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A MORPHOLOGICAL STUDY OF SARGASSUM FILIPENDULA.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXXII.

ETOILE B. SIMONS.

(WITH PLATES X AND XI)

THE family Fucaceae is less understood than its position and prominence in the Phaeophyceae warrant. Many important types have scarcely been considered at all, and, moreover, aside from the comparatively recent cytological studies in the family, few investigations have been conducted with modern methods of technique. The problems of morphology and cytology in the Fucaceae center chiefly around the sexual organs; the peculiar sunken structures in which they are borne, termed conceptacles; the likewise sunken but sterile structures called cryptostomata; and the sporelings.

The present investigation of these structures in *Sargassum filipendula* Ag., a member of perhaps the most highly differentiated genus in the Fucaceae, was undertaken with the hope of filling some of the obvious gaps in our knowledge of this family. It was conducted in the University of Chicago and at the Marine Biological Laboratory, Woods Hole, Massachusetts, under the direction of Professor BRADLEY MOORE DAVIS, who suggested the research to me. It gives me pleasure to express here, both to him and to Professor JOHN MERLE COULTER, my appreciation of valuable suggestions and assistance given me in this work. My acknowledgments are also due the Carnegie Institution for the use of a table at the Marine Biological Laboratory during the summer of 1904.

References to anatomical and morphological work which concern this subject will be given under the topics to which they belong. The early history of the genus with its taxonomic bearing is omitted, as having no place here, but the once credited distribution of Sargassum which was convincingly disproved by KUNTZE ('81) is a matter of history which deserves at least brief mention.

KUNTZE relates that LINNAEUS believed that a vast area of sea was densely covered by Sargassum in active vegetative condition; HUMBOLDT reported that the region surpassed Germany in extent six or seven times; MAURY stated that it equaled the Mississippi valley; and HOECKEL estimated its area to be forty thousand square miles. That these views were generally accepted is well known. They led to instruction regarding a "Sargasso Sea," whose supposed limits were outlined upon maps of the world. KUNTZE, by comparing his own observations and those of other travelers over routes which crossed in different places the outlined area, was able clearly to disprove the existence of such a "sea." Sometimes a voyage was made through the mapped region and little or no Sargassum was seen, and again it appeared somewhat abundantly, but without definite limits or fixed location. Storms which sweep tropical shores, near which attached Sargassum grows abundantly, were found to be in great part accountable for the appearance of the larger quantities of floating Sargassum. KUNTZE obtained no evidence to substantiate the view that floating Sargassum vegetates. It had been believed that floating forms of Sargassum consisted of *S. bacciferum* only, but KUNTZE found several species floating, and observed that the specimens in herbaria which had been collected in mid-ocean and labeled *Sargassum bacciferum* according to general belief, could be referred to various species. He therefore concludes that there is no characteristic floating species. The appearance in mid-ocean of floating masses now and then does not seem strange when the authentic distribution and abundance of attached Sargassum are recalled. According to KJELLMAN ('93) this genus, which includes one hundred fifty species, over half the number belonging to the entire family, is found attached along the coast of all warm seas, reaches north to Cape Cod in the Atlantic, to Japan in the Pacific, and in the south into Australian waters, where it is the most abundant. With the extent of this distribution in mind the presence of floating masses, especially after storms, is to be expected.

MATERIAL AND METHODS.

Material for this study was collected near the shores of Woods Hole, late in July and during August. Plants both in vegetative and in reproductive conditions were abundant. The weak solution of chromacetic acid of Flemming (1 per cent. chromic acid 25^{cc}, 1 per cent. acetic acid 10^{cc}, water 65^{cc}) proved a satisfactory killing and fixing reagent. Microtome sections were cut from paraffin 5 μ in thickness and stained either by iron-alum-haematoxylin after the method of Heidenhain or by safranin and gentian violet. The mucilage on the surface of the plant and in young conceptacles and cryptostomata takes the anilin dyes readily, but is not especially troublesome.

GENERAL MORPHOLOGY AND HISTOLOGY.

The habit of *Sargassum filipendula* is so like that of other species which have been described that it needs but slight attention. This species grows attached to rocks below low water mark, and therefore, unlike Fucus and Ascophyllum, is never exposed to the air. Vegetative plants and reproductive plants bearing all stages of conceptacles are plentiful in summer. Sporelings are abundant also and easily collected, for the discharged eggs and their products, the sporelings, remain attached for some time by mucilage to the surface of reproductive branches near the parent conceptacles.

The stem arises from a small disk-shaped holdfast and passes into long cylindrical branches which bear spirally arranged leaves, berry-like floats, which seem to be modified portions of leaves, as generally stated, and short reproductive branches. This form may attain a height of 60^{cm}, but is commonly shorter. Cryptostomata develop upon stems, leaves, and occasionally also upon reproductive branches in Sargassum, which differs in this respect from Fucus, whose receptacles, according to BOWER, contain no cryptostomata.

KJELLMAN ('93) states that the conceptacles of Sargassum are hermaphrodite. In *Sargassum filipendula* both mature bisexual and unisexual conceptacles are formed. Some conceptacles contain only spermatocysts (antheridia); some, more rarely, contain many spermatocysts and but one or two oocysts (oogonia); and others bear only oocysts. The appearance of a conceptacle devoted to the formation of oocysts differs decidedly from such a structure in Fucus. In

Sargassum the oocyst has no stalk cell. It is an embedded organ, being almost surrounded by wall cells of the conceptacle. As both the size and contents of a conceptacle are dependent upon the activity of wall cells (as described later), this conceptacle in Sargassum is smaller and has fewer sexual organs and paraphyses than the corresponding conceptacle in Fucus. The unisexual tendency in the conceptacle of Sargassum may be due in part to the unproductiveness of the many wall cells which abut upon the embedded oocyst.

The anatomy of the thallus of Sargassum has been studied in four species. In 1876, REINKE reported its development in *Sargassum Boryanum* from a three-sided apical cell situated at the bottom of a pit in the apex of the stem. He stated that the holdfast is composed of rhizoids and that a few intercellular filaments occur in the old parts of the thallus. OLTMANNS ('89) in an anatomical investigation of *Sargassum linifolium* and *S. varians*, likewise described a three-sided apical cell, and in addition gave an account of the origin both of the apical cell of a leaf and of a branch. He believes that the branching in Sargassum holds no relation to dichotomy. He figures an enlarged epidermal cell near the apical cell of the stem, and states that it becomes a three-sided apical cell. This young cell develops an outgrowth in which a second apical cell is soon differentiated, between the first and the stem. The first formed apical cell develops a leaf and the last a branch. OLTMANNS agrees with KUNTZE ('81) that there are all gradations between leaves and floats, and that floats are modified portions of leaves.

In 1892, HANSTEEN published the results of an anatomical and physiological investigation of *Sargassum bacciferum*. He also reported a three-sided apical cell, but did not trace its origin in any structure. He described three kinds of tissues, naming them the assimilating system, the storage system, and the conducting system. The assimilating system, according to HANSTEEN, includes only the outer layer of cells, or epidermis. Its cells are twice as long as broad, have undulating walls, like the epidermal cells in higher plants, and contain "phaeoplasts." The cells of this system add to their own number by radial, and to the cells below by tangential, divisions. The storage system occupies a zone several cells wide between the assimilating system and the innermost tissue which constitutes the conducting system. Most of the cells in the storage system are large. HANSTEEN found them empty in alcoholic material of Sargassum, but he did not doubt their function to be that of storage, because he had found much reserve material in similar cells of living Fucus. The conducting system consists of an axial cylinder of long cells with small diameter and oblique end walls. These cells are believed by HANSTEEN and others to function as sieve tubes. The cells of the three systems communicate by pores.

HANSTEEN observed in the storage cells of *Fucus serratus* and several other types, spherical grains of different sizes, which he named fucosan. He believes that the same structures have been variously considered as fat, proteid, and starch by other observers. The grains do not stain blue with iodin, and are soluble in water. HANSTEEN, who made a chemical analysis to determine their composition, considers them as a carbohydrate with the formula $(C_6H_{10}O_5)_n$. CRATO ('92) described in *Chaetopteris plumosa* spherical or elliptical bladder-like structures which he named physodes. He reported ('93) that they contain phloroglucin as a constant ingredient, function in directing the chemical exchange and transportation of food material within the cell, have motion, and are independent cell organs like the nucleus and chromatophore. CRATO stated further that HANSTEEN had confused various cell contents, and that fucosan grains and physodes are the same. KOCH ('96) denied the presence of phloroglucin in these bodies. In a later paper HANSTEEN (:00) again discusses fucosan grains. He maintains that CRATO'S physodes are fucosan grains, and that they are not independent cell organs but products of the phaeoplast. HANSTEEN has made no further chemical analyses to determine the nature of the bodies, but holds that they surely represent a product of photosynthesis. HANSEN ('95) after an investigation of several forms (*Dictyota dichotoma*, *Taonia alomaria*, *Halyseris polypodioides*, Asperococcus, Hydroclathrus, and Cystoseira), states that the Phaeophyceae contain oil and no starch, and OLTMANNS (:04) expresses the same view. It is seen therefore, that the character of the reserve material in the cells of the Phaeophyceae is still somewhat problematical.

Every stem and leaf structure in *Sargassum filipendula*, as in other species studied, develops through the activities of a three-sided apical cell. The tissue systems described by HANSTEEN are present and each seems to have the function ascribed to it, although without rigidity. Each system, too, has its origin in the group of segments surrounding the apical cell and can be traced very near it. The cells of every system are meristematic in the apical region, but the epidermal cells are apparently the only ones which retain this activity. The cells of any one of the three systems correspond well in general appearance with the similarly placed cells described by HANSTEEN, but an interesting modification was observed in the cells of the conducting system. All are long and of small diameter, but in respect to thickness of walls the tissue is differentiated into two regions. The inner cells have thin walls, while the outer ones have thick walls. The thick-walled cells may be both supporting and conducting in function. The conducting system of a leaf blade consists only of thin-walled tissue. No intercellular filaments, as reported by REINKE, have been found. Sometimes, however, a filamentous alga creeps into the mucilaginous walls of cells near the surface of a leaf or old stem, and gives the appearance of intercellular filaments. As the little alga contains true starch, its cells when stained with iodin present a sharp contrast to the unstained cells of Sargassum. HANSTEEN ('92) figures pores in thin areas consisting of the middle lamella in *Sargassum bacciferum*, and REINKE ('76) represents similar areas but without pores in cell walls of *Fucus vesiculosus*. Such thin areas are common between cells in the tissues of *Sargassum filipendula*, but pores, though probably present, are rarely seen.

The character of the reserve material in Sargassum proved of great interest. Sections from plants which have been preserved in formalin contain much more stored material than tissues which have been kept in alcohol. Preparations, however, which have passed through alcohol, xylol, paraffin, the heat of the bath, etc., still contain within the cells of the epidermis and outer cortex, many bodies which in all probability represent reserve food material. These bodies, which stain readily, vary in size and structure, but are evidently related, for transitional stages can be found between the most extreme forms. Judging by the appearance of the structures, some are intact and others modified. Those which seem intact are spherical, with a diameter which equals or exceeds the length of a chromatophore. Each consists of a more or less homogeneous ground substance and one or more refractive areas which are somewhat centrally placed. The modified structures vary from spheres, whose ground substance has been changed only at the periphery, to swollen masses which have an entirely modified ground substance with an irregular outline. Both the intact and modified bodies may occur within the same cell; but the former and the least modified are more common in epidermal cells, whereas the most modified are in cortical cells. The occurrence of such bodies within epidermal cells where photosynthesis is the most active, suggests that they represent a manufactured food. The varied modifications in the structures indicate the solvent action of the killing fluid, or an intercellular enzyme. As the inner cells contain bodies presenting greater modifications than the epidermal cells, the agent producing the change is apparently applied from within the tissue. If then within, it is probably an enzyme, for a solvent used in the process of killing would attack the contents of epidermal cells, doubtless before any others. The intact bodies may represent a newly formed product, perhaps a carbohydrate, and the modified structures, the product in process of digestion. The bodies do not stand with iodin in any condition. If they are carbohydrate they probably differ as much or more from the starch of higher plants as does inulin. The presence of many small spheres in formalin material and their absence from tissues preserved in alcohol indicates that oil globules are present in the cell, in addition to the structures described above. Future investigations on living material will probably disclose the presence of both oil and a carbohydrate in the Phaeophyceae.

THE ORIGIN AND DEVELOPMENT OF THE CONCEPTACLE.

The conceptacle in the Fucaceae had been but little studied when Bower ('80) gave an account of its development in four genera and six species (*Fucus serratus*, *F. platycarpus*, *F. vesiculosus*, *Ozonthallia nodosa*, *Halidrys siliquosa*, and *Himanthalia lorea*). According to him the development of the conceptacle in every species conforms to one scheme with minor variations.

The "initial cell" of the conceptacle, as stated by Bower, is the terminal cell of a linear series which is produced by a modification of the regular divisions in the segments of the apical cell of a receptacle.

This initial cell, strangely, contributes nothing essential to the conceptacle. It either degenerates directly without having divided at all, or it produces a short filament whose terminal portion degenerates. A cortical cell below the initial is termed by BOWER a "basal cell." This cell and others which adjoin the initial cell laterally, divide and form the walls of the conceptacle from which the sexual organs and paraphyses arise. The initial cell, therefore, according to BOWER, takes no part in the development of the conceptacle, whereas the cells adjacent to the initial produce all that is important, the walls and their products. The prominent features of this scheme for the development of the conceptacle are, it is seen, degeneration of an unimportant initial cell or a part of its filamentous product, and the activity of cells adjacent to the initial in producing the entire conceptacle.

Nearly all contributions in this field since 1880 have been in the main confirmatory of the work of BOWER. VALIANTE ('83) states that the development of the conceptacle in Cystoseira is due to the growth of neighboring tissue, about one or two cells. OLTMANNS ('89) describes the walls of the conceptacle of *Halidrys siliquosa*, *Himanthalia lorea*, and *Ascophyllum nodosum*, as also formed by neighboring cells, with the one exception that in Ascophyllum the initial cell develops a mass of tissue in the base of the conceptacle. This tissue, he reports, shares with the rest of the inner surface formed from neighboring tissue, in developing the sexual organs. As no degeneration of tissue was observed in Ascophyllum, and as its initial cell does contribute some important tissue the development of the conceptacle, this genus presents an exception to a part of the scheme which BOWER reports. Although Sphlachnidium should no longer be included in the Fucaceae, as shown by the Misses MITCHELL and WHITTING ('92), it is of interest to note that these investigators report its conceptacle as developing by the radial division of cells adjacent to a persistent but inconsequential element, which they believe to be homologous with the initial cell of BOWER. GRUBER ('96) states that the conceptacle of *Seirococcus axillaria* is more like that of Halidrys than Ascophyllum, which means, again, that it has an initial cell which contributes nothing of consequence to the conceptacle, whose walls are formed by cells which are adjacent to the initial.

HOLTZ (:03) reports that in the development of the conceptacle of

Pelvetia fastigiata several epidermal cells cut off basal segments which divide transversely until six or more tiers are formed. Over these tiers, one or more epidermal cells break down and a cavity results, which is gradually enlarged by further disintegration of epidermal and meristematic cells. After a time this process ceases, and a "healthy surface" is formed from the deeper meristematic cells. This surface, which comprises the walls of the conceptacle with the exception of the upper part that is formed by "cortical rows" of cells, produces sexual organs and paraphyses. The prominent features which distinguish the conceptacle of Pelvetia from others, as thus described, are the presence of several epidermal or initial cells, the more extended disintegration of tissue, and a difference in the behavior of the basal cells.

The development of the conceptacle in *Sargassum filipendula* is at variance with all the prominent characteristics in the development of the conceptacle as described by BOWER. The initial cell of Sargassum does not break down. It is an active cell which produces the entire conceptacle. As the whole conceptacle is the product of this one cell, adjacent cortical tissue takes no part whatever in the development of the structure. The first indication of the conceptacle is a clearly differentiated epidermal cell which lies near the apical cell of a reproductive branch (*fig. 1*) and constitutes the initial cell of the conceptacle. The upper portion is surrounded laterally by epidermal tissue, whereas its central and basal regions are bounded by cortical. The initial is much larger than any of the cells with which it is in contact and differs much from them in shape. Though it may vary somewhat in length it is always flask-shaped. Its oval bowl, sometimes slightly narrowed at the base, tapers above into an elongated neck whose outer end is flush with the surface. The initial cell is circular in cross section at its apex (*fig. 1a*) and elliptical at its base (*fig. 1b*).

The initial cell never breaks down. On the contrary the development of the conceptacle is initiated by its activity. Its large nucleus divides. Then a curved wall is formed with concave surface above, separating two very unlike cells (*fig. 2*), which form the two-celled stage of the conceptacle. The upper cell, which may be designated the tongue cell, is a long somewhat cylindrical structure; whereas the lower is somewhat conical or wedge-shaped. The initial cell and the two-celled stage of the conceptacle have similar outlines both in longitudinal (*figs. 1* and *2*) and in transverse sections (*figs. 1a, m, b*, and *2a, m, b*). That the lower portion of the tongue cell is surrounded by the upper part of the cell below it is well shown in both longitudinal and transverse sections of the two-celled stage of the conceptacle.

The lower cell of this two-celled structure divides longitudinally into two similar daughter elements, thus producing the three-celled stage of the conceptacle (*fig. 3*). The longitudinal wall reaches to the lower portion of the tongue cell, whose basal portion is surrounded now by two cells instead of by one. The relative position of the three cells is made clearer by an examination of their transverse sections. A cross section near the base of the three-celled structure shows two similar cells (*fig. 3b*). A cross section about midway between the apex and base shows three cells (*figs. 3m* and *3bm*), the tongue cell and the two lower cells which surround its base. A section of the apex is circular in outline and consists of the tongue cell alone (*fig. 3a*). The three-celled stage of the conceptacle is apparently formed occasionally in another way. The two longitudinal sections of an initial cell are shown in *figs. 4, 5*, containing three nuclei but no walls. Two nuclei appear in one section and one in the other. It seems that the nucleus of the initial cell in this instance divided first with its spindle perpendicular to the axis of the cell, and that one of the daughter nuclei divided with its spindle parallel to the axis.

After the three-celled stage, the development of the conceptacle is readily followed. The two lower of the three cells divide longitudinally in various planes. A condition thus results which exhibits five cells in longitudinal median section (*fig. 6*). Four of the five cells are young cells of the recent divisions, and one is the centrally placed tongue cell. Longitudinal divisions continue as before, and a structure showing six or seven cells in longitudinal section is formed (*fig. 7*). The tongue cell is still conspicuous in this and in several succeeding stages. Longitudinal divisions continue as illustrated in *figs. 8, 9, 11*, until the walls of the entire conceptacle are formed. Some of the wall cells begin to develop sexual organs when the conceptacle is very small (*figs. 9, 11*). This activity of the cells, however, does not prevent them from contributing to the growth of the conceptacle. The mouth of the conceptacle is surrounded by a marginal ring of epidermal tissue about one or two cells deep (*figs. 8, 11*). As these cells are not aggressive they may be omitted from further consideration. Excluding this minor detail every portion of the conceptacle is the product of one initial cell. Cortical tissue adjacent to the initial takes no part in its development.

The behavior of the tongue cell is similar to that of the "initial cell" in other forms as reported by BOWER. It may show signs of degeneration (*fig. 8*), may remain inactive for some time (*fig. 11*), or may even divide to form a filament of two or three cells (*figs. 9, 10*). In no case does it contribute to the walls of the conceptacle, but on the contrary after its divisions resembles a paraphysis. The tongue cell is very conspicuous until sexual organs begin to develop, but shortly after their appearance it cannot be distinguished. The upper and lower cells which result from the transverse division of the initial cell (*fig. 2*) correspond in appearance and behavior with the "initial cell" and "basal cell" as described by BOWER and others. It seems probable that BOWER saw both the initial cell and the two-celled stage of the conceptacle, but failing to observe the division in the initial cell, considered the initial and the upper cell of the two-celled stage identical. With this construction, degeneration of the upper or tongue cell was believed to be degeneration of the initial cell itself, and the division of the lower cell of the two-celled stage, a product of the initial cell, was regarded merely as the division of an unrelated cortical cell. A conceptacle developed from cells which happen to be adjacent to a degenerating and unimportant cell would be a very different structure from a conceptacle developed from one active initial cell.

THE ORIGIN AND DEVELOPMENT OF THE CRYPTOSTOMA.

The references embodied in the preceding treatment of the conceptacle constitute the chief source of information bearing upon the cryptostoma. The structure which produces the sexual organs has commonly and naturally been given first attention, but investigators who have studied both, generally agree that the conceptacle and cryptostoma are homologous. Different theories regarding the significance of the cryptostoma have been offered, but no safe generalization can be made until a more extended investigation of both structures has been made in a variety of forms.

Miss BARTON (**'91**) gave an account of the cryptostoma in Turbinaria, stating that an initial cell divides longitudinally, thus forming two daughter elements which produce paraphyses. In demonstration of this two paraphyses are figured arising from the base of a many-celled structure. Miss BARTON does not report the origin of the walls of the cryptostoma, but as the initial cell is believed to develop directly into paraphyses, we may assume that she believed the walls to arise from neighboring tissue in accordance with the views of the earlier writers.

The development of the cryptostoma in Sargassum follows step by step the history of the conceptacle. The initial cell arises near the apical cell of a leaf or vegetative branch. Longitudinal and cross sections of this cell (*figs. 12, 12a, m, b*) show the same form and structure as the longitudinal and cross sections of the initial cell of a conceptacle (*figs. 1, 1a, m, b*). The activities of the two initials are also identical. The initial cell of the cryptostoma divides transversely, forming a two-celled structure (*fig. 13*) which is comparable in every way to the two-celled stage of the conceptacle (*fig. 2*), consisting as it does of a tongue cell and a larger lower cell. The lower cell divides longitudinally. A group of three cells then results (*figs. 14, 15*) which is entirely similar to the three-celled stage of a conceptacle (*fig. 3*). The two lower cells of this three-celled stage then divide longitudinally in one or more planes, forming a structure which shows four or five cells in longitudinal section (*figs. 6, 17*). The center of this structure and of several which follow is occupied by the conspicuous tongue cell (*figs. 16, 17, 18, 19*). Thus by the continued longitudinal divisions of the products of the lower cell of the two-celled stage, the walls of the entire structure are gradually developed. Paraphyses begin to appear in the cryptostoma (*figs. 18, 20*) as early as do the sexual organs in the conceptacle (*fig. 9*). The activity of the wall cells in producing paraphyses, however, does not interfere with their functioning further in developing the cryptostoma (*fig. 21*). Epidermal cells at the mouth of the cryptostoma form here, as in the conceptacle, a marginal ring one or two cells deep (compare *figs. 8* and *19*). The origin of the true walls of the structure, however, may be traced as in the conceptacle to the lower cell resulting from the transverse division of the initial.

The behavior of the tongue cell in the cryptostoma is similar to that of the corresponding element in the conceptacle. Occasionally the tongue cell of the cryptostoma may develop a prominent filament (*fig. 20*), which is clearly identical in structure with a typical paraphysis (*fig. 21*). The young conceptacle and cryptostoma are so alike that they can only be distinguished by their respective positions on fruiting branches or on young vegetative structures, until the appearance of sexual organs in the one and paraphyses in the other defines their mature characters.

The development of the paraphysis is interesting for its regularity. A wall cell enlarges, pushing into the cavity of the cryptostoma, and then divides transversely (*figs. 18, 20*). The upper cell produces the paraphysis, whereas the lower functions in the development of the wall. The growth of the paraphysis results from the transverse divisions of the cell next the wall (*figs. 21, 22*), a method of growth termed *trichothallic*. The development of a paraphysis in the cryptostoma of Sargassum is, therefore, characteristically basipetal, as BARTON ('**91**) found in Turbinaria.

A somewhat advanced paraphysis is composed of three regions. That which adjoins the wall of the cryptostoma consists of the large turgescent meristematically active basal cell (*fig. 22*). The middle region is occupied by six or eight short cells which have so recently been formed that they have not had time to lengthen much. The upper region contains several greatly elongated cells. This region in a mature paraphysis is partly within the cryptostoma and partly without, for fully developed paraphyses extend far beyond the surface of the plant.

A peculiar condition found in many cryptostomata deserves special attention. Structures frequently appear between the paraphyses which seem to bear no relation to them. These are papillae and stalked cells, the former like the papillae which precede male organs in a conceptacle and the latter like the male organs themselves. The stalked cells, although slender and probably always sterile, appear to be spermatocysts no longer functional. This surprising condition is of great interest and importance in relation to the homology and significance of the cryptostoma, a structure formerly believed to contain only paraphyses, but which appears also to have sexual organs or their degenerate representatives. That the cryptostoma and conceptacle are homologous cannot be doubted, since their origin and early development are identical in all details. The occasional appearance of sterile representatives of sexual organs within the cryptostoma further confirms this view of their relationship and strongly supports the theory of BOWER ('80) that the cryptostoma in the Fucaceae is derived from the conceptacle.

The occurrence of conceptacles in special reproductive branches only, the appearance of cryptostomata in both vegetative and reproductive branches, and the development of representatives of sexual organs within the cryptostomata, suggest a line of evolution from plants bearing conceptacles scattered over leaf and branch structures indiscriminately, to the type now under consideration with localization of the conceptacle upon special branches. Certain branches were set apart to bear conceptacles as the conceptacles in all other parts of the plant body were rendered sterile and thus changed into cryptomostata. The presence of sexual organs or their degenerate representatives within a cryptostoma indicates, according to these views, that the process is not carried to its farthest point in Sargassum.

The production of conceptacles upon small special branches only, instead of upon the entire plant, naturally results in fewer conceptacles upon one plant. The conceptacles, however, are much more closely placed than the cryptostomata. On account of their comparatively small size the initials and young conceptacles occupy very little space in the apex of a branch, but farther down on the receptacle the bulging bowls of the developing flask-shaped conceptacles require more and more space, until the mature structures nearly fill the interior of the receptacle and there is only enough intervening tissue to hold the conceptacles together. The cryptostomata, on the other hand, are well scattered upon vegetative branches and mature leaves. The contrast in the placement of cryptostomata with that of conceptacles is, therefore, very marked.

THE SPERMATOCYST.

The male sexual organs (antheridia), which will be called spermatocysts in this paper, according to the terminology of DAVIS (:04), develop from wall cells of the conceptacle in Sargassum as in other forms of the Fucaceae. A wall cell puts forth a papilla (*fig. 23*) which is cut off by a transverse wall (*fig. 24*). The lower cell becomes a part of the wall occupying the place of the cell from which it arose. The upper cell enlarges for a time and then divides, forming the sperm mother-cell or spermatocyst and its stalk (*fig. 24*, at the right.) A stalk cell may have no other relation than that which it bears to the spermatocyst which it supports, or it may function in other ways. It may produce several spermatocysts directly, without individual stalks; it may put forth a papilla which gives rise to a spermatocyst and stalk (*figs. 25, 26*); or it may develop a hair (*fig. 27*). Hairs, however, are comparatively rare within a conceptacle of Sargassum. Owing to the variety of activities which belong to a stalk cell, the growths within a conceptacle lack uniformity. Some structures reach but a little distance above the wall of the conceptacle, whereas others form conspicuous branch systems. Although these systems are prominent in this conceptacle, they are considerably smaller and less dense than the branch systems in a conceptacle of Fucus, and there is far more unoccupied space within the cavity of a conceptacle of Sargassum than of Fucus.

The young spermatocyst contains dense cytoplasm, a centrally placed nucleus and deeply staining granules, the nucleus remaining in a resting condition for a long period. The divisions of the nucleus were not studied in detail. Sixty-four sperms are apparently formed (*figs. 27* and *28*), agreeing, therefore, with the count announced by BEHRENS (**'86**) for *Fucus vesiculosus*. The sperms within the spermatocyst have an elliptical outline, a cytoplasmic ground mass, and a somewhat spirally arranged band, which is probably the nucleus. The discharge of sperms was not seen, but a rent, partly terminal and partly lateral in empty spermatocysts, indicates their mode of escape.

THE OOCYST.

The female sexual organ (oogonium) or oocyst, according to the terminology of DAVIS (**:04**), is peculiar among the Fucaceae, as far as is known, in that it is not borne upon a stalk but is a partially embedded organ (*fig. 31*). The sister cell of the oocyst, instead of developing into a pedicel cell as is usual in this family, functions as one of the wall cells of the conceptacle. The oocyst enlarges greatly, but becomes nearly surrounded by adjacent wall cells.

Its development is simple. A somewhat enlarged wall cell of a young conceptacle divides transversely, forming two cells much alike in size and contents (*fig. 29*). The inner cell, which is the homologue of the stalk cell of the female organ in Fucus, cannot be distinguished from neighboring wall cells shortly after its formation. The outer cell, which has a free surface toward the interior of the conceptacle, increases greatly in size and soon becomes the spherical oocyst. *Fig. 30* represents a young oocyst and its sister cell, already unequal in size. There now follows a long period of growth, during which the oocyst attains a remarkable size, finally containing a great quantity of reserve material, many chromatophores, much cytoplasm, and a large nucleus. The mature organ, drawn under a lower magnification than *fig. 30*, is represented in *fig. 31*. No trace of its sister cell could be found.

The oocyst of Sargassum develops but one egg. The mitosis within the wall cell whose division produces the oocyst is normally the only mitosis in the process of oogenesis. Particular attention was given to this point. The one nucleus of the oocyst remains in a resting condition throughout the entire period of the growth of the cell, and therefore becomes the nucleus of the egg. In the other genera of the Fucaceae, as is well known, there are three mitoses within the oocyst, resulting in eight nuclei. Each of the eight nuclei may become a center for the development of an egg as in Fucus, or some nuclei may degenerate and a less number of eggs be formed, as in Ascophyllum and Pelvetia. It might be supposed from these conditions in the Fucaceae that the oocyst of Sargassum would show similar nuclear divisions and degeneration, but this is not the case. The mitoses characteristic of oogenesis in Fucus are normally suppressed in Sargassum. The tendency in the Fucaceae to reduce the number of eggs produced by an oocyst reaches its culmination, therefore, in Sargassum.

It is interesting to note that Sargassum still gives proof that it belongs to the reduction series which has its beginning in Fucus and allied forms that produce eight eggs in an oocyst. Out of the great number of conceptacles examined, one oocyst was formed which contained two eggs, and two oocysts which contained eight. The oocyst with two eggs was formed in an immature conceptacle that held five normal oocysts. The two eggs appeared fairly vigorous. One of the two oocysts which contained eight eggs was an old conceptacle, from which other sexual elements had apparently long been discharged. The eight together were smaller than one normal mature egg. The other oocyst which contained eight eggs shared a conceptacle with two normal oocysts. It was attached in the side of a conceptacle near the surface of the plant, which for a slight distance was modified in structure as if in response to an injury. It is possible in this case that the wound incited the reversion. The appearance of an oocyst containing more than one egg in Sargassum must be regarded as a rare reversion to the Fucus type.

The resting nucleus of the oocyst is always large, but varies in structure. Sometimes it has few granules and no conspicuous reticulum, whereas at other times it contains many granules and a dense network. The nucleolus is also large in size and variable in structure. At the present time no suggestion can be made to account for the changes in nuclear structure, excepting that they are the concomitants of growth and varying nutritive conditions.

The method of discharge of the egg from the conceptacle of Sargassum is somewhat unlike that reported in Fucus and other genera. In Fucus the outer membrane of the oocyst remains attached to the conceptacle, as explained by THURET, and the eggs escape in a group surrounded by a very delicate inner membrane. In Sargassum the entire oocyst becomes freed from the conceptacle and escapes. In Fucus the inner membrane dissolves or breaks, thereby freeing the naked eggs which it has enclosed. In Sargassum the wall of the oocyst swells, stretches, and sometimes ruptures, but it may persist for a long time, even enveloping later a many-celled sporeling formed within it. The inner membrane enclosing the eggs of Pelvetia is separated from the outer as in Fucus. In Pelvetia, however, as figured by THURET, this membrane persists about the eggs, apparently offering no great resistance to the entrance of sperms. Whether the sperm enters the egg of Sargassum through a break in the oocyst membrane, whether it passes through the membrane, or whether the eggs develop parthenogenetically, is not known. A study of fertilization in Sargassum is surrounded by serious technical difficulties because both eggs and sperms develop upon the same plant, thus making it difficult to isolate the sexual cell.

THE SPORELING.

Many if not all of the eggs of Sargassum on leaving the conceptacle become fastened immediately by the mucilaginous wall of the oocyst, which still surrounds it, to the surface of the reproductive branch. In this position the eggs segment. The first division of the egg in Sargassum does not differentiate a rhizoidal region, as in Fucus and Ascophyllum. Instead, a many-celled ellipsoidal structure is formed, the divisions occurring with mathematical precision. Rhizoids then develop at one end with no apparent relation to a substratum or to gravity, so far as could be observed in fixed material. Sporelings sometimes occur at opposite sides of a branch with rhizoids directed toward the stem, thus showing no relation in the development of rhizoids to gravity; and again, sporelings occur with rhizoids directed away from the branch in various directions, indicating that the parent plant exerts no special influence. It is possible that the attachment of a sporeling upon a plant is so insecure that the direction of its axis may be shifted in the manipulation of material. Otherwise it is difficult to account for the conditions which were observed.

When the many-celled sporeling has reached the condition for rhizoid formation the cells at one pole elongate, thereby giving rise to a tuft of rhizoids of approximately equal length. This mass of rhizoidal filaments finally produces the characteristic disk-shaped holdfast of the mature plant. *Fig. 32* shows a sporeling in about the oldest condition in which it remains attached to the parent plant. No apical cells were found in these sporelings and therefore its differentiation must occur after the sporeling has separated from the parent plant.

The germination of the oospore deserves careful cytological investigation. Many preparations have been made and studied, but further attention will be given the subject before the observations are published. A few conditions may be noted, however. There are numerous radiations at the poles of the early spindles. The asters contain granular inclusions which suggest centrosomes, although their origin and relation to the processes of mitosis have not been traced. Walls following the mitoses are developed somewhat slowly, being formed in part at least by the membranes of contiguous vacuoles.

SUMMARY.

Each stem, branch, and leaf structure develops through the activities of a three-sided apical cell.

The thallus consists of three compact tissues, called for convenience the epidermal, cortical, and conducting tissues. The latter consists of only thin-walled cells in the leaves, but in mature stems contains both thick and thin-walled elements. A ring of thick-walled cells, which may have both a mechanical and conducting function, surrounds the thin-walled conducting cells in the center of the axis.

The tissues normally contain much reserve material, a part of which is oil, and a part, whose nature is undetermined, appears to be a carbohydrate.

Both the conceptacles and cryptostomata originate in a single flask-shaped initial cell which develops the entire structure.

The first division of the initial cell results in two unlike segments: a large lower cell which develops the walls of the conceptacle and cryptostoma; and an upper cell, the tongue cell, which either remains inactive, divides to form a short filament, or degenerates. The "initial cell" of Bower is apparently the tongue cell, a product of the true initial cell.

The conceptacle and cryptostoma are undoubtedly homologous structures. Every stage of development in both structures is the same, from the appearance of the similar initial cells to the development of paraphyses in the cryptostomata and sexual organs in the conceptacle.

The paraphyses are developed basipetally by the division of the lowermost cell in each structure.

Spermatocysts or their degenerate representatives occur in some cryptostomata. Such conditions indicate that the cryptostomata have been derived from conceptacles whose sexual organs have become sterile.

The spermatocysts develop as in other Fucaceae, each finally producing sixty-four sperms which are discharged from a partly terminal and partly lateral rent.

The sister cell of an oocyst does not become a stalk and consequently the oocyst is an embedded structure.

The oocyst normally gives rise to but one egg. The nucleus of the oocyst accordingly becomes the nucleus of the egg.

The oocysts were found containing eight eggs each. These must be considered a rare reversion to the Fucus type.

The entire oocyst of Sargassum, unlike other genera of the Fucaceae which have been studied, is discharged with its enclosed egg. The oocyst wall may break, partially freeing the egg, or it may persist even enveloping a many-celled sporeling.

Segmentation of the egg takes place while it is attached to the surface of the plant by the mucilaginous wall which surrounds it. This segmentation results first in a many-celled undifferentiated ellipsoidal sporeling. Rhizoids develop late at one end of the multicellular sporeling, with no apparent relation to gravity or other stimulus.

Asters, containing granular inclusions suggesting centrosomes, appear at the poles of the spindles in the early mitoses of the segmentation of the egg.

THE UNIVERSITY OF CHICAGO.

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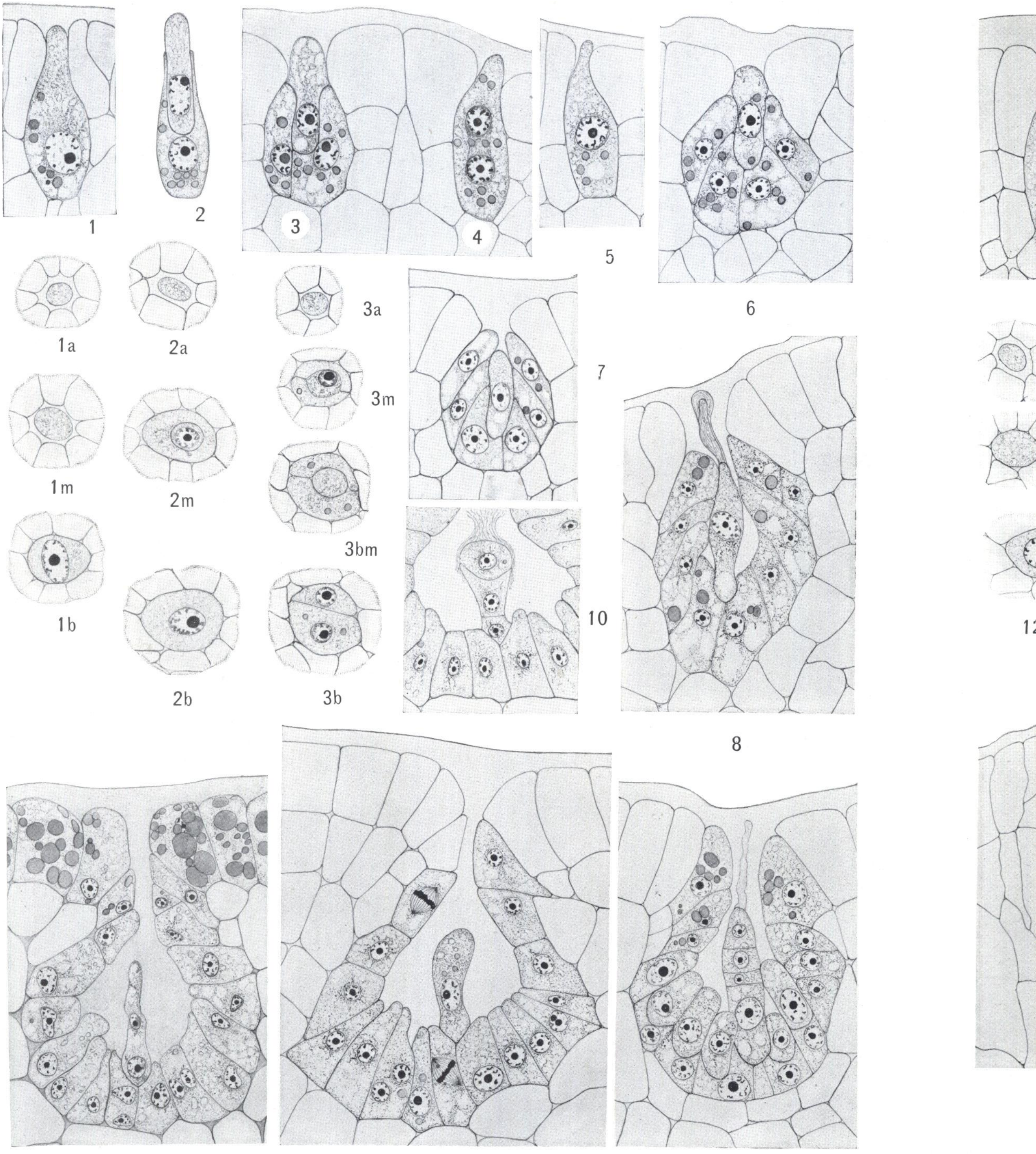

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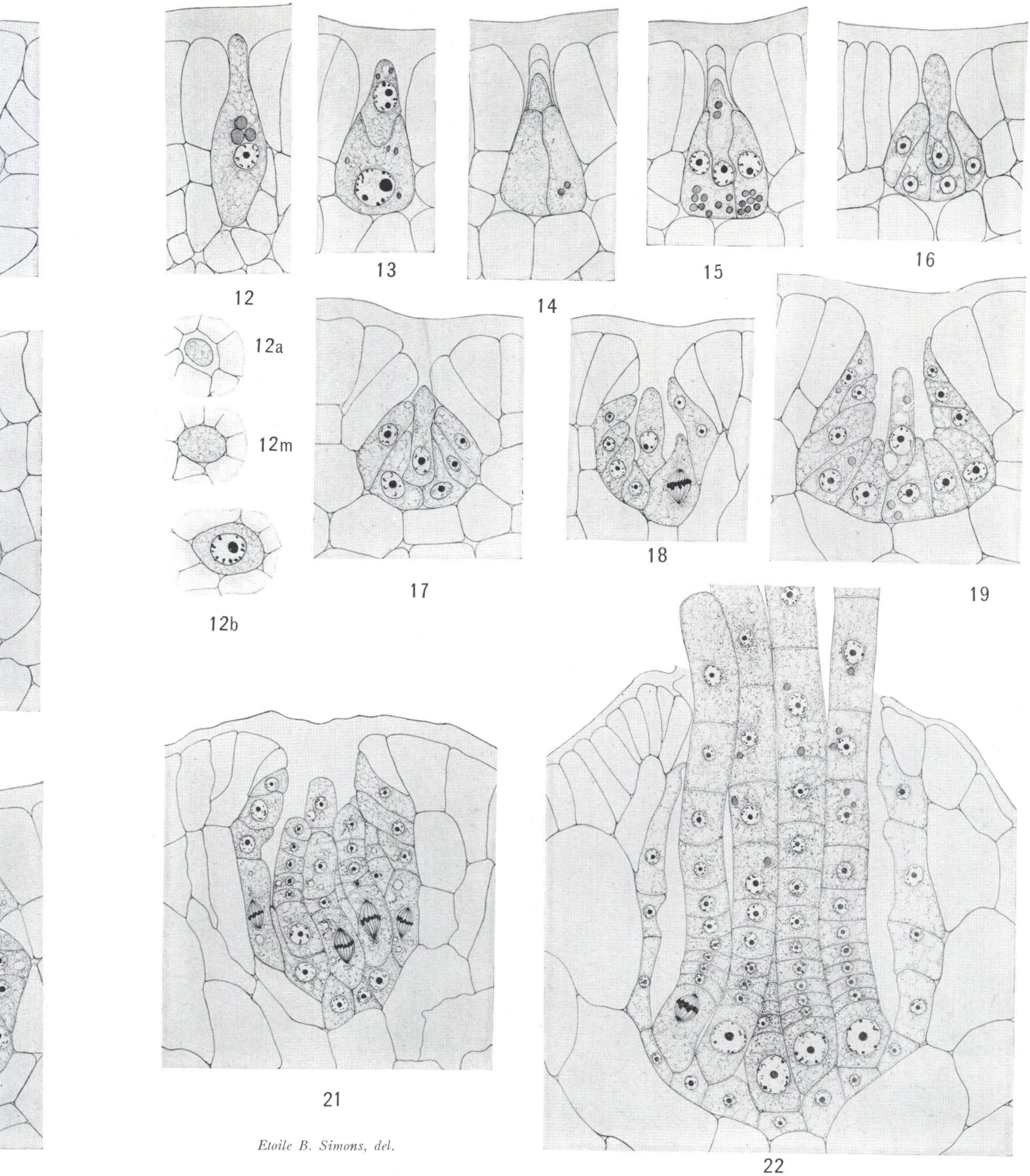
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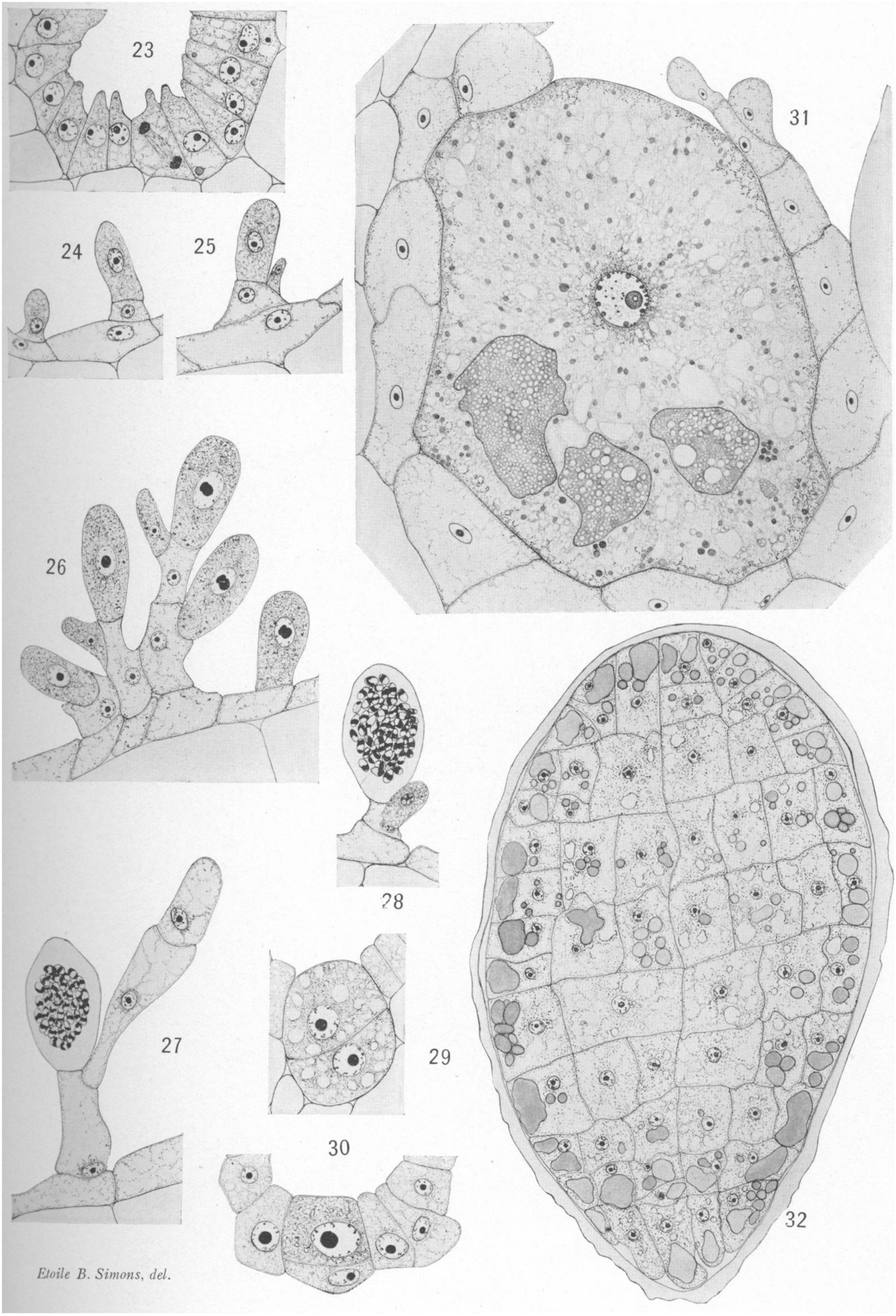

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EXPLANATION OF PLATES X AND XI.

All figures were sketched with a camera and reduced one third in reproduction. *Figs. 1–30* were drawn with Zeiss apochromatic objective 1.5^{mm} and compensating ocular number 4, magnification 1140. *Figs. 31* and *32* were drawn with dry objective, magnification 570.

PLATE X.

Figures 1–11. Development of the conceptacle.

Fig. 1. Initial cells, longitudinal section; *1a*, cross section of apex; *1m*, cross section of median portion; *1b*, cross section of basal portion.

Fig. 2. Two-celled stage, longitudinal section showing the slender upper tongue cell, and a larger lower cell; *2a*, cross section of the apex showing the tongue cell only; *2m*, cross section of the median portion with the centrally placed basal region of the tongue cell surrounded by the upper part of the lower cell; *2b*, cross section of the basal portion showing the lower cell only.

Fig. 3. Three-celled stage, longitudinal section; *3a*, cross section of the apex showing tongue cell only; *3m*, cross section of median portion with the centrally placed lower part of the tongue cell, surrounded by the upper part of its two companion cells; *3bm*, cross section a little below *3m*, showing the same cells; *3b*, cross section of the basal portion showing the two lower cells only.

Figs. 4 and 5. Longitudinal sections of a peculiar trinucleate stage of one conceptacle. The first division of the nucleus of the initial cell must have been with the axis of the spindle perpendicular to that of the cell. *Fig. 5* contains one of the nuclei of the first mitosis and *fig. 4* the products of a division, now in late telophase, of the other nucleus of the first mitosis.

Fig. 6. Longitudinal section of a young conceptacle showing four walls cell and the tongue cell.

Fig. 7. Longitudinal section of a later stage with six similar wall cells and the centrally placed tongue cell.

FIG. 8. Longitudinal section of a more advanced stage illustrating the formation of the cavity of the conceptacle.

FIG. 9. Longitudinal section of a young conceptacle some of whose wall cells are developing papillae. The tongue cell contains two nuclei.

FIG. 10. A filament of three cells formed from the tongue cell.

FIG. 11. Young conceptacle showing simultaneous development of wall cells and papillae.

Figures 12–22. Development of the cryptostoma.

FIG. 12. Initials, longitudinal section; 12*a*, cross section of the apex; 12*m*, cross section of median portion; 12*b*, cross section of basal portion.

FIG. 13. Longitudinal section of the two-celled stage.

FIG. 14. Longitudinal section of the lateral surface of the three-celled stage.

FIG. 15. Longitudinal section of the interior of the same group of cells represented in *fig. 14*.

FIG. 16. Longitudinal section showing four wall cells and the tongue cell.

FIG. 17. Longitudinal section slightly more advanced.

FIG. 18. Longitudinal section of a young cryptostoma beginning to form paraphyses very early.

FIG. 19. Longitudinal section of an older stage which has not yet begun to develop paraphyses.

FIG. 20. Longitudinal section showing five paraphyses developing from wall cells and one from the tongue cell.

FIG. 21. More advanced, illustrating the simultaneous development of paraphyses and wall cells.

FIG. 22. Still more advanced.

PLATE XI.

Fig. 23. The development of papillae which will later give rise to spermatocysts.

FIG. 24. At the left a cell which results from the separation of a papilla from a wall cell. At the right a spermatocyst and stalk which have been formed by the division of a cell similar to the one shown at the left.

FIG. 25. A stalk cell has given rise to a papilla, now separated by a wall.

FIG. 26. A branch system formed through the activity of stalk cells.

FIG. 27. A spermatocyst containing sperms. The stalk cell has developed a hair.

FIG. 28. A mature spermatocyst, the stalk cell pushing out at one side.

FIG. 29. Very young oocyst with its sister cell, which is the homologue of the stalk cell in Fucus.

FIG. 30. Slightly older oocyst and its sister cell already unequal in size.

FIG. 31. A mature embedded oocyst containing many chromatophores and much reserve material.

FIG. 32. A sporeling still attached to the surface of the parent plant. At one pole rhizoids have begun to develop. The old wall of the oocyst surrounds the sporeling.