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Title: Development, Reproductive Morphology, and Cytology of Pelvetiopsis limitata (Setchell) Gardner (Phaeophyta, Fucales)

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Pelvetiopsis limitata (Setchell) Gardner (Phaeophyta, Fucales), collected on the central Oregon coast, was studied by electron microscopy, and by light microscopy using methacrylate embedding and toluidine blue, acid fuchsin, PAS and anilin blue staining. The sperm of both P. limitata and Pelvetia fastigiata (J. Agardh) DeToni (from northern California) are typically fucacean, with a banded proboscis and two flagella, the shorter of which is anterior and bears mastigonemes. Apical growth in Pelvetiopsis limitata is the product of a group of initials. Conceptacle development involves the formation of two tongue cells from the conceptacle initial.

When zygotes are cultured in sterile-filtered sea water at 15 C and 2000 lux light intensity, both Pelveti-

opsis limitata and Pelvetia fastigiata exhibit Fucus-like embryology amd form embryonic apical hairs, although this is delayed several weeks in P. limitata relative to P. fastigiata or Fucus. Continuous light causes developmental abnormalities in P. limitata. Pelvetia fastigiata is found to be so different from the European Pelvetia canaliculata Dcne. et Thuret that consideration of their generic segregation is suggested.

# Development, Reproductive Morphology, and Cytology of *Pelvetiopsis limitata* (Setchell) Gardner (Phaeophyta, Fucales)

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## I. INTRODUCTION

N. L. Gardner (1910, 1913) first recognized a peculiar characteristic in a *Pelvetia*-like plant growing on the Pacific coast of North America that led him to propose a new genus, *Pelvetiopsis*. The distinctive vegetative form of this alga had been recognized earlier by M. A. Howe (1893), and by W. A. Setchell, who had designated it *Pelvetia fastigiata* f. *limitata* Setchell in the Phycotheca Boreali-Americana (Gardner 1910). This earlier designation as a *Pelvetia* led to reports of *Pelvetia fastigiata* (J. Agardh) DeToni occurring in Oregon (Moore 1928, Smith 1944, Fritsch 1945) and British Columbia (Scagel 1957). These records were apparently based on *Pelvetiopsis limitata* determined to be *Pelvetia fastigiata*, in some cases at least, prior to Gardner's 1910 publication (DeCew 1975). However, an exploratory collecting trip made for this study determined that *Pelvetia fastigiata* is not to be found north of Mendocino County, California at the present time. Vegetatively, *Pelvetiopsis limitata* is distinguished from *Pelvetia fastigiata* by its flattened, less terete branches and smaller size (rarely to 15 cm tall, usually 7-10 cm vs. a height of 15-40 cm for *Pelvetia fastigiata*). Gardner made his generic

segregation on the basis of Pelvetiopsis limitata's production of but one viable egg in the oogonium, as opposed to the two formed by Pelvetia. (Although the gametes of the Fucales may be interpreted as specialized spores, it is more convenient as well as consistant with the literature of the group to refer to them as eggs and sperm or antherozoids [Smith 1955].)

There is one unpublished study (Hennigan 1961), but no published detailed investigation of Pelvetiopsis limitata since Gardner's original papers, and no published study of the embryonic development of Pelvetia fastigiata nor of its sperm structure. It has been assumed (Powell 1961) that Pelvetia is ancestral to Pelvetiopsis and that Pelvetia fastigiata and the European Pelvetia canaliculata Dcne. et Thur. are grouped naturally. Manton's (1951, 1952, 1953, 1956, 1964) electron micrographs of the sperm of the Fucales revealed great uniformity in the structure of this cell in the members of the Fucaceae that she examined (Fucus, Ascophyllum, and Pelvetia canaliculata). Subrahmanyan's (1956, 1957a, 1957b) studies of Pelvetia canaliculata described an aberrant pattern of embryonic development and a complete absence of hair-like initial cells, both in the apical growth of the embryo and in the development of the conceptacle, while Moore (1928) and Nienburg (1913) reported two hair-like conceptacle initials in Pelvetia fastigiata. Thus the two Pelvetia species were found to differ from

each other and from Fucus, which has a single hair-like conceptacle initial and develops a hair tuft in the apical groove of the embryo (Fritsch 1945).

More recently, McCully's (1966) use of methacrylate embedding and toluidine blue, acid fuchsin, and periodic acid-Schiff's reaction staining in a study of Fucus permitted a more precise differentiation of cytological details, and revealed a somewhat different mode of apical growth from that described by earlier authors (Fritsch 1945) for Fucus.

Considering the lack of knowledge and contradictory findings concerning characters regarded as being of some taxonomic significance, it seemed that Pelvetiopsis limitata might profitably be examined in regard to its sperm structure, embryonic development, apical growth, and conceptacle formation, with concurrent examination of Pelvetia fastigiata as to sperm structure and embryology, for comparative purposes.

In the following sections "Pelvetiopsis" shall refer to Pelvetiopsis limitata and "Pelvetia" to Pelvetia fastigiata, unless otherwise specified.

## II. MATERIALS AND METHODS

Plants of Pelvetiopsis limitata were collected at Cape Perpetua (Lane County), and Seal Rock and Yaquina Head (Lincoln County), Oregon. Plants of Pelvetia fastigiata were collected at Van Damme State Park (Mendocino County), California. Specimens were kept cool, wrapped loosely in seawater-dampened newspaper inside polyethylene bags, on ice when necessary, while being transported to the laboratory. Reproductive plants were made to shed gametes by the following procedure: plants were rinsed in running tap water at about 12 C until free of associated animals and previously shed gametes, then placed in micropore (2.2 µm) filtered seawater at 5 C or 15 C in light of approximately 2000 lux intensity from cool-white fluorescent tubes for 6-24 h. The embryos resulting from the gametes thus shed were cultured in micropore-filtered seawater at 15 C at the same light intensity as had been used for shedding gametes, in continuous light, or under a 16:8 light-dark cycle.

Material for chromosome squashes was fixed in 1:3 acetic alcohol for 1-24 h, rinsed in tap water, softened in 6% sodium carbonate 1-24 h, rinsed in tap water and stained in warm aceto-carmine (Evans 1966), then mounted in Hoyer's solution. Alternatively, material was fixed in Flemming's weak formula (Taylor 1950) for 24 h, rinsed in seawater, bleached in 3% hydrogen peroxide 4 h, rinsed in tap water

and stained in warm aceto-carmine before mounting in Hoyer's solution.

Material to be sectioned for light microscopy was fixed in 10% acrolein in 0.025 M phosphate buffer, rinsed in buffer, dehydrated in a series of methyl cellosolve-ethanol- n-propanol- n-butanol, then infiltrated with glycol methacrylate (McCully 1966), or fixed in 10% formalin in seawater, rinsed in seawater, dehydrated in an ethanol series (10-30-50-70-90-100%) and infiltrated with butoxyethanol-glycol methacrylate (Polysciences "JB-4" embedding kit). Methacrylate embedded material was polymerized in gelatin capsules capped to exclude oxygen or in polyethylene casting molds sealed with paraffin wax. Glycol methacrylate was polymerized at 60 C, while butoxyethanol glycol methacrylate was polymerized at room temperature, approximately 21 C.

Polymerized plastic blocks were sectioned at 0.5-5 µm using a Porter-Blum MT-1 ultramicrotome with a dry glass knife. The following method was devised to obtain serial sections: the plastic block was trimmed around the specimen to form a rectangular cutting face, then clamped in the chuck of the ultramicrotome. The top and bottom (i.e., horizontal) faces of the block were lightly coated with "Weldwood" contact cement diluted with an equal volume of toluene, and the cement allowed to dry for a few minutes. Sections subsequently cut formed ribbons as in conventional

sectioning of paraffin-embedded tissue. Ribbons thus obtained were floated on distilled water on glass microscope slides and dried on the slides at room temperature, or at 70 C, as the heat appeared to aid adhesion of the sections to the slides.

The ability to form ribbons of methacrylate sections was crucial to the success of the study of the apical cells and conceptacle formation, since it must be established that one is observing a section cut through the center of a structure by examining neighboring sections in the ribbon. This is especially important in determining whether a presumed tongue cell is accompanied in the conceptacle by other erect cells (paraphyses); if so, the conceptacle is too old to provide information concerning its mode of formation.

Sections on slides were stained as for conventional light microscopy with the omission of gradual dehydration procedures or use of transitional solvents; the slides were air dried between steps involving immiscible fluids. The following stains were used: toluidine blue at pH 6.8, acid fuchsin (1% in 1% acetic acid) (McCully 1966), periodic acid-Schiff's reaction (PAS) (Jensen 1962), anilin blue (water soluble, 1% in 1% acetic acid) and fast green (Johansen 1940). Background staining of the methacrylate matrix was removed by washing in 100% ethanol (Polysciences Data Sheet No. 123).

Photomicrographs were made with a Zeiss Standard

microscope equipped with the following oil-immersion objectives: Achromat 16X, 0.40 N.A.; Achromat 40X, 0.85 N.A.; Neofluar 63X, 1.25 N.A.; Neofluar 100X, 1.30 N.A., and a Leica M1 35 mm camera using Kodak SO-410 monochrome photomicrographic film developed in D-19 for four minutes, or Kodachrome II Professional Film (Type A) processed by Kodak. A Leitz Makam $3\frac{1}{4}$ x $4\frac{1}{4}$-in cut-film camera using Kodak Plus-X film developed in DK-50 for 4.5-6 minutes was also used.

Material for electron microscopy (of sectioned structures) was fixed in 10% acrolein or 10% glutaraldehyde in 0.025 M phosphate buffer 1-24 h and postfixed in 1% osmium tetroxide in buffer 24 h. Dehydration was in an acetone series, with or without propylene oxide, followed by infiltration in Araldite or Spurr's Epon, gradually replacing the transition solvent. In vacuo infiltration was sometimes employed. Polymerization was at 60-70 C. Material was sectioned on a Porter-Blum MT-1 ultramicrotome with glass or diamond knives. Staining was in urynal acetate followed by lead citrate (Dawes 1971).

Sperm were prepared for electron microscopy by being shed as described above, centrifuged at 1000 x g for one minute to remove eggs and debris, decanted and recentrifuged at 10,000 x g for five minutes to form a pellet, mixed into warm 3% agar which was then solidified and fixed in buffered 10% acrolein, then prepared for sectioning as described above.

Alternatively, shed sperm were concentrated in a clinical centrifuge at 1500 x g and put dropwise on formvar coated grids and fixed in osmium tetroxide vapor, dried, rinsed in distilled water and shadowcast with chromium.

All specimens for the electron microscope were suspended on formvar- or carbon-coated copper grids, and observed on an RCA EMU-2, EMU-3, or Phillips EM 300 electron microscope.

# III. RESULTS

## Staining Reactions

The effect of toluidine blue staining on Pelvetiopsis limitata was apparently identical to that obtained by McCully (1966) in her study of Fucus: the alginic acid, found in cells, extracellular mucilage, the cell walls, and cuticle of the epidermis is stained purple, while polyphenols in vesicles in cells are stained blue to green. Plastids are not stained, nor are nuclei except those of actively dividing cells, which may stain light blue or light purple. The periodic acid-Schiff's reaction (PAS) stained red both cellulose (said by McCully to be present only in small quantity) and alginic acid, found mostly in the cell walls and cuticle (McCully 1966). Acid fuchsin stained the nuclei red, but weakly, and the plastids more strongly. Fast green is a general cytoplasmic stain. The polyphenol-containing vesicles, which stain blue to green in toluidine blue, do not stain in acid fuchsin, PAS, or fast green, but remain their natural golden-yellow color.

It was found that water-soluble anilin blue could be substituted for acid fuchsin and used as a counterstain for PAS; the most distinctive differentiation of cells resulted from application of PAS reaction followed by 15 minutes in anilin blue. This treatment yields a brilliant differentiation of the cell walls and mucilaginous cuticle in red,

nuclei and plastids in deep blue, and the polyphenol-containing vesicles golden-yellow.

## Apical Growth

Branch tips of Pelvetiopsis grow by means of divisions within a group of apical initials. These initials give rise to cells by anticlinal and periclinal divisions. Cells arising by anticlinal division form new epidermis that matures in appearance as it emerges from the apical furrow, while cells arising from periclinal division add to the length of the branch, mostly by subsequent elongation.

The initial cells are characterized by thin walls staining weakly in PAS, a centrally located nucleus, numerous small, scattered plastids, and a few small polyphenol-containing vesicles. As the maturing epidermal cells are pushed out of the apical furrow they increasingly resemble the mature epidermal cells, which are covered by a PAS-positive cuticle and form a palisade layer on the surface of the branch. Mature epidermal cells possess thick, intensely PAS-staining walls, a nucleus situated at the proximal end of the cell in a surrounding cup of plastids, and many conspicuous polyphenolic vesicles in the central and distal portions of the cell. Viewed in longitudinal section (Fig. 1), the apical initials occur in a row of four to eight cells usually larger than their daughter cells. Viewed in cross-section (Fig. 2), the apical cells appear

as a cluster of cells in two adjacent ranks, each cell about 30 x 60 μm, separated by very thin walls and containing scattered plastids. Daughter cells surrounding the apical cells are smaller, approximately 20 x 20 μm, with thicker walls, peripherally positioned plastids, and larger, more numerous polyphenolic vesicles.

## Development of the Conceptacle

Development of a conceptacle begins in the apical furrow among epidermal cells that have not fully matured. An invagination is formed by growth of cells surrounding the conceptacle initial, which sits at the bottom of the cavity thus formed. The conceptacle initial is not distinguishable from its neighbors until after the formation of this distinct invagination of the epidermis; by this time the developing conceptacle has been pushed out of the confines of the apical groove by further apical growth.

The conceptacle initial is first recognizable as a "basal cell", surmounted by a "tongue cell" (Fig. 3) that protrudes into the cavity. This tongue cell, the product of a transverse division of the initial, may divide longitudinally to produce a second (Fig. 4), and rarely a third tongue cell, each attached to a basal cell produced by longitudinal division of the initial. Division products of the basal cells also form the lining of the floor of the conceptacle, and like the tongue cells they are character-

ized by a centrally positioned nucleus (as opposed to the proximally situated nucleus of epidermal cells) and a lesser number of polyphenol-containing vesicles, of reduced size. These features distinguish the products of the conceptacle initial from epidermal cells that initially surround them. In the mature conceptacle (Fig. 5) the lining of the structure consists exclusively of cells of this type except near the ostiole.

## Sexual Reproduction

The conceptacles of Pelvetiopsis limitata are hermaphroditic. The oogonia are borne on stalk cells which arise from cells of the conceptacle wall. Antheridia may be borne similarly or may be borne on a branched filament. Sterile filaments (paraphyses) are present, which do not protrude from the ostiole. There are no sterile conceptacles in mature portions of the thallus, either opening to the exterior (cryptostomata) or completely sunken and closed off from the exterior (caecostomata) such as are found in Fucus distichus (L.) Powell.

The single nucleus of the oogonium undergoes three divisions to form eight haploid nuclei in a common cytoplasm. Seven nuclei move to the base of the oogonium where they become isolated by a wall from the remaining nucleus which is surrounded by most of the cytoplasmic mass of the oogonium. Chromosome counts made during oogonial meiosis

were not definitive, but appeared consistant with the published report of n=32 (Cole 1967).

The mature egg is released by rupture of the outermost oogonial membrane (exochiton) (Fig. 6), whereupon the freed egg is carried out of the conceptacle by the extrusion of mucilage. The three inner membranous layers are removed sequentially and the large and small eggs separate. The larger, functional egg measures approximately 100 μm in diameter after release; the small seven-nucleate egg is approximately 30 μm in diameter. The eggs are packed with polyphenol-containing vesicles, food storage vesicles, and plastids (Fig. 7). Electron microscope examination of the plastids reveals a structure similar to that found in Fucus (McCully 1968), with osmiophilic inclusions between the thylakoids and no pyrenoid present (Fig. 8).

The antheridium undergoes meiosis, succeeded by mitoses to form 64 nuclei. No evidence was found of formation of septa between the developing sperm. The sperm are released by rupture of the antheridial exochiton, whereupon they are extruded from the conceptacle in a mass of mucilage and subsequently separate as the endochiton dissolves.

Sperm of Pelvetiopsis and Pelvetia (Figs. 11, 14) show typical fucacean form, with an anterior flagellum with mastigonemes, a longer, smooth posterior flagellum, and a proboscis (Figs. 9, 10). Light microscopy reveals the body of the sperm to be approximately 5 μm long, but in air-

dried, shadowcast electron microscope preparations the body shrinks to as little as 2 μm and terminal portions of the flagella, and frequently the mastigonemes of the anterior flagellum, are lost. This effect has been attributed to the action of osmium fixation (Russell 1973).

Shadowcast electron microscope preparations show 11 microfibrillar bands (Fig. 12) in the proboscis of Pelvetiopsis, and 12-13 in that of Pelvetia (Fig. 15). Sections of Pelvetiopsis sperm, although poorly fixed, showed the body of the cell consists mostly of the nucleus and four mitochondria. A chloroplast also appeared to be present.

Syngamy in Pelvetiopsis and Pelvetia is essentially the same as in Fucus; upon release of the gametes into the sea, the eggs sink and are followed by the sperm which aggregate at the periphery of the egg. One sperm penetrates the egg membrane, a cellulose wall is formed at the egg surface following plasmogamy and embryonic development begins at karyogamy.

## Development of the Embryo

The fertilized egg of Pelvetiopsis limitata and Pelvetia fastigiata begins rhizoid formation (Fig. 16) before the first division, in the manner of Fucus (Fritsch 1945). The zygote becomes pyriform as the nucleus moves toward the developing bulge, and the first division (Fig. 17) occurs in the plane perpendicular to the axis of the forming rhi-

zoid, usually within 24 h of fertilization. Rhizoid elongation continues as two more divisions (Fig. 18) occur in the same plane as the first, succeeded by a division parallel to the axis of the rhizoid. The rhizoid continues to elongate and begins to branch in as little as three days.

Study of sections of Pelvetiopsis embryos revealed early differentiation of surface and internal cells as well as apical differentiation, which show well in material stained in toluidine blue. A reduced number of polyphenol-containing vesicles in internal cells was evident in embryos as early as four days after fertilization. At the same time, the first evidence of apical differentiation appears, discernable at the end of the embryo opposite the rhizoid as a group of cells of elongated form, the result of anticlinal divisions (Fig. 19), in contrast to the surrounding cubical cells. By day ten following fertilization these apical cells exhibit divisions characteristic of the adult plant in that they are producing daughter cells by periclinal division (Fig. 21). At this stage surface cells have begun to show the polarized arrangement of nucleus, plastids and polyphenolic vesicles (Fig. 20) found in the epidermis of the adult plant.

Such apical differentiation became obvious externally as an apical depression by week three. Apical hair formation (Fig. 22) did not become evident until week eight. By contrast, Pelvetia embryos showed profuse apical hair form-

ation (Fig. 24) by day 17, in keeping with a generally more rapid development as indicated by rhizoid proliferation and more rapid division to form a many-celled embryo.

By the time Pelvetiopsis embryos were producing apical hairs their morphology had become abnormal. This was presumably a result of continuous light (McLachlan 1974), which was being used to maintain rapid growth since embryos had been reported (Hennigan 1961) to lack apical hairs after four months' growth at 250 ft-c under a 12:12 h light-dark cycle. These hairs were few in number (two to three) and quite long (to 4 mm), arising from an apical furrow, or in many cases from an abnormal apical hump (Fig. 23). Pelvetia embryos produced apical hairs in only 17 days, before the morphological abnormalities induced by continuous light had developed. These hairs arose from the apical furrow and were numerous (four to eight), eventually also growing quite long.

Electron microscopy of Pelvetiopsis showed that plasmodesmata arranged in pit fields (Fig. 25) may be found in the cell walls early in the life of the embryo. Their presence in vegetative cells of the mature thallus is apparent in sections observed by light microscopy, and electron microscopy of pit fields (Figs. 26,27) between cells of paraphyses in conceptacles shows structures similar to those reported in Fucus (Bisalputra 1966).

Embryos of both Pelvetiopsis and Pelvetia were kept

in culture as long as four months, but failed to develop to the stage where flattening of the thallus or branching appeared.

The development of several of the smaller, presumably fertilized and thus eight-nucleate eggs of Pelvetiopsis was observed for up to seven weeks, during which the embryos developed branched rhizoids but failed to initiate any division of the body of the embryo (Figs. 29, 30, 31). It seems likely that the multinucleate eggs that showed some embryonic development were indeed fertilized, since sperm were seen aggregating at the surface of these eggs (Fig. 28) as well as the large functional eggs. That sperm should be attracted by the "non-functional" eggs is not too surprising, since the sperm attractant of Fucus serratus and F. vesiculosis eggs has been shown to possess no species specificity and its effect can be mimicked by n-hexane (Cook and Elvidge 1951). The sperm attractant of F. serratus (and possibly most other Fucaceae) is a fairly simple conjugated hydrocarbon (1,3,5-octetriene, formula $C_8H_{12}$ [Muller and Jaenicke 1973]) which may merely leak from the egg, and since the small eggs contain a significant portion of the oogonial cytoplasm, they may produce a significant quantity of sperm attractant.

# IV. DISCUSSION

Pelvetiopsis limitata appears to be a fairly typical member of the Fucaceae which shares some peculiarities with Pelvetia fastigiata. The sperm of both species are of the fucacean type, although shadowcast preparations show a smaller number (11-13) of microfibrillar bands in the proboscis than in Fucus (13), similar to the European Pelvetia canaliculata which has 11 bands (Manton et al. 1953). The significance of the number of bands in the proboscis is unknown. Manton's (1964) report of an internal, seven-banded homologue of the proboscis in the sperm of Cystoseira, Dictyota and the zoospore of Scytosiphon (evidently part of the flagellar root) would suggest that a larger number of bands in the proboscis indicates a more specialized, highly evolved structure; but it is also possible that the number of bands is dependent on the size of the proboscis, the largest yet found being in Ascophyllum nodosum, which has 14-15 bands (Manton et al. 1952). It is unclear how the size of the proboscis may relate to the size of the sperm.

Hennigan (1961) reported the sperm of Pelvetiopsis limitata to be only 2-2.2 μm in length, but the present study found sperm appearing this small by light microscopy only in preparations that were several hours old. After a time the sperm appear to round up and shrink in size; by this time they show only weak swimming motions and are

therefore more easily observed.

The use of certain stains, especially PAS with anilin blue, has permitted a more precise characterization of the differentiation of cells in the plant. Earlier workers (Holz 1903, Subrahmanyan 1956) referred to the golden-yellow bodies in the cells of the Fucales as chromatophores or plastids, but toluidine blue staining and electron microscopy have shown these bodies to be membrane-bound vesicles containing polyphenolic compounds (McCully 1968b). Acid fuchsin or anilin blue staining shows the photosynthetic plastids to be present in the apical initials that were said to be free of "chromatophores", although they are reduced in size and number relative to those in mature cells. A few small polyphenolic vesicles can usually be seen in the initials, and these become more numerous and larger as the segments from the apical cells become epidermal or medullary cells, while the products of the conceptacle initials remain almost devoid of the vesicles. This suggests that the chemicals in these vesicles are not incorporated in the mucilage produced in the conceptacle. McCully (1966) observed the polarized arrangement of the vesicles, plastids, and the nucleus in epidermal cells of Fucus and concluded that the polarization was evidence of a vigorous secretory function for these cells. An alternative interpretation is that in the epidermal cells the plastids and polyphenol-containing vesicles shield the nucleus from bright light,

and that the polyphenolic compounds are for the most part retained in the vesicles in the epidermal cells where they serve as a deterrent to grazing animals (McCully could find no direct evidence of secretion of the contents of the vesicles). The cells of the conceptacle wall and the paraphyses lack the polarized arrangement of organelles found in epidermal cells, for they are situated below the outer surface of the thallus and are not exposed to direct sunlight. Yet these cells are active in secretion of mucilage, which serves to carry the gametes out of the conceptacle when contact with seawater from the incoming tide swells the mucilage.

Apical development in Pelvetiopsis was found to be the product of a group of apical initials, in keeping with McCully's (1966) observations of Fucus but contrary to earlier findings in Fucus and other genera (Fritsch 1945). This discrepancy was attributed by McCully to a possible difference between vigorous growth (multiple apical initials) and slow growth (one apical initial). This is plausible, for the existance of multiple apical hairs in the embryo suggests that several apical intitals might remain active in the adult plant, at least in those still actively growing. Even Subrahmanyan (1956), who claimed to have found a single apical initial with the classical form of a truncated pyramid in Pelvetia canaliculata, admitted "Sometimes it is difficult to distinguish the apical cell

from the segments cut off by it, since all of them resemble one another very closely for some time" (p. 377). That they resemble each other very closely may be evidence that these cells are all sharing the function of initiating apical growth.

However this mode of growth may be interpreted, it should be noted that it cannot be confused with the pattern characteristic of Nizamuddin's (1962) family Serococcaceae, in which there are two or more apical cells obviously separate from each other forming two or more distinct growing points at the apex of each branch.

Development of conceptacles in Pelvetiopsis is similar to that described by Nienburg (1913) and Moore (1928) in Pelvetia fastigiata, which showed an initial dividing longitudinally, followed by transverse divisions so that two tongue cells resulted. Pelvetiopsis in the present study was found to form a tongue cell by a transverse division of the initial, followed by one or occasionally two longitudinal divisions so that two or three tongue cells are formed; the original basal cell (the initial) dividing longitudinally so that each tongue cell has its own basal cell. These two modes of division seem to differ significantly, but it should be noted that Nienburg described occasional oblique first divisions in the formation of the tongue cells in Pelvetia fastigiata, and Moore's illustrations (her figs. 5-7) suggest the division sequence the

present study finds in Pelvetiopsis rather than her interpretation. Hennigan (1961) described but a single tongue cell in Pelvetiopsis arising from transverse division of the initial. Pelvetia canaliculata is described (Subrahmanyan 1957a) as having no tongue cell at all; thus all early divisions of the initial are longitudinal, and this was interpreted as being similar to Nienburg's description of Pelvetia fastigiata with its longitudinal divisions preceding the transverse divisions, which presumably have been eliminated from P. canaliculata. This is thought to be of significance because Fucus is reported to form a single tongue cell by a transverse division of the initial (Fritsch 1945).

The importance of the number of tongue cells formed, or of the plane of the first division of the initial that forms the tongue cell, is perhaps open to question. At the least, it appears that only Pelvetiopsis and Pelvetia fastigiata are known to produce side-by-side tongue cells. The point of broader significance is that there is but one cell per tongue, i.e., no multicelled tongue filament of the type that is found in some genera of the Cystoseiraceae (e.g., Cystoseira, Coccophora) (Fensholt, 1955).

The present study also conclusively disproves Holz's (1903) claim that Pelvetiopsis (he worked with material that he mistakenly thought was Pelvetia fastigiata) formed the conceptacle cavity by a progressive collapse and decay

of a meristematic pad of epidermal cells produced by proliferation of cells originating from an epidermal initial, a conclusion disputed by Nienburg (1913) on the basis of his observations on Pelvetia fastigiata.

Both Pelvetia fastigiata and Pelvetiopsis were found to possess a Fucus type of embryonic development characterized by initiation of a rhizoid prior to the first division of the zygote, and apical development by ephemeral apical hairs. This is in marked contrast to the embryological behavior of Pelvetia canaliculata (Subrahmanyan 1957b, Moss 1974), which resembles that of genera in the Cystoseiraceae and Sargassaceae: the zygote undergoes cytoplasmic cleavages resulting in a spherical multicelled embryo before the simultaneous initiation of several (in the case of P. canaliculata, four) rhizoids. This was interpreted by Subrahmanyan (1957b) to indicate an "affinity with the Sargassaceae" (p.391). But it should be remembered that P. canaliculata is specialized for life in an environment of extreme desiccation (Fritsch 1945, Moss 1974), so that the peculiar delay in rhizoid initiation might be interpreted as being permitted by the adhesion of the embryo to the substrate by the persistant oogonial walls of this species, and necessitated by the embryo having to force its way out of this same protective envelope as growth proceeds, without also tearing itself from the substrate. Four rhizoids would provide a more secure purchase than one in these

trying circumstances.

Another peculiar feature of Pelvetia canaliculata is the report of an absence of apical hairs in the development of the embryo, as well as no tongue cell involved in conceptacle formation (Subrahmanyan 1957a, 1957b). The lack of embryonic apical hairs is apparently unique in the Fucaceae, but this may also be an adaptation to an environment of extreme desiccation, since the presence of hairs would result in a significant increase in evaporative surface area in the minute embryo. The possibility exists that apical hairs have been overlooked by investigators, but the lack of any tongue cell in conceptacle formation indicates that trichothallic structures have been eliminated from development in P. canaliculata. This conflicts with any supposed connection with the Sargassaceae, among whom the occurrence of a tongue cell or filament is apparently universal (Fensholt 1955).

The mode of nuclear extrusion in Pelvetiopsis is noteworthy, because the only other genus in the Fucales known to show the same pattern is the monotypic Hesperophycus, a plant with a distinctly Fucus-like vegetative structure (Gardner 1910, 1913), and presumably a direct descendant of Fucus (Powell 1961). Walker's (1930) report of the diameter of the two eggs of Hesperophycus as measuring only 11 μm and 2.7 μm, which would make the smaller, seven-nucleate egg half the size of the sperm in other Fucaceae,

was evidently the product of a mathematical error, since her Fig. 11 shows the same size relationship between egg and sperm as found in Pelvetiopsis, and at the indicated magnification of the figure, a 110 µm-diameter large egg is pictured. That the Pelvetiopsis-type pattern of nuclear extrusion should occur only in two genera on the Pacific coast of North America, which are apparently of different immediate ancestry, indicates that the number of functional eggs and the pattern of nuclear extrusion are not such conservative characters as has been assumed. An example of this is found in Pelvetia canaliculata, for here extrusion of the supernumerary nuclei is of the type found in Cystoseira, in which there is a centrifugal extrusion from the cytoplasm of the oogonium, so that the nuclei are scattered at the periphery. But P. canaliculata produces two functional eggs (by a transverse division of the oogonium); in the Cystoseiraceae only one egg is functional. The supernumerary nuclei of Pelvetia fastigiata are found in the space between the two eggs, produced by a longitudinal division of the oogonium, so that they remain together rather than being scattered as in P. canaliculata. This pattern in Pelvetia fastigiata presumably underwent a transition, in Pelvetiopsis, to a form in which only one functional egg remains and the seven supernumerary nuclei are aggregated in a small amount of cytoplasm at the base of the oogonium. Variation in the number of functional eggs produced has

been reported in Pelvetiopsis (Hennigan 1961), Pelvetia canaliculata (Subrahmanyan 1957b) and Pelvetia fastigiata (Moore 1928); in this last case, all specimens from one particular location were found to possess four functional eggs. It is evident that not only is the number of functional eggs produced somewhat variable in several species but also that the mode of oogonial cleavage and nuclear extrusion is variable in the genus Pelvetia, and there is not a single reduction series from Fucus (eight eggs) to Hesperophycus and Pelvetiopsis (one egg). Pelvetia canaliculata appears to be so aberrant in several respects (nuclear extrusion, embryology, early apical growth and conceptacle initiation) that an argument can be made for separating it from Pelvetia fastigiata and the very similar (Gardner 1910, Subrahmanyan 1957b) Pelvetia wrightii of Japan. Pelvetia canaliculata is firmly anchored in the Fucaceae however, on the basis of the sperm structure as reported by Manton et al. (1953), although no electron microscopical study has yet been applied to the sperm of Sargassum.

The unusual embryonic development of P. canaliculata should be considered in light of recent findings (McLachlan 1974) that under varying environmental conditions Fucus embryos exhibit marked alteration of their usual pattern of development. Under 20-hour light periods, many embryos formed spherical thalli without rhizoids, and under light-deficient conditions apical hairs were suppressed. It may

be that the harsh conditions encountered by P. canaliculata in the high intertidal have favored selection of embryos with a tendency for such "abnormal" developmental traits.

In this context it may be significant that apical hair formation is delayed in Pelvetiopsis by comparison with Fucus (McLachlan 1974) and Pelvetia fastigiata, requiring eight weeks under culture conditions in which Pelvetia formed apical hairs in as little as 17 days. Since Pelvetiopsis is found higher in the intertidal than Fucus or Pelvetia fastigiata, perhaps it has adopted the strategy of elaborating the basal part of the embryo before the increased evaporative surface of the apical hairs is added, a trend we see brought to its culmination in Pelvetia canaliculata.

## V. SUMMARY OF CONCLUSIONS

*Pelvetiopsis limitata* is a *bona fide* member of the Fucaceae (*sensu* Fritsch), showing resemblance to *Fucus* in its embryonic development, apical growth, and sperm structure, although possessing some peculiar features also to be found in *Pelvetia fastigiata* or *Hesperophycus*. Delay in the formation of apical hairs in the embryo may be an adaptation toward desiccation-resistance.

Manton's (1964) recognition of the unique and consistant sperm structure in the Fucaceae can be considered valid for the Pacific Ocean forms *Pelvetia fastigiata* and *Pelvetiopsis limitata* also. McCully's (1966) finding of multiple apical initials in *Fucus* applies to *Pelvetiopsis* as well. The occurrence of multiple tongue cells in the formation of the conceptacle in *P. fastigiata* (Nienburg 1913, Moore 1928) is repeated in *Pelvetiopsis*.

*Pelvetia fastigiata* appears to be strikingly different from *Pelvetia canaliculata* according to several criteria that may be a basis for their generic segregation.

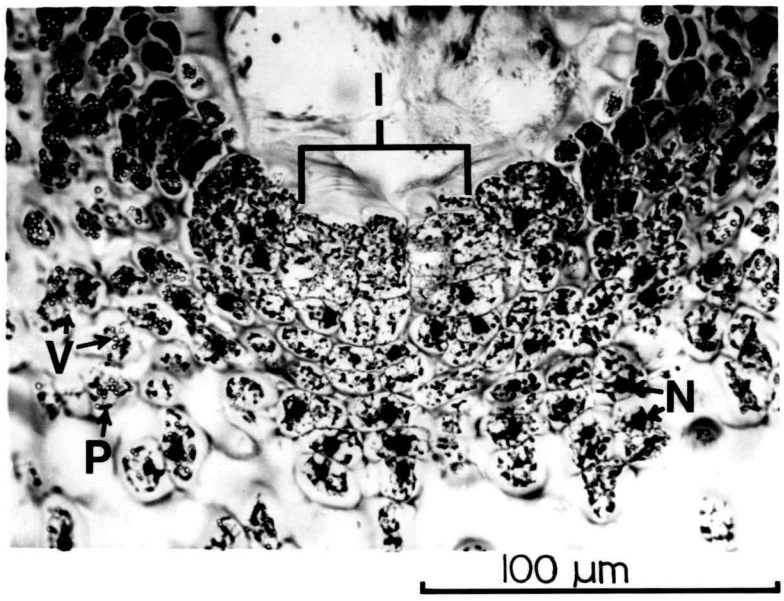
The number of functional eggs formed and embryonic development may, in the Fucaceae, be susceptible to environmental modification to the degree that these characters may not be satisfactory taxonomic discriminators beyond the specific, or at best, generic level.

Fig. 1. Pelvetiopsis: Longitudinal section of a branch apex. PAS-anilin blue staining.

Fig. 2. Pelvetiopsis: Cross-section of a branch apex. PAS-anilin blue staining.

I= initials; N= nucleus; P= plastid; V= vesicle

1

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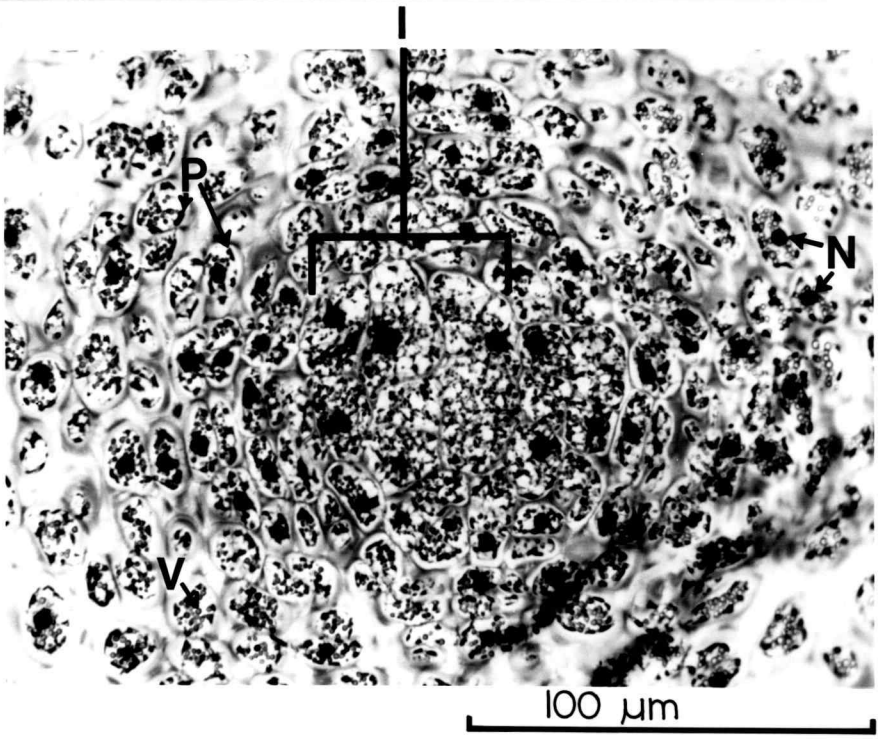


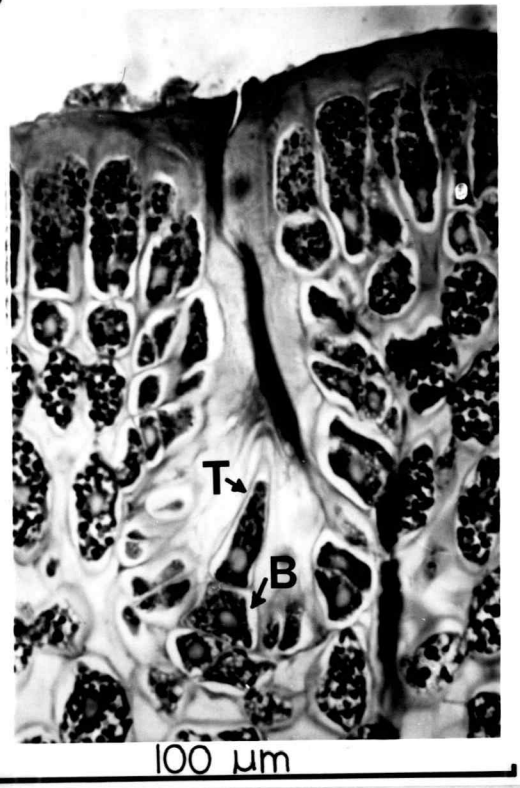
Fig. 3. Pelvetiopsis: Early conceptacle development. PAS-fast green staining.

Fig. 4. Pelvetiopsis: Early conceptacle development. Acid fuchsin-anilin blue staining.

Fig. 5, Pelvetiopsis: Older conceptacle. PAS-anilin blue staining.

B= basal cell; N= nucleus; P= plastid; T= tongue cell V= vesicle.

3

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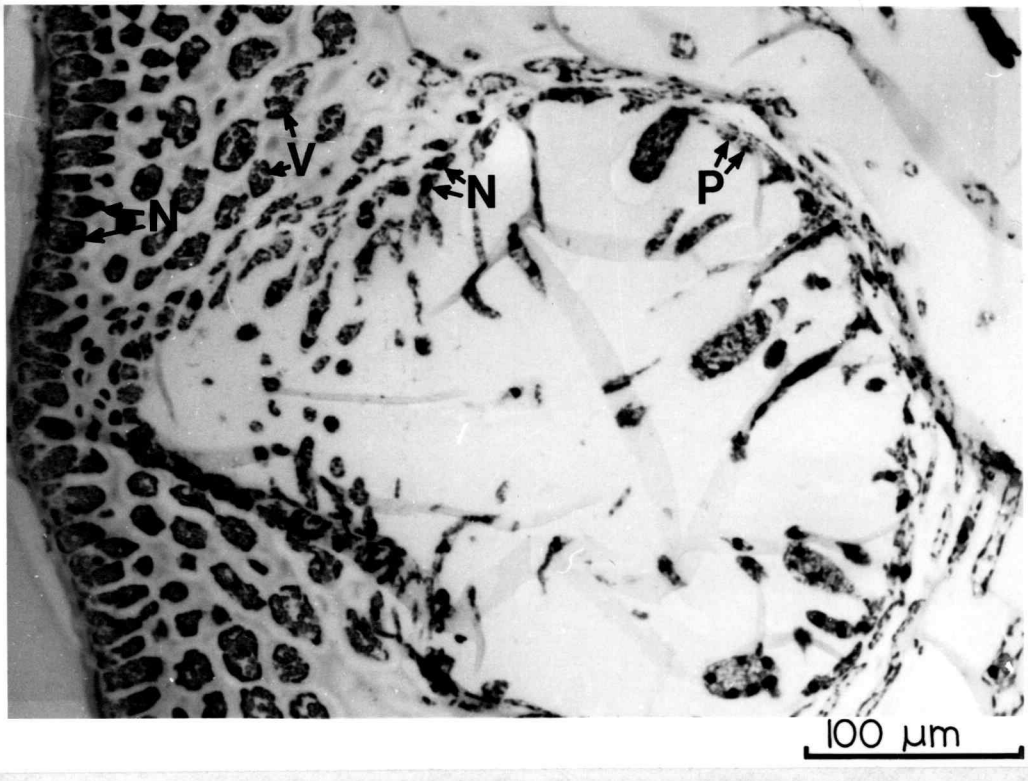

Fig. 6. Pelvetiopsis: Intact, mature oogonium. Three layers of oogonial wall are discernable. Live, unstained preparation.

Fig. 7. Pelvetia: Surface view of egg. Unstained, live preparation.

Fig. 8. Pelvetiopsis: Electron micrograph of egg.

Figs. 9, 10. Pelvetiopsis: Sperm. Live, unstained preparation.

AF= anterior flagellum; E= exochiton; Os= osmiophilic inclusion; P=plastid; PF= posterior flagellum; Pr= proboscis; V= vesicle.

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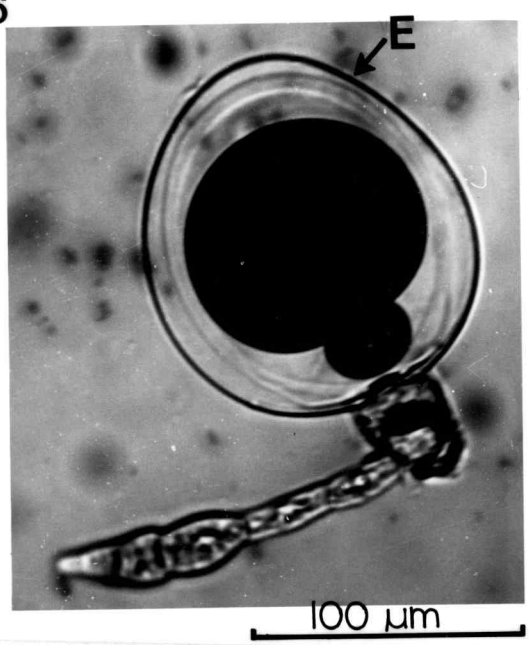


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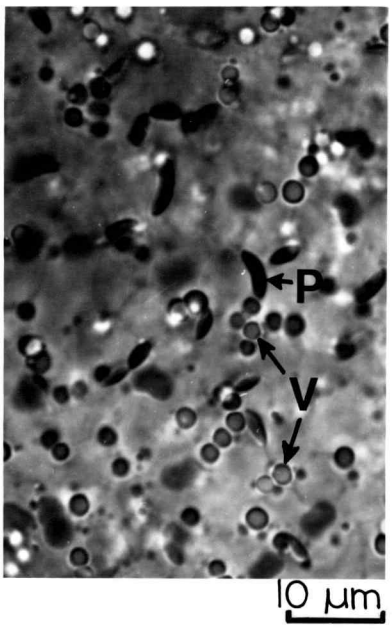


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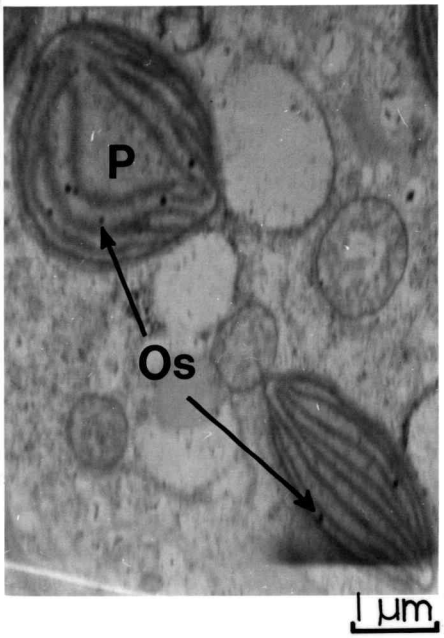


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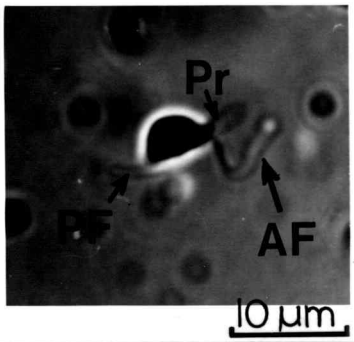


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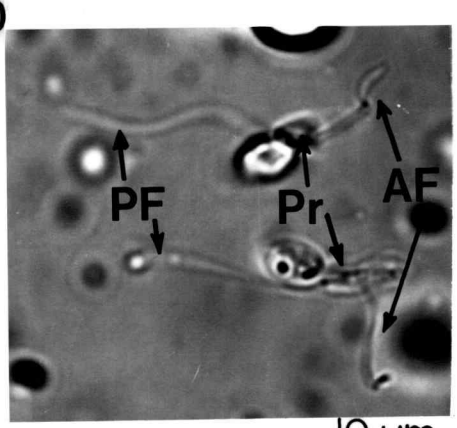

Fig. 11. Pelvetiopsis: Electron micrograph of sperm.

Fig. 12. Detail of proboscis, same specimen as Fig. 11.

Fig. 13. Detail of anterior flagellum, same specimen as Fig. 11.

Fig. 14. Pelvetia: Electron micrograph of sperm.

Shadowcast preparations
M= mastigonemes; Pr= proboscis.

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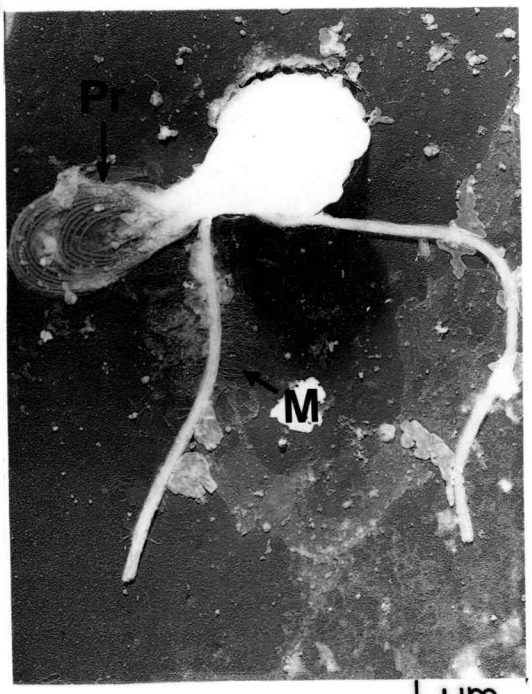


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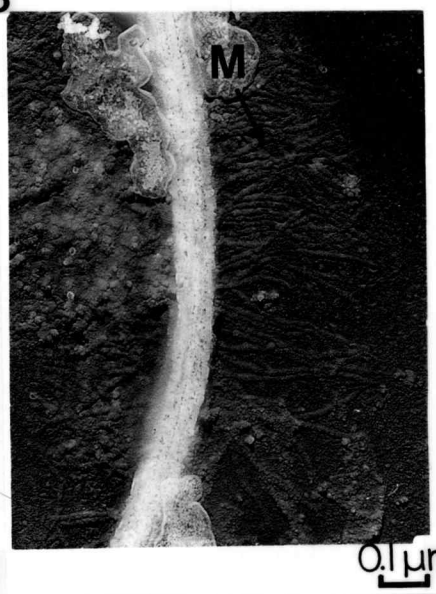


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Fig. 15. Pelvetia: Detail of proboscis, same specimen as Fig. 14.
Shadowcast preparation

Fig. 16. Pelvetiopsis: Zygote; rhizoid initiation prior to first division. Unstained, live preparation.

Fig. 17. Pelvetiopsis: Embryo, shortly before the second division. PAS- anilin blue staining.

Fig. 18. Pelvetiopsis: Embryos, shortly before the third division. PAS- anilin blue staining.

Fig. 19. Pelvetiopsis: Four-day embryos. Note peripheral, dark staining polyphenolic vesicles. Toluidine blue staining.

A= apical differentiation; N= nucleus; RB= rhizoidal bulge; V= vesicle.

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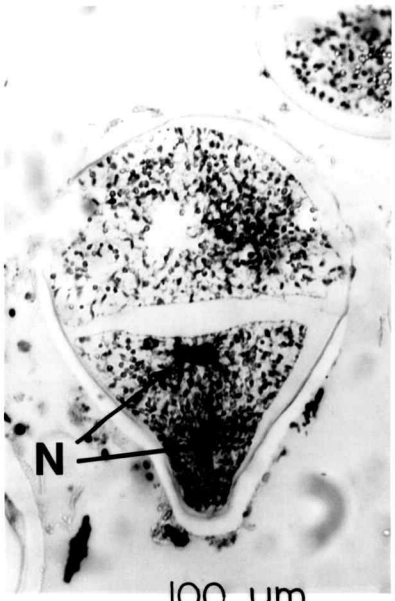


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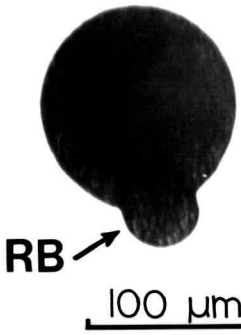


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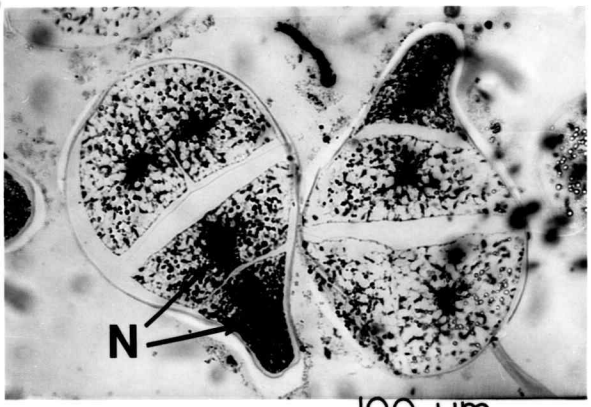


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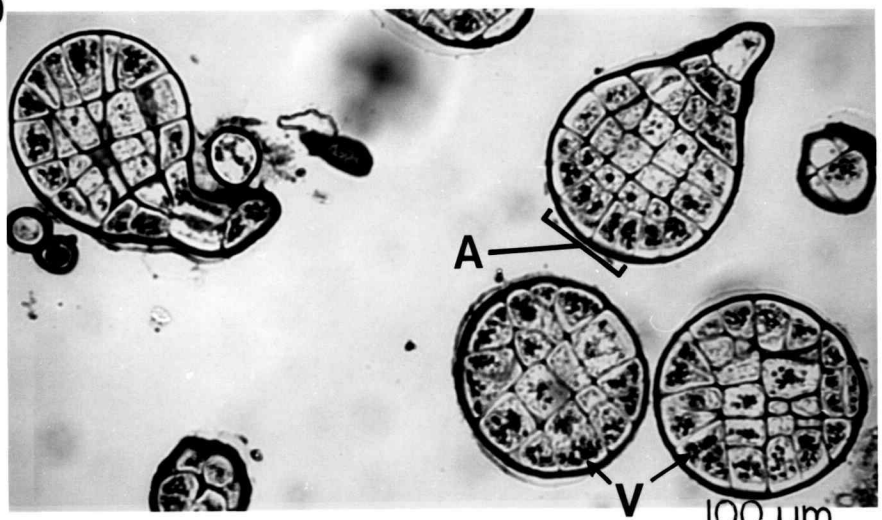

Fig. 20. Pelvetiopsis: Ten-day embryo. Note proximal location of nuclei in peripheral cells. PAS- anilin blue stain.

Fig. 21. Pelvetiopsis: Ten day embryo. Toluidine blue staining.

Fig. 22. Pelvetiopsis: Embryo. Note apical depression and hairs. Unstained, live preparation.

Fig. 23. Pelvetiopsis: Embryo with apical hump, elongate hairs. Unstained, live preparation.

A= apical differentiation; N= nucleus.

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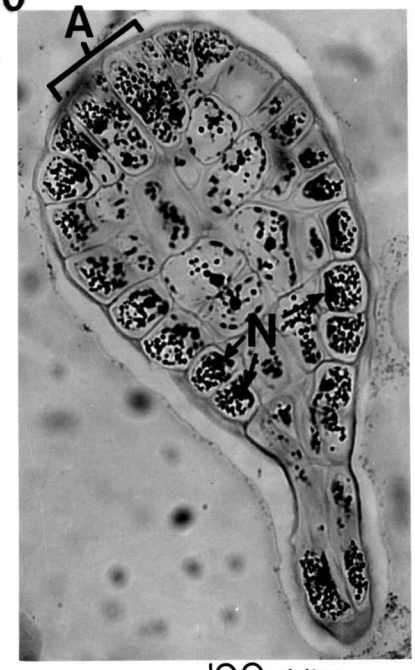


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Fig. 24. Pelvetia: Embryos with apical hairs. Unstained, live preparation.

Fig. 25. Pelvetiopsis: Electron micrograph of pit field in embryo.

Fig. 26. Pelvetiopsis: Electron micrograph of pit field in paraphysis. Arrows indicate cytoplasmic connection.

Fig. 27. Pelvetiopsis: Electron micrograph of pit field in paraphysis. Arrows indicate plasmodesmata showing typical inner ring structure.

PF= pit field.

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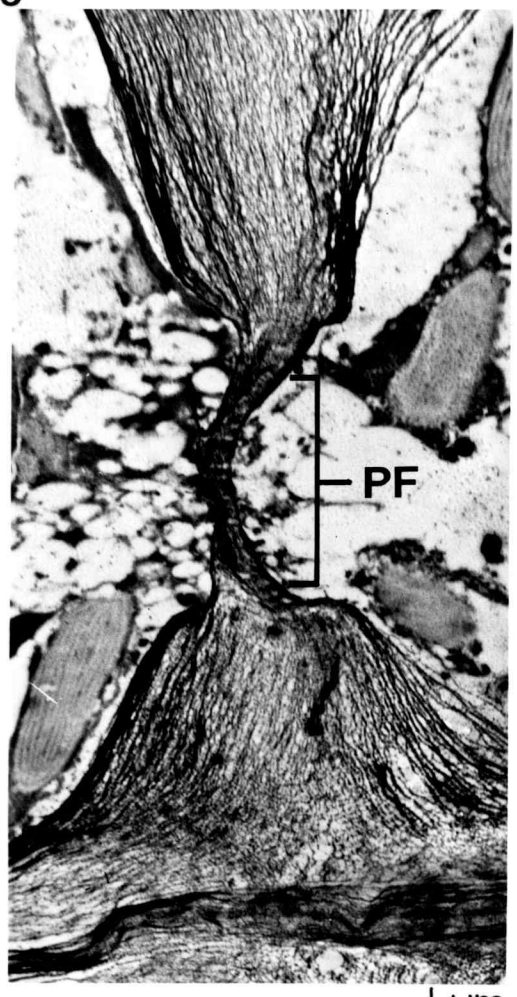


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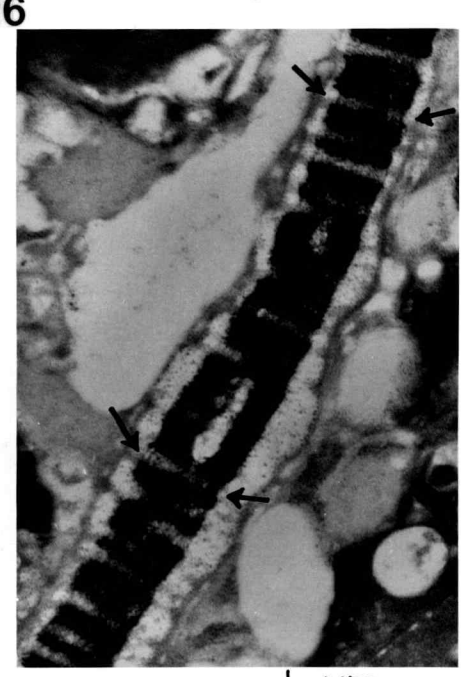


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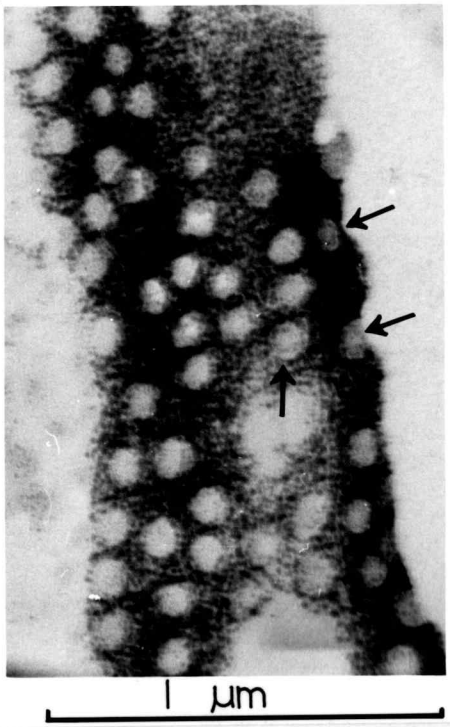

Fig. 28. Pelvetiopsis: Smaller, seven-nucleate egg with sperm. Unstained, live preparation.

Figs. 29-31. Pelvetiopsis: Development of eight-nucleate embryos. Unstained, live preparation.

S= sperm.

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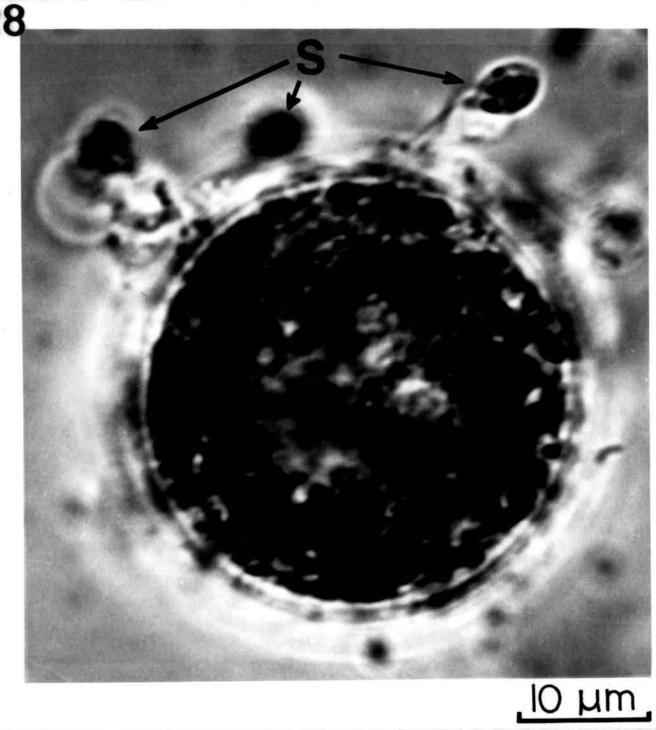


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