styles; acanthostyles are present (echinating the fibers) with only their heads embedded in the spongin. These echinating spicules are extremely numerous and most lie at a ninety-degree angle to the fibers. Palmate isochelas are randomly distributed in the mesenchyme and a few single toxas are also present. Most toxas, however, occur in tracts; these tracts run for various distances in the mesenchyme.

The surface of the sponge is characterized by tufts of thin styles (Pl. 11, fig. 5) which project from it with their points protruding. However, these dermal tufts are not universally present at the surface; in some areas they may be rare and are replaced by thin styles lying in disarray in the dermis. This configuration is particularly common in the areas at the base of and within the conule-like processes. Conule-like processes are pointed processes which are raised above the surrounding surface of the sponge (Pl. 11, fig. 3). They differ from true conules in that the interior of a conule-like process contains a network of spongin fibers rather than a single spongin fiber narrowing to a point. Some dermal tufts are joined at their bases by spongin fibers from the interior. Although there is a decided tendency for thin styles to occur at the surface of the sponge, these spicules also occur in the mesenchyme. The styles which are present within the spongin fibers are a mixture of thin and thick styles. Palmate isochelas are present at the surface of the sponge, distributed randomly.

In addition to the upright colonies described above, a younger colony was collected from an underwater television cable (Pl. 11, fig. 1, 2). This cable had been put in place on May 9, 1962 and the colony of *T. juniperina* was collected on December 18, 1962. A thin basal layer of spongin is present in contact with the substratum. Arising from this basal layer are numerous upright spongin fibers which branch and anastomose, forming a spongin network similar to that present in upright colonies. At the surface true conules are present, as is a dermal skeleton which is like that previously described in adult specimens. However, very few tufts of dermal spicules are present; most thin styles lie in disarray at the surface. Tracts of toxas are present in the mesenchyme, and chelas are distributed randomly. The spicule content of the spongin fibers is like that described above. Although the colony is basically incrusting, at various points short upright shoots are developing and these have the same morphology as described above except that they are not connected with adjacent shoots (Pl. 11, fig. 2). Spicule dimensions and other measurements are similar to those in upright, branching colonies.

B. Adult Histology

Stained sections of *T. juniperina* reveal the following elements (Pl. 11, fig. 6). The mesenchyme contains flagellated chambers and the cell types described in the next section. Exhalant canals are spaces lined by flat epithelial cells. The dermis is a composite structure as in *Microciona,* with various cells as well as strands of fibrous material present in it. Below the dermis are subdermal spaces which are cut off into chambers by dermal columns. These dermal columns con-

sist of tracts of fiber cells which run from the mesenchyme up to the dermis. Within these tracts are thin, dermal styles, and at the base of the dermal columns are spherical masses of cells (Pl. 11, fig. 6). These cell aggregates are composed of two cell types: cell type G-R and cell type S (see description of cell types, p. 52). Dermal columns are not always perpendicular to the dermis; in many areas they join the dermis at various angles and in some cases the angle is so small that the dermal column runs almost parallel to the dermis before joining it. Rudiments of conule-like processes (short pointed processes arising from the dermis, see p. 48) contain only tracts of fiber cells in the interior with no particular orientation in relation to the overlying epidermis. In a few cases dermal columns are joined at their bases by spongin fibers; in these cases there appears to be continuity between the spongin fibers and the tracts of fiber cells.

Tracts of toxas and toxoblasts are present in the mesenchyme. The histology of the young, incrusting colony is similar to that described above with two exceptions: dermal columns and cell aggregates, normally present at the base of dermal columns, are lacking in the incrusting portions and true conules are present (see p. 48).

C. Morphological and Cytochemical Study of the Outgrowth Region

1. Gross aspects of the outgrowth region

Following explantation, cells migrate from the explant to the substratum where they initially establish an undifferentiated area of the outgrowth region. This undifferentiated area, however, is much narrower than in *M. prolifera* and *M. spinosa*. A lower epidermis is present in contact with the substratum, and spicules are normally present in the undifferentiated areas; these spicules are usually single toxas although toxa tracts are also occasionally present.

The differentiated areas contain an upper and a lower epidermis between which are flagellated chambers and mesenchyme. Exhalant canals are difficult to see, owing to the thickness of the tissue. Megascleres and microscleres are present; toxas and toxoblasts occur in tracts. The growing edge of the explant displays periodic regression and particulate matter is ingested by the lower epidermis (see p. 20).

2. Cell types present in the outgrowth region (Measurements of nuclei, cells, and other components are given in Table 19)

The epidermis and ostial openings

The epidermis is made up of cells which are square in outline. Most of these cells have nucleolate nuclei; either one or two nucleoli are present. Some epidermal nuclei, however, lack nucleoli; there is a greater tendency for nucleoli to be present in the cells of the lower epidermis than of the upper epidermis. The lower epidermis is simply an epithelial sheet; the upper epidermis has various other cells associated with it and, in addition, contains ostial openings. Ostia have two or three nuclei associated with them as in *M. prolifera* (see p. 22). The cytoplasm is only vaguely outlined and cell membranes are not evident.

TABLE 19: MEASUREMENTS OF CELLS AND OTHER COMPONENTS IN THALYSIAS JUNIPERINA[1]

Component	Number Measured	Nuclear Diameter	Nucleolar Diameter	Cell Size	Other Features
Epidermal cells	10	3.1-3.9-4.1	0.8-1.0[2]	25.0 x 25.0	
Ostial openings					6.0x6.0 to 19.0x 15.0
Flagellated chambers					20.0 x 20.0
Choanocytes	10	1.8-2.2-2.6	absent	5.0	
Nucleolate cells	10	3.3-3.8-4.4	0.6-1.5	38.0 x 2.0; 12.0 x 6.0	
Cell type C-R	10	1.8-2.0-2.3	absent	29.0 x 3.5; 25.0 x 5.0	Cytoplasmic granules: 0.6-1.4; rod-shaped inclusion: 4.0x1.0 to 7.0x1.0
Cell type S	10	1.9-2.1-2.4	absent	17.8 x 8.0; 20.0 x 8.0	Basophilic cytoplasmic inclusions: 0.6-1.6; Mallory-positive cytoplasmic inclusions: 1.0-1.5
Globoferous cell Type I	10	1.6-1.8-2.0	absent	13.0 x 8.0; 20.0 x 3.0	Basophilic cytoplasmic inclusions: 0.1-0.8; Mallory-positive cytoplasmic inclusions: 1.0-1.7

TABLE 19 (continued)

Component	Number Measured	Nuclear Diameter	Nucleolar Diameter	Cell Size	Other Features
Globoferous cell Type II	Few	approx. 2.5-3.0	absent		Basophilic cytoplasmic inclusions: 1.0; large Mallory-positive cytoplasmic inclusion: 5.0; small Mallory-positive cytoplasmic inclusions: 1.0
Collencytes	Few	approx. 3.0-4.0	absent	25.0 x 3.0	
Toxoblasts	10	2.3x1.5 to 4.0x2.1	absent	20.0 x 5.0	
Cheloblast	10	2.0-2.3-2.6	absent		

[1] Measurements in microns; means underlined. Measurements made on cells, etc. in the outgrowth region of explants. Bouin fixed material.

[2] Nucleoli absent in adult sections.

Surrounding the epidermal nuclei are usually a few small round inclusions and within the cytoplasm are many minute granules (Pl. 12, fig. 1). No mitotic activity has been found in epidermal cells. On a number of occasions, marine bacteria were observed being ingested by the epidermis at the growing edge. Larger particulate matter is also taken up by the lower epidermis.

Choanocytes and flagellated chambers

As in the species previously described, flagellated chambers are composed of groups of choanocytes. The chambers themselves have openings like those in *M. prolifera* (see p. 22). Many mitotic figures have been found in the flagellated chambers. Choanocytes contain anucleolate nuclei.

Nucleolate cells

These cells are extremely numerous in the mesenchyme and show active movement by means of pseudopodia. They contain cytoplasmic inclusions some of which are present in vacuoles; some cells, however, are devoid of inclusions. Nuclei in these cells contain either one or two nucleoli. Many nucleolate cells undergo division in the mesenchyme.

In the differentiated areas of the outgrowth region spongin is present surrounding the rounded ends of some styles and acanthostyles. This spongin is in turn surrounded by a circlet of spongin-secreting, nucleolate cells, as in *M. prolifera* and *spinosa.*

Collencytes

These cells are in all respects similar to nucleolate cells except they lack nucleoli. They are present in the mesenchyme and are usually elongate. They may or may not contain inclusions within their cytoplasm. The size of the nucleus in these cells is similar to that of the nucleolate cells, and their cytoplasm is granular and basophilic.

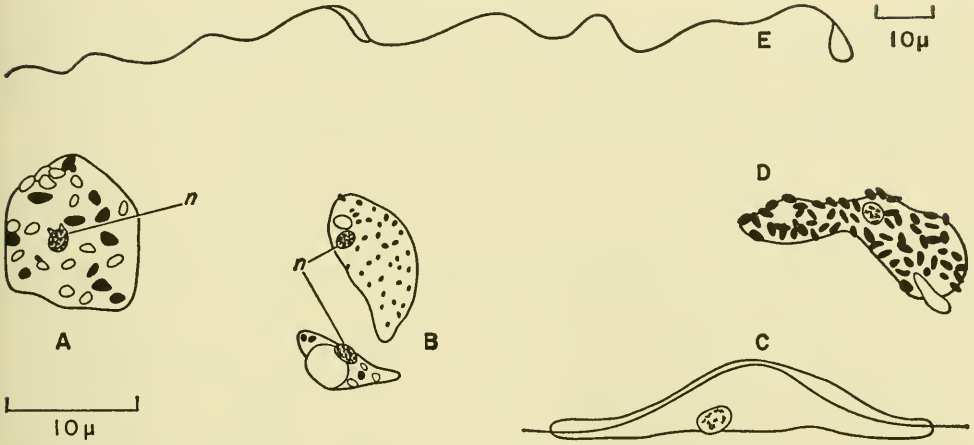
Special cell types

Cell type G-R [Containing granules (G) and a rod-shaped (R) inclusion]

These cells are very often found associated with the upper epidermis in the outgrowth region and are also present in spherical masses in the differentiated (and occasionally in the undifferentiated) areas. They lack mitotic activity and contain in their cytoplasm numerous small granules that are basophilic and, in addition, a single rod-shaped inclusion which stains deep blue in Mallory solution II. Some of this deep blue staining may be due in part to a basophilic reaction. One end of this rod-like inclusion is, in some cells, frayed, giving the appearance of splitting (Text-fig. 4, D). These cells are usually elongate but may also approach a spherical state and their nuclei are anucleolate.

Cell type S [Containing spherical (S) inclusions]

These cells are distributed throughout the mesenchyme and show active movement without the formation of pseudopodia (Pl. 12, fig. 2). They have not



Text-fig. 4. Special cell types and coiled material present in *Thalysias juniperina*. A. Cell type S. Two types of inclusions are present: basophilic inclusions (black) and Mallory-positive inclusions (white). *n* = nucleus. B. Globoferous cells: above, type I and below, type II. Both types contain basophilic inclusions (black) and at least one larger Mallory-positive inclusion (white). *n* = nucleus. C. Toxoblast containing a toxa. D. Cell type G-R containing basophilic granules (black) and a single rod-shaped Mallory-positive inclusion. E. Coiled material present in the mesenchyme. Lower scale for A, B, C, D.

been observed to undergo mitosis. They contain in their cytoplasm approximately 20 spherical inclusions, some of which are basophilic and others of which stain light blue in Mallory solution II. Some cells have a predominance of basophilic inclusions but others have a predominance of Mallory-positive inclusions; intermediate types have some of both types of inclusions. All of these cells contain anucleolate nuclei which appear to be crenated or shrunken. This appearance is due to an irregularity of the nuclear membrane—usually to the pushing in of this membrane on one side (Text-fig. 4, A). These cells have a uniform spherical shape.

Globoferous cells, type I

These cells are morphologically similar to globoferous cells as described in *Microciona* (Text-fig. 4, B). They are, however, very rare in adult sections of *T. juniperina*. In the outgrowth region they occur almost exclusively in the upper epidermis and to a lesser degree in the undifferentiated areas. These cells have not been observed in living material, and their cytochemical characteristics have not been determined because of their scarcity. They are elongate cells and contain anucleolate nuclei which appear crenated. Within the cytoplasm are small basophilic granules; in addition, larger spherical inclusions which stain light blue in Mallory solution II are present. A cell may contain only one of these larger inclusions or it may contain two, three, or many of them. Those which contain a number of larger Mallory-positive inclusions are morphologically similar to cell type G-S in *Axocielita hartmani* (see p. 66).

Globoferous cells, type II

These cells (Text-fig. 4, B) differ very little from the type I cells described above. They are exceedingly rare in the outgrowth region and have been found

in adult sections only in four instances. The small basophilic granules are slightly larger than they are in type I, and the large inclusion, which is Mallory-positive (i.e., stains light blue), is also larger than in type I. There are, in addition, smaller Mallory-positive inclusions present in the cytoplasm. The nucleus is approximately the same size as that in the type I cells.

Toxoblasts

These cells are rod-shaped and most contain toxas within them (Pl. 12, fig. 3) but some have not yet secreted the spicule. The cytoplasm stains a very light blue in Mallory solution II (so light that no internal structures are apparent). When stained with Hale's dialysed iron, however, the cytoplasm is seen to contain irregular granules within a homogeneous cytoplasm; very often empty spaces or vacuoles occur in the cytoplasm. There is a great deal of variation in cell size; longer cells are generally thinner, and these contain toxas which have only a slight bend. Shorter, wider toxoblasts usually contain toxas with a distinct deep arch. The nuclei of these cells are anucleolate and elongate. No mitotic activity has been found in toxoblasts, and none of these cells has been observed to move actively in the mesenchyme.

3. Spiculogenesis in the outgrowth region

Megasclere secretion

In a few cases, nucleolate cells have been found containing a long thin axial thread which is strongly basophilic. Nucleolate cells have also been found which contain immature styles within their cytoplasm. The nuclei of nucleolate cells which are secreting styles are peculiar because they contain, in addition to a nucleolus, chromatin strands and clumps which give the nucleus the appearance of being in prophase.

Chela secretion

Chelas are secreted mainly in the upper epidermis of the outgrowth region. These microscleres are secreted within a cell (cheloblast) which has finely granular cytoplasm and an anucleolate nucleus (refer to Pl. 5, fig. 3).

Toxa secretion

Toxoblasts have already been described in the previous section; toxas are secreted within these cells. Toxas and toxoblasts show a characteristic grouping into tracts in the differentiated areas of the outgrowth region and sometimes also at the growing edge (Pl. 12, fig. 4).

Coiled material

This material is mentioned here because it shows some similarity to the coiled material present around toxas in *Microciona*. It is present throughout the mesenchyme, measures approximately 200.0μ in length, is *not* associated with toxas (Text-fig. 4, E and Pl. 12, fig. 5), and occurs singly, not in tracts.

4. Cytochemistry of the cells in the outgrowth region (see Table 20)

The cell types present in the outgrowth region contain nuclear DNA (Feul-

TABLE 20: CYTOCHEMICAL STAINING RESULTS ON THE CELLS IN THE OUTGROWTH REGION OF THALYSIAS JUNIPERINA

	Feulgen staining	Beta metachromatic staining in azure b bromide	Beta metachromatic staining in azure b bromide following ribonuclease	PAS	PAS following ptyalin	Hale's dialysed iron	Alcian blue	Toluidin blue gamma metachromasia
Interpretation of staining results	- - - - - - - - - - - - - - - - See Table 4 -							
Toxoblasts	N	-	-	+	+	+	+	+[1]
Cell type G-R	N	-	-	+[2]	+[2]	+[2]	+[2]	+[1,2]
Cell type S	N	-	-	+	+	+	+	-
Collencytes	N	C	-			-	-	-
Nucleolate cells	N&Ci	NL&C	-			-	-	-
Epidermal cells	N	NL&C	-			-	-	-
Choanocytes	N	C	-			-	-	-
Coiled material	-	-	-	+	+	-	-	-

N= nuclear; NL = nucleolar; Ci = cytoplasmic inclusions; C = cytoplasmic.

[1] Gamma metachromasia displayed in water mounts directly following staining and in permanent preparations which were dehydrated and mounted.

[2] Rod-shaped inclusion unstained.

gen staining). In addition, some of the cytoplasmic inclusions in nucleolate cells contain Feulgen-positive material; many of these inclusions are present within vacuoles.

RNA is present in the cytoplasm of collencytes, nucleolate cells, epidermal cells, and choanocytes; nucleolar RNA is present in nucleolate cells and most epidermal cells. No detectable amounts of RNA are present in the other cell types. Toxoblasts and cell type G-R contain acid mucopolysaccharide, as borne out by positive staining in Hale's dialysed iron and alcian blue and by gamma metachromasia in toluidin blue (Pearse, 1960). This occurs in the cytoplasm of toxoblasts and in the cytoplasm and small granules of cell type G-R. In the latter case, however, it is difficult to determine whether the staining is solely cytoplasmic or not. The rod-shaped inclusion in cell type G-R is negative in respect to the above stains.

Toxoblasts, cell type G-R, cell type S and coiled material all contain PAS postive, diastase-fast material. This occurs in the cytoplasm of toxoblasts, in the granules and cytoplasm of cell type G-R, and in the inclusions and cytoplasm of cell type S. Cytochemical results on globoferous cells have not been recorded because of the difficulty of finding these cells.

Thalysias schoenus (de Laubenfels, 1936, p. 100) new combination (Pls. 13, 14, Text-fig. 5)

De Laubenfels (1936) originally described this sponge and placed it in the genus *Aulospongus* (Norman, 1878, p. 267). In doing so, he overlooked the presence of microscleres. A study of his specimen (U. S. National Museum specimen number 22404) has shown that there are chelas and toxas present. Thus, this species does not belong in the genus *Aulospongus* but, as will become clear in the following pages, it belongs in the genus *Thalysias*.

A. Skeletal Morphology (Measurements given in Table 21)

The general shape of this sponge is illustrated in Plate 13, figure 1. The interior of the branches consists of a system of anastomosing spongin fibers (Pl. 13, fig. 2) which are not as pronounced as in *T. juniperina* and which contain embedded within them thick (= coring) styles. In addition to the coring styles, echinating acanthostyles are also present, having only their heads embedded in the spongin. Palmate isochelas of two sizes are distributed throughout the mesenchyme and toxas are also present. The latter are more often than not contained in tracts in the mesenchyme.

Microscopically, the surface of this sponge consists of very pronounced tufts of styles (Pl. 13, fig. 3). Each tuft contains approximately 40 thin styles. Conules are lacking and dermal styles rarely lie horizontal in the dermis. In some cases, the spongin fibers in the interior join with the tufts of dermal styles at the base of the tuft. Chelas are numerous and randomly distributed in the dermis.

B. Adult Histology

The mesenchyme of this sponge contains flagellated chambers, exhalant canals, and all of the various cell types described in the following section. At the

TABLE 21: MEASUREMENTS OF SPICULES AND SPONGIN IN THALYSIAS SCHOENUS[1]

Spicules		
Thick (= coring) styles		236.1-322.4-422.2 x 5.5-8.6-14.3
Thin styles		98.8-244.4-353.6 x 1.2-3.5- 5.2
Acanthostyles		42.1- 55.9- 68.5 x 2.4-3.8- 5.5
Toxas		42.8- 93.8-138.0
Palmate isochelas	Small:	4.1- 5.9- 6.2
	Large:	11.3- 12.7- 15.7
Spongin		
Fiber width		20.0 to 60.0
Meshes		40.0 x 60.0 to 200.0 x 350.0

[1]Means (underlined) and extremes of twenty-five spicules in each category; measurements in microns.

surface pronounced dermal columns are present (Pl. 13, fig. 4), arising from the mesenchyme and joining the dermis. These dermal columns consist of fiber cells lying parallel to one another and thin styles; they are perpendicular to the dermis. At the base of the dermal columns are spherical masses of cells consisting of the two cell types, G-R and S, which are described in section C, 2, below (Pl. 12, fig. 4). Below the dermis, which is a composite structure as in *Microciona* and *T. juniperina* (see p. 19), are well formed subdermal spaces.

Larvae have been found in adult sections. Mature larvae possess a flagellated epithelium surrounding a mass of cells in the interior (Pl. 13, fig. 4). The cells within the larvae are toxoblasts, cell types G-R and S, and nucleolate cells. Spicules are also present in the center of the larvae; these include styles, toxas, and chelas. Spherical masses of sperm similar to those previously described in *M. prolifera* (see p. 20) have also been found in the mesenchyme.

C. Morphological and Cytochemical Study of the Outgrowth Region

1. Gross aspects of the outgrowth region

The description of the gross aspects of the outgrowth region of *T. juniperina* is applicable to *T. schoenus*. In the undifferentiated areas, a lower epidermis is present in contact with the substratum; the growing edge displays periodic regression. The differentiated areas of the outgrowth region contain flagellated chambers, canals, an upper epidermis, ostia, spongin, and spicules.

2. Cell types present in the outgrowth region (Measurements of cells, nuclei, and other components are given in Table 22)

The epidermis and ostial openings

The epidermis is similar to that in *T. juniperina,* being composed of epidermal cells many of which contain nucleolate nuclei. The epidermis in this sponge has been observed ingesting diatoms at the growing edge (Pl. 14, fig. 1). The lower epidermis shows a greater tendency to contain nucleoli than the upper epidermis. The upper epidermis is a composite structure consisting of epidermal cells and other cells (nucleolate cells, cheloblasts, cell type S) associated with it. Ostia are also present in the upper epidermis and are associated with nuclei as in *T. juniperina* and *M. prolifera* (see p. 22). Some epidermal nuclei contain a single nucleolus; others contain two nucleoli. No mitotic figures have been observed in epidermal cells.

Choanocytes and flagellated chambers

Flagellated chambers are present in the differentiated areas of the outgrowth region. They contain choanocytes with anucleolate nuclei and flagellae. Flagellated chambers contain two sets of openings as in *M. prolifera* (see p. 22). Many mitotic figures have been found in the choanocytes within flagellated chambers.

Nucleolate cells

These cells are numerous in the mesenchyme and show ameboid movement by means of well-developed pseudopodia. They contain nucleolate nuclei with one or two nucleoli. The cytoplasm of nucleolate cells contains at least a few and usually numerous inclusions, some present within vacuoles. Numerous nucleolate cells have been found undergoing mitosis.

Nucleolate cells form a circlet around the rounded ends of some megascleres and in these cases spongin is present within the ring of nucleolate cells. However, in other cases, these cells secrete spongin along the shaft or around the pointed end of styles.

Collencytes

Collencytes are present in the mesenchyme and contain anucleolate nuclei. They may also contain inclusions in their cytoplasm, although some lack these. Their shape is variable and similar to that of nucleolate cells. The cytoplasm of these cells stains basophilically.

Special cell types

Cell type G-R

These cells are similar to those described under the same term in *T. juniperina.* They contain numerous small cytoplasmic granules and a single rod-shaped inclusion (Text-fig. 5, D). The rod-shaped inclusion is often split at one end. The small granules are basophilic but the larger inclusion stains a deep blue in Mal-

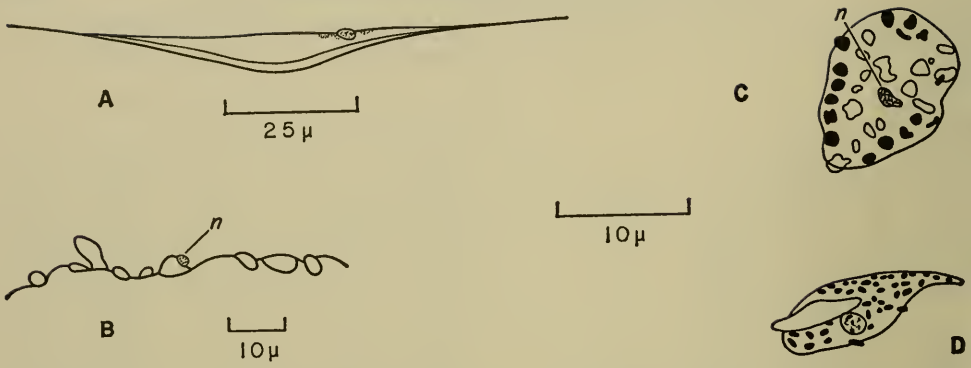
TABLE 22: MEASUREMENTS OF CELLS AND OTHER COMPONENTS IN THALYSIAS SCHOENUS[1]

Component	Number Measured	Nuclear Diameter	Nucleolar Diameter	Cell Size	Other Features
Larval size	Few				214.0x445.0 to 659.0x748.0[2]
Sperm masses	Numerous				100.0 x 60.0
Ostial openings	10				8.0x8.0 to 18.0x18.0
Epidermal cells	10	4.0-4.4-5.0	0.8[3]	30.0x35.0 to 45.0x25.0	
Flagellated chambers	10				15.0x11.0 to 24.0x25.0
Choanocytes	10	1.5-1.9-2.1	absent	5.0	
Nucleolate cells	10	3.5-3.9-4.5	0.5-1.4	14.0 x 8.0	
Cell type G-R	10	1.9-2.0-2.1	absent	14.0 x 2.5	Cytoplasmic granules: 1.0; rod-like inclusion: 8.0x2.0 to 16.0x1.5
Cell type S	10	2.0-2.2-3.0	absent	16.0 x 11.0	Cytoplasmic inclusions: 1.1-1.8
Toxoblasts	10	3.5x2.0 to 4.0x2.0	absent	20.0x5.0 to 35.0x3.0	
Collencytes	Few	approx. 3.0-4.5	absent		
Cheloblasts	Few	approx. 2.0-2.5	absent		

[1] Measurements in microns; means underlined. Measurements of cells, nuclei, etc. in the outgrowth region of explants; Bouin fixed material.

[2] Measurements on living, freed larvae.

[3] Nucleoli absent in adult sections.



Text-fig. 5. Special cell types and coiled material in *Thalysias schoenus*. A. Toxoblast containing a toxa. B. Coiled material. *n* = nucleus. C. Cell type S containing basophilic (black) and Mallory-positive (white) inclusions. *n* = nucleus. D. cell type G-R containing basophilic (black) granules and a single rod-shaped Mallory-positive inclusion.

lory solution II. These cells occur quite commonly in groups at the base of tufts of styles in the differentiated areas.

These cells are usually elongate but some are spherical in shape; they contain anucleolate nuclei. No mitotic figures have been found in them.

Cell type S

These cells are present in the mesenchyme and show movement without the formation of pseudopodia (Pl. 14, fig. 2). They contain spherical, cytoplasmic inclusions some of which stain light blue in Mallory solution II and others of which stain basophilically (Text-fig. 5, C). Some cells contain a predominance of Mallory-positive inclusions; others contain approximately equal numbers of both Mallory-positive and basophilic inclusions. These cells are spherical and contain anucleolate nuclei which give the appearance of being crenated. No mitotic activity has been found in these cells.

Toxoblasts

Toxoblasts are elongate in *T. schoenus* (Text-fig. 5, A). The cytoplasm is relatively homogeneous but does contain a few small inclusions and granules. Nuclei are elongate and anucleolate. Those toxoblasts which contain a toxa within them are usually longer and thinner; they have not been observed to move actively in the mesenchyme or to undergo division. De Laubenfels (1936, p. 101) has described the presence of "slipper-shaped, enucleate cells about 4μ by 12μ" in *schoenus*. He refers to these cells as blue-green algae. Toxoblasts have the same shape as these slipper-shaped cells and from my examination of de Laubenfels specimen (see p. 56) it seems evident that the blue-green algae described by him are toxoblasts.

3. Spiculogenesis in the outgrowth region

Megasclere secretion

Nucleolate cells secrete styles; the latter when they first appear, are immature spicules contained within nucleolate cells—one style per cell. Many early stages

of megasclere secretion have been found in this species; these are (1) nucleolate cells containing a basophilic, axial thread which measures 18.0μ — 100.0μ × 1.0μ — 0.1μ; this thread tapers at one end, coming to a point; (2) nucleolate cells containing an axial thread which has become partially silicified at one end, the silica being laid down on the surface of the thread; (3) nucleolate cells containing axial threads which are silicified along the whole length except for a short region at one end of the thread; and, finally, (4) nucleolate cells containing fully formed immature styles which have only remnants of the axial thread at their tips.

Chela secretion

Chelas are secreted in the upper epidermis of the outgrowth region by cheloblasts which contain anucleolate nuclei and have a finely granular cytoplasm. Both size categories of chelas are secreted in this manner.

Toxa secretion

Toxas are secreted by toxoblasts (see above) in the mesenchyme and show a grouping in long tacts in the differentiated areas of the outgrowth region.

Coiled material

This material is similar to that in *T. juniperina* (Pl. 14, fig. 3) except that it has a small anucleolate nucleus associated with it (Text-fig. 5, B). On some occasions this material occurs in areas which contain toxas; on other occasions it is not associated with these microscleres.

4. Cytochemistry of the cells in the outgrowth region (see Table 23)

All of the cells in the outgrowth region contain nuclear DNA (Feulgen staining). In addition, some of the cytoplasmic inclusions in nucleolate cells contain Feulgen-positive material (= DNA). RNA is present in the cytoplasm of collencytes, nucleolate cells, epidermal cells, and choanocytes and in the nucleolus in nucleolate cells and epidermal cells. No detectable RNA is present in the other cell types.

Toxoblasts and cell type S contain acid mucopolysaccharide as determined by gamma metachromasia in toluidin blue and positive staining in Hale's dialysed iron and alcian blue (Pearse, 1960). This acid mucopolysaccharide occurs in the cytoplasm of toxoblasts and as a network involving both the inclusions and cytoplasm of cell type S. These cells also contain carbohydrate which is not glycogen (= PAS positive). It occurs in the cytoplasm of toxoblasts and as a network involving inclusions and cytoplasm in cell type S. The cytoplasmic granules of cell type G-R and the coiled material also contain non-glycogen carbohydrate.

In the mesenchyme, cell type S appears to secrete acid mucopolysaccharide. This occurs as a homogenous substance around the whole periphery of the cell, spreading out a short distance from the edge of the cell (i.e. approximately 1.0μ).

TABLE 23: CYTOCHEMICAL STAINING RESULTS ON THE CELLS IN THE OUTGROWTH REGION OF *THALYSIAS SCHOENUS*

	Feulgen staining	Beta metachromatic staining in azure b bromide	Beta metachromatic staining in azure b bromide following ribonuclease	PAS	PAS following ptyalin	Hale's dialysed iron	Alcian blue	Toluidin blue gamma metachromasia
Interpretation of staining results	- - - - - - - - - - - - - - S e e	T a b l e 4 - - - - - -						
Toxoblasts	N	-	-	+	+	+	+	+[1]
Cell type G-R	N	-	-	+[2]	+[2]	+	-	-
Cell type S	N	-	-	+	+	+	+	+[1]
Collencytes	N	C	-			-	-	-
Nucleolate cells	N&Ci	NL&C	-			-	-	-
Epidermal cells	N	NL&C	-			-	-	-
Choanocytes	N	C	-			-	-	-
Coiled material	N	-	-	+	+	-	+	+[1,3]

N = nuclear; NL = nucleolar; Ci = cytoplasmic inclusions; C = cytoplasmic.

[1] Gamma metachromasia displayed in water mounts directly following staining and in permanent preparations which were dehydrated and mounted.

[2] Rod-like inclusion unstained.

[3] Extremely weak gamma metachromasia.

GENUS *AXOCIELITA* de Laubenfels, 1936, p. 118

Axocielita hartmani Simpson, 1966
(Pl. 15, Text-figs. 6, 7)

A. Skeletal Morphology (Measurements given in Table 24)

Axocielita hartmani is a red, incrusting sponge which externally looks very much like *M. pennata* (Pl. 15, fig. 2). This species lacks, as far as is known, any observable pattern of radiating canals which are present in some specimens of *M. pennata*. Basally, there is a thin layer of spongin from which upright columns of spongin and thick (= coring) styles arise. These columns contain small amounts

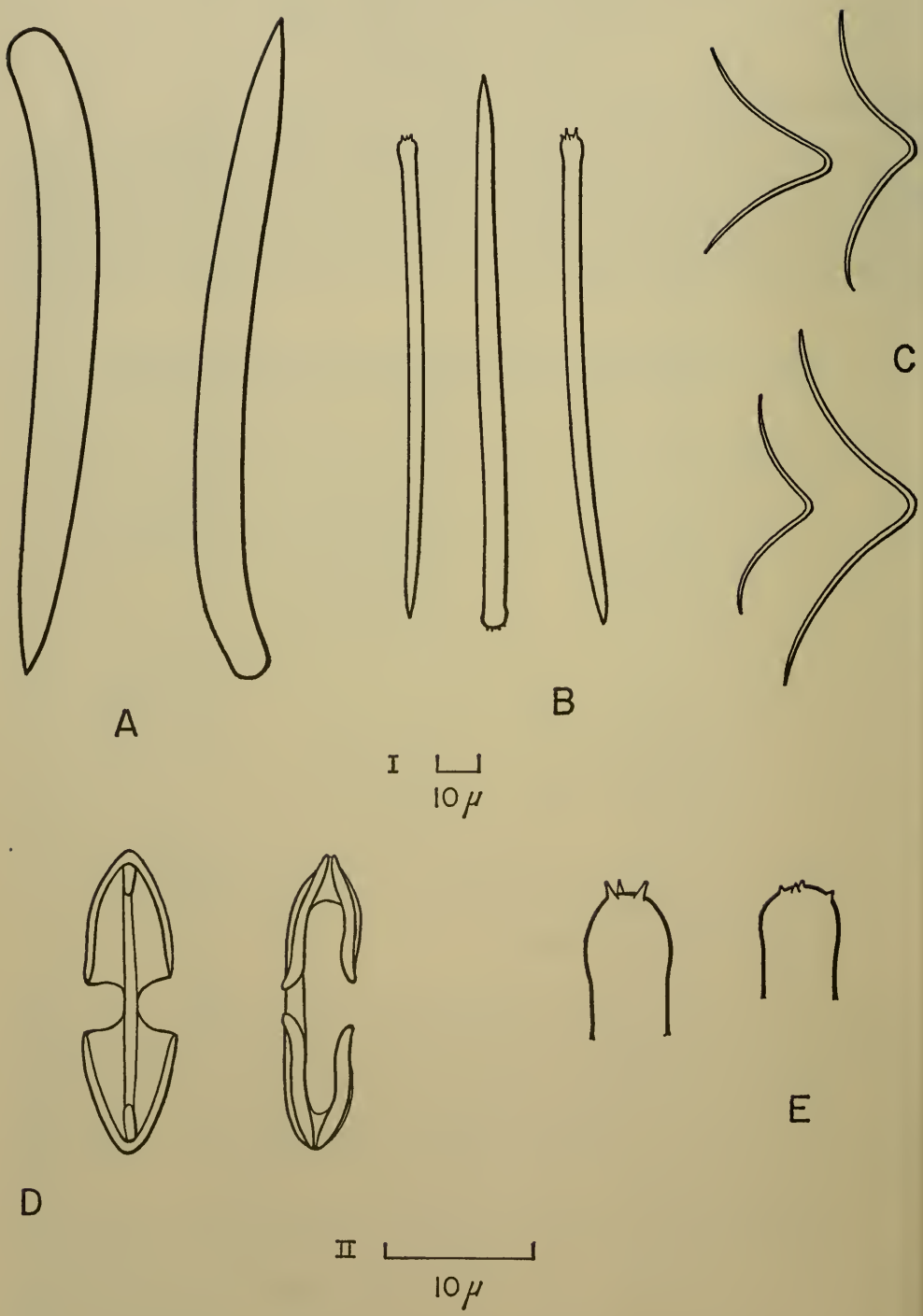
TABLE 24: MEASUREMENTS OF SPICULES AND SPONGIN IN AXOCIELITA HARTMANI[1]

Spicules		
Thick (= coring) styles	156.0-177.8-228.8	x 11.9-15.7-19.0
Thin styles	130.0-154.9-193.4	x 4.8- 5.9-10.2
Palmate isochelas	19.0- 21.7- 23.8	
Toxas	27.3- 69.3-121.4	
Spongin		
Width of columns	50.0 to 70.0	
Width of cross bridges	20.0 to 50.0	
Meshes	irregular	

[1]Means (underlined) and extremes of twenty-five spicules in each category; measurements in microns.

of spongin in which the thick styles are only partially embedded; a few thin styles also occur in the columns. At the surface of the sponge, the upright columns end with a plush of thick styles protruding as in *M. pennata* and *P. illgi*. Short cross bridges the length of one spicule and consisting of either a single thick style or of a bundle of thick styles connect the upright columns. In the mesenchyme thin styles are present as are toxas and palmate isochelas; in a few areas tracts of toxas are present.

At the surface, thin styles with microspined, subtylote heads are present and lie parallel to the surface or stand upright; they are not, however, present in dermal tufts as in *Thalysias*. At the surface where spongin fibers end and where plushes of thick coring styles are present, erect thin styles are usually present also. Palmate isochelas are exceedingly numerous at the surface and a few toxas are also found here. Spicule types are pictured in Text-figure 6.



Text-fig. 6. Spicule types present in *Axocielita hartmani*. A. Thick (= coring) styles. B. Thin styles. C. Toxas. D. Palmate isochelas. E. Microspination present on the heads of thin styles. Scale I: A, B, C. Scale II: D, E.

B. Adult Histology

Nucleolate cells, flagellated chambers, subdermal spaces, exhalant canals, and a dermis containing strands of fibrous material, epidermal cells, nucleolate cells and special cell types are present. Fiber cell tracts (see p. 19) are upright but they lack a distinct tuft of thin styles which is characteristic of dermal columns in *Thalysias*. The special cell types described below are randomly distributed in the mesenchyme.

C. Special Cell Types and Cytochemistry (see Text-fig. 7 and Table 25)

Two of the special cell types present in this sponge may be compared to those in *Thalysias*. Type S [containing spherical (S) inclusions] is very numerous in the dermis and throughout the mesenchyme. The cytoplasm and cytoplasmic in-

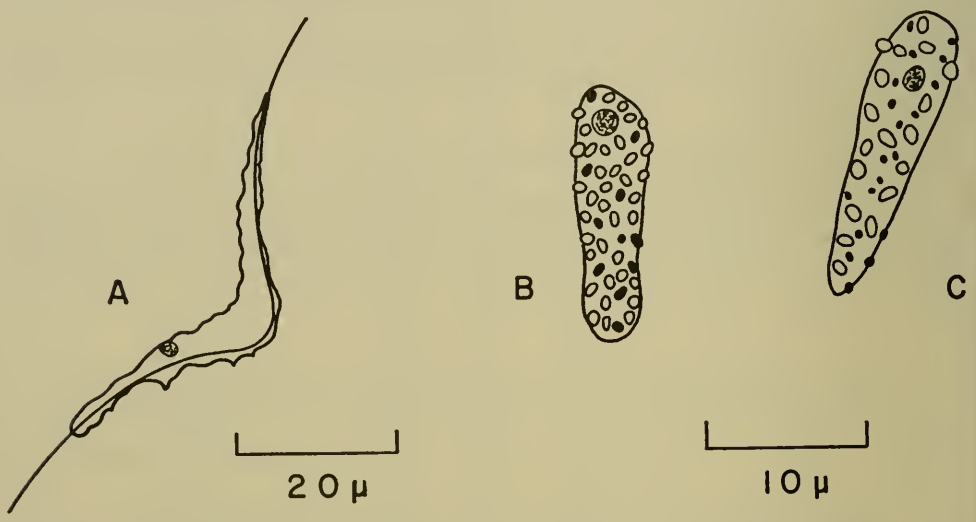
TABLE 25: CHARACTERISTICS OF CELLS IN ADULT SECTIONS OF AXOCIELITA HARTMANI[1]

	Nuclei	Cytoplasm
Toxoblasts	anucleolate; approximately 2.5-3.0 μ in diameter	granular; acid mucopolysaccharide present; cytoplasm weakly PAS positive; each cell secretes one toxa
Cell type S	anucleolate; approximately 2.5-3.0 μ in diameter	inclusions approximately 1.0 μ; some inclusions strongly PAS positive others weakly PAS positive
Cell type G-S	anucleolate; approximately 2.5-3.0 μ in diameter	small granules (0.5 μ) contain acid mucopolysaccharide and are PAS positive; larger inclusions (1.5 μ) are PAS positive
Nucleolate cells	nucleolate; approximately 3.5-4.0 μ in diameter	some inclusions are present in vacuoles
Epidermal cells	anucleolate; approximately 3.0-3.5 μ in diameter	no distinctive organelles
Choanocytes	anucleolate; approximately 2.0 μ in diameter	no distinctive organelles in addition to flagella

[1]Cytochemical staining results restricted to PAS, Hale's dialysed iron, Alcian blue and toluidin blue.

clusions contain non-glycogen carbohydrate (= PAS positive) but acid mucopolysaccharide is absent. Toxoblasts are similar to those in *Thalysias* in that they contain a homogeneous granular cytoplasm which stains positively for acid mucopolysaccharide and gives a weak PAS positive, diastaste fast reaction. An oc-

casional cytoplasmic vacuole is present in these cells as well as the toxa. Toxoblasts in this sponge differ from those in *Thalysias* in that the cell membrane is irregular (ragged) rather than smooth.



Text-fig. 7. Special cell types present in *Axocielita hartmani*. A. Toxoblast containing a toxa. Note the irregularity of the cell membrane. B. Cell type S containing basophilic (black) and Mallory-positive (white) inclusions. C. Cell type G-S containing basophilic (black) granules and Mallory-positive (white) inclusions.

A final special cell type designated as type G-S [containing granules (G) and spherical (S) inclusions] is not present in *Thalysias* although this cell type is similar to globoferous cell type I in *Thalysias* (see p. 53). It contains small basophilic granules and larger Mallory-positive (i.e. blue staining) spherical inclusions. The small granules stain positively for acid mucopolysaccharide and both granules and inclusions contain non-glycogen carbohydrate. RNA distribution has not been recorded, due to the lack of appropriately fixed material.

Genus *CLATHRIA* Schmidt, 1862, p. 57

Clathria sp.
(Pl. 15, Text-fig. 8)

Note on Species Determination

According to Lévi (1960a, p. 61-62), *Clathria coralloides* possesses acanthostyles which have very reduced spination, toxas which have smooth ends, spongin fibers which are yellow, and a growth form which consists of cylindrical upright branches. On the other hand, *Clathria compressa* (also according to Lévi, 1960a, p. 62) possesses acanthostyles with recurved spines which are more numerous on the pointed half of the spicule, toxas which have spines at their ends, a growth form which results in lamellate branches, and small (6.0-9.0μ) chelas. The specimen utilized in this study has characteristics of both *C. coralloides* and *compressa*. I have, therefore, decided for the present to refer to this specimen as *Clathria* sp. rather than to make an arbitrary decision by placing it in either

C. coralloides or *C. compressa*. Both of these species were first described by Schmidt (1862) and *compressa* was later designated as the type species (Schmidt, 1864, p. 35).

A. Skeletal Morphology (Measurements given in Table 26)

This specimen has cylindrical branches much like those of branching colonies of *M. prolifera* (Pl. 15, fig. 3). The branches contain anastomosing tracts of spicules with only small amounts of spongin binding them together. These tracts consist of thick (= coring) styles and acanthostyles both of which may be completely or only partially embedded in the spongin. At the surface, these tracts end with a few thick styles protruding; just below the surface acanthostyles

TABLE 26: MEASUREMENTS OF SPICULES AND SPONGIN IN CLATHRIA SP.[1]

Spicules		
Thick (= coring) styles		202.8-345.3-566.8 x 9.0-10.7-15.5
Thin styles		182.0-303.7-447.2 x 1.4- 3.6- 6.7
Acanthostyles		140.4-153.9-168.5 x 5.2- 8.8-11.7
Toxas	Some with spines:	30.9- 39.5- 59.5
	Always spined:	44.0-106.1-226.1
Palmate isochelas		16.7- 19.0- 20.7
Spongin		
Fiber width		10.0 to 35.0
Meshes		irregular

[1]Means (underlined) and extremes of twenty-five spicules in each category; measurements in microns.

form cross bridges, approximately the length of one spicule, between the tracts rising to the surface. At the surface, numerous thin styles are present which form a confused mass of spicules. Palmate isochelas and toxas are present at the surface as well as within the mesenchyme. In addition thin styles occur scattered throughout the mesenchyme. Both thin and thick styles have microspined heads, and some toxas (always the largest) have spines near the ends of the spicule.

B. Adult Histology

The histology of adult sections of *Clathria* sp. is similar to that of the previously described sponges. Nucleolate cells, exhalant canals, flagellated chambers,

subdermal spaces, a dermis containing strands of fibrous material, epidermal cells, nucleolate cells and special cells, and fiber cell tracts like those in *M. prolifera* (see p. 19) are present. Toxoblasts, rhabdiferous-like cells, nucleolate cell type S, and microgranular cells are present throughout the mesenchyme without special localization. Toxas and toxoblasts tend to occur in groups, but toxas and toxoblasts are so numerous that it is difficult to decide whether to refer to these groups as toxa tracts or not.

C. Special Cell Types (see Text-fig. 8 and Table 27)

Note: The fixative employed for this specimen is unknown and consequently only PAS and toluidin blue staining have been recorded.

Clathria sp. contains four types of special cells. All of these except nucleolate cell type S have small anucleolate nuclei. Nucleolate cell type S [con-

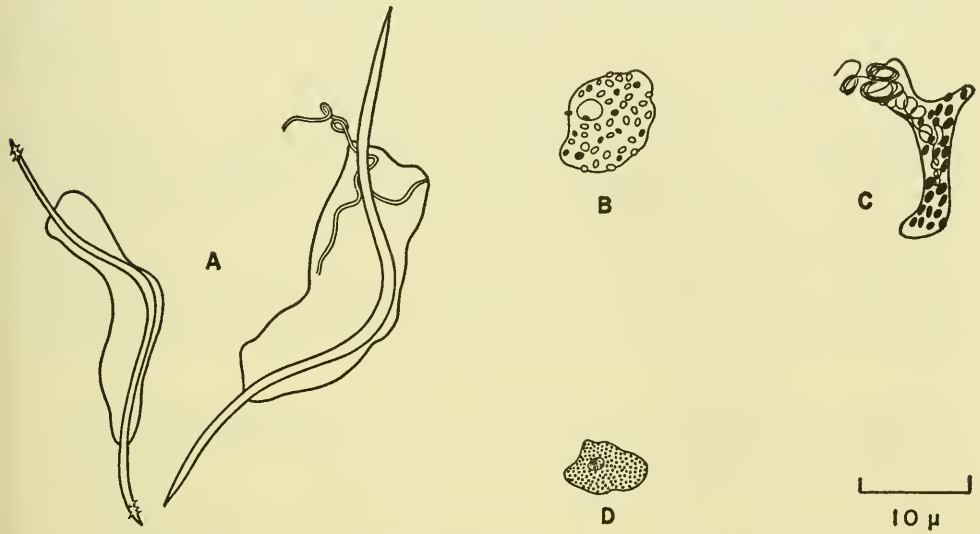
TABLE 27: CHARACTERISTICS OF CELLS IN ADULT SECTIONS OF CLATHRIA SP.[1]

	Nuclei	Cytoplasm
Toxoblasts	anucleolate; approximately 2.5-3.0 μ in diameter	some contain strongly PAS positive coiled material which displays gamma metachromasia in toluidin blue; cytoplasm is granular, weakly PAS positive and displays gamma metachromasia in toluidin blue
Microgranular cells	anucleolate; approximately 2.5-3.0 μ in diameter	numerous minute granules (0.5 μ) are PAS positive
Rhabdiferous-like cells	anucleolate; approximately 2.5-3.0 μ in diameter	coiled material and inclusions (irregular) are gamma metachromatic in toluidin blue; coiled material is strongly PAS positive
Nucleolate cell type S	nucleolate[2]; approximately 3.5-4.0 μ in diameter	some inclusions (1.5 μ) are strongly PAS positive, others are weakly PAS positive; glycogen deposit present
Nucleolate cells	nucleolate[2]; approximately 3.5-4.0 μ in diameter	some inclusions present in vacuoles
Epidermal cells	anucleolate; approximately 3.0-3.5 μ in diameter	no distinctive organelles
Choanocytes	anucleolate; approximately 2.0 μ in diameter	no distinctive organelles in addition to flagella

[1]Staining restricted to PAS, toluidin blue and hematoxylin-Mallory II (or eosin).

[2]Data on RNA staining not available.

taining spherical (S) inclusions] has approximately 20 spherical inclusions like those of cell type S in *Thalysias* but has a nucleolate (RNA data not available) nucleus. In addition, small deposits of glycogen are present throughout the cytoplasm as in glycogenous cell type S in *Raphidophlus;* the cytoplasmic inclusions contain non-glycogen carbohydrate. Microgranular cells [containing minute granules] have numerous basophilic cytoplasmic granules. These granules are strongly PAS positive and diastase-fast.



Text-fig. 8. Special cell types present in *Clathria* sp. A. Toxoblasts containing toxas. Note coiled material in toxoblast on the right and spines on the tips of one toxa. Nuclei not pictured. B. Nucleolate cell type S containing basophilic (black) and Mallory-positive (white) inclusions. Cytoplasmic glycogen deposits are present but are not pictured. Note the presence of a nucleolus (see text). C. Rhabdiferous-like cell containing coiled material and basophilic inclusions. Nucleus is not pictured. D. Microgranular cell containing numerous small basophilic granules and an anucleolate nucleus.

Toxoblasts are present but some toxoblasts containing toxas have, in their cytoplasm, coiled material which occurs around the toxa and extends out of the cell into the mesenchyme (see Text-fig. 8). This material is strongly PAS positive (= non-glycogen carbohydrate) as is the cytoplasm of the toxoblast. In addition, both toxoblast cytoplasm and coiled material stain gamma metachromatically in toluidin blue. Finally, rhabdiferous-like cells are present in *Clathria* sp. These cells contain spherical and irregular inclusions which stain gamma metachromatically in toluidin blue but are PAS negative, and coiled material which is present in a tangled mass. This coiled material is strongly PAS positive as well as gamma metachromatic in toluidin blue. Toxas have been found within this mat of coiled material in a *few* rhabdiferous-like cells; these toxas are always small and lack spines. It is not clear whether toxas are secreted by rhabdiferous-like cells or whether the association of toxas with them is accidental. The vast majority of rhabdiferous-like cells do not contain toxas. Rhabdiferous-like cells occur throughout the mesenchyme and some of them release small amounts of gamma metachromatic material. In a few cases the coiled material in these

cells is stretched out and extends into the mesenchyme. These cells differ in two respects from rhabdiferous cells in *Microciona:* first, they release only small amounts of material into the mesenchyme; secondly, the coiled material is strongly PAS positive and is thus distinct from the remaining cytoplasmic inclusions.

Genus *RHAPHIDOPHLUS* Ehlers, 1870, p. 31

Rhaphidophlus cervicornis Thiele, 1903, p. 959 (Pl. 15)

A. Skeletal Morphology (Measurements given in Table 28)

Rhaphidophlus cervicornis is an upright sponge which produces smooth, cylindrical branches (Pl. 15, fig. 4). In the center of these branches is a network of very stout, anastomosing spongin fibers which contain completely embedded within them smooth coring styles; echinating the fibers with only their heads embedded are acanthostyles. Palmate isochelas, toxas (many in tracts), and styles are present in the surrounding mesenchyme. There is no distinct class of thin styles in this species. Enclosing the central region is a specialized ectosome which contains an irregular network of much thinner spongin fibers. The thin, ectoso-

TABLE 28: MEASUREMENTS OF SPICULES AND SPONGIN IN RHAPHIDOPHLUS CERVICORNIS[1]

Spicules	
Styles[2] (= coring)	135.2-246.5-312.0 x 2.4-4.8-7.6
Acanthostyles	54.3- 64.7- 73.3 x 2.3-4.9-5.9
Toxas	33.8- 44.9- 55.9
Palmate isochelas	11.4- 12.4- 13.6
Spongin	
Width of thick fibers	160.0 to 185.0
Mesh size of thick fibers	200.0 x 500.0 to 150.0 x 270.0
Width of thin, ectosomal fibers	15.0 to 50.0

[1] Means (underlined) and extremes of twenty-five spicules in each category; measurements in microns.

[2] Thirty-five spicules measured.

mal spongin fibers contain primarily coring styles with only a few echinating acanthostyles. Thin, ectosomal spongin fibers merge with the stout fibers of the central region. At the surface of the sponge, the thin spongin fibers are perpendicular and end in a small plush of protruding coring styles. At the point

where thin spongin fibers merge with the central stout fibers there is present a great abundance of echinating acanthostyles; these spicules form a distinct plush. In the ectosome, palmate isochelas, toxas (some in tracts), and styles are present interstitially.

B. Adult Histology

Nucleolate cells, flagellated chambers, exhalant canals, narrow subdermal spaces, and a dermis with fibrous strands of material are present. The mesenchyme is extremely compact in the ectosome; globoferous cells and glycogenous cell type S are both very numerous in the ectosome, particularly in the areas around the subdermal spaces. Glycogenous cell type S is also present in spherical masses in the area where the ectosome merges with the central region.

The thin, ectosomal spongin fibers contain, in addition to a homogeneous material, long, thin fiber cells lying parallel to one another. At intervals, the thin spongin fibers give off branches which run up to the dermis.

TABLE 29: CHARACTERISTICS OF CELLS IN ADULT SECTIONS OF RHAPHIDOPHLUS CERVICORNIS[1]

	Nuclei	Cytoplasm
Toxoblasts	anucleolate; approximately 2.5-3.0 μ in diameter	granular; mildly PAS positive; gamma metachromatic in toluidin blue
Globoferous cells	anucleolate; approximately 2.5-3.0 μ in diameter	small inclusions (1.0 μ) are PAS positive and gamma metachromatic in toluidin blue; larger inclusion (3.0 μ) is strongly PAS positive
Glycogenous cell type S	anucleolate; approximately 2.5-3.0 μ in diameter	some inclusions (1.5 μ) are strongly PAS positive others are weakly PAS positive; glycogen deposits present
Nucleolate cells	nucleolate[2]; approximately 3.5-4.0 μ in diameter	some inclusions present in vacuoles
Epidermal cells	anucleolate; approximately 3.0-3.5 μ in diameter	no distinctive organelles
Choanocytes	anucleolate; approximately 2.0 μ in diameter	no distinctive organelles in addition to flagella

[1]Staining restricted to PAS, toluidin blue and hematoxylin-Mallory II (or eosin).

[2]Data on RNA not available.

C. Special Cell Types (see Table 29 and Pl. 17)

Note: The fixative employed for this sponge is unknown and consequently only PAS and toluidin blue staining results have been recorded.

Three special cell types are present in *Rhaphidophlus cervicornis.* Globoferous cells contain small cytoplasmic granules which stain gamma metachromatically and a single larger spherical inclusion. Both the small granules and the large inclusion contain non-glycogen carbohydrate (= PAS positive). Globoferous cells are, as mentioned above, most abundant in the ectosomal region. Toxoblasts are present and contain, in addition to one toxa per cell, a granular cytoplasm which stains gamma metachromatically in toluidin blue and is mildly PAS positive (= non-glycogen carbohydrate). Finally, glycogenous cell type S [containing spherical (S) inclusions] is present. These cells contain spherical inclusions which are PAS positive; some of these inclusions are strongly basophilic in iron hematoxylin. Small deposits of cytoplasmic glycogen are also present. All of the special cell types contain small, anucleolate nuclei.

FAMILY TEDANIIDAE

Genus *TEDANIA* Gray, 1867, p. 520

Tedania ignis (Duchassaing and Michelotti, 1864, p. 83) de Laubenfels, 1936, p. 89 (Pl. 16, Text-figs. 9, 10)

A. Skeletal Morphology (Measurements given in Table 30)

Tedania ignis may be incrusting or massive. Upright colonies (Pl. 16, fig. 1) consist of vase-like portions (also sometimes referred to as oscular chimneys) of the sponge which have a single opening at the end of the upright portion. Incrusting colonies are characterized by oscular chimneys which are only slightly raised.

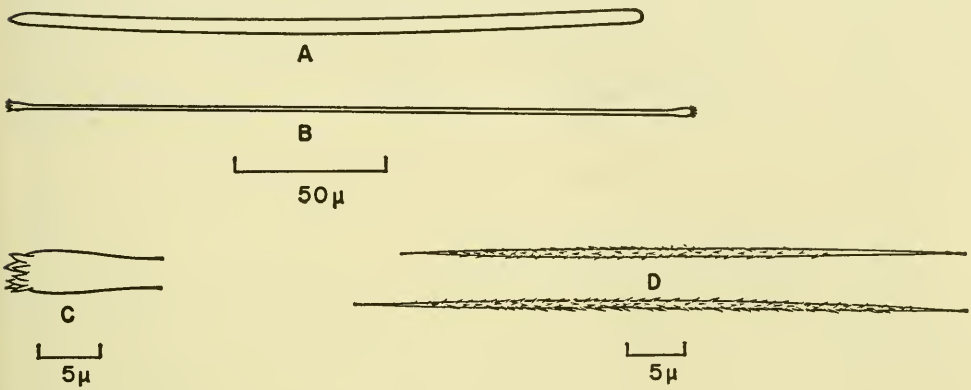
TABLE 30: MEASUREMENTS OF SPICULES AND SPONGIN IN TEDANIA IGNIS[1]

Spicules	
Tylotes	204.9-232.9-254.8 x 2.4-3.4-4.3
Styles (= coring)	197.6-231.9-265.2 x 3.6-4.5-5.2
Raphides	44.0-131.6-197.0
Spongin	
Fiber width	20.0 to 60.0
Meshes	irregular

[1] Means (underlined) and extremes of twenty-five spicules in each category; measurements in microns.

The surface of the sponge contains groups of tylotes (with microspined heads) many of which are erect, although many of these dermal spicules are

unoriented. Also present in the dermis are numerous microspined raphides which usually lie parallel to the surface. Within the sponge is an irregular reticulation of coring styles which are joined together by very small amounts of spongin; some tracts of styles lack any detectable amount of spongin. There is no distinct class of thin styles. In the mesenchyme of the sponge, many raphides occur in tracts although others are randomly distributed. A few styles may also occur randomly distributed in the dermis. The spicule types present in this sponge are pictured in Text-figure 9.



Text-fig. 9. Spicule types present in *Tedania ignis*. A. Style. B. Tylote. C. Spination present on the heads of tylotes. D. Microspined raphides.

B. Adult Histology

The mesenchyme of *T. ignis* shows no particular differences from the mesenchyme of the sponges described previously. Flagellated chambers are present as are exhalant canals and all of the cell types described in the next section. However, cell type S-R lacks the rod-shaped inclusion which is characteristic of it in the outgrowth region. In all other respects however it is identical to type S-R (see p. 77). In the adult, this cell type is mainly associated with the canal linings and the dermis. The dermis is a composite structure with fibrous material associated with it; below it are well-developed subdermal spaces partitioned off by strips of mesenchyme which run up to the dermis. A few fiber cells (see p. 19) are present in these partitions. The exhalant canal system in this sponge is particularly extensive and takes up almost half of the area occupied by the sponge tissue. Tracts of styles and lophocytes are present in some areas of the mesenchyme (see p. 74).

Sperm have been found in the mesenchyme in spherical masses. In addition, gemmule-like larvae, surrounded by an epithelium, have been found in the mesenchyme; none of these contain a flagellated epithelium, however. They consist of a spherical mass of cells many of which contain nucleolate nuclei. Numerous mitotic figures are present in these cells. Colonies were sampled from September through January and no mature larvae were found. Wilson (1894) reported the occurrence of asexually produced larvae in *T. brucei* (which appears to be a synonym for *T. ignis*). He concluded that these larvae are formed

from gemmules which in turn are produced by the aggregation of cells in the adult tissue. The gemmule-like larvae of *T. ignis* which have been found in the present study are similar to what Wilson refers to as gemmules, prior to their transformation into mature larvae. I have chosen to refer to them as gemmule-like larvae rather than as gemmules for the following reasons: (1) no evidence of their origin from cell aggregates has been found in the present study, and (2) mature sperm have been found in the tissue along with them. These observations indicate a sexual origin of the gemmule-like larvae. Although this does not remove the possibility of an asexual origin, it does bring Wilson's conclusion into question. It thus appears to me best not to employ the term gemmule for these reproductive bodies because this implies an asexual origin, which is actually the case for encapsulated, overwintering gemmules of the fresh-water sponges (see, for example, Leveaux, 1939).

C. Morphological and Cytochemical Study of the Outgrowth Region

1. Gross aspects of the outgrowth region

In the outgrowth region, spicule secretion is extremely abundant; raphides tend to be the first spicules to appear in the undifferentiated areas. Tylotes appear in groups in the upper epidermis where raphides are also most numerous. Long tracts which consist of styles, tylotes, and a few raphides are also present in the outgrowth region. These tracts contain, in addition, numerous lophocytes (see p. 76). The differentiated areas of the outgrowth region contain flagellated chambers, canals, an upper epidermis and patches of spongin around some syles. Periodic regression (see p. 20) occurs at the growing edge and particulate matter is ingested by the lower epidermis during forward movement.

2. Cell types present in the outgrowth region (Measurements of cells, nuclei, and other components are given in Table 31)

The epidermis and ostial openings

The epidermis consists of cells many of which contain nucleoli; epidermal cells in the lower epidermis contain nucleolate nuclei more often than those in the upper epidermis. Surrounding the nucleus are usually a few small inclusions. No mitotic activity has been found in epidermal cells.

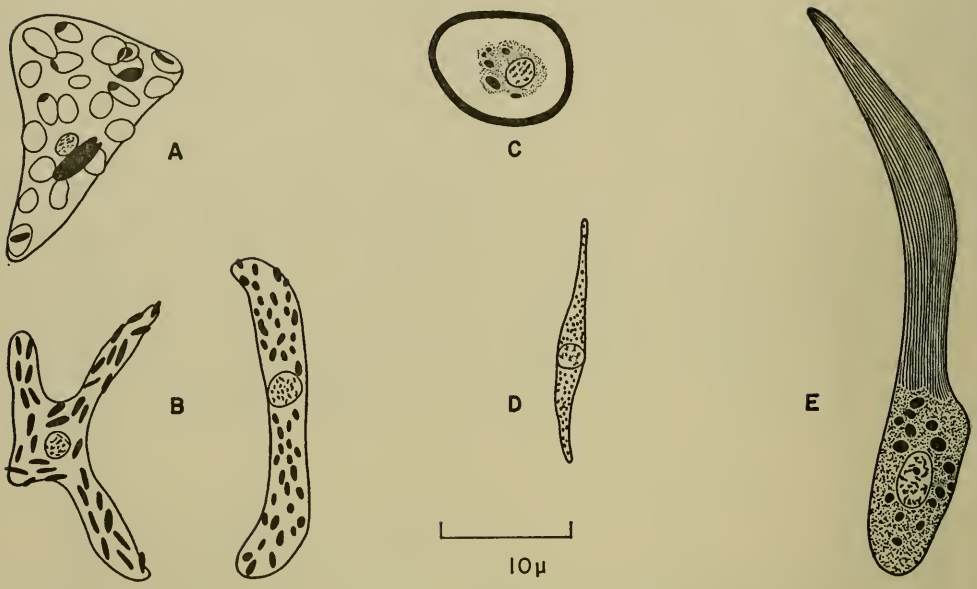
In the upper epidermis ostial openings are present. These have one, two, or three nuclei associated with them as in *M. prolifera* (see p. 22). Around the rim of the openings are thin strands of material; cell boundaries are not distinct so that it is not possible to decide whether ostial openings are formed by one or more cells.

In addition to these characteristics, there are also present in the lower epidermis epidermal cells which have a thick band of material surrounding them (Text-fig. 10, C). This material stains light blue in Mallory solution II; these cells contain spherical inclusions near the nucleus. The upper epidermis is a composite structure containing nucleolate cells, special cell types, ostia, raphides and epidermal cells.

TABLE 31: MEASUREMENTS OF CELLS AND OTHER COMPONENTS IN TEDANIA IGNIS[1]

Component	Number Measured	Nuclear Diameter	Nucleolar Diameter	Cell Size	Other Features
Size of gemmule-like larvae	Numerous				420.0 x 250.0
Sperm masses					40.0x40.0 to 70.0x60.0
Epidermal cells	10	3.7-4.0-4.5	0.8[2]	3.0 x 30.0	
Ostial openings					3.5 to 14.0
Nucleolate cells	10	3.8-4.2-5.2	0.9-1.3		45.0x3.0; 25.0x8.0
Lophocytes		see nucleolate cells	see nucleolate cells		Fibrillar extension of cell: 45.0
Choanocytes	10	2.1-2.6-3.0	absent	6.0	
Flagellated chambers	10				15.0x15.0 to 25.0x28.0
Cell type G	20	2.0-2.5-3.0	absent	25.0 x 4.0	Cytoplasmic granules: 0.5-1.7
Cell type S-R	10	2.0-2.4-3.0	absent	30.0 x 5.0	Cytoplasmic inclusions: 2.7-3.1
Microgranular cells	10	1.5-2.3-3.0	absent	17.0 x 2.0	Cytoplasmic granules: 0.5
Collencytes	Few	approx. 3.5-4.0	absent	40.0 x 2.5	
Raphidoblast	10	2.3-2.6-2.9	absent		

[1]Measurements in microns; means underlined. Measurements made in the outgrowth region of explants; Bouin fixed material. [2]Nucleoli absent in adult sections.



Text-fig. 10. Special cell types present in *Tedania ignis*. A. Cell type S-R containing Mallory-positive inclusions most of which contain a small basophilic (black) area and a single rod-shaped basophilic inclusion. B. Cell type G containing either rod-shaped (to the left) or spherical (to the right) basophilic granules. C. Special type of epidermal cell possessing a thick band of material surrounding it. D. Microgranular cell with small basophilic granules. E. Lophocyte containing a nucleolate nucleus, cytoplasmic vacuolar inclusions, and a long fibrillar extention of the cell.

Nucleolate cells

These cells are similar in all respects to nucleolate cells in the other sponges described. They are numerous in the mesenchyme and show ameboid movement by means of well-developed pseudopodia; they contain nucleolate nuclei. In the cytoplasm are a variable number of inclusions some of which are present in vacuoles. Groups of nucleolate cells secrete spongin around styles at either end or along the shaft. Numerous mitotic figures are present in nucleolate cells.

Lophocytes

These cells which occur in the mesenchyme are similar to nucleolate cells in all respects except they possess long, fibrillar extensions up to 45μ in length (Text-fig. 10, E). Some lophocytes have these fibrillar extensions at both ends of the cells. The extensions of these cells stain light blue in Mallory solution II.

Flagellated chambers and choanocytes

Flagellated chambers are present in the differentiated areas of the outgrowth region and contain the same types of openings as in *M. prolifera* (see p. 22). Choanocytes within the flagellated chambers have anucleolate nuclei. Many mitotic figures are present within the flagellated chambers.

Collencytes

These cells are similar in all respects to nucleolate cells except that they lack

nucleoli. They contain a variable number of inclusions within their cytoplasm. Their nuclei are the same size as nucleolate cells, but the cell shape is usually elongate.

Special cell types

Cell type G [Containing granules (G)]

These cells contain numerous granules in their cytoplasm which are either round or rod-shaped or both (Text-fig. 10, B) and which stain basophilically. These cells occur at the growing edge of the outgrowth region and are also present throughout the mesenchyme and in the upper epidermis; they contain anucleolate nuclei. No mitosis has been found in these cells.

Cell type S-R [Containing spherical (S) inclusions and a single rod-shaped (R) inclusion]

These cells are very numerous in the upper epidermis where they form almost a continuous sheet in many areas. They contain spherical inclusions within their cytoplasm; a small area within most of these inclusions stains basophilically in iron hematoxylin (Text-fig. 10, A). In addition to the spherical inclusions there is also present a single rod-shaped inclusion which stains deep blue in Mallory solution II. Nuclei in these cells are anucleolate and no mitotic figures have been found.

Microgranular cells

These cells are similar to the cells described under the same term in *M. spinosa* (see p. 31). They contain numerous, small, basophilic granules in their cytoplasm (Text-fig. 10, D). These cells are present mainly in the upper epidermis and contain anucleolate nuclei. No mitotic figures have been found in these cells.

3. Spiculogensis in the outgrowth region

Megasclere secretion

Immature tylotes or styles have not been found within single nucleolate cells. The initial stage of style and tylote secretion must be very brief. However, mature spicules are present with two or three nucleolate cells present along their length.

Raphide secretion

These microscleres are secreted mainly in the upper epidermis. When they appear they are present within a cell which is closely applied to the upper epidermis and which has finely granular cytoplasm and an anucleolate nucleus. In many cases two raphides lying side by side occur within the same cell.

4. Cytochemistry of the cells in the outgrowth region (see Table 32)

The cells in the outgrowth region contain nuclear DNA (Feulgen staining); some of the inclusions within the nucleolate cells also contain Feulgen-positive material. Cytoplasmic RNA is present in collencytes, nucleolate cells, lopho-

TABLE 32: CYTOCHEMICAL STAINING RESULTS ON THE CELLS IN THE OUTGROWTH REGION OF TEDANIA IGNIS

	Feulgen staining	Beta metachromatic staining in azure b bromide	Beta metachromatic staining in azure b bromide following ribonuclease	PAS	PAS following ptyalin	Hale's dialysed iron	Alcian blue	Toluidin blue gamma metachromasia
Interpretation of staining results	See Table 4							
Cell type S-R	N	-	-	+[1]	+[1]	+[1]	+[1]	+[1,2]
Cell type G	N	-	-	+	+	+	+	+[2]
Collencytes	N	C	-			-	-	-
Nucleolate cells	N&Ci	NL&C	-			-	-	-
Lophocytes	N	NL&C	-	+[3]	+[3]	-	-	-
Epidermal cells	N	NL&C	-	+[4]	+[4]	-	-	-
Choanocytes	N	C	-			-	-	-

N = nuclear; NL = nucleolar; Ci = cytoplasmic inclusions; C = cytoplasmic.

[1] Rod-shaped inclusion fails to stain.

[2] Gamma metachromasia displayed both in water mounts directly following staining and in permanent preparations which were dehydrated and mounted.

[3] Cytoplasmic inclusions and fibrillar extensions are very strongly positive.

[4] Thick band of material around some epidermal cells and cytoplasmic inclusions within these cells are strongly positive.

cytes, epidermal cells, and choanocytes; in addition, nucleolar RNA is present in nucleolate cells, epidermal cells and lophocytes. No detectable RNA is present in the other cell types.

Non-glycogen carbohydrate (= PAS positive) is present in the cytoplasm and inclusions of cell types S-R and G. In addition, the fibrillar extensions and the cytoplasmic inclusions of lophocytes are strongly PAS positive. Those epidermal cells which have a thick band of material surrounding them show the presence of carbohydrate (PAS positive, diastase fast staining) in the cytoplasmic inclusions and in the band of surrounding material.

Cell types S-R and G both contain acid mucopolysaccharide, as borne out by positive staining in Hale's dialysed iron and alcian blue, and gamma metachromasia in toluidin blue (Pearse, 1960). This occurs in the inclusions and cytoplasm of both cell types. The rod-like inclusion in type S-R is, however, negative in respect to these staining criteria. Cytochemical staining results on microgranular cells have not been recorded because their intimate contact with epidermal cells makes interpretation unreliable.

Tedania suctoria Schmidt, 1870, p. 43
(Pl. 16)

A. Skeletal Morphology (Measurements in Table 33)

Tedania suctoria grows as a thick incrustation and contains on the surface many upright papillae (Pl. 16, fig. 2). These papillae contain numerous smooth tylotes oriented at various angles to the surface. A few styles are also present in the interior of the papillae and some of these are present in loose tracts. There is only a single size category of styles present. Microspined raphides are randomly

TABLE 33: MEASUREMENTS OF SPICULES IN TEDANIA SUCTORIA[1]

Spicules	
Styles (= coring)	254.8-343.2-375.4 x 5.0-7.4-9.5
Tylotes	260.0-300.6-324.5 x 2.1-3.1-4.5
Raphides	62.4-161.2-197.6
Spongin	irregular

1 Means (underlined) and extremes of twenty-five spicules in each category; measurements in microns.

distributed in the papillae and are numerous at the surface. At the surface of the sponge smooth tylotes are located mainly in tufts but some occur unoriented. Numerous raphides, some standing erect, are also present at the surface. In the

interior of the sponge is a confused mass of styles, some of which are located in short tracts. Spongin is present in very small quantities around styles. There is a slight tendency for the endosomal styles to form a reticulate pattern. Many raphides are present in the mesenchyme in tracts.

B. Adult Histology

A characteristic feature of *T. suctoria* is the presence of a thick (30.0 to 70.0μ), well-developed dermis containing a homogeneous material (in the form of fibers) which forms an irregular network in the dermis. Also present in the dermis are numerous nucleolate cells and cell type S. Fiber cell tracts (see p. 19) containing tylotes run up to the dermis at irregular intervals. Flagellated chambers, exhalant canals, subdermal spaces, and nucleolate cells are present in the mesenchyme as in the previously described sponges.

The papillae contain the same elements as described above except that the exhalant canals are much more extensive. Lundbeck (1910, p. 1) has described two types of papillae, inhalant and exhalant, in *T. suctoria*. In the present study, no differences have been found in the histology of papillae. However, a more extensive study of this sponge, aimed at elucidating differences in the structure of papillae, is needed in order to clarify Lundbeck's observations, since a second type of papilla may have been overlooked in my study.

C. Special Cell Types (see Table 34 and Plate 17)

Two special cell types are present in *T. suctoria*. Cell type S [containing

TABLE 34: CHARACTERISTICS OF CELLS IN ADULT SECTIONS OF TEDANIA SUCTORIA[1]

	Nuclei	Cytoplasm
Cell type S	anucleolate; approximately 2.5-3.0 μ in diameter	inclusions (1.5 μ) are PAS positive and contain acid mucopolysaccharide
Cell type G	anucleolate; approximately 2.5-3.0 μ in diameter	granules (0.5 μ) are PAS positive and may (see text) contain acid mucopolysaccharide
Nucleolate cells	nucleolate[2]; approximately 3.5-4.0 μ in diameter	some inclusions are present in vacuoles
Epidermal cells	anucleolate[2]; approximately 3.0-3.5 μ in diameter	no distinctive organelles
Choanocytes	anucleolate; approximately 2.0 μ in diameter	no distinctive organelles in addition to flagella

[1]Cytochemical staining restricted to PAS, Alcian blue, Hale's dialysed iron and toluidin blue.

[2]Data on RNA not available.

spherical (S) inclusions] possesses 10 to 20 spherical inclusions which do not stain basophilically in hematoxylin but do contain acid mucopolysaccharide. In addition, these inclusions are PAS positive, diastase-fast (= non-glycogen carbohydrate). Cell type G [containing granules (G)] has numerous granules which are mildly PAS positive (diastase-fast) and which may contain acid mucopolysaccharide (staining in alcian blue is very weak). Both cell types contain small anucleolate nuclei. RNA distribution has not been recorded, due to the lack of appropriately fixed material.

GENUS *LISSODENDORYX* Topsent, 1894, p. 35

Lissodendoryx isodictyalis (Carter, 1882, p. 285)
Topsent, 1897, p. 456; type species of *Lissodendoryx*
(Pl. 16, Text-figs. 11, 12)

A. Skeletal Morphology (Measurements given in Table 35)

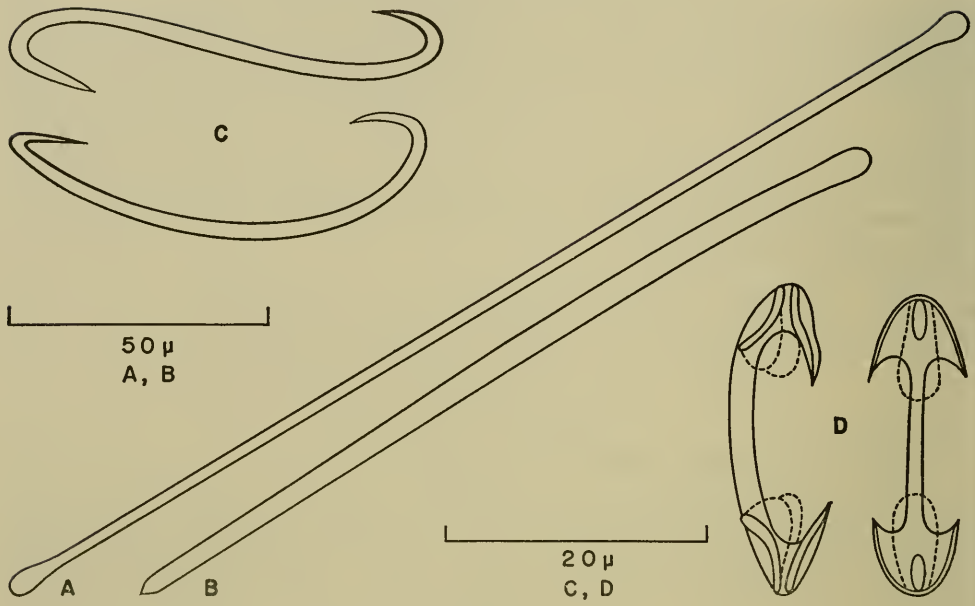
Lissodendoryx isodictyalis is a massive sponge, containing upright, vase-shaped portions (commonly referred to as oscular chimneys) which have a single opening at the top (Pl. 16, fig. 3.) In this respect it is very similar to *Tedania ignis*. At the surface are groups of smooth tylotes either lying parallel to the

TABLE 35: MEASUREMENTS OF SPICULES AND SPONGIN IN LISSODENDORYX ISODICTYALIS[1]

Spicules	
Styles (= coring)	153.9-174.7-192.4 x 2.9-4.0-4.8
Tylotes	199.7-220.5-243.4 x 2.4-2.6-3.6
Arcuate isochelas	9.5- 20.7- 28.6
Sigmas	14.3- 31.4- 39.3
Spongin	
Width	5.0 to 25.0
Meshes	100.0 to 200.0

[1] Means (underlined) and extremes of twenty-five spicules in each category; measurements in microns.

surface or lying at various angles to it. No pattern of tufts of tylotes as seen in *T. ignis* is present. Numerous arcuate isochelas and sigmas are also present at the surface. In the interior of the sponge is an isodictyal reticulation of styles with only small amounts of spongin binding the spicules together; arcuate isochelas and sigmas are also present; the former are very abundant around exhalant canals. The spicule types present in this sponge are pictured in Text-figure 11.



Text-fig. 11. Spicule types present in *Lissodendoryx isodictyalis*. A. Tylote. B. Style. C. Sigmas. D. Arcuate isochelas. Dotted structures visible only in some spicules.

B. Adult Histology

The histology of *L. isodictyalis* is similar to that of *T. ignis*. Exhalant canals are very extensive and subdermal spaces are large. Flagellated chambers, nucleolate cells and a dermis, containing anucleolate epidermal cells, strands of fibrous material, nucleolate cells and special cell types are present. In the mesenchyme can be found short tracts consisting of styles, tylotes, lophocyte-like nucleolate cells, and cell types G and S-LS. Cell types G and S-LS are also randomly distributed in the mesenchyme. Spherical masses of sperm are present in the mesenchyme and are surrounded by an epithelium as in *M. prolifera* (see p. 20).

C. Special Cell Types and Cytochemistry (see Text-fig. 12 and Tables 36 and 37)

Two types of special cells, types G and S-LS, are present in *L. isodictyalis*. Cell type G [containing granules (G)] is similar in morphology and displays the same cytochemistry as it does in *T. ignis*. Cell type S-LS [containing spherical (S) inclusions and a larger spherical (LS) inclusion] possesses spherical cytoplasmic inclusions some of which stain basophilically in hematoxylin and others of which are Mallory-positive (light blue staining). Some cells of type S-LS contain only Mallory-positive inclusions. Some of both the former and latter contain in addition a single larger spherical inclusion which is basophilic (Text-fig. 12, A). Lophocyte-like nucleolate cells are similar to lophocytes in *T. ignis* in that they occur in tracts in the mesenchyme and have a long extension of the cell which lies parallel to the tract. However, they lack strongly PAS positive fibrils within the extended portion of the cell (see Table 32). Cell types G and S-LS lack detectable RNA and contain small anucleolate nuclei. Cytoplasmic RNA is present

TABLE 36: MEASUREMENTS OF CELLS AND OTHER COMPONENTS IN LISSODENDORYX ISODICTYALIS[1]

Component	Number Measured	Nuclear Diameter	Nucleolar Diameter	Cell Size	Other Features
Nucleolate cells	10	2.9-3.7-4.5	0.3-1.0-1.5	11.0 x 6.0	
Epidermal cells	Few	same size range as nucleolate cells	absent	?	
Flagellated chambers	10				11.0x17.0 to 25.0x23.0
Choanocytes	10	1.4-1.8-2.5	absent	5.0	
Cell type G	10	1.4-2.1-2.7	absent	9.0 x 4.0	Cytoplasmic granules: 0.8
Cell type S-LS	10	1.3-2.0-2.9	absent	17.0 x 3.0	Cytoplasmic inclusions: 1.5-2.0; larger inclusion: 2.0-2.3-3.1
Sperm masses	Numerous				40.0 to 70.0

[1] Measurements in microns; means underlined. Measurements made on adult sections fixed in Bouin.

TABLE 37: CYTOCHEMICAL STAINING RESULTS ON THE CELLS IN ADULT SECTIONS OF LISSODENDORYX ISODICTYALIS

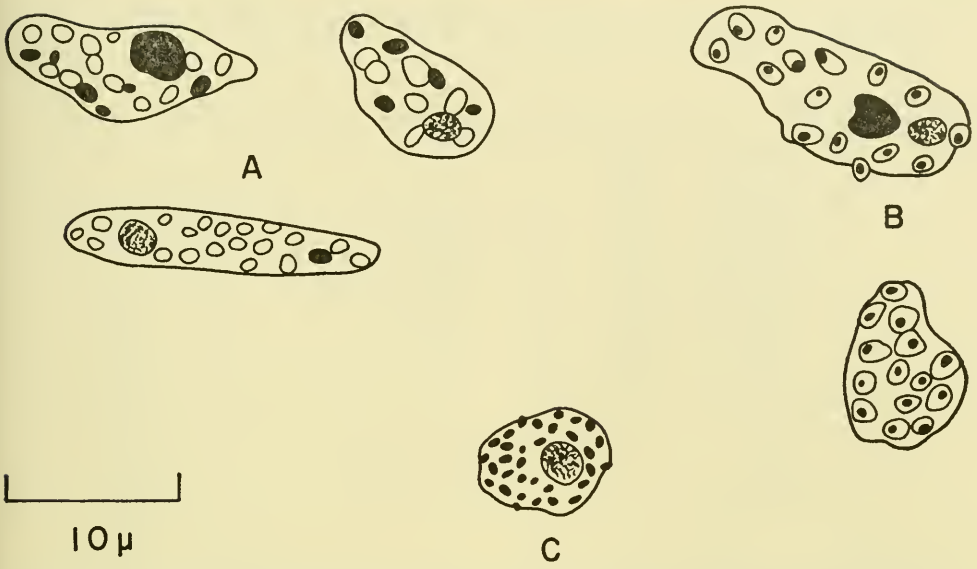
	Feulgen staining	Beta metachromatic staining in azure b bromide	Beta metachromatic staining in azure b bromide following ribonuclease	PAS	PAS following ptyalin	Hale's dialysed iron	Alcian blue	Toluidin blue gamma metachromasia
Interpretation of staining results	See Table 4							
Cell type G	N	-	-	+	+	+	+	+[1]
Cell type S-LS	N	-	-	+[2]	+[2]	+[2]	+[2]	+[1,2]
Nucleolate cells	N&Ci	NL&C	-			-	-	-
Epidermal cells	N	C	-			-	-	-
Choanocytes	N	C	-			-	-	-
Lophocyte-like nucleolate cells	N	NL&C	-	±[3]	±[3]	-	-	-

N = nuclear; NL = nucleolar; Ci = cytoplasmic inclusions; C = cytoplasmic.

1 Gamma metachromasia displayed in water mounts directly following staining and in permanent preparations which were dehydrated and mounted.

2 Larger spherical inclusion when present does not stain.

3 PAS staining is weak or absent in the long extension of these cells.



Text-fig. 12. Special cell types present in *Lissodendoryx isodictyalis* and *Lissodendoryx carolinensis*. *L. isodictyalis* contains cells pictured in A and C. *L. carolinensis* contains cells pictured in A, B, and C. A. Cell type S-LS. To the left and above is a cell containing a single large basophilic (black) inclusion, and smaller inclusions some of which are basophilic (black) and others of which are Mallory-positive (white). Nucleus is not pictured. To the right and above is a cell which lacks the larger basophilic inclusion. Below is a cell which contains only a single basophilic inclusion (black) which is the same size as the remaining inclusions. B. Cell type S-LS var. *ignis*. Above is a cell containing a large basophilic (black) inclusion and smaller Mallory-positive (white) inclusions each of which contains a basophilic granule or area. (Compare with text-figure 10, A.) Below is a cell which lacks the larger basophilic inclusion. Nucleus is not pictured. C. Cell type G containing numerous basophilic granules.

in nucleolate cells, lophocyte-like nucleolate cells, choanocytes, and epidermal cells; nucleolar RNA is present in nucleolate cells, and lophocyte-like nucleolate cells (either one or two nucleoli per cell). Collencytes have not been found in *L. isodictyalis*. Cell types G and S-LS both contain acid mucopolysaccharide within cytoplasmic inclusions and the cytoplasm. The larger spherical inclusion in type S-LS lacks positive staining for this material. Both cell types also contain non-glycogen carbohydrate. This occurs both in the inclusions and cytoplasm of type G and in the smaller inclusions and cytoplasm of type S-LS.

Lissodendoryx carolinensis, Wilson, 1912, p. 11
(Pl. 16, Text-fig. 12)

A. Skeletal Morphology (Measurements given in Table 38)

The specimens of *L. carolinensis* used in this study are incrusting with irregular protuberances on the surface (Pl. 16, fig. 4). At the surface are groups of smooth tylotes, some standing erect and others lying in confusion. Groups of tylotes occasionally are located just below the surface where they are perpendicular to it; tracts of styles, of which there is only a single size category, from the interior also occasionally reach the surface. In the interior of the sponge is an

TABLE 38: MEASUREMENTS OF SPICULES AND SPONGIN IN LISSODENDORYX CAROLINENSIS[1]

Spicules	
Styles (= coring)	144.5-159.1-218.4 x 2.8-5.2-7.1
Tylotes	163.3-174.7-191.4 x 2.4-3.8-5.0
Arcuate isochelas	13.6- 20.5- 26.7
Sigmas	21.2- 28.8- 38.1
Spongin	
Width	5.0 to 40.0
Meshes	100.0 to 150.0

[1] Means (underlined) and extremes of twenty-five spicules in each category; measurements in microns.

isodictyal reticulation of styles with only small amounts of spongin present. Arcuate isochelas and sigmas are present at the surface and throughout the mesenchyme, both being most numerous around exhalant canals and in the dermis.

B. Adult Histology

The exhalant canals and subdermal spaces in *L. carolinensis* are extensive, as they are in *L. isodictyalis,* but within the sponge the tissue is more compact and the exhalant canals are not so numerous. Flagellated chambers, nucleolate cells, and a dermis containing anucleolate epidermal cells, nucleolate cells, special cell types, and fibrous material, are present. Cell types G, S-LS, and S-LS var. *ignis* are present throughout the mesenchyme and in the dermis. At irregular intervals there are fiber cell tracts (see p. 19) which run up to the dermis at various angles and contain, in addition to fiber cells, tylotes.

C. Special Cell Types and Cytochemistry (see Text-fig. 12 and Tables 37 and 39)

The morphology and cytochemistry of the special cell types, G and S-LS, in *L. carolinensis* are like those in *L. isodictyalis* (see Text-fig. 12, A, C; Tables 36, 37, and 39). However, there is a variant of cell type S-LS present (see Text-fig. 12, B). This additional cell type, S-LS var. *ignis* is like cell type S-LS except that it contains, in addition to spherical cytoplasmic inclusions and a single larger basophilic inclusion, small basophilic areas which are contained within each spherical inclusion—one per inclusion—as is found in *Tedania ignis* in cell type S-R. The cytochemistry of cell type S-LS var. *ignis* is like that of cell type S-R in *Tedania ignis* (see Table 32).

TABLE 39: MEASUREMENTS OF CELLS AND OTHER COMPONENTS IN LISSODENDORYX CAROLINENSIS[1]

Component	Number Measured	Nuclear Diameter	Nucleolar Diameter	Cell Size	Other Features
Nucleolate cells	10	3.1-3.9-4.5	0.5-1.1-1.6	12.0 x 6.0	
Epidermal cells	Few	same size range as nucleolate cells	absent	?	
Flagellated chambers	10				10.0x20.0 to 27.0x30.0
Choanocytes	10	1.0-1.3-1.5	absent	5.0	
Cell type G	10	2.0-2.2-2.5	absent	10.0 x 5.0	Cytoplasmic granules: 0.5-1.0
Cell type S-LS	10	2.0-2.3-2.6	absent	23.0 x 4.0	Cytoplasmic inclusions: 1.5-2.0; large inclusion: 2.9-3.1-3.5
Cell type S-LS var. ignis	10	1.6-2.4-3.0	absent	13.0 x 5.0	Cytoplasmic inclusions: 1.0-1.6; granules within inclusions: 0.3; large inclusion: 1.5-2.9-3.5

[1] Measurements in microns; means underlined. Measurements made on adult sections fixed in Bouin.

Sections of *L. carolinensis* stained with toluidin blue reveal groups of thin fibrous strands of gamma metachromatic material present in the mesenchyme and dermis. This material is not present in *L. isodictyalis.* Lophocyte-like nucleolate cells are absent in *carolinensis.* RNA distribution is the same as that in *L. isodictyalis.*

DISCUSSION

The results of this study will be treated in the following discussion, first, by summarizing those features of the sponges studied which are either common to all of them or which are of taxonomic importance only if they are correlated with many other characteristics. Secondly, each genus will be discussed in terms of (1) the characteristics which delimit it, (2) the known degree of variability of these characteristics and (3) its relationship to other genera. Third, a critical evaluation of the presently employed taxonomic characters (skeletal morphology and external features) will be made in light of the results of the present study. Finally, a brief review of the taxonomic employment of non-classical characteristics among the sponges and other animal groups will be presented in order to better define the importance of this study.

COMMON FEATURES IN THE SPONGES STUDIED
(see Table 40)

Nucleolate cells (Pl. 4, fig. 3) are present in all of the species studied in this work and have been reported by other investigators to be present in all other Demospongiae which have been studied histologically. In the nine species in which RNA distribution has been recorded, nucleolate cells contain cytoplasmic and nucleolar RNA. In addition, nucleolate cells contain cytoplasmic inclusions, some of which are present in vacuoles and some of which are Feulgen-positive (= DNA). These cells show ameboid movement by means of well-developed pseudopodia, are present throughout the mesenchyme, and undergo mitosis.

The presence of Feulgen-positive inclusions within vacuoles in these cells is taken to mean that they ingest and/or digest particulate food. Borojevic and Lévi (1964) and Borojevic (1966) have shown, using the electron microscope, that these types of inclusions are present within cytoplasmic vacuoles (= phagosomes). Other workers (Kilian, 1952; Van Tright, 1919; Van Weel, 1949; Pourbaix, 1933) have found that particulate food is ingested and/or digested by nucleolate cells in a variety of Demospongiae. The precise mechanism by which food is taken up by these cells has not been ascertained in the present study. Pourbaix (1933) and Van Tright (1919) have found that the means of ingestion of particulate food varies from species to species depending upon the size of the food particles and the size of the flagellated chambers.

In the outgrowth region of explants, nucleolate cells have been found to secrete styles (Pl. 3, fig. 4) in *M. prolifera* and *M. spinosa* and in *Thalysias juniperina* and *T. schoenus*. However, in *M. prolifera* nucleolate cells containing an axial thread have not been found. Axial threads are present in the other three species and have been reported to represent the initial stage of megasclere secretion in other sponges (Schröder, 1936; Lévi, 1963; Minchin, 1910). In *T. juniperina* the scleroblast nucleus appears to be in prophase. In four of the sponges studied by explantation (*M. prolifera, M. spinosa, T. juniperina* and *T. schoenus*) the secretion of palmate isochelas occurs mainly in the upper epidermis by small, anucleolate cells, cheloblasts (Pl. 5, fig. 3). Raphides in *Tedania ignis* are secreted in a manner very similar to palmate isochelas. Toxas are

TABLE 40: COMMON FEATURES OF THE SPONGES STUDIED

Feature	Observed in:
Nucleolate cells containing cytoplasmic and nucleolar RNA, and DNA in some cytoplasmic inclusions	*M. prolifera*, *M. spinosa*, *M. seriata*, *M. atrasanguinea*, *T. juniperina*, *T. schoenus*, *T. ignis*, *L. isodictyalis*, *L. carolinensis*
Style secretion initiated by a single nucleolate cell	*M. prolifera*, *M. spinosa*, *T. juniperina*, *T. schoenus*
Axial threads in nucleolate cells	*M. spinosa*, *T. juniperina*, *T. schoenus*
Palmate isochela secretion by anucleolate cells mainly in upper epidermis	*M. prolifera*, *M. spinosa*, *T. juniperina*, *T. schoenus*
Spongin secretion by nucleolate cells	*M. prolifera*, *M. spinosa*, *T. juniperina*, *T. schoenus*, *T. ignis*
Presence of collencytes	*M. spinosa*, *T. juniperina*, *T. schoenus*, *T. ignis*
Nucleolate epidermal cells in outgrowth region containing cytoplasmic and nucleolar RNA	*M. prolifera*, *M. spinosa*, *T. juniperina*, *T. schoenus*, *T. ignis*
Adult anucleolate, epidermal cells containing cytoplasmic RNA	*M. prolifera*, *M. spinosa*, *M. seriata*, *M. atrasanguinea*, *T. juniperina*, *T. schoenus*, *T. ignis*, *L. isodictyalis*, *L. carolinensis*
Ingestion of particulate material by epidermal cells in the outgrowth region	*M. prolifera*, *M. spinosa*, *T. juniperina*, *T. schoenus*, *T. ignis*
Ostia (number of cells forming a single ostium not clear)	*M. prolifera*, *M. spinosa*, *T. juniperina*, *T. schoenus*, *T. ignis*
Dermis containing fiber cells, strands of fibrous material, nucleolate cells and special cell types	All species; *M. spinosa*, *M. seriata*, and *L. isodictyalis* apparently lack fiber cells
Choanocytes with cytoplasmic RNA	*M. prolifera*, *M. spinosa*, *M. seriata*, *M. atrasanguinea*, *T. juniperina*, *T. schoenus*, *T. ignis*, *L. isodictyalis*, *L. carolinensis*
Two kinds of openings in flagellated chambers	*M. prolifera*, *M. spinosa*, *T. juniperina*, *T. schoenus*, *T. ignis*
Sperm masses enclosed in an epithelium in mesenchyme	*M. prolifera*, *M. spinosa*, *M. seriata*, *P. illgi*, *T. schoenus*, *T. ignis*, *L. isodictyalis*
Mature larva containing flagellated epithelium and major cell types	*M. prolifera*, *M. spinosa*, *T. schoenus*
Canals lined by epithelial cells	All species
Subdermal spaces	All species

secreted in one of two ways (Pl. 5, fig. 4; Pl. 8, fig. 3; Pl. 12, fig. 3). Differences in the method of toxa secretion are discussed on page 102.

In the outgrowth region of explants nucleolate cells have been found to secrete spongin (in *M. prolifera, M. spinosa; Thalysias juniperina, T. schoenus; Tedania ignis*). This is accomplished by groups of nucleolate cells which encircle the rounded ends of megascleres (Pl. 3, fig. 5). In *Thalysias schoenus* and *Tedania ignis* this configuration is varied slightly in some cases: nucleolate cells often aggregate around the shaft and/or pointed end of the spicule. The secretion of spongin by nucleolate cells has also been reported in other marine Demospongiae by Tuzet (1932) for *Reniera elegans* and *Reniera simulans* and by Lévi (1960b) for *Microciona (= Ophlitaspongia) seriata.* In these cases however, rather than circlets of nucleolate cells, these workers have found a lining up of cells in tracts.

Collencytes are present in *M. spinosa, T. juniperina* and *T. schoenus* and *Tedania ignis.* They contain cytoplasmic RNA and inclusions and differ from nucleolate cells in that they lack nucleoli. In addition, the cytoplasmic inclusions do not contain DNA. These cells are also present in some other marine Demospongiae (Fauré-Fremiet, 1931; Paris, 1960).

Epidermal cells (Pl. 7, fig. 1; Pl. 12, fig. 1; Pl. 14, fig. 1) are present in all of the sponges investigated. Basal epidermal cells in *M. prolifera* (Pl. 4, fig. 1) do not have visibly defined cell membranes, either in the living state or in fixed and stained material. This finding is peculiar to *M. prolifera,* since in four other species examined by phase contrast microscopy (*M. spinosa, T. juniperina* and *T. schoenus,* and *Tedania ignis*) distinct membranes are present. The presence of well-defined epidermal cell membranes in the latter species implies that epidermal cells in *M. prolifera* do possess membranes but that these require special staining techniques to make them visible. Fauré-Fremiet (1931) found that epidermal cell membranes were visible only after special staining in *Ficulina ficus.* Rasmont (personal communication), Bagby (1966), and Borojevic and Lévi (1964), with the use of the electron microscope, have found distinct cell membranes in epidermal cells in a number of sponges. Wilson and Penney (1930) have claimed that the epidermis in *M. prolifera* (and in other Demospongiae) is syncytial. Cells in the lower epidermis in the outgrowth region contain nucleoli more often than those in the upper epidermis. Epidermal cells in the outgrowth region are similar to nucleolate cells in that they contain nucleolar and cytoplasmic RNA. Adult epidermal cells lack nucleolar RNA.

The lower epidermis in the outgrowth region of explants ingests particulate material. The ingestion of particulate matter by the epidermis at the edge of the outgrowth region (Pl. 14, fig. 1) is of some interest. Since the growing edge displays periodic regression, particulate matter once again builds up in the area previously occupied by the edge. This means that when forward movement of the growing edge resumes, a new supply of particulate matter is available for ingestion. This phenomenon is certainly of importance to incrusting colonies of *M. prolifera* and *M. spinosa.* However, since the two species of *Thalysias* are branching, not incrusting, this process cannot be of major importance to these sponges. It may serve as a partial means of food procurement for the basal attachment area of these sponges. Kilian (1952) and Van Weel (1949) have found that epidermal cells ingest particulate food in fresh-water sponges.

The upper epidermis contains ostia, but whether or not these are formed by a single cell cannot be stated with any assurance (Pl. 3, fig. 1). Fauré-Fremiet (1931) has described ostial openings in *Ficulina ficus* as being formed by single cells, while ostia in the calcareous sponges (cf., for example, Prenant, 1925) are formed by single, epidermal cells, porocytes. It seems likely that single cells might also form ostia in the sponges studied, but further investigation is needed to support this contention. In addition to ostia, the upper epidermis in the outgrowth region has nucleolate and special cells associated with it; in the adult, it is a composite structure containing long thin strands of fibrous material, which form layers, and usually fiber cells which are organized in tracts (Pl. 2, fig. 2), as well as nucleolate cells and other special cell types.

Choanocytes show the same characteristics in all the sponges studied; they contain cytoplasmic RNA (10 species studied) and undergo mitosis. In the five species studied by explantation, flagellated chambers contain two kinds of openings (Pl. 3, figs. 2 and 3), one produced by spaces between the choanocytes and the other by a single cell.

In the species in which they have been observed (*M. prolifera, M. spinosa, M. seriata, Plocamilla illgi, T. schoenus, Tedania ignis, L. isodictyalis)* sperm occur in spherical masses in the mesenchyme (Pl. 2, fig. 4). The origin of sperm is unknown. This method of sperm production is present in many other Demospongiae (Lévi, 1956; Leveaux, 1942; Tuzet, 1932; Brien and Govaert-Mallebrancke, 1958)) and clearly differs from the keratosan sponges in which sperm are produced by choanocytes within the flagellated chambers (Tuzet and Pavans de Ceccatty, 1958; Hartman, personal communication). Eggs in *M. prolifera* and *M. spinosa* contain nucleolate nuclei and cytoplasmic inclusions. In other Demospongiae eggs are similar to those in *M. prolifera* and *M. spinosa* (Brien and Govaert-Mallebrancke, 1958; Lévi, 1956).

In the three species (*M. prolifera, M. spinosa* and *T. schoenus*) in which mature larvae were observed they are similar (Pl. 2, fig. 1; Pl. 6, fig. 3). All contain a flagellated epithelium, a central mass of cells consisting of the major cell types present in the adult sponge, and spicules. The cell types present in the mature larvae do not add any new characteristics to those in adult sections. Larvae in other Demospongiae (Lévi, 1956; Ali, 1956; Wilson, 1894; Meewis, 1939a, 1939b, 1941) have the same general structure except that in some cases flagellated cells are absent at one pole of the larva. The lack of flagellae appears not to be a characteristic of a particular group of sponges, but rather one which varies from species to species. Lévi (1956) has shown that the type of larva, either parenchymella or amphiblastula, and the incubation or release of eggs is of significance for separating the Demospongiae into two subclasses. The larvae of the sponges studied here are of the incubated, parenchymella type, and this is what one would expect to find on the basis of Lévi's proposed subclasses (Lévi, 1956).

The adult mesenchyme of all the sponges investigated is similar. It contains flagellated chambers, exhalant canals lined by an epithelium, and subdermal spaces (Pl. 2, fig. 1; Pl. 6, fig. 3; Pl. 9, fig. 4; Pl. 11, fig. 6; Pl. 13, fig. 4). Nucleolate cells are present throughout the mesenchyme, as are spongin fibers.

In addition to the above elements, all of these sponges contain special cell types which lack detectable RNA and mitotic activity, but which contain non-

glycogen carbohydrate, small anucleolate nuclei and a distinct assemblage of cytoplasmic organelles. Lévi (1964) has recently found in an electron micrographic study of the larva of *Mycale contarenii* that two particular cell types, both of which contain characteristic cytoplasmic inclusions, lack ribosomes and well-developed nucleoi. These two cell types thus appear to fit into the category of special cell types. At least one of the cell types described by Lévi (1964) contains large amounts of acid mucopolysaccharide. Dr. Ross F. Nigrelli and Dr. Martin F. Stempien (personal communication) of the New York Aquarium, Department of Marine Biochemistry and Ecology, have recently found that the distribution of acid mucopolysaccharide may be of significance for defining orders of the class Demospongiae. The results of the present study support their preliminary finding that the order Poecilosclerida is characterized by species which contain large amounts of acid mucopolysaccharide contained within "amebocytes" (their designation). Of the sponges within orders other than the Poecilosclerida which Nigrelli and Stempien studied, none was characterized by having large quantities of acid mucopolysaccharide confined to "amebocytes." This characteristic may prove to be of great taxonomic significance for defining the order Poecilosclerida.

It is concluded in this study that the special cell types in sponges are constant elements within the sponge and are well suited for use as systematic characteristics. In *M. prolifera* the special cell types are present in all phases of the life cycle except in undifferentiated, immature larvae. They are present in this sponge throughout the mesenchyme both in incrusting and branching colonies, in mature larvae, in overwintering colonies (Simpson, 1968) and in the outgrowth region of explants. In *Thalysias juniperina,* the special cell types are present in young incrusting colonies as well as in mature branching ones. Mature larvae of *M. spinosa* and *Thalysias schoenus* contain the same special cell types that are present in adult colonies. Finally, in all of the species studied by explantation the same special cell types present in the adult are also present in the outgrowth region.

Although the presence of a category of special cell types in the sponges studied is a feature common to all of them, differences in cell structure within this category of cells are among the most distinctive characteristics which are of importance for defining taxa and deriving systematic relationships. In the following sections, differences or similarities in the special cell types in conjunction with all other characters will be employed to delineate taxa and to suggest taxonomic relationships.

COMPARISON OF THE SPECIES STUDIED AND THEIR TAXONOMIC POSITION

The Family Clathriidae
(Pl. 17)

The Genera *Microciona* and *Plocamilla*

In this study the genus *Microciona* includes sponges with the following characteristics (see Table 41): (1) spongin fibers containing coring styles, many or most of which are only partially embedded within spongin; (2) toxas present in

the tissue, some occurring in tracts; (3) coiled material present around toxas and occurring in tracts when toxas are also present in tracts; (4) gray cells containing numerous cytoplasmic granules and glycogen deposits; (5) globoferous cells containing cytoplasmic inclusions and at least one larger spherical inclusion, acid mucopolysaccharide usually present in the smaller inclusions; (6) rhabdiferous cells containing acid mucopolysaccharide within spherical, rod-shaped, coiled, and irregular inclusions and releasing this material into the mesenchyme; (7) fiber cell tracts usually present, containing thin styles and meeting the dermis at various angles; (8) the elements discussed under the heading, Common Features in the Sponges Studied, i.e., nucleolate cells, subdermal spaces, etc.

Among some of the above-listed elements there is variation. In *M. spinosa* the coiled material around toxas which are not present in tracts contains acid mucopolysaccharide; coiled material in the other species does not contain acid mucopolysaccharide. Microgranular cells are present in *M. spinosa* and *M. seriata* but not in the other species. The size of the inclusions in globoferous cells and of the granules in gray cells is larger in *M. seriata, M. pennata,* and *M. spinosa* than in *M. atrasanguinea* and *M. prolifera.* The globoferous cells in *M. prolifera* lack acid mucopolysaccharide, although it is present in these cells in the other four species. Toxa tracts and tracts of coiled material are present in *M. atrasanguinea* and *M. spinosa.* In the latter, tracts of coiled material are very well-developed and are a major element in the mesenchyme. Fiber cell tracts and a dermal skeleton are absent in *M. spinosa* and *M. seriata,* but present in the other species. *M. seriata* contains groups of globoferous cells in adult sections. Such groups are absent in the other species, although *spinosa* has globoferous cells present in tracts in the outgrowth region of explants.

Recently, Borojevic and Lévi (1964), in an electron microscopic study of *Microciona (= Ophlitaspongia) seriata,* have confirmed the presence of gray cells, globoferous cells, and rhabdiferous cells and have found endoplasmic reticulum, ribosomes and nucleoli (= RNA) lacking in these cells. They have, in addition, described microgranular cells in this sponge. The large spherical inclusion in the globoferous cells has been found by these workers to contain "bonnets alignés." This material becomes oriented following its initial appearance. Bagby (1964) has described precisely the same type of structure in globoferous cells in *M. prolifera,* although he refers to these cells as "crystalloid cells." Borojevic and Lévi (1964) have disputed the existence of globoferous cells in *M. prolifera* because they found that these cells in *M. seriata* contained only a single, large, spherical inclusion and lacked any smaller cytoplasmic inclusions. I have examined adult sections of *M. seriata* and found globoferous cells with both a single large inclusion and smaller inclusions, as well as globoferous cells with only the large inclusion (Pl. 10, figs. 1 and 2). Borojevic and Lévi (1964) examined the cells in *M. seriata* after they were dissociated, not in the adult. Thus, it appears that in *M. seriata* globoferous cells in reaggregation bodies lack the smaller cytoplasmic inclusions that are present in some of these cells in the adult. Wilson and Penney (1930) have found that, when the cells of *M. prolifera* are dissociated, the globoferous cells likewise lose their characteristic smaller inclusions.

Concerning other characteristics, variability is the rule rather than the ex-

ception (see Table 41). In incrusting colonies, separate non-anastomosing spongin fibers and acanthostyles are present in *M. atrasanguinea* and *M. prolifera.* Conules are present in *M. spinosa* but are absent in the other species. There are no spines on the heads of coring (thick) styles or thin styles in *M. spinosa* and *M. seriata;* palmate isochelas are absent in *M. seriata* and *M. pennata.* Distinct and regularly arranged oscular openings are present in *M. seriata* but not in the other species. Upright branches are produced by *M. prolifera* and *M. spinosa* (de Laubenfels, 1936, p. 113, has found ramous specimens of *M. spinosa*). Only *M. seriata* contains a prominent, and very regular, trellised pattern of spongin; the pattern in *M. pennata* is similar but only small amounts of spongin are present. These variable characteristics are summarized in Table 41. As can be seen from the Table, these characteristics overlap in complex ways so that the drawing of distinct lines which separate one or more species from the rest is not possible, except in one case. The presence of acanthostyles and non-anastomosing spongin fibers is limited to *M. atrasanguinea* and *M. prolifera.* These characters could be used to separate these two species from the remaining three. However, *M. prolifera* produces anastomosing spongin in upright branches and in some areas of incrusting colonies. In addition, *M. prolifera* differs from *M. atrasanguinea* by lacking acid mucopolysaccharide in the globoferous cells and by not possessing tracts of coiled material, whereas both of these characteristics are present in *M. atrasanguinea* and *M. spinosa.* It is thus concluded that these distinctions are useful only on a subgeneric level if they are to be employed at all. Different combinations of the variable characteristics in Table 41, therefore, are considered as elements which display species differences; the amount of overlap indicates the degree of relationship—e.g. *M. prolifera* is more closely related to *M. atrasanguinea* than it is to *M. seriata.*

These findings show that some skeletal elements (coring styles and toxas) plus the presence of certain special cell types (Pl. 17) can define the genus *Microciona.* Overlying these characteristics are variations in spicule type, spongin arrangement, fiber cell tracts and dermal skeleton, toxa tracts, etc. Since *seriata* is the type species of *Ophlitaspongia* (see Lévi, 1960a, p. 58, 64), the genus *Ophlitaspongia* (first described in 1866) must: (1) drop in synonomy to *Microciona* (described in 1864), of which the type species is *atrasanguinea,* or (2) must be redefined with a new type species, or (3) must be held in abeyance until data on other species is available. I feel that the third alternative is the appropriate one, at least for the time being. Since *pennata* also possesses the non-variable characters listed in Table 41, it belongs in the genus *Microciona.*

The relationship of the genus *Plocamilla* to *Microciona* is of interest because *Plocamilla* differs in skeletal features from *Microciona* by possessing a basal, isodictyal reticulation of acanthostrongyles; de Laubenfels (1936) has utilized this difference to place *Plocamilla* in a separate family thus implying a relatively distant taxonomic relationship to *Microciona.*

No conclusions concerning the fate of the genus *Plocamilla* can be drawn until the type species and additional species have been studied. The special cell types present in *P. illgi* are the same as those in *Microciona,* except that some gray cells contain a network of cytoplasmic material in addition to glycogen (see Pl. 17). Rhabdiferous cells in *P. illgi* not only contain acid mucopolysaccharide and

TABLE 41: CHARACTERISTICS OF THE SPECIES OF MICROCIONA AND PLOCAMILLA ILLGI

	atrasanguinea	prolifera	spinosa	seriata	pennata	Plocamilla illgi
NON-VARIABLE CHARACTERS						
Coring styles	+	+	+	+	+	+
Toxas	+	+	+	+	+	+
Coiled material around toxas	+	+	+	+	+	+
Gray cells	+	+	+	+	+	+[1]
Globoferous cells	+	+	+	+	+	+
Rhabdiferous cells	+	+	+	+	+	+
VARIABLE CHARACTERS						
AMPS[2] in coiled material	-	-	+	-	-	-
Microgranular cells	-	-	+	+[3]	-	-
AMPS in globoferous cells	+	-	+	+	+	?
Groups of globoferous cells	-	-	+[4]	+	-	+
Fiber cell tracts and dermal skeleton	+	+	-	-	+	+
Tracts of coiled material and toxa tracts	+	-	+[5]	-	-	+
Non-anastomosing spongin fibers	+	+[6]	-	-	-	+[7]
Acanthostyles	+	+	-	-	-	+
Conules	-	-	+	-	-	-
Spines on head of coring and thin styles	+	+	-	-	+	+

TABLE 41: CHARACTERISTICS OF THE SPECIES OF MICROCIONA AND PLOCAMILLA ILLGI (continued)

	atrasanguinea	prolifera	spinosa	seriata	pennata	Plocamilla illgi
Palmate isochelas	+	+	+	-	-	+
Acanthostrongyles	-	-	-	-	-	+
Basal, isodictyal reticulation of spongin	-	-	-	-	-	+
Distinct and regularly arranged oscula	-	-	-	+	-	-
Upright branches	-	+	+[8]	-	-	-
Trellised spongin	-	-	-	+	+[9]	-
Inclusions in globoferous cells and granules in gray cells larger	-	-	+	+	+	-

[1]Some gray cells contain a network of cytoplasmic material, others contain a network and small granules, a third variant contains only granules. All variants contain glycogen.

[2]AMPS = acid mucopolysaccharide.

[3]Microgranular cells have been found by Borojevic and Levi (1964).

[4]Globoferous cells present in tracts in the outgrowth region of explants.

[5]Tracts of coiled material exceedingly well developed.

[6]Spongin fibers anastomose in branches and in some areas of incrusting colonies.

[7]Separate, upright spongin fibers as in atrasanguinea arise from a basal, isodictyal reticulation of acanthostrongyles.

[8]de Laubenfels (1936, p. 113) has found branching colonies of spinosa.

[9]Only small amounts of spongin present with short cross bridges.

permeate the mesenchyme but also abut one another, forming a continuous network in the sponge tissue. Furthermore, globoferous cells in *P. illgi* are grouped as in *M. seriata* and many contain an oblong, larger inclusion rather than a spherical one. Toxa tracts and tracts of coiled material are present, as well as fiber cell tracts containing thin styles. This sponge is very closely related to *Microciona* and it is concluded that *P. illgi* and *Microciona* had a common origin. In this connection, *P. illgi* appears more closely related to *M. atrasanguinea* than to the other four species of *Microciona,* although it also shares a number of variable characteristics in common with the latter four species (see Table 41).

The Genus *Thalysias*

One of many reasons (see also Introduction, p. 13) for undertaking this study was to make a thorough comparison of *M. prolifera* and *T. juniperina.* De Laubenfels (1936, p. 104) suggested that the genus *Thalysias* possibly ought to drop in synonymy to *Microciona* because the only difference between these genera was the presence of a better-developed dermal skeleton (tufts of dermal styles) in *Thalysias.* Since de Laubenfels (1936) did not have young incrusting colonies of *T. juniperina* to study, he stated that if these proved to have separate, upright fibers as do incrusting colonies of *prolifera,* then the genus *Thalysias* should drop in synonymy to *Microciona.* Hartman (1955) and then Wells *et al.* (1960) decided that the differences between *M. prolifera* and *T. juniperina* were so small that *T. juniperina* should be placed into *Microciona.*

The present work establishes: (1) that young incrusting colonies of *T. juniperina* contain anastomosing spongin fibers, not separate, upright fibers as in incrusting colonies of *prolifera;* and (2) that there is a great divergence in nonskeletal characteristics between *Thalysias* and *Microciona* (compare Tables 41 and 42; see Pl. 17). Thus, it is concluded that the genus *Thalysias* should be retained as distinct from *Microciona.*

A further problem has been raised in respect to the genus *Thalysias.* Lévi (1960a) has concluded that *Thalysias* does not merit retention first because two other genera, *Clathria* and *Rhaphidophlus,* overlap *Thalysias* and also because de Laubenfels (1936 and 1954) was undecided as to the actual delimitation of the genus. Furthermore, Lévi (1960a) states that the original description of *Thalysias* by Duchassaing and Michelotti in 1864 is so general that it cannot be used to define a genus. However, de Laubenfels (as stated in Hartman, 1955, p. 173) restudied Duchassaing and Michelotti's original specimens of *T. juniperina* and found them to be the same as specimens which he collected in the Dry Tortugas. Thus, de Laubenfels' account (de Laubenfels, 1936, p. 105) of *T. juniperina,* which he sets up as the type species of *Thalysias,* gives us a proper description of the genus. Furthermore, as will become apparent in the following pages, all three genera are distinct and should be retained.

In the present study, the species *juniperina* and *schoenus* have been shown to belong to the same genus because they both contain all of the characteristics listed in Table 42. As is evident from comparing Tables 41 and 42, the nonskeletal characteristics, in particular the special cell types, in *Thalysias* are very different from those in *Microciona* (Pl. 17). The skeletal characteristics within the genus *Thalysias* do not show as much variation as in *Microciona;* this could

TABLE 42: CHARACTERISTICS OF TWO SPECIES OF THE GENUS *Thalysias*

Skeletal	
Endosome:	(1) anastomosing spongin fibers which contain coring styles and echinating acanthostyles
	(2) some toxas present in bundles which form tracts in the tissue; others free in the tissue
	(3) palmate isochelas randomly distributed in the tissue
Ectosome:	(1) palmate isochelas randomly distributed
	(2) some dermal styles present in tufts
Non-Skeletal	
Special cell types:	(1) type G–R with anucleolate nucleus; small, basophilic granules in the cytoplasm; and a single, rod-shaped cytoplasmic inclusion
	(2) type S with anucleolate nucleus and 20 or so cytoplasmic inclusions, some of which are basophilic
	(3) toxoblasts which may or may not contain toxas and which are usually present in tracts
Dermal columns:	tracts of fiber cells which contain dermal styles; at least some of which are perpendicular to the dermis and form dermal tufts
Cell aggregates:	spherical masses of cell type G–R and S present at the base of dermal columns

be due to one of two reasons. First, only two species of *Thalysias,* not five, as in *Microciona,* have been examined. If only *M. prolifera* and *M. atrasanguinea* had been studied, the degree of known variability of skeletal characteristics would be much less. Secondly, it may be that the species of the genus *Thalysias* are more uniform in skeletal morphology. Judging from the results of the study of the five species of *Microciona,* the latter possibility seems more remote than the former.

The number of non-skeletal differences between *T. juniperina* and *T. schoenus,* as listed as Table 43, is greater than has been utilized by other workers for species separation in other sponges; the absence of globoferous cells in *T. schoenus* is the most outstanding difference. This situation is similar to the presence of microgranular cells in *M. spinosa* and *M. seriata* and their absence in *M. atrasanguinea, M. prolifera,* and *M. pennata.* The presence of acid mucopolysaccharide in cell type G-R in *T. juniperina* but in cell type S in *T. schoenus* is puzzling. In *T. schoenus* cell type S appears to secrete this material into the mesenchyme in the immediate vicinity of the cell; in *T. juniperina* cell type G-R appears not to have a secretory function. These differences plus those in spicule dimensions and shape (i.e. toxas) show that *juniperina* and *schoenus* have diverged from one another quite significantly but that the similarities in structure (Table 42) demand the placement of both species within the same genus.

Differences and Similarities between *Axocielita hartmani, Clathria* sp., and *Rhaphidophlus cervicornis* as Compared to the Genera *Thalysias* and *Microciona*

Axocielita hartmani shares a number of characteristics in common with *Thalysias* (see Table 44). Differences between them are on approximately the same level as differences between *Plocamilla illgi* and *Microciona* (Pl. 17).

TABLE 43: DIFFERENCES BETWEEN *Thalysias juniperina* AND *Thalysias schoenus*

	T. juniperina	*T. schoenus*
Skeletal Characteristics		
Spicule and spongin dimensions	compare Tables 18 and 21	
Conule-like processes	present	absent
Dermal styles	some present in tufts	all present in tufts
Non-Skeletal Characteristics		
Dermal columns	some perpendicular	all perpendicular
Globoferous cells	present	absent
Nuclear size of epidermal cells[1]	3.1–*3.9*–4.1	4.0–*4.4*–5.0
Acid mucopolysaccharide	cell type G–R	cell type S
Megascleroblast nucleus	prophase	interphase
Spongin secretion	around rounded end of megasclere	around rounded end, pointed end, or shaft of megasclere
Coiled material	not associated with a nucleus	associated with a nucleus
Larvae	not present from Nov. to Jan.	present from Nov. to Jan.

[1] In microns.

For *Clathria* sp. (see Table 44), the most obvious similarity to *Thalysias* is the presence of toxoblasts which contain gamma metachromatic material (Pl. 17). Nucleolate cell type S found in *Clathria* sp. is similar to cell type S as found in *Thalysias* but contains glycogen deposits and a small nucleolus. Since RNA data is unavailable for *Clathria* sp., it is not known whether this is a true nucleolus (i.e. containing RNA) or not. If it is, this would be the only finding thus far of RNA in a special cell type. Rhabdiferous-like cells in *Clathria* sp. are similar to rhabdiferous cells in *Microciona* because they contain spherical and irregular cytoplasmic inclusions which display gamma metachromasia in toluidin blue, and they release small amounts of this material into the mesenchyme. Coiled material around toxas is present in *Clathria* sp. but is always within toxoblasts or rhabdiferous-like cells. This finding, plus the finding in *M. prolifera* and *M. spinosa* of coiled material which appears to be derived from rhabdiferous cells and of coiled material within rhabdiferous cells, leads to the conclusion that the rhabdiferous cells in the genus *Microciona* are responsible for the production of coiled material in which toxas are secreted. This conclusion is further supported by the occurrence of rhabdiferous cells within tracts of coiled material in the outgrowth region of *M. spinosa* and the occurrence of a higher concentration of these cells in areas which contain toxa tracts in *M. atrasanguinea.*

Rhaphidophlus cervicornis differs markedly from the other sponges studied by containing a specialized and extensive ectosomal region with a secondary spongin fiber system in it. But the presence of toxoblasts relates this sponge to *Thalysias, Axocielita hartmani,* and *Clathria* sp.; the presence of glycogen in cell type S shows similarity to *Clathria* sp. (Pl. 17), and the occurrence of these cells in aggregates suggests a relationship to *Thalysias* (see Table 44).

TABLE 44: CHARACTERISTICS OF THALYSIAS, AXOCIELITA HARTMANI, CLATHRIA SP., AND RHAPHIDOPHLUS CERVICORNIS

	Thalysias	A. hartmani	Clathria sp.	R. cervicornis
Coring styles	+	+	+	+
Acanthostyles	+	-	+	+
Palmate isochelas	+	+	+	+
Toxas	+	+	+	+
Toxa tracts	+	+	-	+
Secondary spongin system in ectosome	-	-	-	+
Fiber cell tracts	produce dermal columns	present but no dermal columns produced		
Cell aggregates	+	-	-	+
Toxoblasts	+	+	+	+
Coiled material in toxoblast	-	-	+	-
Cell type S	+	+	-	with glycogen
Nucleolate cell type S	-	-	+, with glycogen	-
Globoferous cells	+ or -	-	-	+
Cell type G-R	+	-	-	-
Microgranular cells	-	-	+	-
Cell type G-S	-	+	-	-
Rhabdiferous-like cells	-	-	+	-

The Taxonomic Relationship of *Microciona, Thalysias, Plocamilla, Axocielita, Clathria,* and *Rhaphidophlus*

Clearly, the species of *Microciona* and *Plocamilla illgi* when compared to *Thalysias* prove to be quite distinct (compare Tables 41 and 42). In the latter, glycogen is absent in the special cells, toxas are secreted within toxoblasts without the intervention of coiled material, fiber cell tracts are organized into dermal columns, and cell aggregates are present at the base of dermal columns. *Axocielita hartmani* is closely related to *Thalysias* although it lacks dermal columns, cell aggregates, cell type G-R and acanthostyles. In addition it contains one special cell type which is divergent (type G-S, see Table 44). *Clathria* sp. and *Rhaphidophlus cervicornis* possess some characteristics intermediate between the above two groups. Both contain glycogen deposits in cells similar to cell type S in *Thalysias* and *Axocielita hartmani. R. cervicornis* contains cell aggregates similar to those in *Thalysias,* while *Clathria* sp. possesses rhabdiferous-like cells and toxoblasts which contain coiled material. Both lack dermal columns and *R. cervicornis* contains a highly specialized ectosome.

Rhabdiferous cells, toxoblasts, coiled material, and intracellular glycogen deposits can be conveniently utilized as a basis for deriving the relationship of these sponges. Two distinct lines are evident, as well as an intermediate one. On one line, the secretion of acid mucopolysaccharide has become the dominant function of rhabdiferous cells with the probable production of coiled material (see p. 100) in which toxas are secreted as an additional function—this is the *Microciona-Plocamilla* line. On a second line, the secretion of toxas within toxoblasts has become the only function of "rhabdiferous" cells—this is the *Thalysias-Axocielita* line. *R. cervicornis* fits into the *Thalysias* line but overlaps the *Microciona* line by the possession of intracellular glycogen and globoferous cells. *Clathria* sp. could fit into either line, but is further removed from the *Microciona* line by the difference in morphology of the glycogen-containing cells, differences in the additional special cell types, and the lack of a dominant secretory pattern in rhabdiferous-like cells.

Coiled material which is associated with toxas has been found only in those species which contain intracellular glycogen deposits. It is indicated, therefore, that there is a direct connection between these elements. However, the presence of glycogen but the absence of coiled material in *R. cervicornis* reveals that the presence of glycogen does not necessarily imply the presence of coiled material. The relationship of these species as derived above is presented in Text-figure 13.

The Taxonomic Placement of Species with the Family Clathriidae

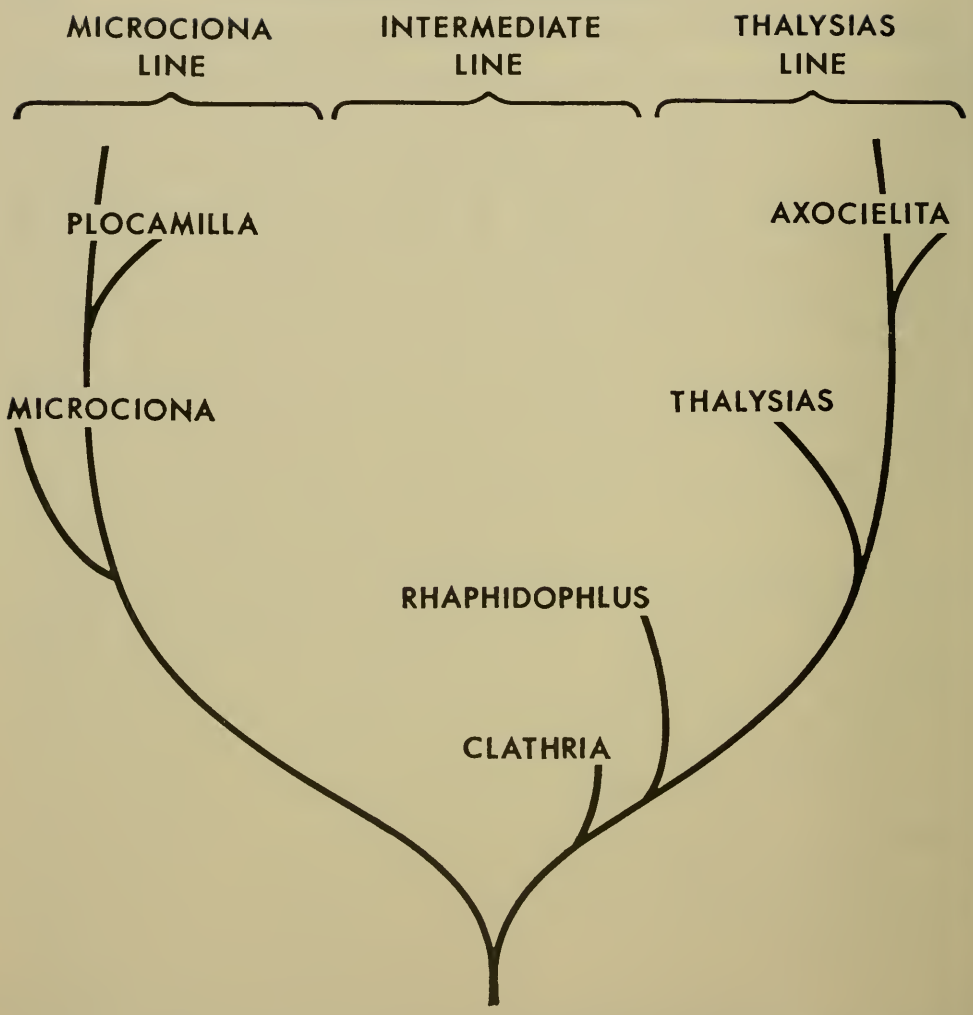
The present work supports Lévi's (1960a) recent redefining of the family Clathriidae in which he includes all of the species dealt with here. This family includes, according to him, many sponges which possess coring styles, echinating acanthostyles, chelas, toxas, and dermal styles. Acanthostyles and chelas may be absent and a special isodictyal reticulation of megascleres may be present. Concerning generic placement and the relationship of these species, the decisions of Lévi (1960a) and of other systematists, based only upon skeletal characteristics and growth form, are not upheld. Table 45 presents those characteristics which have been previously employed by systematists.

TABLE 45: SKELETAL CHARACTERISTICS OF THE SPECIES IN THE FAMILY CLATHRIIDAE

	M. atrasanguinea	*M. prolifera*	*M. spinosa*	*M. seriata*	*M. pennata*	*P. illgi*	*T. juniperina*	*T. schoenus*	*A. hartmani*	*Clathria* sp.	*R. cervicornis*
Coring styles	+	+	+[1]	+[1]	+[1]	+	+	+	+	+	+
Toxas	+	+	+	+	+	+	+	+	+	+	+
Palmate isochelas	+	+	+	-	-	+	+	+	+	+	+
Dermal styles	+	+	-	-	+	+	+	+	+	+	+
Dermal styles in tufts	-	-	-	-	-	-	+	+	-	-	+
Dermal styles not in tufts	+	+	-	-	+	+	+	-	+	+	-
Acanthostyles	+	+	-	-	-	+	+	+	-	+	+
Spongin separate fibers	+	+	-[2]	-	-	+[3]	-	-	-	-	-
anastomosing fibers	-	+[4]	+	+	+	+[5]	+	+	+	+	+
Growth form incrusting	+	+	+	+	+	+	-	-	+	-	-
branching	-	+[6]	+[7]	-	-	-	+	+	-	+	+
Basal, isodictyal reticulation	-	-	-	-	-	+	-	-	-	-	-

[1] Some of the coring styles are considered by de Laubenfels (1936, p. 112) to be echinating.
[2] Conules present.
[3] Separate fibers arise from basal reticulation.
[4] Spongin anastomoses in branches.
[5] Spongin anastomoses basally.
[6] Branches produced by some colonies.
[7] de Laubenfels (1936, p. 113) has found branching specimens.

Vosmaer (1935), after studying a whole series of sponges which are skeletally related to *M. prolifera,* came to the conclusion that all of the characteristics employed were intergrading and that all forms, including most of those species studied in this work, should be placed within a single species, *M. prolifera,* (Table 46). Within *M. prolifera* Vosmaer recognized five groups, or tropi, as he referred to them. These are listed in Table 46 with the pertinent species in each. Vosmaer's separation of *R. cervicornis* from *spinosa, seriata, atrasanguinea,* and *prolifera* has been upheld in this work, as well as his close placement of *seriata* and *spinosa.* However, cytological data demand that *atrasanguinea* and *prolifera* should be placed with *seriata* and *spinosa,* and that one of Vosmaer's two



Text-fig. 13. Diagrammatic representation of the relationship of the genera within the family Clathriidae as derived in the present study. *Clathria* and *Rhaphidophlus* are more closely related to the *Thalysias* line than to the *Microciona* line although they possess a number of characteristics common to both lines. The position of genera reflects taxonomic relationship and is not related to a time scale.

species of *Clathria* (depending upon the final disposition of the specimen of *Clathria* sp. utilized in the present study, this could be either *compressa* or *coralloides)* should be placed with *R. cervicornis.* The placement of *P. illgi* by Vosmaer (1935) is not known. However, the lumping of all of these sponges into a single species is categorically rejected. Each of the species studied here has a distinct set of characteristics; this consists of both skeletal and non-skeletal characters, as well as the size of various elements (see Table 41 and 43 for example).

Topsent (1928) places all but one of the species into the family Clathriidae, omitting *P. illgi* because of its basal reticulation of acanthostrongyles. He places the genus *Plocamilla* in the family Plocamiidae. Lévi (1960a) is the only systematist who would place all of these species into the same family (see Table 46);

TABLE 46: THE TAXONOMIC PLACEMENT OF SPECIES WITHIN THE FAMILY CLATHRIIDAE

CONCLUSIONS FROM THIS STUDY	VOSMAER (1935, p. 653)
Order: Poecilosclerida	*Microciona prolifera*
Family: Clathriidae	Tropus *senata:*
Microciona-like species:	*Microciona prolifera*
Microciona atrasanguinea	*Clathria compressa*
Microciona (= *Clathria*[1]) *prolifera*	Tropus *stylata:*
Microciona (= *Ophlitaspongia*[1]) *seriata*	*Clathria coralloides*
Microciona (= *Ophlitaspongia*[1]) *pennata*	*Ophlitaspongia seriata*
Microciona (= *Axociella*[2]) *spinosa*	*Microciona spinosa*
Plocamilla illgi	Tropus *tegens:*
Intermediate group:	*Microciona atrasanguinea*
Clathria sp.	Tropus *spinosa:*
Rhaphidophlus (= *Thalysias*[2] or *Clathria*[3]) *cervicornis*	*Rhaphidophlus cervicornis*
Thalysias-like species:	? ? ?
Thalysias (= *Microciona*[4]) *juniperina*	? ? ?
Thalysias (= *Rhaphidophlus*[1]) *schoenus*	*Plocamilla illgi*
Axocielita (= *Ophlitaspongia*[1]) *hartmani*	

LÉVI (1960a)	DE LAUBENFELS (1936, pp. 75–81; 103–122)
Family: Clathriidae	Order: Poecilosclerida
Microciona atrasanguinea	Plocamiiformes
Clathria prolifera	Family: Plocamiidae
Clathria sp.	*Plocamilla* (= *Holoplocamia*) *illgi*
Clathria or *Rhaphidophlus juniperina*[5]	Microcioniformes
Rhaphidophlus cervicornis	Family: Microcionidae
Rhaphidophlus schoenus	*Microciona atrasanguinea*
	Microciona prolifera
Ophlitaspongia seriata	*Thalysias juniperina*
Ophlitaspongia pennata	*Thalysias schoenus*[7]
Ophlitaspongia spinosa[6]	*Thalysias cervicornis*[8]
Ophlitaspongia hartmani[6]	*Clathria* sp.[9]
	Family: Ophlitaspongiidae
Plocamilla illgi	*Ophlitaspongia seriata*
	Ophlitaspongia pennata
	Axociella spinosa
	Axocielita hartmani

[1] According to Lévi (1960a).
[2] According to de Laubenfels (1936).
[3] According to Bergquist (1965).
[4] According to Hartman (1955).
[5] Lévi (1960a) would probably place *juniperina* in *Clathria* but it is also possible that he would place it in *Rhaphidophlus*.
[6] Probable generic placement by Lévi (1960a) who states on p. 60 that the establishment of a separate series of genera all of which lack chelas is ". . . ce qui me parait completement superflu . . ."
[7] de Laubenfels (1936, p. 105) would have placed *schoenus* in *Thalysias* had he known about the presence of chelas and toxas in this sponge.
[8] According to de Laubenfels (1954, p. 135).
[9] de Laubenfels (1954, pp. 140–141) revived the genus *Clathria* after having previously synonomized it.

however, he does not recognize the genus *Thalysias* (see p. 98). This leaves for Lévi (1960a) three genera, *Microciona, Clathria,* and *Rhaphidophlus,* all of which according to him contain coring styles, echinating acanthostyles, dermal styles, toxas, and palmate isochelas. Lévi (1960a) feels that the genus *Microciona* should contain only incrusting species which possess separate, upright, non-anastomosing spongin fibers. *Clathria* on the other hand should consist of species which are branching, and which contain anastomosing spongin fibers and dermal styles which are not present in tufts. *Rhaphidophlus* should include sponges like *Clathria* except the dermal styles are present in tufts. This point of view has led Lévi to place the species under consideration into genera as shown in Table 46.

It is obvious from the results of my study that his decisions are not given support. First, my results indicate that the type species, *seriata,* of the genus *Ophlitaspongia* as well as *pennata* should be placed in *Microciona.* Secondly, the species *hartmani* belongs in a separate genus, *Axocielita,* close to *Thalysias.* Thirdly, *P. illgi* belongs close to *Microciona.* The species *prolifera* belongs in the genus *Microciona,* and *juniperina* and *schoenus* belong in the genus *Thalysias,* separate from *Rhaphidophlus* and *Clathria.* Thus, on the basis of skeletal characteristics (see Table 45), the taxonomic relationship as well as the generic placement of these species by Lévi is not supported. My work establishes that *Thalysias* is a valid genus and is distinct from *Microciona, Clathria,* and *Rhaphidophlus;* the genera *Clathria* and *Rhaphidophlus* have been shown to be meaningful in terms of non-skeletal characteristics as well. According to skeletal characteristics and growth form (see Table 45) Lévi places *prolifera* in *Clathria,* and *schoenus, cervicornis,* and possibly *juniperina* in *Rhaphidophlus* (see Table 46).

De Laubenfels has pushed the utilization of skeletal characteristics to the extreme (see Table 46). He placed the genus *Plocamilla* in a new, separate suborder, the Plocamiiformes, in the family Plocamiidae which contains sponges in which ". . . there is a main skeleton of diactinal spicules more or less reticulate in arrangement. . . . To them are added auxiliary monactinal spicules. . . ." (1936, p. 75). The species *seriata, pennata, spinosa,* and *hartmani* are grouped by de Laubenfels (1936, p. 112) in the family Ophlitaspongiidae which ". . . differs from the Microcionidae, on paper at least, chiefly in that the fibers are echinated by smooth rather than spiny styles." Finally, *Microciona, Clathria* and *Thalysias* are placed by him in the family Microcionidae. A comparison of de Laubenfels' classification with the present findings shows strong divergency. Species in *Axocielita* are separated from those in *Axociella* by de Laubenfels because of their incrusting mode of growth without the production of upright branches (see Table 45). Although a generic separation of *hartmani* and *spinosa* is upheld by the present study, it is done so not on the basis of growth form but on the basis of cytological features. De Laubenfels (1954) did not recognize the genus *Rhaphidophlus* and thus placed *cervicornis* along with *juniperina* into *Thalysias.* In addition, he separated *Axociella* and *Axocielita* from *Ophlitaspongia* because of the presence of palmate isochelas, a procedure which has been shown to be misleading on the basis of cytological features. On the basis of the four species studied (*seriata, pennata, spinosa* and *hartmani*), de Laubenfels' family Ophlitaspongiidae, has been found to be without taxonomic significance.

Concluding Remarks

The genera which have been discussed up to this point have been delimited in this study primarily on the basis of histological and cytological characteristics (Pl. 17). This is most clear from the comparison of the genus *Microciona* with *Thalysias*. The morphology of the special cell types has served as the primary basis for this procedure. The distribution of acid mucopolysaccharide within the special cells, though of primary significance (i.e. in rhabdiferous cells and toxoblasts), must, in some cases, be interpreted liberally. In the two species of *Thalysias,* for instance, it occurs in different cell types as well as within toxoblasts in both. In *M. prolifera,* globoferous cells lack acid mucopolysaccharide, which is present in these cells in the remaining species of *Microciona*. Globoferous cells are a characteristic feature of the *Microciona* line, but they also occur in *Rhaphidophlus cervicornis* and *Thalysias juniperina*. This points out how misleading the employment of a single characteristic (in this case globoferous cells) can be if a great deal of weight is given to it and emphasizes the necessity of correlating characters in order to arrive at a meaningful taxonomy [see quote from Petrunkevitch (1933) at the beginning of this work].

In addition to the special cell types (Pl. 17), histological features such as the presence or absence of cell aggregates and dermal columns must be employed. Some skeletal characteristics have also been utilized (i.e. styles and toxas in *Microciona;* styles, toxas, palmate isochelas, acanthostyles for *Thalysias*). However, as has been pointed out for *Thalysias* (see p. 98), the extent of variation in spicule types within genera as defined here (i.e. on the basis of non-skeletal *and* skeletal characters) cannot be fully ascertained until additional species are studied cytologically and histologically. The important point is that the sum total of characters (both skeletal and non-skeletal) must be taken into consideration for the defining of genera. It is thus concluded that precise generic definitions based primarily on spicule types do not lead to a natural classification. This is amply demonstrated by the occurrence of the same spicule types, spicule localization, and spongin arrangement in *M. spinosa* and *Axocielita hartmani,* in which the cell types are divergent on every count; the same holds true for *Thalysias* and *M. prolifera* (compare Tables 41 and 44).

The Family Tedaniidae: *Tedania* and *Lissodendoryx* (Pl. 17)

The Genus *Tedania*

As is evident from the descriptions of *T. ignis* and *T. suctoria,* these sponges are quite different from each other; the differences are summarized in Table 47. Cell type S in *suctoria* is similar to type S-R in *ignis,* and type G is present in both sponges (see Pl. 17). *T. suctoria* has a specialized thick dermis and upright papillae, but *T. ignis* possesses lophocytes, many of which are present in tracts, and a special type of epidermal cell. These differences are clearly sufficient to place *suctoria* in a separate genus. However, the type species of *Tedania, nigrescens* (see de Laubenfels, 1936, p. 90), has not been studied cytologically, and until this has been accomplished it is premature to establish a new genus at this time. It is suggested that *suctoria* be referred to a separate group (possibly a subgenus)

TABLE 47: CHARACTERISTICS OF TEDANIA IGNIS AND TEDANIA SUCTORIA

	ignis	suctoria
Cell type S-R	+	-[1]
Cell type S	-[2]	+
Cell type G	+	+
Lophocytes	+	-
Microgranular cells	+	-
Special epidermal cell	+	?[3]
Special, thick dermis	-	+
Papillae	-	+
Fiber cell tracts	-	+
Coring styles, dermal tylotes, raphides	+	+
Spicule dimensions	See Tables 30 and 33	
Spines on tylotes	+	-

[1] Similarity to type S-R shown by type S in the possession of cytoplasmic inclusions which contain acid mucopolysaccharide.

[2] Similarity to type S as in one (1) above.

[3] Special epidermal cells can only be observed in the outgrowth region of explants. Data not available for suctoria.

within the genus *Tedania* until the non-skeletal characteristics of *nigrescens* have been elucidated.

Lophocytes have been described in a fresh-water haplosclerid sponge, *Ephydatia fluviatilis* (Ankel and Wintermann-Kilian, 1952 and 1953); in two choristidian sponges, *Pachymatisma johnstonia* (Tuzet and Pavans de Ceccatty, 1953) and *Chondrosia reinformis* (Pavans de Ceccatty, 1957); and in a hadromeridan sponge, *Tethya lyncurium* (Tuzet and Paris, 1957). It appears that this type of cell, which according to Pavans de Ceccatty (1957) secretes material which is incorporated into the ground substance of the mesenchyme, is not restricted to any particular group of sponges; therefore, its presence must be correlated with other characteristics if it is to be used for taxonomic purposes.

The Genus *Lissodendoryx*

The two species of *Lissodendoryx* studied here have been previously merged by Hartman (1958a, p. 41) and Wells *et al.* (1960, p. 212) in *L. isodictyalis* on the basis of skeletal morphology. The present study has shown that there are distinct non-skeletal differences between *L. isodictyalis* and *L. carolinensis* (see Table 48).

These differences have been utilized in this study to revive Wilson's original separation of these species (Wilson, 1912, p. 11).

Although the species *carolinensis* differs externally from *isodictyalis* in being incrusting rather than massive, this distinction appears to me, as it did to Hartman (1958a) and Wells *et al.* (1960), to be of little value, since young colonies of *isodictyalis* must also be incrusting. The most outstanding differences between these species are the presence of cell type S-LS var. *ignis* in *carolinensis* (Pl. 17)

TABLE 48: CHARACTERISTICS OF LISSODENDORYX ISODICTYALIS AND LISSODENDORYX CAROLINENSIS

	isodictyalis	carolinensis
Cell type G	+	+
Cell type S-LS	+	+
Cell type S-LS var. ignis	-	+
Fiber cell tracts	-	+
Coring styles, dermal tylotes, arcuate isochelas, sigmas	+	+
Thin, fibrous strands of material in mesenchyme	-	+
Tracts containing lophocyte-like nucleolate cells	+	-
Spicule dimensions	See Tables 35 and 38	

and its absence in *isodictyalis,* and the presence of tracts of cells containing lophocyte-like nucleolate cells in *isodictyalis* and their absence in *carolinensis.* In addition, the exhalant canal system is more extensive in *isodictyalis* and the mesenchyme less compact. Differences in spicule dimensions reveal longer, thinner styles in *isodictyalis.* Sigmas and chelas show no distinct differences, although Hartman (1958a, p. 41) has found that *carolinensis* tends to have larger sigmas and smaller chelas than *isodictyalis.* The present specimens, however, do not show this tendency (see Tables 35 and 38). Fiber cells, although present in *isodictyalis,* are not organized into fiber cell tracts as they are in *carolinensis.*

Conclusions concerning the Relationship of *Tedania* and *Lissodendoryx*

In addition to the similarity in megasclere content and localization, *T. ignis* shows similarity to both species of *Lissodendoryx* but in different elements. Cell type S-LS var. *ignis* in *carolinensis* is like type S-R in *ignis* (Pl. 17). Tracts of lophocytes in *ignis* are similar to tracts of lophocyte-like nucleolate cells in *isodictyalis.* The latter two sponges both contain extensive exhalant canals also. *T. suctoria* is a divergent species which is more closely related to *T. ignis* than to the two species of *Lissodendoryx.* Data on other sponges is needed in order to

comprehend more fully the relationship of *T. suctoria* to the other three species.

The analysis of these two genera has shown that skeletal characteristics can, in some cases, be a reliable set of elements which reflect underlying similarities. The generic placement of these species as derived here, with the exception of the divergency of *T. suctoria,* is the same as that of other systematists, and the placement of both genera in the family Tedaniidae, as de Laubenfels (1936, p. 89 and 93) has done, is upheld. The separation of *Tedania* and *Lissodendoryx* in different families (which in terms of de Laubenfels' classification are roughly equal to orders) as clearly would have been done by Ridley and Dendy (1887) is rejected. This would have been done by these workers because of the absence of chelas in *Tedania* but their presence in *Lissodendoryx.*

Although the species of *Tedania* and *Lissodendoryx* are distinct in skeletal morphology from the clathriid sponges, they show a greater affinity for the *Thalysias* line than for the *Microciona* line and the intermediate line of the Clathriidae. There is a lack of intracellular glycogen and cells with small cytoplasmic granules (type G) are present. These two genera cannot presently be placed in the family Clathriidae because this family is defined on the basis of skeletal characteristics (see p. 102) which are quite different from those in *Tedania* and *Lissodendoryx.* However, the results of the comparison of *Lissodendoryx* and *Tedania* indicate that the *Microciona* line in the family Clathriidae is a highly specialized group of sponges which has originated from within the Clathriidae, possibly from forms similar to *Clathria* sp.

AN EVALUATION OF SKELETAL MORPHOLOGY AND EXTERNAL CHARACTERISTICS AS A BASIS FOR SPONGE SYSTEMATICS

The foregoing discussion and taxonomic utilization of the results of this study now permit a more general evaluation of the employment of skeletal characteristics as a basis for sponge taxonomy.

Species Separations

First, in respect to species separation, in the case of *L. isodictyalis* and *L. carolinensis* skeletal characteristics and external features can be employed only with difficulty for elucidating species differences. Spicule dimensions and localization do not yield striking differences for this purpose; growth form is a highly variable element (cf. for example Hartman, 1958a) and, in the absence of other differences, may likewise be unconvincing as a basis for species separation. In a second case, that of *Clathria* sp., skeletal and external characteristics are insufficient for the purpose of placing specimens in previously described species. Table 49 summarizes skeletal and other data on the specimen utilized in this study and the data collected by Lévi (1960a) for two species of *Clathria, compressa* and *coralloides.* It is obvious that one cannot make a decision as to which of these two species the present specimen belongs. Hartman (personal communication) has found that situations similar to this occur not infrequently among the Porifera. However, in some cases (i.e. *Thalysias juniperina* and *schoenus*) where skeletal characteristics and external features have both diverged, they can serve as a sound basis for establishing separate species. A few workers have employed non-skeletal features (histological, larval, etc.) for species separation. Lévi (1956)

TABLE 49: A COMPARISON OF SKELETAL CHARACTERISTICS AND GROWTH FORM IN CLATHRIA SP. WITH CLATHRIA COMPRESSA AND CLATHRIA CORALLOIDES[1]

	Clathria sp.	compressa	coralloides
Growth form	Cylindrical branches	Lamellate branches	Cylindrical branches
Spongin fibers	Colorless	? (presumably colorless)	Yellow
Spines on acanthostyles	Moderately developed along whole spicule	Spines recurved; numerous at pointed end	Spination reduced
Coring styles	Divergent; most not totally embedded in spongin. Microspined heads	Divergent; microspined heads	Completely embedded; no spines
Toxas	Spined tips on some; always on largest forms	Spined tips	No spines
Spicule dimensions[2]			
Toxas	Some with spines: 30.9-39.5-59.5 Always spined: 44.0-106.1-226.1	40.0-65.0 80.0-120.0-170.0	80.0-120.0
Palmate isochelas	16.7-19.0-20.7	6.0-9.0	13.0-14.0; 16.0-20.0
Acanthostyles	140.4-153.9-168.5 x 5.2-8.8-11.7	75.0-130.0 x 7.0-9.0	80.0-120.0 x ?
Thick styles	202.8-345.3-556.8 x 9.0-10.7-15.5	130.0-450.0 x 10.0-18.0	200.0-500.0 x 12.0-15.0
Thin styles	182.0-303.7-447.2 x 1.4-3.6-6.7	110.0-250.0 x 4.0-6.0	200.0-550.0 x 2.0-5.0

[1]Data from the present study for Clathria sp. and from Levi (1960a) for Clathria compressa and coralloides.

[2]For Clathria sp., twenty-five spicules in each category measured; means (underlined) and extremes. Measurements in microns.

used differences in larval metamorphosis and spermatogenesis to separate two species of *Halisarca;* in these species, since there are no skeletal elements present, one must resort to other characteristics. Brien and Govaert-Mallebrancke (1958) have established two species of *Spongilla* based upon differences in spicule size and arrangement correlated with the presence in one of the species of a greater number of cells containing zoochlorellae and a greater abundance of a specific cell type. Tuzet (1932) has shown that two species of *Reniera* (? = *Haliclona*) differ from each other in the method by which spongin is secreted and in the size of the cells. In the latter two cases species separations based solely upon skeletal and external features would have been very difficult if not impossible.

Generic Delimitation

In respect to the delimitation of genera, skeletal characteristics may reflect underlying similarities (i.e. *Lissodendoryx* and *Tedania*) or they may be highly misleading (i.e. *M. spinosa* and *A. hartmani*). Reference to de Laubenfels' classification (de Laubenfels, 1936) as compared to the present one (see Table 46) of the family Clathriidae illustrates vividly just how misleading skeletal characteristics can be when they alone are employed rigorously. *A. hartmani* and *M. spinosa,* as previously pointed out (see p. 107) contain the same spicule types and spicule localization. However, identity of skeletal characters does not reflect a similar underlying structure of the special cell types (Pl. 17). This is also true for *Thalysias juniperina* and *M. prolifera* (see p. 102). Differences in microsclere content involving the presence or absence of palmate isochelas may (*M. seriata* and *A. hartmani*) or may not (*M. spinosa* and *M. seriata*) be of significance for generic separation. Similarly, the presence or absence of one type of megasclere, acanthostyles, may (*M. spinosa* and *Thalysias*) or may not (*M. spinosa* and *M. prolifera*) imply divergent underlying characteristics.

Equally misleading may be growth form. The production of upright branches in *M. prolifera* is of significance as a species characteristic but is totally misleading when combined with skeletal characteristics as a basis for establishing generic relationships. On the basis of growth form and skeletal morphology, *M. prolifera* is intermediate between *Thalysias* and *Microciona* (as defined by de Laubenfels, 1936, and Lévi, 1960a). This conclusion has been shown to be erroneous (see p. 98). Sarà (1959) has described two new species of *Microciona*-like sponges from the Mediterranean Sea, both of which are incrusting; he thus placed both in the genus *Microciona.* Melone (1963) has more recently found branching specimens of both of these species, and, on this basis, has placed them in the genus *Clathria.* There is no way of deciding in which of these genera the species belong without examining them cytologically.

The production of true conules in *M. spinosa* is not of significance for generic separation (see Table 41 and p. 93), and the quantity of spongin present is in the case of *M. seriata* and *M. pennata* only of value for species separation. In addition, the anastomosis of spongin fibers *(M. pennata)* as compared to separate upright fibers *(M. atrasanguinea)* is not of generic significance (see Table 41 and p. 93). The presence of distinct oscular openings arranged in a regular pattern (*M. seriata*) is of value for species separation only. In two species of *Microciona, spinosa* and *seriata,* a distinct dermal skeleton is wanting, and this characteristic is likewise not of value for generic distinction. However, the organization of fiber cell tracts into dermal columns (in *Thalysias*) which produce tufts of dermal styles, and the presence of tufts of dermal styles in *Rhaphidophlus cervicornis,* may prove, when employed as a generality, to be of value for establishing generic relationships. The study of additional species will either uphold or reject this. Judging from the variation of the dermal skeleton in *Microciona,* it would not be surprising to find the same type of variation among additional species of *Thalysias* and *Rhaphidophlus.* The degree of correspondence between skeletal and histological and cytological characters will have to be worked out in each instance until definite trends can be recognized.

Family Placement

As for family placement of genera, the addition of a basal reticulation of acanthostrongyles (*Plocamilla illgi*) to a skeletal morphology like that of *M. atrasanguinea* does not necessarily reflect a change in underlying features. The absence of echinating acanthostyles (*M. spinosa, M. seriata,* and *M. pennata*) is not a meaningful family distinction either. It is indicated that sponges containing toxas which either are secreted within toxoblasts with small anucleolate nuclei, no RNA, an oblong shape, and cytoplasmic acid mucopolysaccharide or are secreted within coiled material which is strongly PAS-positive, should be transferred to the family Clathriidae. Based on my material it can be predicted that sponges which secrete toxas in these two ways will also contain special cell types which are similar to others in at least some of the clathriid species which have already been studied.

This suggestion of placing toxa-containing sponges into the family Clathriidae requires that we be able to recognize toxas, as defined above, when they are present in a sponge. In the present study, toxas have been found to have a varied morphology; some of them are almost straight (*Thalysias schoenus*). The latter approximate raphides in their morphology. Raphides in *Tedania ignis* are secreted in a manner similar to palmate isochelas in *Microciona* and *Thalysias* and are thus a separate spicule type, distinct from toxas. It may be necessary in some cases to have data on the mode of secretion of a toxa-like spicule before any decision can be made as to whether to refer to it as a toxa. For example, in *Callyspongia repens* Little (1963) has found ". . . microxeas of which many are bent to resemble toxas so closely as to be mistaken for them." Other authors have referred to raphides and microxeas which could conceivably, in some cases, actually be toxas.

A final word concerning the employment of only skeletal characteristics is in order. Namely, in the absence of additional characteristics, the various schemes of classifying the clathriid sponges studied here (see Table 46), as well as other schemes dealing with different groups of sponges, can each be defended equally well—Lévi's redefining of the Clathriidae (1960a) could be by-passed and de Laubenfels' (1936) or Vosmaer's (1935) system accepted. The purpose of this study has been to evaluate the degree of correspondence between skeletal characteristics and histological and cytological features and then to utilize all available data in deriving a natural classification.

THE UTILIZATION OF NON-SKELETAL CHARACTERISTICS AMONG THE PORIFERA BY OTHER WORKERS

This work has employed histological and cytological features to aid in the establishment of a taxonomy of clathriid sponges. A few workers in the past have also used non-skeletal characteristics in an attempt to derive more meaningful classifications of other groups of sponges.

Bidder (1898) suggested the taxonomic use of non-skeletal characteristics among the Calcarea, and Hartman (1958b) has reevaluated their employment in light of more recent data. Hartman has reenforced Bidder's original separation of the class Calcarea into two subclasses, Calcaronea and Calcinea, on the basis of the type of larva produced, the position of the choanocyte nucleus, the type

and orientation of spicules, etc. Vacelet (1961) has found that two genera in the subclass Calcinea contain characteristics of both subclasses and that a third genus shares most of its characters in common with the "wrong" subclass. Vacelet (1961) therefore moved the family in which these genera had been placed into the Calcaronea and stated: "This mixture of characteristics, added to the common points of all these sponges, are in disagreement with Hartman's classification" (Vacelet, 1961, p. 47). Sarà (1963) has also found a case of disagreement in one character and concluded that Hartman's subclasses are invalid. Burton (1963), using a third case of disagreement, also rejects Hartman's system. This argument is like saying that since *Clathria* sp. contains both toxoblasts and coiled material, the characteristics used in the present study for deriving relationships and delimiting genera are invalid. As Hartman (1964) has pointed out, and as I have confirmed in this study, the finding of species or genera with overlapping characteristics does not imply that the utilization of these characteristics is invalid. Rather, it implies that within certain genera or groups these characteristics vary randomly, resulting in intermediate forms of one kind or another. Those who reject taxonomic schemes which contain intermediate types do so, it seems to me, in order to establish distinct, non-overlapping groups. Among the Porifera, these groups have been defined by Vosmaer (1935) and Burton (1963) primarily on the basis of skeletal characteristics without concern as to what the rest of the animal is like. Indeed, Burton (1963) rejects the employment of other types of characteristics. This decision implies that similar skeletal characteristics among sponges always reflect the existence of the same non-skeletal attributes. Thus, Burton (1963) and Vosmaer (1935) have utilized skeletal attributes to set up species categories each of which contains a great deal of variability, but each of which is also a distinct, non-overlapping entity. This has been done without evaluating the degree of correspondence between skeletal elements and other characteristics and, in the case of Burton (1963), by rejecting the employment of additional characteristics as suggested by Hartman (1958b). It seems quite clear that some (if not many) of Burton's species will turn out to be comparable to the species complex *M. prolifera* of Vosmaer (1935, see p. 103).

A second case is that of Sollas (1888) who employed non-skeletal characteristics in conjunction with skeletal elements for delimiting genera and families among the Tetractinellida. In addition to skeletal elements, he distinguished the tetractinellids as having a "concrescence of choanocytes" (Sollas, 1888, p. ci) which involves the coalescence of choanocyte collars to form a secondary lining in the flagellated chambers. In addition, the type of flagellated chambers, either eurypylous or aphodal, and the type of mesenchyme present (collenchymatous or sarcenchymatous) were employed by Sollas (1888) as family characteristics. For the delimitation of genera, the presence or absence of a cortex was used as well as other histological features and skeletal elements. The classification of the tetractinellids according to Sollas (1888) has been generally accepted (cf. for example, Topsent, 1928) although de Laubenfels (1936), relying almost exclusively upon skeletal elements, has rearranged many genera in new families. The non-skeletal characteristics (concresence, type of mesenchyme, etc.) employed by Sollas have, unfortunately, not been found to be of significance among the poecilosclerid sponges studied here. However, Sollas' work shows that non-

skeletal features are important in establishing a taxonomy of the Tetractinellida.

Sollas (1888) strongly emphasized the utilization of concrescent choanocytes in defining the Tetractinellida. He found that concrescence is absent among suberitid and tethyid sponges and thus placed them outside of the Tetractinellida. Topsent (1900) followed Sollas by placing the Suberitidae and Tethyidae in the order Hadromerida, separate from the Tetractinellida. However, de Laubenfels (1936) has removed the Tethyidae from the Hadromerida and placed them in a separate order, the Epipolasida. Recently, Paris (1960) has found that there is a very strong serological similarity between *Suberites domuncula* and *Tethya lyncurium,* and on this basis has suggested that the family Tethyidae be dropped and the members of it be placed in the Suberitidae in the order Hadromerida.

Bergmann (1949 and 1962) has isolated and identified sterols from a number of marine sponges. Among his findings is the discovery that a number of species (including *Suberites domuncula*) in the family Suberitidae all contain dextrorotary, saturated sterols; and in this respect they differ from species in other families of de Laubenfels' (1936) Hadromerida but are similar to two families in the order Halichondrida. In addition, Bergmann (1962) has found in a species of *Tethya actinia,* that saturated sterols are absent. Thus, according to the work of Bergmann, the Suberitidae are a distinct family of the order Hadromerida and are, in terms of saturated sterols, related to two families (the Halichondriidae and Hymeniacidonidae) in the order Halichondrida. According to Paris (1960), *Tethya lyncurium* and possibly other members of the Tethyidae are serologically very close to *Suberites domuncula,* but at least one species of *Tethya* has been found by Bergmann (1962) to differ from the Suberitidae by lacking saturated sterols. These findings support the decisions of Topsent (1900) to place both the Tethyidae and Suberitidae in the order Hadromerida but to maintain them as separate families.

The point of this discussion is one which has been previously made. Without an analysis of additional characteristics, there is no basis for deciding which scheme, that of de Laubenfels (1936) or that of Topsent (1900), is more likely to reflect a natural classification of the tethyid and suberitid sponges. Obviously, more work needs to be done in order to support the decision of Topsent (1900), but the work of Bergmann (1949, 1962), Paris (1960), and Sollas (1888) supports his views and leads the way for additional verification of his decisions.

THE USE OF ADDITIONAL TAXONOMIC CHARACTERS IN OTHER GROUPS OF ANIMALS

It is appropriate to end this discussion by referring to work on characteristics not previously employed for taxonomic purposes in groups of animals other than the Porifera. This will place the present work in better perspective in terms of its biological value and will also reenforce a number of broad generalizations which are inherent in the foregoing discussion.

Petrunkevitch (1933) has investigated the internal anatomy of a large number of spiders and by employing these anatomical characteristics taxonomically he has reconstructed the relationship of families. These characteristics include the structure of the respiratory system, the number of ostia present in the heart, the structure of coxal and maxillary glands, as well as those features which had been

previously employed for establishing family relationships. His study resulted in numerous changes in the grouping and thus the relationship of families (compare pp. 357-359 of Petrunkevitch, 1933, with pp. 13-14 of Petrunkevitch, 1928) as well as in elucidating the types of morphological (and indirectly, physiological) variation which has occurred among these animals. In some cases, however, family affinities remained for the most part unchanged. This work thus supports to some degree the utilization of characteristics previously employed (i.e. those utilized by Petrunkevitch, 1928) but at the same time argues against a rigorous employment of *only* these characteristics on the family level.

Mossman (1953) has studied the structure of the male genital tract of members of the Sciuridae (squirrels) and has found in two genera that there is a great deal of divergence from other members in the group in the size of Cowper's gland and the absence of a bulbar gland and penile duct. The inclusion of these genera within the subfamily Sciurinae is thus strongly questioned by Mossman (1953, p. 293). In addition, smaller variations in the male genital tract were shown to be of significance for generic distinction. Differences in the female genital system were found to be generally less pronounced and were of use primarily in separating taxa higher than genera, although in some cases the microscopic structure of the ovary was found useful in distinguishing genera. On the level of families, the microscopic structure of the ovary is similar within families: "All of the mustelids examined have a very similar and typical interstitial cell pattern . . ." (Mossman, 1953, p. 294). The study of fetal membranes by Mossman (1953) in various groups of mammals has led in some instances to similarities between groups which, on the basis of other criteria, are not considered to be close phylogenetically. This last-mentioned finding is of much interest since it establishes new ideas concerning the possible relationship of higher categories of mammals.

Numerous studies have been made of chromosome morphology and number (see M. J. D. White, 1954). In many cases, identity or similarity in these elements (White, 1954, pp. 171-197) has been found within genera or higher taxa which, on the basis of other characteristics, have been considered to be closely related. These findings thus support the use of previously employed characteristics. However, numerous cases of divergence within genera, particularly in chromosome number, have been found (White, 1954, pp. 197-203). White (1954, p. 197) points out the importance of and problems involved in such cases:

> "In considering problems of caryotype evolution particular interest naturally attaches to those instances where closely related species or subspecies possess widely different chromosome numbers. The findings of such a case should, however, always prompt the asking of the question: are these forms actually as closely related as they have been regarded in the past? In some instances the answering of this question may lead to a revision of the taxonomic status of the forms under consideration. Even so, however, there are a good many instances where morphologically very similar species possess quite different caryotypes, even involving considerable differences in chromosome number. . . . It is unfortunate that so few of these cases have been subjected to a really critical analysis."

Not all studies of this sort have been concerned with morphological characteristics; many have involved biochemical and serological aspects (for a review of recent work in this area, see Leone, 1964). Leone (1954) has investigated the serological relationship of six groups of decapod Crustacea. The results of his work were in general agreement with the accepted taxonomic relationship of these groups. In one instance, however, a strong serological similarity was found between two species, *Dromia vulgaris* and *Panulirus argus,* which are placed in different groups. Although Leone (1954) did not interpret this result, it is indicated that the relationship between these species may be much closer than previously thought. Bertini and Rathe (1962) have recently examined the electrophoretic characteristics of hemoglobin in a number of anurans and have found general agreement between their results and the previously accepted relationship of these animals. However, in one instance a large degree of divergence was found between two (*bufonina* and *tucumana*) of four species, all of which are placed in the genus *Pleurodema.* Dessauer *et al.* (1962) have investigated the transferrins in the plasma of a large number of amphibians and reptiles. In terms of the characteristics of these compounds, some groups (i.e. Salientia and Caudata) can be separated, but other groups (i.e. families of Sauria, lizards, and Serpentes, snakes) show no distinct separation on this basis. Some evidence was found which supports the idea of the origin of snakes from the Gekkonidae (lizards). Within a number of genera of lizards, species were found to be very similar. Haslewood and Ogan (1959) have examined the chemical composition of the bile salts of the West African cutting-grass and have compared them to the South American coypu. Both of these species are placed by G. G. Simpson (1945) in the same superfamily, though Wood (1955) separates them in different groups. Haslewood and Ogan (1959) have found that these two species contain quite different and distinct bile salts, and they conclude, therefore, that the separation of them in different groups by Wood (1955) is valid.

No attempt has been made to present a large quantity of work of the sort discussed above. Rather, a limited number of papers have been chosen in order to exemplify certain elements common to all of them. Numerous other investigations have been carried out on many other animal groups and could have been used in the present discussion. It is clear that in those cases in which additional characteristics were looked at and were found to conform to a previously accepted taxonomic scheme, the characters previously employed are given greater validity in terms of establishing a natural classification. In some cases these additional characteristics have themselves proven to be useful for species and subspecies distinction as well as for distinguishing higher categories (see for example, Mossman, 1953). More often, additional characteristics show some degree of divergence from previous schemes and thus (as White, 1954, has pointed out) force a reevaluation of the basis previously employed for the taxonomy of the particular group under consideration. In all of the work mentioned above, at least one case of divergence from the accepted taxonomy has been found. The meaning of the divergence may be related either to the nature of the particular additional character investigated (which could be an independently variable one) or to the basis of the accepted taxonomy. In either case, the result of this type of work is to stimulate, or renew, interest in certain aspects of the morphol-

ogy, physiology, and biochemistry of the animals involved. The results of this stimulated research may lead to the finding of new structures, processes, biochemical pathways, etc. Taking one example from the present study, the finding of a class of RNA-negative cells in a number of marine sponges raises the question of whether protein synthesis is lacking in these cells or whether it is carried out in other ways not involving RNA. Finally, this type of work adds to our knowledge of the biology of the animals in question, allowing us to arrive at a more natural taxonomy for the group and giving us added insight into the types of variation, in morphology and physiology, which a particular group of animals has undergone.

CONCLUSIONS

Cytochemical and histological investigation of fifteen species of marine poecilosclerid sponges has revealed that the following elements are common to all species: a composite dermis, subdermal spaces, flagellated chambers, exhalant canals, nucleolate cells, and special cell types. In the five species which were studied by explantation, the following additional elements were found in all: (1) two sets of openings in the flagellated chambers, one of which is formed by a single cell and the other by spaces between the choanocytes, (2) palmate isochela secretion mainly in the upper epidermis by small anucleolate cells, (3) secretion of megascleres and spongin by nucleolate cells, (4) the presence of Feulgen-positive (= DNA) inclusions within cytoplasmic vacuoles in nucleolate cells, (5) ostial openings in the upper epidermis, (6) mitotic activity in nucleolate cells and choanocytes, (7) temporary regression of the growing edge of the outgrowth region, and (8) the intake of particulate matter by the epidermis at the growing edge of the outgrowth region. In those species in which they were found, sperm and larvae occur in the mesenchyme surrounded by an epithelium. Immature larvae consist of nucleolate cells; mature larvae contain a flagellated epithelium surrounding a mass of nucleolate cells and special cell types. In the adult, RNA is present in epidermal cells, nucleolate cells, and choanocytes, but not in the special cell types. The latter contain anucleolate nuclei, non-glycogen carbohydrate, and characteristic cytoplasmic organelles, but lack mitotic activity. At least one of the special cell types contains acid mucopolysaccharide.

The morphology of the special cell types has been utilized in conjunction with various histological features and skeletal characteristics for defining genera and establishing taxonomic relationships. The results of this analysis have shown that skeletal characteristics and growth form can be highly misleading regarding generic placement and, more important, in establishing relationships between species and genera. The following elements have been found *not* to be of significance for generic delimitation in the poecilosclerid sponges examined: (1) palmate isochelas, (2) acanthostyles, (3) the quantity of spongin present, (4) separate versus anastomosing spongin fibers, (5) dermal skeleton, (6) spines on the heads of styles, (7) conules or conule-like processes, (8) distinct oscular openings, and (9) the production of upright branches. Of more far-reaching importance is the finding that the whole spicule complement (including spicule localization) may be the same in two sponges but may not reflect the presence of similar cytological characteristics in both (i.e. *Thalysias juniperina* and *Microciona prolifera; Microciona spinosa* and *Axocielita hartmani*). Skeletal characteristics have also been found in one case (i.e. *Lissodendoryx isodictyalis* and *L. carolinensis*) to be an insufficient basis for species separation and for the placement of specimens (i.e. *Clathria* sp.) within previously described species. Skeletal characteristics have, however, been found to be of use in a general way for defining families (i.e. the family Clathriidae).

In the present study, genera in the family Clathriidae fall into one of three categories: the *Microciona* line, the *Thalysias* line, or an intermediate line. Two species of *Tedania* and two of *Lissodendoryx* show greater affinity for the *Thal-*

ysias line in the Clathriidae. It is concluded, therefore, that the *Microciona* line is a highly specialized one which originated from within the clathriid sponges possibly from forms similar to *Clathria* sp., which is in some respects intermediate between the *Microciona* line and the *Thalysias* line.

The employment of the following two skeletal characteristics for family distinction has been found to be misleading: (1) the presence of acanthostyles which echinate the spongin fibers, and (2) the presence of a basal, isodictyal reticulation of acanthostrongyles.

Among the sponges, the number of characteristics available for taxonomic purposes is small, even when considering the whole animal. Burton's (1963) insistence upon the utilization of skeletal characteristics and his rejection of other characters for the taxonomy of sponges leads to a biologically indefensible position. This point of view glosses over differences in cytological and histological characteristics which in the present study have been found to be of primary significance for deriving systematic relationships. The study of characteristics which have not been previously employed for taxonomic purposes in the sponges (and in other groups of animals) presents new ideas concerning the relationships of those groups investigated and may lead to the finding of new structures and processes.

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PLATE 1

Figure 1. Surface view of an incrusting colony of *Microciona prolifera.* Numerous minute pores are just visible. Colony is growing on a rock substratum. Marker represents 5 mm.

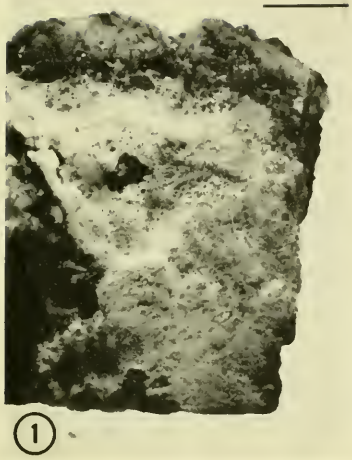
Figure 2. Surface view of an incrusting colony of *M. prolifera.* Note the upright shoots which are at an early stage of development. Marker represents 5 mm.

Figures 3 and 4. Branching colonies of *M. prolifera.* In figure 3, a number of branches have fused to form a flat, lamellate branch. Markers represent 1 cm.

Figure 5. Hand section made perpendicular to the surface (= cross section) of an incrusting colony of *M. prolifera;* stained with basic fuchsin. A: basal layer of spongin; B: upright spongin fibers; C: surface of the sponge. Marker represents 100 μ.

Figure 6. Hand section of the interior of a branch of *M. prolifera* made parallel to the surface (= longitudinal section). Stained with basic fuchsin. Note the anastomosing spongin fibers and the tendency of the fibers to branch upward. The tip of the branch is at the top of the photograph. Marker represents 250 μ.

Figure 7. Hand section of a branch of *M. prolifera* made perpendicular to the surface (= cross section); stained with basic fuchsin. Note the central location of the spongin fibers and the branches (at A) given off which run to the surface. Marker represents 150 μ.



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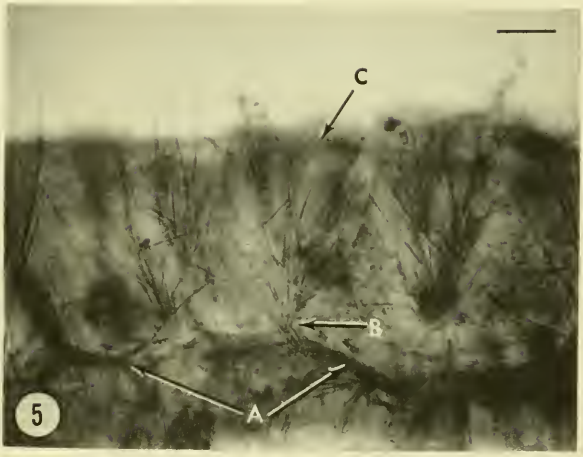

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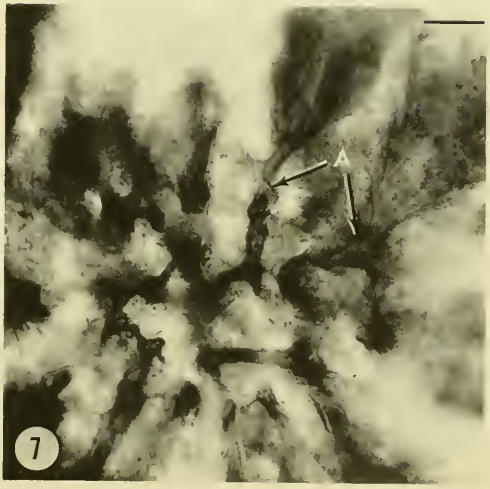

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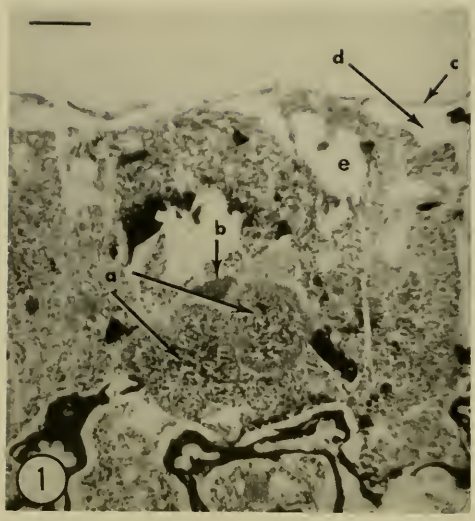
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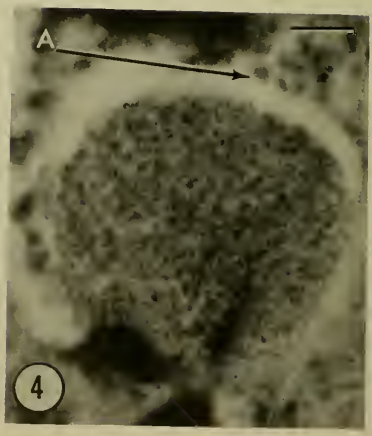
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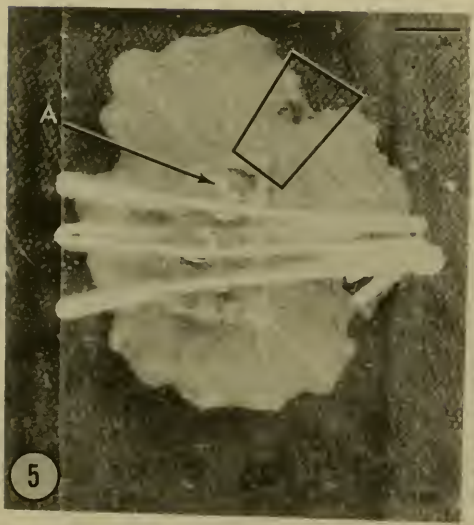
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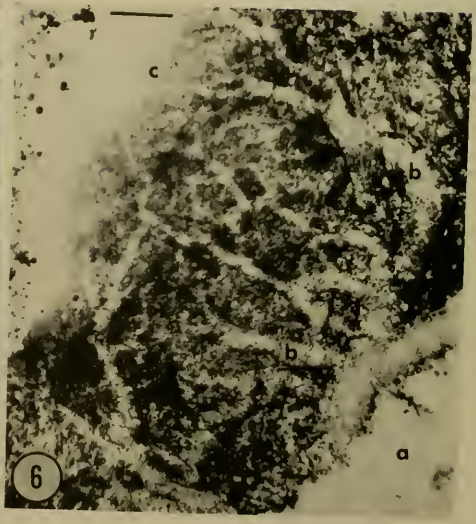
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PLATE 2

Figure 1. A section of an adult colony of *Microciona prolifera*. Note the dermal membrane (at **c**) and below it the subdermal space (at **d**). An exhalant canal is present at **e**. Darkly staining material is spongin. Flagellated chambers and mesenchyme are evident. Two larvae (at **a**) are present in the mesenchyme. Note the flagellated epithelium of the larvae and, at **b**, the presence of an irregularly shaped sperm mass. Sectioned at 15 μ. Bouin, iron hematoxylin, Mallory II. Marker represents 140 μ.

Figure 2. Fiber cell tract present below the dermis in *M. prolifera*. Note the thin fiber cells which run from the mesenchyme (at **a**) up to the dermis (at **b**). Sectioned at 15 μ. Bouin, iron hematoxylin, Mallory II. Marker represents 50 μ.

Figure 3. Mature egg present in the mesenchyme of *M. prolifera*. Note the large nucleolate nucleus and the cytoplasmic inclusion. Compare the size of this egg with the nucleolate cell at **a**. Bouin, iron hematoxylin, Mallory II. Marker represents 25 μ.

Figure 4. Sperm mass present in the mesenchyme of *M. prolifera*. Dark material above and below is spongin. An epithelial lining is present at A. Bouin, iron hematoxylin, Mallory II. Marker represents 25 μ.

Figure 5. Top view of a live explant (A), including the outgrowth region, of *M. prolifera* tied to a glass slide. Note the prominent exhalant canals in the outgrowth region. The blocked out area represents a region similar to that pictured in figure 6 on this plate. Marker represents 4 mm.

Figure 6. Top view of a fixed and stained area of the outgrowth region of *M. prolifera* similar to that blocked out in figure 5 on this plate. Before removal, the edge of the original explant was located in the clear area (**a**). The exhalant canals (**b**) are evident. In the darkly staining areas between them are located the flagellated chambers and mesenchyme. At the growing edge (**c**) the outgrowth region is thin and undifferentiated. Note the presence of particulate matter at the growing edge. Kahle, iron hematoxylin, Mallory II. Marker represents 300 μ.

PLATE 3

Figure 1. Ostial openings present in the upper epidermis of the outgrowth region of *Microciona prolifera*. The openings can be seen at A. Note the presence of a single nucleus associated with some ostia and the presence of epidermal nuclei and other cells. Kahle, iron hematoxylin, Mallory II. Marker represents 45 μ.

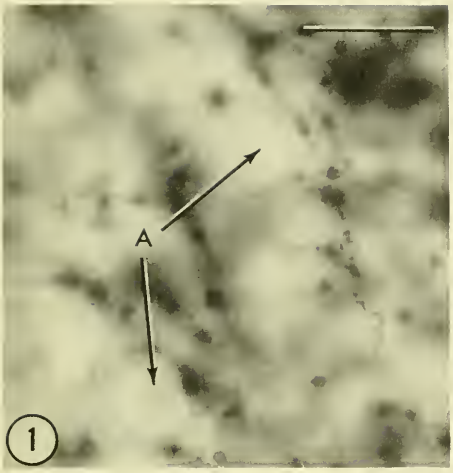
Figure 2. One side of a flagellated chamber in a differentiated area of the outgrowth region of *M. prolifera*. The spaces between the choanocytes are evident. Kahle, iron hematoxylin, Mallory II. Marker represents 15 μ.

Figure 3. The other side of the flagellated chamber pictured in figure 2 on this plate. Note the nucleus associated with the opening. The rim of the opening at A is below the field of focus. Kahle, iron hematoxylin, Mallory II. Marker represents 15 μ.

Figure 4. Nucleolate cell secreting a style in the outgrowth region of *M. prolifera*. Note the nucleolate nucleus at **a** and the presence of cytoplasm surrounding the spicule at **b**. Kahle, iron hematoxylin, Mallory II. Marker represents 10 μ.

Figure 5. An aggregate of nucleolate cells surrounding the rounded end of a style in a differentiated area of the outgrowth region of *M. prolifera*. Spongin is secreted by these cells. Kahle, iron hematoxylin, Mallory II. Marker represents 10 μ.

Figure 6. Erupting rhabdiferous cell in a differentiated area of the outgrowth region of *M. prolifera*. The presence of fibrous material and spherical inclusions is evident. The material in this cell has separated into two halves except at A. To the left is the remnant of a rhabdiferous cell which has released most of its contents. Carnoy, azure b bromide. Marker represents 10 μ.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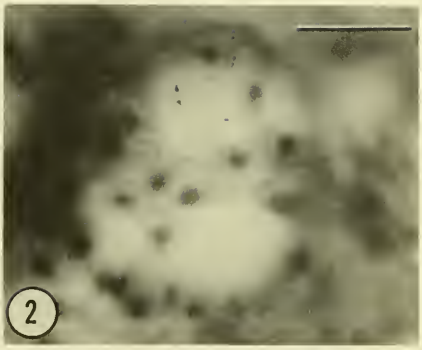
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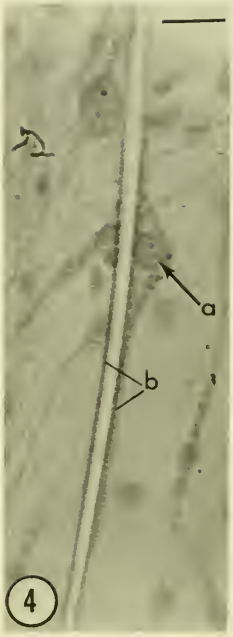
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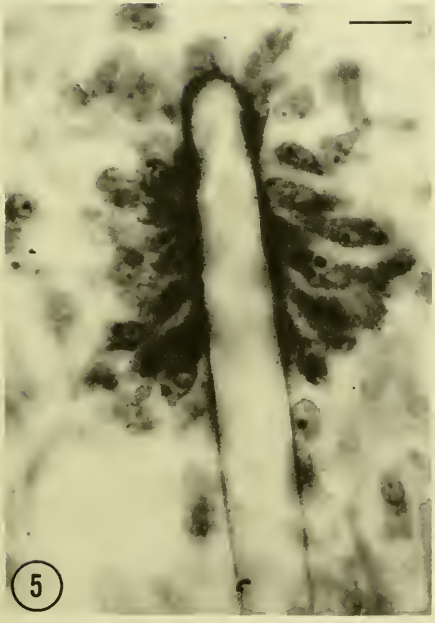
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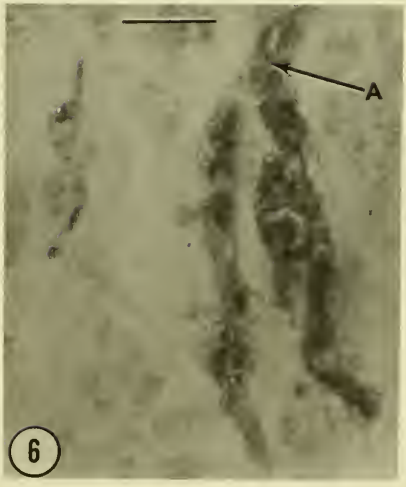
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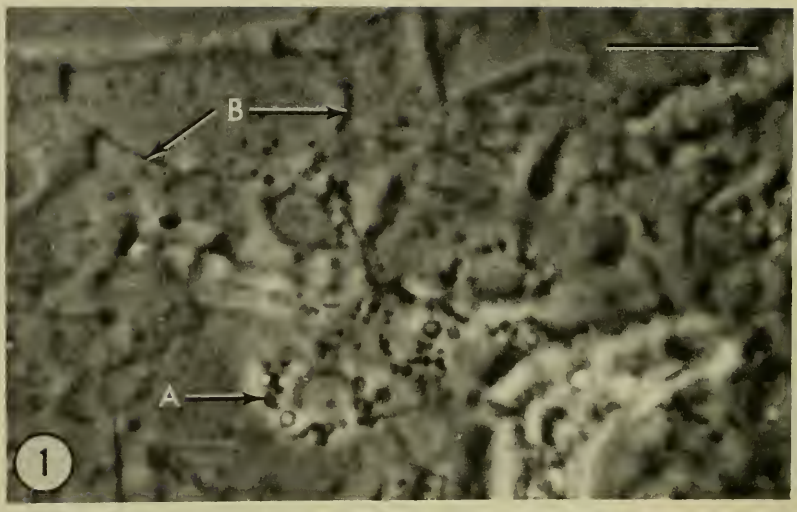
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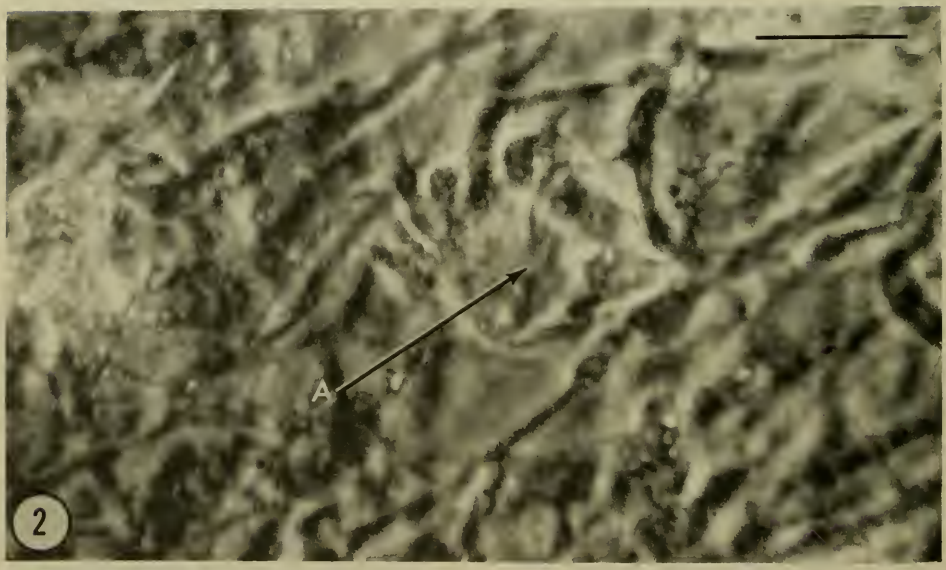
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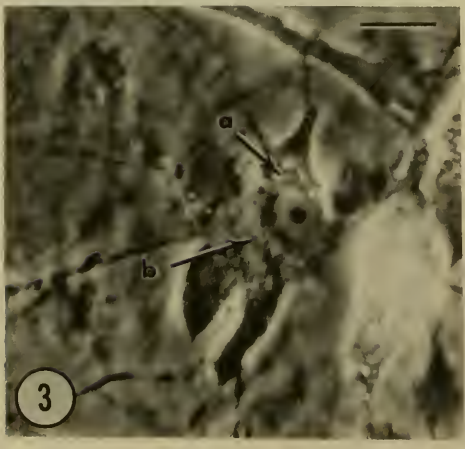
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PLATE 4

Figure 1. Phase contrast photomicrograph of the epidermis present in contact with the coverslip near the growing edge of the outgrowth region of *Microciona prolifera*. Note the inclusions (A) surrounding the nuclei and the presence of a small nucleolus. Cytoplasmic thickenings can be seen and at B give the appearance of being a limiting membrane. Live. Marker represents 10 μ.

Figure 2. A young flagellated chamber in an area of the outgrowth region of *M. prolifera* which was undergoing differentiation. The lumen of the chamber is at A. Live, phase contrast. Marker represents 12 μ.

Figure 3. Phase contrast photomicrograph of a nucleolate cell of *M. prolifera* moving through the mesenchyme in an undifferentiated area of the outgrowth region. Note the nucleolate nucleus and the presence of prominent pseudopodia. Above the nucleus, at a, are a number of cytoplasmic inclusions. At b a few of the minute granules present throughout the cytoplasm are visible. Live. Marker represents 10 μ.

Figure 4. Phase contrast photomicrograph of two gray cells present at the edge of an explant of *M. prolifera*. Note the numerous and highly refractile granules present throughout the cytoplasm. The bright area in the center of each cell (A) is the area in which the nucleus is located. At B is the cytoplasmic area which is free of granules and which contains glycogen. Live. Marker represents 10 μ.

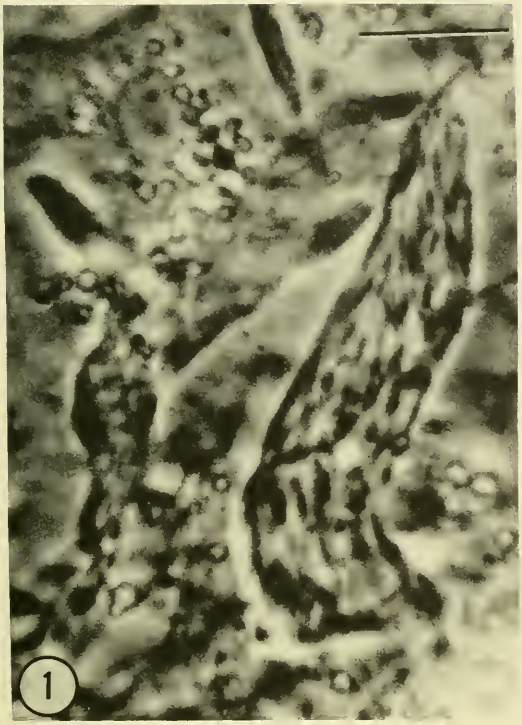
PLATE 5

Figure 1. Phase contrast photomicrograph of rhabdiferous cells present in the outgrowth region of *Microciona prolifera*. The cell to the left is below the focal plane. Note the presence of rod-shaped inclusions. Live. Marker represents 10 μ.

Figure 2. Phase contrast photomicrograph of a globoferous cell in an undifferentiated area of the outgrowth region of *M. prolifera*. A single large spherical inclusion is present at A and numerous smaller inclusions are present throughout the cytoplasm. Live. Marker represents 10 μ.

Figure 3. Phase contrast photomicrograph of the secretion of a chela in the outgrowth region of *M. prolifera*. Note the cytoplasm surrounding the chela. A and A′ are the ends of the spicule. Live. Marker represents 11 μ.

Figure 4. Toxa secretion in an undifferentiated area of the outgrowth region of *M. prolifera*. Note the material at A which is coiled around the toxa and extends out into the mesenchyme. Live, phase contrast. Marker represents 11 μ.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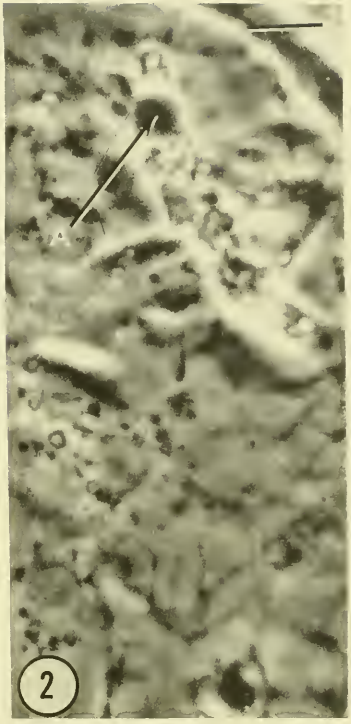
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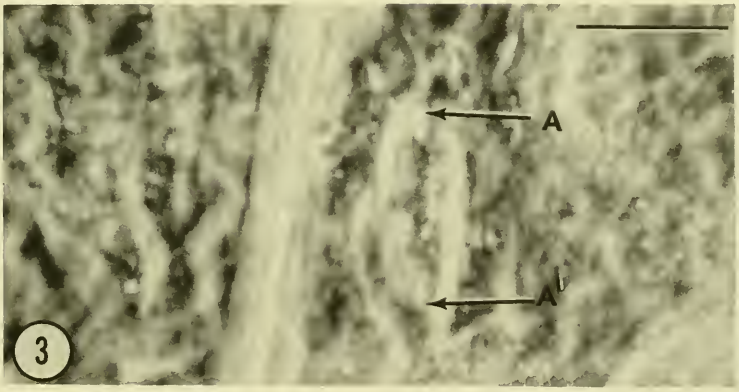
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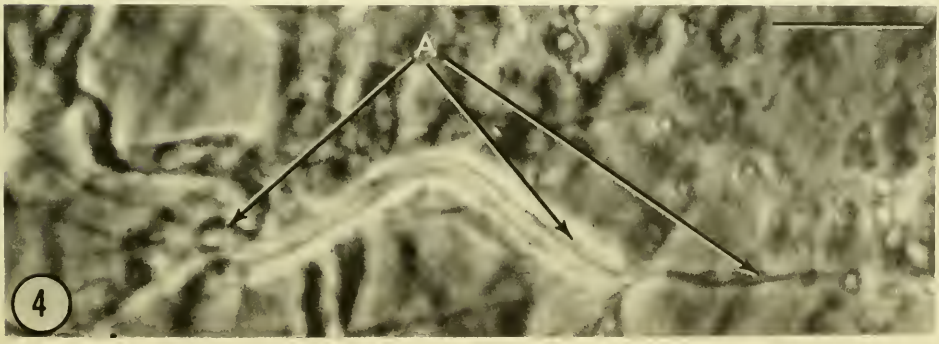
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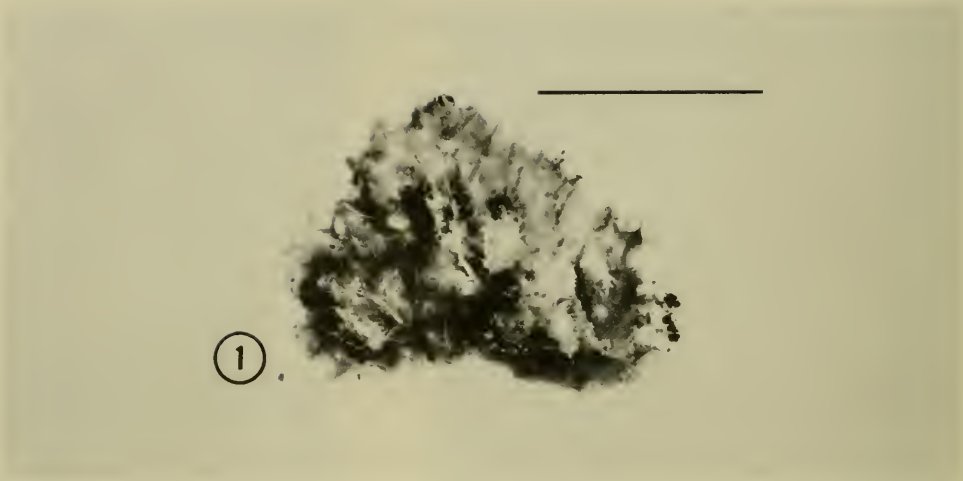

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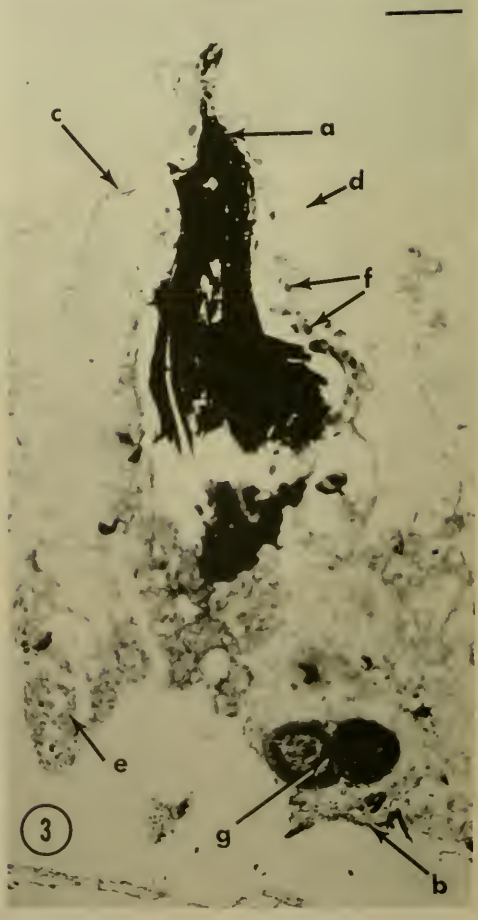


3

PLATE 6

Figure 1. A portion of a colony of *Microciona spinosa*. Note the presence of conules (short, pointed processes) at the surface. Marker represents 1 cm.

Figure 2. Hand section of *M. spinosa* made perpendicular to the surface. Part of the basal layer of spongin is seen at **a**. The integrity of individual spongin fibers which form conules at the surface (at **b**) can be seen, as well as the fusion of one fiber with another (at **c**). Bouin; stained with basic fuchsin. Marker represents 200 μ.

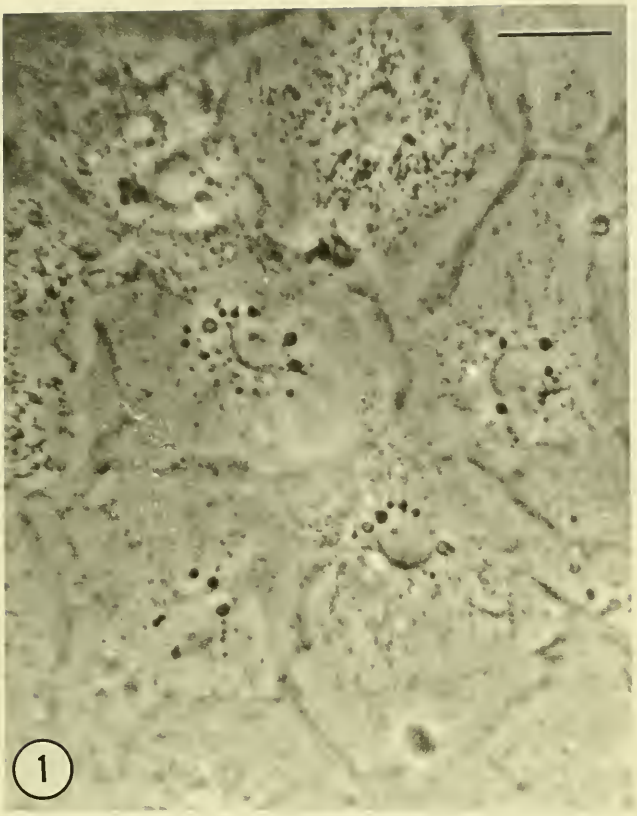
Figure 3. Microtome section of *M. spinosa* perpendicular to the surface. Part of a spongin fiber can be seen forming a conule at the surface at **a**. The presence of bands of spongin which make up the spongin fiber can also be seen. Remnants of the basal layer of spongin are at **b**. The dermal membrane, at **c** (disrupted in some areas by sectioning), subdermal space (**d**), and mesenchyme (**e**) can be seen. Small dark masses at **f** are sperm. Two mature larvae are present in the mesenchyme at **g**. Sectioned at 15 μ. Bouin, iron hematoxylin, Mallory II. Marker represents 200 μ.

PLATE 7

Figure 1. Phase contrast photomicrograph of the epidermis of *Microciona spinosa* present in contact with the substratum in the outgrowth region. Note the distinct cell membranes, nucleolate nuclei, and inclusions around the nuclei. Compare with Plate 4, figure 1; Plate 12, figure 1; and Plate 14, figure 1. Live. Marker represents 10 μ.

Figure 2. Phase contrast photomicrograph of a gray cell of *M. spinosa* present in an undifferentiated area of the outgrowth region. Note the numerous cytoplasmic granules but the lack of well-defined pseudopodia. The nucleus is located in the bright area at A. At B a small area of cytoplasm which is free of cytoplasmic granules can be seen; this area contains glycogen. Compare with Plate 4, figure 4. Live. Marker represents 10 μ.

Figure 3. Phase contrast photomicrograph of the secretion of a toxa in an unpresent in an undifferentiated area of the outgrowth region. Note the numerous rod-shaped inclusions. The cell nucleus is not visible. Compare with Plate 5, figure 1. Live. Marker represents 10 μ.



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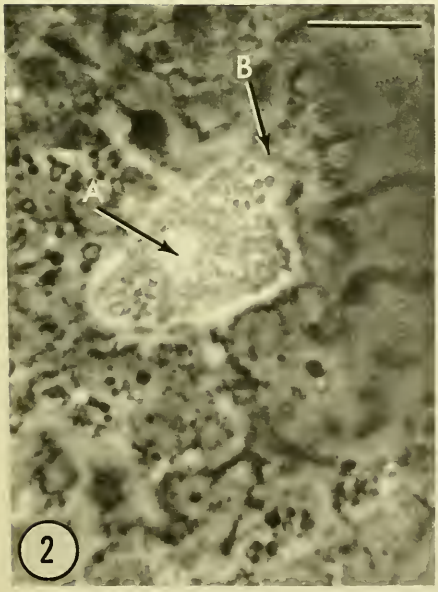

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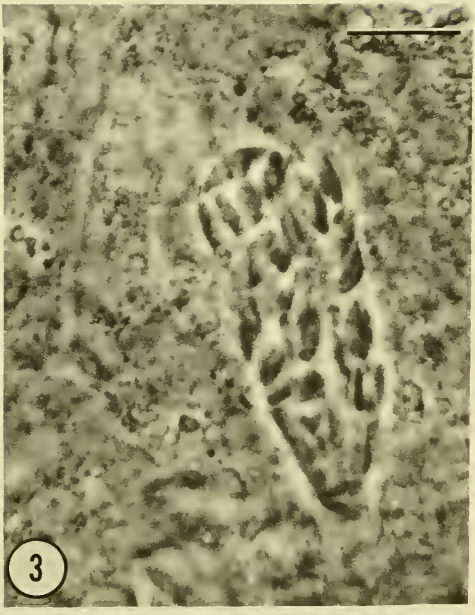
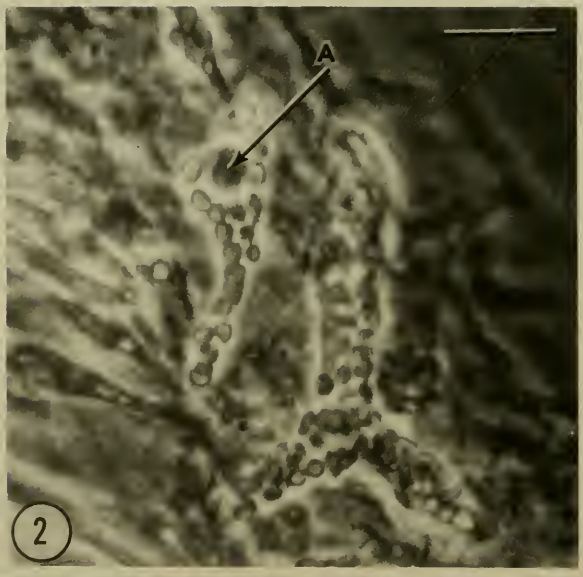


3


A
1


A
2


b
a
a
3

PLATE 8

Figure 1. Phase contrast photomicrograph of a portion of coiled material present within a tract in the outgrowth region of *Microciona spinosa*. Two rhabdiferous cells (at A) can be seen, one of which is closely associated with the coiled material. Toxas are present in this material but are not visible. Live. Marker represents 10 μ.

Figure 2. Phase contrast photomicrograph of two globoferous cells present at the edge of the outgrowth region in *M. spinosa*. In one of these cells the large spherical inclusion is visible at A. Note the cytoplasmic inclusions and the elongate shape of the cells. Compare with Plate 5, figure 2. Live. Marker represents 10 μ.

Figure 3. Phase contrast photomicrograph of the secretion of a toxa in an undifferentiated area of the outgrowth region in *M. spinosa*. The ends of the toxa are at a and the arch in the center of the toxa is at b. Compare with Plate 5, figure 4. Live. Marker represents 10 μ.

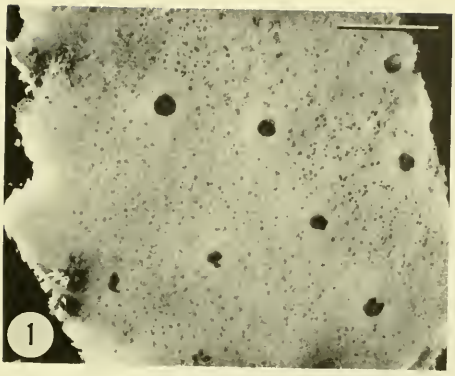
PLATE 9

Figure 1. Surface view of *Microciona seriata*. Note the presence of prominent oscular openings and numerous, much smaller incurrent pores. Specimen photographed in alcohol. Marker represents 5 mm.

Figure 2. Hand section of *M. seriata* cut perpendicular to the surface and stained with basic fuchsin. Note the ascending tracts of spicules and spongin. Basal layer of spongin is at the bottom of the photograph. Marker represents 300 μ.

Figure 3. A higher-power view of part of the hand section of *M. seriata* pictured in figure 2 on this plate. The surface of the sponge is at the top of the photograph. Note the anastomosis of spongin. Marker represents 116 μ.

Figure 4. Microtome section of *M. seriata*. The surface of the sponge is at the top of the photograph. At **a** is an oscular opening seen in cross section. At **b** are large exhalant canals which lead into the oscular opening. Sectioned at 15 μ. Bouin, iron hematoxylin, eosin. Marker represents 116 μ.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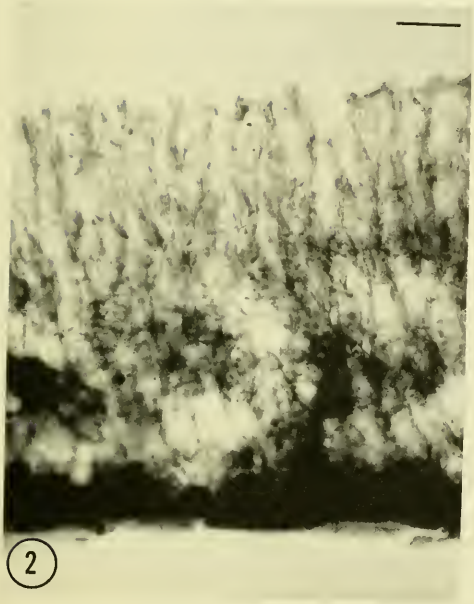
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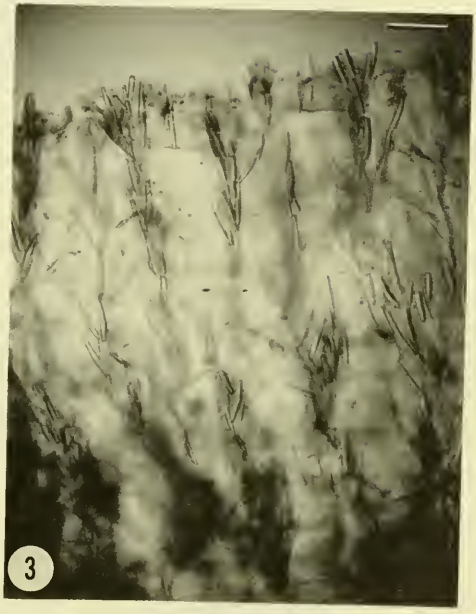
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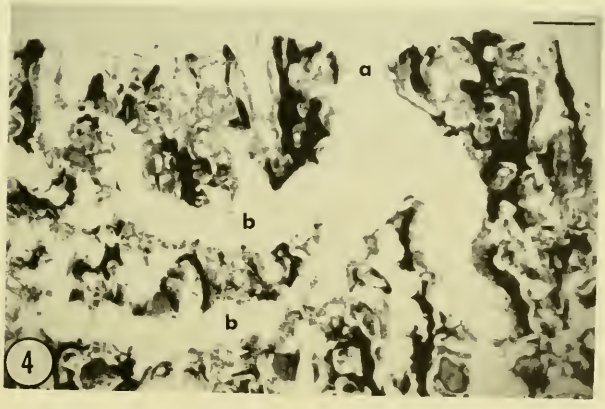
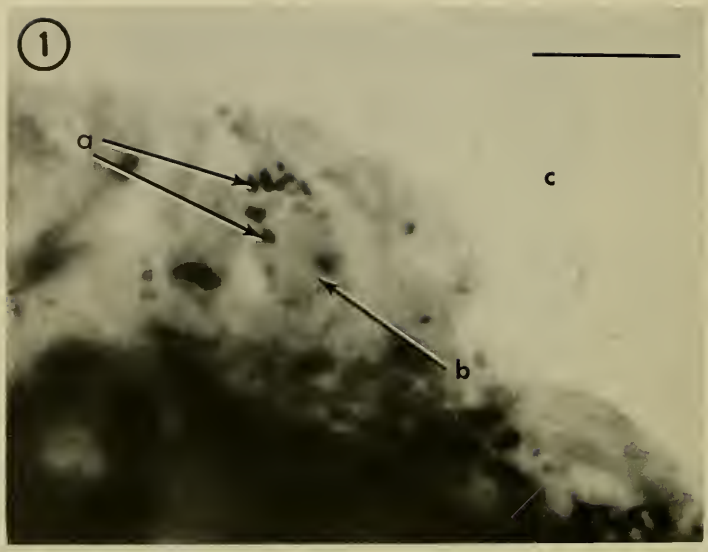

a
b
b
4


1
a
b
c

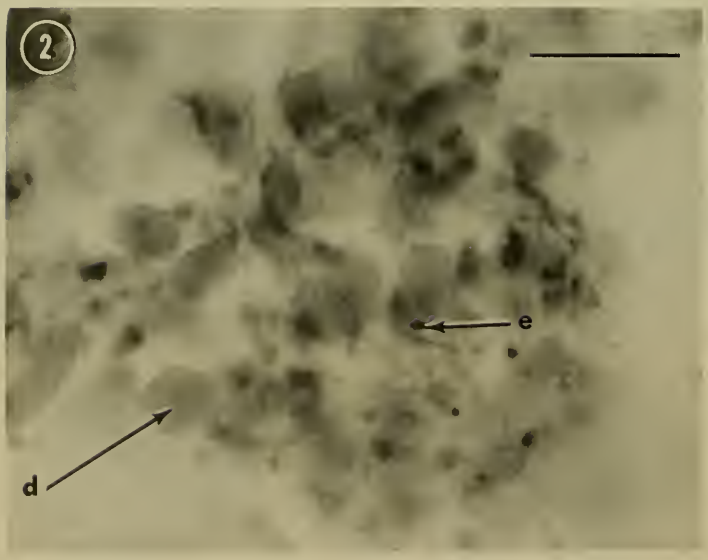

2
d
e

PLATE 10

Figure 1. A histological section of *Microciona seriata* showing the presence of a globoferous cell in the mesenchyme. The cell contains a number of small basophilic inclusions (at a) and a single large inclusion (at b). The cell nucleus is not visible. The large empty space (c) is an exhalant canal. Sectioned at 10 μ, fixed in Bouin and stained with iron hematoxylin and eosin. Scale represents 11 μ.

Figure 2. A different area of the same histological section of figure 1, this plate. A group of globoferous cells is seen in the mesenchyme. Most of these cells contain only a single large inclusion (at d) and lack the smaller inclusions. A few cells, however, do contain one or two smaller inclusions (at e). Sectioned at 10 μ, fixed in Bouin and stained with iron hematoxylin and eosin. Scale represents 11 μ.

PLATE 11

Figure 1. Surface view of a portion of a young incrusting colony of *Thalysias juniperina*. Note the presence of conules arising from the surface (see text). Marker represents 1 cm.

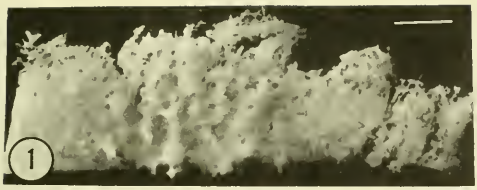
Figure 2. Side view of a portion of a young incrusting colony of *T. juniperina*. Note the upright shoot. Compare with figure 3 on this plate. Marker represents 1 cm.

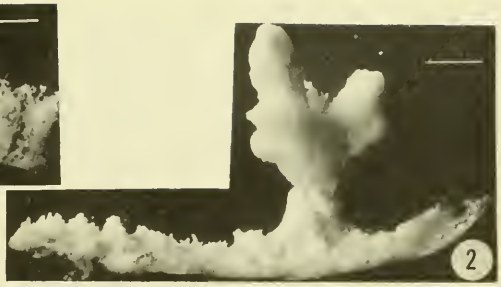
Figure 3. Part of an adult colony of *T. juniperina*. The basal portion of the sponge is at the bottom of the photograph. Note the well-developed conule-like processes (pointed protuberances) at the surface. Marker represents 1 cm.

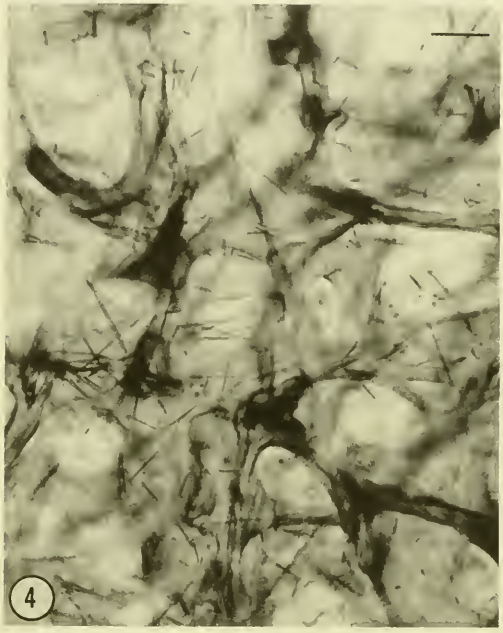
Figure 4. Hand section of the interior of a branch of *T. juniperina* showing the anastomosis of spongin fibers. Compare with Plate 13, figure 2. Bouin; stained with basic fuchsin. Marker represents 125 μ.

Figure 5. Hand section of the surface of *T. juniperina* made parallel to the surface. Note the presence of dermal styles which are not present in tufts at **a** and those which are present in tufts at **b**. Compare with Plate 13, figure 3. Bouin; stained with basic fuchsin. Marker represents 125 μ.

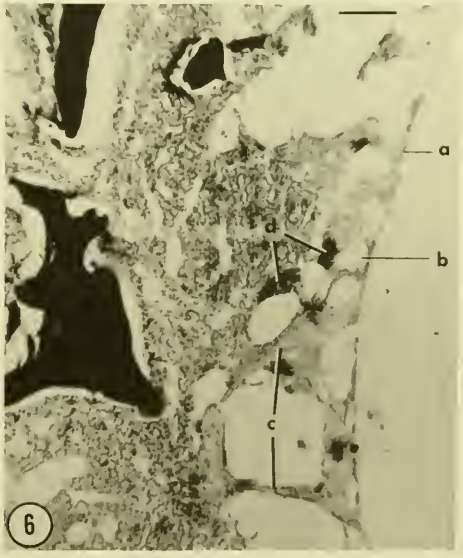
Figure 6. Microtome section (= cross section) of the outer portion of a branch of *T. juniperina*. The very darkly staining material is spongin. The dermal membrane is present at **a** and below it is the subdermal space at **b**. Dermal columns at **c** run up to the surface, and associated with them, usually at the base, are aggregates of cell types S and G-R at **d** (see text, p. 52). Mesenchyme, flagellated chambers, and exhalant canals can also be seen. Compare with Plate 13, figure 4. Sectioned at 15 μ. Bouin, iron hematoxylin, Mallory II. Marker represents 125 μ.

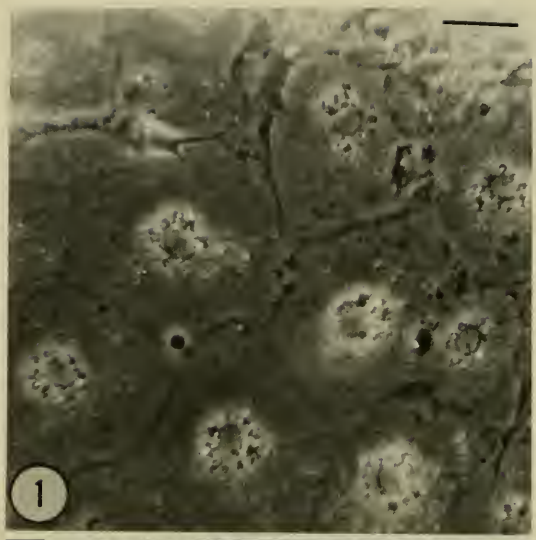







b
c


a
d
b
c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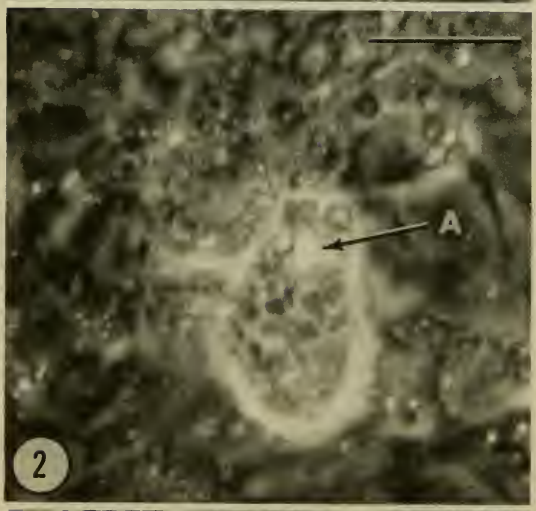
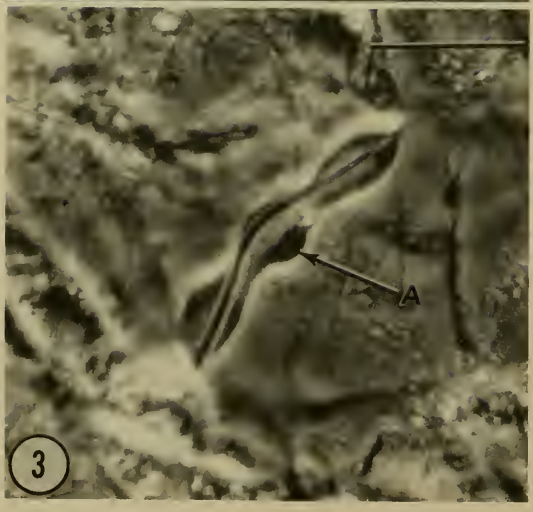

A
2


A
3


4

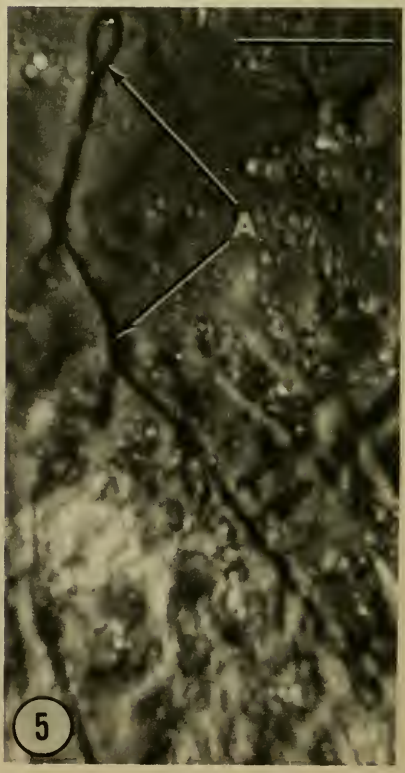

A
5

PLATE 12

Figure 1. Phase contract photomicrograph of the basal epidermis in the outgrowth region in *Thalysias juniperina*. Note the distinct cell membranes, nucleolate nuclei, and cytoplasmic inclusions. Live. Marker represents 11 μ.

Figure 2. Phase contrast photomicrograph of cell type S in *T. juniperina* present in an undifferentiated area of the outgrowth region. Note the presence of cytoplasmic inclusions and, at A, the bright area which contains the nucleus. Compare with Plate 14, figure 2. Live. Marker represents 11 μ.

Figure 3. Phase contrast photomicrograph of a toxoblast secreting a toxa in an undifferentiated area of the outgrowth region of *T. juniperina*. The dark, elongate nucleus is at A. Compare with Plate 5, figure 4 and Plate 8, figure 3 in which toxa secretion occurs in association with coiled material rather than within toxoblasts. Marker represents 11 μ.

Figure 4. An initial grouping of toxas and toxoblasts at the edge of the outgrowth region in *T. juniperina*. Four toxas contained within toxoblasts can be seen. Live. Marker represents 11 μ.

Figure 5. Phase contrast photomicrograph of coiled material (A) in *T. juniperina* present in the mesenchyme of the outgrowth region. Compare with Plate 14, figure 3. Live. Marker represents 11 μ.

PLATE 13

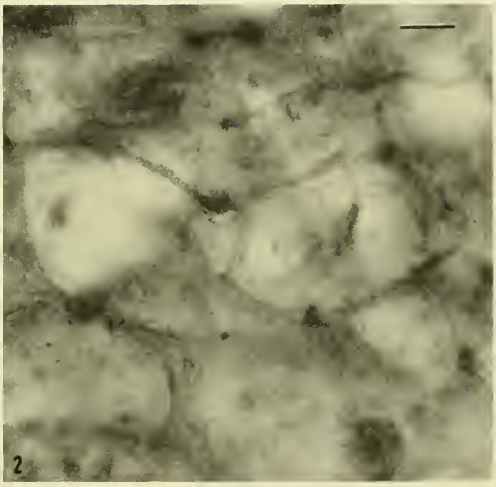
Figure 1. A specimen of *Thalysias schoenus*. Note the smooth surface and the massive appearance of the specimen due to the fusion of branches. Compare with Plate 11, figure 3. Marker represents 1 cm.

Figure 2. Hand section of the interior of a branch of *T. schoenus* showing the anastomosis of spongin fibers. Compare with Plate 11, figure 4. Bouin; stained with basic fuchsin. Marker represents 140 μ.

Figure 3. Hand section of the surface of *T. schoenus* made parallel to the surface. Note the presence of dermal styles in tufts and compare with Plate 11, figure 5. Bouin; stained with basic fuchsin. Marker represents 140 μ.

Figure 4. Microtome section of the outer portion of a branch of *T. schoenus* showing a mature larva at **a** surrounded by an epithelium at **b**. Distinct dermal columns at **c** are seen, and associated with them at **d** are aggregates of cell types S and G-R. Compare with Plate 11, figure 6. Sectioned at 15 μ. Bouin, iron hematoxylin, Mallory II. Marker represents 130 μ.

1



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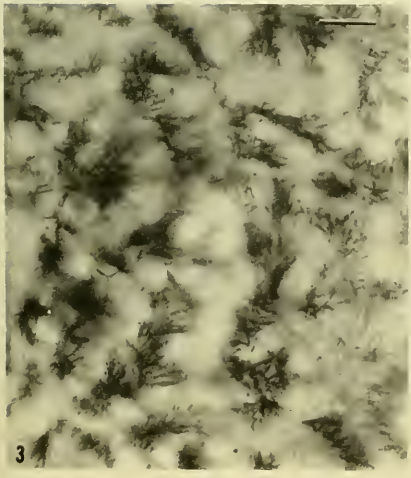

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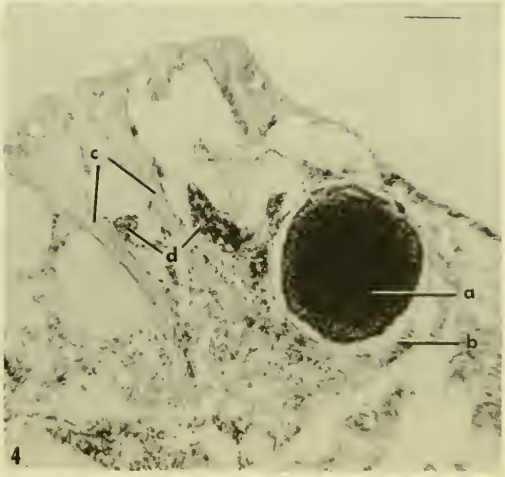

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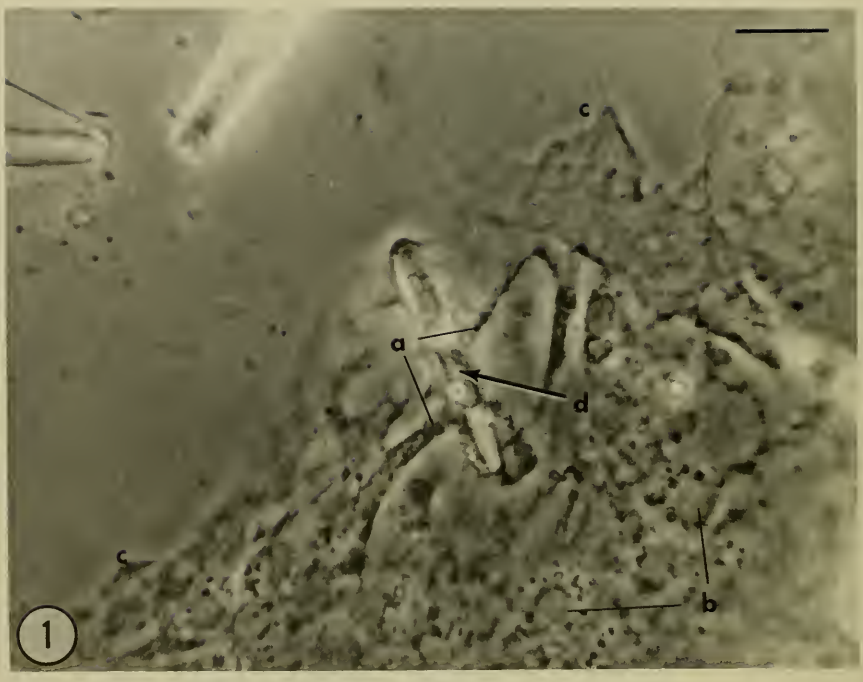
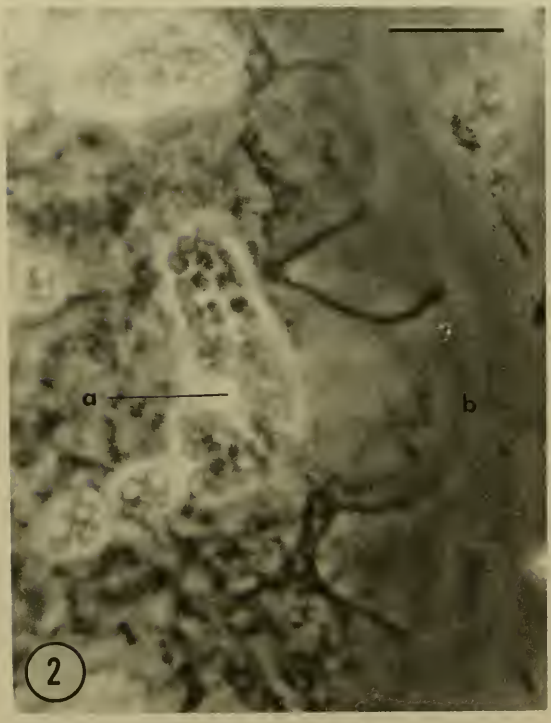

c
a
d
c
b
1


a
b
2


a
a
3

PLATE 14

Figure 1. Phase contrast photomicrograph of the ingestion of a diatom (d) at the edge of the outgrowth region of *Thalysias schoenus*. Note the extensions of the epidermal cells which are engulfing the diatom at a. Epidermal nuclei can be seen at b. At c is the outer edge of the outgrowth region present in contact with the coverslip. Live. Marker represents 10 μ.

Figure 2. Phase contrast photomicrograph of cell type S in *T. schoenus* present at the edge of the outgrowth region. Note the cytoplasmic inclusions and the bright area at a where the nucleus is located. The edge of the outgrowth region is at b. Compare with Plate 12, figure 2. Live. Marker represents 8 μ.

Figure 3. Phase contrast photomicrograph of coiled material in *T. schoenus* which is present near the edge of the outgrowth region. The ends of this material are at a. Compare with Plate 12, figure 5. Live. Marker represents 15 μ.

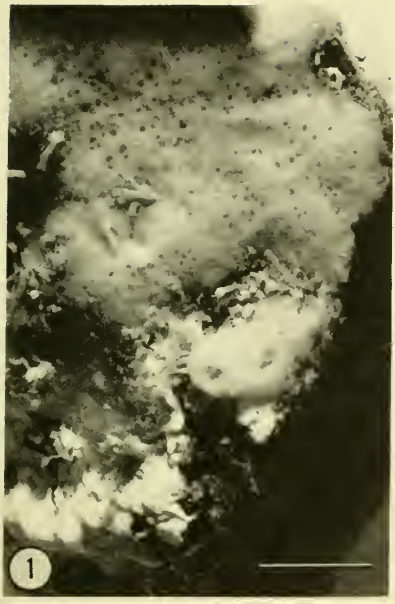
PLATE 15

Figure 1. Surface view of part of a colony of *Microciona pennata*. Colony is growing on a rock substratum seen at the lower right. Note the numerous pores present at the surface. Marker represents 5 mm.

Figure 2. Surface view of the holotype of *Axocielita hartmani*. Oscular openings are either spherical or elongate. A piece of the colony in the center was removed for analysis. Compare with figure 1 on this plate and with Plate 9, figure 1 and Plate 1, figure 1. Marker represents 1.5 cm.

Figure 3. The specimen of *Clathria* sp. used in this study. Compare with Plate 1, figures 3 and 4; Plate 11, figure 3; and Plate 13, figure 1. Marker represents 1.5 cm.

Figure 4. The specimen of *Rhaphidophlus cervicornis* used in this study. Compare with figure 3 on this plate. Marker represents 3 cm.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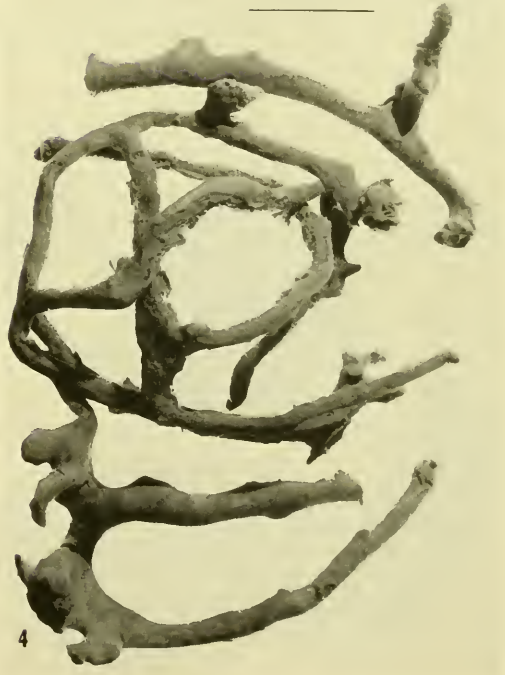
3


2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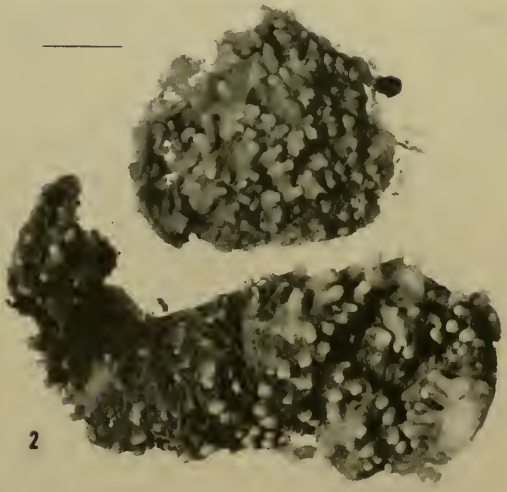

2

4

PLATE 16

Figure 1. Part of a colony of *Tedania ignis*. Note the presence of a prominent oscular "chimney." Marker represents 2 cm.

Figure 2. Surface view of *T. suctoria*. Numerous papillae are evident at the surface. Marker represents 1 cm.

Figure 3. Part of a colony of *Lissodendoryx isodictyalis*. Note the oscular "chimneys" and compare with figure 1 on this plate. Marker represents 2 cm.

Figure 4. Surface view (above) and side view (below) of *L. carolinensis*. Note the presence of surface protuberances and compare with figure 3 on this plate. Marker represents 2 cm.

PLATE 17

Semi-diagrammatic drawings of the special cell types and the mode of toxa secretion in the sponges studied. All cells are not drawn to the same scale but the relative sizes of cell organelles are maintained. Inclusions and granules which are filled-in (i.e., black) are basophilic in iron hematoxylin while those which are not filled-in (i.e., white) are not basophilic. The cytochemistry of cell organelles is given in the Results section under the appropriate species. Areas within cells which are labeled G contain glycogen. Cytochemically detectable RNA is absent in these cells with possibly one exception, i.e., cell type S in *Clathria* sp. may (data not available) contain nucleolar RNA.