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CYTOLOGICAL INVESTIGATIONS OF THE GENUS ALARIA  
GREVILLE, AS IT OCCURS ON THE WEST COAST OF  
NORTH AMERICA.

ABSTRACT

Although the taxonomy of the brown algal genus Alaria Greville of the order Laminariales has recently been illucidated, neither the morphological nor the cytological aspects of the life-cycles of the species occurring on the west coast of North America has been investigated. Therefore a major part of this present project has been to establish the morphological and cytological phases of several species of Alaria. Since the earliest investigations into the cytology of the Laminariales the concepts of the nuclear division processes have not changed as they have in higher organisms. A supplementary objective has therefore been the application of modern techniques and interpretations to nuclear divisions in the genus Alaria.

During 1964-67 samples of Alaria marginata Postels et Ruprecht, Alaria nana Schrader, Alaria tenuifolia Setchell, Alaria taeniata Kjellman, Alaria fistulosa Postels et Ruprecht and Alaria grandifolia J. Agardh were collected from the west coast of North America, from Cape St. Elias, Alaska to Pescadero Point, California. Cultures of these species were established from spore suspensions and maintained under controlled conditions until young sporophytes were produced. From these cultures it has been demonstrated that the life-cycles of all six species show an alternation of heteromorphic generations: macroscopic sporophytic and microscopic dioecious gametophytic generations. It has also been established that there is a corresponding chromosomal alternation of generations, the sporophyte being diploid and the gametophyte, haploid.

The development of male and femal gametophytes, the production of gametangia, fertilisation, and the early developmental stages of the young sporophytes

have been examined. The similarities and differences between these phases in Alaria and other members of the Laminariales have been investigated and found to be similar except for the germination of zoospores of A. marginata, the formation of the egg cells in all the Alaria spp. and the occurrence of a possible "fertilisation pore" and "tube" in the eggs of A. taeniata. The occurrence of parthenogenesis and the production of malformed haploid "parthenosporophytes" are reported.

Meiosis in the immature zoosporangia and mitosis in gametophytes and young sporophytes have been observed, compared with these processes in other members of the Laminariales, and found to be similar in a number of cases. However, the concepts of meiosis held by many earlier authors are not applicable to this process in Alaria. A haploid chromosome number of approximately 14 alternates with a diploid number of approximately 28 in A. marginata, A. nana, A. tenuifolia, A. fistulosa and A. taeniata. The haploid number of A. grandifolia is approximately 24. Consequently only A. grandifolia can possibly be distinguished on the basis of chromosome numbers.

The methods used in laminarian cytology and the difficulties involved in counting the extremely small chromosomes of the members of this order have been critically discussed.

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Although the taxonomy of the brown algal genus Alaria Greville of the order Laminariales has recently been elucidated, neither the morphological nor the cytological aspects of the life-cycles of the species occurring on the west coast of North America has been investigated. Therefore a major part of this present project has been to establish the morphological and cytological phases of several species of Alaria. Since the earliest investigations into the cytology of the Laminariales the concepts of the nuclear division processes have not changed as they have in higher organisms. A supplementary objective has therefore been the application of modern techniques and interpretations to nuclear divisions in the genus Alaria.

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The development of male and female gametophytes, the production of gametangia, fertilisation, and the early developmental stages of the young sporophytes have been examined. The similarities and differences between these phases in Alaria and other members of the Laminariales have been investigated and found to be similar except for the germination of zoospores of A. marginata, the formation of the egg cells in all the Alaria spp. and the occurrence of a possible "fertilisation pore" and "tube" in the eggs of A. taeniata. The occurrence of parthenogenesis and the production of malformed haploid "parthenosporophytes" are reported.

Meiosis in the immature zoosporangia and mitosis in gametophytes and young sporophytes have been observed, compared with these processes in other members of the Laminariales, and found to be similar in a number of cases. However, the concepts of meiosis held by many earlier authors are not applicable to this process in Alaria. A haploid chromosome number of approximately 14 alternates with a diploid number of approximately 28 in A. marginata, A. nana, A. tenuifolia, A. fistulosa and A. taeniata. The haploid number of A. grandifolia is approximately 24. Consequently only A. grandifolia can possibly be

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The methods used in laminarian cytology and the difficulties involved in counting the extremely small chromosomes of the members of this order have been critically discussed.

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## INTRODUCTION

Ideally a total understanding of the biology of any species and its relationship with other similar species demands a knowledge of all phases of its existence. However, the scope of the various aspects of biology is so vast and biological research is so compartmented that in practice it is necessary to deal with each specialised field individually.

A relatively large amount of information has been published concerning the Laminariales, an order of the Phaeophyta. This is perhaps because of the economic importance of several genera of this order (Scagel, 1966). Taxonomic studies were obviously the first to be made, and initially these were based almost completely upon the gross morphological characteristics of the plants concerned. Subsequent taxonomic studies have unfortunately also been based upon incomplete information, which has resulted in the weighting of certain characteristics over others. The life histories of many species of the Laminariales have been examined morphologically and in almost all cases it has been shown that an heteromorphic alternation of generations exists, incorporating dioecious gametophytic generations. A few species have been investigated cytologically. Others have not been studied at all.

The taxonomy of the genus Alaria Greville, a member of the Laminariales, has recently been investigated (Widdowson, 1964).

However, there have been no studies made of the life-history or cytology of this genus, as it occurs on the west coast of North America. Since the time of Kylin's (1918) earliest investigations into nuclear divisions in the Laminariales there has been little change in the concepts of the division processes. Although Naylor (1956) and Evans (1963b, 1965) have applied modern cytological techniques to nuclear divisions in certain members of the order, there is still an obvious need for the application of these to many more species. Consequently the present project was undertaken to establish the life histories of several Alaria species morphologically and cytologically, and to make a detailed comparative study of meiosis and mitosis within the genus. The species most thoroughly examined were those native to the shores of British Columbia. It was also proposed to determine chromosome numbers and to compare these numbers with others of the Alariaceae and the Laminariales as a whole.

## LITERATURE REVIEW

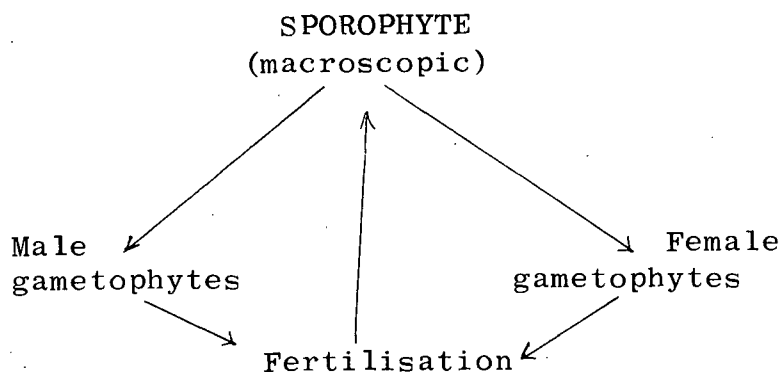
### a. General survey of the life-cycle of the Laminariales:

In 1850 Thuret first observed the liberation and germination of zoospores in Sacchoriza bulbosa. Since that time a considerable amount of research has been conducted on the Laminariales, and a well defined picture of the life-cycle typical of this order has emerged. The species in which the life-cycle has been investigated are listed in Table 1.

Thuret (1850) had correctly interpreted the product of the unilocular sporangia as zoospores. However, Drew (1910) regarded these sporangia in Laminaria digitata and Laminaria saccharina as gametangia noting the "fusion" of non-motile "gametes". Sauvageau (1915a, 1915b, 1916a, 1916b, 1916c) and Kylin (1916), working independently on Laminaria flexicaulis, Laminaria saccharina, Sacchoriza bulbosa and Alaria esculenta and Laminaria digitata, observed two morphological types of gametophyte, some of which had empty cells. Although neither investigator was able to recognise antherozoids or fertilisation, they suspected that the two gametophytic types represented the differentiation of male and female sexes and that fertilisation occurs in this generation. In 1918 Kylin established that reduction division takes place in the unilocular sporangia of the macroscopic plants of Chorda filum and Williams (1921) observed fertilisation in Laminaria and Chorda. There followed Schreiber's

(1930) observation that sex is probably genetically determined in Laminaria saccharina, since the male and female gametophytes are formed in equal numbers from a single unilocular sporangium. Therefore, by the early 1930's it had been quite firmly established that the laminarian life-cycle involves an alternation of heteromorphic generations, a conspicuous sporophyte and microscopic male and female gametophytes (Text Fig. 1).

Text figure 1. Diagrammatic laminarian life-cycle.



A considerable amount of investigation has been conducted on the morphological phases of the laminarian life-cycle. The development of the zoosporangium has been thoroughly examined in Undaria undarioides, Eckloniopsis radicata, Ecklonia cava (Nishibayashi & Inoh, 1960a ; Ohmori, 1967), Alaria valida, Alaria crassifolia, Laminaria yendoana, Laminaria cicherioides and Eisenia bicyclis (Ohmori & Inoh, 1963; Ohmori, 1967), Chorda filum, Undaria pinnatifida, Laminaria japonica



(Nishibayashi & Inoh, 1958; Ohmori, 1967), Laminaria longipedalis, Laminaria angustata, Costaria costata, Ecklonia stolonifera, Ecklonia kurome and Alaria angusta (Ohmori, 1967). Most workers have described the liberated zoospores as pyriform with two laterally inserted flagella of unequal length. The longer flagellum projects anteriorly and the shorter, posteriorly. In Laminaria saccharina it has been shown that the anterior flagellum is tinselated and the posterior whiplash (Manton & Clarke, 1951). After a short period of activity the zoospores come to rest, lose their flagella, and secrete a rigid wall around themselves. Each spore then produces a germ tube and the initial nuclear division occurs. Both daughter nuclei migrate into the tube or one may remain in the spore case as the germ tube is cut off by a cell wall. Mitosis continues and a multi-cellular gametophyte develops. The antheridia produced on the male gametophyte may be either terminal or both terminal and intercalary. A single biflagellate antherozoid is liberated from each antheridium. Each cell of the female gametophyte seems to be a potential oogonium. It is quite a common phenomenon for the female gametophyte to develop no further than one or two cells before oogonium formation. A single egg is extruded from each oogonium, and remains attached to the oogonium. The process of fertilisation has been observed in a number of species, but very rarely has it been investigated

cytologically. The nuclear cytology of this process has been observed in Pterygophora californica (McKay, 1933), Eisenia arborea (Hollenberg, 1939), Macrocystis integrifolia (Cole, 1959) and Nereocystis luetkeana (Kemp & Cole, 1961). Both McKay (1933) and Hollenberg (1939) noted that nuclear fusion occurs while both nuclei are in prophase. However, in Nereocystis luetkeana fertilisation occurs while the nuclei of the egg and the antherozoid are in interphase, and involves a complete dissolution of the nuclear membranes (Kemp & Cole, 1961).

Following fertilisation the zygote divides mitotically and develops into the young sporophyte. A uniseriate filament of four to twelve cells is first produced. Division in a second plane then results in the formation of a monostromatic blade. The basal cells of this blade give rise to rhizoids which initially remain in the empty oogonium. Division in a third plane produces a distroma, within which is laid down the precortical and premedullary tissue. This sequence has been described for Costaria turneri, Undaria pinnatifida and Laminaria (Yendo, 1911).

A number of deviations from an alternation of diploid sporophyte and haploid male and female gametophytes have been recorded in the Laminariales. In Chorda tomentosa (Sundene, 1963) it is reported that the gametophytic generation is monoecious. The gametophytes of this species give rise to both

oogonia and antheridia, although no antherozoids have actually been observed. It has also been demonstrated that in this species sporophytes can be produced from uni-gametophytic cultures (Sundene, 1963). This observation does not, however, preclude the possibility of parthenogenesis, which is often stated as taking place in the Laminariales. In Laminaria digitata and Laminaria hyperborea the formation of sporophytes without fertilisation of the egg has been reported by Shreiber (1930). This phenomenon has also been reported in Laminaria religiosa, Laminaria japonica, Laminaria angustata, Laminaria angustata var. longissima, Laminaria ochotensis, Laminaria diabolica, Alariacrassifolia, Undaria pinnatifida and Arthrothamnus bifidus by Yabu (1964a) and in Nereocystis luetkeana by Kemp and Cole (1961). Only in Nereocystis luetkeana was it shown that these sporophytic plants possess an haploid chromosome complement.

In the Laminariales it appears that reproductive isolation is not a sound criterion on which to base the definition of species, since relatively free inter-crossing has been shown between a number of species. Yabu (1964a) demonstrated that crossing occurred between Laminaria religiosa, Laminaria japonica and Laminaria angustata, and between Laminaria japonica and Laminaria diabolica. Prior to this Sundene (1958) had successfully crossed varieties of Laminaria digitata, but not separate species. However, no chromosomal evidence has been

presented to substantiate any of the crossing experiments conducted.

Investigations of the cytological aspects of the laminarian life-cycle were initiated by Kylin (1918). His account of meiosis as it occurs in zoosporangium of Chorda filum closely resembles modern day descriptions. In meiosis the interphase nucleus is either reticulate or granular. A reticulate state is described in Laminaria angustata (Nishibayashi & Inoh, 1956; Ohmori, 1967), Chorda filum (Nishibayashi & Inoh, 1961a, 1961b; Ohmori, 1967), Costaria costata (Nishibayashi & Inoh, 1957; Ohmori, 1967), Undaria undarioides (Nishibayashi & Inoh, 1960b; Ohmori, 1967), Ecklonia stolonifera, Ecklonia cava (Ohmori, 1965, 1967), Undaria pinnatifida (Inoh & Nishibayashi, 1955, 1960; Ohmori, 1967), Laminaria longipedalis, Laminaria yendoana, Eisenia bicyclis, Ecklonia kurome, Eckloniopsis radicata (Ohmori, 1967), Pterygophora californica (McKay, 1933), Egregia menziesii (Myers, 1928) and Laminaria flexicaulis and Laminaria saccharina (Magne, 1953). Evans (1965) indicates a granular, interphase condition in Laminaria saccharina, Laminaria digitata, Laminaria hyperborea, Laminaria ochroleuca, Alaria esculenta, Chorda filum and Sacchoriza polyschides as do Kemp & Cole (1961) in Nereocystis luetkeana. There is usually one distinct nucleolus, although the occasional occurrence of two has been reported (Nishibayashi & Inoh, 1957, 1960b, 1960c, 1961a, 1961b;

Ohmori, 1965, 1967; Yabu, 1957, 1958, 1964b, 1965).

At the onset of prophase I the nucleus enlarges. A reticulum either becomes more distinct or, where a granular interphase has been observed, appears for the first time (Evans, 1965; Kemp & Cole, 1961). According to most workers the chromonemata then aggregate in a mass of loops, which concentrates on one side of the nucleus. However, Evans (1965) states that this aggregation occurs only very occasionally in Laminaria saccharina, Laminaria digitata, Laminaria hyperborea, Laminaria ochroleuca, Alaria esculenta, Chorda filum and Sacchoriza polyschides. A description of the zygotene stage is seldom presented. It appears that the congregation of chromonemata to one side of the nucleus and the synaptic stage are considered synonymous (Hollenberg, 1939; Inoh & Nishibayashi, 1954, 1955, 1960; McKay, 1933; Myers, 1928; Nishibayashi & Inoh, 1956, 1960b, 1960c, 1961a, 1961b; Ohmori, 1965, 1967; Yabu, 1957, 1958, 1964b, 1965; Yabu & Tokida, 1963). Evans (1965) describes the appearance of "knots" at this stage of division. In Nereocystis luetkeana the result of this process is compared with "synizesis" as it occurs in higher plants, although the actual process of pairing could not be observed (Kemp & Cole, 1961).

Pachytene and diplotene of meiosis are most often described as a "spireme" stage. However, this concept is questioned in Nereocystis luetkeana due to the impracticability of observing

the continuous nature of the chromatin (Kemp & Cole, 1961). The spireme thickens towards the onset of diakinesis (Hollenberg 1939; Inoh & Nishibayashi, 1954, 1955, 1960; McKay, 1933; Myers, 1928; Nishibayashi & Inoh, 1956, 1960b, 1960c, 1961a, 1961b; Ohmori, 1965, 1967; Yabu, 1957, 1958, 1964b, 1965; Yabu & Tokida, 1963), although Yabu and Tokida (1963) have reported a resting stage prior to diakinesis, associated with a reduced stainability of the chromatin. By diakinesis the nucleolus and nuclear membrane have generally disappeared, although in Costaria costata (Nishibayashi & Inoh, 1957), Laminaria digitata, Laminaria saccharina, Laminaria hyperborea, Laminaria ochroleuca, Alaria esculenta, Chorda filum and Sacchoriza polyschides (Evans, 1965) the nuclear membrane can persist until the chromosomes are aligned in metaphase I. X-, Y-, O-, V- and II-shaped bivalent configurations are described in diakinesis in Laminaria angustata (Nishibayashi & Inoh, 1956; Ohmori, 1967), Costaria costata (Nishibayashi & Inoh, 1957; Ohmori, 1967), Undaria undarioides (Nishibayashi & Inoh, 1960b, 1960c; Ohmori, 1967), Chorda filum (Nishibayashi & Inoh, 1961a, 1961b; Evans, 1965; Ohmori, 1967), Ecklonia stolonifera and Ecklonia cava (Ohmori, 1965, 1967), Undaria pinnatifida (Inoh & Nishibayashi, 1954, 1955, 1960; Ohmori, 1967), Pterygophora californica (McKay, 1933), Nereocystis luetkeana (Kemp & Cole, 1961) Ecklonia radicata, Laminaria longipedalis, Laminaria yendoana,

Eisenia bicyclis, Ecklonia kurome (Ohmori, 1967), Laminaria digitata, Laminaria saccharina, Laminaria hyperborea, Laminaria ochroleuca, Alaria esculenta, Chorda filum and Sacchoriza polyschides (Evans, 1965). Diakinesis marks the end of the meiotic prophase, and the chromosomes then amass to form the equatorial plate of metaphase I. Spindles appear at this stage and centrosome-like bodies have been reported in all the species studied except Eisenia arborea (Hollenberg, 1939) Egregia menziesii (Myers, 1928), Laminaria flexicaulis and Laminaria saccharina (Magne, 1953), Undaria undarioides (Nishibayashi & Inoh, 1960b, 1960c; Ohmori, 1967), Chorda filum (Nishibayashi & Inoh, 1961a, 1961b; Ohmori, 1967), Laminaria longipadalis, Ecklonia kurome (Ohmori, 1967) and the seven species studied by Evans (1965). Only one centrosome has been recorded in the first division of meiosis in Laminaria japonica (Abe, 1939), Arthrothamnus bifidus (Yabu & Tokida, 1963) and Nereocystis luetkeana (Kemp & Cole, 1961).

During anaphase I the two tightly associated chromosome masses separate and move towards the poles. At this stage in Nereocystis luetkeana (Kemp & Cole, 1961) the presence of some bridges indicates a minor degree of chromosomal non-disjunction. In telophase I nuclear membranes and nucleoli appear again. In Laminaria digitata, Laminaria saccharina, Laminaria hyperborea, Laminaria ochroleuca, Chorda filum, Alaria

esculenta and Sacchoriza polyschides prophase II appears to follow telophase I very quickly, since there is a scarcity of binucleate cells (Evans, 1965).

The second division in the sporangium is equational. Where chromosome counts have been possible at this stage they have been used to confirm the fact that the initial division is reductional. Mitotic divisions follow until 32 nuclei are produced. In Pterygophora californica (McKay, 1933) and Eisenia arborea (Hollemborg, 1939) 64 nuclei have been observed on occasion. In Chorda filum the final number of nuclei is 16 (Nishibayashi & Inoh, 1961a, 1961b; Ohmori, 1967) and in Sacchoriza bulbosa it is 128 (Sauvageau, 1915a).

Mitosis has been most commonly described as it occurs in the gametophyte and developmental stages of the young sporophyte. In Laminaria flexicaulis and Laminaria saccharina (Magne, 1953) and Chorda filum (Kylin, 1918) mitosis has been followed in meristoderm cells. McKay (1933) studied mitosis in the inner cortical cells of Pterygophora californica. Perhaps the most complete descriptions of mitosis are those of Naylor (1956) and Evans (1965) in Laminaria digitata, Laminaria saccharina, Laminaria hyperborea, Laminaria ochroleuca, Alaria esculenta, Chorda filum and Sacchoriza polyschides. The interphase nucleus is granular with a distinct nucleolus. Both Naylor (1956) and Evans (1965) have shown that the interphase nuclei



of the male and female gametophytes and the young sporophytes have different stainabilities. The highest stainability exists in the male gametophyte and the lowest in the female gametophyte. An intermediate stainability was found in the young sporophytes. At the onset of the mitotic prophase the nucleus enlarges. This increase in size is most obvious in the division prior to egg formation in the female gametophyte (Evans, 1965; Naylor, 1956). In prophase a reticulum of beaded threads appears. These threads contract during prophase until tiny spheres (Naylor, 1956) or rods (Evans, 1965) remain. By this prometaphase stage the nucleolus has disappeared and the nuclear membrane has broken down. At metaphase the chromosomes are tightly associated, forming a metaphase plate. Spindles have been observed (Naylor, 1956), and it is possible to distinguish chromatids at this stage only (Evans, 1965). In anaphase the chromosomes move apart in tightly associated masses. Bridges and lagging chromosomes have been observed at this stage (Naylor, 1956). In telophase there is a reversion to the granular state, accompanied by the reappearance of nucleoli and nuclear membranes.

Walker (1954), describing mitosis in Laminaria digitata gametophytes and young sporophytes, introduced a theory of endomitosis. He claimed that the nuclear membrane breaks down and the chromosomes multiply in the cytoplasm. No other worker has reported any such phenomenon. Although

multinucleate cells have been observed in some young sporophytes, it has been assumed that these abnormalities would not survive to maturity (Kemp & Cole, 1961).

There are relatively few accounts of the morphology of the chromosomes of the Laminariales, perhaps because of their exceedingly small size. The relative sizes of the chromosomes of both the haploid and diploid complements are reported for Egregia menziesii (Myers, 1928). The eight chromosomes of the haploid complement were measured at mitotic metaphase in the sporangium; five are quite large, two are smaller and one is smaller still. Four of the chromosomes of the diploid complement at anaphase in somatic mitosis are very small and twelve are much larger. McKay (1933) reported the actual sizes of chromosomes, as measured at metaphase in diploid somatic cells, in Pterygophora californica. She observed three pairs of rod-shaped chromosomes, the largest of which measured 0.8 microns, nine pairs of ovoid chromosomes measuring 0.3 - 0.6 microns, and one pair of minute spheroid chromosomes, too close to the ultimate resolution of the light microscope to measure. In Laminaria digitata, Laminaria saccharina, Laminaria hyperborea, Laminaria ochroleuca, Alaria esculenta and Chorda filum the chromosomes of the gametophytic generation, as measured at mitotic metaphase, range in size from 0.4 to 1.7 microns (Evans, 1963a, 1965). One chromosome in the female gametophyte is 1.3 to 1.7 microns long.

Such a chromosome could not be found in the male gametophyte. The implication behind such an observation is obviously that heteromorphic sex chromosomes exist in these species. To establish this clearly requires a meiotic configuration showing the pairing of unequal chromosomes. No truly conclusive configuration has been found (Evans, 1965). In Sacchoriza polyschides, however, a much larger chromosome has been recorded. In this species the chromosomes of the female gametophyte can be grouped according to size: three are 0.4 - 0.7 microns; the majority, 0.8 - 1.0 microns; three, 1.1 - 2.6 microns; and one, 4.0 - 6.0 microns (Evans, 1965). No chromosome has been accounted for in the male gametophytes which are as large as 4.0 - 6.0 microns. In diakinesis this large chromosome has been observed paired to a smaller. From these observations Evans (1965) concluded that an X/Y sex mechanism exists in Sacchoriza polyschides.

Chromosome numbers in the Laminariales have shown a wide diversity since the initial cytological investigation of Kylin (1918). This diversity is spread throughout a number of species, and in some cases even in a single species. Chromosome counts which exist at the time of this research are presented in Table II. Kemp & Cole (1961) have tentatively suggested that perhaps a polyploid series around the number ten might exist in the Laminariales, but that much more information is required before any conclusive statement can be made.

b. The genus Alaria Greville

The genus Alaria, characterised by "fronds membranaceous, furnished with a percurrent cartilaginous midrib, the stem pinnated with distinct leaflets", (Greville, 1830), has for some considerable time presented a major problem in the taxonomy of the Laminariales. However, Widdowson (1964) has applied numerical methods to the taxonomy of the genus, and following extensive population studies was able to confirm the identity of ten species. There are four other species which Widdowson (1964) was not able to study to the same extent. The 14 species of the genus are as follows :

Alaria angustata Kjellman

Alaria crassifolia Kjellman

Alaria crispa Kjellman

Alaria esculenta (L.) Greville

Alaria fistulosa Postels et Ruprecht

Alaria marginata Postels et Ruprecht

Alaria nana Schrader

Alaria praelonga Kjellman

Alaria taeniata Kjellman

Alaria tenuifolia Setchell

Alaria grandifolia\* J. Agardh

Alaria ochotensis\* Yendo

Alaria paradisea\* (Miyabe et Nagai)

Alaria pylaii\* (Bory) Greville.

(\* studied less extensively).

Except for taxonomic and distributional records of this genus, very few investigations have been conducted. Chromosome numbers have been recorded for three species (Table II). A typical alternation of generations has been established in Alaria esculenta (Sauvageau, 1916b, 1916c; Evans, 1965), and Alaria crassifolia (Yabu, 1964a). Yabu (1964a) has reported the formation of parthenosporophytes in Alaria crassifolia, and the possibility of an X/Y sex mechanism has been proposed, but not substantiated, for Alaria esculenta (Evans, 1965). An "Alaria-type" ontogeny of the zoosporangia has been shown in Alaria crassifolia by Nishibayashi & Inoh (1958), Ohmori & Inoh (1963) and Ohmori (1967). According to these authors the meristoderm cell of the sporophyll divides to give rise to an outer cell and a basal cell. The outer cell produces a paraphysis, and the basal cell first produces another paraphysis and then the zoosporangial mother-cell. However, this is not consistent throughout the genus. In A. valida (-A. marginata (Widdowson, 1964)) the basal cell gives rise directly to the zoosporangial mother-cell (Ohmori & Inoh, 1963; Ohmori, 1967). This is a

"Laminaria-type" ontogeny, and has been shown to occur in A. crassifolia also (Ohmori & Inoh, 1963; Ohmori, 1967). The formation of the zoosporangia has been described in A. fistulosa (Kibbe, 1915), but not with the same accuracy.

## MATERIALS AND METHODS

### a. Description of the macroscopic plants: (Text, Fig. 2)

The Alaria plant is characterised by a distal blade and midrib, and a proximal stipe attached to the substrate by haptera. At the junction of the blade and the stipe there is an intercalary meristem. Below the intercalary meristem is the rachis zone, marked by the attachment of sporophylls and remnants of sporophylls. The sporophyll typically consists of a fertile sorus areas on both surfaces surrounded by a sterile areas. The sori, formed in late spring or early summer, are generally restricted to the sporophylls, although one population of Alaria tenuifolia has been found in which the basal area of the blade also possesses fertile tissue. The growth of Alaria is mainly restricted to the intercalary meristem. However a certain amount of diffuse growth presumably occurs from the meristoderm layer of cells over the surface of the whole plant.

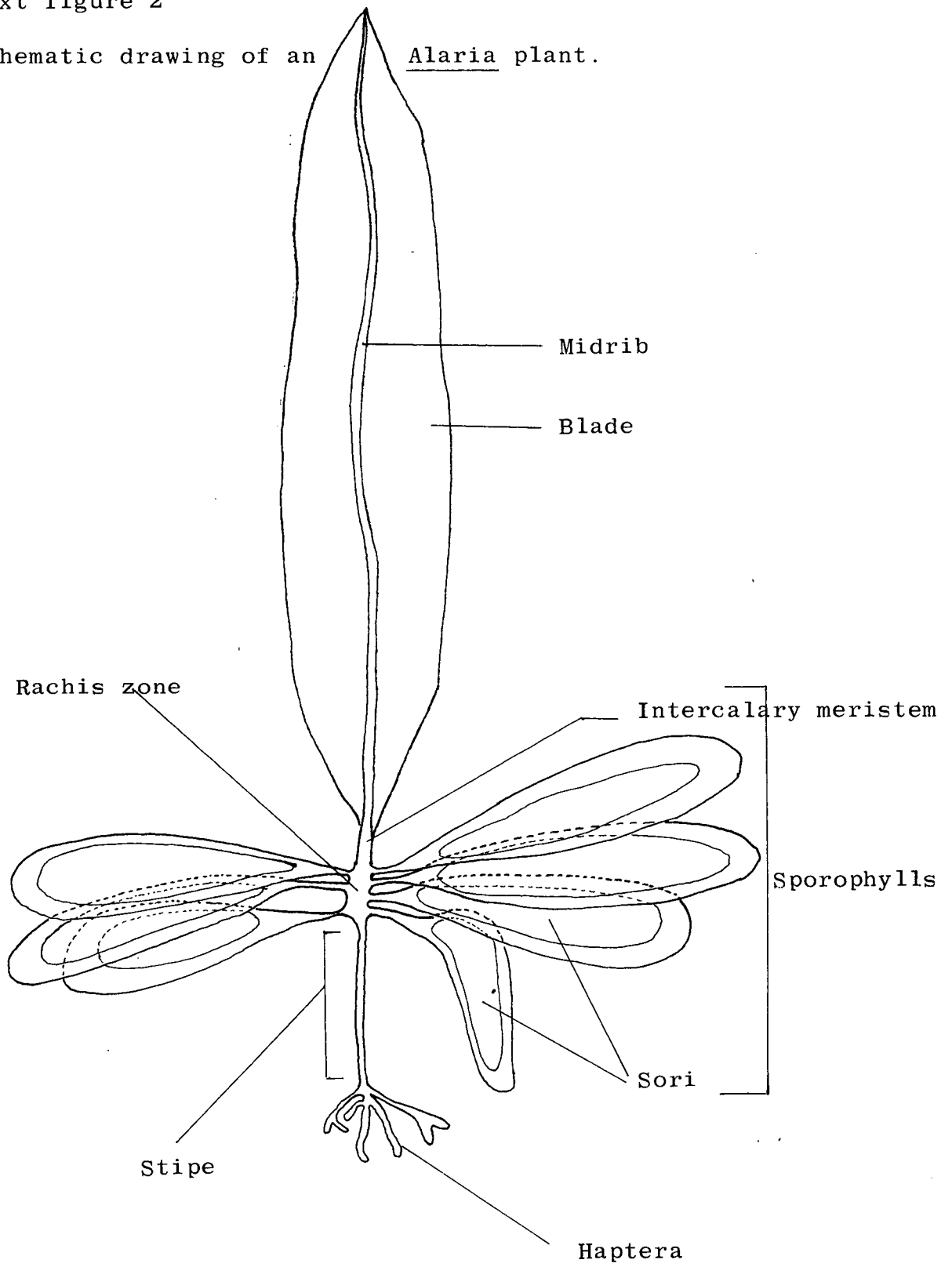
The genus Alaria is extremely commonly represented in the low intertidal and sub-tidal zones of the North East Pacific. There is no evident northern limit to the genus, while the 20 ° C isotherm of maximum sea temperature appears to be the southern limit (Widdowson, 1964).

### b. The identification of separate species.

In Widdowson's investigation of the taxonomy of Alaria,

Text figure 2

Schematic drawing of an Alaria plant.





thirteen measurements were incorporated. However, from the results obtained, the author constructed a key based upon stipe length and shape, sporophyll width and shape, mode of attachment of sporophylls and shape of the rachis (Widdowson, 1964). This key (Appendix I) has been used throughout the current study for the identification of Alaria plants. When collecting material for cytological investigation, at least thirty plants were used from each population before an identification was made. Identifications were always made on wet fertile plants.

The species which have been identified and used in this project are;

Alaria marginata Postels et Ruprecht

Alaria nana Schrader

Alaria tenuifolia Setchell

Alaria taeniata Kjellman

Alaria fistulosa Posels et Ruprecht

Alaria grandifolia J. Agardh

Habit drawings of these six species are presented in Figures 1 - 6.

A. marginata, A. nana and A. tenuifolia have been treated more extensively than A. taeniata, A. fistulosa and A. grandifolia, because of their local abundance on the coast of British Columbia.

In almost all cases the location of these species has corresponded with documented records of their distribution. The material identified as Alaria grandifolia, however, did not. This species has previously been recorded only in Labrador, Greenland and Japan (Widdowson, 1964), and yet the plants named Alaria grandifolia in this project were collected on Coronation Island, Alaska. Voucher specimens of the six species studied are recorded in Table VIII.

c. The collection of material (Figure 7.)

In obtaining material for cytological investigation each species was collected from a variety of locations. In this way a number of morphological forms of each species, except Alaria grandifolia, was obtained. Collections were made at the following locations:

<u>Alaria marginata</u>	Wiffin Spit, Sooke, Vancouver Island, B.C.
	Glacier Point, Vancouver Island, B.C.
	Jordan River, Vancouver Island, B.C.
	Beaver Point, Saltspring Island, B.C.
	Indian Beach, Ecola State Park, Oregon, USA.
	Short Sands Beach, Oregon, U.S.A.
	Volga Island, Alaska, U.S.A.
<u>Alaria nana</u>	Long Beach, Vancouver Island, B.C.
	Glacier Point, Vancouver Island, B.C.

Wigham Island, Alaska, U.S.A.

Cape Muzon, Alaska, U.S.A.

Pescadero Point, California, U.S.A.

Alaria tenuifolia Goose Island, Alaska, U.S.A.

Brockton Point, Vancouver, B.C.

Alaria fistulosa Cape Muzon, Alaska, U.S.A.

Port Conclusion, Alaska, U.S.A.

Alaria grandifolia Coronation Island, Alaska, U.S.A.

Alaria taeniata Wigham Island, Alaska, U.S.A.

Volga Island, Alaska, U.S.A.

Cape Spencer, Cross Road, Alaska, U.S.A.

Where possible, fixation of material was carried out in the field, and living material transferred directly to the laboratory for culturing. The most effective method of transporting live material for short distances was by wrapping freshly collected plants in paper, dampened with sea water, and placing them in a darkened refrigerated box. Over long periods of time, such as those involved in transporting plants from Alaska, this method was not used. Instead, plants were maintained in large marine plywood aquaria boxes filled with sea water, which was changed regularly.

d. Culturing Methods.

Gametophytic cultures were established for all species

collected. The method used for the initiation of cultures was that of Hollenberg (1939). Fertile sorus material was cut into small pieces. These were rinsed in sterile sea water and blotted dry. After a short period of time the pieces were immersed in a culture medium, in 500 ml. crystallising dishes. Once spore release had been established, the pieces of sorus were removed. Several c.c. of the remaining suspension of motile spores was then pipetted into culture containers. These culture containers were 12" x 6" x 4" "plexiglass" boxes, in which racks of either cover-glasses or microslides were set. The slides and cover-glasses provided a large settling area for the spores, and subsequently permitted very convenient study of the developing plants.

Three different culture media were used during this project; Erdschreiber medium (Starr, 1956), a controlled enrichment medium, and a modified ASP artificial medium (West, personal communication) (Appendix II). Basically, the Erdschreiber and controlled enrichment media are supplemented sea water. In this project the sea water was obtained from the vicinity of Glacier Point, Vancouver Island, to ensure a high salinity. The Erdschreiber supplement consists in part of a soil extract, whereas the controlled enrichment medium has a defined supplement. The artificial medium of West (Personal communication) is a modification of the ASP medium of Provasoli,

McLaughlin and Droop (1957) and allows absolute control of the constituents of cultures. Of the sea water solutions the controlled enrichment medium was preferred, since the gametophytes grown in Erdschreiber showed a wide diversity in development and morphology. This diversity might be accounted for by the unknown, and possibly variable, composition of the soil extract. Ultimately West's artificial medium proved to be superior and was used extensively for all six species of Alaria examined.

Cultures were maintained at 10 C. in a refrigerated culture chamber, under a light intensity of approximately 200 foot candles emitted from a single cool white fluorescent tube (Sylvania F48T12CW). The photo-period was altered throughout this project to correspond approximately with seasonal conditions. Since two constant temperature culture chambers (5 C. and 10 C.) were available for the culturing of Alaria gametophytes, a preliminary experiment was conducted on Alaria grandifolia and Alaria fistulosa to ascertain which was the more suitable temperature. One culture of each species was maintained at 5 C. and the other at 10 C. All other environmental conditions were identical. The cultures at 10 C. showed significantly faster vegetative growth, which provided sufficient somatic divisions for investigation (Figs. 256 & 257). The 10 C. culture of Alaria fistulosa also produced young sporophytes within 30 days,

whereas the 5 C. culture never did produce sporophytes. For the purposes of this project a considerable amount of vegetative growth and the production of antheridia, oogonia and sporophytes have been used as the criteria for determining the success of cultures.

e. Fixation methods.

An ideal fixative kills and preserves living cells without disturbing their internal structure or external arrangement. Such a solution must act quickly and have rapid penetration properties. It was found that for the fixation of the macroscopic sorus material and the microscopic gametophytes and young sporophytes of Alaria, Carnoy's 3:1 absolute ethanol:glacial acetic acid fixing solution has a number of advantages. Not only does it fix material quickly, but bleaches out the chloroplast pigments. In algal cytology this is essential, otherwise the chloroplasts tend to mask the nuclei completely. The 3:1 fixing solution does, however, also have some disadvantages. It hardens material to the extent that a squash technique cannot be used without the inclusion of a softening step. It also has a tendency to distort the initial fragile stages of the gametophytic generation and occasionally causes the interphase nucleus to collapse.

For the fixation of the fragile development stages it became

necessary to employ a less violent fixative. A medium chromic acid-acetic acid fixative was used, viz:

10% aqueous chromic acid	7.0 c.c.
10% aqueous acetic acid	10.0 c.c.
Filtered sea water	to 100 c.c.

f. Mordanting and Staining methods;

Some stains can be applied after certain fixations, without the inclusion of a mordanting procedure. For example crystal violet can be applied directly after fixation in a chromic acid solution. It was found in Alaria, as in other members of the Laminariales, that an intermediate mordanting step is required after ethanol:acetic acid fixation and prior to an aceto-carmin or haemotoxylin stain. Without this step nuclei stained very poorly and individual chromosomes could hardly be distinguished. For the staining of chromosomes in Alaria a number of mordanting procedures were used with differing success:

1. Ferric acetate. A saturated solution of ferric acetate in 45% acetic acid has been used as a mordant in conjunction with an aceto-carmin stain. It has been added to the fixative (5-10 drops/100 c.c. fixative), applied as a separate step for 10 to 20 minutes between fixation and staining, or has been added to the stain (5-10 drops/100 c.c. stain).

2. Ferric chloride. A 4% solution of ferric chloride in 45%

acetic acid has been used as a post fixation step. This step was applied with some success prior to an aceto-carmin staining procedure.

3. Ferric ammonium sulphate. A 2% aqueous solution of ferric ammonium sulphate has been used prior to an acetic-haemotoxylin stain in a modification of Wittmann's staining procedure (1962).

4. Ferric chloride/EDTA. The most successful mordanting procedure was a modification of the technique used by McClaren (1967) for the staining of meiotic chromosomes in Basidiomycetes. A 4% aqueous solution of ferric chloride was saturated with ethylenediaminetetraacetic acid (EDTA). The resulting solution was used to mordant fixed material for 24 hours prior to an aceto-carmin staining procedure. An acetic-haemotoxylin stain was also applied after this mordant, but with limited success.

The staining of the microscopic stages and sorus material of Alaria has necessitated somewhat different techniques. For the examination of sorus material a squash method was used in preference to the sectioning methods most often used in the past. Since the sorus material has thick cell walls and had been hardened by the ethanol:acetic acid fixation it was necessary to soften it before squashing. Three softening techniques were used after the mordanting of material and prior to staining:

1. A 6% aqueous solution of sodium bicarbonate (Naylor, 1957).



2. A solution of iodic acid, aluminum alum and chrome alum in absolute ethanol and concentrated hydrochloric acid (Wittmann, 1962).

3. A one molar aqueous solution of lithium chloride as used by Evans (1963b).

The third method proved to be very effective and has been widely used throughout this project. The mordanted, softened sorus material was then squashed in a drop of Belling's acetocarmine (Darlington & LaCour, 1962) on a slide. Squashing was carried out under a cover-glass using a flexible "plexiglass" rod. Heat was applied gently during the squashing procedure in order to speed up the differentiation and penetration of the stain. Such preparations were made permanent by floating off the cover-glass in 95% ethanol. The squashed material generally remained attached to the cover-glass, which was then passed through several changes of absolute ethanol, and mounted on a slide with Euparal. This procedure stained nuclei very clearly and allowed observation of division figures. Throughout this project it has been used in preference to all others, although the acetic-haemotoxylin procedure of Wittmann (1962) did yield results. The major disadvantage to the latter method was a serious tendency for the stain to become progressively intensified to the extent that nuclear detail became obliterated. It has also been found that haemotoxylin stains, such as those used by all Japanese workers on the Laminariales, are not necessarily nuclei-specific.

The gametophytic and microscopic sporophytic stages of Alaria were similarly stained with aceto-carmin, but without squashing. Squashing of this material made it extremely difficult to distinguish male and female gametophytes and young sporophytes.

The complete procedures which were used predominantly in this project are presented in Appendix III.

g. Pretreatments:

In the initial stages of this research it was noted that gametophytic nuclear divisions appeared very infrequently in the stained preparations. This suggested that either nuclear divisions were occurring extremely quickly thereby making it difficult to fix material in the process of dividing; or in fact they occurred very seldom, creating the same problem. This second possibility was difficult to accept since the number of cells in the cultured plants increased very rapidly. One other possibility is that division was partially synchronous and occurred at a particular time during the day. Such a phenomenon, however, was not observed during several 24 hour periods during which material was fixed at thirty-minute intervals.

Several pretreatments were applied in an attempt to either increase the rate of division (Bactophytohemagglutinin M) or halt divisions at metaphase (colchicine and paradichlorobenzene). Combinations of these pretreatments were also applied (Appendix

IV). Regrettably these pretreatments seemingly had no affect on the division rate. Colchicine pretreatments have previously been applied to members of the Phaeophyta by Levan & Levring (1942), Evans (1966) and Roberts (1966) with little or no success, but this represents the first application of Bactophytohemagglutinin M and paradichlorobenzene to the plants.

h. Photographic methods:

All photographs were taken with Kodak "Panatomic X" film, developed in Edwal "FG7" developer. Prints were made on Ilford bromide paper (Numbers 3, 4 and 5) and developed in Kodak "D 11" developer. Photographs were taken using a Leica DBD camera with a Lietz Wetzlar x1 extension tube mounted on a Nikon S-Ke research microscope.

## RESULTS

### a. Meiosis and the formation of meiospores:

The sorus of Alaria is characteristically composed of unilocular sporangia and sterile paraphyses, both of which arise from the meristoderm layer of cells. The first nuclear division in immature unilocular sporangial is reductional. Meiosis has been studied in the immature zoosporangia of A.marginata, A.nana, A.tenuifolia, A.fistulosa and A.grandifolia, and was found to be similar in all five species. There was insufficient material available to examine this process thoroughly in A.taeniata.

The dimensions of the immature zoosporangia prior to the onset of meiosis are approximately 10 x 25 microns. The interphase nucleus appears granular (Figs 8 & 9) and measures 8-10 microns in diameter. There is no obvious reticulum connecting the heterochromatic granules (chromomeres) at this stage. A single nucleolus and distinct nuclear membrane were visible in only a few preparations of all the species studied. At the onset of division the chromomeres become more condensed and have an increased stainability with aceto-carmin. At this stage there is some evidence of thread-like connections between the chromomeres (Figs. 10 & 11). However, the resultant beaded threads cannot be discerned as distinct chromosomes. This stage is interpreted as leptotene. The following stage, zygotene, when homologous chromosomes pair, cannot be clearly delimited in

Alaria species. Although there is some indication of the pairing of chromomeres (Figs. 12, 13, 14 & 15), there is often only one instance of pairing visible in a zygotene nucleus. Added to this difficulty is the fact that the chromomeres at this stage are only 0.2-0.5 microns in diameter and cannot readily be distinguished under the light microscope. Since the centromeres cannot be distinguished at zygotene it is impossible to state at which region of the chromonemata synapsis commences. Pachytene is marked by the increased size of the paired chromomeres and the overall condensation of the chromosomes (Figs. 16, 17, 18, 19, 20, 21, 22, 23, 24 & 25). The chromosomes still appear as beaded threads, but the beads are now fewer and much larger while the threads are much shorter. There is seldom any indication of the double structure of the bivalents at this stage. This is the earliest time at which chromosome counts can be obtained, although it has been found to be more desirable to restrict counting to a later stage of prophase or even metaphase I. Due to an abundance of pachytene configurations in squash preparations it seems that this stage may last some considerable time. This is especially so in A.tenuifolia where pachytene may in fact represent a resting stage in the division process, although there is no decreased stainability.

The diplotene stage is perhaps the most difficult to distinguish due to the exceedingly small size of the chromosomes. Typical diakinesis configurations are also rarely distinguishable.

Consequently, diplotene and diakinesis have been regarded simply as prometaphase (Figs. 26, 27, 28 & 29). X-, V- and Y-shaped configurations have been recorded in a few preparations and have been used to delimit diakinesis more accurately (Figs. 139 & 140). During diplotene and diakinesis centromeres are distinguishable for the first time, but only in the larger chromosomes. The nucleoli and nuclear membranes have completely disappeared by diakinesis.

Following the prometaphase stages the bivalents align themselves in an equatorial plate (Figs. 30 & 31). At metaphase I spindle apparatus have been distinguished in a number of preparations, but centrioles have never been seen. The homologues then move to opposite poles in anaphase I. During telophase I the daughter nuclei revert to an interphase condition. Due to the scarcity of binucleate sporangial cells in A.marginata, A.nana and A.tenuifolia it appears that this interphase condition lasts for only a short time before the equational division of meiosis occurs.

Prophase II commences with the condensation of distinct chromosomes (Fig. 32). These chromosomes are then aligned in plates at metaphase II (Fig. 33). During anaphase II the daughter chromosomes separate in tightly associated masses to their respective poles (Fig. 34). Following telophase II a four-nucleate sporangial cell is produced, each nucleus of which

is presumed to be haploid. Three further mitotic divisions occur producing 32 nuclei in the sporangium (Figs. 35, 36, 37, 40 & 41). After these 32 nuclei have passed into an interphase condition the cytoplasm of the sporangial cell is cleaved in such a way that 32 rounded spores are produced, each with a single nucleus and chloroplast. Although 32 spores per unilocular sporangium is undoubtedly the most frequently occurring number, occasionally sporangia with 16 and 64 spores have been observed in all the Alaria species examined. The dimensions of the mature sporangium are approximately 10 x 45 microns.

It has been possible to obtain chromosome counts at late prophase stages during the mitotic divisions in the sporangium (Figs. 38 & 39). These counts have, as a rule, matched the bivalent counts made at prometaphase I and metaphase I of meiosis and the chromosome counts made during mitotic divisions in the gametophytic generations. However, for the actual confirmation of a chromosomal alternation of generations in this genus, chromosome counts had to be obtained from vegetative sporophytic cells. The actual counts are presented in section "f" of the results.

In a single sorus it is possible to distinguish all stages of the development of sporangia. Sporangia do not mature in a synchronised manner. Further, it appears that there is no synchrony of nuclear divisions between sporangia. Sporangial material fixed and examined at set intervals over 24 hour periods

shows a random range of division stages. Such observations were, however, only made on A.marginata, A.nana and A.tenuifolia, and fixation was always carried out on material living under artificial culture conditions. Within a single sporangium there is synchronised division of the nuclei.

b. Development of gametophytes and the formation of gametangia:

Gametophytic development and the formation of antheridia and oogonia were followed in A.marginata, A.nana, A.tenuifolia, A.fistulosa, A.taeniata and A.grandifolia, and were found to be similar.

At maturity the zoospores are forcefully liberated from the unilocular sporangia. The empty sporangia then either break away or degenerate extremely quickly. The zoospores (Fig. 42) are elongate (approximately 8 x 4 microns), tapering anteriorly. Depending on the orientation of these cells under the microscope, some appear to be somewhat pyriform in shape. They are laterally biflagellate, the anterior flagellum being the longer of the two. The zoospores remain actively motile for a variable length of time before losing their flagella and rounding up (Fig. 43). Rounded-up spores of A.marginata have been observed within minutes of liberation in the same suspension in which motile zoospores have been noted after 26 hours. The diameters of rounded-up spores have been measured for all the species examined and approximate 5 microns. No size difference is apparent, either amongst the spores of one species or the spores



of all the species.

The non-motile spores germinate by producing a small protuberance (Fig. 44) which subsequently elongates to become a distinct germination tube. The spore nucleus divides mitotically and one of the daughter nuclei migrates into the germination tube (Fig. 45) where it is cut off by a cell wall, forming the initial cell of the gametophyte (Fig. 46). The remaining nucleus degenerates in A.nana, A.tenuifolia, A.fistulosa and A.taeniata. In A.marginata, however, it divides again (Fig.47) and one of the daughter nuclei of this division is cut off in the formation of the second cell of the gametophyte (Fig. 48). The remaining nucleus then degenerates within the empty spore case. Progressive mitotic divisions subsequently give rise to uniseriate, branched, filamentous gametophytes, the cells of which remain uninucleate.

All the species of Alaria examined are dioecious, forming morphologically distinct male and female gametophytes (Figs. 49 & 50). The female gametophyte consistently has fewer and larger cells than the male. The frequency of male and female gametophytes has been recorded in cultures established from uniform spore suspensions, and it appears that the frequency of each is approximately 50% (Table III). Since 7,500 gametophytes were counted, this could indicate that the sex of the gametophytes is genotypically determined.

The gametophytes mature after a variable length of time. Gametangia may be formed either when the gametophyte is only one or two cells long or after it has produced a considerable number of cells. The factors responsible for the initiation of antheridium and oogonium production are not clearly understood. During the course of this project it has been noted that gametangia are readily produced in cultures in which the medium has remained unchanged for a considerable time, and that cultures established in May and June produce gametangia without the prior establishment of extensive gametophytic systems. The vegetative growth of gametophytes on which gametangia are produced is apparently curtailed.

Any cell of the female gametophyte is a potential oogonium. The first indications of oogonium production are an increased cytoplasmic density within the potential gametangium, the production of irregular protuberances from the cell, and an increase in cell volume (Fig. 49). A single mitotic division occurs within the oogonium (Figs. 54 & 55). One of the daughter nuclei degenerates and the other becomes the nucleus of the egg. Each oogonium produces a single egg cell (Fig. 56). The egg cell is extruded through a rupture in one of the irregular protuberances of the oogonium. Once extruded, the egg remains attached to the "neck" of the oogonium (Fig. 57), and it is in this position that fertilisation takes place.

Antheridium production involves numerous divisions of a single cell of the male gametophyte. The result of these divisions is a cluster of cells rather than a uniseriate filament. Each cell of these clusters is an antheridium. The cell wall of each antheridium becomes thickened proximally, leaving distal thin areas, which eventually rupture with the release of the antherozoids. The antheridial clusters may be intercalary (Fig. 51), or terminal, or, as in A.taeniata, terminal on short lateral stalk filaments (Fig. 52). The antherozoids are released singly from the antheridia. Although the shape of the antherozoids is seemingly quite variable, and ranges from an elongate form to a spherical form, the most common is undoubtedly elongate (Fig. 53), with two unequal flagella inserted in a lateral position. The anterior flagellum is consistently longer than the posterior.

c. Fertilisation:

Fertilisation is heterogamous, involving the syngamy of the large (15-20 microns in diameter), labile, spherical eggs and small (5 x 3 microns) biflagellate antherozoids. Although several antherozoids have been seen swimming in the vicinity of extruded egg cells, only one actually fuses with each egg. Once an antherozoid has become associated with an egg cell its flagella are apparently shed and plasmogamy occurs. A

fertilisation papilla remains on the surface of the egg, marking the point at which fusion took place (Figs. 58 & 59). In A.taeniata a fertilisation pore and tube appear to be produced within the cytoplasm of the egg just prior to karyogamy (Fig.57). This phenomenon has not been observed in any other species examined. During plasmogamy the nuclei of both sex cells remain in an interphase condition, so that the result of plasmogamy is a binucleate cell in which both nuclei are in a resting stage (Fig. 60). Karyogamy then occurs with the fusion of the two resting nuclei and the zygote is established.

d. Development of the young sporophyte:

The elongation of the zygote (Figs. 62, 63 & 64), which is still attached to the "neck" of the oogonium at this stage, marks the initiation of its germination. The elongation is terminated by a mitotic division giving rise to a two-celled sporophyte (Fig. 65). The ontogeny of the young sporophyte most commonly involves the production of a uniseriate filament (Figs. 66 & 70) and the subsequent division in a second plane to produce a biseriate filament. This secondary division may occur when the filament is only three or four cells long (Figs. 67 & 68), or it may be delayed until the uniseriate filament is ten or more cells long (Fig. 71). Invariably the basal cell of the filament assumes the role of a rhizoidal cell, initially

acting as a means of anchoring the young sporophyte to the empty oogonium. Before dividing this rhizoidal cell may become quite branched (Fig. 74). The biseriate filament then divides in a third plane, assuming the parenchymatous habit of a distromatic blade (Figs. 72 & 73). The development of this distromatic blade has not been followed beyond a length of 4 - 5 m.m.

There is one major abnormality in the process of sporophytic development. This is the occurrence of parthenogenesis, and the production of embryonic stages of sporophytes without fertilisation taking place. There are two ways in which "parthenosporophytes" are formed. Some are formed within the oogonium (Fig. 68), in which case they can be readily detected, while others are produced subsequent to the extrusion of the egg, and cannot be readily distinguished from normal sporophytes. The only method of accurately determining whether the latter type is parthenogenetic in origin is by examination of its chromosome complement. This has been done in cultured material of A.marginata, A.nana, A.tenuifolia, A.fistulosa and A.taeniata (Figs. 247, 248, 249, 250, 251 & 252), and in fact it appears that the chromosome complement of suspected "parthenosporophytes" is haploid.

e. Mitosis in Gametophytes and young Sporophytes:

The mitotic process has been studied in male and female gametophytes and extremely young sporophytes of A.marginata, A.nana, A.tenuifolia, A.fistulosa and A.taeniata and is quite uniform for all species. The cultured plants of A.grandifolia did not respond to the staining techniques, making it impossible to investigate them cytologically.

Interphase nuclei have a granular appearance (Fig. 75). The diameter of the male interphase nucleus varies from 2.5 to 4.0 microns; the female from 5.0 to 6.0 microns, and the sporophyte nucleus from 6.0 to 8.0 microns. A single nucleolus and nuclear membrane are visible at this stage. Several larger heterochromatic bodies are often discernable amongst the granules of the interphase nucleus. As the interphase nucleus moves into mitotic prophase it becomes reticular. The granules appear to be linked together by fine chromatic threads (Fig. 76). At this initial stage of prophase, nucleoli are seldom observed. The contraction and condensation of the chromatic threads give rise to larger granules and the first indications of distinct chromosomes (Fig. 77). In a few preparations of Alaria marginata, A.nana and A.tenuifolia distinct chromatids have been observed, although in most cases these are not distinguishable, presumably on account of their exceedingly small size (Figs. 78 & 79). Prophase continues with the progressive contraction of the chromosomes (Fig. 80), until they appear as

distinct separate bodies (Figs. 81, 82, 83 & 84) in a prometaphase stage. At this stage it is possible to distinguish the centromeres of the larger chromosomes, and chromosome counts can be made. The chromosomes then align themselves in an extremely tightly associated metaphase plate (Fig. 84). The chromosomes are so tightly compacted together at this stage that it has proved a very unsatisfactory source of chromosome counts. The separation of crescent-shaped chromatid masses in anaphase is also a very tightly compacted stage of mitosis. In anaphase configurations (Fig. 86) separate daughter chromosomes cannot be distinguished at all. Neither spindles nor centrioles have been observed during mitosis. In telophase a new cell wall is laid down and the resultant nuclei revert to an interphase condition.

f. Chromosome numbers and size:

The haploid chromosome numbers for A.marginata, A.nana, A. tenuifolia, A.fistulosa, A.taeniata and A.grandifolia have been obtained from diplotene, diakinesis and metaphase I configurations of meiosis (Table IV). In A.marginata bivalent counts range from 13 to 16 (Figs. 87,88,89,90,91,92,93,94,95,96, 97 & 98); in A.nana from 12 to 16 (Figs. 99,100,101,102,103,104, 105,106,107,108,109 & 110); in A.tenuifolia from 12 to 18 (Figs. 111,112,113,114,115,116,117,118,119,120,121 & 122);

in A.fistulosa from 12 to 16 (Figs. 123,124,125,126,127,128,129, 130,131,132,133 & 134); in A.grandifolia from 17 to 26 (Figs. 139,140,141,142,143,144,145,146,147,148,149,150,151,152,153,154, 155,156,157 & 158) and in A.taeniata, where only two counts have been made due to a lack of suitable material, the haploid number is 14 (Figs. 135, 136, 137 & 138). In A.marginata, A.nana, A.tenuifolia and A.fistulosa the modal number is 14, whereas in A.grandifolia it is 24 (fig. 253). In A.tenuifolia two counts of 21 and 26 were omitted from the computations on the basis that they probably represent asynaptic or partially asynaptic configurations.

Chromosome counts have also been made from prometaphase stages of mitosis in the gametophytes of all species but A.grandifolia (Table V). In A.marginata these counts ranged from 13 to 16 (Figs. 159,160,161,162,163,164,165,166,167 & 168); in A.nana from 12 to 17 (Figs. 169,170,171,172,173,174,175 & 176); in A.tenuifolia from 12 to 17 (Figs. 177,178,179,180,181 & 182); in A.taeniata from 12 to 18 (Figs. 183,184,185,186,187,188,189, 190 & 191) and in A.fistulosa from 12 to 17 (Figs. 192,193,194, 195,196,197 & 198). The modal number for all of these species is 14 (Fig. 254).

The diploid chromosome complements of young cultured sporophytes have been determined from prometaphase stages of mitosis for all species except A.grandifolia (Table VI). In



A.marginata the numbers have ranged from 26 to 29 (Figs. 204,205, 206,207,208,209,210,211,212 & 213); in A.nana from 26 to 30 (Figs. 214,215,216,217,218,219,220,221,222 & 223); in A.tenuifolia from 24 to 31 (Figs. 224,225,226,227,228,229,230 & 231); in A.taeniata from 24 to 31 (Figs. 232,233,234,235,236,237,238,239, 240 & 241) and in A.fistulosa from 23 to 31 (Figs. 242,243,244, 245 & 246). In all cases the modal number is 28. (Fig. 255).

From these chromosome counts it appears that the haploid number is approximately 14 and the diploid number approximately 28 for A.marginata, A.nana, A.tenuifolia, A.fistulosa and A.taeniata. In A.grandifolia the haploid number is approximately 24, although this number could only be confirmed from meiotic material (Table VII).

The chromosomes representing the haploid complement of all six species can be grouped approximately into three size groups, when measured at prometaphase I. In A.marginata, A.nana, A.tenuifolia, A.fistulosa and A.taeniata six chromosomes are 0.5 to 1.0 microns, four, 1.25 to 1.6 microns and four, 1.8 to 2.0 microns. In A.grandifolia eleven chromosomes are 0.5 to 1.0 microns, nine, 1.2-1.6 microns and four, 1.8 to 2.0 microns.

## DISCUSSION

In A.marginata, A.nana, A.tenuifolia, A.fistulosa, A.taeniata and A.grandifolia the immature zoosporangia are the site of meiosis. This corresponds with every other investigation on members of the Laminariales since the time of Kylin's initial description of meiosis in Chorda filum in 1918. However, descriptions of the meiotic process differ widely. Some of the differences may reflect dissimilarities between species; others, the different techniques and interpretations used by various authors. For example Kylin (1918), Myers (1928), McKay (1933), Hollenberg (1939) and all the Japanese workers in this field have used a haemotoxylin staining reaction for the examination of chromosomes and division stages. During this current project it was found that haemotoxylin is not a specific nuclear stain, since it not only stains chromosomes and nuclei but also chloroplasts and other miscellaneous cytoplasmic intrusions. A Feulgen technique was first introduced to the cytological studies of the Laminariales by Walker (1952) and Magne (1953). Although this procedure undoubtedly results in a DNA specific staining reaction, it is extremely difficult to use on the Laminariales and has been superceded by the application of Belling's (1926) aceto-carmin technique by Naylor (1956), Kemp & Cole (1961), Cole (1962) and Evans (1963a, 1965). In addition, all authors in this field, with the exception of Evans

(1965), have relied upon sectioned material for the examination of meiotic stages. This method has obvious limitations and has been superseded by a squash technique used in this current project in conjunction with a softening procedure.

The interphase nuclei of the sporangial mother-cells of Alaria spp. appear granular, as do those of Nereocystis luetkeana (Kemp & Cole 1961), Laminaria digitata, Laminaria saccharina, Laminaria hyperborea, Laminaria ochroleuca, Alaria esculenta, Chorda filum and Sacchoriza polyschides (Evans, 1965). Other authors have reported a reticular interphase nucleus. The fact that nuclear membranes and nucleoli are seldom visible at this stage in Alaria may be explained by the rigorous squashing technique used. In the majority of cases in which nuclear membranes and nucleoli have been reported as easily visible at interphase, a sectioning technique has been used.

In most species studied up until this present time the synaptic zygotene stage has been considered synonymous with the congregation of chromonemata to one side of the nucleus, and although chromatin "knots" have been noted by Evans (1965), the actual process of pairing has never been described. In Alaria spp. the presence of this unilateral congregation of chromatin<sup>2</sup> loops has not been observed, nor have chromatin "knots". These differences between zygotene in Alaria and zygotene in other members of the Laminariales might possibly indicate that either

the arduous squash technique used on Alaria has obliterated the unilateral congregation of loops or that their absence indicates a genetic difference between Alaria spp. and other Laminarian species. In Alaria, however, there is convincing evidence of the pairing of chromomeres, presumed to be homologous chromosomes, even although it cannot be said at which region of the chromosomes pairing commences or at what rate it occurs.

Today the concept that chromosomes are joined end-to-end in pachytene and diplotene in a "spireme" is neither cytologically nor genetically conceivable (Darlington, 1965). This is especially so in Laminaria angustata (Nishibayashi & Inoh, 1956; Ohmori, 1967), Costaria costata (Nishibayashi & Inoh, 1957; Ohmori, 1967), Undaria undarioides (Nishibayashi & Inoh, 1960b, 1960c; Ohmori, 1967), Chorda filum (Evans, 1965; Nishibayashi & Inoh, 1961a, 1961b; Ohmori, 1967), Ecklonia stolonifera and Ecklonia cava (Ohmori, 1965, 1967), Undaria pinnatifida (Inoh & Nishibayashi, 1954, 1955, 1960; Ohmori, 1967), Pterygophora californica (McKay, 1933), Ecklonia radicata, Laminaria longipedalis, Laminaria yendoana, Eisenia bicyclis, Ecklonia kurome (Ohmori, 1967), where "O"-shaped bivalents have been described in diakinesis. Although the concept of a "spireme" in pachytene and diplotene has been seriously questioned in Nereocystis luetkeana by Kemp & Cole (1961) on the basis of the

impracticability of observing the continuous nature of the chromatin, every other author from the time of Kylin's initial description of meiosis in Chorda filum (1918) has either maintained that a spireme exists (Abe, 1939; Inoh & Nishibayashi, 1954, 1955, 1960; McKay, 1933; Myers, 1928; Nishibayashi & Inoh, 1956, 1957, 1960b, 1960c, 1961a, 1961b; Ohmori, 1965, 1967; Yabu, 1957, 1958, 1964b, 1965; Yabu & Tokida, 1963), or has failed to describe these phases of meiotic prophase completely (Evans, 1965). In the species of Alaria studied in this project there is no reason at all to suspect that a spireme exists in pachytene and diplotene, since there is a condensation of distinct chromosomes during these stages. Although it is impossible to say exactly how much contraction of the chromosomes occurs during prophase I, it is considerable. The partial separation of the homologues, described in diplotene bivalents of higher organisms, has not been seen in Alaria, due presumably to the small size of the chromosomes. Although X-, Y- and V-shaped bivalent configurations, similar to those reported by every other author in this field, have occasionally been observed in Alaria spp., diplotene and diakinesis are most often indistinguishable and have been regarded simply as prometaphase I. Centromeres can be distinguished in the larger chromosomes from constrictions between chromomeres at this stage.

Metaphase I has been reported as a tightly compacted stage of meiosis by Nishibayashi & Inoh (1956, 1960a, 1961a), Inoh &

Nishibayashi (1954, 1955, 1960), Ohmori (1965, 1967), Yabu (1957, 1958, 1964b, 1965), Yabu & Tokida (1963), Magne (1953) and Kemp & Cole (1961). Chromosomes in the species studied by these authors have been so tightly associated at metaphase I that chromosome counts could not be made. The squash technique used by Evans (1965) on Laminaria digitata, Laminaria saccharina, Laminaria hyperborea, Laminaria ochroleuca, Alaria esculenta, Chorda filum and Sacchoriza polyschides and by the author in this current project on A.marginata, A.nana, A.tenuifolia, A.fistulosa, A.taeniata and A.grandifolia has, however, successfully flattened and separated metaphase I bivalents, so that chromosome counts could be made at this stage. A number of the so-called metaphase I configurations described by Evans (1965) are extremely similar to prometaphase I and even earlier prophase I squashes of Alaria spp.. Metaphase I bivalents in the Alaria species characteristically have the "dumbell" shape previously reported at metaphase I in the species studied by Nishibayashi & Inoh (1956, 1957, 1960a, 1960b, 1960c, 1961a, 1961b), Inoh & Nishibayashi (1954, 1955, 1960), Ohmori (1965, 1967), Yabu (1957, 1958, 1964b, 1965), Yabu & Tokida (1963), Magne (1953) and Kemp & Cole (1961). This shape is not reported by Evans (1965), further substantiating the possibility that he has been describing prophase I chromosomes as metaphase bivalents.

Although the orientation of the spindle apparatus can be inferred from the position of the metaphase plate in Alaria spp., a spindle has very seldom been seen. The appearance of spindles may simply be a function of the staining reaction used, since all workers using haematoxylin report their presence. They have also reported either one or two "centrosomes" at the spindle poles. These structures were not observed at all by Evans (1965), and were observed very occasionally by Kemp & Cole (1961) but were interpreted as division abnormalities. They have not been observed at all in the Alaria species studied in this project. It should be stated that before the presence or absence of spindles and centrosomes can be confirmed, ultra-structure studies of the nucleus of the Laminariales are required.

Anaphase I and telophase I apparently follow the same pattern in Alaria spp. as in other members of the Laminariales, with the exception that indications of non-disjunction have not been observed in these species as they have in Nereocystis luetkeana (Kemp & Cole, 1961). Prophase II follows telophase I very quickly after a very brief interphase stage in A. marginata, A. nana and A. tenuifolia. This is in agreement with the observation of Evans (1965) in Alaria esculenta, Chorda filum, Sacchoriza polyschides and 4 spp. of Laminaria and of Kemp & Cole (1961) on Nereocystis luetkeana. Whether or not this is true for other members of the order is not known, since no other author has commented on the speed of division at all. The

equational meiotic division and subsequent mitotic divisions in the aporangia of the Alaria spp. appear to be no different from the equivalent divisions in other members of the Laminariales that have been studied cytologically. In the Alaria spp. divisions in a single sporangium are synchronous while in Nereocystis luetkeana this is not necessarily so (Kemp & Cole, 1961). With the exception of Chorda filum, which has 16, (Nishibayashi & Inoh, 1961a, 1961b; Ohmori, 1967) and Sacchoriza bulbosa, which has 128, (Sauvageau, 1915a) the final number of nuclei (and spores) in each sporangium is 32 in the Laminariales.

The occurrence of rounded-up spores in suspensions of freshly liberated zoospores of Alaria spp. has not been previously reported for any member of the Laminariales. A possible interpretation of this phenomenon is that some zoospores are liberated prior to the formation of flagella. This may either be an inherent characteristic of some zoospores or it may simply occur due to the rupturing of immature sporangia. The latter may be caused by the bursting of neighbouring mature cells or the turgor changes induced artificially to promote spore release in the laboratory.

The germination of zoospores of A.marginata, A.nana, A.tenuifolia, A.fistulosa, A.taeniata and A.grandifolia by way of a germ tube is quite consistent with other members of the



Laminariales as is the single mitotic division in the spore case of all the Alaria spp., except A.marginata. In A.marginata two divisions occur in the spore case, a single daughter nucleus of each division being cut off in the initial two cells of the gametophyte.

The production of morphologically distinct male and female gametophytes in Alaria spp. also occurs in all other members of the order except Chorda tomentosa, which has a monoecious gametophytic generation (Sundene, 1963). The proportions of male and female gametophytes formed from uniform spore suspensions of all the Alaria spp. would seem to indicate that their sex is genotypically determined. However, such a statement can only be made with serious reservations. Before definite genotypic determination of sex can be established it must be demonstrated that the products of single sporangium produce 50% male and female gametophytes.

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In Alaria species it has been determined that at 5 C. the development of the gametophytes and the formation of gametangia are inhibited. It has also been demonstrated that there may be a seasonal effect on the production of gametangia, similar to that reported by McKay (1933) for Pterygophora californica, and that nutrient depletion may effect the formation of gametangia. In addition it has been found that the vegetative growth of Alaria gametophytes is curtailed by the production of gametangia.

Although the literature concerning the environmental regimes that effect gametophyte growth and the production of gametangia is often conflicting, the observations on Alaria are in agreement with Kain's (1964) general observation that the ranges of each environmental factor are greater for the support of vegetative growth than for the production of gametangia. Since gametophytic growth and the production of gametangia are obviously closely associated in Alaria spp., it might be construed that the early production of gametangia and the consequential termination of gametophytic growth reflects a tendency towards the loss of the gametophyte generation altogether. Svedelius (1927) maintained that the life cycles of the Phaeophyta represent a phylogenetic series from an alternation of isomorphic generations (Ectocarpales) through an alternation of heteromorphic generations (Laminariales) to a life-cycle in which there was no gametophytic generation at all (Fucales). Therefore, based upon the assumption that such a phenetic series actually reflects phylogeny, it might be argued that this reduction of the gametophyte generation reflects the transition from a Laminarian-type life-cycle to a Fucales-type life-cycle. However, before such a hypothesis can be substantiated in the absence of a good fossil record, it is necessary to know the exact physiological effect of environmental variables on growth of the gametophyte and the production of gametangia. Further, it would be desirable to know the history of these environmental factors

in the oceans. In the Phaeophyta ontogeny may well recapitulate phylogeny, but not necessarily so.

The formation of oogonia and antheridia in A.marginata, A.nana, A.tenuifolia, A.fistulosa, A.taeniata and A.grandifolia is indistinguishable from these processes in other members of the Laminariales. However, the actual process of egg formation is quite different, since a mitotic division in the oogonium precedes egg formation, one of the resultant nuclei being incorporated into the egg cell. In Pterygophora californica (McKay, 1933), Egregia menziesii (Myers, 1928) and Nereocystis luetkeana (Kemp & Cole, 1961), for example, the entire contents of the oogonium are rounded up as the egg cell without a nuclear division. Fertilisation in A.marginata, A.nana, A.tenuifolia, A.fistulosa and A.taeniata occurs while both male and female nuclei are in interphase. This is consistent with the observations of Kemp & Cole (1961) on Nereocystis luetkeana. In Pterygophora californica (McKay, 1933) and Eisenia arborea (Hollenberg, 1939), however, this process occurs while the nuclei are in prophase. The occurrence of a "fertilisation pore" and "tube" in the egg cells of Alaria taeniata is unique. Neither have been reported in any other member of the Laminariales. It is possible that the "fertilisation pore" is in fact a "micropyle" and the "tube" represents the pathway followed by the male nucleus preceding

karyogamy. On the other hand both may simply be remnants of incomplete cytoplasmic cleavage during the division preceding egg formation.

The early developmental stages of the young sporophytes of the Alaria spp. studied in this project are very similar to those described for other species of the Laminariales. Similarly the mitotic process as it occurs in the gametophytes and young sporophytes of these species corresponds with the descriptions of mitosis made by Naylor (1956), Kemp & Cole (1961) and Evans (1965).

Previously the occurrence of parthenogenesis has been described in Laminaria digitata and Laminaria hyperborea by Schreiber (1930), in Laminaria religiosa, Laminaria japonica, Laminaria angustata, Laminaria angustata var. longissima, Laminaria ochotensis, Laminaria diabolica, Alaria crassifolia, Undaria pinnatifida and Arthrothamnus bifidus by Yabu (1964a) and in Nereocystis luetkeana by Kemp & Cole (1961). In the current project parthenosporophytes have been observed in all Alaria species and, as in Nereocystis luetkeana by Kemp & Cole (1961), these abnormal sporophytes are haploid. It is not known whether the occurrence of parthenosporophytes is a naturally occurring phenomenon or whether it is restricted to cultured plants. If they occurred under normal field conditions, achieved maturity and were able to sporulate then their presence might assume substantial significance. However,

since solely mitotic sporangia have never been reported in mature plants, and since the proportions of male and female gametophytes in cultures established from one sporophyte are indicative of a normal meiotic segregation, it appears most likely that these young parthenosporophytes do not mature at all. Nevertheless since no author has attempted to culture these abnormalities to maturity it is obvious that further investigations are required to determine their fate.

Chromosome numbers throughout the Laminariales show a wide range which may reflect genetic differences between species. On the other hand, since widely conflicting counts for the same species do occur in the literature, they may reflect the possible discrepancies detailed below, and a certain amount of subjectivity that must necessarily be applied to the counting of extremely small chromosomes. From Table II it can be seen that the haploid number of c. 14 in A.marginata, A.nana, A.renuifolia, A.fistulosa and A.taeniata is considerably lower than those of most other members of the Laminariales, whereas the haploid number of c.24 for A.grandifolia more closely approximates the haploid numbers of other species of the order. The numbers that are reported here for Alaria spp. do not conform with numbers of other members of the genus. However, assuming that the different chromosome numbers do reflect genetic differences between species and not discrepancies in technique, it is conceivable that a polyploid series around the numbers 13 or 14

exists in the genus Alaria. Such a polyploid series may even apply to the family Alariaceae, or to the entire order. Kemp & Cole (1961) have tentatively suggested that a polyploid series around the number 10 might exist in the Laminariales. These authors emphasised, and it should be mentioned again, that before such a system can be fully accepted it is necessary that most numbers be confirmed and many more species examined. It should also be recognised that most of the possible sources<sup>of error</sup> in counting chromosomes would tend to produce a falsely high number. Perhaps, as has been suggested by Cole (1967), the actual chromosome numbers of the Laminariales are lower than those reported.

The small size of the chromosomes of the Laminariales, and the extreme difficulty involved in separating them successfully, makes it very difficult to distinguish each chromosome separately. In addition to this are the problems involved in distinguishing chromatids from chromosomes, when both simply appear as tiny chromatic masses almost at the ultimate resolving power of the light microscope. Similarly there is difficulty in distinguishing the arms of a chromosome on either side of the centromere from two entire chromosomes lying close together, and the homologues of a bivalent at meiosis, from two bivalents lying together.

In obtaining chromosome numbers many authors have not defined

clearly what they are counting as chromosomes in terms of the above alternatives. There has been a tendency for authors to simply count each chromatin mass as a separate chromosome. What is more confusing is the fact that often the stage of division, at which counts are made, is not clearly defined, or is even indefinable because of the small size of the chromosomes. This obviously leads to discrepancy - for example if a count were made at early prophase it would probably be of chromomeres whereas at count made at very late prophase would be of entire chromosomes or bivalents. In this current study of Alaria, chromosomes have been counted at either metaphase or very definite late prophase stages to avoid these possible discrepancies. In mitotic counts two chromatin bodies lying in juxtaposition have been regarded as parts of the one chromosome - either arms of the one chromosome or chromatids. In meiosis, also, chromatin bodies in close association with one another have been regarded as parts of a single bivalent.

In Alaria, as in other members of the Laminariales that have been examined cytologically, most of the chromosomes fall within the size range 0.5 to 2.0 microns at late prophase stages of meiosis and mitosis (Evans, 1965; McKay, 1933). An exception to this has been demonstrated in Sacchoriza polyschides (Evans, 1965) with the discovery of heteromorphic sex chromosomes in which the X chromosome is 4-6 microns long and is much longer than the Y. Although Evans (1965) suspects a similar mechanism in

Laminaria digitata, Laminaria saccharina, Laminaria hyperborea, Laminaria ochroleuca, Chorda filum and Alaria esculenta, it is not at all obvious from his measurements and micrographs. No apparent X/Y sex chromosomes have been detected at all in any of the Alaria spp. studied in this project.

Although A.marginata, A.nana, A.tenuifolia, A.fistulosa, A.taeniata, and A.grandifolia are morphologically distinguishable (Widdowson, 1964), the only distinction between species that can be made on the basis of chromosome numbers is that A.grandifolia has a different haploid chromosome number from A.marginata, A.nana, A.tenuifolia, A.fistulosa and A.taeniata. Not only are the chromosome numbers of these five species the same, but the chromosome size and the actual division processes are indistinguishable. Although there are some small developmental differences between these species, such as the germination of zoospores in A.marginata and the presence of a possible "fertilisation pore" and "tube" in the egg cells of A.taeniata, it is apparent that ecological investigations are urgently required if a complete understanding of the genus is to be obtained.



## SUMMARY

The morphological and cytological phases of the life-cycles of Alaria marginata, Alaria nana, Alaria tenuifolia, Alaria fistulosa, Alaria taeniata and Alaria grandifolia have been investigated. The morphological life-cycles of all six species are represented by an alternation of macroscopic sporophytic generations with microscopic dioecious gametophytic generations. Chromosomal alternations correspond with the morphological in that the sporophyte is diploid and the gametophyte, haploid. The first and second divisions in the immature zoosporangia of mature sporophytes are meiotic. Meiosis has been critically investigated in all six species and compared with this process in other members of the Laminariales. In a number of cases it was found to be similar, although the concepts of the division processes presented by a number of authors were found to be questionable. In addition to this the majority of authors have completely omitted comprehensive descriptions of meiosis and mitosis.

The development of gametophytes and gametangia, the process of fertilisation and the development of the young sporophytes have been followed. Gametophytic development is similar to that in other members of the Laminariales, although the germination of zoospores of A. marginata is apparently unique in that it involves two mitotic divisions instead of the normal one division in the spore case. The male and female

gametophytes are morphologically distinct and genotypic sex determination is suspected. Fertilisation is oogamous as in other members of the order, involving the fusion of biflagellate antherozoids with extruded non-motile egg cells. Egg formation is somewhat different, however, since it involves a mitotic division within the oogonium prior to the differentiation of the egg. In addition the presence of a possible "fertilisation pore" and "tube" in the eggs of A.taeniata is apparently unique. The development of young sporophytes from the zygotes of Alaria spp. is indistinguishable from this process in other members of the Laminariales. The occurrence of parthenogenesis and the formation of haploid "parthenosporophytes" have been demonstrated.

The haploid numbers of the six species of Alaria have been obtained from diplotene, diakinesis and metaphase I stages of meiosis. The haploid number of A.marginata, A.nana, A.tenuifolia, A.fistulosa and A.taeniata is approximately 14, whereas in A.grandifolia it is approximately 24. In all species, except A.grandifolia the haploid number has also been confirmed from prometaphase stages of mitosis in the gametophytes. A diploid number of approximately 28 has been obtained from mitotic divisions in the young sporophytes of A.marginata, A.nana, A.tenuifolia, A.fistulosa and A.raeniata. The use of nuclear cytology in the taxonomy of these members of the genus has obvious

limitations. Only A. grandifolia could be distinguished on the basis of chromosome number.

The chromosome numbers and sizes of the Alaria species have been compared and contrasted with those of other members of the Laminariales. In addition to this the methods that have been used in the cytological investigations of the Laminariales and the problems involved in the distinction of chromosomes from chromatids and from bivalents have been discussed.

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Appendix I

Key to the species of the genus *Alaria* (Widdowson, 1964)

Dimensions are taken from wet fertile plants of the species, and treated statistically.

1. Midrib fistulose (Furnished with air chambers) at intervals..... A. *fistulosa*
1. Midrib solid throughout..... 2
2. At least some lateral branching (fertile or sterile) with midrib..... A. *paradisea*
2. Lateral branches completely devoid of midribs, usually fertile..... 3
3. Stipe virtually non-existent; lower trunk bearing two ranks of persistent sporophyll petioles or adapted hapters..... A. *ochotensis*
3. Stipe present..... 4.
4. Stipe more than 15.4 cm long..... 5.
4. Stipe less than 15.4 cm long..... 6.
5. Sporophylls less than 2.8 cm wide..... A. *grandifolia*
5. Sporophylls more than 2.8 cm wide..... A. *tenuifolia*
6. Stipe less than 3.0 cm long..... A. *taeniata*
6. Stipe more than 3.0 cm long..... 7.
7. Sporophylls more than 2.8 cm wide..... 8.
7. Sporophylls less than 2.8 cm wide..... 10.
8. Stipe cylindrical, 0.3 cm or less wide..... A. *marginata*
8. Stipe sometimes flattened, 0.3 cm or more wide. 9.

9. Sporophylls less than 4.5 cm wide..... A. praelonga
9. Sporophylls more than 4.5 cm wide..... A. pylaii
10. Sporophylls tending to be fasciculate with  
more than one rank on each side..... 11.
10. Sporophylls one-ranked at point of  
attachment..... 12.
11. Sporophylls cylindrical in cross section close  
to the petiole very thick and narrow..... A. crispa
11. Sporophylls flattened in cross-section..... A. angusta
12. Rachis evenly tapering distally..... A. nana
12. Rachis not tapering although sometimes  
constricted at transition zone..... 13.
13. Two kinds of sporophylls; one, most common  
in summer, dark, thick, sterile; the other,  
more common in winter, lighter in colour,  
thin and fertile..... A. crassifolia
13. Sporophylls usually all one kind; if some are  
thickened, of unequal thickness in different  
parts..... A. esculenta.



Appendix II.

Semi-artificial and artificial culture media (Starr, 1956)

1. "Erdschreiber" Medium

To prepare 2 litre of this medium, 2 litres of sea water were filtered three times through a 12" glass column, packed with glass wool. This filtered sea water was then heated to 70 °C. and allowed to cool. To prepare a soil extract, 500 gms of garden soil were brought to the boil in one litre of tap water and simmered for 40 minutes. After allowing the soil to settle it was filtered through two layers of number 2 Whatman Filter Paper. 100cc of this extract was then brought to the boil and when just off the boil 0.020 gms.  $\text{NaNO}_3$  and 0.040 gms of  $\text{Na}_2\text{HPO}_4$  were added. Finally the soil extract, with added salts, was added to the cold filtered sea water and allowed to cool again, after which the medium was ready for use.

All glassware was heat sterilised and the prepared medium was stored in dark glass bottles at 10 °C.

2. Controlled enrichment medium.

For the preparation of this medium, vitamins, nitrate and organic phosphate were added to sterilised sea water in the following concentrations:

$\text{NaNO}_3$ .....	0.3 gm/l
Na Glycerophosphate.....	0.1 gm/l
Vitamin $\text{B}_{12}$ .....	0.00001 mg/l

Biotin..... 0.00001 mg/l

Thiamine..... 1.0 mg/l

3. Modified ASP medium (West, personal communication).

Stock solutions:

No. 1. NaCl

Weigh out 240 gms. and dissolve in 1 litre of double distilled (DD) water. Add 100 ml/l medium.

No. 2.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

Dissolve 40 gms in 200 ml DD water.

Add 20 ml/l medium.

No. 3. Basal salts

Dissolve 7 gms KCl, 2 gms CaCl and 3 gms  $\text{NaNO}_3$  separately in DD water and make up to 100 ml. Add 10 ml/l medium.

No. 4. Vitamin B<sub>12</sub>

Dissolve 0.1 mg in 100 ml DD water. Add 1 ml of this solution to 99 ml DD water. Add 1 ml of this final dilution to each litre of medium.

No. 5. Biotin

Same as for vitamin B<sub>12</sub>

No. 6. Thiamine

Dissolve 100 mg of thiamine in 100 ml DD water, and add 1 ml/l medium.

No. 7. Trace metals

Weigh out 5 gms NTA (nitrilo-triacetic acid) in 50 ml DD water

(Dilute NaOH).

- A. Dissolve 10 gms  $\text{FeCl}_3$  in 100 ml DD water.
- B. Dissolve 2.5 gms  $\text{ZnCl}_2$  in 100 ml DD water.
- C. Dissolve 10 gms  $\text{MnCl}_2$  in 100 ml DD water.
- D. Dissolve 100 mg  $\text{CuCl}$  in 100 ml DD water.
- E. Dissolve 1 gm  $\text{H}_3\text{BO}_4$  in 100 ml water.
- F. Dissolve 2.5 gms  $\text{Na}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$  in 100 ml DD water.
- G. Dissolve 100 mg  $\text{CoCl}_2$  in 100 ml DD water.

Add 1 ml of each to the NTA, except the  $\text{BO}_3$  - add 10 ml of this solution, and bring to 100 ml with DD water. Add 1-3 ml of this solution to each litre of medium. Next adjust the pH of the medium to 7.5 - 7.8 and add stock solutions 8 and 9.

No. 8. Hydroxy-methylamino-methane (Tris.)

Dissolve 10 gms of Tris. buffer to 100 ml DD water and add 10 ml/1 of medium.

No. 9. Sodium Glycerophosphate

Dissolve 1 gm in 100 ml DD water and add 10 ml/1 of medium.

### Appendix III

#### Fixing, mordanting, softening and staining procedures.

##### 1. Aceto-carminé squash technique for sorus material.

- A. Section sorus into approximately 1 mm x 5 mm pieces.
- B. Fix in 3:1 absolute ethanol:glacial acetic acid for 12 - 24 hours until completely discoloured.
- C. Wash thoroughly in distilled water. (Material can be stored for a short period at this stage in a refrigerator.)
- D. Mordant in  $\text{FeCl}_3$ /EDTA (ethylenediaminetetraacetic acid) solution for 24 hours.
- E. Wash thoroughly in distilled water.
- F. Place in 1 M Lithium Chloride for 15 minutes-2 hours.
- G. Place in distilled water for 6 hours.
- H. Squash in a drop of aceto-carminé.
- I. Heat gently and continue to irrigate slide with aceto-carminé. (Darlington & LaCour, 1962).
- J. Float off cover-glass in 95% ethanol.
- K. Pass cover-glass with attached material into 100% ethanol for 15 min..
- L. Mount cover-glass on a slide with Euparal.

##### 2. Aceto-carminé staining technique for gametophytes and microscopic sporophytes.

- A. Place slides or cover-glasses with attached material in

3:1 absolute ethanol:glacial acetic acid fixative for  
24 hours.

B. Wash well in distilled water.

C. Mordant in  $\text{FeCl}_3$ /EDTA solution for 24 hours.

D. Wash thoroughly in distilled water.

E. Stain in warm aceto-carmin.

F. Differentiate stain briefly in 45% acetic acid.

G. Dehydrate completely in 100% ethanol.

H. Mount with Euparal.

### 3. Acetic-iron-haematoxylin technique for sorus material.

(after Wittmann, 1962)

A. Fix in 3:1 fixative for 12 - 24 hours, until completely  
discoloured.

B. Mordant and macerate in the following solution for 10  
minutes:

Iodic acid..... 3 gms.

Aluminium alum..... 3 gms.

Chrome alum..... 3 gms.

95% ethanol..... 90 c.c.

Concentrated HCl..... 90 c.c.

C. Post fix in 3:1 fixative for 10 minutes.

D. Squash in acetic-iron-haematoxylin

Haematoxylin..... 2 gms.

Iron alum..... 0.5 gms.

45% acetic acid..... 50 c.c.

- E. Heat gently during squashing and continue to irrigate slide with stain.
- F. Differentiate stain briefly in a 2% solution of iron alum in 45% acetic acid.
- G. Float off cover-glass in 95% ethanol.
- H. Pass cover-glass with attached material into 100% ethanol for 15 minutes.
- I. Mount on a slide with Euparal.

## Appendix IV

### Pretreatment procedures.

#### 1. Colchicine in modified ASP culture medium.

Cultures were established in the following concentrations of colchicine in culture medium: 2%, 1%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, 0.05% and 0.01%. Material was fixed from all concentrations after 5 minutes, 10 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 24 hours and 48 hours.

#### 2. Paradichlorobenzene in modified ASP medium.

Two cultures were established in modified ASP culture medium. In one culture the medium was 100% saturated with paradichlorobenzene, and in the other it was 50% saturated. Material was fixed from both cultures after 5 minutes, 10 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 24 hours and 48 hours.

#### 3. Bacto-phytohemagglutinin M in modified ASP medium.

Cultures were established in 1%, 0.5%, 0.25%, 0.1%, 0.05%, and 0.01% Bacto-phytohemagglutinin M, and material was fixed from each culture after 5 minutes, 10 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 24 hours and 48 hours.

#### 4. Bacto-phytohemagglutinin M in a 0.1% solution of colchicine in modified ASP culture medium.

The concentrations of Bacto-phytohemagglutinin M were 1.0%, 0.5%, 0.25%, 0.1%, 0.05% and 0.01%. Fixations were again made after 5 minutes, 10 minutes, 30 minutes, 1 hour, 2 hours, 4 hours,

8 hours, 12 hours, 24 hours and 48 hours.

All four pretreatments were applied to established gametophyte cultures of Alaria marginata (10 days old) and actively germinating zoospores of Alaria nana (48 hours old).



Appendix V

Tables I - VIII

Table I

MEMBERS OF THE LAMINARIALES IN WHICH THE LIFE CYCLE HAS BEEN  
INVESTIGATED

<u>SPECIES</u>	<u>AUTHOR</u>
<b>CHORDACEAE</b>	
<u>Chorda filum</u>	Kylin, 1918
	Kanda, 1938
	Evans, 1965
<u>Chorda tomentosa</u>	Sundene, 1963
<u>Chorda</u> sp.	Williams, 1921
<b>LAMINARIACEAE</b>	
<u>Laminaria angustata</u>	Kanda, 1941a
	Yabu, 1964a
<u>Laminaria angustata</u> var, <u>longissima</u>	Yabu, 1964a
<u>Laminaria yendoana</u>	Kanda, 1938
<u>Laminaria cichorioides</u>	Kanda, 1938
<u>Laminaria digitata</u>	Drew, 1910
	Kylin, 1916
	Schreiber, 1930
	Harries, 1932
	Walker, 1954
	Naylor, 1956
	Sundene, 1958, 1962
	Evans, 1965

Laminaria flexicaulis

Sauvageau, 1916a, 1916b

Laminaria ochroleuca

Naylor, 1956

Evans, 1965

Laminaria saccharina

Drew, 1910

Sauvageau, 1916a, 1916b

Kuckuck, 1917

Pascher, 1918

Schreiber, 1930

Harries, 1932

Naylor, 1956

Evans, 1965

Laminaria hyperborea

Schreiber, 1930

Evans, 1965

Laminaria cloustoni

Harries, 1932

Laminaria japonica

Kanda, 1936

Yabu, 1964a

Laminaria sinclairii

Myers, 1925

Laminaria diabolica

Yabu, 1964a

Laminaria religios

Yabu, 1964a

Laminaria ochotensis

Yabu, 1964a

Laminaria yezoensis

Kanda, 1938

Laminaria pallida

Papenfuss, 1942

Laminaria sp.

Yendo, 1911

Williams, 1921

Sacchoriza polyschides

Evans, 1965

Sacchoriza bulbosa

Thuret, 1850

Sauvageau, 1915a, 1915b

Pleurophycus gardneri

Angst, 1929

Agarum eribrosum

Kanda, 1941a

Costaria costata

Angst, 1927

Kanda, 1936

Costaria turneri

Yendo, 1911

Kjellmaniella crassifolia

Kanda, 1938

Arthrothamnus bifidus

Kanda, 1936

Yabu, 1964a

#### LESSONIACEAE

Nereocystis luetkeana

Hartge, 1928

Kemp & Cole, 1961

Postelsia palmaeformis

Myers, 1925

Macrocystis integrifolia

Walker, 1952

Cole, 1959, 1967

Macrocystis pyrifera

Brandt, 1923

Delf & Levyn, 1926

Levyns, 1933

Papenfuss, 1942

Neushul, 1963

Pelagophycus porra

Herbst & Johnstone, 1937

ALARIACEAE

Alaria crassifolia

Kanda, 1936

Yabu, 1964

Alaria esculenta

Sauvageau, 1916b, 1916c

Evans, 1965

Pterygophora californica

McKay, 1933

Undaria undarioides

Segi & Kida, 1957, 1958

Undaria pinnatifida

Yendo, 1911

Kanda, 1936

Undaria pinnatifida f. distans

Yabu, 1964

Undaria peterseniana

Kanda, 1941a

Ecklonia cava

Kanda, 1941b

Ecklonia stolonifera

Kanda, 1941b

Ecklonia maxima

Papenfuss, 1942

Eckloniopsis radicata

Kanda, 1941b

Eisenia bicyclis

Kanda, 1941b

Eisenia arborea

Clare & Herbst, 1938

Hollenberg, 1939

Egregia menziesii

Myers, 1928

Table II  
Chromosome numbers in the Laminariales

SPECIES	DIVISION		STAIN			METHOD		N	2N	AUTHOR	
	M	M	F	A	H	S	S				
	E	I	E	C	A	Q	E				
	I	T	U	E	E	U	C				
	O	O	L	T	M	A	T				
	S	S	G	O	O	S	I				
	I	I	E	C	T	H	O				
	S	S	N	A	O		N				
				R	X						
				M	Y						
				I	L						
				N	I						
				E	N						
<u>Chorda filum</u>	x	x			x		x	20	40	Kylin, 1918	
	x				x		x	30		Nishibayashi & Inoh, 1961a, 1961b.	
	x				x		x	c.30		Ohmori, 1967	
		x		x		x		c.30		Evans, 1963a	
<u>Laminaria angustata</u>	x	x		x		x		c.28	c.56	Evans, 1965	
	x				x		x	22		Nishibayashi & Inoh, 1956	
	x				x		x	c.22		Ohmori, 1967	
<u>Laminaria angustata</u> var.											
<u>longissima</u>	x				x		x	30		Yabu, 1965	
<u>Laminaria digitata</u>		x	x				x		16	Walker, 1954	
		x	x	x			x	27-31		Naylor, 1956	
		x		x			x	31		Evans, 1963a	
	x	x		x			x	31	62	Evans, 1965	
<u>Laminaria flexicaulis</u>	x	x	x				x	13		Magne, 1953	
<u>Laminaria ochroleuca</u>		x	x	x			x	27-31		Naylor, 1956	
		x		x			x	31		Evans, 1963a	

Table II/Continued

SPECIES	DIVISION		STAIN		METHOD		N	2N	AUTHOR
<u>Laminaria ochroleuca</u>	x	x	x		x		31	62	Evans, 1965
<u>Laminaria saccharina</u>	x	x	x			x	13		Magne, 1953
		x	x		x		27-31		Naylor, 1956
		x			x		31		Evans, 1963a
<u>Laminaria hyperborea</u>	x	x	x		x		31	62	Evans, 1965
		x	x		x		31		Evans, 1963a
	x	x	x		x		31	62	Evans, 1965
<u>Laminaria cloustoni</u>		x	x		x		11	22	Walker (unpublished)*
<u>Laminaria japonica</u>	x			x		x	c.22		Abe, 1939
<u>Laminaria diabolica</u>	x			x		x	22		Yabu, 1958
<u>Laminaria longipedalis</u>	x			x		x	28-32		Ohmori, 1967
<u>Laminaria yendoana</u>	x			x		x	27-31		Ohmori, 1967
<u>Sacchoriza polyschides</u>		x	x		x		c.30		Evans, 1963a
	x	x	x		x		31	62	Evans, 1965
<u>Agarum cribrosum</u>	x			x		x	22		Yabu, 1964b
<u>Costaria costata</u>	x			x		x	c.30		Nishibayashi & Inoh, 1957
	x			x		x	27-31		Ohmori, 1967
<u>Kjellmaniella gyrata</u>	x			x		x	22		Yabu, 1965
<u>Arthrothamnus bifidus</u>	x			x		x	22		Yabu & Tokida, 1963
<u>Nereocystis luetkeana</u>	x	x	x		x	x	31	58-62	Kemp & Cole, 1961
<u>Macrocystis integrifolia</u>		x	x		x		16	32	Walker, 1952
		x	x		x		14-16	28-32	Cole, 1967
<u>Alaria crassifolia</u>	x			x		x	22		Yabu, 1957
<u>Alaria praelonga</u>	x			x		x	22		Yabu, 1964b
<u>Alaria esculenta</u>		x	x		x		c.30		Evans, 1963a
	x	x	x		x		28	56	Evans, 1965
<u>Pterygophora californica</u>									
	x	x		x		x	13	26	McKay, 1933
<u>Undaria undarioides</u>	x			x		x	29		Nishibayashi & Inoh, 1960a
	x			x		x	c.30		Ohmori, 1967

Table II/ continued

SPECIES	DIVISION		STAIN	METHOD	N	2N	AUTHOR
<u>Undaria pinnatifida</u>	x		x	x	22		Inoh & Nishibayashi, 1954
	x		x	x	c.30		Inoh & Nishibayashi, 1955, 1960a
	x		x	x	c.30		Ohmori, 1967
<u>Ecklonia cava</u>	x		x	x	c.30		Ohmori, 1965
	x		x	x	29-32		Ohmori, 1967
<u>Ecklonia stolonifera</u>	x		x	x	c.34		Ohmori, 1965
	x		x	x	30-32		Ohmori, 1967
<u>Eisenia arborea</u>	x	x	x	x	15	30	Hollenberg, 1939
<u>Eisenia bicyclis</u>	x		x	x	27+		Ohmori, 1967
<u>Egregia menziesii</u>	x	x	x	x	8	16	Myers, 1928

\* in Walker, 1952



Table III

Frequency of occurrence of Male and Female gametophytes in cultures established from uniform spore suspensions. (All cultures approximately one month old at time of counting.)

	Number of Male Gametophytes	Number of Female Gametophytes
<u>A. marginata</u>		
Culture 1	262	238
Culture 2	257	243
Culture 3	281	219
Total	800	700
PERCENTAGE	53.3%	46.7%
<u>A. nana</u>		
Culture 1	241	259
Culture 2	268	232
Culture 3	251	249
Total	760	740
PERCENTAGE	50.7%	49.3%
<u>A. tenuifolia</u>		
Culture 1	270	230
Culture 2	244	256
Culture 3	262	238
Total	776	724
PERCENTAGE	51.7%	48.3%
<u>A. fistulosa</u>		
Culture 1	251	249
Culture 2	263	237
Culture 3	254	246
Total	768	732
PERCENTAGE	51.2%	48.8%
<u>A. taeniata</u>		
Culture 1	228	272
Culture 2	248	252
Culture 3	260	240
Total	736	764
PERCENTAGE	49.1%	50.9%

Table III/Continued

	Number of Male Gametophytes	Number of Female Gametophytes
<u>A. grandifolia</u>		
Culture 1	288	212
Culture 2	246	254
Culture 3	267	233
Total	801	699
PERCENTAGE	53.4%	46.6%

Table IV

Bivalent counts made during meiosis at diplotene, diakinesis or metaphase I.

SPECIES	COUNTS
<u>Alaria marginata</u>	13, 13, 14(Figs. 87 & 88), 14 (Figs 89 & 90), 14(Figs. 91, 92 & 93), 14(Figs.97 & 98), 14, 14, 14, 14, 14, 14, 14, 15, 15, 16(Figs. 94, 95 & 96), 16.
<u>Alaria nana</u>	12(Figs.102, 103 & 104), 12(Figs. 108, 109 & 110) 12, 13, 14(Figs. 105 106 & 107), 14, 14, 14, 15, 16.
<u>Alaria tenuifolia</u>	12(Figs. 118, 119 & 120), 12, 13 (Figs. 111 & 112), 13(Figs. 121 & 122), 13, 13, 13, 14(Figs. 113 & 114), 14, 14, 14, 14, 14, 14, 14, 14, 15, 15, 16, 16, 18, 20, 21 (Figs. 115, 116 & 117).
<u>Alaria fistulosa</u>	12(Figs. 123, 124 & 125), 12(Figs. 126 & 127), 13(Figs. 128 & 129), 13(Figs. 130 & 131), 13(Figs. 132, 133 & 134), 13, 14, 14, 14, 14, 14, 14, 15, 16.
<u>Alaria taeniata</u>	14(Figs. 135 & 136), 14(Figs. 137 & 138).
<u>Alaria grandifolia</u>	17(Figs. 155, 156, 157 & 158), 20, 24(Figs. 144, 145, & 146), 24(Figs. 147, 148, 149, 150, 151 & 152), 24, 24, 24, 25(Figs. 139 & 140), 25(Figs. 153 & 154), 25, 26(Figs. 141, 142 & 143), 26.

Table V

Haploid chromosome counts made at prometaphase of mitosis in male and female gametophytes.

SPECIES	COUNTS
<u>Alaria marginata</u>	13 (Figs. 165 & 166), 13, 13, 14 (Figs. 159, 160 & 161), 14 (Figs. 167 & 168), 14, 14, 14, 14, 15, 15, 15, 15, 16 (Figs. 162, 163 & 164), 16.
<u>Alaria nana</u>	12, 14 (Figs. 174, 175 & 176), 14, 14, 14, 14, 14, 15 (Figs. 169 & 170), 15 (Figs. 171, 172 & 173), 17.
<u>Alaria tenuifolia</u>	12, 13 (Figs. 179 & 180), 13 (Figs. 181 & 182), 13, 13, 14, 14, 14, 14, 14, 14, 15 (Figs. 177 & 178), 15, 16, 17.
<u>Alaria taeniata</u>	12 (Figs. 187, 188, 189, 190 & 191) 13, 14, 14, 14, 15 (Figs. 183, 184, 185 & 186), 18.
<u>Alaria fistulosa</u>	12, 12, 13, 13, 14 (Figs. 192, 193, 194 & 195), 14 (Figs. 196, 197 & 198), 14, 14, 14, 14, 14, 15, 16, 17.

Table VI

Diploid chromosome counts made at prometaphase of mitosis in very young sporophytes.

SPECIES	COUNTS
<u>Alaria marginata</u>	26 (Figs. 210, 211, 212 & 213), 26, 26, 28 (Figs. 204, 205, 206, 207, 208 & 209), 28, 28, 28, 28, 28, 29.
<u>Alaria nana</u>	23 (Figs. 220, 221, 222 & 223), 26, 26, 28 (Figs. 214, 215, 216, 217, 218 & 219), 28, 28, 28, 28, 28, 30.
<u>Alaria tenuifolia</u>	24 (Figs. 224, 225, 226 & 227), 24 (Figs. 228, 229, 230 & 231), 26, 28, 28, 28, 28, 28, 30, 31.
<u>Alaria taeniata</u>	24 (Figs. 238, 239, 240 & 241), 24 (Figs. 238, 239, 240 & 241), 24, 26 (Figs. 232, 233, 234, 235, 236 & 237), 26, 26, 28, 28, 28, 28, 28, 28, 28, 29, 30, 31.
<u>Alaria fistulosa</u>	23 (Figs. 242, 243, 244, 245, & 246), 25, 28, 28, 28, 30, 31.

Table VII

Chromosome numbers of six species of the genus, Alaria.

Species	M	G M	S M
	e	a i	p i
	i	m t	o t
	o	e o	r o
	s	t s	o s
	i	o i	p i
	s	p s	h s
		h	y
		y	t
		t	e
		e	
<u>A. marginata</u>	c.14	c. 14	c. 28
<u>A. nana</u>	c.14	c. 14	c. 28
<u>A. tenuifolia</u>	c.14	c. 14	c. 28
<u>A. fistulosa</u>	c.14	c. 14	c. 28
<u>A. taeniata</u>	C.14*	c. 14	c. 28
<u>A. grandifolia</u>	c.24	-	-

\* - Based on two observations only.

Table VIII

Voucher specimens of Alaria used in investigation.

<u>Species</u>	<u>Location</u>	<u>Herbarium and No.</u>
<u>Alaria marginata</u>	Wiffin Spit, Sooke, Vancouver Island	UBC 29468 UBC 29469
	Glacier Point Vancouver Island	UBC 12534
	Jordan River Vancouver Island	UBC 29831
	Beaver Point Saltspring Island	UBC 29832
	Indian Beach, Ecola State Park, Oregon	UBC 21619 UBC 24912
	Short Sands Beach, Oregon.	UBC 24656 UBC 24911
	Volga Island Alaska.	UBC 25151 UBC 27946 UBC 25139 UBC 12165
	Glacier Point, Vancouver Island	UBC 13060 UBC 12370 UBC 12633
	Pescadero Point, San Mateo Co., California	UBC 24568
	Wigham Island, Alaska.	UBC 20952
<u>Alaria tenuifolia</u>	Goose Island, Alaska.	UBC 20788 UBC 20785
	Brockton Point, Vancouver.	UBC 11513

<u>Species</u>	<u>Location</u>	<u>Herbarium and No.</u>
<u>Alaria fistulosa</u>	Cape Muzon, Alaska	UBC 20799
	Port Conclusion.	UBC 20709
	Alaska	UBC 20588
<u>Alaria taeniata</u>	Wigham Island,	UBC 21007
	Alaska.	
	Volga Island,	UBC 23076
	Alaska.	UBC 23078
<u>Alaria grandifolia</u>	Cape Spencer, Cross	
	Road, Alaska.	UBC 20783
	Aats Bay, Coronation	
	Island, Alaska.	UBC 27948
		UBC 25146
		UBC 23677

UBC : Phycological Herbarium of the University of British  
Columbia.



**Appendix VI**

**Plates 1 - 36**

Plate 1

Figure 1: Habit drawing of Alaria marginata Postels  
et Ruprecht.

x 0.5

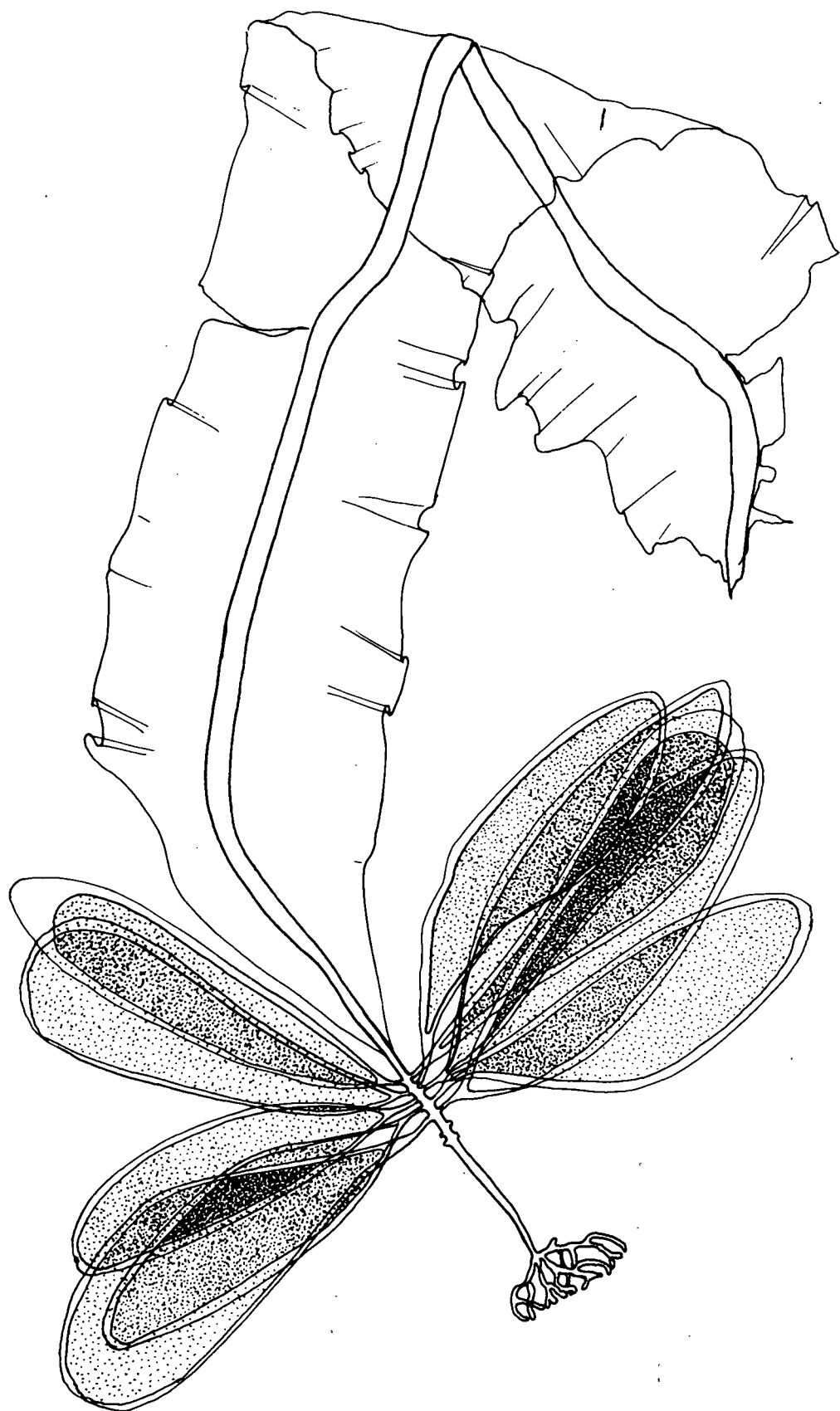


Plate 2

Figure 2: Habit drawing of Alaria nana Schrader.

Actual size.

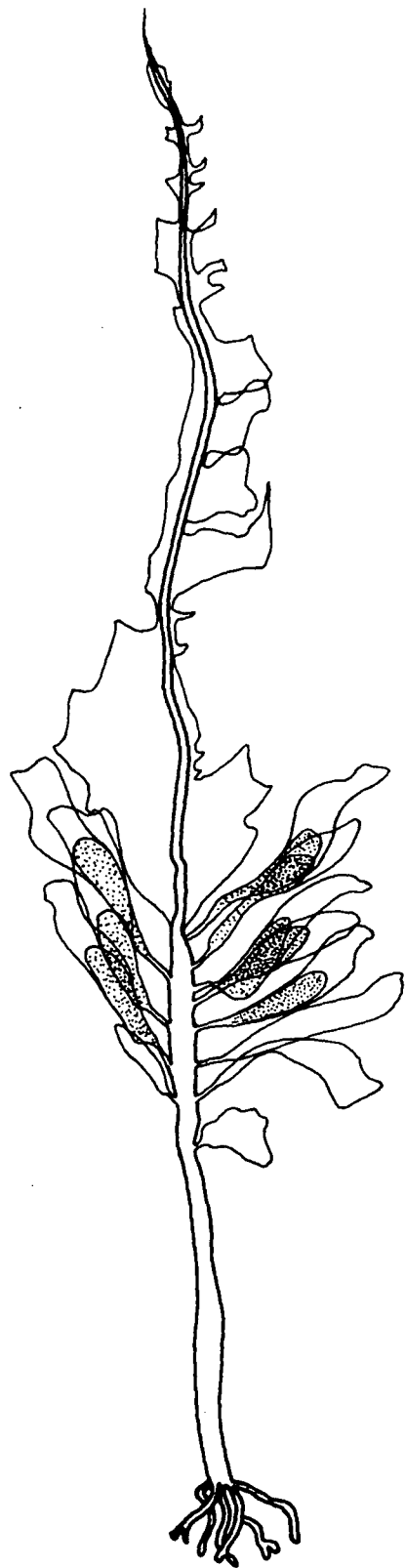


Plate 3

Figure 3: Habit drawing of Alaria tenuifolia Setchell.

x 0.5

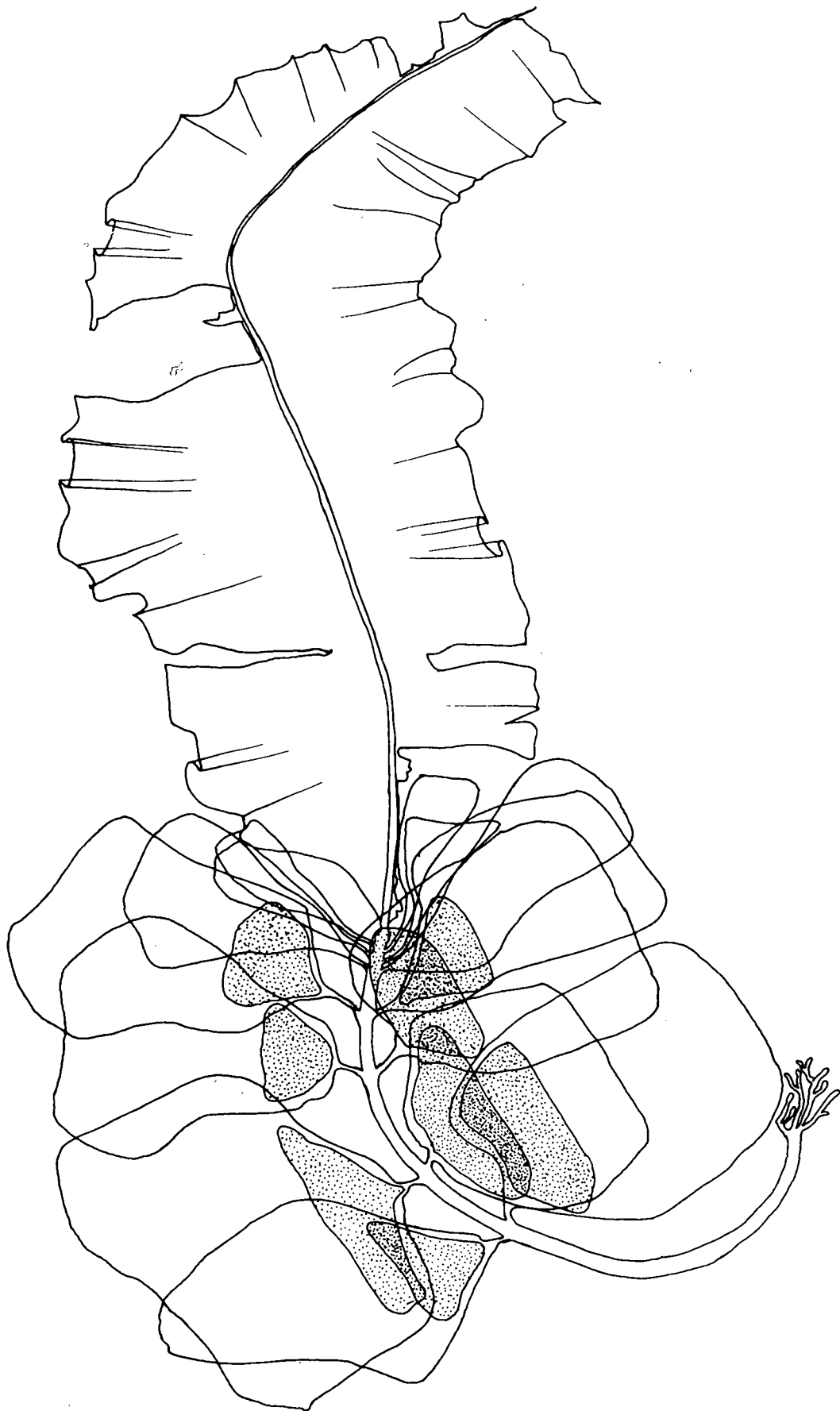


Plate 4

Figure 4: Habit drawing of Alaria fistulosa Postels et  
Ruprecht.

x 0.5



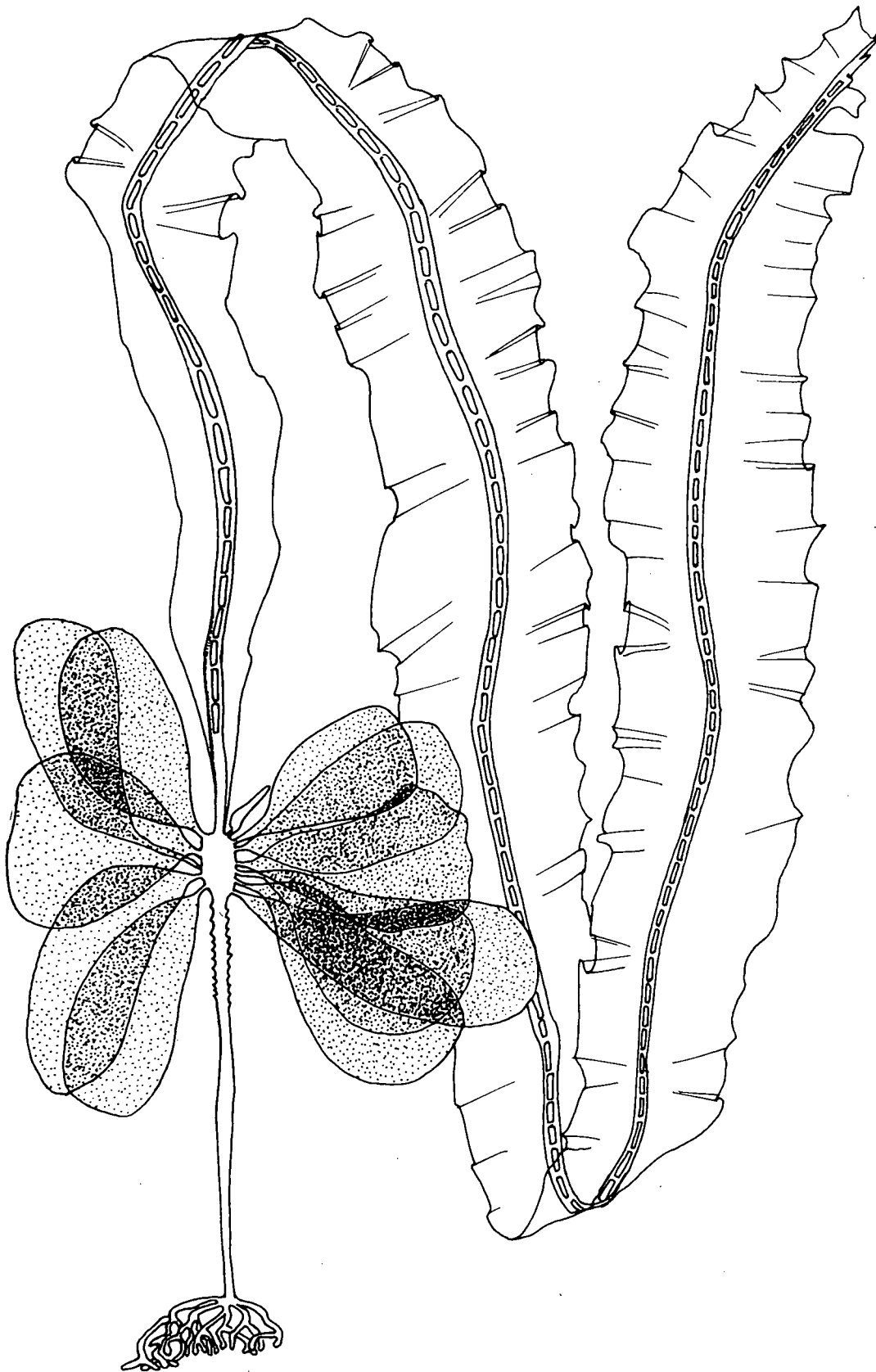


Plate 5

Figure 5: Habit drawing of Alaria taeniata Kjellman.

x 0.75

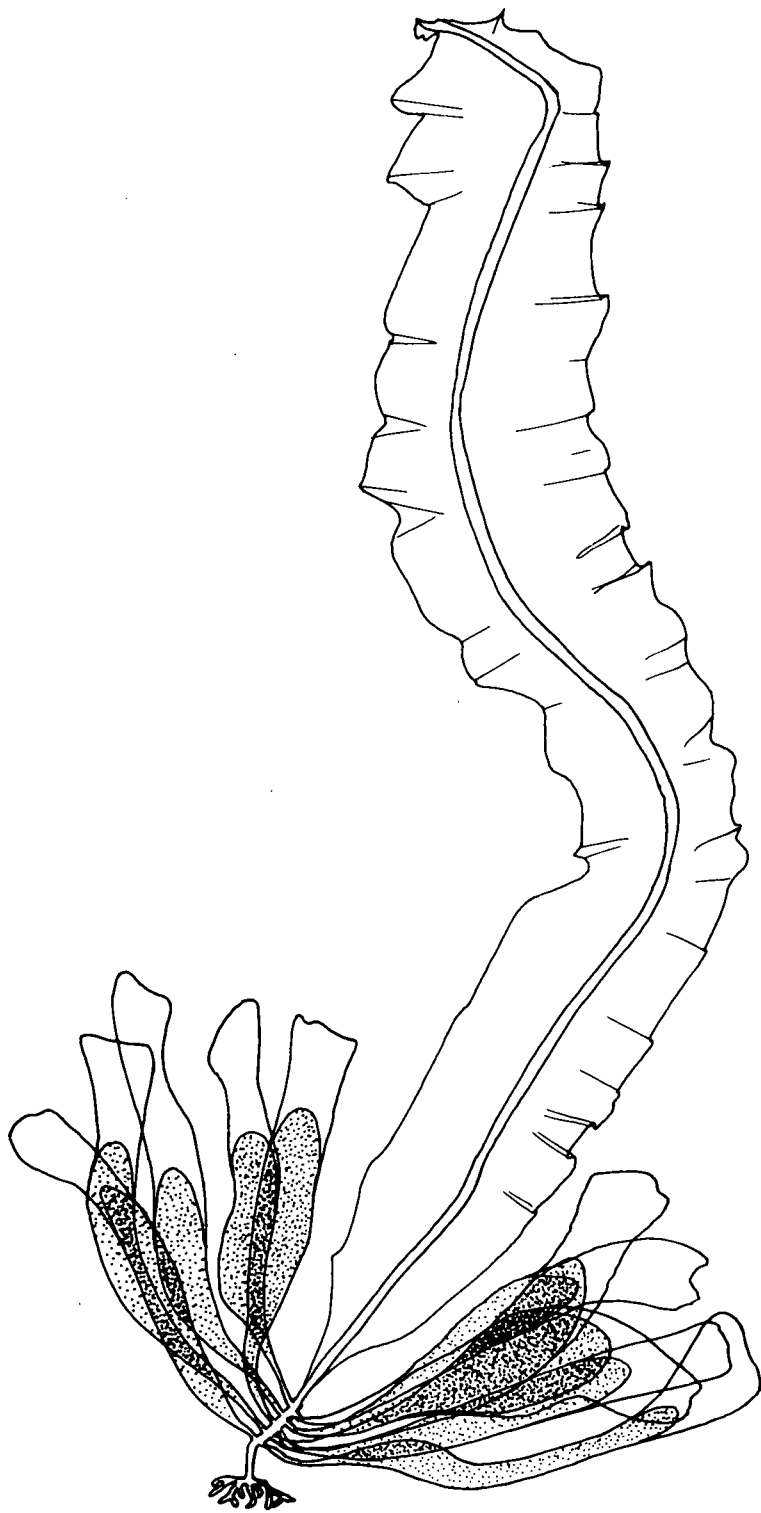


Plate 6

Figure 6: Habit drawing of Alaria grandifolia  
J. Agardh.

x 0.75



Plate 7

Figure 7: Collecting stations on the Pacific coast of North America from which specimens of Alaria were obtained.

Scale: 1:12,500,000

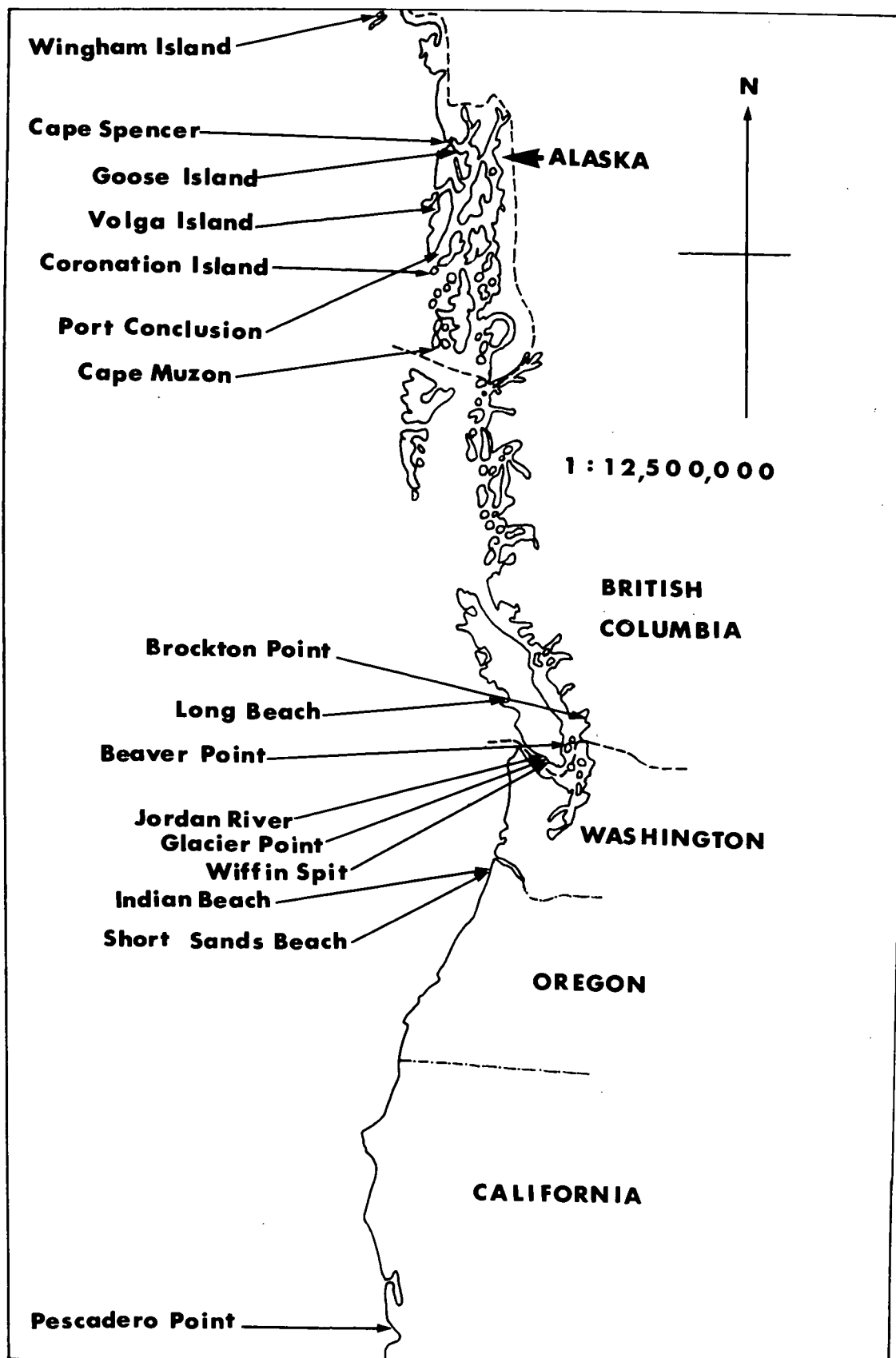


Plate 8

Figure 8: Interphase nucleus of an immature sporangium of A. marginata. x 4,000

Figure 9: Diagrammatic representation of Fig. 8.

Figure 10: Leptotene of meiosis in an immature zoosporangium of A. marginata. x 4,000

Figure 11: Diagrammatic representation of Fig. 10.

Figure 12: Zygotene of meiosis in an immature zoosporangium of Alaria marginata. x 4,000

Figure 13: Diagrammatic representation of Fig. 12.

Figure 14: Zygotene of meiosis in an immature zoosporangium of A. marginata. x 4,000

Figure 15: Diagrammatic representation of Fig. 14.

Figure 16: Pachytene chromosomes in an immature zoosporangium of A. marginata. x 4,000

Figure 17: Diagrammatic representation of Fig. 16.

Figure 18: Pachytene chromosomes in an immature zoosporangium of A. nana. x 4,000

Figure 19: Diagrammatic representation of Fig. 18.

Legend: Nc - Nucleolus  
Pc - Pairing chromomeres  
R - Beaded thread of reticulum.



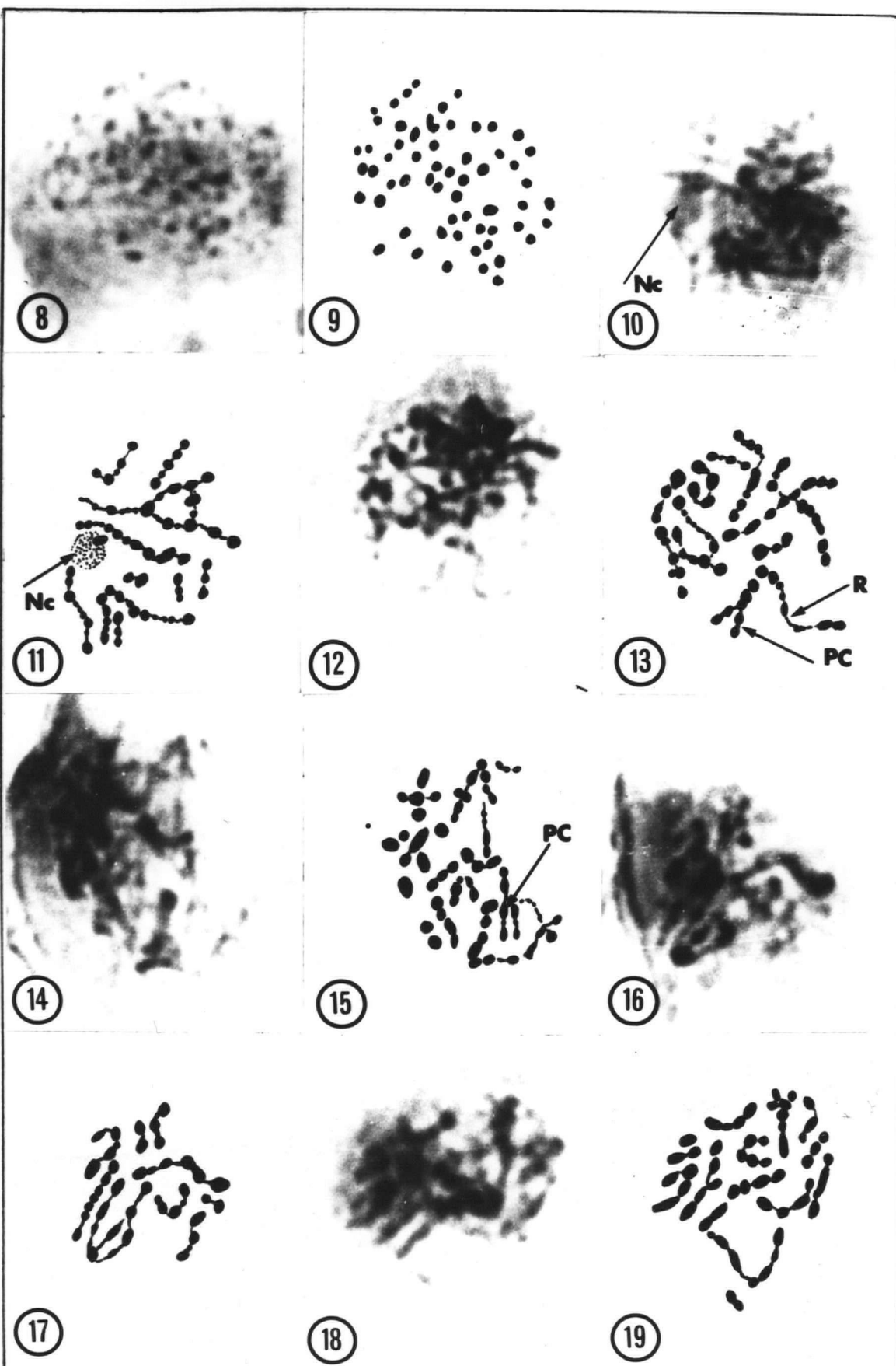


Plate 9

- Figure 20: Pachytene chromosomes in an immature zoosporangium of A. tenuifolia. x 4,000
- Figure 21: Diagrammatic representation of Fig. 20.
- Figure 22: Pachytene chromosomes in an immature zoosporangium of A. fistulosa. x 4,000
- Figure 23: Diagrammatic representation of Fig. 22.
- Figure 24: Pachytene chromosomes in an immature zoosporangium of A. grandifolia. x 4,000
- Figure 25: Diagrammatic representation of Fig. 24.
- Figure 26: Diplotene chromosomes in an immature zoosporangium of A. grandifolia. x 4,000
- Figure 27: Diagrammatic representation of Fig. 26.
- Figure 28: Diakinesis chromosomes in an immature zoosporangium of A. nana. x 4,000
- Figure 29: Diagrammatic representation of Fig. 28.
- Figure 30: Metaphase I equatorial plate showing 13 bivalents in A. fistulosa. x 4,000
- Figure 31: Diagrammatic representation of Fig. 30.

Legend: Nc - Nucleolus  
C - Centromere

z

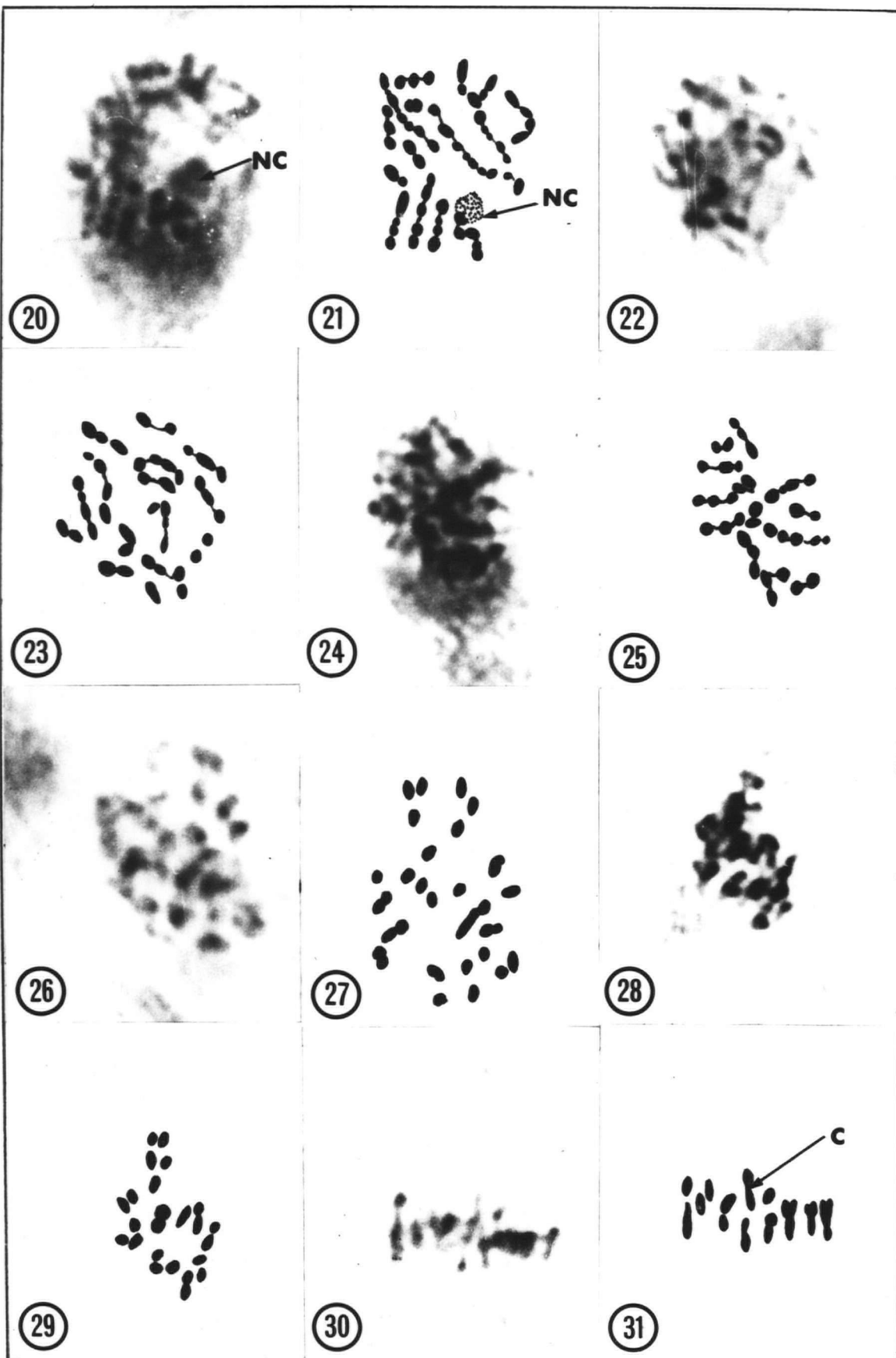


Plate 10

- Figure 32: Prophase II in a young zoosporangium of A. nana. x 3,000
- Figure 33: Metaphase II in a young zoosporangium of A. tenuifolia. x 3,000
- Figure 34: Anaphase II in a young zoosporangium of A. nana. x 2,300
- Figure 35: Prophase of four-nucleate sporangium of A. nana. x 2,500
- Figure 36: Prophase in eight-nucleate sporangium of A. nana. x 2,000
- Figure 37: Mitotic metaphase plates in eight-nucleate sporangium of A. nana (four metaphase plates shown). x 2,000
- Figure 38: Late prophase configurations in eight-nucleate sporangium, showing distinct chromosomes. A. grandifolia. x 3,000
- Figure 39: Late prophase configurations in eight-nucleate sporangium of A. marginata, showing distinct chromosomes. x 3,000
- Figure 40: Sixteen-nucleate sporangium of A. marginata. x 2,500
- Figure 41: Thirty-two-nucleate sporangium of A. nana. x 2,000



Plate 11

- Figure 42: Biflagellate motile zoospore of A. marginata.  
x 1,000
- Figure 43: Rounded-up, non-motile zoospore of A. marginata.  
x 1,000
- Figure 44: Initial stage of germination of zoospore of  
A. marginata. x 1,000
- Figure 45: Production of germination tube, and migration  
of nucleus into the tube in A. marginata.  
x 1,000
- Figure 46: The formation of the initial cell of the  
gametophyte of A. taeniata. x 1,000
- Figure 47: Second division of the spore nucleus in A.  
marginata. x 1,000
- Figure 48: Two-cell gametophyte of A. marginata. x 1,000
- Figure 49: Female gametophytes of A. marginata. Some cells  
have produced protuberances typical to oogonia.  
x 1,000

Legend: SC - Spore case  
GT - Germination tube  
CY = cytoplasm  
V - Central vacuole  
N - Nucleus  
OP - Oogonial protuberance

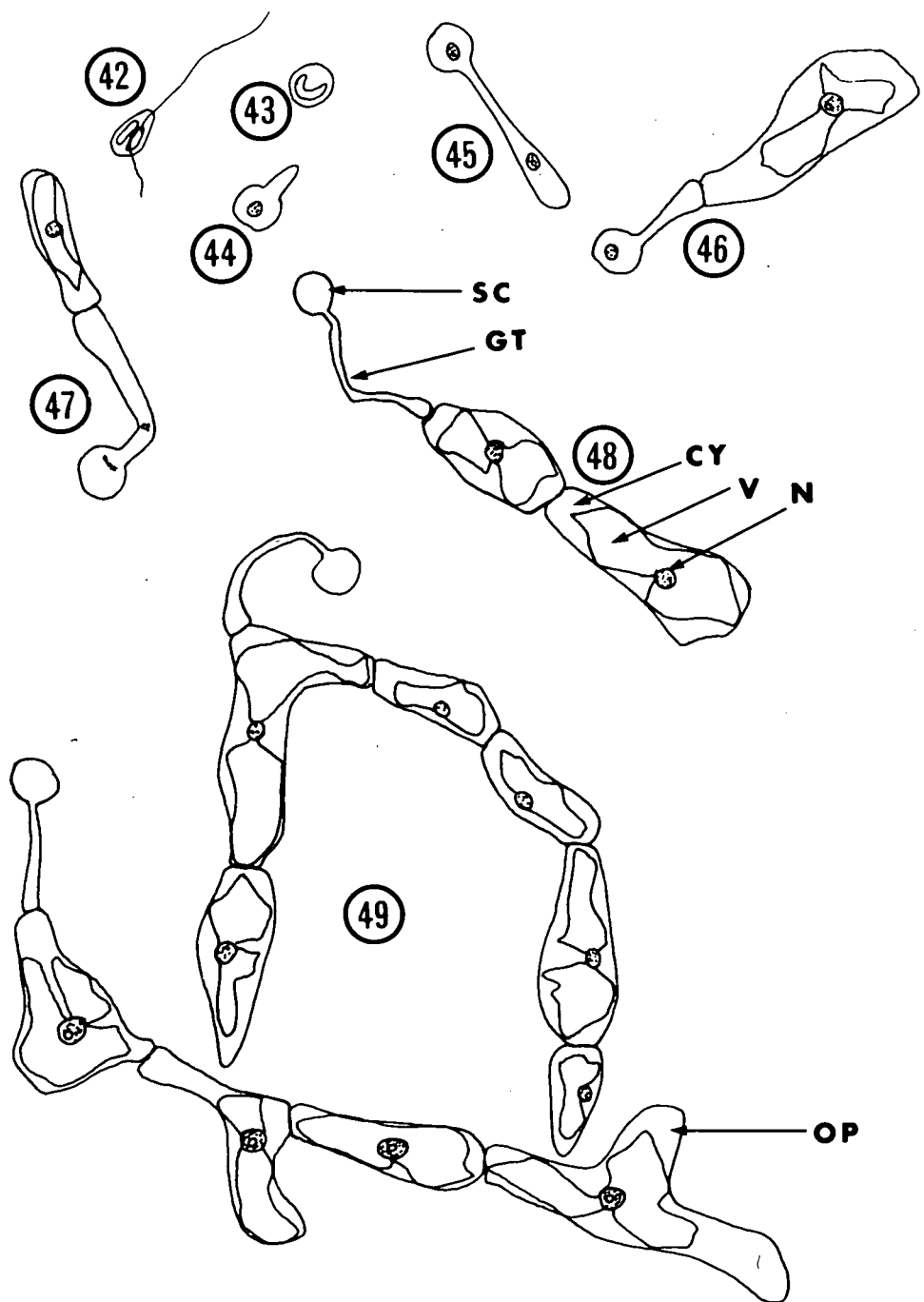


Plate 12

Figure 50: Male gametophyte of A. marginata. x 1,000

Figure 51: Male gametophyte of A. marginata, bearing  
intercalary antheridia. x 1,000

Figure 52: Male gametophyte of A. taeniata, bearing  
intercalary stalked clusters of antheridia.  
x 1,000

Figure 53: Biflagellate antherozoids of A. marginata.  
x 1,000

Legend: A - Antheridia



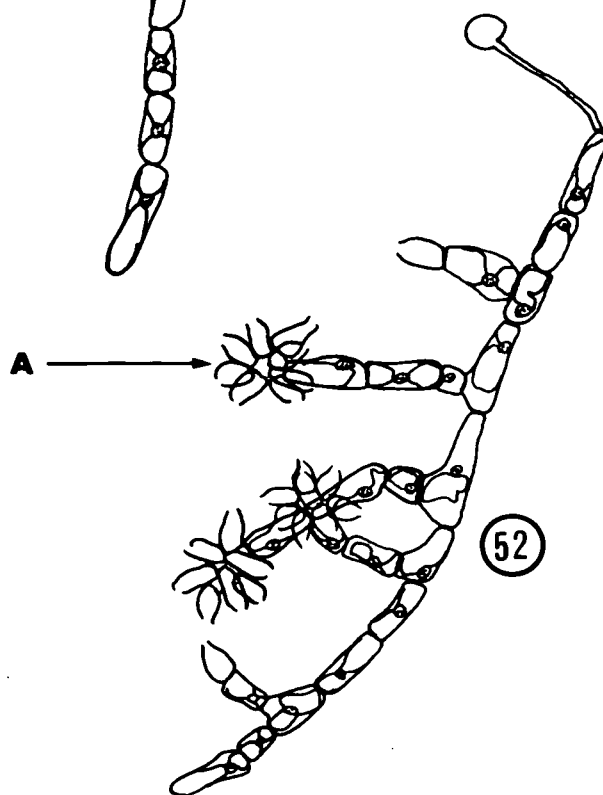
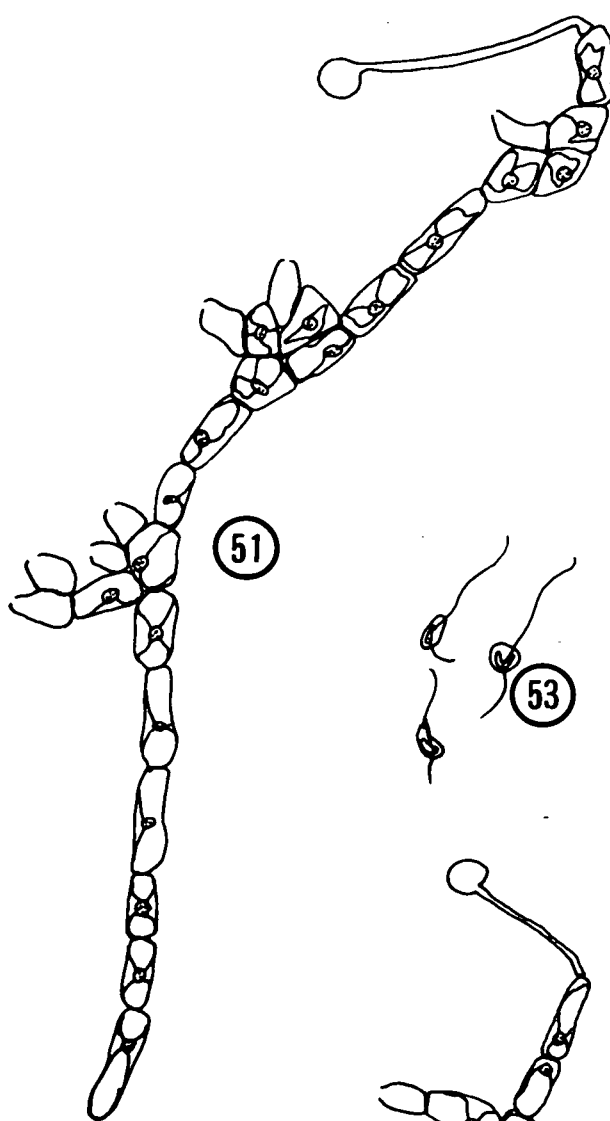
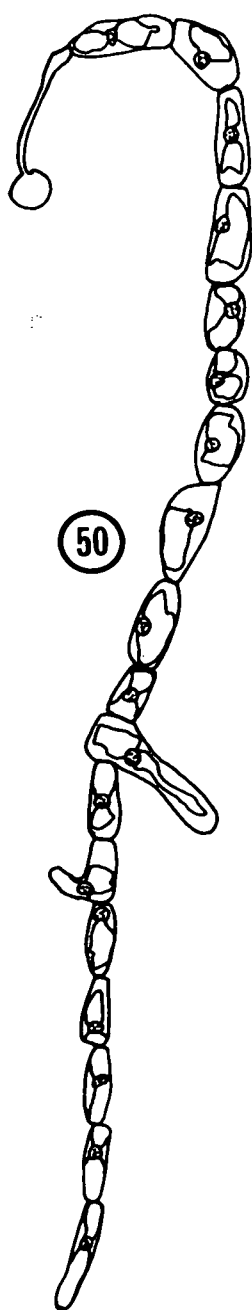


Plate 13

- Figure 54: Oogonium of A. marginata. Cytoplasm has drawn away from the cell wall. x 3,000
- Figure 55: Mitotic division prior to the formation of the egg in an oogonium of A. marginata. x 3,000
- Figure 56: Extrusion of the egg from the oogonium in A. marginata. x 3,000
- Figure 57: Extruded egg cell of A. taeniata, showing "fertilisation pore" and "fertilisation tube". x 3,000
- Figure 58: Extruded egg of A. marginata showing attachment of antherozoid. x 3,000
- Figure 59: Extruded egg of A. taeniata showing the point of attachment of the antherozoid. x 3,000

Legend: FT - "Fertilisation tube"  
FP - "Fertilisation pore"  
ON - Oogonial "neck"  
AP - Antherozoid papilla

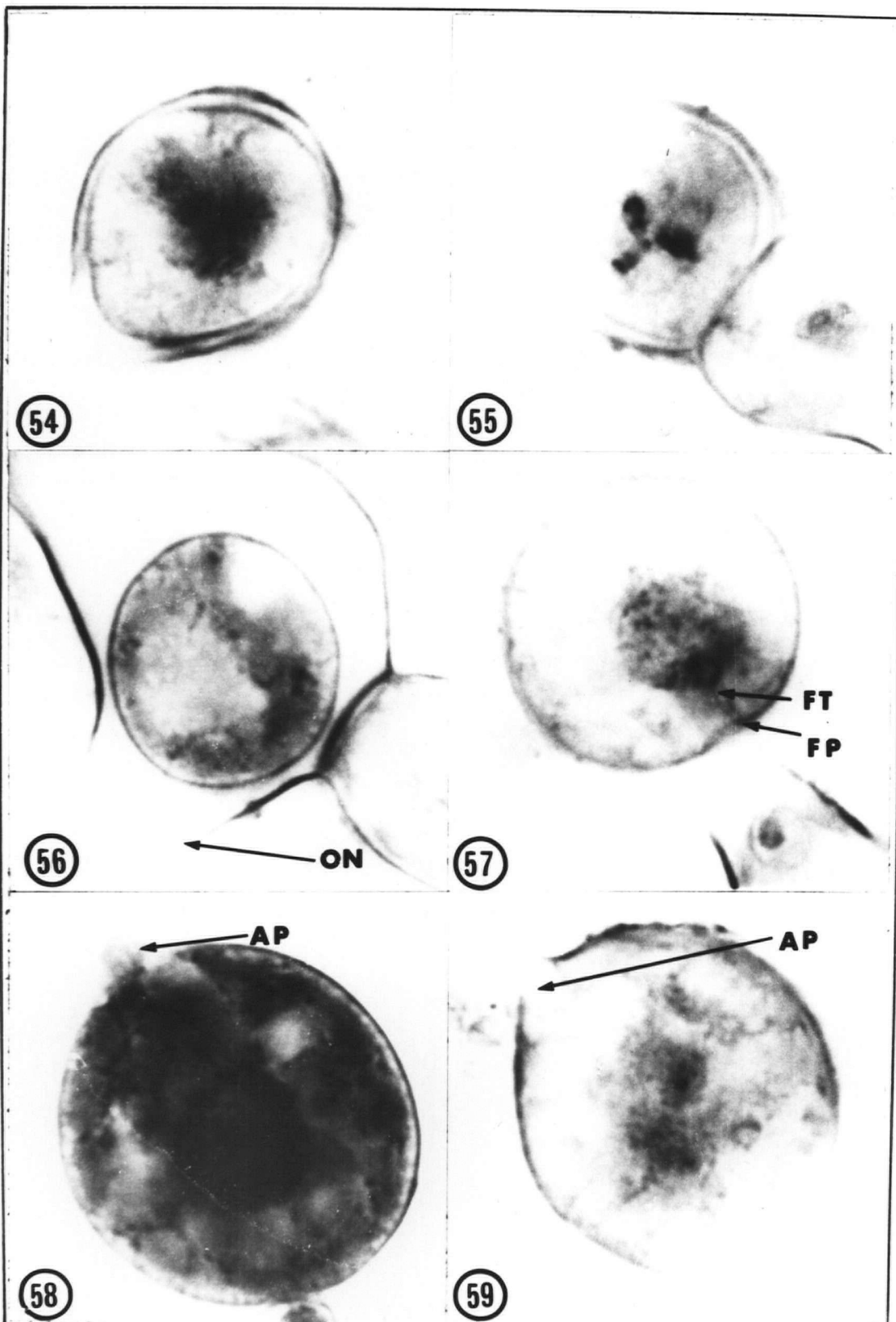


Plate 14

Figure 60: Binucleate egg cell after plasmogamy in A. marginata. x 3,000

Figure 61: Karyogamy in A. marginata. x 3,000

Figure 62: Elongation of the zygote of A. marginata prior to its germination. x 3,000

Figure 63: Elongation of the zygote of A. taeniata prior to its germination. x 3,000

Figure 64: Elongation of the zygote in A. nana. x 3,000

Figure 65: Two-celled sporophyte of A. nana. x 3,000

Legend: N - Nucleus  
Nc - Nucleolus

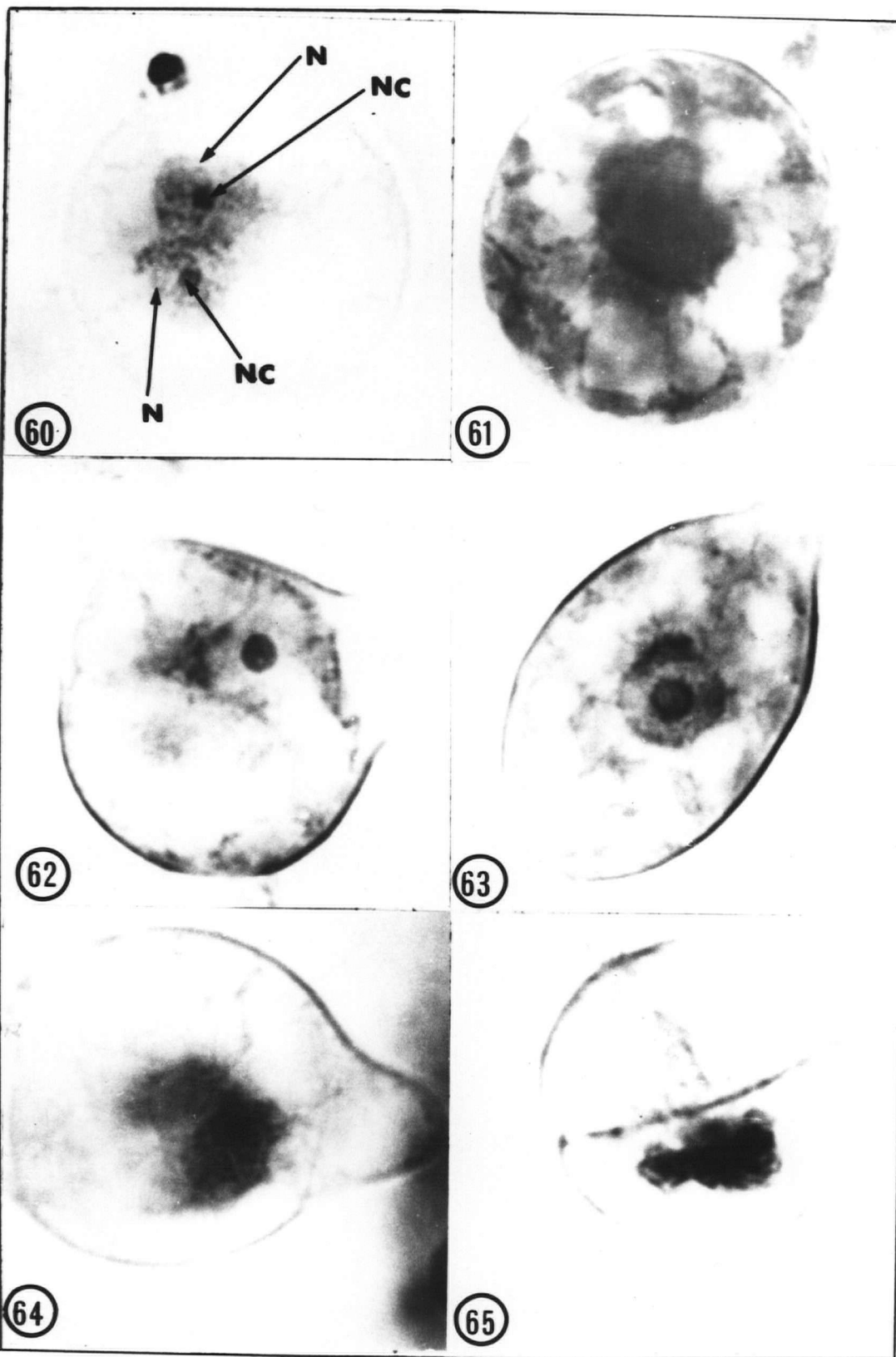


Plate 15

- Figure 66: Four-celled sporophyte of A. marginata.  
x 1,000
- Figure 67: Formation of a biseriate filament at the three-celled stage in A. tenuifolia. x 1,000
- Figure 68: Formation of a biseriate sporophyte filament at the four-celled stage in A. marginata.  
x 1,000
- Figure 69: Formation of a parthenosporophyte within an oogonium of A. taeniata. x 1,000
- Figure 70: Nine-celled uniseriate sporophyte of A. marginata showing the formation of a rhizoidal basal cell.  
x 1,000
- Figure 71: Formation of a biseriate young sporophyte at ten cell stage in A. nana. x 1,000

Legend: N - Nucleus

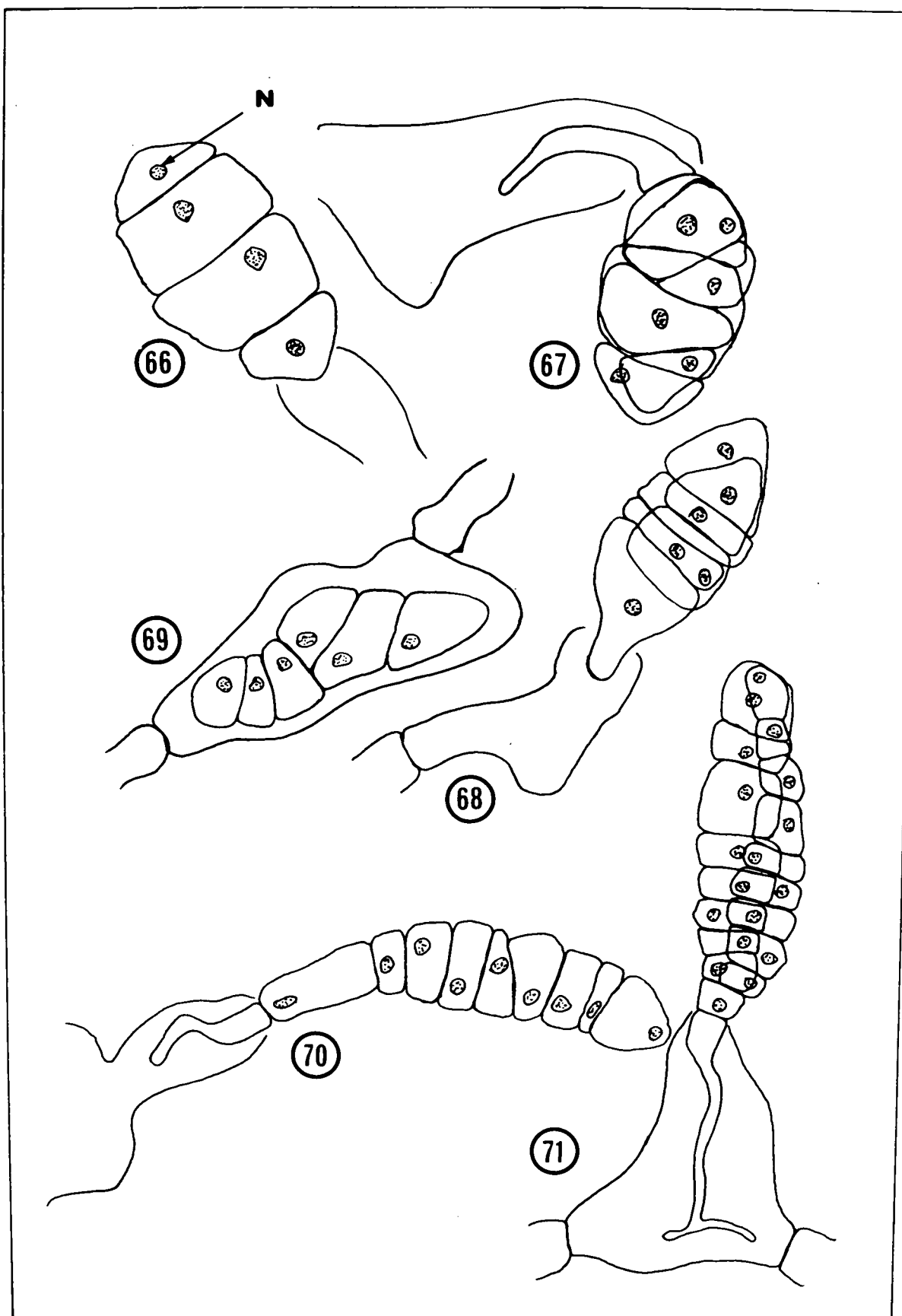


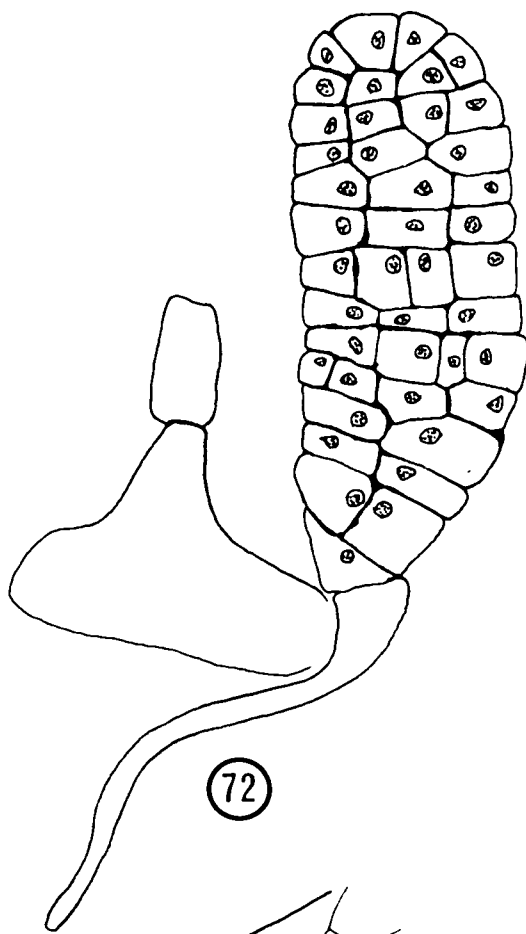
Plate 16

Figure 72: Young sporophyte of A. marginata still attached to empty oogonium. x 1,000

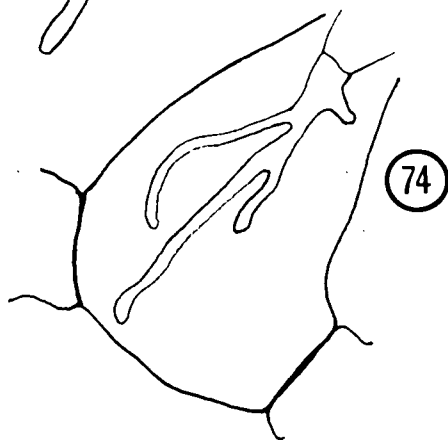
Figure 73: Young sporophyte of A. tenuifolia. x 1,000

Figure 74: Branched rhizoidal basal cell of young sporophyte. x 1,000

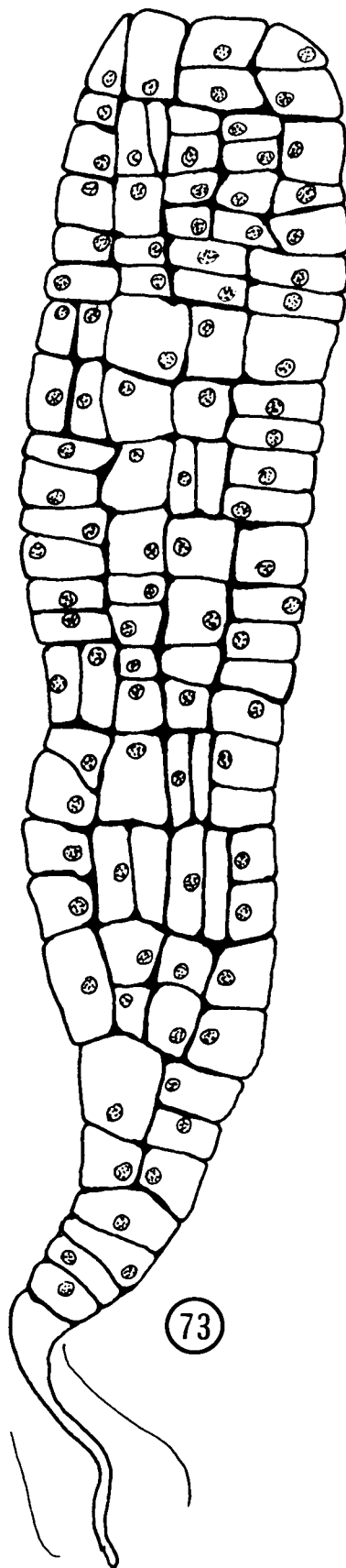




72



74



73

Plate 17

- Figure 75: Granular interphase nucleus in zygote of A. taeniata. x 3,000
- Figure 76: Reticular early prophase stage of mitosis in female gametophyte of A. marginata. x 3,000
- Figure 77: Mid-prophase of mitosis in female gametophyte of A. marginata showing the condensation of distinct chromosomes. x 3,000
- Figure 78: Mid-prophase of mitosis in female gametophyte of A. tenuifolia showing pairs of chromatids. x 4,000
- Figure 79: Diagrammatic representation of Fig. 78.
- Figure 80: Late prophase of mitosis in male gametophyte of A. fistulosa. x 4,000
- Figure 81: Late prophase of mitosis in female gametophyte of A. fistulosa showing distinct chromosomes. x 4,000
- Figure 82: Three progressive focal views of late prophase
- Figure 83: of mitosis in female gametophyte of A. taeniata.
- Figure 84: Centromeres can be distinguished at this stage. x 4,000
- Figure 85: Metaphase plate of chromosomes in mitosis of male gametophyte of A. marginata. x 3,000
- Figure 86: Mitotic anaphase in germination tube of A. nana. x 3,000

Legend:    N - Nucleus  
            Nc - Nucleolus  
            HG - Heterochromatic granules  
            CD - Chromatids  
            CM - Centromere

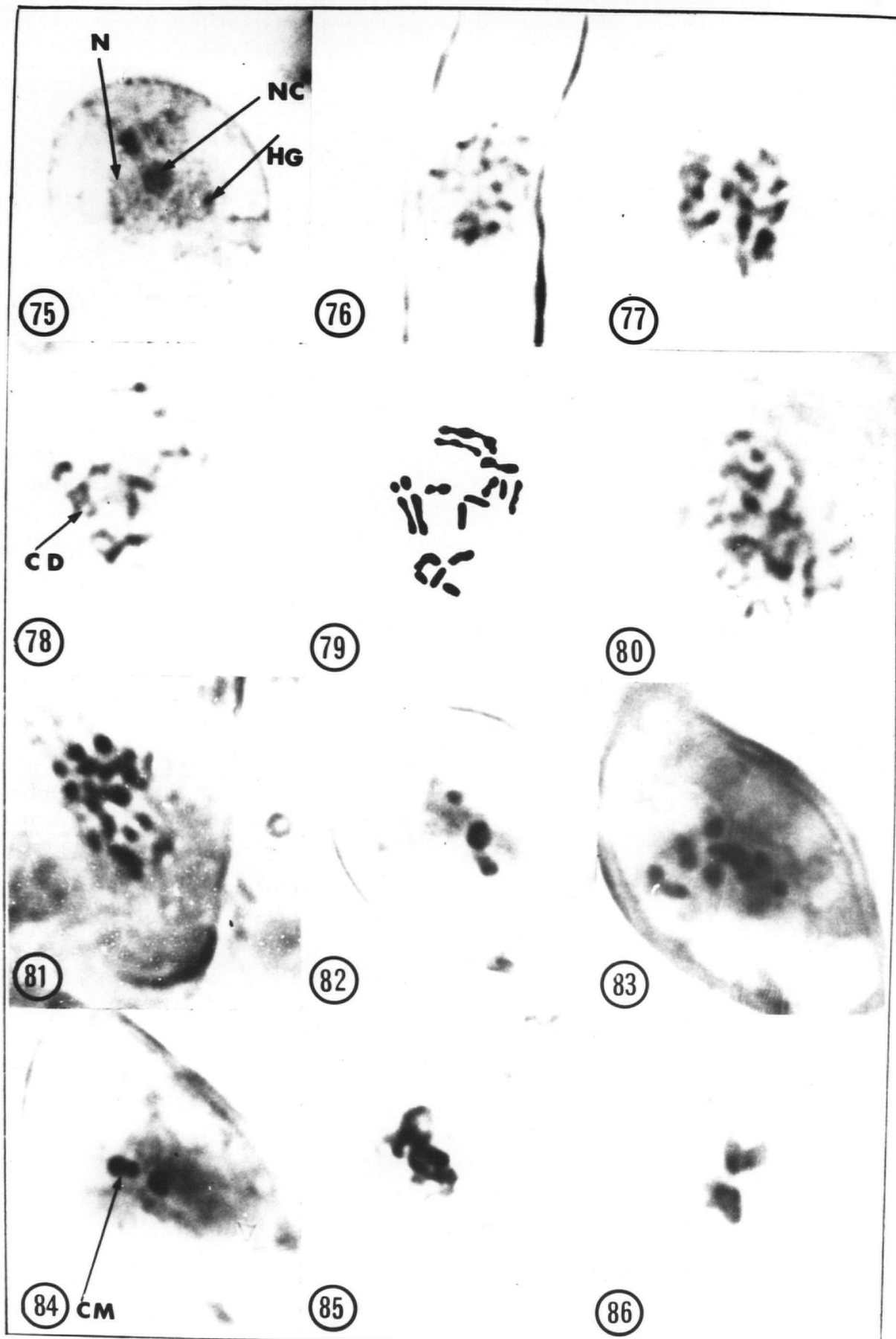


Plate 18

- Figure 87: Diplotene configuration of bivalents in an immature zoosporangium of A. marginata. 14 bivalents can be counted. x 4,000
- Figure 88: Diagrammatic representation of Fig. 87.
- Figure 89: Prometaphase stage of meiosis in A. marginata in which 14 bivalents can be counted. x 4,000
- Figure 90: Diagrammatic representation of Fig. 89.
- Figure 91: Progressive focal views of polar aspect of
- Figure 92: metaphase I in an immature zoosporangium of A. marginata. 14 bivalents. x 4,000
- Figure 93: Diagrammatic representation of Figs. 91 & 92.
- Figure 94: Progressive focal views of diplotene/diakinesis
- Figure 95: configuration in an immature zoosporangium of A. marginata. 16 bivalents. x 4,000
- Figure 96: Diagrammatic representation of Figs 94 & 95.
- Figure 97: Metaphase I plate in an immature zoosporangium of A. marginata, showing 14 bivalents. x 4,000
- Figure 98: Diagrammatic representation of Fig. 97.

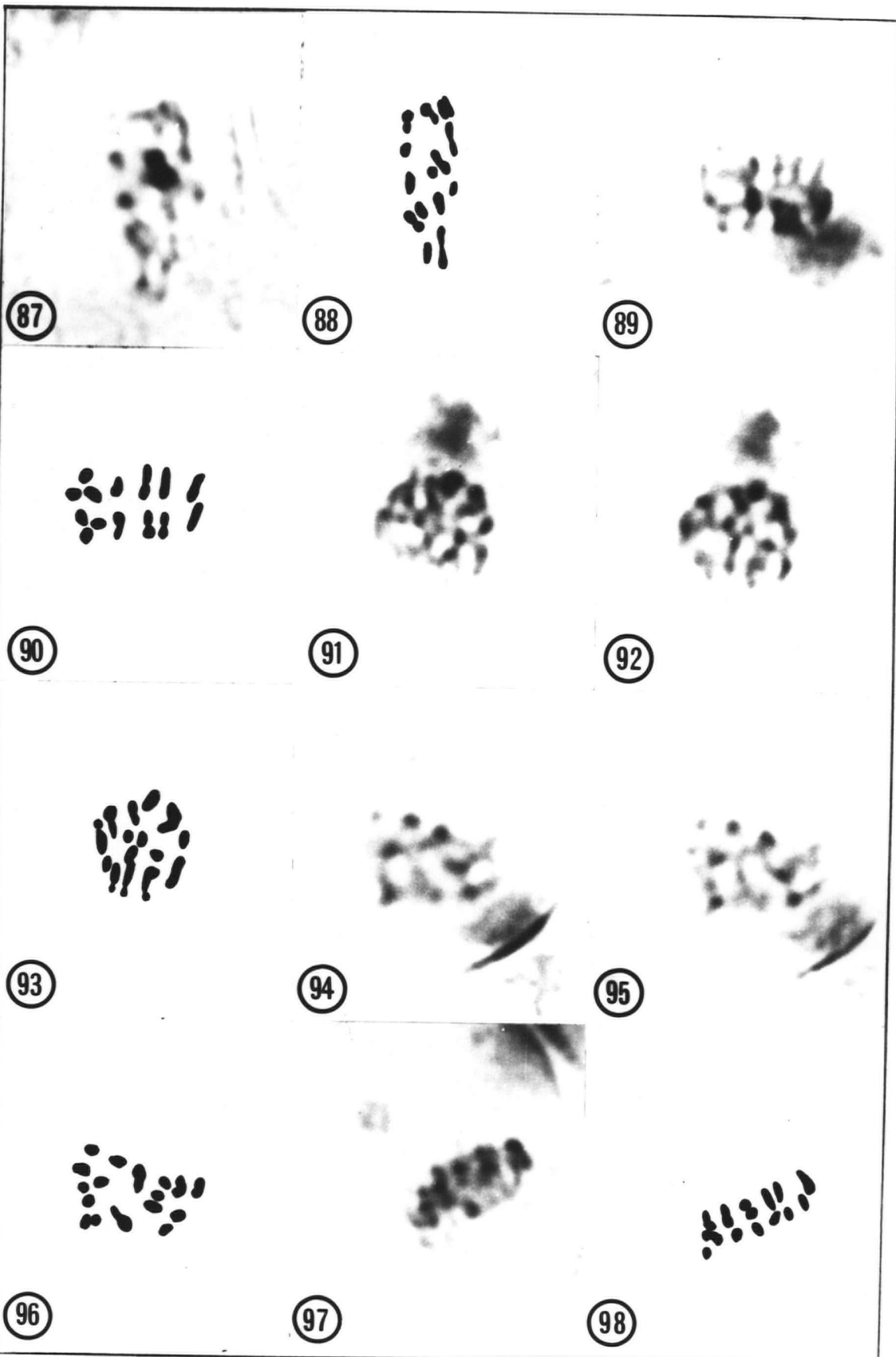


Plate 19

- Figure 99: Progressive focal views of a polar aspect of  
Figure 100: metaphase I in an immature zoosporangium of  
A. nana. 15 bivalents. x 4,000  
Figure 101: Diagrammatic representation of Figs. 99 & 100.
- Figure 102: Diplotene configuration in immature zoospor-  
Figure 103: angium of A. nana, showing 12 bivalents.  
x 4,000  
Figure 104: Diagrammatic representation of Figs. 102 & 103.
- Figure 105: Progressive focal views of prometaphase I  
Figure 106: in an immature zoosporangium of A. nana.  
12 bivalents. x 4,000  
Figure 107: Diagrammatic representation of Figs. 105 & 106.
- Figure 108: Progressive focal views of prometaphase I  
Figure 109: in an immature zoosporangium of A. nana.  
12 bivalents. x 4,000  
Figure 110: Diagrammatic representation of Figs. 108 & 109.

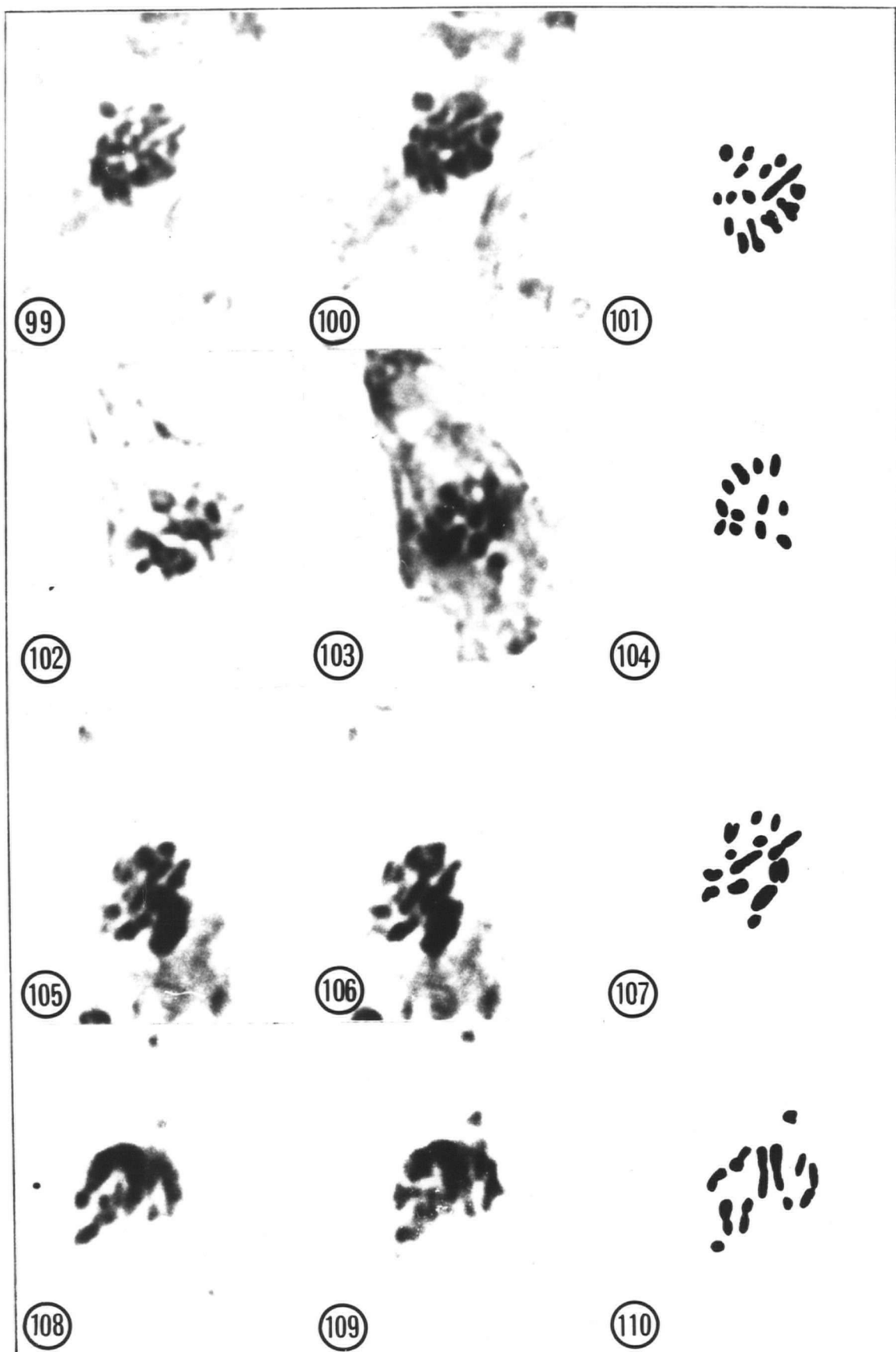


Plate 20

- Figure 111: Metaphase plate in meiosis I in an immature zoosporangium of A. tenuifolia. 13 bivalents. x 4,000
- Figure 112: Diagrammatic representation of Fig. 111.
- Figure 113: Metaphase I configuration in an immature zoosporangium of A. tenuifolia. 14 bivalents. x 4,000
- Figure 114: Diagrammatic representation of Fig. 113.
- Figure 115: Asynaptic stage of late prophase I in an immature zoosporangium of A. tenuifolia, at which 21 chromosomes can be counted. x 4,000
- Figure 116: Diagrammatic representation of Figs. 115 & 116.
- Figure 118: Progressive focal views of metaphase I configuration in an immature zoosporangium of A. tenuifolia. 12 bivalents. x 4,000
- Figure 119: Diagrammatic representation of Figs. 118 & 119.
- Figure 120: Diagrammatic representation of Figs. 118 & 119.
- Figure 121: Side view of metaphase I plate of bivalents in an immature zoosporangium of A. tenuifolia. 13 bivalents. x 4,000
- Figure 122: Diagrammatic representation of Fig. 121.



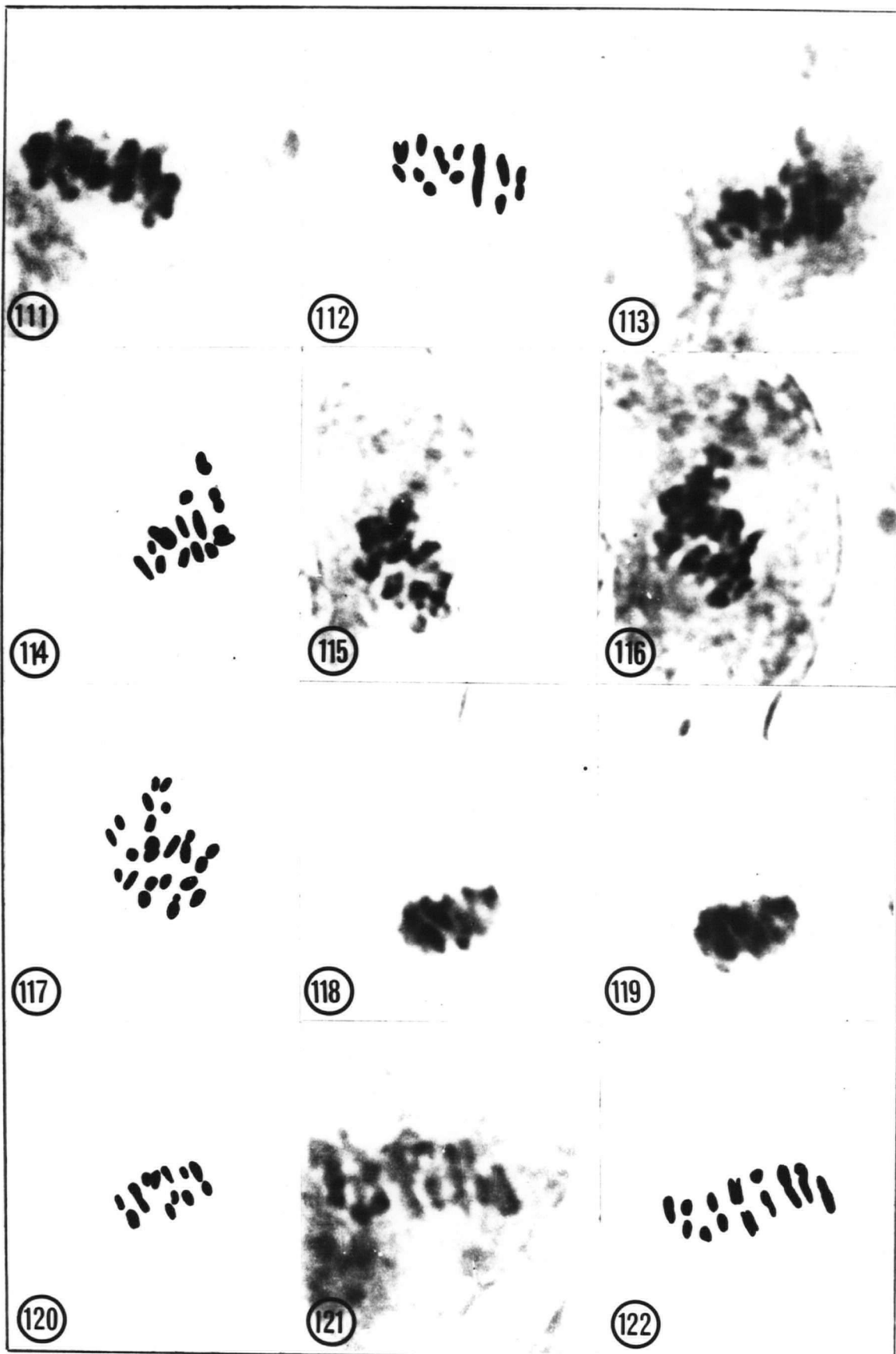


Plate 21

- Figure 123: Progressive focal views of metaphase I in  
Figure 124: an immature zoosporangium of A. fistulosa.  
12 bivalents. x 4,000  
Figure 125: Diagrammatic representation of Figs. 123 & 124.
- Figure 126: Metaphase I plate of bivalents in an immature  
zoosporangium of A. fistulosa. 12 bivalents.  
x 4,000  
Figure 127: Diagrammatic representation of Fig. 126.
- Figure 128: Metaphase I plate of bivalents in an immature  
zoosporangium of A. fistulosa. 13 bivalents.  
x 4,000  
Figure 129: Diagrammatic representation of Fig. 128.
- Figure 130: Metaphase I plate of bivalents in an immature  
zoosporangium of A. fistulosa. 13 bivalents.  
x 4,000  
Figure 131: Diagrammatic representation of Fig. 130.
- Figure 132: Progressive focal views of a metaphase I  
Figure 133: plate of bivalents in an immature zoosporangium  
of A. fistulosa. 13 bivalents. x 4,000  
Figure 134: Diagrammatic representation of Figs. 132 & 133.

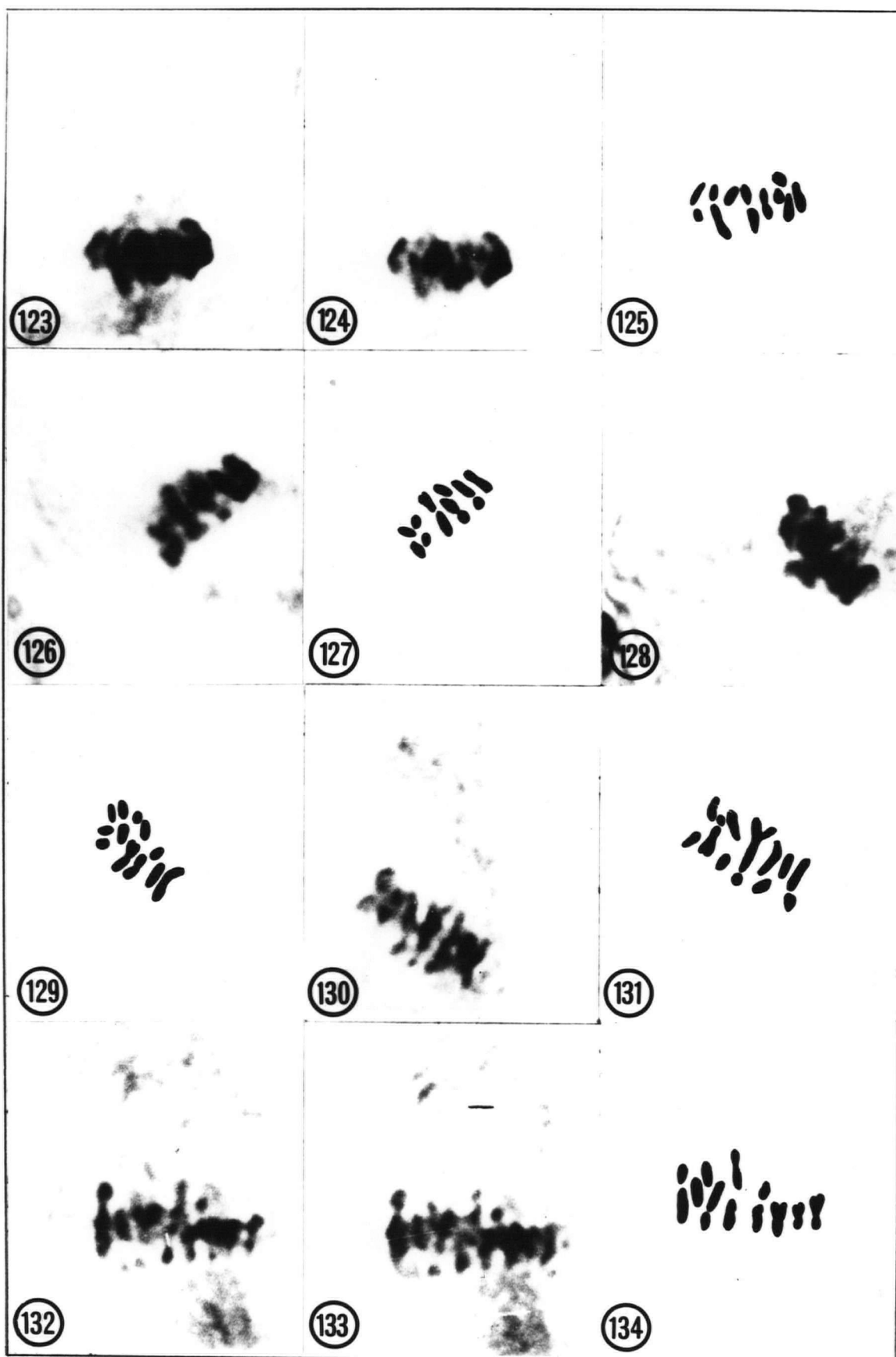


Plate 22

- Figure 135: Diplotene configuration in an immature zoosporangium of A. taeniata. 14 bivalents.  
x 4,000
- Figure 136: Diagrammatic representation of Fig. 135.
- Figure 137: Diakinesis configuration in an immature zoosporangium of A. taeniata. 14 bivalents.  
x 4,000
- Figure 138: Diagrammatic representation of Fig. 137.
- Figure 139: Diakinesis configuration in an immature zoosporangium of A. grandifolia. 25 bivalents.  
x 4,000
- Figure 140: Diagrammatic representation of Fig. 139.
- Figure 141: Progressive focal views of diplotene of meiosis  
Figure 142: in an immature zoosporangium of A. grandifolia.  
26 bivalents. x 4,000
- Figure 143: Diagrammatic representation of Figs. 141 & 142.
- Figure 144: Progressive focal views of a diplotene configuration in an immature zoosporangium of  
Figure 145: A. grandifolia. 24 bivalents. x 4,000
- Figure 146: Diagrammatic representation of Figs. 144 & 145.

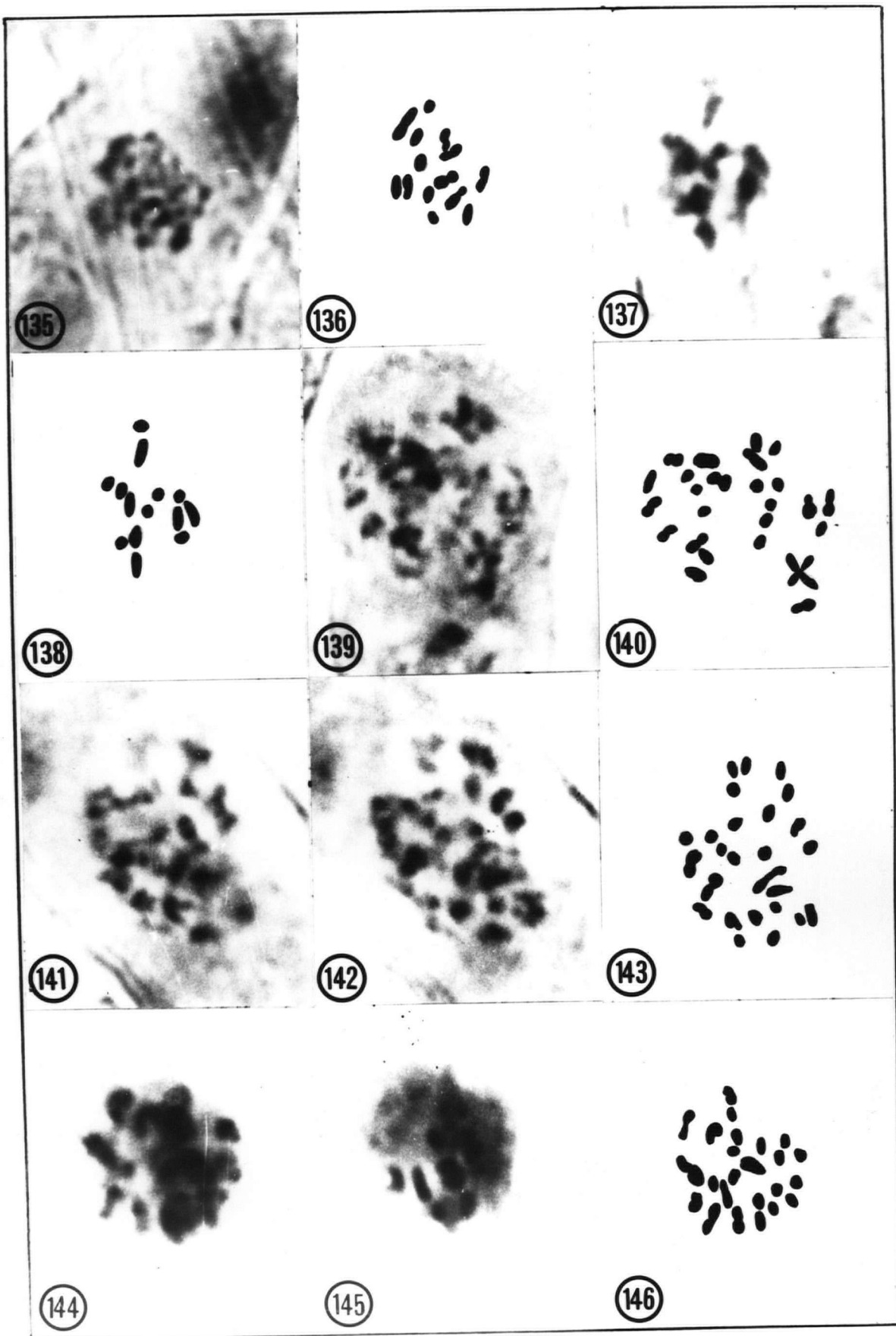


Plate 23

Figure 147:

Figure 148: Progressive focal views of a diakinesis con-

Figure 149: figuration in an immature zoosporangium of

Figure 150: A. grandifolia. 24 bivalents. x 4,000

Figure 151:

Figure 152: Diagrammatic representation of Figs. 147, 148, 149, 150 & 151.

Figure 153: Diplotene configuration in an immature zoosporangium of A. grandifolia. 25 bivalents. x 4,000

Figure 154: Diagrammatic representation of Fig. 153.

Figure 155: Progressive focal views of a diplotene con-

Figure 156: figuration in an immature zoosporangium of

Figure 157: A. grandifolia. 17 bivalents. x 4,000

Figure 158: Diagrammatic representation of Figs. 155, 156 & 157.

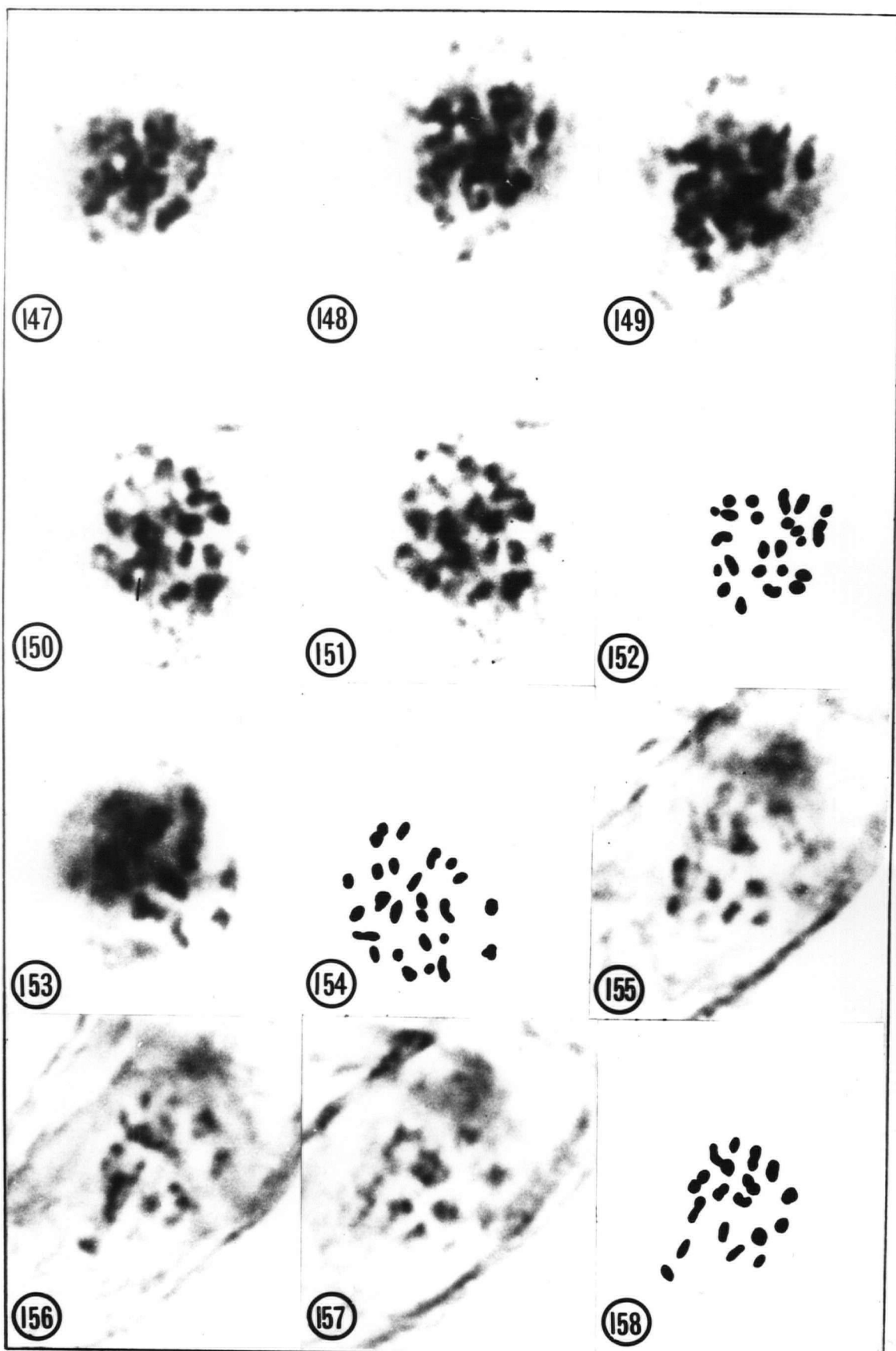


Plate 24

- Figure 159: Progressive focal views of late prophase of  
Figure 160: mitosis in a male gametophyte of A. marginata.  
14 chromosomes. x 4,000  
Figure 161: Diagrammatic representation of Figs. 159 & 160.
- Figure 162: Progressive focal views of late prophase of  
Figure 163: mitosis in a female gametophyte of A. marginata.  
16 chromosomes. x 4,000  
Figure 164: Diagrammatic representation of Figs. 162 & 163.
- Figure 165: Late prophase of mitosis in a males gameto-  
phyte of A. marginata. 13 chromosomes. x 4,000  
Figure 166: Diagrammatic representation of Fig. 165.
- Figure 167: Late prophase of mitosis in a female gameto-  
phyte of A. marginata. 14 chromosomes. x 4,000  
Figure 168: Diagrammatic representation of Fig. 167.
- Figure 169: Late prophase of mitosis in a female gameto-  
phyte of A. nana. 15 chromosomes. x 4,000  
Figure 170: Diagrammatic representation of Fig. 169.



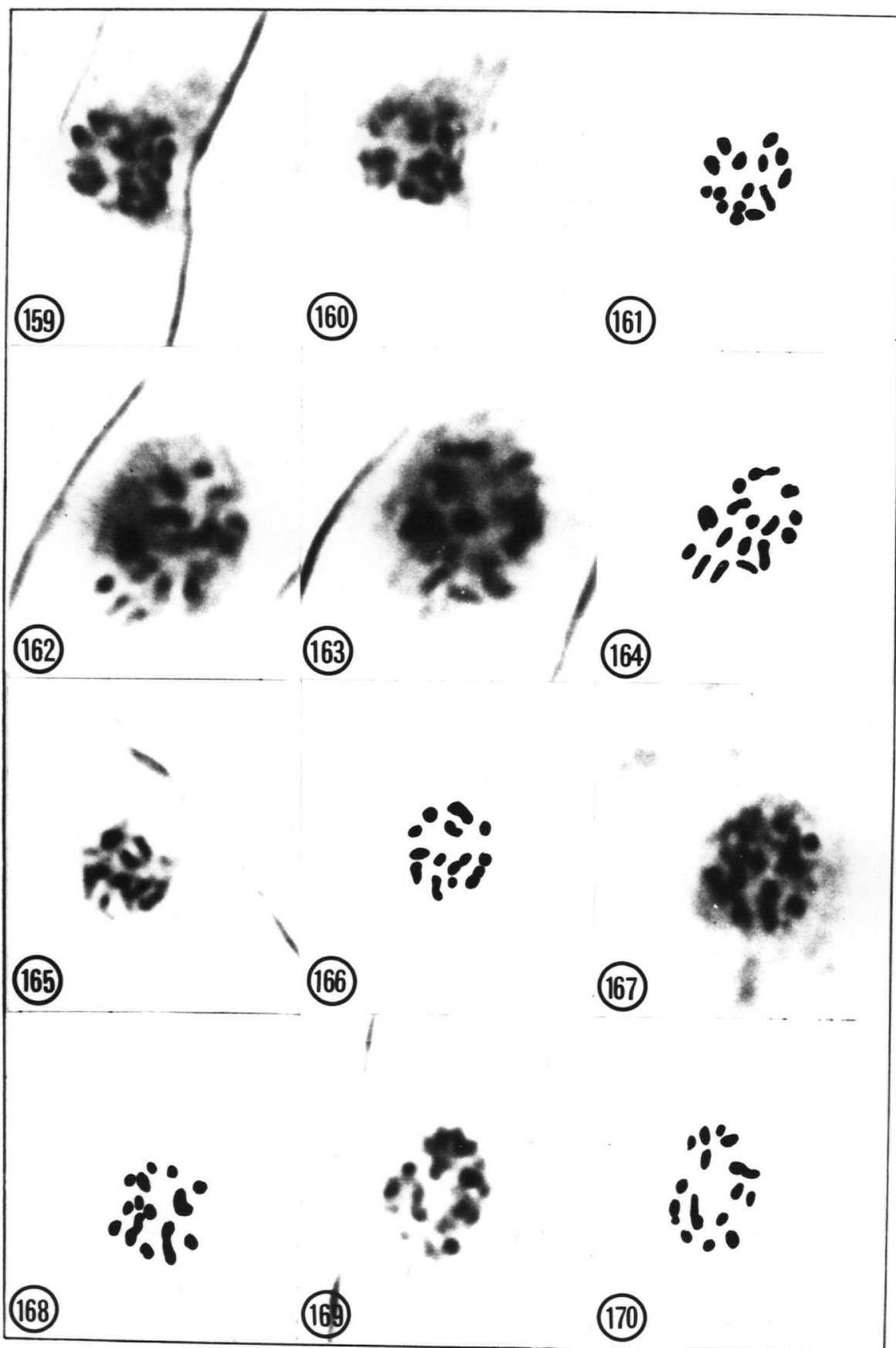


Plate 25

- Figure 171: Progressive focal views of late prophase of  
Figure 172: mitosis in a female gametophyte of A. nana.  
15 Chromosomes. x 4,000  
Figure 173: Diagrammatic representation of Figs. 171 & 172.
- Figure 174: Progressive focal views of late prophase of  
Figure 175: mitosis in a male gametophyte of A. nana.  
14 chromosomes. x 4,000  
Figure 176: Diagrammatic representation of Figs. 174 & 175.
- Figure 177: Late prophase of mitosis in a female gameto-  
phyte of A. tenuifolia. 15 chromosomes. x 4,000  
Figure 178: Diagrammatic representation of Fig. 177.
- Figure 179: Late prophase of mitosis in a male gameto-  
phyte of A. tenuifolia. 13 chromosomes. x 4,000  
Figure 180: Diagrammatic representation of Fig. 179.
- Figure 181: Late prophase of mitosis in a male gameto-  
phyte of A. tenuifolia. 13 chromosomes. x 4,000  
Figure 182: Diagrammatic representation of Fig. 181.

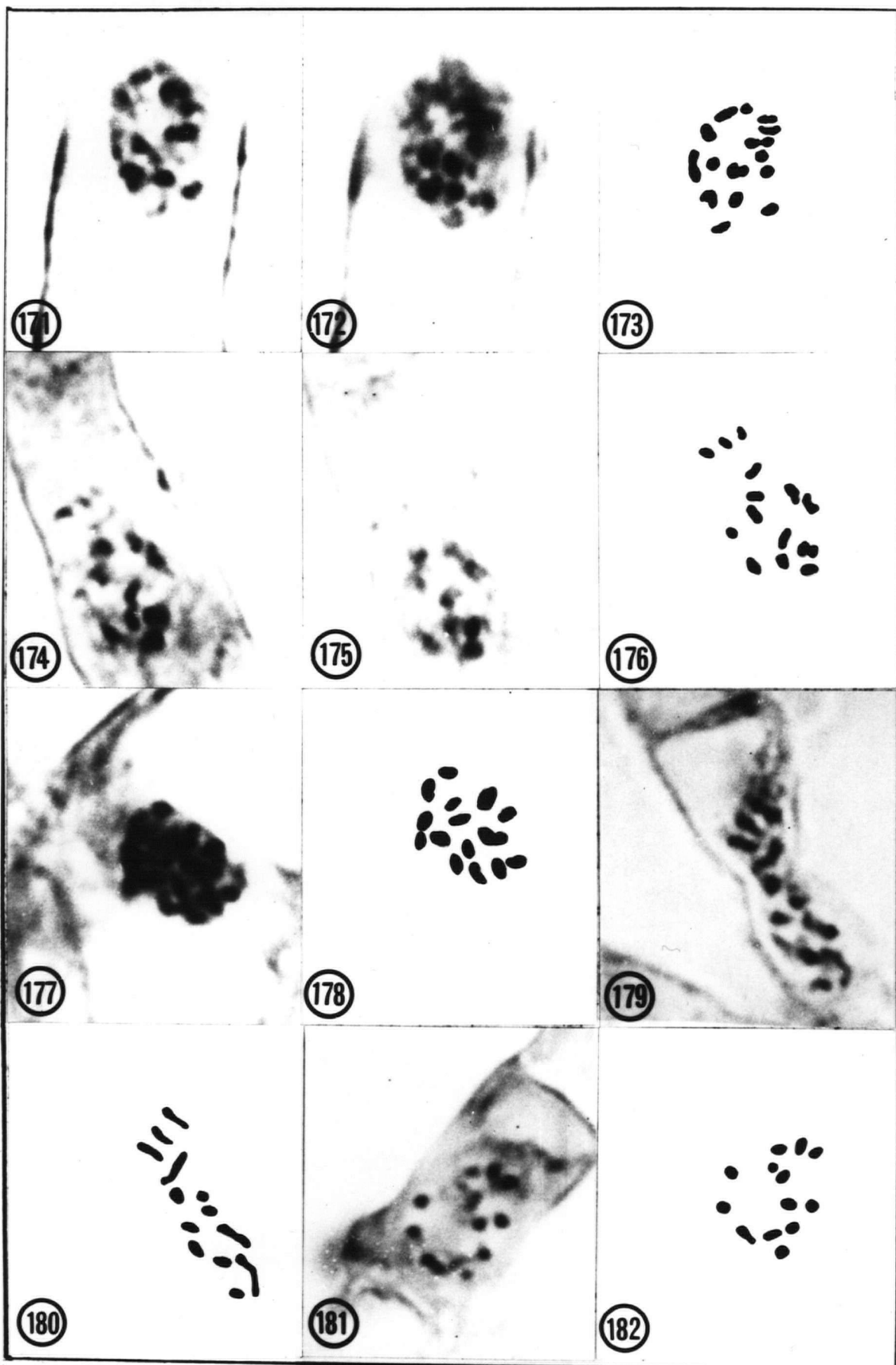


Plate 26

- Figure 183: Progressive focal views of late prophase of  
Figure 184: mitosis in a female gametophyte of A. taeniata.  
Figure 185: 15 chromosomes. x 4,000
- Figure 186: Diagrammatic representation of Figs. 183, 184  
& 185.
- Figure 187: Progressive focal views of late prophase of  
Figure 188: mitosis in a male gametophyte of A. taeniata.  
Figure 189: 12 chromosomes. x 4,000  
Figure 190:
- Figure 191: Diagrammatic representation of Figs. 187, 188,  
189 & 190.
- Figure 192: Progressive focal views of late prophase of  
Figure 193: mitosis in a female gametophyte of A. fistulosa.  
Figure 194: 14 chromosomes. x 4,000
- Figure 195: A diagrammatic representation of Figs. 192, 193  
& 194.
- Figure 196: Progressive focal views of late prophase of  
Figure 197: mitosis in a female gametophyte of A. fistulosa.  
14 chromosomes. x 4,000
- Figure 198: Diagrammatic representation of Figs. 196 & 197.

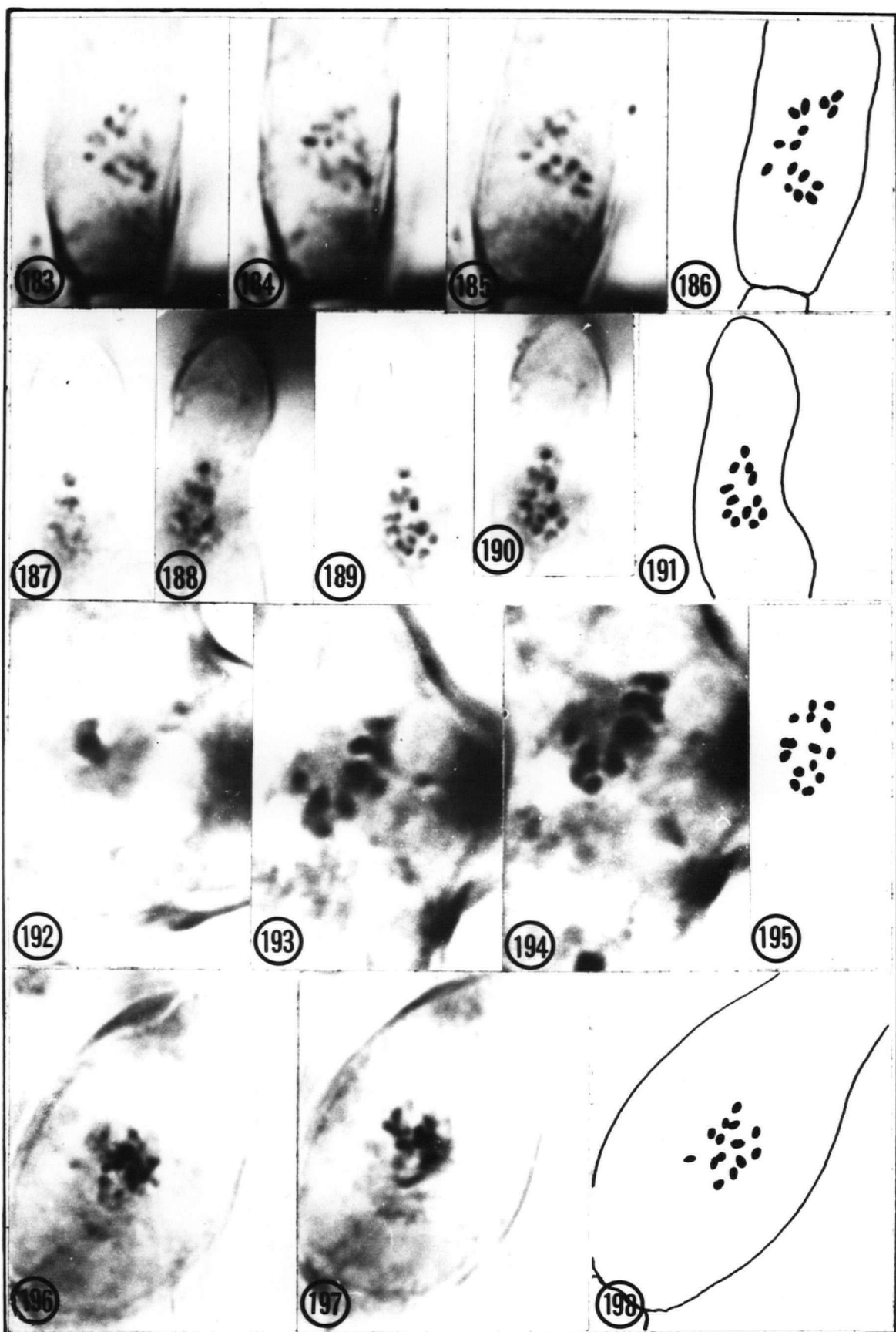


Plate 27

Figure 199: Progressive focal views of late prophase of  
Figure 200: the division of the female gametophyte of A.  
Figure 201: taeniata prior to egg formation. 19 chromosomes.  
Figure 202: x 4,000

Figure 203: Diagrammatic representation of Figs. 199, 200,  
201, & 202.

Figure 204: Progressive focal views of late prophase of  
Figure 205: mitosis in the first division of the zygote  
Figure 206: of A. marginata. 28 chromosomes. x 4,000  
Figure 207:  
Figure 208:

Figure 209: Diagrammatic representation of Figs. 204, 205,  
206, 207 & 208.

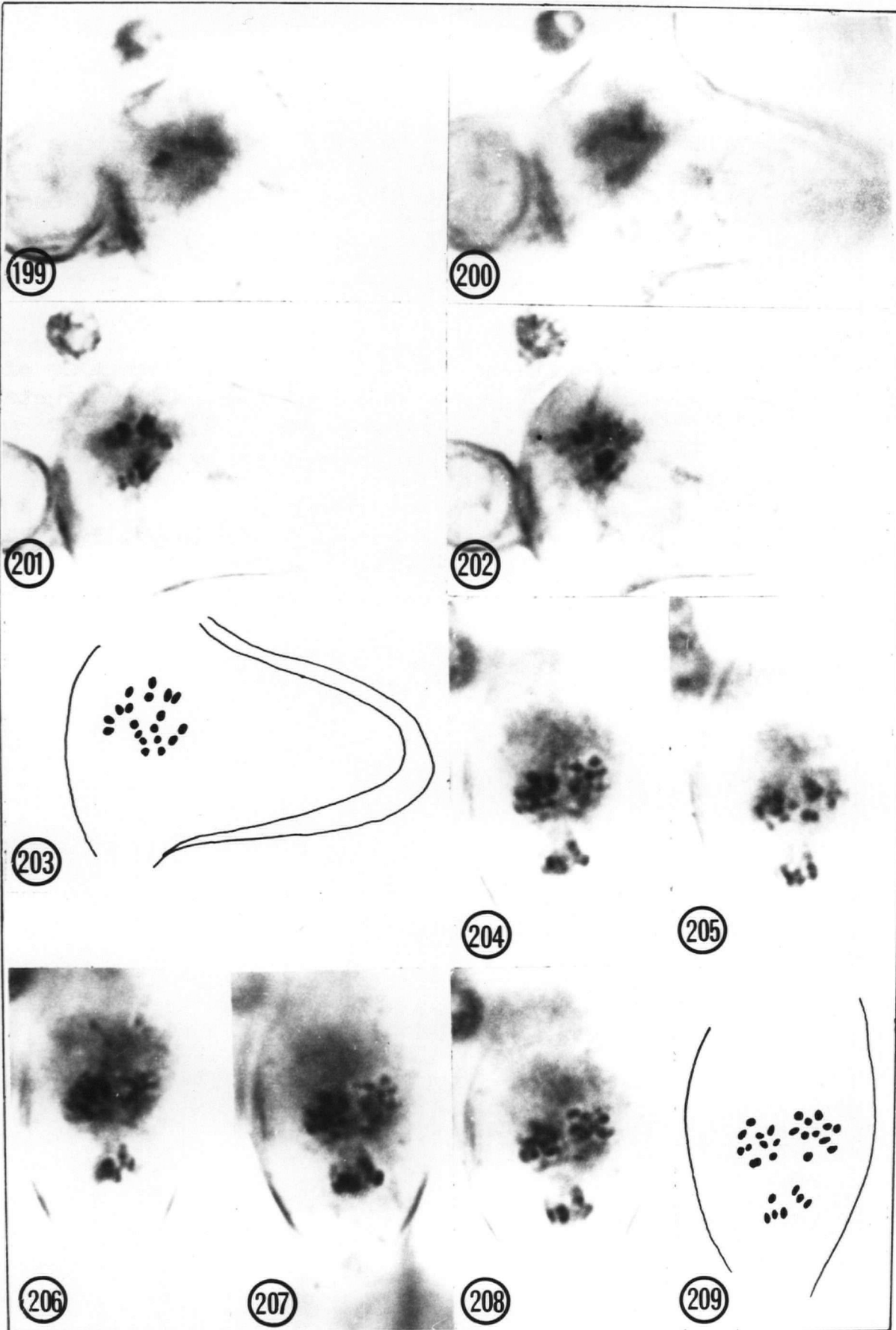


Plate 28

Figure 210: Progressive focal views of late mitotic prophase  
Figure 211: in the first division of the zygote in A. marginata.  
Figure 212: 26 chromosomes. x 4,000

Figure 213: Diagrammatic representation of Figs. 210, 211,  
& 212.

Figure 214: Progressive focal views of late mitotic prophase  
Figure 215: in the first division of the zygote of A. marginata.  
Figure 216: 28 chromosomes. x 4,000  
Figure 217:  
Figure 218:

Figure 219: Diagrammatic representation of Figs. 214, 215,  
216, 217 & 218.



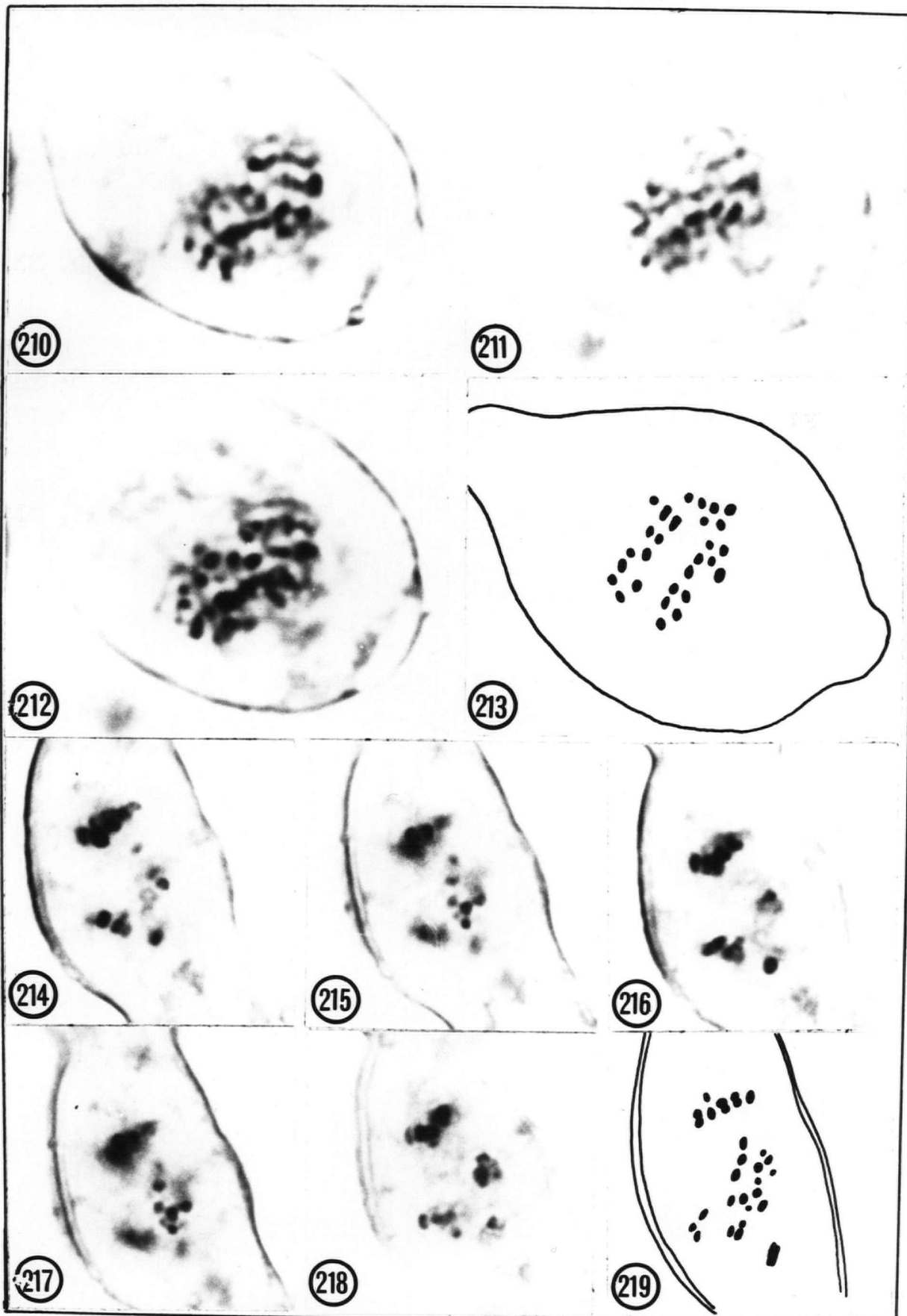


Plate 29

- Figure: 220: Progressive focal views of late mitotic prophase  
Figure 221: in the first division of the zygote in A. nana.  
Figure 222: 23 chromosomes. x 4,000
- Figure 223 : Diagrammatic representation of Figs. 220, 221  
& 222.
- Figure 224: Progressive focal views of late mitotic  
Figure 225: prophase in the first division of the zygote  
Figure 226 : in A. tenuifolia. 24 chromosomes. x 4,000
- Figure 227: Diagrammatic representation of Figs.  
224, 225 & 226.
- Figure 228: Progressive focal views of late mitotic  
Figure 229: prophase in the first division of the zygote in  
Figure 230: A. tenuifolia. 24 chromosomes. x 4,000
- Figure 231: Diagrammatic representation of Figs. 228,  
229 & 230.

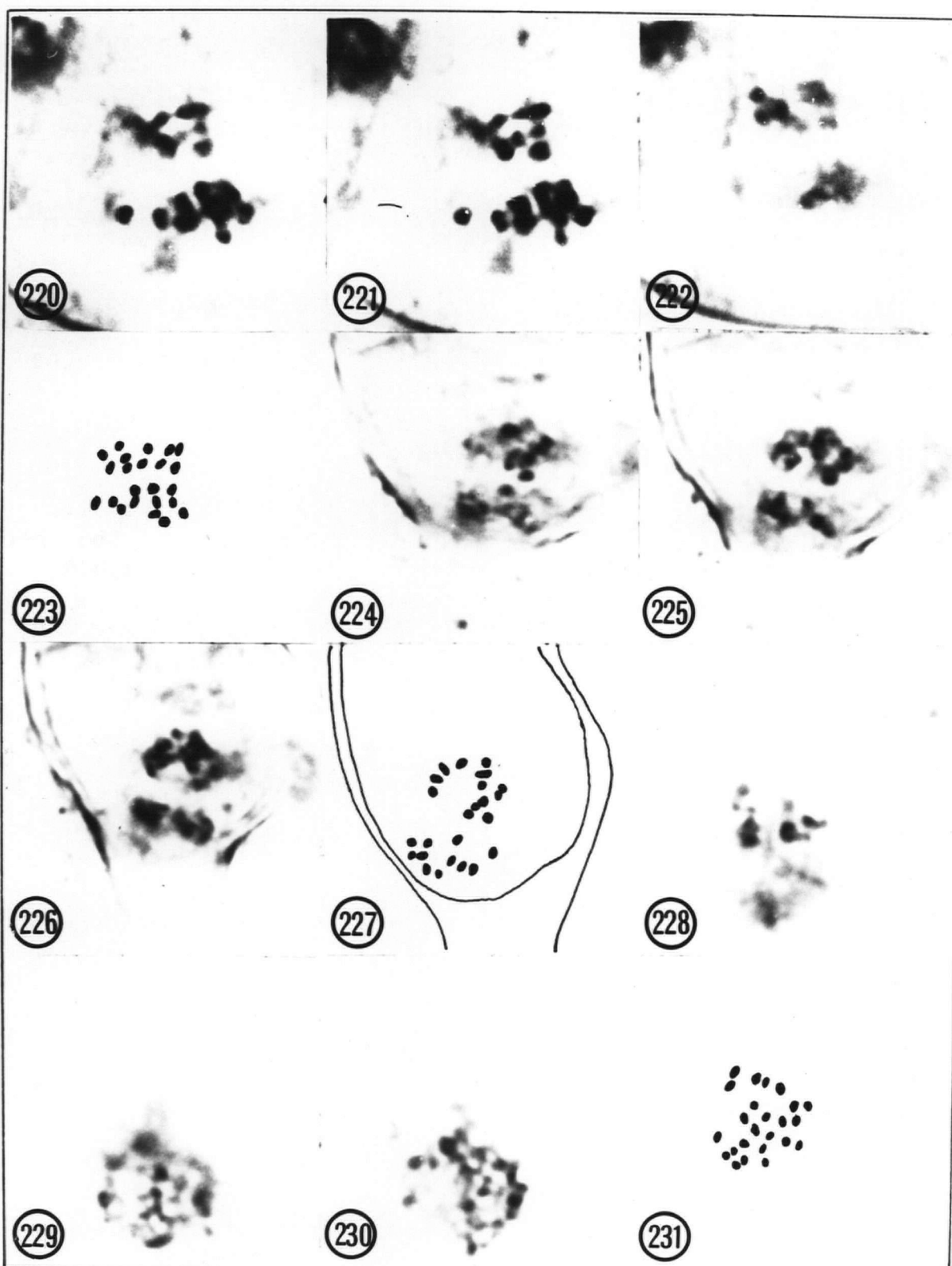


Plate 30

- Figure 232: Late mitotic prophase in a three-celled sporophyte of A. taeniata (progressive focal views).  
Figure 233: phyte of A. taeniata (progressive focal views).  
Figure 234: 26 chromosomes. x 4,000  
Figure 235:  
Figure 236:
- Figure 237: Diagrammatic representation of Figs. 232, 233, 234, 235 & 236.
- Figure 238: Late mitotic prophase in a binucleate sporophytic cell of A. taeniata (progressive focal views).  
Figure 239: phytic cell of A. taeniata (progressive focal views).  
Figure 240: 24 chromosomes visible in both complements. x 4,000
- Figure 241: Diagrammatic representation of Figs. 238, 239 & 240.

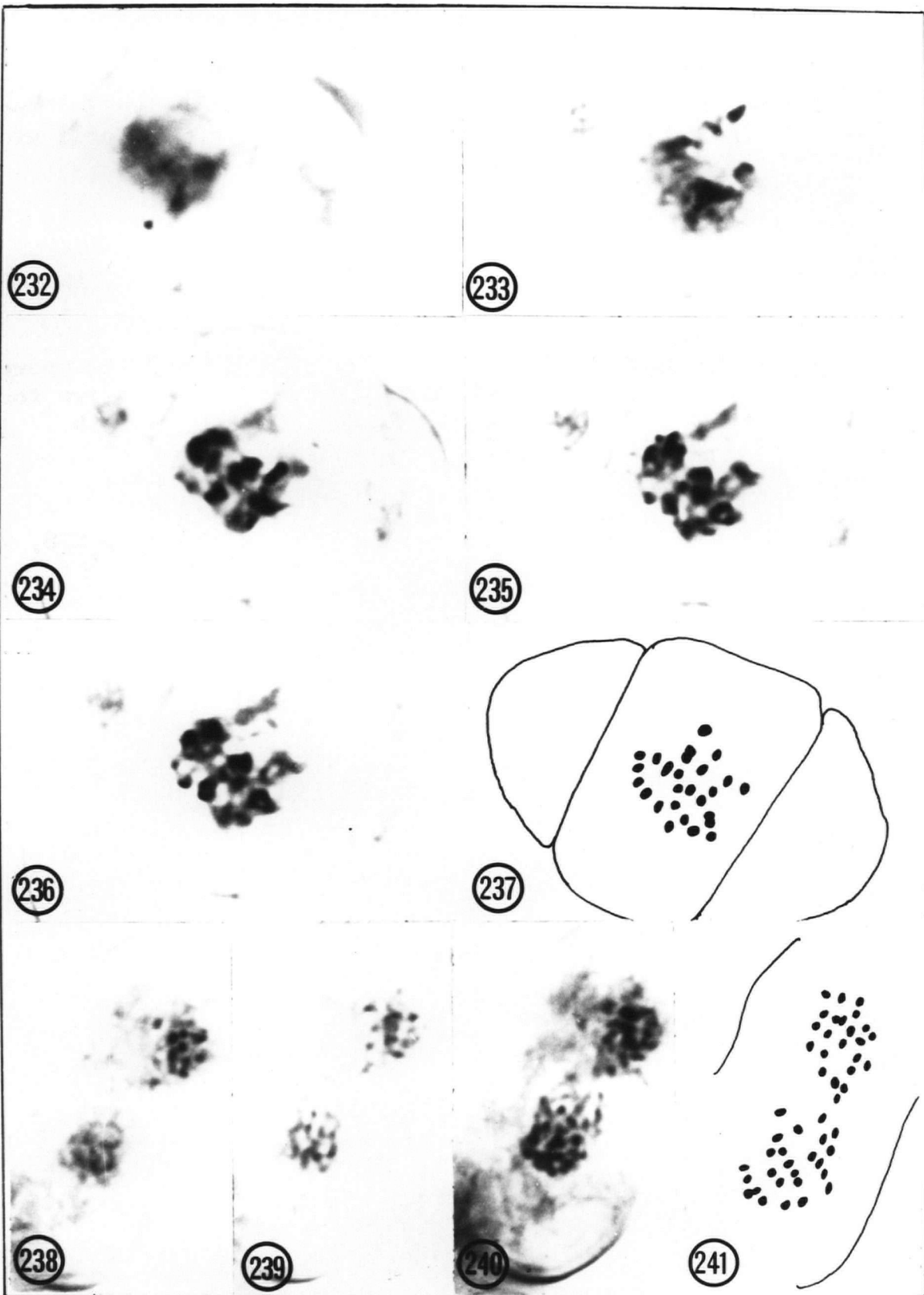


Plate 31

- Figure 242: Late mitotic prophase in the initial division  
Figure 243: of the zygote of A. fistulosa (progressive focal  
Figure 244: views). 23 chromosomes. x 4,000  
Figure 245:
- Figure 246: Diagrammatic representation of Figs. 242, 243,  
244 & 245.
- Figure 247: Late mitotic prophase in a two-celled partheno-  
Figure 248: sporophyte of A. taeniata. 12 chromosomes.  
Figure 249: x 4,000  
Figure 250:  
Figure 251:
- Figure 252: Diagrammatic representation of Figs. 247, 248,  
249, 250 & 251.

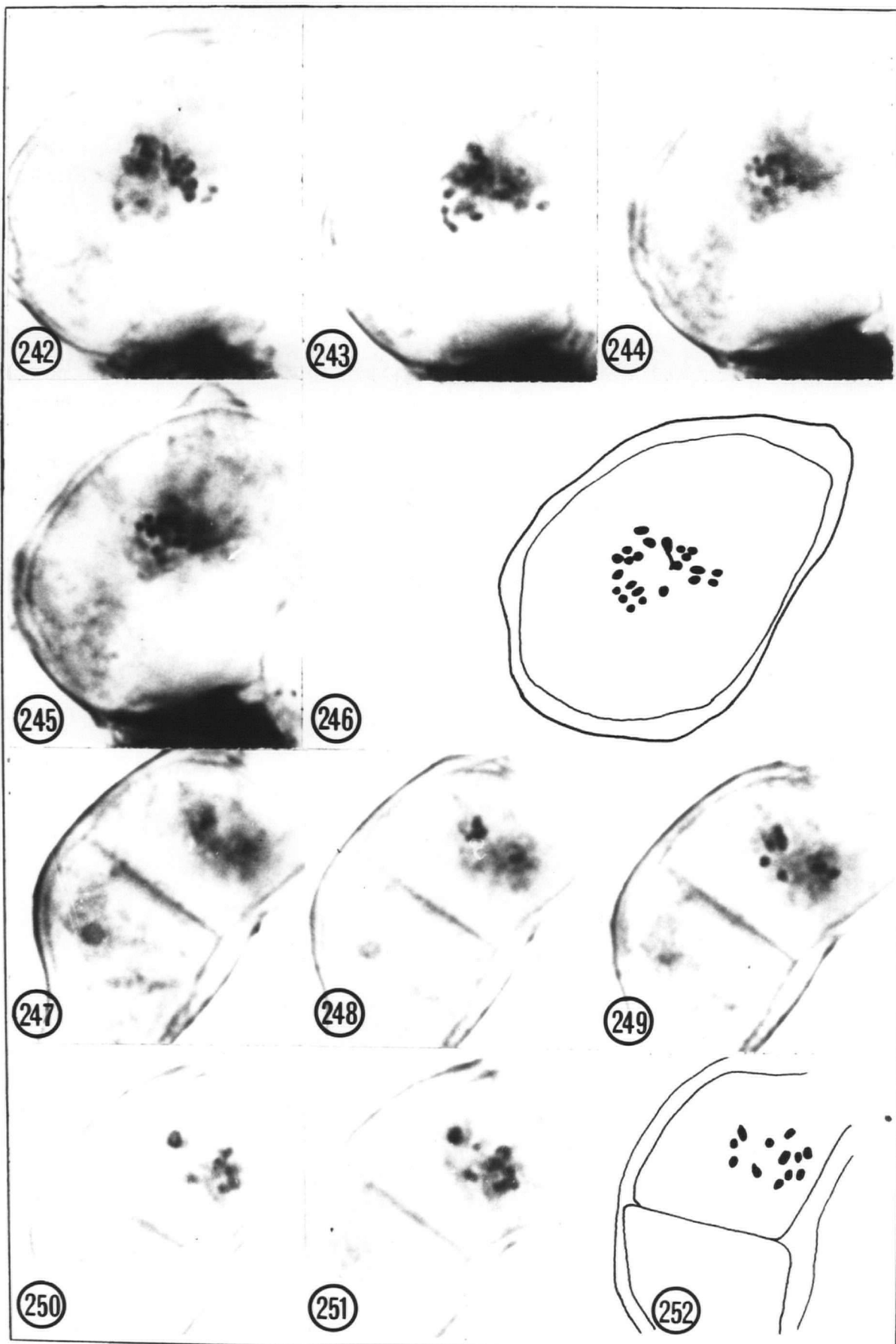


Plate 32

Figure 253:



FIGURE 253

Graphic representation of chromosome counts made during meiosis in Alaria spp.

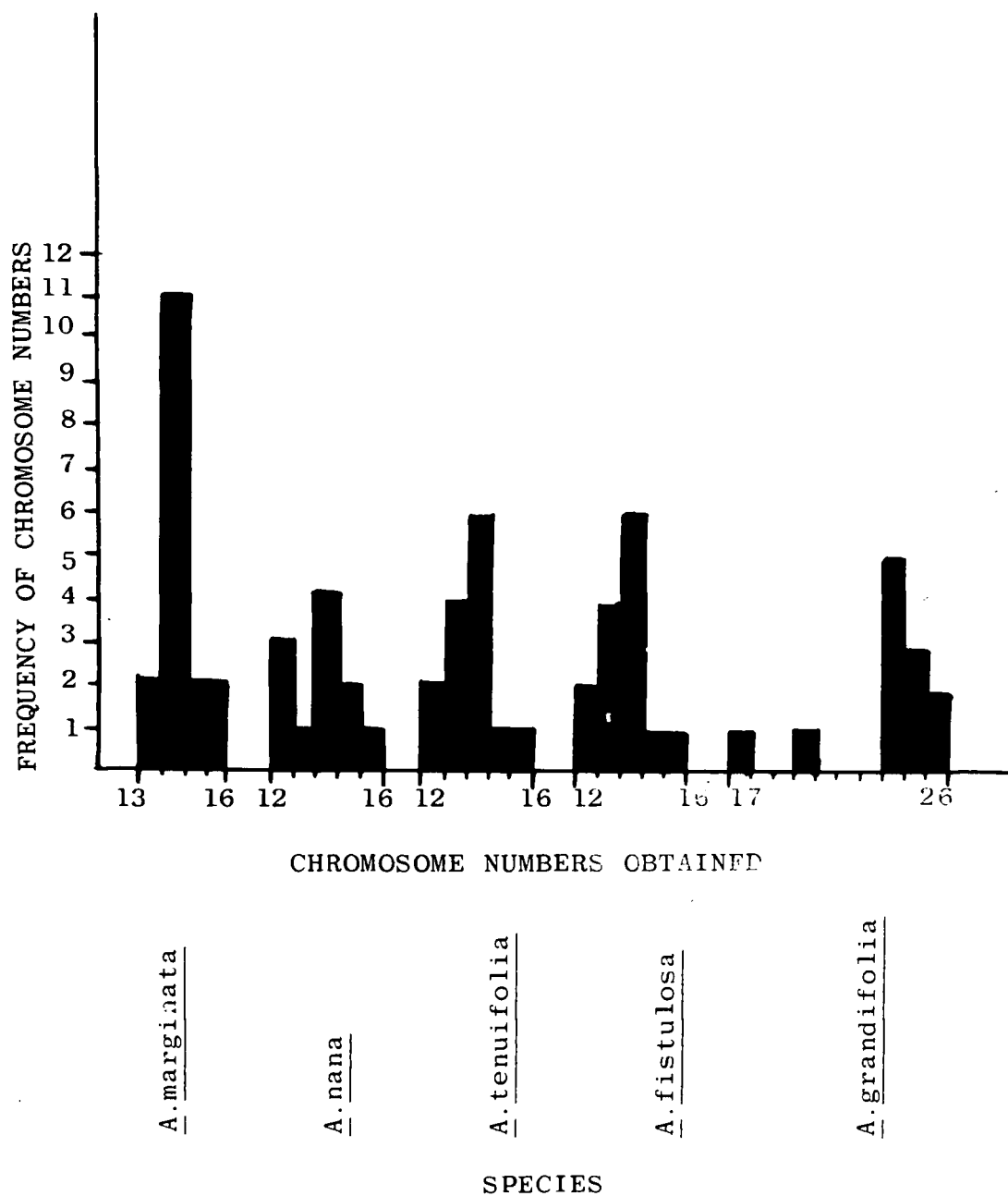


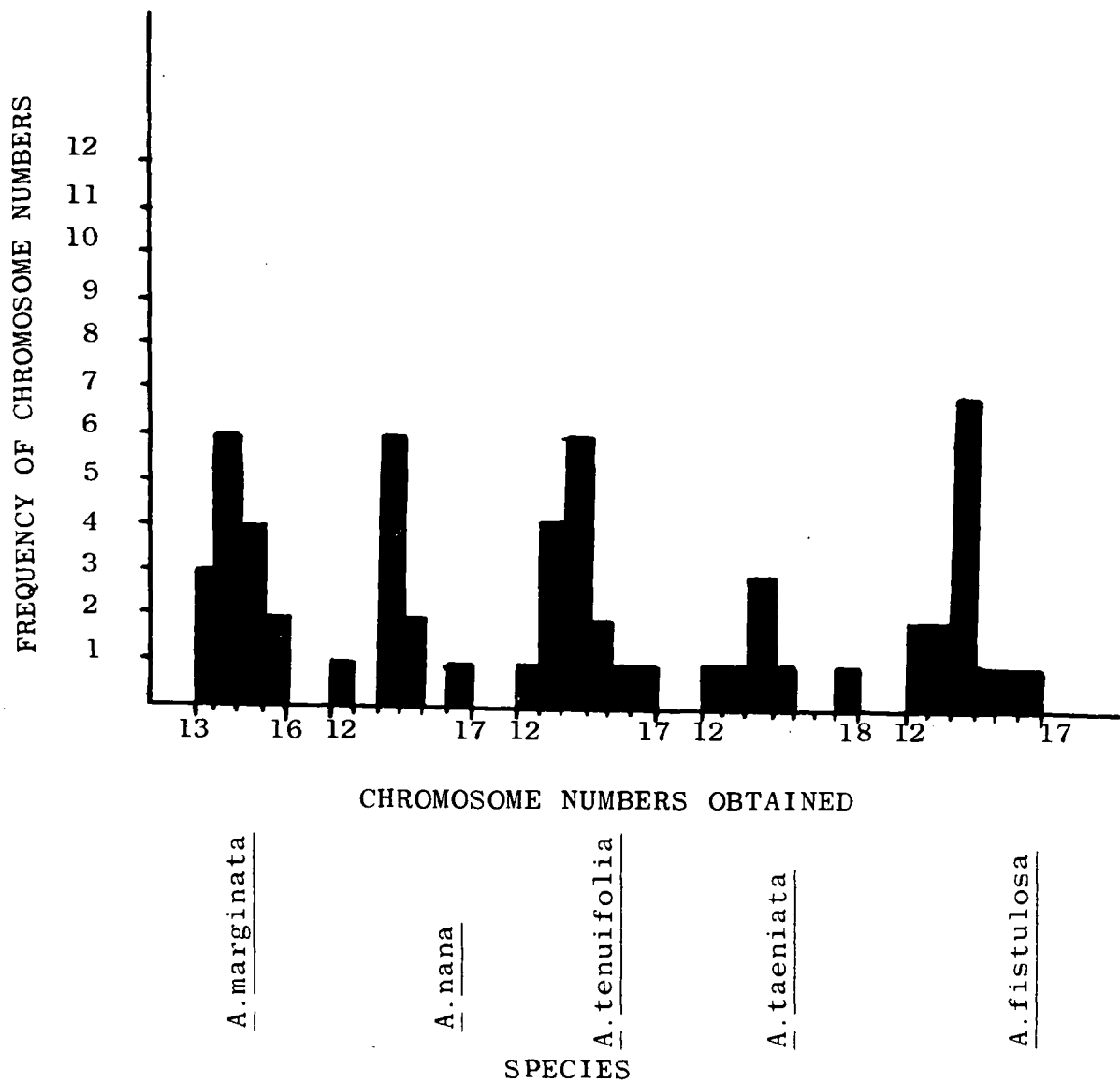
Plate 33

Figure 254:

5

FIGURE 254

Graphic representation of chromosome counts made during mitosis in male and female gametophytes of Alaria spp.



**Plate 34**

Figure 255:

FIGURE 255

Graphic representation of chromosome counts made during mitosis in young cultured sporophytes of Alaria spp.

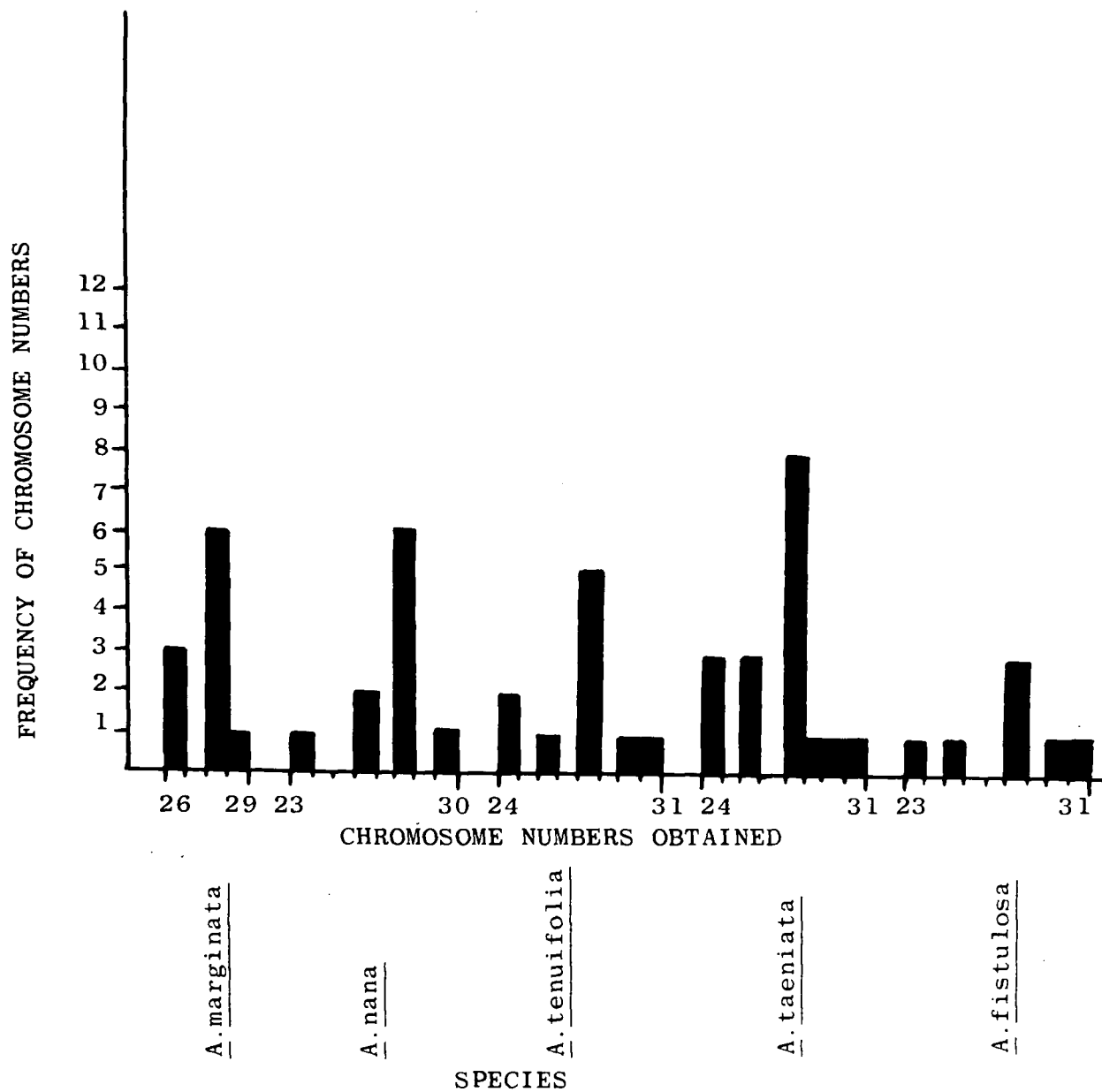


Plate 35

Figure 256: The affect of temperature on gametophytic growth in A. grandifolia.

Graphic representation of range of gametophyte size and mean size.

- o
- A - Male gametophytes at 10 °C.
  - B - Female gametophytes at 10 °C.
  - C - Male gametophytes at 5°C.
  - D - Female gametophytes at 5°C.

Figure 256

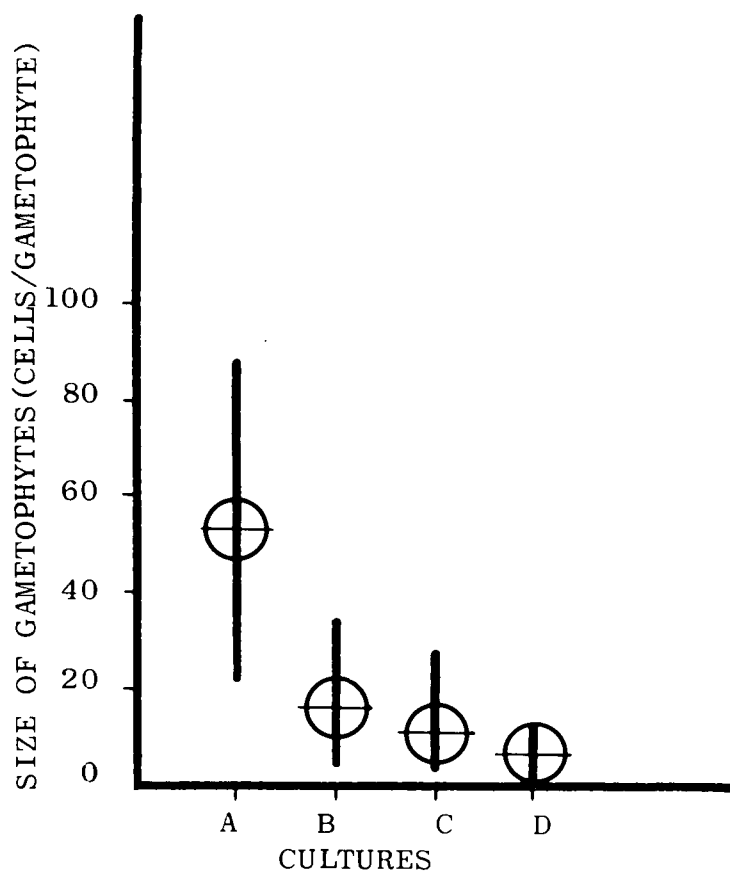


Plate 36

Figure 257: The affect of temperature on gametophytic growth in A. fistulosa.

Graphic representation of range of gametophyte size and mean size.

- A - Male gametophytes at 10°C.
- B - Female gametophytes at 10°C.
- C - Male gametophytes at 5°C.
- D - Female gametophytes at 5°C.



Figure 257

