A CYTOMORPHOLOGICAL STUDY OF *PELVETIOPSIS LIMITATA* (SETCHELL) GARDNER

by

CAROL AILEEN HENNIGAN

B.Sc., Mount Saint Vincent College, 1958

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in the Department

of

BIOLOGY AND BOTANY

We accept this thesis as conforming to the

required standard

THE UNIVERSITY OF BRITISH COLUMBIA

March, 1961

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Biology and Botany

The University of British Columbia,
Vancouver 8, Canada.

Date March 27, 1961.

ABSTRACT

A cytomorphological study of Pelvetiopsis limitata (Setchell) Gardner confirmed a cytological alternation of generations in the plant. Approximately thirty-two chromosomes in the haploid phase alternate with sixty-four chromosomes in the diploid phase.

Pelvetiopsis is oogamous and monoecious. Antheridia and oogonia are produced in flask-shaped conceptacles. Nuclear division is regular in developing gametangia. Reduction of chromosome number appears to take place during the first nuclear division in the antheridium and oogonium. No centrosomes or chromophilous spherules are discernible during nuclear division. In the oogonium the two divisions of the meiotic sequence are followed by a mitotic division producing eight nuclei. Two eggs of unequal size are formed in the oogonium. The larger egg is uninucleate and functional, while the smaller is seven-nucleate. At maturity 4 to 16 chromocenters may be observed in the nucleus of the large egg prior to its liberation. Before the release of the large egg from the oogonium approximately 32 chromosomes differentiate as the nucleus enters mitotic prophase. The smaller egg formed in the oogonium usually disintegrates after extrusion from the conceptacle. The inner layers of the wall of the oogonium are soon discarded after liberation. Aberrant eggs occur. Two eggs of equal size may form or there may be as many as 5 eggs of various sizes. Binucleate eggs are sometimes present.

In the antheridium 4 mitotic divisions follow meiosis producing 64 nuclei. The cytoplasm differentiates around each nucleus and 64 spermatozoids are formed. These are released by the rupture of the outer layer, and by gelatinization of the inner layer of the wall of the antheridium. Approximately 30 - 32 chromosomes were observed at the second nuclear division in the antheridial initial.

Oogamy in Pelvetiopsis restores the diploid complement of approximately 64 chromosomes. Fertilization occurs after the gametes have been liberated from the conceptacle. The egg is naked at the time of fertilization. The first division of the zygote is transverse to the plane in which the rhizoid develops. One, sometimes two, primary rhizoids form. Several other septate rhizoids develop later.

The anatomy of the thallus of Pelvetiopsis is similar to other Fucaceae. Cryptostomata are rare and caecostomata are absent.

Sterilized filtered seawater cooled to 6° to 9°C. provides a suitable medium in which Pelvetiopsis limitata may be grown in culture. A minimum light intensity of 250 foot-candles promotes satisfactory development.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

The author wishes to express her gratitude to the following organizations and individuals for assistance in making possible the research reported in this thesis: to the Archbishop McCarthy Assembly, Fourth Degree Knights of Columbus, Halifax, Nova Scotia for award of a post graduate scholarship (1958 - 1959); to the National Research Council for award of a bursary (1959 - 1960) and summer supplement (1960); to Dr. K. M. Cole for her invaluable criticism; to Dr. R.F. Scagel for his helpful suggestions; to Mr. T. B. Widdowson for his assistance in collecting experimental material; and to the Department of Biology and Botany of the University of British Columbia for use of facilities.

A Cytomorphological Study of *Pelvetiopsis limitata* (Setchell) Gardner.

A. Introduction

1. Objectives of the Investigation.

Although many members of the Fucales have been investigated thoroughly, there is very little literature concerning *Pelvetiopsis limitata* (Setchell) Gardner. Since the development of antheridia and oogonia of *Pelvetiopsis* has not been thoroughly discussed, a cytological study of the life cycle of one form, *Pelvetiopsis limitata* f. *limitata*, has been undertaken. The data collected are compared to those presented by other investigators for various other members of the family Fucaceae and the order Fucales. An attempt is made to establish the position of *Pelvetiopsis limitata* amongst related genera with regard to its cytological and morphological characteristics in order that the life cycle may be better understood.

2. *Pelvetiopsis* and related genera.

The genus *Pelvetiopsis* was established by Gardner in 1910. *Pelvetiopsis* was merely a new combination for the plant Setchell had first described in 1905 as *Pelvetia fastigiata* f. *limitata* (Collins, Holden and Setchell, 1905). In this early paper, Gardner described briefly the morphology of the plant. Further morphological details were later presented (Gardner, 1913; Setchell and Gardner, 1925). A somewhat superficial description of the vegetative tissues and reproductive structures had been made earlier by Holtz (1903) who identified the plant as *Pelvetia fastigiata* (J. Ag.) DeToni.

Although Pelvetiopsis and Pelvetia are similar in external vegetative characteristics, cytoplasmic divisions within the oogonium occur in a different manner in the two genera (Gardner, 1910, 1913). Pelvetiopsis resembles Hesperophycus harveyanus in its sexual development, but the two plants are quite different in gross morphology (Gardner, 1913). Thus it can be seen that the establishment of the genus Pelvetiopsis as distinct from other genera was necessary.

The genus Pelvetiopsis includes two species, P. limitata (Setchell) Gardner, and P. arborescens Gardner (Gardner, 1910, 1940). P. limitata has two recognized forms, P. limitata f. lata (Gardner, 1913) and P. limitata f. limitata (Gardner, 1913; Scagel, 1957). Pelvetiopsis limitata f. limitata occurs from Hope Island, B.C. to Carmel, California (Scagel, 1957). P. limitata f. lata grows in the area from Tomales Point to Monterey, California (Smith, 1944). P. arborescens is known only from Carmel, California (Gardner, 1940).

Pelvetiopsis limitata f. limitata is a small plant, 7 - 12 cm. high when mature. It is olive to brownish green in color, and bushy in habit (Fig. 1 and 2). The plant is differentiated into three areas, the holdfast, the stipitate region and the branches. The holdfast is irregularly conical in shape and about 6 - 9 mm. in diameter. One or more stipes may arise from the holdfast. There is no midrib. Each branch consists of a lower flattened portion arising from the repeatedly dichotomous stipitate region and a distally extended portion containing mucilage. The receptacles are usually bilobed. The receptacles become much enlarged during reproductive periods. The gametangia are borne in conceptacles located within the receptacles.

Related genera of the Fucaceae mentioned in the following discussion include Ascophyllum, Fucus, Hesperophycus, Pelvetia, Phyllospora and Xiphophora.

Included also are a few representative genera from other families: Hormosira and Notheia from the Notheiaceae; Bifurcaria, Cystophyllum, Cystoseira and Halidrys from the Cystoseiraceae; Carpophyllum, Coccophora, Sargassum and Turbinaria from the Sargassaceae; and Himanthalia from the monotypic Himanthaliaceae.

B. Literature Survey

1. General

Members of the Fucales do not produce spores, and have only a cytological alternation of generations; in these respects the Fucales are comparable to the Metazoa (Strasburger, 1897; Farmer and Williams, 1898; Yamanouchi, 1909; Svedelius, 1929). In the Fucales the extremely reduced haploid generation develops within the conceptacles of the diploid generation. The diploid chromosome number is restored at fertilization and maintained in the macroscopic generation. The haploid generation is represented by only the few nuclei in the oogonium and antheridium produced by meiosis and the mitotic division succeeding it (Fig. 3). Such haploid nuclei occur in the antheridium from the two- to sixty-four-nucleate stage, in the oogonium from the two- to eight-nucleate stage, and in the mature spermatozoids and eggs (Yamanouchi, 1909). Smith (1956), however, maintains that the thalli of the Fucales are sporophytes. According to his interpretation of the life cycle, spores which are produced in unilocular sporangia function as gametes.

2. Conceptacle formation

In the Fucaceae, conceptacles are initiated within receptacles located on the upper limits of the dichotomies. There are about 150 to 200 conceptacles in each branch of the bilobed receptacles in Pelvetiopsis limitata (Holtz, 1903).

Several workers have described the development of the conceptacle and its contents. Bower (1880) was the first to suggest that the conceptacle of Fucus has its origin from an epidermal cell of the receptacle; he wrongly concluded, however, that the initial cell later disintegrates and cortical cells complete the conceptacle. Holtz (1903) also concluded that cells from the

cortex of Pelvetiopsis limitata participate in the development of the conceptacle.

It was soon established (Nienburg, 1913) that the cortex is not involved in conceptacle formation of Fucales, but that the conceptacle is initiated by a single epidermal cell which becomes sunken in the thallus. Further study showed that the flask-shaped conceptacle initial cell divides into two cells by a transverse or curved wall. The outer cell so formed is known as the tongue cell, the inner cell is known as the basal cell. The first division of the conceptacle initial of Pelvetia canaliculata, however, is longitudinal and is followed by a transverse division producing two basal cells (Nienburg, 1913; Moore, 1928). Nienburg (1913) considered the tongue cell filament, which occurs in some of the Fucales, to be a manifestation of vestigial trichothallic growth. In the Fucaceae, the tongue cell grows no further, and does not contribute to the formation of the conceptacle.

3. Cryptostomata and caecostomata.

It was noted by Simons (1906) that conceptacles and hairpits (cavities in the receptacle containing hair-like structures) of Sargassum filipendula are homologous structures. Both structures are derived from individual epidermal cells. It was noted in Fucus that hairpits are capable of becoming sexual (Roe, 1916). Cryptostomata (sterile hairpits) have been observed in Fucus vesiculosus (Baker and Bohling, 1916), Sargassum filipendula (Simons, 1906), S. tenerrimum (Rao, 1946), Turbinaria turbinata (Blomquist, 1945), occasionally in Pelvetia fastigiata (Moore, 1928), and rarely in Fucus parksii (Gardner, 1940). In Notheia anomala, cryptostomata have been observed in the young plant, but they later disappear as the plant matures (Williams, 1923). No cryptostomata have been noted in Ascophyllum nodosum (Fritsch, 1945),

Bifurcaria tuberculata (Rees, 1933), Halidrys spp. (Fritsch, 1945) or Pelvetia canaliculata (Gardner, 1910). Cryptostomata have been reported to occur in young plants of Pelvetiopsis limitata (Gardner, 1913). Rao (1946) saw oogonial initial cells occurring in what he considered to be cryptostomata in Sargassum tenerrimum and concluded that these represent degenerate and non-functional conceptacles.

Caecostomata (completely closed cavities which lack hairs) are closely related to cryptostomata and conceptacles (Powell, 1957b). Gardner (1922) noted caecostomata in Fucus furcatus (= F. distichus subsp. edentatus (De la Pyl.) Powell). Since that time caecostomata have been found in all North American sub-species of Fucus distichus, and are now considered to be more common in the Atlantic sub-species (Powell, 1957a, 1957b). There are no caecostomata in the California species Fucus parksii (Gardner, 1940).

4. Reproductive structures.

Bower (1880) noted that male and female reproductive structures are morphologically identical in their early development. In Pelvetiopsis limitata as in other Fucaceae it has been noted that the antheridia and oogonia develop from cells which form the wall of the conceptacle (Holtz, 1903). Williams (1923) suggested that the oogonium is sporangial in nature and that Notheia anomala is always diploid. Neither she nor Mitchell (1893) found any indication of antheridia or of reduction division in the oogonium of Notheia. Williams (1923) seems to have been unaware of the work of Barton (1899) in which antheridia were identified.

4a. Production of eggs

In the Fucales the oogonia are usually stalked, and are seldom borne on paraphyses (Thuret, 1854). In some species, such as Sargassum tenerrimum, there is no stalk cell (Rao, 1946). Each oogonium of Fucales species has a wall composed of at least three layers before liberation of eggs takes place (Thuret and Bornet, 1878; Farmer and Williams, 1898). The outer portion of the oogonial wall of Pelvetia fastigiata is pectic, and the inner portion contains cellulose (Moore, 1928). Holtz (1903) claimed that the oogonial wall of Pelvetiopsis limitata has only two layers. It has been reported that discharged Fucus oogonia have five-layered walls (Resuhr, 1935). Three of these layers are firm, and they alternate with two softer layers. In Pelvetia canaliculata (Subrahmanyan, 1957b) an outer layer of the oogonial wall and four inner layers have been identified. The outer layer gelatinizes after the oogonium is released into the sea. From her studies of stained material Mitchell (1941) concluded that there are at least five layers in some regions of the wall of the oogonium of Xiphophora. Naylor (1954), however, studied living specimens of Xiphophora and concluded that there are only three layers in the oogonial wall.

The most primitive form of egg development is found in the genus Fucus where eight eggs are produced (Thuret, 1854; Thuret and Bornet, 1878). In all members of the Fucales, the nucleus of the oogonial initial divides three times producing eight potential egg nuclei. The first two divisions are reductional (Strasburger,1897; Farmer and Williams, 1898) and comprise the meiotic sequence (Yamanouchi, 1909). Following a short period of rest, the third nuclear division ensues (Farmer and Williams, 1898). This last division is mitotic (Yamanouchi, 1909). Not all of these resulting eight nuclei

differentiate into eggs within the mature oogonium (Oltmanns, 1889b; Thuret and Bornet, 1878). In most genera fewer eggs are produced. In Pelvetia two eggs are produced. In P. canaliculata the division of the oogonium into two eggs is parallel to the longer axis of the oogonium, and the six supernumerary nuclei are cast out on the surface (Oltmanns, 1889b). In P. fastigiata and P. wrightii the division of the oogonium into two eggs is parallel or oblique to the shorter axis of the oogonium and the extra nuclei are cast out between the eggs (Yendo, 1907; Gardner, 1910; Moore, 1928). Details on the number of eggs produced in certain other Fucales are presented in Table I.

Three nuclear divisions were observed in oogonia of Hesperophycus harveyanus and Pelvetiopsis limitata (Gardner, 1910). Gardner claimed that the mitotic division is not simultaneous in all nuclei in the oogonium. Of the eight resulting nuclei, seven migrate to that end of the egg which is closest to the conceptacle wall, and a septum forms, cutting them off from the larger, now uninucleate, portion. Thus, two egg cells are formed, one large and uninucleate, the other smaller with seven nuclei. Holtz (1903) was able to follow only the division of the oogonial mother cell nucleus to the four-nucleate stage in Pelvetiopsis limitata. He thought that the two eggs which form in the oogonium are equal in size, and are separated by a transverse wall. Both eggs escape from the oogonium. The two eggs of Pelvetia spp. are retained within a thick-walled mucilaginous oogonium which protects them from dry physical conditions enabling the plant to successfully reproduce in the upper intertidal zone (Oltmanns, 1889a; Isaac, 1933).

Some of the earliest chromosomal studies of Fucus and related genera were made by Farmer and Williams in 1896 and 1898. In their classic work they reported that the haploid number of chromosomes was approximately 10 - 12 in the oogonia of Fucus serratus and F. vesiculosus. They considered the number to be between 26 - 30 in the oogonium of Ascophyllum nodosum and 14 - 15 in the egg itself.

At late prophase of first division meiosis, Yamanouchi (1909) counted 64 chromosomes in oogonia of Fucus vesiculosus. At metaphase of the third division 32 chromosomes were observed, and also in the four nuclei at the end of meiosis II (Yamanouchi, 1909). Kunieda (1926, 1928) claimed that 16 was the haploid complement of chromosomes in Sargassum horneri oogonia, but it was later shown by Okabe (1929a) that there are 32 chromosomes. Nuclear division in the somatic cells was described in a later paper, and the diploid number was found to be 64 (Okabe, 1930). The results of other investigations of the chromosome numbers of various Fucales are presented in Table II.

The cytology of the Fucales, particularly that of the Sargassaceae, has been investigated by several workers. The first nuclear division in the oogonium of Sargassum is reductional (Tahara and Shimotomai, 1926). Synapsis and diakinesis are typical in Sargassum horneri (Okabe, 1929a). In meiotic divisions the nuclear membrane usually disappears when the metaphase plate forms. This is distinct from the mitotic divisions in the young embryo where the nuclear membrane does not disappear until anaphase (Okabe, 1930). There is no septum discernible at the rarely observed two-nucleate stage; the spindle apparatus in intranuclear (Shimotomai, 1928). The second division of the meiotic sequence follows soon after the first division is completed. The two daughter nuclei divide simultaneously in Sargassum (Tahara and Shimotomai, 1926).

4b. Production of Spermatozoids.

The antheridium, like the oogonium, arises as a projection from one of the cells which form the wall of the conceptacle. The antheridium is sometimes stalked as in Sargassum horneri (Kunieda, 1928), Fucus spp., Ascophyllum nodosum, Pelvetia spp., and Himanthalia sp. (Thuret and Bornet, 1878). In Pelvetiopsis the antheridia may be borne singly on a basal hair, or located on a branching hair. One to six antheridia may be found on each branching hair (Holtz, 1903). Antheridia of Ascophyllum, Fucus, Himanthalia (Thuret and Bornet, 1878), Hormosira (Osborn, 1949), and Pelvetia (Isaac, 1933) have two-layered walls, but those of Bifurcaria, Cystoseira and Halidrys have only one layer (Thuret and Bornet, 1878).

Meiotic and mitotic divisions within the antheridia of Fucales have been clearly outlined by several investigators. The first two divisions of the antheridial initial nucleus comprise the meiotic sequence. In Sargassum piluliferum a synizesis-like figure appears in late prophase of the first nuclear division (Inoh and Hiroe, 1954a). Typically, four mitotic divisions follow meiosis in the antheridia of Fucales. This has been demonstrated in Cystophyllum spp. (Shimotomai, 1928), Fucus vesiculosus (Yamanouchi, 1909), Pelvetia canaliculata (Subrahmanyan, 1957a), P. wrightii (Yabu and Imai, 1957), Phyllospora comosa (Williams, 1923), Sargassum horneri (Kunieda, 1926, 1928), S. piluliferum (Inoh and Hiroe, 1954c) and S. tenerrimum (Rao, 1946). Kunieda (1926) reported that these divisions are simultaneous in S. horneri. At the thirty-two to sixty-four-nucleate stages in Pelvetia canaliculata no cross-walls are formed in the cytoplasm (Subrahmanyan, 1957a). The formation of cross-walls in antheridial cytoplasm has been reported in Fucus (Yamanouchi, 1909; Kylin,1916; Richard, 1932), and Pelvetia fastigiata (Moore, 1928).

Smith (1944) appears to be the only investigator who claims that there are one-hundred twenty-eight, rather than sixty-four "microspores" (spermatozoids) formed in the "microsporangium" (antheridium) of Pelvetiopsis limitata and Pelvetia fastigiata. Holtz (1903) presumed that there were sixty-four nuclei produced in the antheridium of Pelvetiopsis although he counted approximately only forty himself.

The spermatozoid of Fucus is uninucleate. Guignard (1889) was among the first to see the spermatozoid of Fucus, which he described as a pear-shaped cell. It was first thought that a thin cytoplasmic envelope surrounded the nucleus (Kylin, 1916, 1920; Richard, 1932). Kylin reported that the main body of the spermatozoid has a blepharoplast, plastomere and chromatophore. It has been demonstrated recently that contrary to previous thought, the nucleus does not occupy most of the body of the spermatozoid (Manton and Clarke, 1951).

The mature spermatozoid is 4 - 5 microns long and 2.3 - 2.5 microns wide (Kylin, 1916). Two flagella of unequal length are laterally inserted in the cytoplasm. The shorter flagellum is directed forward (Richard, 1932). The longer of the two flagella is directed posteriorly. In Hormosira banksii the anterior flagellum is 10 microns long, and the other, 13.5 microns (Osborn, 1949). Manton and Clarke (1951) in their remarkable electron microscope study demonstrated the tinsellation of the anterior flagellum of Fucus serratus.

The first count of the number of chromosomes in the spermatozoid nucleus of Fucus was made by Strasburger (1897). He estimated that there were 30 chromosomes in the spermatozoid nucleus of Fucus platycarpus (F. spiralis L. var. platycarpus). Yamanouchi (1909) counted 32 chromosomes

in the spermatozoid nucleus of Fucus vesiculosus, as also did Yabu and Imai(1957) in the mitotic divisions following meiosis in the antheridial nuclei of Pelvetia wrightii. Subrahmanyan (1957a), however, reported 22 chromosomes as the haploid number in the antheridial nucleus of P. canaliculata.

5. Liberation of eggs and spermatozoids.

The mechanism responsible for liberation of sexual products is not clear. If receptacles of Pelvetiopsis are dried somewhat and then placed in sea water, liberation of eggs and spermatozoids will ensue (Gardner, 1910). Turgor pressure and the pressure of the swelling mucilage of the inner layers of the oogonial wall may be the vital factors (Oltmanns, 1889a; Resuhr, 1935). Pierce (1902) stated that liberation in Fucus was induced by mechanical pressure which developed within the plant itself, and was not dependent on drying followed by immersion. In Pelvetia fastigiata gametes are shed if plants are brought into the light after a period of darkness. As many as 10,000 eggs per receptacle have been released in a half-hour period (Jaffe, 1954).

Liberation of the egg fascinated many of the early workers such as Thuret (1854) and Farmer and Williams (1898). Release of the eggs of Fucus begins with the rupture of the exochiton, the outermost layer of the oogonial wall (Thuret, 1854). The mesochiton (medial layer) then bursts and slides back over the eggs, and the endochiton (the innermost layer surrounding the egg) dissolves. According to Subrahmanyan (1957b), the entire oogonium of Pelvetia canaliculata is released from the conceptacle, although Delf (1935) reported that in other Fucales the exochiton may rupture while the oogonium is still attached.

The spermatozoids are released by the rupture of the exochiton while the antheridium is still attached to the stalk cell. The endochiton dissolves after discharge from the conceptacle into the sea. In Hesperophycus harveyanus, however, the entire antheridium is released into the surrounding medium when the conceptacles are induced to liberate. Immature as well as mature antheridia are released (Walker, 1931).

6. Fertilization

Fertilization usually occurs after the eggs are released from the oogonium. In Pelvetia canaliculata, however, fertilization takes place within the oogonium (Subrahmanyan, 1957b). The eggs of several species increase in size after their release.

In Fucus spp. spermatozoids often surround the egg in numbers large enough to cause it to rotate at 40 - 50 gyrations per minute (Thuret, 1954; Farmer and Williams, 1898). One spermatozoid penetrates the egg cell membrane and the male nucleus slips through the membrane of the egg nucleus (Yamanouchi, 1909). Soon after this the fertilization membrane forms. The fertilization membrane of eggs of Fucus spiralis and F. vesiculosus is known from the studies of Levring (1947). The spermatozoid which fertilizes the egg causes a contraction of the surface of the egg. He postulated that the membrane of the unfertilized egg acts as a base upon which the fertilization membrane forms, and is a part of it.

Fertilization in Fucus may occur up to one hour after the spermatozoid has penetrated the egg (Yamanouchi, 1909). It has been reported that a second centrosome is associated with the entrance of the spermatozoid for it appears at the spot where the spermatozoid entered the egg (Strasburger, 1897; Yamanouchi, 1909). Farmer and Williams (1898) claimed that the centrosomes

which occur in the fertilized oogonia of Fucus serratus, F. vesiculosus, and Ascophyllum nodosum are not associated with the spermatozoid.

Polyspermy has been observed in Ascophyllum, Fucus and Hesperophycus (Farmer and Williams, 1898; Yamanouchi, 1909; Walker, 1931). Damman (1931) observed spindles with three and four poles in Fucus and suggested that these are evidence that polyspermy has taken place.

7. Hybridization

The possibility that hybridization occurs between certain algae has been investigated. As early as 1854 Thuret introduced hybrid formation by fertilizing eggs of Fucus vesiculosus with Fucus serratus spermatozoids. In 1908, Sauvageau reported the occurrence of the hybrid in nature. This cross and its reciprocal have since been made with over 90% successful germinations in both cases (Burrows and Lodge, 1951). In 1899, Williams reported that a hybrid had been induced in the laboratory between Fucus serratus and Ascophyllum nodosum. Whether or not this hybrid occurs in nature is not known as there are no further details on the appearance, structure, or behavior of the intergeneric hybrid. Whitaker (1931) established that there is no cross-fertilization between F. vesiculosus and A. nodosum, nor between F. evanescens and F. vesiculosus. A cross between F. platycarpus (= F. spiralis L.) and F. ceranoides was found in nature (Gard, 1910), and it was noted that the female organs in the hybrid were abnormal. Hybridization has also been reported as occurring between F. platycarpus (= F. spiralis) and F. lutarius (Sauvageau, 1909), and between F. spiralis and F. vesiculosus (Burrows and Lodge, 1951).

8. Embryo development

Farmer and Williams (1898) claimed that division of the Fucus zygote occurs twenty to twenty-four hours after the spermatozoid has penetrated the egg, although in some instances only thirteen hours elapse in F. serratus. The newly fertilized egg elongates and divides transversely to the plane of the developing rhizoid (Decaisne and Thuret, 1845; Oltmanns, 1889a). Three transverse walls are formed in the Fucus zygote dividing it into four cells. The cell distal to the differentiating rhizoid then divides into four cells by two longitudinal divisions (Oltmanns, 1889a; Nienburg, 1931). Segmentation of the embryo is fairly uniform in Fucus spp., and differs from other genera (Thuret, 1854; Oltmanns, 1889b; Nienburg, 1910).

Polarity of the egg is determined at first division (Whitaker, 1931). In most cases the nucleus of the fertilized egg divides first and the rhizoid then appears. However, sometimes the rhizoid appears first and the zygote assumes a pear-shape before karyokinesis occurs (Farmer and Williams, 1898).

Abe (1938) studied the division of the fertilized egg of Sargassum. He discovered that further division of the zygote nucleus takes place at the periphery of the cell rather than at the center. The segmentation divisions are not all simultaneous in the embryo of Sargassum piluliferum (Inoh and Hiroe, 1954a). The number of primary rhizoids varies in different species. The number of cells participating in rhizoid formation is related to the size of the egg (Inoh, 1932).

The upper cells of the embryos of Fucales undergo further anticlinal and periclinal divisions after the formation of a central core of cells and a peripheral layer (Oltmanns, 1889b; Nienburg, 1931). Although LeTouzé (1912)

claimed that there were only two tissues in the thallus, there are three distinct regions in the mature plant, the epidermis, the cortex and the medulla (Fritsch, 1945).

The apical meristem of members of the Fucales has its origin in a single cell of the thallus (Woodworth, 1888). In Fucus, a cell at the apex of the young plant sinks into the thallus as the cells around it continue to divide, forming a groove (Oltmanns, 1889b). The cell becomes differentiated into a hair with meristematic activity (Nienburg, 1931). Other cells in the apical depression then produce hairs (Nienburg, 1931), giving a tufted appearance to the young embryo. All of these hairs eventually disintegrate with the exception of the basal cell of the first-formed hair which becomes the apical cell (Oltmanns, 1889b). In other families of the Fucales, the apical initial cell remains three-sided. In the Fucaceae, however, it becomes four-sided at an early stage (Woodworth, 1888; Oltmanns 1889b). In Pelvetia and Ascophyllum there are no hairs formed in the apical groove (Oltmanns, 1889b).

Increase in width is accomplished by divisions of the much less-active cells of the epidermis, or meristoderm, as it is sometimes known (Oltmanns, 1889b). The dichotomies of Fucus arise as the result of the division of the apical initial into two lateral initials, each of which then assumes the function of an apical cell. In Ascophyllum where the thallus is much branched, several initials are cut off from derivatives of the apical initial. The growth and division of the surrounding cells cause them to become sunken in the thallus. These lateral initial cells then grow and divide, thus giving rise to the branches of the thallus (Oltmanns, 1922).

C. Materials and Methods

Specimens of Pelvetiopsis limitata f. limitata were collected near Botany Beach, Port Renfrew, British Columbia in June and November, 1959, and in May 1960. Material was also obtained from Clayoquot, B.C. during the summer of 1959, and from Long Beach and Amphitrite Point, B.C. in March, 1960. Some plants from each collection were kept in culture in the cold chamber at the University of British Columbia. Other plants were preserved in one of the following solutions:- (a) a 10% solution of formalin in sea water; (b) 3:1 fix --- 3 parts 95% ethyl alcohol to one part glacial acetic acid; (c) a modified (Fensholt, 1955) Karpetchenko's solution of chrom-acetic formalin, consisting of 2 solutions mixed in equal proportions just prior to use. When material fixed for 8 to 24 hours in 3:1 solution was not used immediately it was stored in 70% ethyl alcohol to which a small amount of ferric acetate had been added.

Mature and immature plants were cultured in a cold chamber in which the temperature was maintained at 6° - 9°C., with 12 hours of light and 12 hours of darkness per 24-hour day. Each plant was located so that it would be exposed to the same intensity of light each day. Different sections of the culture area provided light intensities of 16, 50, 125, and 250 foot-candles. Unsterilized filtered sea water was used initially, but filtered sea water, sterilized in an autoclave at a presure of 15 pounds per square inch for 20 minutes, and cooled to 6° - 9°C., provided a more suitable medium. Sea water was changed in the cultures every 5 - 7 days. Fungal contamination was controlled by quickly dipping infected plants in 95% ethyl alcohol, or in a weak solution of calcium hypochlorite.

In order to determine if material containing antheridia and oogonia was undergoing nuclear division and was profitable for future study, hand sections were made, both of living and fixed material, according to the technique outlined by Naylor (1957). When it was decided that the specimens were suitable for further examination, killed material was embedded in Tissuemat with a melting point 56° - 58°C. Embedded material was sectioned with a rotary microtome. Sections varying in thickness from 4 - 12 microns were mounted on glass slides. The mounted sections were dewaxed, hydrated in a xylene, ethanol, water series, then stained, dehydrated and mounted in gum damar from xylene.

Several stains were employed, among them, acetic-lacmoid, aceto-carmine, and Heidenhain's haematoxylin. A Feulgen technique as outlined by Naylor (1958) was also attempted. A satisfactory procedure for sectioned material was a modified aceto-carmine technique in which both the mordant, 2% ferric alum (ferric ammonium sulfate), and the aceto-carmine were heated to 60°C. The slides were mordanted 5 - 15 minutes, dipped in distilled water to remove excess mordant, then stained in the warm aceto-carmine for 10 - 20 minutes.

Fertile conceptacles were encouraged to liberate according to the method of Gardner (1910). The receptacles were permitted to dry for a few hours and were then immersed in sea water. The liberated oogonia were collected on slides. The mucilaginous nature of the conceptacle contents caused the eggs to adhere to the glass. These slides were then kept in culture for further observation, or else fixed in 3:1 alcohol-acetic-solution.

Photomicrographs were taken using Kodak High Contrast Copy 35 mm. film, which was developed in Kodak D-11, and fixed in Amfix. Prints were made on Kodabromide F-3 single weight paper, developed in Cobrol for 2 minutes and fixed in Amfix. Photographs of camera lucida drawings were made with Adox R 14 120 film, and printed on Kodak velox F-3 paper.

D. Observations

1. Cultures

Specimens of Pelvetiopsis limitata f. limitata grew favorably when cultured in filtered, sterilized sea water in a cold chamber where the temperature was maintained at 6° - 9°C. at the University of British Columbia. Those plants exposed to the highest light intensity (250 foot-candles) were healthier than plants grown in the same chamber which received only 16, 50 or 125 foot-candles illumination. Growth rates of specimens exposed to lower light intensities were not satisfactory and the contamination by diatoms and fungi were encouraged.

2. Anatomy of the thallus

In the mature receptacles of Pelvetiopsis limitata three distinct tissues are present, the epidermis, cortex and medulla (Fig. 4 and 5). The epidermis is one layer thick, and is protected by a mucilaginous layer, the "cuticle". The cells of the epidermis appear rectangular in cross-section (Fig. 5). They contain many ovoid chromatophores and fucosan vesicles. In a mature part of the receptacle the epidermal cell averages 28 x 16 microns in size and possesses a nucleus of about 5 microns in diameter.

The cells of the cortex are closely compressed and appear somewhat rounded in cross-section, with five to six sides (Fig. 5 and 6). The cortex is four to five cells thick and ovoid chromatophores are particularly abundant in the outer two layers of the cortex. These cells vary in size from 16 x 12 microns to 28 x 22 microns. Most cortical cells are about 25 x 20 microns with nuclei from 5 to 6 microns in diameter. Approximately 64 chromosomes were seen in one cell (Fig. 7 and 8).

There is no clear distinction between cortical and medullary tissue. The change is gradual rather than abrupt. The medullary cells form a network, although there are no hyphae in the receptacle medulla. The cells of the medulla are elongated vertically (Fig. 9 and 10) and have the appearance of sieve-tubes of higher plants. Simple pit connections occur (Fig. 9). There is abundant mucilage in the intercellular spaces. The nuclei of medullary cells are about 6 microns in diameter.

3. Conceptacle formation

Many conceptacles are embedded within the receptacles of Pelvetiopsis limitata (Fig. 11). During periods of fertility the receptacles become swollen and bloated, and sometimes are larger than the remainder of the plant. In a single cross-section of the receptacle of a fruiting specimen, as many as thirteen conceptacles may be observed; the average number per cross-section is about six.

The conceptacle is initiated, as in other Fucaceae, by a single epidermal cell. The conceptacle initial has a retarded rate of cell division, and becomes embedded in the thallus, remaining behind when the neighboring cells adjoining it grow and divide. As the surrounding cells continue their growth, they separate from the conceptacle initial. A groove is thus formed with the initial cell at the base (Fig. 12). The initial cell divides transversely, forming an inner base cell and an outer tongue cell. The upper cell does not divide any further. It may detach from the basal cell and disintegrate later. The cells lining the floor of the conceptacle are derived from the initial cell. The wall of the conceptacle may be three or four cells thick as the result of cell division (Fig. 11). The nuclei of the wall cells are approximately 5 microns in diameter.

The mature conceptacle is a flask-shaped cavity which contains oogonia, antheridia and paraphyses (hair filaments) (Fig. 11). These paraphyses are particularly abundant at the ostiole, but do not project through it.

Cryptostomata were observed in only a few instances, and in very immature receptacles. All samples containing these cryptostomata also contained conceptacles with mature oogonia and antheridia. No caecostomata were observed.

4. Oogonia and antheridia

Oogonia and antheridia of Pelvetiopsis limitata were seen in both living and fixed material from all collections. Oogonia and antheridia arise as papillae from cells which form a part of the conceptacle wall. (Fig. 13 and 14). The cell nucleus divides mitotically and one daughter nucleus remains in the lower part of the papilla while the other assumes a position near the distal end (Fig. 15 and 51). A cross-wall then develops between them. The lower cell thus formed becomes the stalk cell, and the upper cell becomes an oogonial or antheridial mother cell.

4a. Oogonia

A study of fixed material collected in the summer of 1959 at Port Renfrew, B.C. yielded observations which were not paralleled in any preceding or subsequent collection. The eggs of this material were at a much more mature stage than those of any other material examined, and details of nuclear activity prior to fertilization were noted.

Differentiation of the oogonium of Pelvetiopsis limitata begins as the oogonial initial cell which is attached to the wall of the conceptacle, enlarges. The nucleus may increase from 6.4 up to 15.5 microns in diameter.

Two divisions of the nucleus ensue, presumably comprising the meiotic sequence. This cannot be stated definitely as no chromosome count could be made during the first division. As few oogonia were seen in the two-nucleate stage it was concluded that these nuclear divisions take place in rapid sequence (Fig. 16 - 20). There is a brief resting stage between the first and second nuclear divisions when the nuclear membrane reforms (Fig. 17). At this resting stage the cytoplasm is very dense, and the chromatin material in the interphase nucleus is very weakly stained. The chromatin is diffuse at this stage, and individual chromosomes can no longer be distinguished. In some cases a single nucleolus is the only deeply staining body observable in the nucleus.

The second nuclear division of meiosis occurs simultaneously in both of the nuclei in the oogonium (Fig. 18). The nuclear membrane disappears early in prophase II, and the chromosomes pass rapidly from this stage into metaphase. No chromophilous spherule was observed. The chromosomes are very condensed at this stage, and form the plate in the equatorial region in a regular manner. The metaphase II spindle structure is about 10 microns from pole to pole of the two simultaneously dividing nuclei (Fig. 19 and 20). The equatorial plate is 7.2 microns at its widest point. In metaphase II the two nuclei are not orientated with regard to one another in the oogonium. The spindle fibers are very distinct. No centrosomes occur, and there is no indication of astral ray formation. There is no precocious movement of chromosomes towards the polar regions. At the metaphase II stage of division the oogonium measures 35.8 x 23.6 microns.

No septa are formed at either the two-nucleate or four-nucleate stage in the developing oogonium. There is a brief resting period at the four-nucleate stage prior to the final division into eight nuclei (Fig. 21). At this time the nuclei are in close association. They stain very lightly with aceto-carmine and become very translucent. The nucleolus is the most distinct body in the nucleus at this stage.

The third division within the oogonium is mitotic and results in the formation of eight nuclei (Fig. 22). At this time once again the nuclei are closely associated. At first they stain darkly with aceto-carmine, then the chromatin tends to become almost invisible as seven of the nuclei migrate to the end of the oogonium closest to the stalk cell (Fig. 23). During division stages of the nuclei, the cytoplasm of the oogonium occasionally absorbs some of the stain.

A cross-wall forms in the basal part of the oogonium (Fig. 24 and 25). The plane of cleavage is usually perpendicular to the longer axis of the oogonium, but it is sometimes oblique (Fig. 44). It first appears as a thin line in the central region of the lower quarter of the oogonium. This cross-wall gradually thickens and eventually extends across the lower part of the oogonium. As the oogonium matures the two eggs produced separate from one another where the cross-wall formed (Fig. 27 - 29). The space between the two eggs may be 3.1 microns wide. The larger egg formed by the cleavage of the cytoplasm has an eccentrically placed nucleus 10 microns in diameter. The smaller egg contains seven nuclei of equal size, ranging from 5.7 - 10 microns in diameter, and averaging 6.8 microns.

The small egg is limited in volume and the seven nuclei are necessarily closely situated to one another within the cell (Fig. 30). Often a single nucleolus is discernible in each of the nuclei. The nucleolus may be 1.9 - 2.1 microns in diameter.

Sometimes the stalk cell of the mature oogonium is partially embedded in the wall of the conceptacle. The stalk cell averages 31 x 16 microns (Fig. 25). The wall of the oogonium has at least four and perhaps five layers (Fig. 26). The outer wall, or exochiton, is firm, and the other layers are thinner. The size of a mature oogonium varies from 102.3 x 55.8 microns to 111.6 x 69.8 microns. There may be as few as one, or as many as thirty-two oogonia per conceptacle. The average number per conceptacle is six oogonia.

The eggs of Pelvetiopsis limitata vary in size before and after liberation from the conceptacle. Before its release, the large mature egg is 71.3 x 49.6 microns to 86.8 x 64 microns, while the smaller egg is from 15.5 x 26.4 microns to 21.7 x 37.2 microns. While it is still contained and compressed within the oogonium in the conceptacle, the smaller seven-nucleate egg often appears wedge-shaped in longitudinal section (Fig. 25). After its release from the oogonium the large uni-nucleate egg may increase in size from 90 to an average diameter of 108 microns (Fig. 75). The large egg may be as much as 155 microns in diameter after its liberation. The nucleus may increase from 9 - 16 microns in diameter. After liberation the smaller egg is less compressed, becomes rounded (Fig. 28 and 29), and increases from 33 - 50 microns in diameter, averaging about 43 microns. The nuclei of the seven-nucleate eggs are 5.6 - 7.8 microns in diameter both before and after liberation (Fig. 30).

The nucleus of the mature egg migrates from the center of the oogonium while it is in the resting condition (Fig. 31), and is still attached to the stalk cell. When it reaches the periphery of the egg , several granular, densely staining bodies may be observed (Fig. 32 - 37). The number of these chromocenters varies from 4 - 16. The size of these particles varies; there may be discerned several smaller, dot-like granules as well as one or two larger bodies (Fig. 33). There is a tendency of these clumps of chromatin to exhibit polarization similar to synizesis (Fig. 33 and 34). A number of distinct granules was also seen (Fig. 34 - 37). At a later stage a number of chromosomes appear as the egg enters prophase in preparation for division even before fertilization occurs. Approximately 32 chromosomes were counted in the nuclei of mature eggs, by careful examination of the material, and from photomicrographs which were taken at several levels in the plane of vision (Fig. 38 and 39).

While cleavage of the cytoplasm within the oogonium usually results in the formation of a large uninucleate egg and a smaller egg with seven nuclei, aberrant forms have been noted. In some oogonia two equally-sized eggs were formed (Fig. 40 - 42). Three, four and possibly five eggs of various sizes have also been observed in one oogonium (Fig. 45 - 50). In addition, binucleate eggs occur (Fig. 43 and 44).

4b. Antheridia

The antheridium of Pelvetiopsis limitata is initiated in the same way as the oogonium. It may be recognized at an early stage in its development by its affinity for aceto-carmine stain. The antheridium is stalked, arising singly from the wall of the conceptacle, or on branched paraphyses (Fig. 51 - 53).

The wall of the antheridium is composed of two layers which are united at the base of the antheridium. The mature antheridium is from 34.1 x 17.0 microns up to 57.2 x 23.0 microns in size. At the distal end of the antheridium there is a space of 4 - 7 microns between the wall and the cytoplasm in which the nuclei are embedded. In the maturing antheridium this space has the appearance of an apical cap.

Antheridia are more plentiful than oogonia in the conceptacle. Sometimes the antheridia are small and immature while the eggs appear to be ready for liberation. As many as thirteen antheridia may be observed on the same branch system, but five per hair is the average number (Fig. 74).

The first three divisions in the antheridium are very similar to those in the oogonium. The first and second divisions follow rapidly on one another and are probably meiosis I and II (Fig. 54 - 59). Before division the antheridial initial nucleus measures 5.0 - 6.2 microns in diameter. The metaphase I spindle apparatus is about 10 microns long. The chromosomes are very small and are compactly arranged. At prophase II the nuclei are 4.2 - 5.4 microns in diameter. At metaphase of second division approximately 30 chromosomes may be counted in polar view (Fig. 58 and 59). No chromophilous spherules or chromocenters were observed in connection with divisions in the antheridium. The chromosomes of the nuclei of the oogonium and antheridium can be counted with accuracy only at metaphase II in polar view, and in the preparatory prophase of the mature egg. At metaphase the chromosomes form a solid plate which is small in area.

After the formation of four nuclei in the antheridium, no resting stage occurs. Each of the nuclei measures approximately 4.6 microns in diameter. The four nuclei divide simultaneously and equationally into eight nuclei about 4 microns in diameter (Fig. 60 and 61). Further divisions follow from eight to sixteen, sixteen to thirty-two, thirty-two to sixty-four nuclei (Fig. 62 - 72). The size of the nuclei in the antheridium varies at different stages of development. They are largest at the one-nucleate stage, and decrease with each successive division, becoming smallest at the sixty-four-nucleate stage, when they are about 2 microns in diameter.

Division of the nuclei is usually, but not always, simultaneous (Fig. 56). In anaphase of mitosis, the chromosomes separate into two groups (Fig. 65). These groups of chromosomes are often curved at the outer edge of the spindle apparatus, giving a crescent-shaped appearance as the chromosomes are pulled to the poles. No individual chromosome was observed pulling away from the main group. In anaphase each of these clumps has the appearance of a flat plate with curved edges. Cross-walls do not form in the cytoplasm after the nuclear divisions have taken place. Cross-walls are not distinguishable at the thirty-two and sixty-four-nucleate stages. The sixty-four nuclei and surrounding cytoplasm differentiate into spermatozoids within the antheridium before release of gametes takes place.

The spermatozoid of Pelvetiopsis limitata is about 2.0 - 2.2 microns long. It has a pear-shaped body composed largely of nucleus. A chromatophore can be distinguished. No eyespot was seen. There are two flagella of unequal size which are laterally inserted (Fig. 73). The shorter flagellum is directed anteriorly. It could not be discerned if the flagella are tinsellated.

5. Liberation of gametes

Gametes of Pelvetiopsis limitata are liberated in groups at different times. When receptacles, which have been kept in the dark and dried for four to twelve hours, are immersed in sea water, liberation of eggs and spermatozoids takes place. Some immature as well as mature gametes are released. The exochiton of the oogonium bursts and the two unequal eggs are released from the conceptacle with the inner layers of the oogonial wall persisting. They pass through the ostiole one at a time. A second layer pulls back over the eggs and is discarded. The third layer of the wall ruptures. The fourth membrane dissolves and the two eggs are released (Fig. 29, 30 and 75). The two eggs then separate from one another. The freed eggs in some cases attach to the outer wall of the receptacle.

The spermatozoids are released from the antheridium by the rupture of the exochiton and are expelled from the conceptacle in a jelly-like mass. The endochiton dissolves and the spermatozoids swim away.

6. Fertilization

Living material of Pelvetiopsis limitata was induced to liberate gametes and examined in order to study fertilization. In some cases gyration of the egg is discernible in the process. One spermatozoid may enter the egg, and within half an hour a fertilization membrane forms. There are no centrosomes formed in the egg which could be correlated with the entrance of the sperm.

7. Embryo development

The first division of the fertilized egg of Pelvetiopsis limitata occurs within twenty-four hours after the release of gametes. The zygotes sink to the bottom of the culture medium and attach to slides previously placed there. There is a tendency for embryos to associate in groups. In a few cases embryos were observed growing on the receptacle surface.

Polarity of the zygote is determined at the first nuclear division. The zygote becomes pyriform before division of the cytoplasm takes place (Fig. 76). The first two cytoplasmic divisions following karyokinesis are perpendicular to the plane of the developing rhizoid (Fig. 77). Another division in the same plane follows. Then the upper cell of the apical part of the embryo divides anticlinally by two divisions into four cells (Fig. 78) forming an eight-celled embryo. Vertical division of the uppermost cell may occur at the two-, three-, or five-cell stage. In some instances the first division of the zygote occurs before the primary rhizoid appears.

A primary rhizoid initial is usually differentiated before the zygote undergoes cytokinesis (Fig. 76). The rhizoid divides a few times before more rhizoids appear (Fig. 80). Within the first four days following fertilization the embryo undergoes periclinal and anticlinal divisions which cause it to elongate (Fig. 79 and 80).

Although at first there is only one rhizoid in Pelvetiopsis limitata usually seven more may be found at a later stage (Fig. 81).

In older embryos there is sometimes a bifurcation or trifurcation of one or more of these rhizoids (Fig. 80 and 81). In some cases two primary rhizoids appear (Fig. 82). In normal embryos, rhizoids grow on the side of the embryo which is farthest removed from the source of illumination. When several embryos are grouped together this is not observed.

As cell division occurs, and the embryo grows, it loses its spherical shape and becomes elongated. The upper end of the embryo always remains larger (Fig. 79 and 80). Embryos raised to the age of four months in the cold chamber under previously mentioned culture conditions do not show any hairs in the apical distal region, although an apical groove is distinguishable. At this stage it is impossible to determine the number of rhizoids as they are all branched and much intermeshed.

The upper cells of the rhizoid closest to the main body of the maturing embryo divide periclinally. The cells of the embryo are much elongated. The nucleus is about 6 microns in diameter and the cell contents are less concentrated than those in the cells of the main body of the embryo. Often the walls separating the cells of the rhizoids are oblique rather than at right angles to the longitudinal axis of the embryo (Fig. 79). Each cell of the upper surface of the embryo has a centrally-located nucleus and dense cytoplasm containing chromatophores and fucosan vesicles.

Unusual growth of the seven-nucleate egg of Pelvetiopsis limitata occurs. This so-called "non-functional" egg usually disintegrates after extrusion from the oogonium. In some cases, however, it appeared to have divided to produce an abnormal embryo (Fig. 83), which did not persist past the five-cell stage.

E. Discussion

1. Culture conditions and natural environment

The slow growth of Pelvetiopsis kept in culture in the cold chamber with a constant temperature of 6° - 9°C. is believed to be atypical. It is reasonable to expect that the organism would not thrive under culture conditions as it would have done under natural conditions. In its natural environment Pelvetiopsis is exposed to a maximum light intensity of 7500 foot-candles, whereas the highest light intensity in culture was 250 foot-candles. Factors such as the rise and fall of the tides, ocean spray and seasonal variation could not be imitated in the cold chamber. In its habitat, Pelvetiopsis is submerged only 1% - 5% of the time (Widdowson, personal communication, 1959), however, in culture it was submerged for the major part of its existence. Since Pelvetiopsis grows in the higher intertidal zone, maximum emergence is favored to the extent that the organism may almost be considered to be more terrestrial than marine.

2. Early development of the conceptacle initial

In Pelvetiopsis, as in other Fucaceae, the conceptacle initial is a flask-shaped cell. In most Fucales the conceptacle initial usually divides by a transverse division into an outer tongue cell and an inner base cell. Pelvetiopsis limitata is very similar to Fucus in that the tongue cell does not elongate or divide to form a filament (Nienburg, 1913). Pelvetiopsis differs from Pelvetia fastigiata in that the first division of the conceptacle initial into two cells is transverse rather than vertical and no tongue cell is formed (Moore, 1928).

3. Mitosis and meiosis

Mitosis and meiosis in somatic and reproductive cells appear to be quite regular in Pelvetiopsis as in other Fucales. The metaphase plate is very small and compact. The division of the chromosomes takes place without any apparent abnormalities. No laggards or precocious chromosomes were observed. Astral rays and centrosomes, typical of a more primitive state, are lacking in the nuclear divisions of the oogonium and antheridium, indicating an advancement on the evolutionary scale.

Centrosomes have been observed in the nuclei of developing oogonia of Ascophyllum nodosum (Farmer and Williams, 1898), Cystophyllum sisymbrioides (Shimotomai, 1928), Fucus evanescens (Inoh, 1935), F. serratus, F. vesiculosus (Strasburger, 1897), Pelvetia wrightii (Inoh, 1935), Sargassum filipendula (Simons, 1906), S. horneri (Okabe, 1929a), and S. enerve (Tahara and Shimotomai, 1926). They have also been observed in antheridia of Hizikia fusiformis (Inoh and Hiroe, 1954b) and Sargassum horneri (Hiroe and Inoh, 1954a) and embryos of S. horneri (Okabe, 1930) and Pelvetia canaliculata (Subrahmanyan, 1957b). No centrosomes, however, have been seen in the antheridia of Sargassum confusum (Abe, 1933), S. piluliferum (Inoh and Hiroe, 1954c), or S. tortile (Hiroe and Inoh, 1956), in the oogonia of Coccophora langsdorfii (Tahara, 1929) and Hesperophycus harveyanus (Walker, 1931) or in the embryo of Sargassum piluliferum (Inoh and Hiroe, 1954a). Walker (1931) reported observation of astral rays in Hesperophycus.

The so-called "chromophilous spherule" was not seen in the nuclei of antheridia or oogonia of Pelvetiopsis. Although chromophilous spherules have not been seen in oogonia of Fucus evanescens (Inoh, 1935), or in antheridia of Halidrys siliquosa (Naylor, 1958), they have been observed in contact with the nucleolus and aggregating chromosomes in some other species. Chromophilous

spherules have been seen in dividing oogonia of Coccophora langsdorfii (Tahara, 1929), Carpophyllum flexuosum (Dawson, 1940), Halidrys siliquosa (Naylor, 1958) and Sargassum horneri (Okabe, 1929a). These structures have also been reported in antheridia of Sargassum piluliferum (Inoh and Hiroe, 1954c), S. horneri (Hiroe and Inoh, 1954a), and Hizikia fusiformis (Inoh and Hiroe, 1954b).

4. Chromocenters in the mature egg

The many dark, densely-staining bodies within the membrane of resting nuclei of Pelvetiopsis are probably chromocenters. Chromocenters are portions of the chromatin network which stain during interphase. Darlington and LaCour (1941) observed very similar bodies in the liliaceous species Fritillaria pudica. These granules exhibiting heteropycnosis are composed of heterochromatin, and are believed to represent inert parts of chromosomes. Naylor (1958) noted the similarity of her observations on Halidrys siliquosa to those of Darlington and LaCour. Photomicrographs of Pelvetiopsis showing chromocenters are very similar to Naylor's photomicrographs of Halidrys.

Chromocenters were observed only in oogonia of Pelvetiopsis collected in November, 1959, at Port Renfrew. It is possible, therefore, that the egg nucleus remains in the resting stage in the late fall months. The nucleus of the mature functional egg then enters prophase preparing for the first division of the zygote before the spermatozoid enters. This is in agreement with the observations made on Halidrys (Naylor, 1958). A condition similar to this exists in Pinus spp. where the egg nucleus enters the preliminary stages of mitosis before the pollen tube reaches the egg (Ferguson, 1901). In both Pelvetiopsis and Halidrys the number of chromocenters

varies and is not related to the number of chromosomes. The author's investigations, however, show several differences between Pelvetiopsis and Halidrys. In Pelvetiopsis, unlike Halidrys, there are no chromocenters in the antheridial nuclei. The chromocenters persist in the oogonium of Pelvetiopsis until prophase, whereas in the oogonium of Halidrys they disappear before prophase begins. In Halidrys 2 - 9 chromocenters were distinguishable, while 4- 16 were seen in Pelvetiopsis.

5. Variations in the production of eggs

The number of eggs produced in Pelvetiopsis may vary from two to five, and the eggs may also differ in size. These observations have been paralleled in Pelvetia (Gardner, 1910), and in Ascophyllum (Farmer and Williams, 1896). In Pelvetia spp., two eggs of equal size are usually formed, but three, four and five eggs are known in Pelvetia fastigiata (Gardner, 1910; Moore, 1928). Four eggs have also been seen in a tetrad in P. canaliculata and P. wrightii (Gardner, 1910; Yabu and Imai, 1957). Binucleate eggs occur in Pelvetia as well as in Pelvetiopsis (Gardner, 1910). In Ascophyllum four eggs are usually produced, but a fifth was seen by Farmer and Williams (1896).

There are two possible explanations which may account for the observation of two eggs of equal size in the oogonium. Sometimes the cleft between the two eggs of Pelvetiopsis is oblique rather than horizontal. In longitudinal section, only a part of the oogonium is observable, and there is only one view of the oblique cleft between the eggs. In this situation the cleft might appear to divide the oogonial contents into two equal eggs. In reality, one may be examining a section which has passed through the upper end of the oblique division between the two eggs. If serial sections have been

made, this situation can be corrected by observing preceeding and subsequent sections. As this, however, does not account for the appearance of three and four eggs in the oogonium of Pelvetiopsis, it is more probable that these are aberrant forms of eggs.

The variations from the accepted normal condition of two unequally-sized eggs in Pelvetiopsis might, on first glance, be considered as an error in division of the cytoplasm caused by some external factor. Every one of these "abnormalities" occurred in all experimental material which was examined, but most frequently in cultured material. It is suggested, therefore, that these unusual oogonial divisions are phenotypic differences only resulting from various responses of genotypes to environmental conditions. Environmental agents such as light, heat, nourishment, and substrate, may contribute to the establishment of threshold conditions by which a character, such as the tendency to form extra cross-walls in the oogonium, may be expressed. Not enough data are known to substantiate these ideas, and they are beyond the scope of this investigation. It is suggested that controlled experiments and statistical analysis of samples of Pelvetiopsis would be of value in elucidating the cause or causes of this phenomenon. It should not be assumed that all variations in cytokinesis are the results of external modifying agents, however, cytokinesis is more susceptible to environmental changes than karyokinesis. As far as the author has been able to discern, little is known of the criteria of analysing genetic variations among the marine algae.

6. Comparison of gametangia and gametes of Pelvetiopsis limitata with other species of Fucales.

The development of oogonia in the Fucales is similar until the eight-nucleate stage. The first two nuclear divisions in an oogonial initial

cell of a member of the Fucales comprise the meiotic sequence and are followed by a mitotic division to produce eight nuclei. The oogonial wall is composed of at least three layers and four were seen in Pelvetiopsis. Pelvetiopsis (Fig. 84) and Hesperophycus are the only genera in which two eggs of unequal size are produced (Gardner, 1910). In other genera as few as one and as many as eight eggs are produced (see Table 1). The occurrence of a small "non-functional" egg with seven nuclei in Pelvetiopsis can be compared to the extrusion of supernumerary nuclei in Pelvetia and Ascophyllum (Oltmanns, 1889a).

The oogonia and eggs of Pelvetiopsis are of medium size compared to those of related species of the Fucales (Tables IV and V). The oogonium is smaller than that occurring in Sargassum (Fensholt, 1955), Turbinaria (Blomquist, 1945), Hormosira (Osborn, 1949), and Carpophyllum (Dawson, 1940) spp., but larger than Cystoseira (Fensholt, 1955) and Notheia spp. (Barton, 1899). The large egg of Pelvetiopsis before liberation is about the size of a Fucus egg, being 87 x 64 microns. The large egg increases to as much as 155 microns, and the smaller up to 50 microns in diameter after their release. Both large and small eggs are greater in size and volume than the related Hesperophycus harveyanus in which the large egg measures 11 microns in diameter, and the smaller, 2.6 microns (Walker, 1931).

The eggs are liberated in much the same fashion in all genera. The release of the large egg of Pelvetiopsis from the inner membranes of the oogonium wall is very similar to Himanthalia (Gibb, 1937). In both genera after the two outer layers of the wall of the oogonium have disappeared and only the inner layer persists, a protuberance develops on one side of the periphery of the remaining oogonial membrane. A small rupture occurs and the cell contents pour through the opening leaving the membrane behind (Gibb, 1937).

The development of antheridia in Pelvetiopsis (Fig. 84) as in other Fucales follows the same sequence in all genera. The size of antheridia seems very similar in many genera. Antheridia of Pelvetiopsis, Bifurcaria, and Hormosira are between 30 - 57 microns long x 17 - 23 microns wide, while those of Himanthalia are longer and narrower (Table VI).

In the Fucales (Yamanouchi, 1909) as in Pelvetiopsis the first two nuclear divisions of the antheridial initial nucleus are meiotic, followed by four mitotic divisions producing sixty-four nuclei. Each spermatozoid is formed by the modification of one of these sixty-four nuclei and the cytoplasm surrounding it. The spermatozoids show considerable variation in size amongst the various genera (Table VI). The length of the spermatozoid of Pelvetiopsis (2 - 2.2 microns), is amongst the smallest recorded in the Fucaceae, and is more similar to the spermatozoid of the distantly-related Halidrys in size than to the closely-related Fucus.

Spermatozoids of Pelvetiopsis do not have eyespots. Eyespots have been observed in spermatozoids of Fucus spp. (Kylin, 1916, 1920), Coccophora langsdorfii (Shimotomai, 1928) and Pelvetia canaliculata (Subrahmanyan, 1957b) but not in Cystoseira spp. (Sauvageau, 1911).

In most genera of the Fucaceae, including Pelvetiopsis, the release of spermatozoids from the two-layered antheridial wall begins with the rupture of the exochiton while the antheridium is still in the conceptacle. In Hesperophycus, however, it occurs after the antheridium has escaped from the conceptacle (Walker, 1931). In most Fucaceae (Thuret & Bornet, 1878), as in Pelvetiopsis, the inner wall of the antheridium then gelatinizes and the spermatozoids are freed.

7. Development of the liberated eggs and spermatozoids

The liberation of gametes from the conceptacles of Pelvetiopsis seems to be a reaction to 2 stimuli, light and water (Oltmanns, 1889a; Pierce, 1902; Resuhr, 1935). As soon as the eggs are liberated they begin to swell. In remarking that the eggs of some species are larger than others, Inoh (1932) suggested that those species with larger eggs are higher in the evolutionary scale. The swelling of the liberated egg is probably of advantage in at least two ways. It exposes a larger surface to which the spermatozoid may be attracted, thus increasing the probability that fertilization will take place. Also as the fertilized eggs contain chromatophores there will be an increase in photosynthesis and a subsequent increased food supply.

As has been observed in Fucus serratus (Manton and Clarke, 1951), the spermatozoids of Pelvetiopsis limitata are not motile when they are first released from the antheridium. Within a very brief period the flagella uncoil and the spermatozoid is able to move about freely.

In most Fucales fertilization occurs soon after sexual products have been released. In Pelvetia canaliculata, Subrahmanyan (1957b) has observed fertilization taking place within the oogonium. Spermatozoids of Pelvetiopsis cluster about an egg, one penetrates the cell membrane, and its nucleus fuses with the egg nucleus as in Fucus (Yamanouchi, 1909).

The fertilized eggs of Pelvetiopsis develop in the same manner as other Fucaceae. It seems that more than one cell is capable of responding to conditions which favor the growth of rhizoids. Usually one primary rhizoid occurs in Pelvetiopsis but sometimes two form. In this latter case, it is suggested that some factor or factors was altered in the early growth of the first rhizoid. It has been found that several agents may influence the

developmental axis of embryos of the Fucaceae. Farmer and Williams (1898) made the basic observation that rhizoids of Fucus were formed on the side of the embryo which was farthest away from the source of illumination. Proximity to nearby eggs causes the embryo rhizoids to grow towards these neighbouring cells (Whitaker, 1931). It has also been found that temperature gradients can affect the polarity of eggs which have been grown in the dark; the rhizoids appear on the warm side of the zygotes (Lowrance, 1937). Centrifugation of fertilized eggs of Pelvetia fastigiata and Fucus serratus grown in the dark causes some to form rhizoids near the centrifugal poles (Lowrance and Whitaker, 1940). Jaffe (1958) found that zygotes of Pelvetia and Fucus have a "polarotropic response". Rhizoids will grow 90° - 135° away from a source of polarized light. As many as 50% of the embryos raised under these conditions have rhizoids growing at both poles. Other factors affecting polarity include unilateral light, pH gradient, and electrical current (duBuy and Olson, 1937). It has been established that polarity of the egg is not affected by the spermatozoid in Sargassum piluliferum, as eggs can develop parthenogenetically after treatment with butylic acid and calcium carbonate in sea water (Hiroe and Inoh, 1954).

In Table III the number of primary rhizoids formed in various genera is recorded. If larger than one or two, the number appears to be a multiple of four. The number of primary rhizoids is directly correlated with the size of the egg and complexity of thallus structure (Inoh, 1932). In this respect Pelvetiopsis has a more primitive type of development as only one primary rhizoid, sometimes two, occurs. Other Fucaceae may have as many as four primary rhizoids. In the Cystoseiraceae, the number may be as high as thirty-two.

In some unusual cases, the small egg of Pelvetiopsis developed a rhizoid. In those embryos examined, usually only two or three nuclei could be discerned in the main body cell of the embryo. Perhaps this state follows fusion of the spermatozoid nucleus with one of the seven nuclei of the small egg before the disintegration of all of the six remaining nuclei. This condition would be somewhat analogous to that in Sargassum spp. where the spermatozoid nucleus fuses with one of the eight nuclei of the large egg and the other seven disintegrate (Kunieda, 1926; Tahara and Shimotomai, 1926). Two other possibilities exist. The small egg might undergo parthenogenetic development, and most of the nuclei disintegrate with the exception of the one or ones functioning. The other possibility is that spermatozoid nucleus may not yet have fused with the egg nucleus. Unfortunately no chromosome counts are available to confirm or deny these postulations. As this abnormal embryo soon aborts and dies, it may be stated that the small eggs cannot grow under the standard culture conditions employed in this investigation.

8. The Systematic Position of Pelvetiopsis

Pelvetiopsis is closely related to three genera in the Fucaceae, Pelvetia, Hesperophycus and Fucus (Table VII). In gross morphological structure, Pelvetiopsis is similar to Pelvetia in the shape of the thallus and lack of a midrib. Cryptostomata found occasionally on immature Pelvetiopsis sometimes occur in Pelvetia fastigiata, but not at all in P. canaliculata. In Pelvetia as well as in Pelvetiopsis there may be one or two primary rhizoids, except in Pelvetia wrightii when there are commonly four (Inoh, 1935). Cytologically Pelvetiopsis and Pelvetia have similar features in oogonial development. The septa form transversely or obliquely except in the oogonium

of P. wrightii where they form vertically (Yendo, 1907). Differences in the two genera occur. The thallus of Pelvetiopsis is much smaller than Pelvetia and is lighter in color. There are two eggs of unequal size, one is uninucleate while the other is seven-nucleate (Gardner, 1913), while in Pelvetia (Oltmanns, 1889a), both eggs are equal in size and uninucleate. In Pelvetia the six supernumerary nuclei are extruded from the cytoplasm (Oltmanns 1889a; Gardner, 1910). Centrosomes occur in Pelvetia (Subrahmanyan, 1957b), but in Pelvetiopsis no centrosomes were observed. Although chromosome counts of 2n = 64 have been recorded in Pelvetia wrightii and Pelvetiopsis limitata, a count of 2n = 44 has been reported for P. canaliculata (Tables II, VII).

The cytomorphological aspects of the life cycle of Pelvetiopsis are similar to Hesperophycus in several ways (Table VII). There is one primary rhizoid formed in both genera. The cross-walls which divide the contents of the oogonium into 2 unequally-sized eggs are transverse and sometimes oblique. In both genera the large egg has one nucleus and the smaller egg has seven nuclei. No centrosomes have been observed in dividing nuclei of either genus. Several important differences exist between these two genera. The thallus of Pelvetiopsis is shorter and lighter in colour than Hesperophycus. Also, Hesperophycus has a midrib and plentiful cryptostomata (Walker, 1931). Both of these structures are lacking in Pelvetiopsis. The two eggs of Pelvetiopsis are much larger than those of Hesperophycus, finally the chromosome numbers of these two species differ, as 2n = approximately 64 in Pelvetiopsis (Fig. 7 and 8) while 2n = 14 - 18 in Hesperophycus (Tables II, VII).

Pelvetiopsis is much like Fucus with regard to the light tan color and small size of the thallus, the occurrence of one primary rhizoid (sometimes two) in the embryo, and the diploid chromosome complement of approximately 64. However, the dissimilarities are much more striking. In Pelvetiopsis there are no midribs or caecostomata, and a few cryptostomata, all of which are found in Fucus. Eight eggs of equal size occur in oogonia of Fucus (Thuret and Bornet, 1878; Oltmanns 1889a,b), whereas only two unequally-sized eggs form in Pelvetiopsis. There are no centrosomes in dividing nuclei of Pelvetiopsis. In Fucus the spermatozoids are larger than those found in Pelvetiopsis (Table VII).

From these points it can be seen that Pelvetiopsis is most like Pelvetia from which Gardner (1910) separated it with regard to the gross morphology of the plant. The cytological structure of the thallus is similar to that of Hesperophycus. The relationship to Fucus is more remote. Therefore, it can be seen that Pelvetiopsis is clearly related to Pelvetia, Hesperophycus, and Fucus in more than one respect, but it is definitely a distinct entity.

F. Summary

A cytomorphological study of Pelvetiopsis limitata was undertaken using both living and fixed material. Mature plants and embryos were kept in culture in sterilized filtered sea water in a cold chamber where the temperature varied from 6° - 9°C. Growth of plants was most suitable with a light intensity of 250 foot-candles. Sectioned material stained with warm aceto-carmine proved most satisfactory for cytological study.

Conceptacle formation is initiated by a single epidermal cell, as in other Fucaceae. There are no caecostomata or cryptostomata in the mature plant. There are three tissues in the thallus, the epidermis, cortex, and medulla.

The life cycle is similar to that of other Fucales. Oogonia and antheridia are formed in the conceptacles and the gametes are formed within the oogonia and antheridia. The oogonia grow attached to the wall of the conceptacle by a stalk cell. Three nuclear divisions, the first two of which are probably meiotic, occur in the oogonium producing eight nuclei. There is a brief resting stage between each division. Two unequal eggs are formed in the oogonium. The larger egg formed is uninucleate while the smaller egg has seven nuclei. Both eggs are liberated from the oogonium and increase in size. Approximately 32 chromosomes were counted in the nucleus of the mature uninucleate egg. Aberrations, such as the occurrence of two equally-sized eggs, and of three and four eggs of unequal size were found.

The antheridia grow singly from a stalk cell, or from a branching paraphysis. Meiosis probably takes place during the first two divisions of the antheridial initial cell. Approximately 30 - 32 chromosomes were counted

at metaphase of the second division in the antheridium. Each antheridial initial undergoes division six times to produce sixty-four nuclei. The last four divisions are mitotic. The sixty-four nuclei and surrounding cytoplasm are modified into spermatozoids within the antheridium.

Mature gametangia were induced to liberate, and it was noted that immature as well as mature eggs and spermatozoids were released. The mature egg is naked at the time fertilization takes place. Fertilization of the egg by one spermatozoid restores the 2n chromosome number. There is, therefore, only a cytological alternation of generations in Pelvetiopsis limitata. The first cytoplasmic division of the zygote occurs after polarization has been established. The first three cross-walls formed in the zygote are usually transverse to the developing rhizoid, and are soon followed by cell divisions in the other two planes when the primary rhizoid has become two or three cells long.

The mature thallus is diploid. Approximately 64 chromosomes were counted in cells of the cortex.

G. Literature Cited

Abe, K. 1933. Mitosen im Antheridium von Sargassum confusum Ag. Sci. Repts. Tôhoku Imp. Univ. Ser. IV, 8 (3): 259 - 262.

_____ 1938. Über die Befruchtung und die ihr folgende erst Kernteilung bei Sargassum. Sci. Repts. Tôhoku Imp. Univ. Ser. IV, 13 (3): 253 - 257.

Baker, S.M. and Bohling, M.H. 1916. On the Brown Seaweeds of the Salt Marsh. II. Their Systematic Relationships, Morphology and Ecology. Journ. Linn. Soc. London, Bot. 43: 325 - 380.

Barton, E.S. 1899. On Notheia anomala Harv. et Bail. Journ. Linn. Soc. London, Bot. 34: 417 - 425.

Beams, H.W. 1937. The Air-Turbine Ultracentrifuge Together with Some Results Ultracentrifuging the Eggs of Fucus serratus. Journ. Mar. Biol. Ass. U.K. 21 (2): 571 - 578.

Blomquist, H.L. 1945. Development of Reproductive Structures in the Brown Alga Turbinaria turbinata. Bot. Gaz. 106: 290 - 304.

Bower, F.O. 1880. On the development of the Conceptacle in the Fucaceae. Quart. Journ. Micros. Sci. 20: 36 - 49.

Burrows, E. and Lodge, S. 1951. Autecology and the Species Problem in Fucus. Journ. Mar. Biol. Ass. U.K. 30 (1): 161 - 176.

Collins, F., Holden, H. and Setchell, W. 1905. Phycotheca Boreali - Americana. CXIV. Exs. 1238.

Damman, H. 1931. Aus dem Nachlass von Hans Kniep: Experimentelle Erzeugung von rieseneiern bei Fucus und deren Entwicklung nach Befruchtung. Ber. deutsch. bot. Ges. 49 (8): 392 - 402.

Darlington, C.D. and LaCour, L. 1941. The Detection of Inert Genes. Journal Hered. 22: 115 - 121.

Dawson, A.E.E. 1940. Studies on the Fucales of New Zealand. II. Observations on the Female Frond of Carpophyllum flexuosum (Esp.) Grev. = Carpophyllum Phyllanthus (Turn.) Hook. et Harv.

Decaisne, J. and Thuret, G. 1845. Recherches sur les anthéridies et les spores de quelques Fucus. Ann. Sci. nat. (Bot.) Ser. 3, 3: 1 - 15.

Delf, E.M. 1935. Liberation of Oogonia in Bifurcaria and Other Members of the Fucaceae. New Phytol. 34: 245 - 259.

duBuy, H.G. and Olson, R.A. 1937. The Presence of Growth - Regulators During the Early Development of Fucus. Am. Journ. Bot. 24: 609 - 611.

Farmer, J.B. and Williams, J.L. 1896. On Fertilisation and the Segmentation of the Spore in Fucus. Ann. Bot. 10: 479 - 487.

Farmer, J.B. and Williams, J.L. 1898. Contributions to Our Knowledge of the Fucaceae: Their Life-History and Cytology. Phil. Trans. Roy. Soc. London B. 190: 623 - 645.

Fensholt, D.E. 1955. An Emendation of the Genus Cystophyllum (Fucales). Am. Journ. Bot. 42 (3): 305 - 322.

Ferguson, M. 1901. The Development of the Egg and Fertilization in Pinus Strobus. Ann. Bot. 15(59): 435 - 479.

Fritsch, F.E. 1945. Structure and Reproduction of the Algae. Vol. II. Cambridge Univ. Press. pp. 322 - 396.

Gard, M. 1910. Sur un hybride des Fucus platycarpus et Fucus ceranoides. C.R. Acad. Sci., Paris 151: 888 - 890.

Gardner, N.L. 1910. Variations in Nuclear Extrusion Among the Fucaceae. Univ. Cal. Publ. Bot. 4: 121 - 136.

_____ 1913. New Fucaceae. Univ. Cal. Publ. Bot. 4: 317 - 374.

_____ 1922. The Genus Fucus on the Pacific Coast of North America. Univ. Cal. Publ. Bot. 10: 1 - 180.

_____ 1940. New Species of Melanophyceae from the Pacific Coast of North America. Univ. Cal. Bot. 19 (8): 267 - 286.

Getman, M.R. 1914. Oogenesis in Hormosira. Bot. Gaz. 58: 264 - 270.

Gibb, D.C. 1937. Observations on Himanthalia lorea (L.) Lyngb. Journ. Linn. Soc. London, Bot. 51: 11 - 21.

Gruber, E. 1896. Ueber Aufbau und Entwicklung einiger Fucaceen. Bibl. Bot. 38: 34 pp.

Guignard, L. 1889. Développement et constitution des anthérozoides. Rev. gén. Bot. 1: 136 - 145.

Hiroe, M. and Inoh, S. 1954a. Cytological Studies on the Fucaceous Plants. IV. On the Mitotic Division in the Antheridium of Sargassum horneri (Turn.) Ag. Bot. Mag. Tokyo. 67. 190 - 192.

_____ and _____ 1954b. Cytological Studies on the Fucaceous Plants. V. On the Mitotic Division of the Embryo of Sargassum patens C. Ag. Biol. Journ. Okayama Univ. 2 (1): 1 - 6.

_____ and _____ 1954c. Artificial Parthenogenesis in Sargassum piluliferum. Bot. Mag. Tokyo 67: 271 - 274.

Hiroe, M. and Inoh, S. 1956. On the Meiotic Division in the Antheridium of Sargassum tortile C. Ag. La Kromosomo 27 - 28: 942 - 947.

Holtz, F.L. 1903. Observations on Pelvetia. Minn. Bot. Studies Ser. 3. Part I. pp. 23 - 45.

Inoh, S. 1932. Embryological Studies on Sargassum and Cystophyllum. Journ. Fac. Sci. Hokkaido Imp. Univ. Ser. V, I (4): 126 - 133.

_____ 1935. Embryological Studies on Pelvetia wrightii Yendo and Fucus evanescens Ag. Journ. Fac. Sci. Hokkaido Imp. Univ. Ser. V, 5: 6 - 23.

Inoh, S. and Hiroe, M. 1954a. Cytological Studies on the Fucaceous Plants. I. On the Somatic Mitosis in the Embryo of Sargassum piluliferum C. Ag. La Kromosomo 21: 760 - 763.

_____ and _____ 1954b. Cytological Studies on the Fucaceous Plants. II. On the Meiotic Division in the Antheridium of Hizikia fusiformis Okamura. La Kromosomo 21: 764 - 766.

_____ and _____ 1954c. Cytological Studies on the Fucaceous Plants. III. On the Meiotic Division in the Antheridium of Sargassum piluliferum C. Ag. La Kromosomo 21: 767 - 769.

Isaac, W.E. 1933. Some Observations and Experiments on the Drought Resistance of Pelvetia canaliculata. Ann. Bot. 47 - (186): 343 - 348.

Jaffe, L. 1954. Stimulation of the Discharge of Gametangia From a Brown Alga by a Change from Light to Darkness. Nature 174: 743.

_____ 1958. Tropistic Responses of Zygotes of the Fucaceae to Polarized Light. Exptl. Cell Res. 15 (2): 282 - 299.

Kunieda, H. 1926. On the Mitosis and Fertilization in Sargassum Hörneri Ag. Bot. Mag. 40 (478): 545 - 550.

_____ 1928. On the Development of the Sexual Organs and Embryogeny in Sargassum Horneri Ag. Journ. Coll. Agric. 9: 383 - 396.

_____ and Suto, S. 1940. On the Fertilization in Sargassum horneri Ag. Jap. Journ. Bot. 11 (1): 141 - 146.

Kylin, H. 1916. Ueber den Bau der Spermatozoiden der Fucaceen. Ber. deuts. bot. Ges. 34: 194 - 201.

_____ 1920. Bemerkungen über den Bau der Spermatozoiden der Fucaceen. Ber. deutsch. bot. Ges. 38: 74 - 78.

Laing, E.M. 1941. A Note on Bifurcaria laevigata (Kutz.) Delf & Mitch. Journ. Bot. 79: 145 - 156.

LeTouzé, M. 1912. Contribution à l'étude histologique des Fucacées. Rev. gén. Bot. 24: 33 - 47.

Levring, T. 1947. Remarks on the Surface Layers and the Formation of the Fertilization Membrane in Fucus Eggs. Acta. Medd. Got. Bot. Trädgård 17: 97 - 105.

Lowrance, E.W. 1937. Effects of Temperature Gradients on Polarity of Eggs of Fucus furcatus. Journ. Cell. and Comp. Physiol. 10: 321 - 337.

_____ and Whitaker, D.M. 1940. Determination of Polarity in Pelvetia Eggs by Centrifuging. Growth 4 (1): 73 - 76.

Manton, I. and Clarke, B. 1951. An Electron Microscope Study of the Spermatozoid of Fucus serratus. Ann. Bot. n.s. 15 (60): 461 - 471.

Mitchell, M.O. 1893. Notheia anomala Bail. and Harv. Phycol. Mem. Part II. 36 - 37.

Mitchell, M. 1941. Studies on the Fucales of New Zealand. III. Some investigations on Xiphophora chondrophylla. (R. Br. ex. Turner) Harv. Journ. Bot. 79: 49 - 56, 65 - 71.

Moore, L.B. 1928. Pelvetia fastigiata. Bot. Gaz. 86 (4): 419 - 434

Moss, B.L. and Elliot, E. 1957. Observations on the Cytology of Halidrys siliquosa. Ann. Bot. n.s. 21: 143 - 151.

Naylor, M. 1954. A note on Xiphophora chondrophylla var. maxima J. Ag. New Phytol. 53: 155 - 159.

_____ 1957. An Acetocarmine Squash Technique for the Fucales. Nature 180: 46.

_____ 1958. The cytology of Halidrys siliquosa. Ann. Bot. 22 (86): 205 - 217.

Nienburg, W. 1910. Die Oögonentwicklung bei Cystoseira und Sargassum. Flora 101: 167 - 180.

_____ 1913. Die Konzeptakelentwicklung bei den Fucaceen. Zeits. Bot. 5: 1 - 27.

_____ 1929. Zur Entwicklungsgeschichte der Fucus -Keimlinge. Ber. deuts. bot. Ges. 47 (8): 527 - 529.

_____ 1931. Die Entwicklung der Keimlinge von Fucus vesiculosus und ihre Bedeutung für die Phylogenie der Phaeophyceen. Wiss. Meeresunters. Kiel, N.F., 21: 49 - 63.

Okabe, S. 1929a. Meiosis im Oogonium von Sargassum Horneri (Turn.) Ag. Sci. Repts. Tôhoku Imp. Univ. IV, 4 (4): 661 - 669.

_____ 1929b. Rhizoidenentwicklung im Embryo von Cystophyllum. Sci. Repts. Tôhoku Imp. Univ. IV, 4: 591 - 595.

_____ 1930. Mitosis im keimenden Embryo von Sargassum Horneri (Turn.) Ag. Sci. Repts. Tôhoku Imp. Univ. Ser. IV, 5 (4): 757 - 762.

Oltmanns, F. 1889a. Beiträge zur vergleichen den Entwickelungsgeschichte der Fucaceen. Sitzungsberichte der Königlich Preussischen Akademie der Wissenschaften zu Berlin. 585 - 599.

_____ 1889b. Beiträge zur Kenntnis der Fucaceen. Bibl. Bot. 14. 100 pp.

_____ 1922. Morphologie und Biologie der Algen. 2 Edit. II, Jena.

Osborn, J. 1949. The Structure and Life History of Hormosira banksii (Turner) Decaisne. Trans. Roy. Soc. N.Z. 77: 47 - 71.

Pierce, G.J. 1902, Extrusion of the Gametes in Fucus. Torreya 2: 134 - 137.

Powell, H.T. 1957a. Studies in the Genus Fucus L. I. Fucus distichus L. emend. Powell. Journ. Mar. Biol. Ass. U.K. 36 (2): 407 - 432.

_____ 1957b. Studies in the Genus Fucus L. II. Distribution and Ecology of Forms of Fucus distichus L. emend. Powell in Britain and Ireland. Journ. Mar. Biol. Ass. U.K. 36: 663 - 693.

Rao, C.S.P. 1946. A Contribution to the Morphology and Life History of Sargassum tenerrimum J. Ag. Proc. Indian Acad. Sci. Section B 23 (1): 39 - 51.

Rees, E.M. 1933. Some Observations on Bifurcaria tuberculata Stackh. Ann. Bot. 47: 101 - 115.

Resuhr, B. 1935. Über den Bau und den Öffnungsmechanismus der Fucus-oogonien. Flora 129 (3): 336 - 346.

Richard, J. 1932. Origine et Structure du Spermatozoide de Fucus. C.R. Soc. Biol. 110 (21): 436 - 438.

Roe, M.L. 1916. The Development of the Conceptacle in Fucus. Bot. Gaz. 61: 231 - 246.

Sauvageau, C. 1908. Sur deux Fucus récoltés à Arcachon (Fucus platycarpus et F. lutarius). Bull. Stat. Biol. Arcachon, 11: 65 - 224.

_____ 1909. Sur l'hybride des Fucus vesiculosus et Fucus serratus. C.R. Soc. Biol., 67: 832 - 833.

_____ 1911. Sur les Cystoseira à anthérozoïdes sans point rouge. C.R. Soc. Biol. 71: 472 - 473.

Scagel, R.F. 1947. An Annotated List of the Marine Algae of British Columbia and Northern Washington. Nat. Mus. Canada. Biol. Ser. Bull. 150.

Setchell, W.A. and Gardner, N.L. 1925. The Marine Algae of the Pacific Coast of North America. Part III•Melanophyceae. Univ. Cal. Publ. Bot. 8 (3): 383 - 898.

Shimotomai, N. 1928. Karyokinese im Oögonium von Cystophyllum sisymbrioides J. Ag. Sci. Repts. Tôhoku Imp. Univ. Ser. IV, 3 (4): 577 - 579.

Simons, E. 1906. A Morphological Study of Sargassum filipendula. Bot. Gaz. 41: 161 - 182.

Smith, G.M. 1944. Marine Algae of the Monterey Peninsula. Stanford Univ. Press., Cal., U.S.A.

_____ 1956. Cryptogamic Botany. Vol. I. McGraw-Hill, New York. pp. 264 - 271.

Strasburger, E. 1897. Kerntheilung und Befruchtung bei Fucus. Jahrb. wiss. Bot. 30: 351 - 374.

Subrahmanyan, R. 1956. Observations on the Anatomy, Cytology, Development of the Reproductive Structures, Fertilization and Embryology of Pelvetia canaliculata Dcne. et Thur. Part 1. Anatomy of the Thallus and Somatic Mitosis. Journ. Ind. Bot. Soc. 35 (4): 374 - 390.

_____ 1957a. Observations on the Anatomy, Cytology, Development of the Reproductive Structures, Fertilization and Embryology of Pelvetia canaliculata Dcne. et Thur. Part II. Development of Conceptacles, Reproductive Structures and Meiotic Division of the Nucleus during Gametogenesis. Journ. Ind. Bot. Soc. 36 (1): 12 - 34.

_____ 1957b. Observations on the Anatomy, Cytology, Development of the Reproductive Structures, Fertilization and Embryology of Pelvetia canaliculata Dcne. et Thur. Part III. The Liberation of Reproductive Bodies, Fertilization and Embryology. Journ. Ind. Bot. Soc. 36 (3): 373 - 395.

Svedelius, N. 1929. An Evaluation of the Structural Evidence for Genetic Relationships in Plants. Algae. Proc. Int. Congress Plant Sci. I: 457 - 471.

Tahara, M. 1929. Oögenesis in Coccophora langsdorfii (Turn.) Grev. Sci. Repts. Tôhoku Imp. Univ. IV, 4: 551 - 556.

_____ und Shimotomai, N. 1926. Mitosen bei Sargassum. Sci. Repts. Tôhoku Imp. Univ. IV, 1 (3): 189 - 192.

Thuret, G. 1854. Recherches sur la fécondation des Fucacées suives d'observations sur les anthéridies des Algues. Ann. Sci. nat. Bot. ser. IV, 2: 197 - 214.

Thuret, G. and Bornet, E. 1878. Études Phycologiques. Paris.

Tomita, K. 1932. Befruchtung und Kernteilung bei Coccophora langsdorfii (Turn.) Grev. Sci. Repts. Tôhoku Imp. Univ. IV, 7: 43 - 47.

Walker, R.I. 1931. Fertilization and Embryo Development in Hesperophycus Harveyanus. La Cellule 40 (2): 175 - 188.

Whitaker, D.M. 1931. Some Observations on the Eggs of Fucus and upon Their Mutual Influence in the Determination of the Developmental Axis. Biol. Bull. Mar. Biol. Lab. 61 (3): 294 - 308.

Williams, J.L. 1899. New Fucus Hybrids. Ann. Bot. 13: 187 - 188.

Williams, M.M. 1923. A Contribution to Our Knowledge of the Fucaceae. Proc. Linn. Soc. New South Wales 48: 634 - 646.

Woodworth, W.M. 1888. The Apical Cell of Fucus. Ann. Bot. 1: 203 - 211.

Yabu, H. and Imai, A. 1957. On Nuclear Division in the Antheridium of Fucus evanescens and Pelvetia wrightii, and on the Four-Egged Oogonium of Pelvetia Wrightii. Bull. Jap. Soc. Phycol. 5: 44 - 49.

Yamanouchi, S. 1909. Mitosis in Fucus. Bot. Gaz. 47: 173 - 197.

Yendo, K. 1907. The Fucaceae of Japan. Journ. Coll. Sci. Imp. Univ., Tokyo, Japan. Vol. 21: 1 - 174.

H. Tables

Table I

Comparison of Oogonial Characteristics of Certain Fucales

Species	No. Nuclei Produced	No. Nuclei Abort	No. Eggs Formed	No. Eggs Functional	Reference
FUCACEAE:					
Pelvetiopsis limitata	8	0	2u	1	Gardner, 1910, 1913
Ascophyllum nodosum	8	4	4e	4	Oltmanns, 1889b Farmer and Williams, 1898. Thuret and Bornet, 1878
Fucus spp.	8	0	8e	8	Farmer and Williams, 1898. Oltmanns, 1889a,b. Thuret and Bornet, 1878.
Hesperophycus harveyanus	8	0	2u 7s	1	Gardner, 1910.
Pelvetia canaliculata	8	6	2e	2	Thuret and Bornet, 1878. Oltmanns, 1889a.
Pelvetia fastigiata	8	6	2e	2	Gardner, 1910. Moore, 1928.
Pelvetia wrightii	8	6	2e 4r	2	Inoh, 1935.
Phyllospora comosa	8	7	1	1	Williams, 1923.
Xiphophora chondrophylla	8	4	4e	4	Barton, 1899. Mitchell, 1941.

Table I cont.

Species	No. Nuclei Produced	No. Nuclei Abort	No. Eggs Formed	No. Eggs Functional	Reference
CYSTOSEIRACEAE:					
Bifurcaria tuberculata	8	7	1	1	Rees, 1933. Thuret and Bornet, 1878.
Bifurcaria laevigata	8	4	4e	4b	Laing, 1941
Cystoseira osmundacea	8	7	1	1	Gardner, 1910
Halidrys siliquosa	8	7	1	1	Naylor, 1958
NOTHEIACEAE:					
Hormosira banksii	8	4	4e	4	Gruber, 1896 Getman, 1914.
Notheia anomala	8	0	8e	8	Barton, 1899 Williams, 1923.
SARGASSACEAE:					
Sargassum, many spp.	8	7	1	1	Kunieda, 1926, 1928. Tahara and Shimotomai, 1926 Simons, 1906 Abe, 1933.
Turbinaria turbinata	8	7	1	1	Blomquist, 1945.

Abbreviations:

b .... binucleate eggs occur
e .... equal division
r .... rarely
u .... unequal division
7s ... smaller egg has 7 nuclei

Table II

Chromosome Counts of Fucales

Species	2n	n♂	n♀	Reference
FUCACEAE:				
Ascophyllum nodosum	26-30	--	14-15	Farmer and Williams, 1898
Fucus evanescens	--	--	32	Inoh, 1935.
	--	32	--	Yabu and Imai, 1957.
Fucus platycarpus (= *F. spiralis*)	--	30	30	Strasburger, 1897.
Fucus serratus	--	30	30	Strasburger, 1897.
	26-28	--	14-15	Farmer and Williams, 1898
Fucus vesiculosus	64	32	32	Yamanouchi, 1909.
Hesperophycus harveyanus	14-18	--	--	Walker, 1931.
Pelvetia canaliculata	44ap.	20 +	22	Subrahmanyan, 1956, 1957a, 1957b.
Pelvetia wrightii	--	--	32	Inoh, 1935.
	--	32	--	Yabu and Imai, 1957.
HIMANTHALIACEAE:				
Himanthalia lorea	28	--	--	Farmer and Williams, 1898
NOTHEIACEAE:				
Hormosira banksii	24	12	--	Osborn, 1949.
CYSTOSEIRACEAE:				
Cystophyllum sisymbrioides	--	--	32	Shimotomai, 1928.

Table II Cont.

Species	2n	n♂	n♀	Reference
Halidrys siliquosa	--	--	8	Moss and Elliot, 1957
	55+	30ap.	28ap.	Naylor, 1958
SARGASSACEAE:				
Coccophora langsdorfii	64	--	--	Tomita, 1932
	--	--	32	Tahara, 1929
Hizikia fusiformis	--	32	--	Inoh and Hiroe, 1954b
Sargassum confusum	--	32	--	Abe, 1933
Sargassum enerve	--	--	32ap.	Tahara and Shimotomai, 1926
Sargassum horneri	32	16	16	Kunieda, 1926, 1928
	--	--	32	Okabe, 1929a
	64	--	--	Okabe, 1930
	--	32	--	Hiroe and Inoh, 1954a
Sargassum patens	64	--	--	Hiroe and Inoh, 1954b
Sargassum piluliferum	64	--	--	Inoh and Hiroe, 1954a
	--	32	--	Inoh and Hiroe, 1954c
Sargassum tortile	--	32	--	Hiroe and Inoh, 1956

ap. = approximately

Table III

The Number of Primary Rhizoids Formed in Embryos of Certain Fucales

Species	Number	Reference
FUCACEAE:		
Ascophyllum nodosum	2	Oltmanns, 1889b
Fucus evanescens	1	Oltmanns, 1889b
Fucus serratus	1	Inoh, 1935
Fucus vesiculosus	1 (2)b	Thuret and Bornet, 1878 Oltmanns, 1889a,b
Pelvetia canaliculata	4	Oltmanns, 1889b
Pelvetia wrightii	1 (2)	Inoh, 1935
CYSTOSEIRACEAE:		
Cystoseira barbata	4b	Inoh, 1935
Cystophyllum hakodatense	4	Inoh, 1935
Cystophyllum sisymbrioides	32	Okabe, 1929b
SARGASSACEAE:		
Sargassum confusum	8	Inoh, 1932
Sargassum hemiphyllum	8	Inoh, 1932
Sargassum horneri	8	Inoh, 1932
Sargassum enerve	16	Inoh, 1932
Sargassum patens	16	Inoh, 1932
Sargassum piluliferum	16	Inoh, 1932
Sargassum tortile	16	Inoh, 1932

Table III cont.

The Number of Primary Rhizoids Formed in Embryos of Various Fucales

Species	Number	Reference
SARGASSACEAE:		
Turbinaria turbinata	4,8,16,or 32	Blomquist, 1945.

Numbers in brackets () refer to the number of primary rhizoids which may occur.

b ... may be bifurcated.

Table IV

Oogonium Size in Certain Fucales

Species	Size (in microns)	Reference
Sargassum muticum	176 x 140	Fensholt, 1955
Turbinaria turbinata	174 x 140	Blomquist, 1945
Carpophyllum flexuosum	188 x 137	Dawson, 1940
Hormosira banksii	160 x 110	Osborn, 1949
Cystoseira geminata	71 - 75[1]	Fensholt, 1955
Cystoseira osmundacea	70 - 75[1]	Fensholt, 1955
Notheia anomala	75 x 20	Barton, 1899

[1] . . . diameter

Table V

Egg Size in Certain Fucales

Species	Size (in microns)	Reference
Himanthalia lorea	300 - 500	Gibb, 1937
Sargassum microantheum	384 x 275	Inoh, 1932
Cystophyllum sisymbrioides	321 x 229	Inoh, 1935
Sargassum horneri	264 x 198	Inoh, 1932
Carpophyllum flexuosum	150 x 110 to 178 x 137	Dawson, 1940
Sargassum hemiphyllum	125 x 105	Inoh, 1932
Pelvetia canaliculata	111 - 137[1]	Subrahmanyan, 1957b
Pelvetia wrightii	84[1]	Inoh, 1935
Pelvetia fastigiata	82 - 113[1]	Lowrance and Whitaker, 1940
Hormosira banksii	64 - 71[1] to 126 x 112	Osborn, 1949
Fucus furcatus	65 - 90[1]	Lowrance and Whitaker, 1940
Fucus serratus	60 - 90[1]	Beams, 1937
Fucus vesiculosus	52 - 70[1]	Whitaker, 1931
Fucus evanescens	60[1]	Inoh, 1935
Hesperophycus harveyanus	11[1] (L) 2.6[1] (S)	Walker, 1931

1 . . . diameter

(L) . . Large egg

(S) . . Small egg

Table VI

Antheridium and Spermatozoid Size in Certain Fucales

Species	Antheridium Size	Spermatozoid Size	Reference
Sargassum horneri	. . .	6 (7) long	Kunieda and Suto, 1940
Hormosira banksii	42 x 17 (20)	5 (6) x 2	Osborn, 1949
Bifurcaria tuberculata	30 (40) long	. . .	Rees, 1933
Fucus spp.	. . .	4 (5) x 2.3 (2.5)	Kylin, 1916
Himanthalia lorea	42 (60) x 9 (12)	3 (4)	Gibb, 1937
Halidrys siliquosa	. . .	2[1]	Naylor, 1958

[1] . . . diameter

Brackets ( ) indicate maximum measurement

Table VII

Comparison of Data on Pelvetiopsis with Data Reported in the Literature on Pelvetia, Hesperophycus and Fucus.

Character	Pelvetiopsis	Pelvetia	Hesperophycus	Fucus	Reference
Gross size (in centimeters)	7 - 12	Pf - 15 - 40 Pw - 70	20 - 40	7 - 12 some spp.	Setchell & Gardner, 1925 Smith, 1944 Inoh, 1935
Color	Light tan	Dark green	Dark green-brown	Light	Smith, 1944
Midrib	None	None	Present	Present	Gardner, 1910
Primary Rhizoids	1 (2 sometimes)	Pc - 4 Pw 1 sometimes 2	1	1 sometimes	Oltmanns, 1889b Walker, 1931 Inoh, 1935
Cryptostomata	In young parts	Pf - present Pc - none	Present	Present	Gardner, 1910 Baker & Bohling, 1916 Moore, 1928
Caecostomata	None	. . .	. . .	Present some spp.	Gardner, 1940 Powell, 1957b
Size of spermatozoids (in microns)	2 - 2.2	. . .	. . .	4 (5) x 2.3 (2.5)	Kylin, 1916

Table VII cont'd.

Character	Pelvetiopsis	Pelvetia	Hesperophycus	Fucus	Reference
Size of eggs (in microns)	90 (155)[1] L 33 (50)[1] S	80 (120) x 113 (160)	11[1] L 2.6[1] S	52 - 90[1]	Walker, 1931 Inoh, 1935 Beams, 1937 Subrahmanyan, 1957b
Cross walls in oogonium	Transverse, sometimes oblique	Pc, Pf Transverse Pw vertical	Transverse, sometimes oblique	Octagonal	Oltmanns, 1889a Yendo, 1907 Gardner, 1910 Moore, 1928
Number of eggs formed	2	2	2	8	Oltmanns, 1889a Gardner, 1910
Fate of supernumerary nuclei	Expelled in small egg	Expelled from cytoplasm	Expelled in small egg	None present	Oltmanns, 1889a Gardner, 1910
Centrosomes	None	Present	None, but astral rays present	Present	Yamanouchi, 1909 Walker, 1931 Subrahmanyan, 1957b
Chromosome number	2n = 64	Pc - 2n = 44 Pw - 2n = 64	2n = 14 - 18	2n = 64	Yamanouchi, 1909 Walker, 1931 Subrahmanyan, 1957b

Pc . . . . . Pelvetia canaliculata
Pf . . . . . Pelvetia fastigiata
Pw . . . . . Pelvetia wrightii
L . . . . . Large egg
S . . . . . Small egg
1 . . . . . Diameter

I. Figures

Abbreviations used in Figures

Apical Cap .....AC

Cortex C

Cuticle..... CU

Epidermis ..... E

Medulla ..... MD

Membrane ..... MM

Metaphase ..... M

Metaphase Plate ..... MP

Nucleolus ..... NU

Nucleus ..... N

Pit ..... P

Rhizoid ..... R

Septum ..... S, SE

Stalk Cell ..... SC

Fig. 1 - 2. Habitat and Morphology of *Pelvetiopsis limitata*.

Fig. 1 - *Pelvetiopsis limitata* f. *limitata* at Port Renfrew, B.C., June, 1959. x 1/5. Fig. 2 - Mature *P. limitata*. x 2/3.

Fig. 1 - 2. from living material.

FIG. 1 - 2

HABITAT AND MORPHOLOGY OF

PELVETIOPSIS LIMITATA

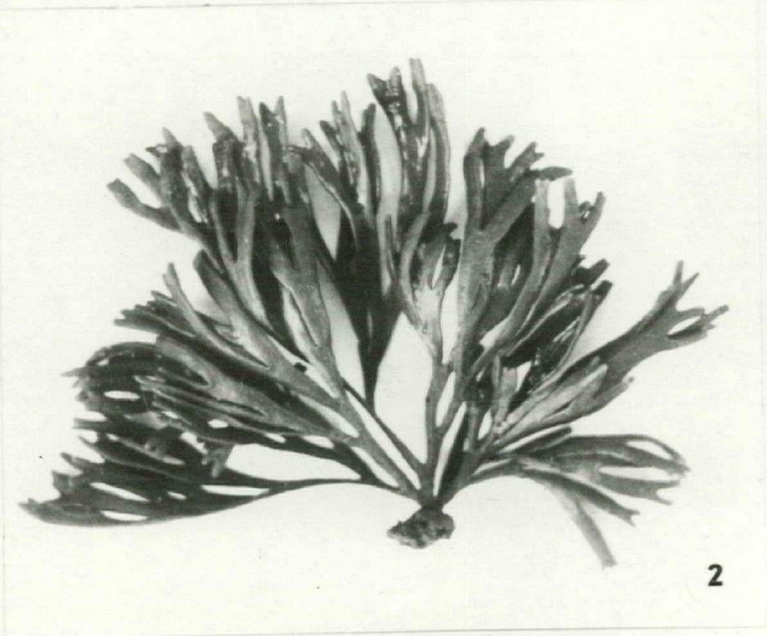


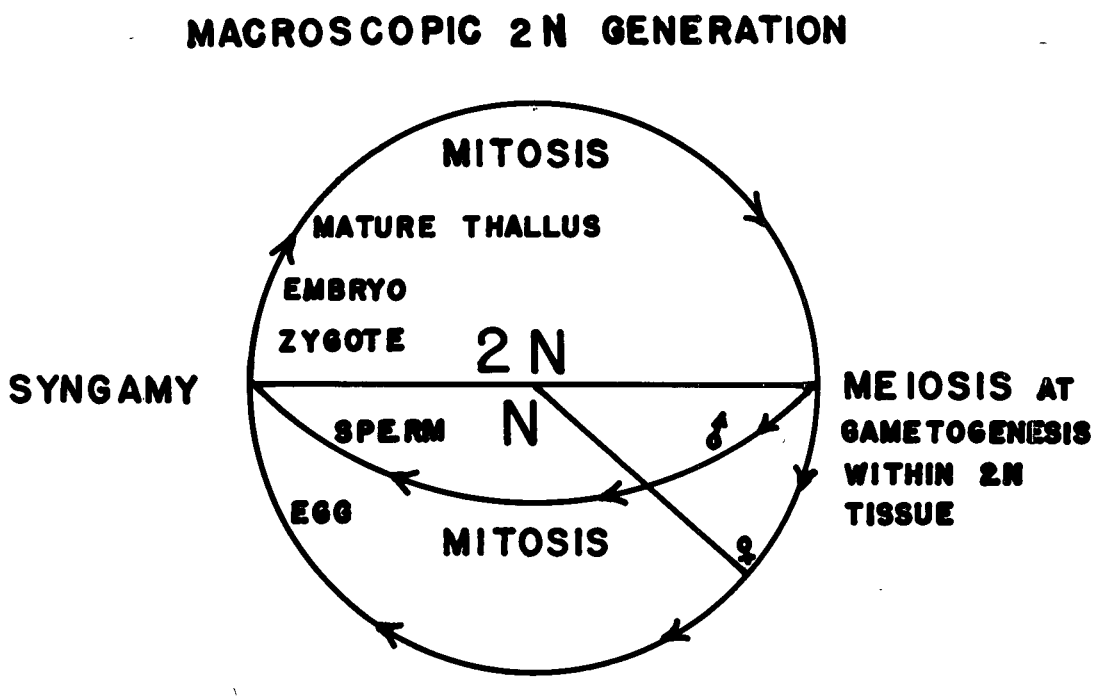
Fig. 3. Generalized Fucales Life Cycle.

A macroscopic diploid generation alternates with a microscopic haploid generation which produces gametes. There is no free-living haploid generation.

FIG. 3

GENERALIZED FUCALES

LIFE CYCLE



[1]- 64 SPERMATOZOIDS PRODUCED

1-8 EGGS FORMED

Fig. 4 - 12. Anatomy of the Thallus of Pelvetiopsis limitata.

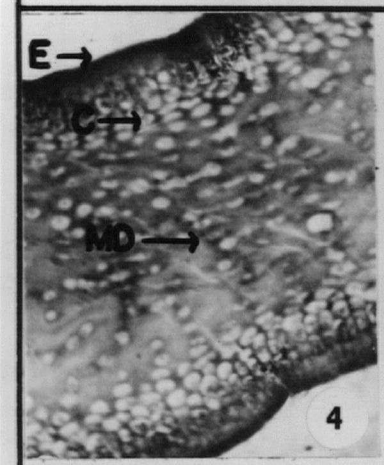
Fig. 4 - Cross section of thallus showing epidermis, cortex and medulla. x 48. Fig. 5 - Surface layers of the thallus. x 430. Fig. 6 - Cortical cell with chomatin material visible. x 2000. Fig. 7 - Mitotic prophase in a cell of the cortex. Approximately 64 chromosomes visible. x 2200. Fig. 8 - Camera lucida drawing of Fig. 7 showing the positions of the chromosomes. x 2200. Fig. 9 - Longitudinal section of medulla showing elongated cells with pit connections. x 1100. Fig. 10 - Camera lucida drawing of cells of the medulla showing longitudinal elongation. x 400. Fig. 11 - Conceptacle containing gametangia. x 100. Fig. 12 - Young conceptacle with remnant of initial cell at base, indicated by arrow. x 500.

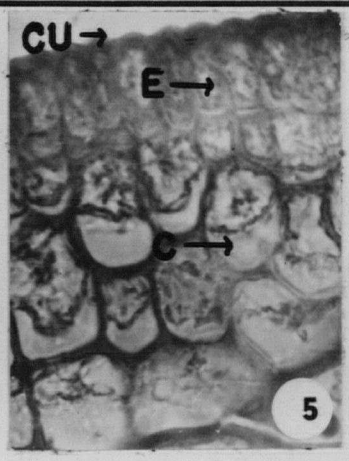
Fig. 4 - 12 from fixed material.

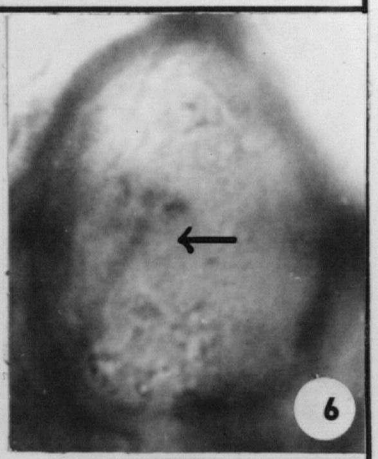
FIG. 4 - 12

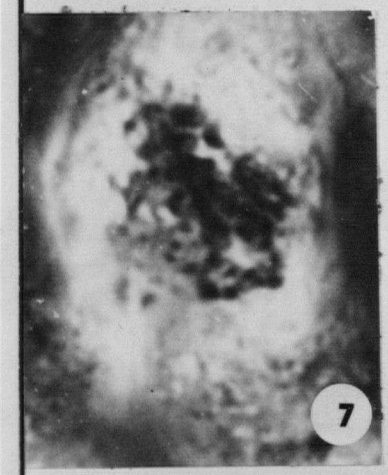
ANATOMY OF THE THALLUS OF

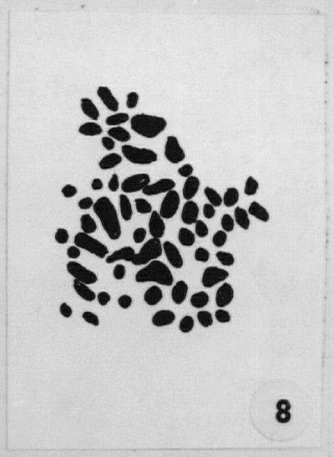
PELVETIOPSIS LIMITATA

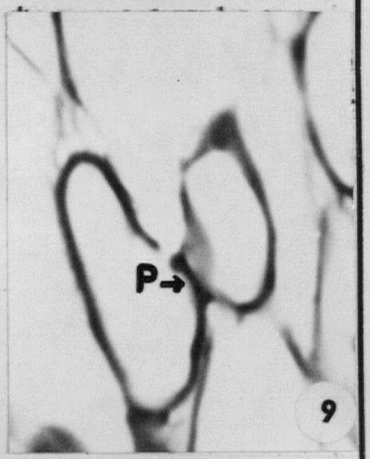


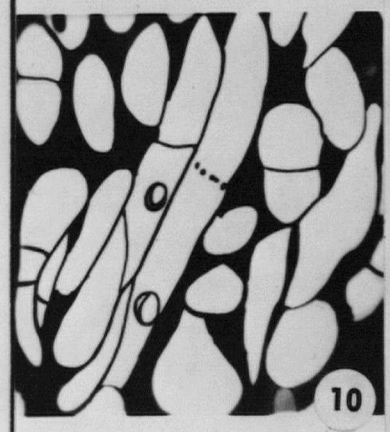












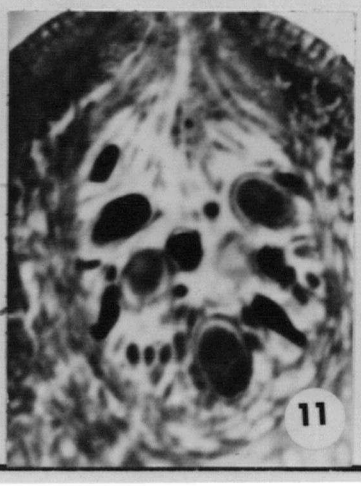


Fig. 13 - 21. Development of Eggs in Pelvetiopsis limitata.

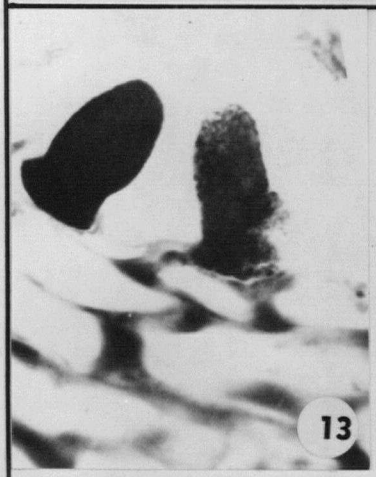
Fig. 13 - Papillae of conceptacle wall cells. x 800. Fig. 14 - Papilla showing nucleus. x 1300. Fig. 15 - Mitotic division of cell has produced two nuclei. Top nucleus is the oogonial initial. x 1000. Fig. 16 - Metaphase of first nuclear division in the oogonial initial (probably meiosis I). x 3150. Fig. 17 - Resting stage. The two nuclei are stained very lightly. x 670. Fig. 18 - Camera lucida drawing of metaphase of second division (probably meiosis II) in the oogonium. Division is simultaneous. x 1540. Fig. 19 - Metaphase II nucleus as in right of Fig. 18. x 5600. Fig. 20 - Metaphase II nucleus as in left Fig. 18. x 5600. Fig. 21 - Interphase between meiosis II and mitosis. Nuclei are grouped and stain lightly. Nucleolus visible. x 670.

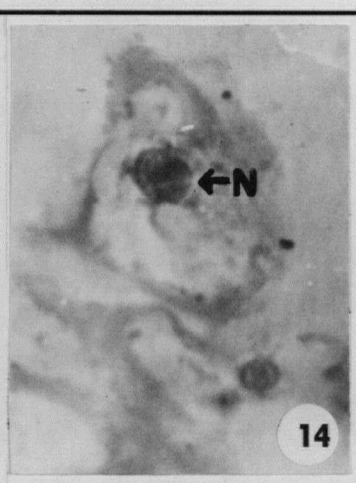
Fig. 13 - 21 from fixed material.

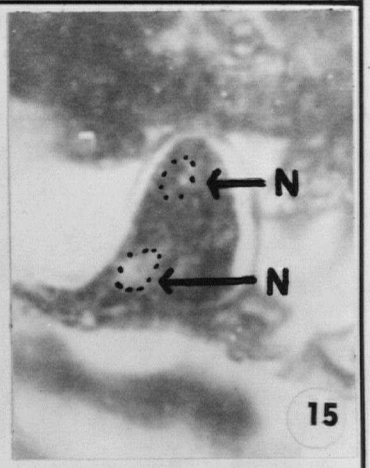
FIG. 13 -21

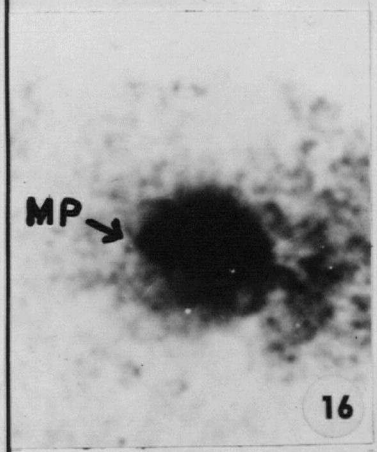
DEVELOPMENT OF EGGS IN

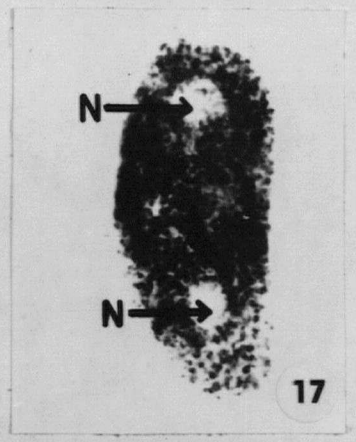
PELVETIOPSIS LIMITATA

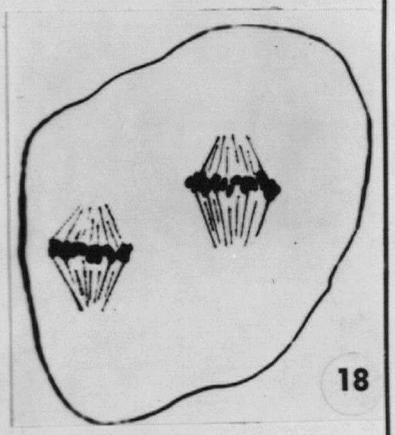


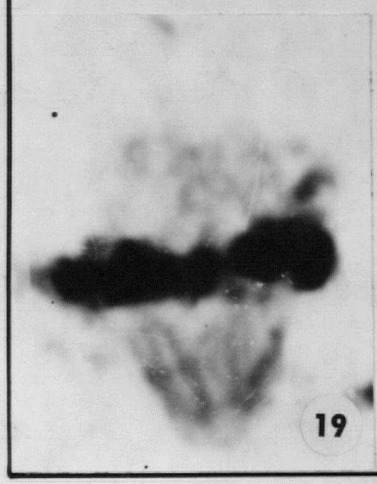












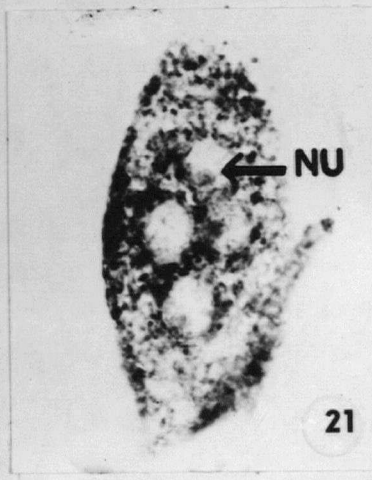


Fig. 22 - 30. Development of Eggs in Pelvetiopsis limitata.

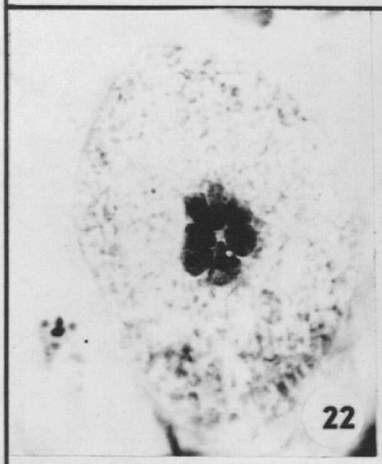
Fig. 22 - Eight nuclei in close association in the oogonium after mitosis. x 840. Fig. 23 - Nuclei migrating to end of oogonium closest to stalk cell (indicated by arrow). x 530. Fig. 24 - Seven nuclei at the lower end of the oogonium, and one nucleus is centrally situated. x 590. Fig. 25 - Maturing eggs in oogonium. Note septum between eggs. x 410. Fig. 26 - At least four layers are present in the wall of the oogonium. x 400. Fig. 27 - Large and small eggs. Two nuclei are visible in the smaller egg. x 880. Fig. 28 - Two eggs shortly after their release from the conceptacle. Smaller egg is flattened. x 370. Fig. 29 - Eggs surrounded by inner wall membranes. The smaller egg has become rounded. x 375. Fig. 30 - Small egg with seven nuclei visible. x 1125.

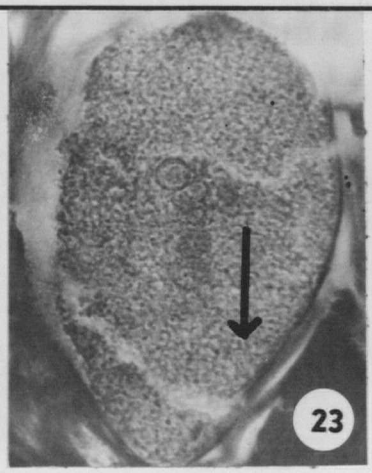
Fig. 22 - 27 and Fig. 30 from fixed material. Fig. 28, 29 from living material.

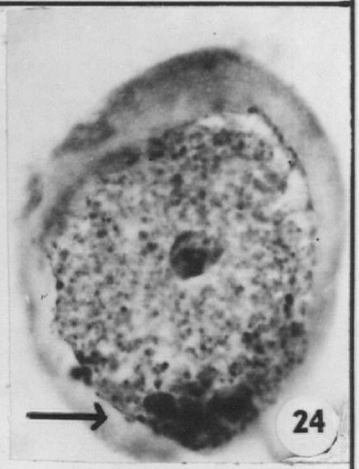
FIG. 22 - 30

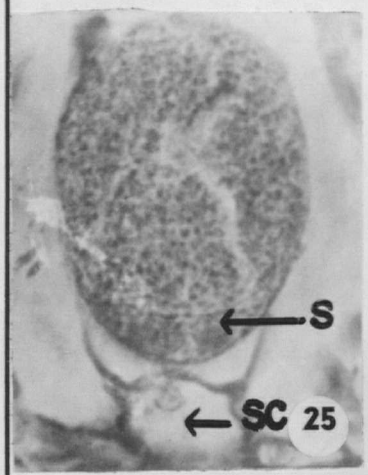
DEVELOPMENT OF EGGS IN

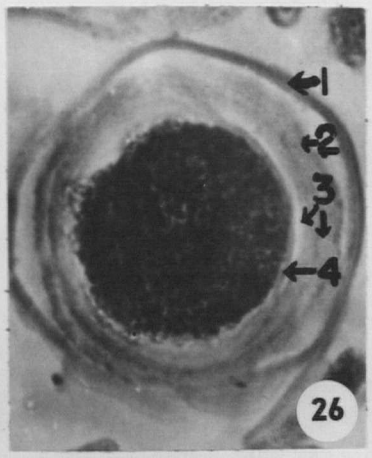
PELVETIOPSIS LIMITATA

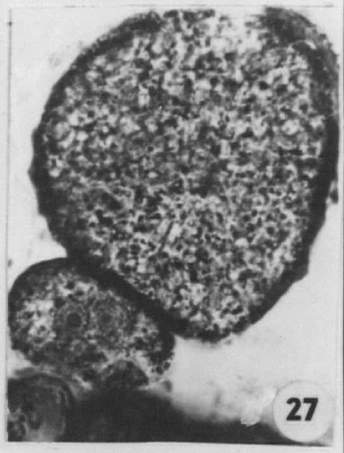


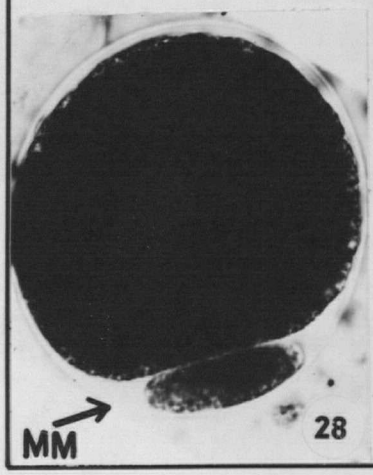


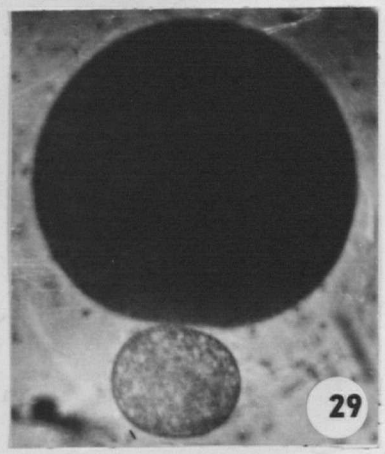












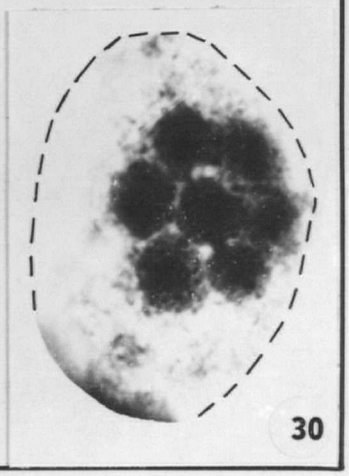


Fig. 31 - 39. Chromocenters in the Egg of Pelvetiopsis limitata.

Fig. 31 - Threads of chromatin network visible while the nucleus is in the center of the egg. x 5160. Fig. 32 - Condensed chromatin threads. x 4850. Fig. 33 - Mass of chromatin material, and 3 - 4 chromocenters above it. Nucleus is now situated near the distal periphery of the egg. x 4750. Fig. 34 - 7 chromocenters visible. x 4850. Fig. 35 - 8 chromocenters visible. x 4850. Fig. 36 - Nucleus contains about 11 chromocenters. x 4850. Fig. 37 - About 16 chromocenters. x 4850. Fig. 38 - Egg nucleus with some of its 32 chromosomes visible. x 5840. Fig. 39 - Composite drawing of Fig. 38, showing 32 chromosomes. x 5840.

Fig. 31 - 39 from fixed material.

FIG. 31 - 39

CHROMOCENTERS IN THE EGG

OF PELVETIOPSIS LIMITATA

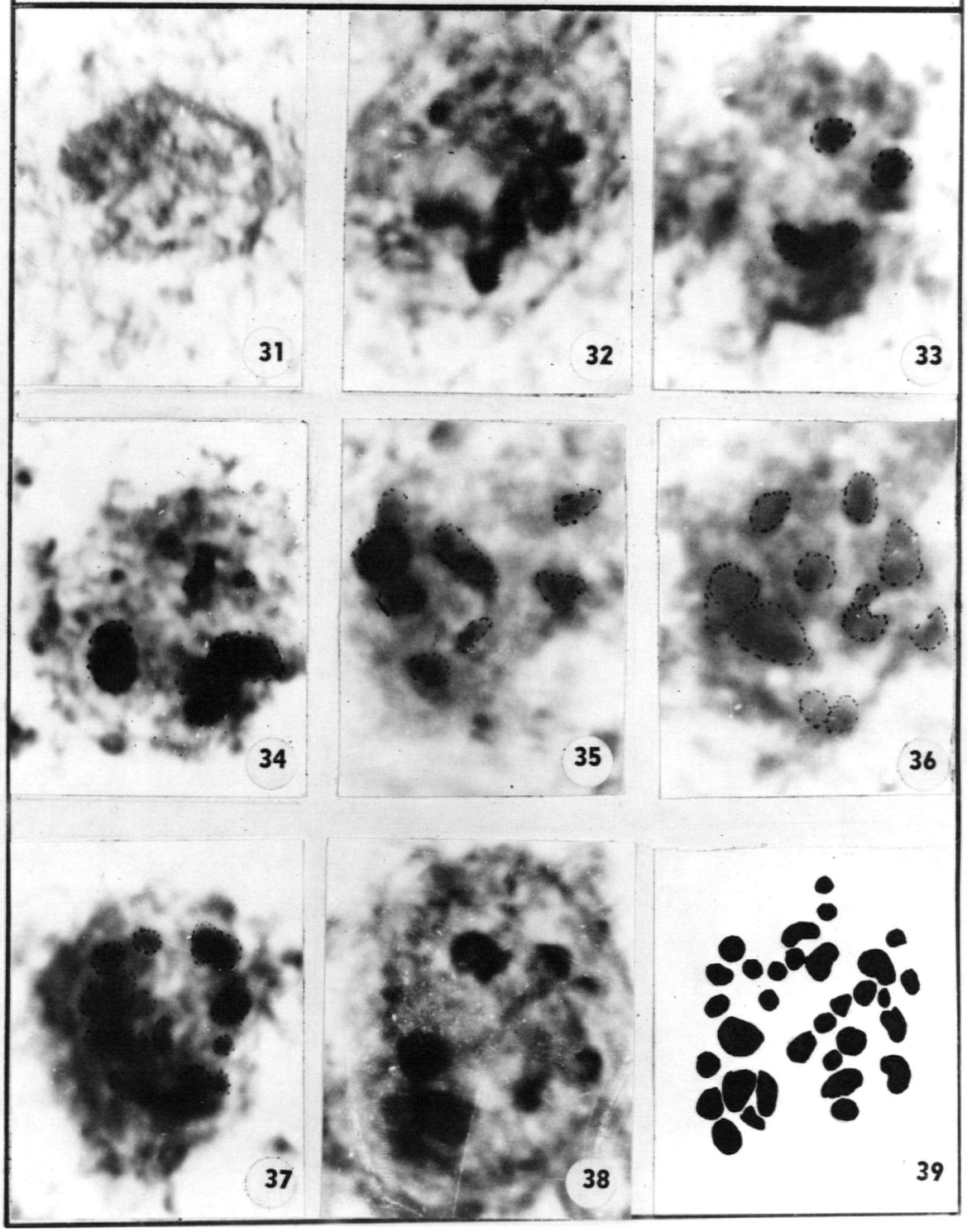


Fig. 40 - 50. Aberrant Eggs Formed in Pelvetiopsis limitata.

Fig. 40 - Two equal eggs, note vertical septum. x 1100. Fig. 41 - Two equal eggs. 3 nuclei may be observed in lower egg. x 590. Fig. 42 - Two equal eggs. Lower egg is elongated. Both appear to be uninucleate. x 580. Fig. 43 - Binucleate egg. x 540. Fig. 44 - Oblique septum forming. Large egg is binucleate. x 610. Fig. 45 - Three eggs developing, lower two are smaller. x 580. Fig. 46 - Four eggs. x 580. Fig. 47 - Four eggs, nuclei visible in three of them. x 565. Fig. 48 - Different focus of Fig. 47, showing that one of the eggs is probably binucleate (indicated by arrow). x 565. Fig. 49 - Three eggs. Two lines of cleavage cut across the egg similar to Fig. 47 and 48, but there does not appear to be a vertical septum. x 580. Fig. 50 - Four, perhaps five eggs. Numbers refer to septa. x 630.

Fig. 40 - 50 from fixed material.

FIG. 40 – 50

ABERRANT EGGS FORMED IN

PELVETIOPSIS LIMITATA

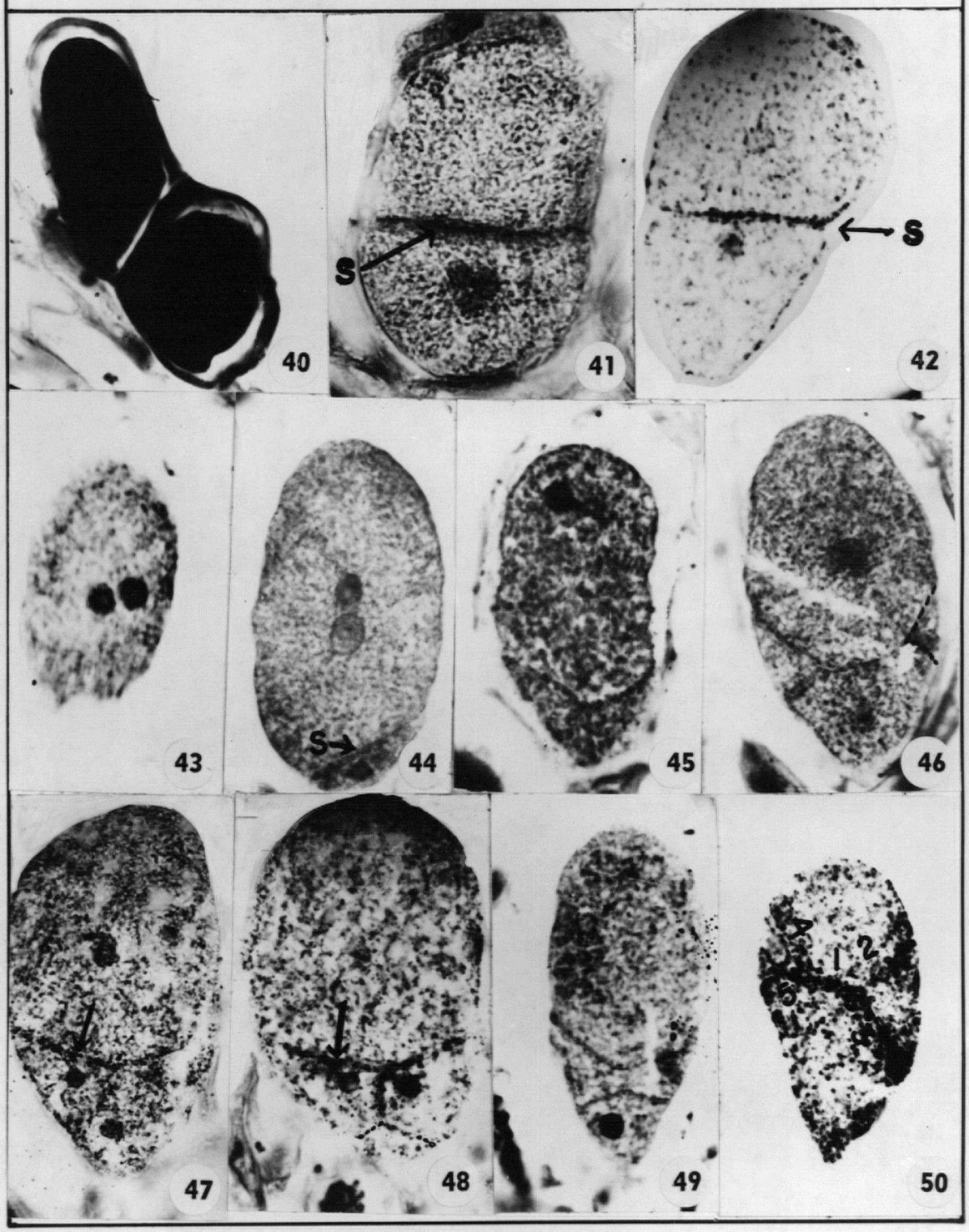


Fig. 51 - 62. Development of Spermatozoids in Pelvetiopsis limitata

Fig. 51 - Wall cell of conceptacle has given rise to two nuclei. The upper nucleus is the antheridial initial. x 1690. Fig. 52 - The nucleus indicated by the arrow is undergoing division to form a new antheridium, which will branch to the right of the hair filament. x 1780. Fig. 53 - Uninucleate antheridial initial showing the stalk cell. x 1210. Fig. 54 - Metaphase of the first nuclear division in the antheridium (probably meiosis I). x 3200. Fig. 55 - Two nuclei in antheridial initial cell. x 1430. Fig. 56 - Second nuclear division in the antheridium (probably meiosis II). One nucleus appears to be in prophase while the other is in metaphase. x 1860. Fig. 57 - Metaphase of second nuclear division, probably meiosis II. x 3200. Fig. 58 - Meiosis II. Approximately 30 chromosomes visible in polar view of metaphase on right. x 2200. Fig. 59 - Camera lucida drawing of Fig. 58, with 30 chromosomes shown on right. x 2800. Fig. 60 - Division III. Four nuclei in mitotic prophase. x 3040. Fig. 61 - Eight-nucleate stage in the antheridium. x 1400. Fig. 62 - Metaphase of Division IV from eight to sixteen nuclei. x 1860.

Fig. 51 - 62 from fixed material.

FIG. 51 - 62

DEVELOPMENT OF SPERMATOZOIDS

IN PELVETIOPSIS LIMITATA

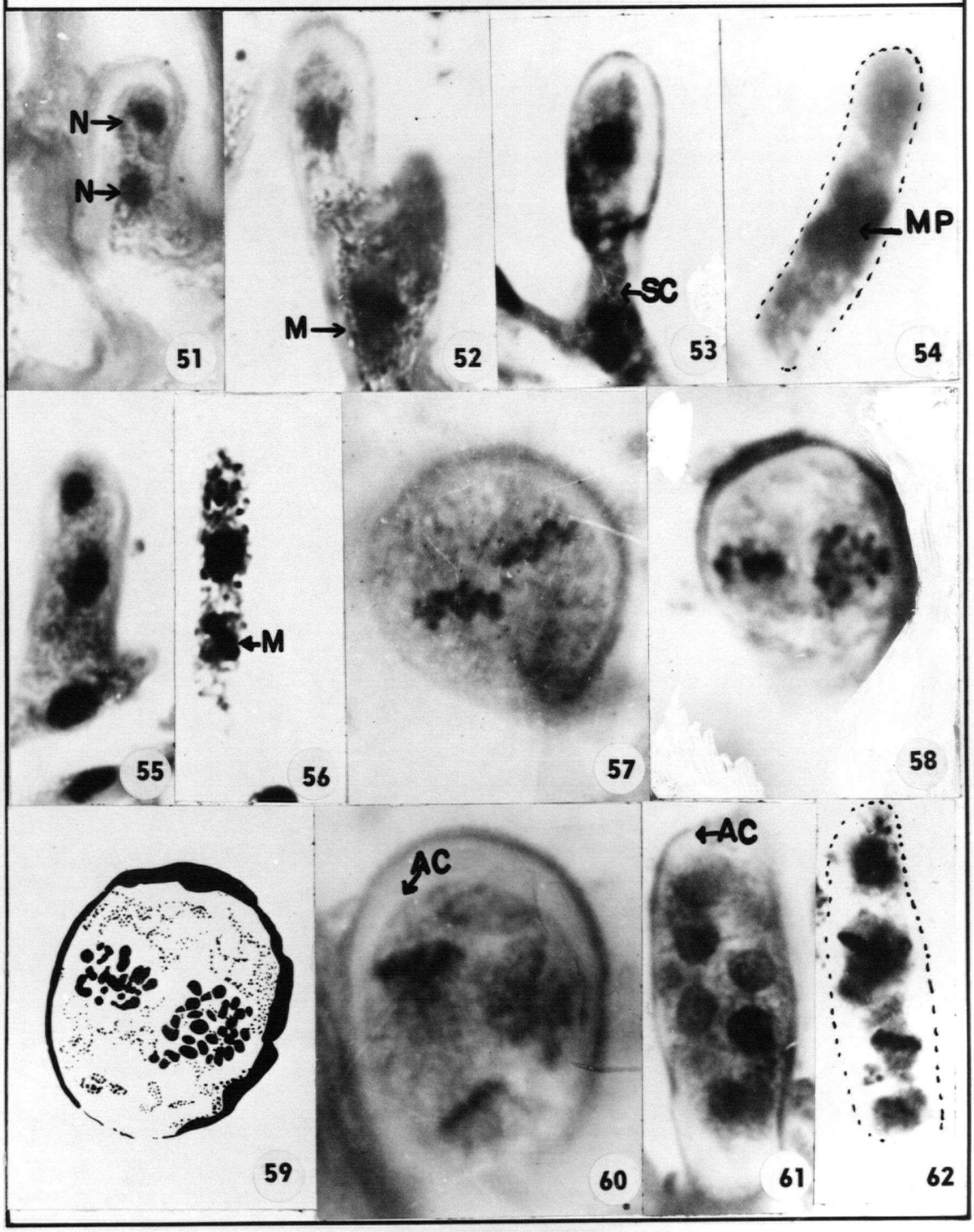


Fig. 63 - 74. Development of Spermatozoids in Pelvetiopsis limitata.

Fig. 63 - Eight nuclei in mitotic metaphase in antheridium, some in polar view. Antheridium is not so elongated as in Fig. 60. x 2380. Fig. 64 - Eight nuclei in metaphase, top four in polar view. x 2380. Fig. 65 - Eight nuclei in anaphase. Note crescent effect at edge of spindle. x 2300. Fig. 66 - Sixteen-nucleate stage. x 1825. Fig. 67 - Sixteen nuclei in prophase. x 1665. Fig. 68 - Sixteen nuclei in metaphase. x 2000. Fig. 69 - Sixteen nuclei dividing, some of the lower nuclei appear to be in anaphase. Note apical cap. x 2000. Fig. 70 - Thirty-two-nucleate stage. x 2400. Fig. 71 - Camera lucida drawing of thirty-two nuclei dividing. x 3200. Fig. 72 - 64 nuclei. x 1165. Fig. 73 - Drawing of liberated spermatozoid with shorter anterior flagellum. x 3180. Fig. 74 - Antheridia arising on a branching hair system from the wall of the conceptacle. x 400.

Fig. 63 - 72 and Fig. 74 from fixed material. Fig. 73 from living material.

FIG. 63 - 74

DEVELOPMENT OF SPERMATOZOIDS IN PELVETIOPSIS LIMITATA

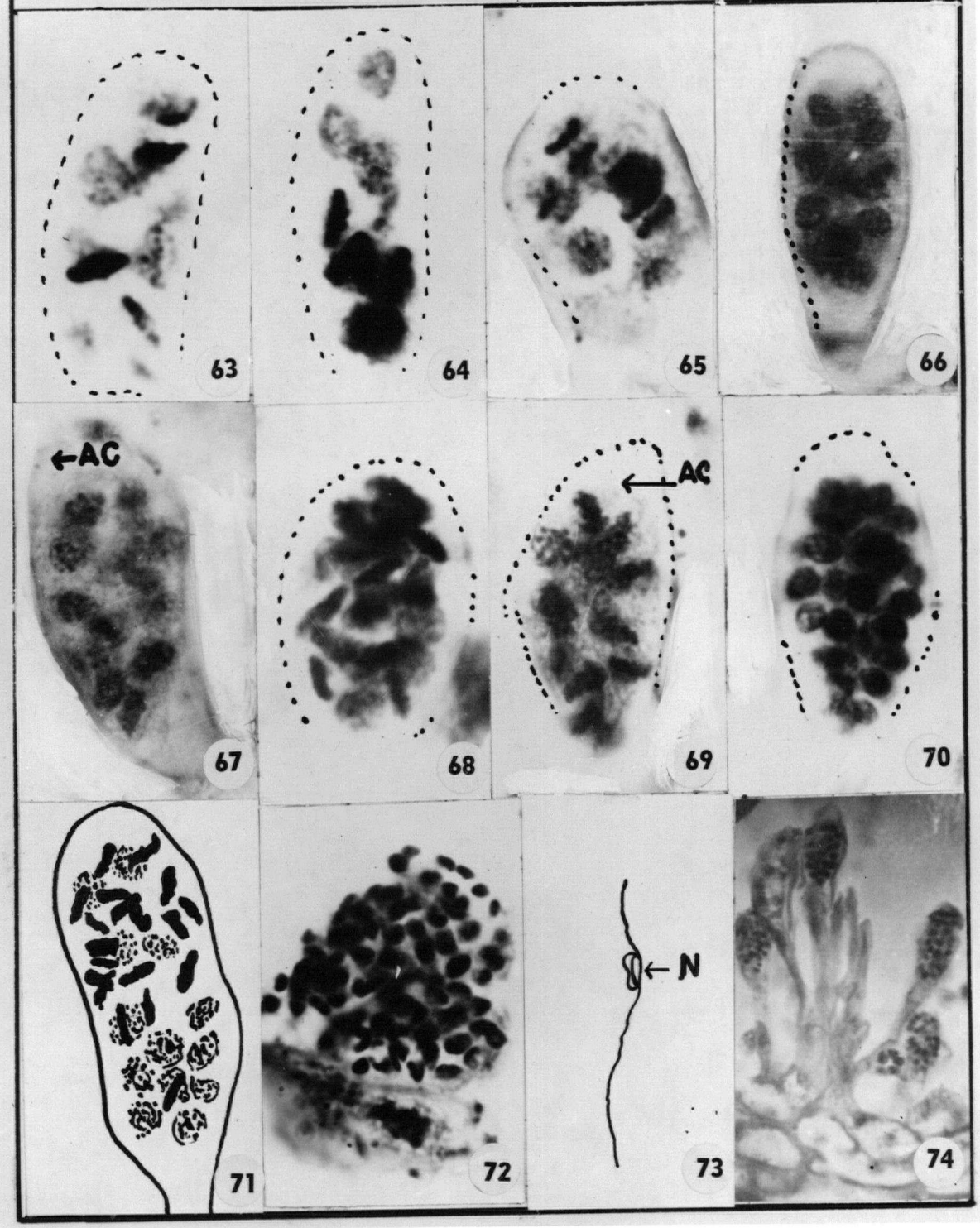


Fig. 75 - 83. Embryology of Pelvetiopsis limitata.

Fig. 75 - Liberated naked egg 10 minutes after liberation from the conceptacle. x 390. Fig. 76 - Pear-shaped zygote, 24 hours after liberation. No division of the cytoplasm has taken place. x 390. Fig. 77 - Line drawing of 3-celled zygote, 1 day old. Septa (SE^1, SE^2) have formed. A primary rhizoid initial has been established below SE^2. x 297. Fig. 78 - Line drawing showing division of young embryo by 3 transverse walls. Top cell has divided vertically by 2 divisions into 4 cells, 2 of which are shown here, 2 days old. x 290. Fig. 79 - Line drawing showing further growth in the embryo, 3 days old. x 290. Fig. 80 - Line drawing showing a second rhizoid appearing, 4 days old. x 210. Fig. 81 - Normal embryo with several rhizoids, one month old. x 135. Fig. 82 - Embryo with rhizoids growing from two areas, one month old. x 135. Fig. 83 - Line drawing of abnormal embryo with 3 nuclei in upper cell, 5 days old. x 516.

Fig. 75 - 76 from living material. Fig. 77 - 83 from both living and fixed material.

FIG. 75 - 83

EMBRYOLOGY OF

PELVETIOPSIS LIMITATA

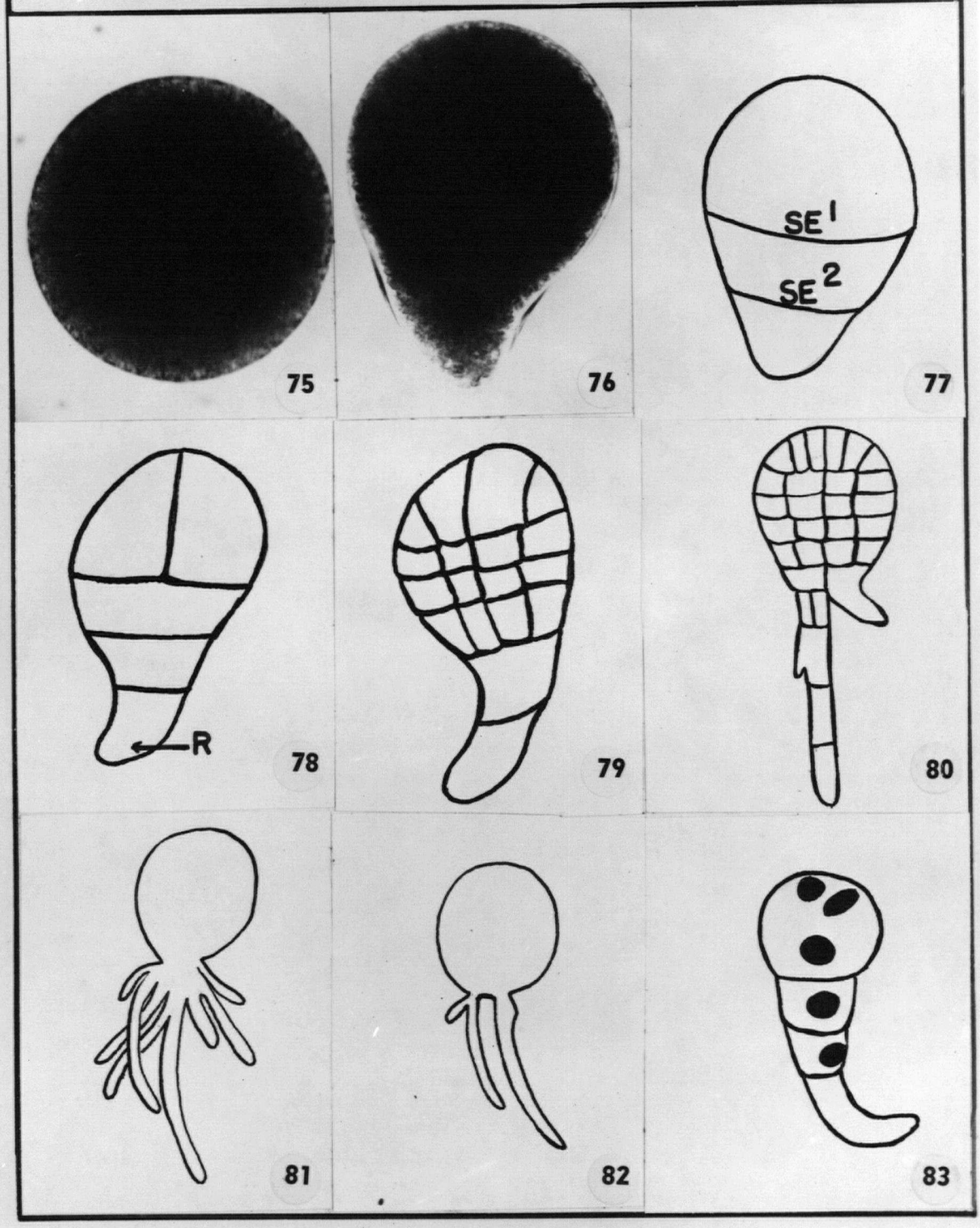


Fig. 84. Life History of Pelvetiopsis limitata (Setchell) Gardner.

The mature plant with a diploid chromosome complement of 64 (i) has many receptacles (ii) producing gametangia in the flask-shaped conceptacles (iii). The first two nuclear divisions in the oogonium comprise the meiotic sequence which reduces the chromosome number to approximately 32 (iv, v, vi). These are followed by a mitotic division which produces 8 nuclei in the oogonium (vii). Seven nuclei migrate to one end of the oogonium and are cut off from the larger, now uninucleate portion as a septum develops (viii, ix, x). Two eggs are formed. The larger one is functional (xi), while the smaller egg (xii) soon aborts. In the antheridium the first 2 divisions are probably meiotic (xiii, xiv) reducing the chromosome number to approximately 30 - 32. Four mitotic divisions follow (xv, xvi, xvii, xviii, ix) which produce 64 nuclei. Sixty-four spermatozoids are released into the surrounding medium by rupture of the antheridium wall. One sperm nucleus (xx) fuses with one egg nucleus restoring the the original chromosome number of approximately 64 (xxi). The embryo develops (xxii) and grows into a new plant, thus completing the life cycle (i).

Magnifications of drawings: i (x 7/10); ii (x1); iii (x25); iv, vi (x250); v (x140); vii, viii, ix (x200); x, xii (x280); xi, xxi (x166); xiii, xiv, xv, xvi (x600); xvii, xviii, xix (x700); xx (x1600); xxii (x 70).

FIG. 84

LIFE HISTORY OF PELVETIOPSIS LIMITATA (SETCHELL) GARDNER