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BIOLOGY OF OPHIOGLOSSUM L.

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ABSTRACT

Ophioglossales are the natural group of early vascular plants which exhibit the most simple and most complicated combinations of characters comparable to bryophytes, pteridophytes, progymnosperms, gymnosperms and angiosperms. Essentially, pteridophytes these plants are often referred and classified as ferns. However, there are some fundamental differences which should not justify their present alliance. The chief "genetic loss" in plants of this group can be presumed to be the loss of capability of producing sclerenchyma. Also, the sporangia are unlike ferns; they do not have an annulus and are supplied with vascular tissue. Additionally, absence of circinate vernation and presence of periderm (in about 22% of *Ophioglossum* population) make them "unlike ferns".

The conventionally recognised three genera, *Botrychium, Helminthostachys* and *Ophioglossum* constitute a single family Ophioglossaceae of the order Ophioglossales. Nevertheless, intergeneric differences are so pronounced that recognition of three separate families viz. Botrychiaceae, Helminthostachyaceae and Ophioglossaceae by some taxonomists are quite justified. *Botrychium and Ophioglossum* are further divided to have subgenera; *Botrychium has Sceptridium, Eubotrychium* and *Osmundopteris*, while *Ophioglossum* has two, viz. *Ophioglossum* and *Ophioderma*.

Population cytogenetic studies have been carried out chiefly from the localities where more than one species of *Ophiglossum* grow. Repeated meiotic studies have also been carried out from populations of single or isolated species of *Ophioglossum* and monotypic *Helminthostachys*. Numerous teratologies of genetic importance have been described. Role of natural selection is being assessed. Lately, a new specis *O. eliminatum* is being suspected to have been arisen by natural hybridization and chromosomal elimination. *O. eliminatum* has the lowest count no = 90 in the genus.

Molecular genetic data generated in many laboratories have proved that the genus *Ophioglossum* in particular, is highly suitable for studies in evolutionary genetics and we can decipher highly complicated evolutionary mechanisms of the entire group of plants. The cotwin control approach on vegetatively reproducing plants connected with stolons until maturity have opened up a new technique of experimental controls for certain sets of experiments. In fact now we need to take use of all possible morphological, biochemical and molecular genetic techniques to understand evolutionary mechanisms.

Key words; Ophioglossales. Natural Variations. Morphogenetic Variation and Evolution. Speciation in *Ophioglossum*, cotwin control approach.

Introduction

The Ophioglossales represent the most primitive of the pteridophytes having strange combination of characteristics of simple and complex nature. It is bewildering that on one hand this group, in particular *Ophioglossum*, exhibits features comparable with bryophytes to angiosperms and on the other hand, the group is totally absent in fossil beds; even it has no directally among the living plants. As a matter of fact, the entire group which consists or one order Ophioglossales, one representative family Ophioglossaceae comprising three main genera *Botrychium* (30 spp). *Helminthostachys* (1 sp) and *Ophioglossum* (40 spp) offer a unique challenge to morphologists, cytologists and evolutionists.

1. Systematics and Taxonomy

Number of Genera and Species

There has always been a controversy regardig the exact number of species of *Botrychium*, and *Ophioglossum* (Eames, 1936; Foster and Gifford, 1959; Bierhorst, 1971; Sporne, 1970). Majority of taxonomists and morphologists seem to agree that number of species should be reduced. For the sake of taxonomic convenience and based on anatomical studies, Japanese, workers led by Nishida, divided the genus *Botrychium* into three subgenera viz. *Sceptridium*, *Eubotrychium* and *Japanobotrychium*, and constituted the family Botrychiaceae (see Nishida, 1952; Panigrahi & Dixit, 1969,1972). Copeland (1947) mentions *Rhizoglossum* with 1 sp and Pichi-sermolli (1959) recognizes *Cheiroglossa* with 2 spp., as two other genera in addition to *Botychium*, *Helminthostachys* and *Ophioglossum*. Some workers however consider *Cheiroglossa* and *Rhizoglossum* as the sub genera of *Ophioglossum*.

1.1 Families and Genera

There has also been a controversy regarding the number of families and genera in the group of plants. Besides conventional consideration of only one family Ophioglossaceae with three major genera *Helminthostachys, Ophioglossum*, and Botrychium, Pichi-sermolli's classification, propounded in 1977, is getting attention by pteridologists. According to this approach there are three families and seven genera:

I. Helminthostachyaceae
    Helminthostachys
II. Botrychiaceae
    Botrychium
    Botryphus
III. Ophioglossaceae
    Cheiroglossa
    Ophioderma
    Ophioglossum
    Rhizoglossum

Family : Ophioglossaceae

Perennial herbs, terrestrial or epiphytic, but not growing in water; rhizome subterranean, short fleshy not scaly erect or creeping, with a cluster of stout fibrous roots and bearing at the apex a fertile spike/ sporophyll and one to several sterile blades/tropophylls, the latter enclosing a leaf bud in its sheathing base; the leaves erect or pendant (never circinate), venation free or netted; the lamina stalked or sessile, simple, lobed or variously decompound, the fertile segments simple, racemose or paniculate but always representing segments of a typically partly sterile frond and bearing sporangia, embedded in or seated upon a stalked spike which is usually erect; the sporangium without any annulus and opening by a longitudinal or transverse slit and thus, bivalvate, spores numerous per sporangium, trilete and circular; the gametophytes subterranean, tuberous, micorrhizic and without chlorophyll.

A family of three distinct genera, viz: *Botrychium, Helminthostachys* and *Ophioglossum* (Figs.1-3), all of which are

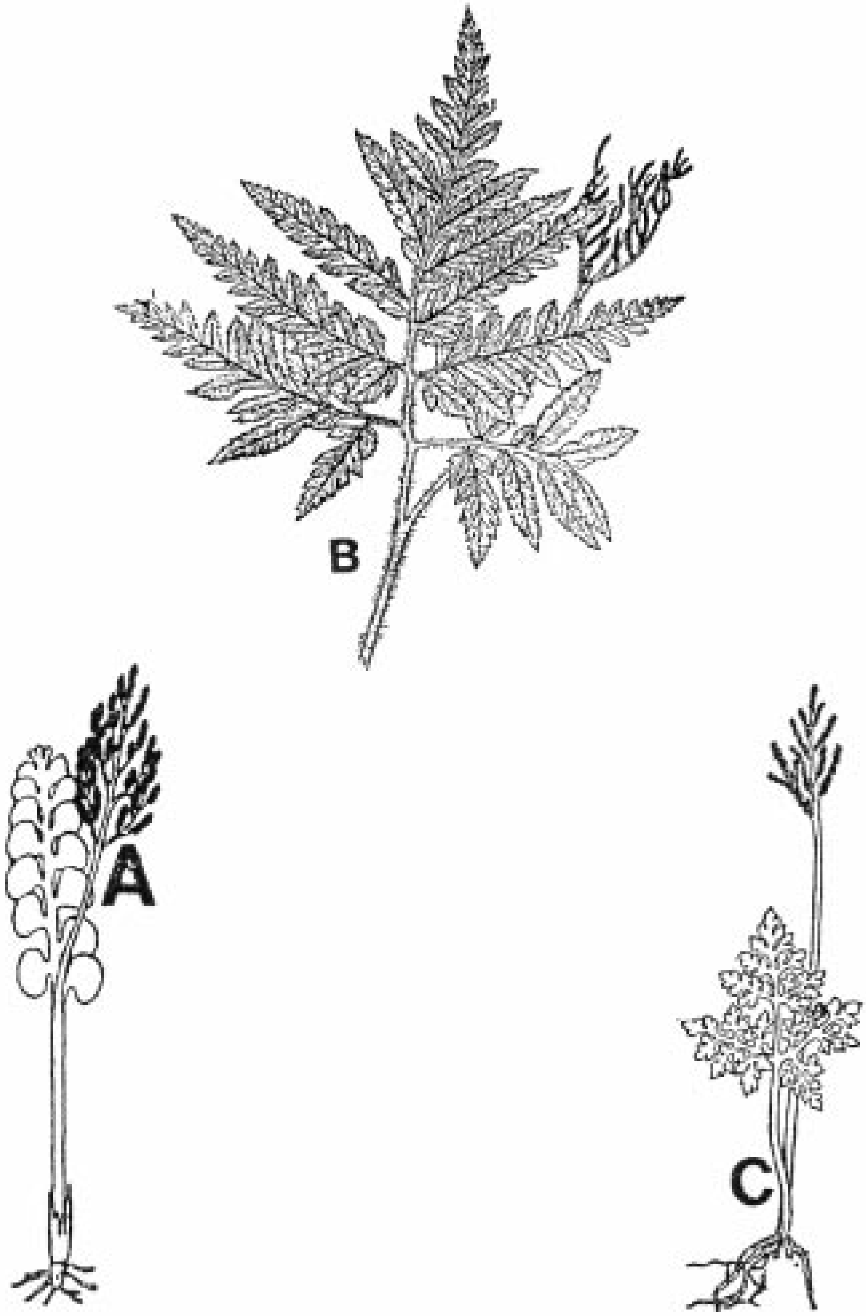


Fig. 1 — Different species of *Botrychium*. A. *B. lunaria*. B. B. *daucifolium*. C. B. *virginianum*. (courtesy: Fern's handbook).

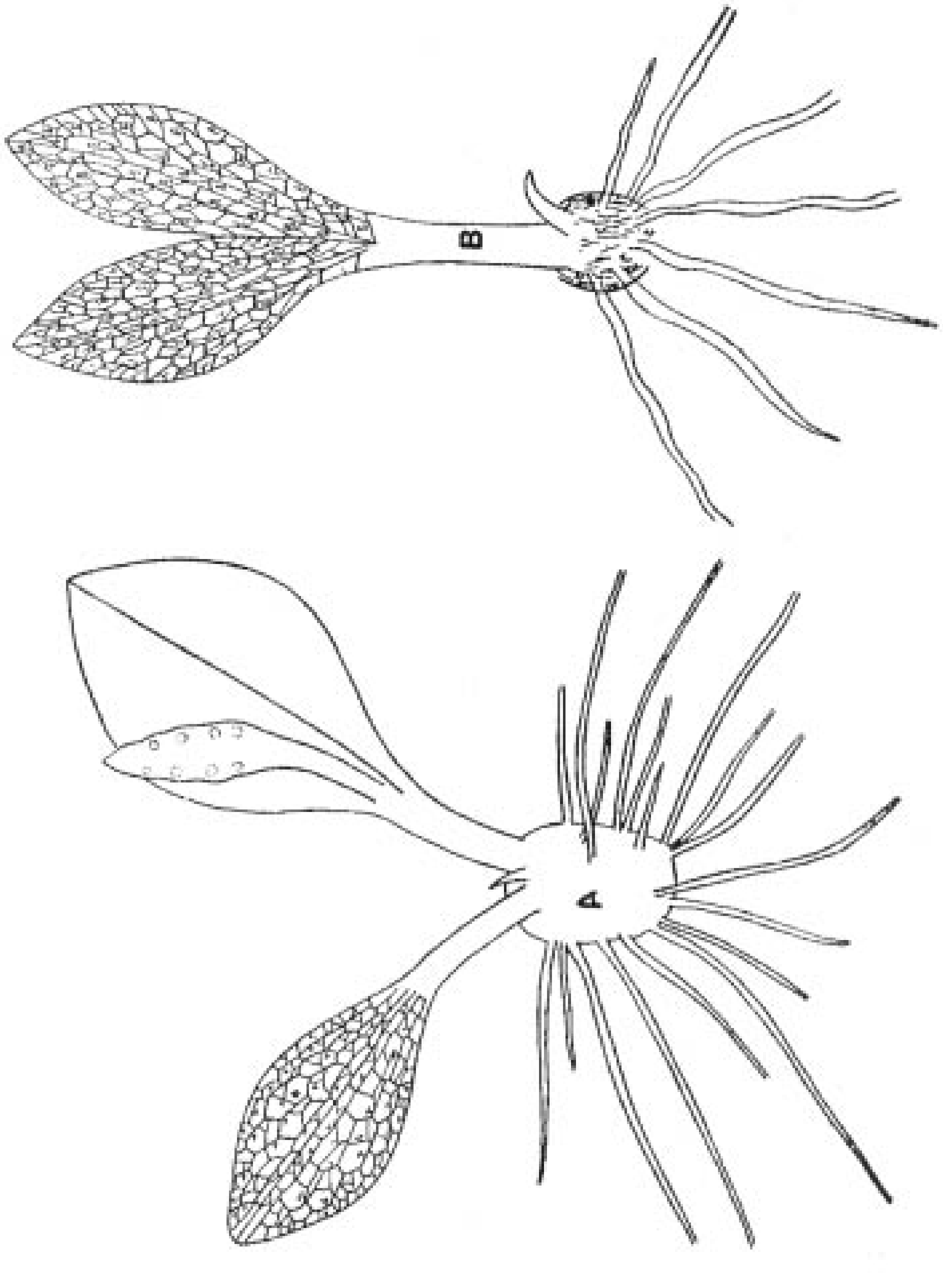


Fig. 2A & B — Abnormal plants of *Ophioglossum. A* shows feebly developed spike while *B* shows bifurcated tropophyll without a spike.

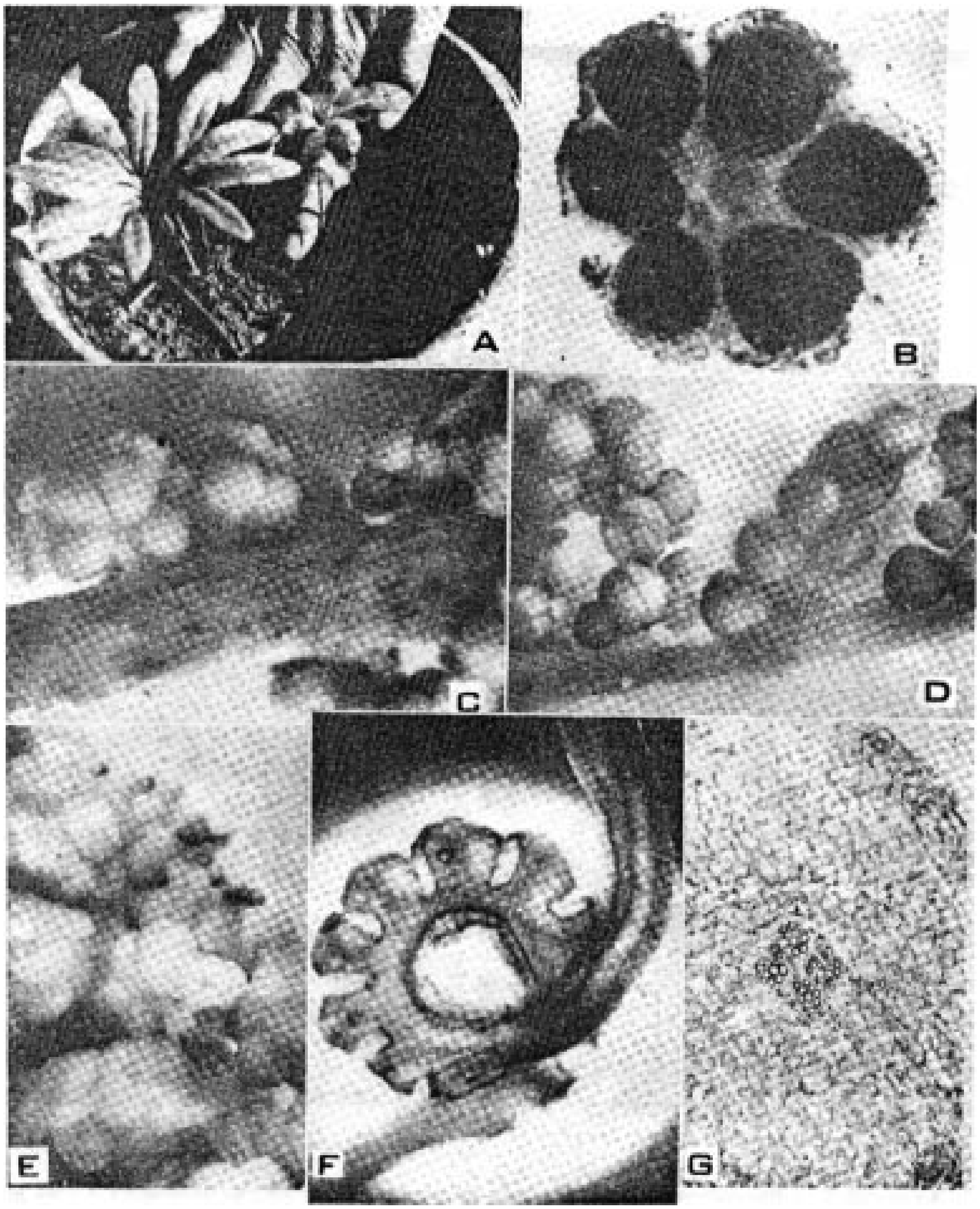


Fig. 3 — A Potted plant of *Helminthostachys Zeylanica.* Note the variation in leaf forking. B. A. whole glycerine mount of young rosette of *sporangia of Helminthostachys* (× 120). G. A. portion of mature spike of *Helminthostachys.* Note the clusters of sporangia borne on a pedicel (sporangiophore). Note the margins of laccinate outgrowths as seen in side view (× 25). D. Arrangement of globular sporangia on a portion of spike of *Botrychium virginianum.* Note the line of dehiscence of the sporangia (× 25). E. Cluster of sporangia on a portion of spike of *O. eliminatum* showing leafy and (near label). G. A. transverse section of the root of *Helminthostachys zeylanica*, a tetrarch root (× 5).

widely distributed in the continents including india.

1.2 DIAGNOSTIC FEATURES AND DISTRIBUTION OF GENERA

A. Venation free, both the sterile and fertile segments compound (rarely simple); spike simple, racemose or paniculate bearing distinct sessile or stalked sporangia.

B. Sterile segments pinnate or decompound, the fertile segments pinnate with several spreading branches; sporangium dehiscing by a transverse slit ...I. *Botrychium*

B. Sterile segments palmate with several separate leaflets; fertile segments compact with many short branches; sporangia dehiscing by a longitudinal slit...II. *Helminthostachys*.

A. Venation reticulate; sterile and fertile segments simple, entire (or with few lobes); spike simple terete, erect or pendulous, sporangia half or full embedded in two lateral rows ... III. *Ophioglossum*.

I. *Botrychium Swartz*

A genus of about 30 species (Claussen, 1938), Botrychium is found in all parts of the world growing in meadows and on humus covered soil in moist shady areas ranging from 1500 to 4000 M in altitude. Plants 15-80 cm high; common stalk 4-8 cm long; sterile stalk 4-20 cm long; sterile blade deltoid, tripinnate to quadripinnatifid, stalk of the pinnae 0.5-2 cm long and with ultimate segments 2-6 X 3-4 mm, apex acutish, veins simple or forked; fertile stalk 10-24 cm long; fertile spike ofter compound, 3-6 cm long; spores trilete with circular outline, triangular in polar view, 20-38 µm in diameter and with verrucose exine. Fertile, August to November.

Claussen(1938) recognizes three subgenera in *Botrychium*, viz: *Sceptridium, Eubotrychium* and *Osmundopteris*, they are distinguished from each other as follows.

Key

I. Sterile blades oblong to broadly deltoid, variable in texture but rarely membraneous. Bud hairy or smooth, completely enclosed by the sheathing base of the stalk.

II. Sterile blades rather large, ternately decompound, long stalked to sessile, usually inserted towards the base of the plant, at times arising rather higher up; the buds commonly hairy, rarely almost glabrous. ... A.subgenus : *Sceptridium*

III. Sterile blades usually small, pinnately or palmately divided, rarely simple, sessile or short stalked, parting from the common stalk at various heights, buds glabrous ... B... subgenus: *Eubotrychium*

I. Sterile blades large, deltoid, much divided with membranous texture. Buds hairy, partially exposed by the sheathing base of the stalk which is open on one side ...C Subgenus: *Osmundopteris* (= *Japanobotrychium* = *Botrypus*)

In India, following six species are, recorded belonging to the major genus *Botrychium* :

1. *Botrychium multifidum*
2. *B. ternatum*
3. *B. daucifolium*
4. *(A)* *B. lunaria var. lunaria*
   (B) *B. lunaria var. onondag*anse

5. *(A)* *B. lanuginosum var. lanuginosum*

   (B) *B. lanuginosum var. leptostachyum = Botrypus*

6. *B. virginianum*

II *Helminthostachys* Kaulfuss

Grows in open grassy swamps in forests and also along river banks, on moist alluvial sandy soil or on blackish mud between 200 and 1000 m altitude. Plants green to purplish in colour, 30-43 cm high; rhizome thick, fleshy, creeping and bearing many thick fleshy roots which become brittle when dry; common stalk fleshy, 20-30 cm long, sterile frond consisting of sessile, palmately tripartite (Fig. 2) lamina with a stalked fertile spike, and all those frond parts separate from the apex of the common stalk; divisions of the sterile blade 10-30x2-3 cm, oblong-lanceolate in shape, usually acute, rarely obtuse at the apex, cuneate at base, margin entire or slightly and irregularly toothed, midrib grooved above and raised below, veins fine, close arising obliquely from the midrib and once or twice forked; fertile spike longer than the sterile blade and stalk being 5-12 cm long, fruiting spike green to brown in colour, 4-8 cm long and bearing crowded short lateral branches, each with a sessile group of round sporangia and small sterile lobes at the apex spores trilete with circular outline 30-35 µm in diameter and with reticulate exine. Fertile: September–February.

Distribution: Ceylon, Malaya Peninsula, China, Japan, Phlippines, Solomon Island, New Caledonia, New Guinea and Australia (cf. Claussen, 1938). In India, the genus is found in Assam — Animadas s.n. (Assam); Lebang, Sibsagar Mikir hills, Gauhati; Motharguri, Sandal Bhell and adjoining sal forests; Singri; Sylhet; Khasi and Jaintia hills; Nongpoh.Uttar Pradesh — Baharaich; Abdullagunj, Nishangraha — Uraital, Gorakhpur: Kusmi forests and Ramgarh forest, Bhagalpur, Bihar; South India, near Trivandrum.

III. *Ophioglossum* Linn.

The generic name is derived from two Greek words which mean snake tongue, the fertile frond having the appearance of a snake's tongue.

Plants measure 1.5 cm to 25 cm or more; often mesophytic rarely epiphytic; rhizome usually short erect, bearing few fronds, each frond often accompanied with an adaxially placed fertile spike; adventitious roots arise in bunch from the rhizome. Frond, simple usually entire more or less fleshy with reticulate venation; spike consists of lateral, sunken round sporangia, each sporangium containing a few hundred to few thousand spores, spores monolets, trilete and or alete measuring 20-25 µm in diam.

Ophioglossums show the highest grade of polyploidy (2n =180 to >1400 chromosomes) in the whole biological system. The genus comprising of nearly 30 species is cosmopolitan in distribution (Nishida, 1959, Nishida and Kurita, 1969). Many new species are being added (Khandelwal and Goswami, 1984, Zeach *et. al.*, 1998; He, 2001).

Key to the sub-genera

1. Strobilus mostly with a sterile apex; clear demarcation between ropophyll and common stalk, tropophyll not or only at the base adnate to the fertile stalk;

venation mostly with at least a few secondary veinlets, leaf trace with a single strand ... subgenus *Ophioglossum*.

2. Strobilus with a fertile apex, tropophyll gradually attenuate into a common stalk (stalk often hardly discernible); tropophyall conspicuously adnate to the fertile stalk; venation consisting of the primary veins only ... subgenus: *Ophioderma*.

TABLE 1 — SPECIES OF OPHIOGLOSSUM FOUNDIN INDIA

	Present Teratment
1.	*O. costatum R. Br.*
2.	*O. gramineum Willd. var. gramineum*
2. (a)	*O. gramineum Willd. var. majus (v.A. v.R.) Wief.*
3.	*O. lusitanicum Linn.*
4.	*O. thermale Kumarov var. nipponicum (Miyabe et Kudo) Nishida*
5.	*O. nudicaule Linn. f.*
5. (a)	*O. nudicaule Linn. f. var. macrorrhizun (kze) Claussen*
6.	*O. polyphyllum A. Br. apud Seubert.*
7.	*O. vulgatum Linn.*
8.	*O. reticulatum Linn. f reticulatum.*
8. (a)	*O. reticulatum Linn. f. complicatum (Miq). Wiet*
8. (b)	*O. reticulatum Linn. f. dilatatum (Miq.) Wief.*
9.	*O. petiolatum Hooker*
10.	*O. pendulum Linn. f pendulum.*
11.	*O. eliminatum Khandelwal et Goswami*
12.	*O. oleosum Khandelwal*

2. Morphology of the Sporophyte

2.1 Brief Description of Sporophytes of Three Genera

Botrychium

Botrychium commonly known as grape fern, or moonwort, has about 30 species in the world flora. The plant possesses subterraean rhizome with adventitious roots. The roots are fleshy and mycorrhizal. The frond stipe is fleshy and consists of two parts: the vegetative lamina with open dichotomous venation and the fertile spike with large sporangia present at the margins of the fertile segment. Branching of the leaf is sometimes correlated with pinnate branching of the spike (Fig. 1).

Species of *Botrychium* as also of other allied genera *Helminthostachys* and *Ophioglossum* produce a large number of spores per sporangium (1 to 6 thousands). The prothallia of all genera are subterranean, fleshy, mycorrhizal with rhizoids except the gametophytes of *Ophioglossum* which do not show rhizoids at any stage of development.

Helminthostachys

Based on morphological and anatomical studies (Boodle, 1899; Bower, 1926, 1935, Ogura, 1938; Nishida, 1957), *Helminthostachys zeylanica* is placed with *Botrychium* and *Ophioglossum* in the order *Ophioglossales*. However, unlike the other two genera, the spike of *Helminthostachys* is considered to be 'diffused' in nature. And also, it is difficult as yet to offer a morphological explanation of the rosette of vegetative laciniae (Fig. 3, C and E) produced by the sporangiophore, a structure which is unique in the entire plant kingdom (Scott, 1923; Bower, 1926, 1935). Sporne (1970) and Bierhorst (1971) compare these growths with those found in the Carboniferous fern *Botryopteris antiqua* (Scott, 1923).

Based on morphological, anatomical and cytological studies of specimens studied from Kerala, Gorakhpur and

Bhagalpur, Goswami and Khandelwal (1980) have suggested the need for extensive study as the population structure of this monotypic genus revealed variations of morphogenetic importance. For example, above mentioned flaps of sporangiophore have always been figured by all writers, following Bower (1926), as dentate and leaf, structures with stiff margins. In contrast, these flaps were observed as spongy and fleshy structures with smooth margins, their outer portion being parenchymatous and the central portion containing spongy cells (Fig.3C).

Ophioglossum

The genus *Ophioglossum* was discovered in 1620 by Bauhin. Linnaeus (1753) validated the generic status and included it in his 'Species Plantarum'. *Ophioglossum* is a straightforwardly simple plant. Fibrous roots are attached to the solid bulbous corm of 2-10 cm in diameter; corm produces a sessile or pedunculated leaf (tropophyll) which bears an adaxial fertile origan, the spike except in *O. palmatum* where several spikes originate (Fig. 4D) from the peduncle. *O. palmatum* and *O. pedunculatum* are large epiphytic species; the remaining 30-35 species are cosmopolitan, rainy season small herbaceous terrestrials, ranging from a few centimetres to more than 25 cm (epiphytic *O. pendulum* reaches 2.7 m; Eames 1936).

Plants of *Ophioglossum* species appear soon after the first showers in various parts of India and occupy highly variable habitats.

Ophioglossum leaves simple or lobed are characterised by reticulate venation (4 A, B) while those of related genera *Botrychium* and Helminthostachys have open dichotomous venation. Peculiarly enough *O. Kawamurae*, *O. simplex*, *O. lineare* and *O. ramosi* do not have tropophyll (see Nishida 1953). Two plants were also observed at Bhopal in the population group of *O. gramineum* which were devoid of sterile blade. The spike is linear laving sunken sporangia on both the sides.

2.2 Stomata

Pant and Khare (1971) have studied the mode of development of stomata in species of *Ophioglossum, Botrychium*, and *Helminthostachys*. Basically of piper type, the development is perigenous in all the three genera. Frequently the neighbouring cells of perigene are divided by radial walls before or after the formation of guard cells and increase in their number. The meristemoid by two successive walls formed at right angles to each other cutting off two mesogene cells before the meristemoid functions as the guard cell mother cell. The guard cells of a mature stomata are surrounded by complete rings of subsidiary and encircling cells, each ring consisting of several cells. In *Helminthostachys zeylanica* the marginal cells of lower epidermis form dome-like papillae not found in other genera of the group. Similarly, the guard cells in *O. palmatum* show 'lipped projections' (Khandelwal and Goswami, 1977) not observed elsewhere. This is very interesting to mention that stomata of *O. palmatum* (epiphytic giant species, found in USA) resemble more closely with *Helminthostachys zeylanica* than with any other species of *Ophioglossum* or *Botrychium*. Almost all species of *Ophioglossum* possess stomata on both sides of leaf (tropophyll; Mahabale, 1962; Pant and Khare, 1971) except

O.palmatum where stomata are not found on the upper surface. This feature is also observed in H. zeylanica and B. tematum (Khandelwal and Goswami, 1977). The size of epidermal cells (273 x 27µm) and the stomata (110 x 69 µm) in *O. palmatum* are the largest among ophioglossales.

New epidermal features :

As far as known the epidermal features described herein on the leaf (tropophyll) peelings of *Ophioglossum eliminatum* Khandelwal and Goswami are new to the botanical literature. The appearance of this new species of *Ophioglossum* has been understood to have taken place after natural hybridisation (Goswami and Khandelwal 1980; Khandelwal and Goswami 1984) and during the selection for survival, this species has exhibited numerous teratologies of evolutionary significance.

The plants of O. eliminatum which bear healthy spikes, exhibit regular meiosis (ninety bivalents) and in majority, spores are normal, grow under shady moist areas while the plants growing on forest road sides and or towards exposed areas are either sterile or their spikes and tropophylls shrivel and never reach maturity. At that time, it was speculated that hybrid population is exhibiting selection by way of varied expressions (Goswami, 1983).

In order to find out the basic similarities and also differences for comparison of this new species with the other species of the area we made a comparable approach for epidermal features as per techniqures described earlier for six species of the genus (Khandelwal and Goswami, 1977). These plants, which are suspected to be hybrids possess very high stomatal frequency, when compared to species growing in the same area (Table 8). There is a remarkable difference in the stomatal index of this species when compared with *O. costatum* and *O. nudicaule* with which *O. eleiminatum* resembles most.

The tropophylls (sterile leaves) have further shown the presence of two unusual features viz.: (a) Twin stomata, which are bodily fused from several sides (Fig. 14) and (bP) "hyadothode-like" structures/ openings scattered randomly over the upper surface. The last feature (Fig. 15) is comparable to none of the structures described so far from either surface of any leaf. These structures can neither be regarded as "dismantled" stomata nor even hydathodes which are normally present at the tip ends of a mid vein. On the young leaves, these structures

TABLE 8—STOMATAL FREQUENCY IN SIX SPECIES OF OPHIOGLOSSUM
(Average of both surfaces)

	No. of leaves studied	Stomatal index (average)	Leaves with Twin (fused) stomata and tear apertures	Chromosome number
1. *O. costatum*	207	10.5	—	n=120
2. *O. gramineum*	210	10.2	—	n=120
3. *O. thermale*	150	8.5	—	n=360
4. *O. nudicaule*	300	12.4	—	n=120
5. *O. lusitanicum*	125	13.2	—	n=240
6. *O. eliminatum*	400	19.5	0.92	n=90

appear as a demarcated area of thin walled parenchymatous cells surrounded by thick walled elongated cells appearing like irregular palisade tissue. When the leaf matures the thinner cells are "teared apart" resulting in an opening (Fig. 16) which becomes broad like a canal, obviously, bringing faster release of water vapours. As a matter of fact these lezves must have an accelerated mechanism of evaporation of water vapours and minerals. Needless to mention, these plants have a differential selection pressure; the plants which grow in shady moist areas survive, bear normal spikes/spores while the plants exposed to open atmosphere might loose their water and minerals at a very fast rate resulting in early death of plants. This indicates that *O. eliminatum* characterised with n=90 chromosomes possesses in its 93% tropophylls, highly unusual "twin stomata" and "tear apertures" or canals (Fig. 4) which result in excessive loss of water. Thus plants which grow in moist shady areas survive while exposed ones are being eliminated. These new structures also explain how *Anabena* cells enter into the mesophyll cells of Ophioglossum tropophylls (Fig. (5).. We have surveyed leaf peels of four species of *Ophioglossum* but only these plants which have shown such features exhibit intracellular *Anabena*. This is another outstanding. feature. Under fluorescence, after staining the blue green cells look like "pearls".

The genetic mechanism resulting in evolution of these features has offered O. eliminatum are extremely rare features, probably never recorded elsewhere in any living or a fossil plant.

Fungal infection: Report of fungal infection has been extremely rare on any members of *Ophioglossales*. Among the species occurring in India *Curvularia* has been reported to infect *O. vulgatum*, while we have collected leaves of *O. vulgatum*, *O.nudicaule* and *O. polyphyllum* infected with *Fusarium* species. Entry of a demataceous fungus was observed from the leaf seedlings of a small plant of *O. palmatum* which was kept alive for about ten days in the laboratory.

2.3 Spike and tropophyll

The nature of the spike is a century old moot question. Chrysler (1941) has reviewed the early literature on the topic, and he advocated the view propounded by Roeper (1859) and Holle (1857) that the fertile spike represents two fused basal pinnae of a leaf, of which the rest of the pinnae are sterile. Zimmermann (1942) has interpreted the fertile spike as being 'telomic' in nature and has suggested that both the fertile and sterile segments of the frond represent modified branch systems. Chrysler (1945) was impressed by the telome theory (Zimmermann, 1930) and mentioned that the shoots of *Botrychium* appeared to be of a dichotomous construction. This view was further elaborated by Nishida (1952, 1957) who described both segments of the frond as 'derived telomes and the common stalk of the two as a 'mesome'. Troll (1933) had however opposed the brach like nature of the spike and proposed that the frond represents a peltate leaf with both the sterile and fertile segments being basically parts of the same leaf. Although many views regarding the nature of *Ophioglossacean* spike have been adavnced, exact information on its phylogenetic development may not be obtained due to the complete lack of fossil evidence (Surange, 1966). I however suggest that variables within the population structure which have been repeatedly assigned to involvement of genetic mechanisms speak of past and future relatives. Surveys of natural populations conducted over several years have indicated that the genomes of *Helminthostachys* and *Ophioglossum* (in particular) are exerting detectable changes leading to evolution of the respective genera. Some of the interesting abnormalities are presented below and their significance is discussed at the end. Morphogenetic significance of teratologies is discussed separately. This is worth mentioning here that present data support pinna nature of the spike.

Tropophyll

Chrysler (1925) while discussing the 'pinna nature' of fertile spike of Ophioglossaceae, considered the occurrence of sporangia on tropophylls (sterile fronds) of *Botrychium* and *Helminthostachys* to be of evolutionary significance. Certain abnormal features of tropophylls are presented hereunder, which are also abundant in populations of *Ophioglossum.*

(a) *Sporangia on tropophyll* : The tropophyll is essentially a sterile region, but recently, sporangia have been observed on their margins (Goswami and Khandelwal, 1973a).

(b) *Lack of tropophyll :* Nishida mentioned six species of *Ophioglossum* known to lack tropophyll. These are *O. simplex, O. inermedium, O. linerae, O. ramosii, O. Kawamurae and O. nipponicum*. None of the Indian species belong to this category except three plants of *O. gramineum* collected in 1983 on hill tops at Bhopal.

(c) *Forked tropophyll* : Forked tropophyll have been observed in *O. vulgatum* (Vasistha, 1927), *O.costatum* and *O. petiolatum*. It is recorded that such plants do not bear spikes (Fig 2B).

Spike (Fig. 5)

The spike of *Ophioglossum* is simple or occasionally equally or unequally dichotomously branched. The sporangia are fused laterally into elongate synangia. But each has an independent vascular supply. The fertile spike which bears the sporangia is borne adaxially on the sterile frond at variable distances from the stem. By and large, the spikes are quite conservative and hence abnormalities in the distribution of sporangia are the most important. (Bower, 1926). Some of them are quite important.

(a) *Branched or forked spike:* Few branched spikes were observed by Bower (1926) in *O. pendulum* and *O. palmatum*, Nishida (1957) in *O. nipponicum* Mahabale (1962) in *O. pendunculosum* sensu Prantl, non Desv.(= *O.petiolatum* Hook; Panigrahi and Dixit, (1969); Pant and Khare (1971) in *O. reticulatum* and *O. gramineum* and recently by Goswami and Khandelwal (1973a) in *O. costatum, O.nudicaule*, *O. gramineum*, *O. lusitanicum* and also in *O. eliminatum*. This appears to be the commonest abnormality because it is frequently recorded in species found in Britain, Japan and India. A plant of *O. lusitanicum* exhibiting the origin of two spikes from two twisted interlocked petiole has been a rare observation. Partial branching is also seen in *Helminthostachys*.

(b) *Twisted spike:* Twisted spikes have been reported in *O. costatum, O. vulgatum* and *O. nudicaule*. This is recorded to be due to a large number of anucleate spores in the sporangia situated in the middle of spikes thus making upper and lower halves heavier than the middle portion.

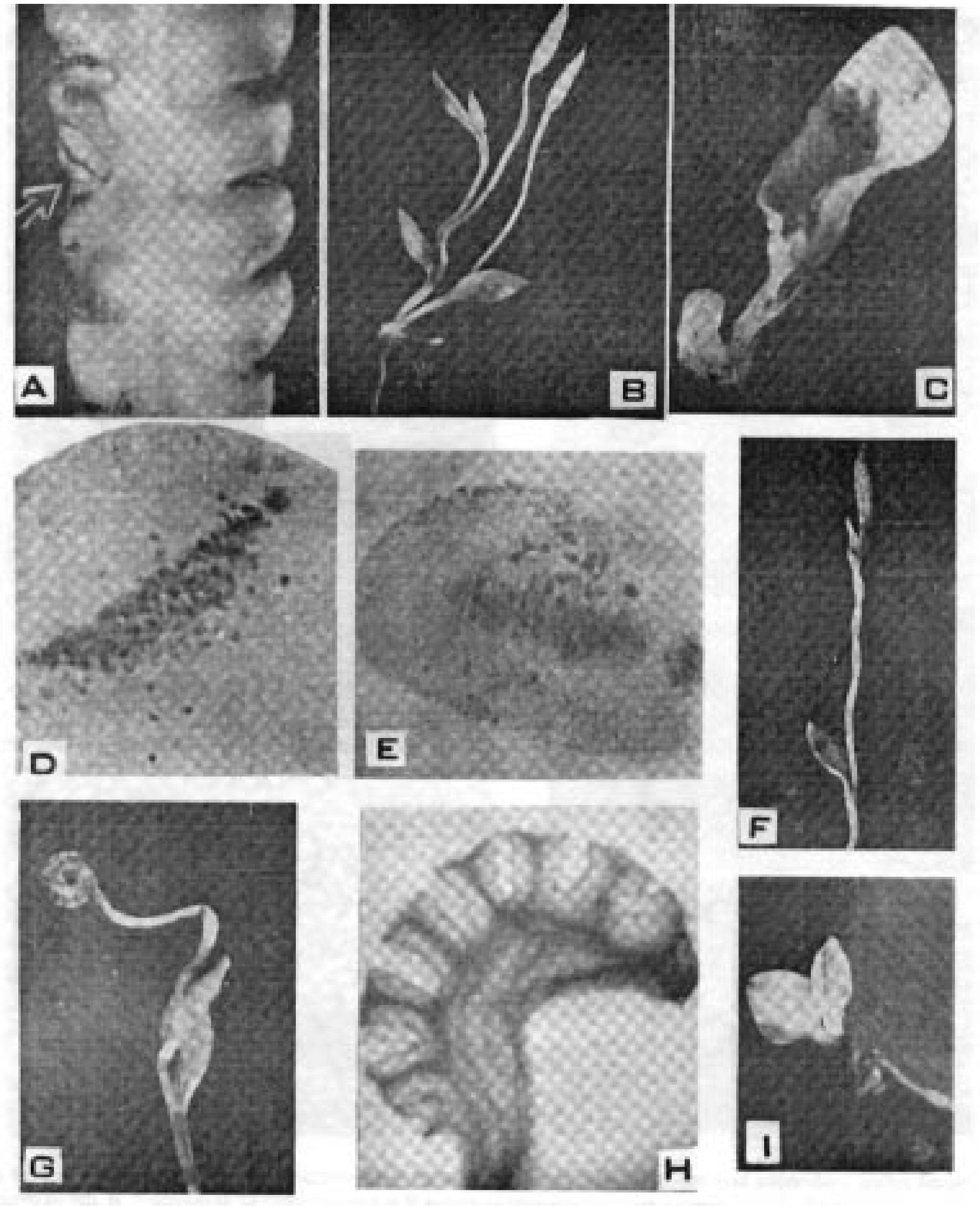


Fig. 5 — A. A mature and dehisced spike. Note the bifid slit indicating the presence of more than one sporangium in a socket. B. *O. iusitanicum* showing normal and trifurcated petiole bearing three spikes (× 1). C. an enlarged funnel shaped tropophyll of *O. eliminatum* (× 2). D. Lagging bivalents and univalents at metaphase I in *O. eliminatum* (× 1700). E. Highly irregular segregation at early metaphase I in *O. eliminatum* (× 1700). F. Two spikes originate from two twisted interlocked petiole in *O. Iusitanicum* (× 2) G. A circinately coiled spike (extremely rare feature) orginating from a deformed hafe sterile (× 2) in *O. Iusitanicum.* H. A fan shaped half fertile and half sterile spike showing unusual venation in *O. eliminatum.* The upper fertile portion shows perfect dehiscing and vasculature below the sporangial seat (× 4) I. an unusual plant of *O. Iusitanicum* showing stunted spike (× 1).

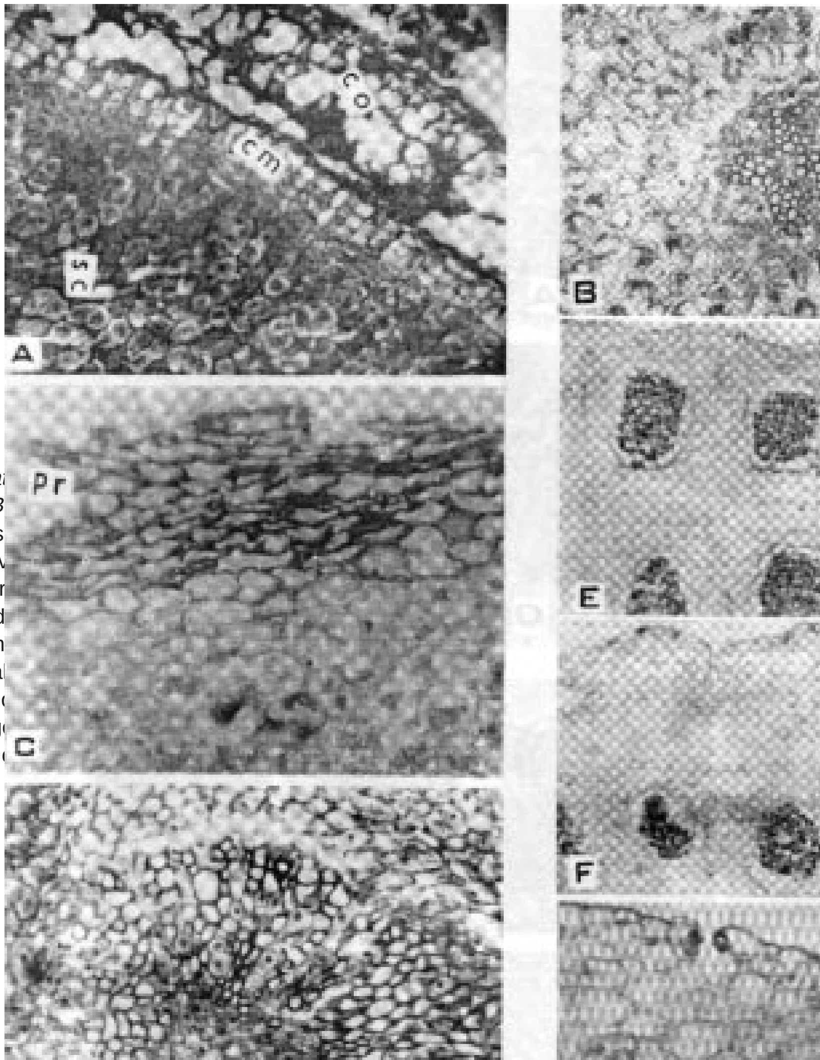


Fig. 6 — A. Transverse section of rhizome of B. *terna*
at maturity (x 30). B. Transverse section of a root of *B*
section of *H. Zeylanica* showing periderm (pr.), the s
Transverse section of *B. ternatum* showing secondary v
of a spike of *O. eliminatum* showing sporangia with spor
and annulus like opening slit formed from long thick and
section of one sided fertile spike showing presence of n
sporangia on the other. Sporangial areas can be visual
20). G. An enlarged portion of stomatal and slit area. N
wall layers and mode of arrangements of cells are sugg
not as a structural part

(c) *Displaced spike/sporangia:* (i) A few spikes of *O. costatum* show fertile sporangia even at the tip (Goswami and Khandelwal, 1973a). This feature is, otherwise common within only *O. pendulum, O.intermedium* and *O. simplex* none of which is recorded from India.

(ii) Rather infrequently, certain spikes show more than one sporangia in a 'socket' in case of *O. costatum, O. eliminatum* (Fig. 5 A) and *O. gramineum* exhibiting thereby 'fission' or failure in fusion to form synangium (see Goswami, 1978).

(iii) A single plant, resembling *O.costatum*, exhibited broad, flattened leafy petiole with ill developed venation (Fig. 2A). An unhealthy spike originated from the flat tropophyll at a displaced position. This specimen reminds an idea as if a 'spike' were a modified fertile leaf segment.

(iv) A mature spike originated from the twisted petiole in *O. lusitanicum* (Fig. 5F).

(v) Few plants of *O. eliminatum* show that a healthy mature spike can originate from the ill developed feat.

(d) *Abnormal spikes:* (i) *Leafy spike:* The plants of *O. costatum* and *O. eliminatum* demonstrated leafy spike borne on healthy petiole. This possessed outlines of sporangial positions without much differentiation of sporogenous tissue.

(ii) *Spike with there sided synangia:* A portion of single spike showing the dehisced condition on one side and two rows showing early development on other side. Note the shattering of vein like structure on the lower area (Fig. 5H).

(iii) *One sided curved spike:* Extremely interesting specimens of *Ophiolossum eliminatum* have been collected which show 'circinately coiled spikes' exhibiting unilateral, growth of sporangia. The other side of the spike did not develop (Figs.3 F and 6 F).

(iv) *Mature spikes with sterile sporangia:* Sterile sporangia may occur on normal spike in various positions.

(v) Inverted spike: A few plants belonging to *O.eliminatum* possessed inverted spikes (due to bending of petiole).

2.4 Sporangia and spores

Spores

All genera of Ophioglossales are homosporous. Common characteristics of spores of various species are: spores free, anisopolar, radiosymmetrical and chlorophyllous.

Detailed studies on spore morphology have revealed following interesting abnormal features:

(i) *Dimorphic spores:* The occurrence of spores differing in the type of laessurae in one and the same sporangium (alete and monolete) has been reported recently in *O. reticulatum* (Pant and Khare, 1971), *O. vulgatum* (monolete and trilete), *O. costatum* (alete, monolete and trilete) and *O. eliminatum* (alete, monolete, bilete and trilete). More recently, spores differing in wall layers, area of contact and shape of the laessurae have been detected in *O. costatum* strain 'B' *O. vulgatum*, *O. nudicaule* and *O. eliminotum*. For example, in *O. costatum* there are two types, the first type of spores show 4 layered exine, clear intine, straight laessurae and thickly ornamented are of contact; while the second type of spores A posses inseparable intine, sinuous laessurae, and thin and smooth area of contact.

(2) Joint spores (Fig.7): Joined inseparable spores have now been recorded in *Ophioglossum*. Prior to this, such a feature was known only in *Isoetes* among the Pteridophytes and in certain bryophytes like

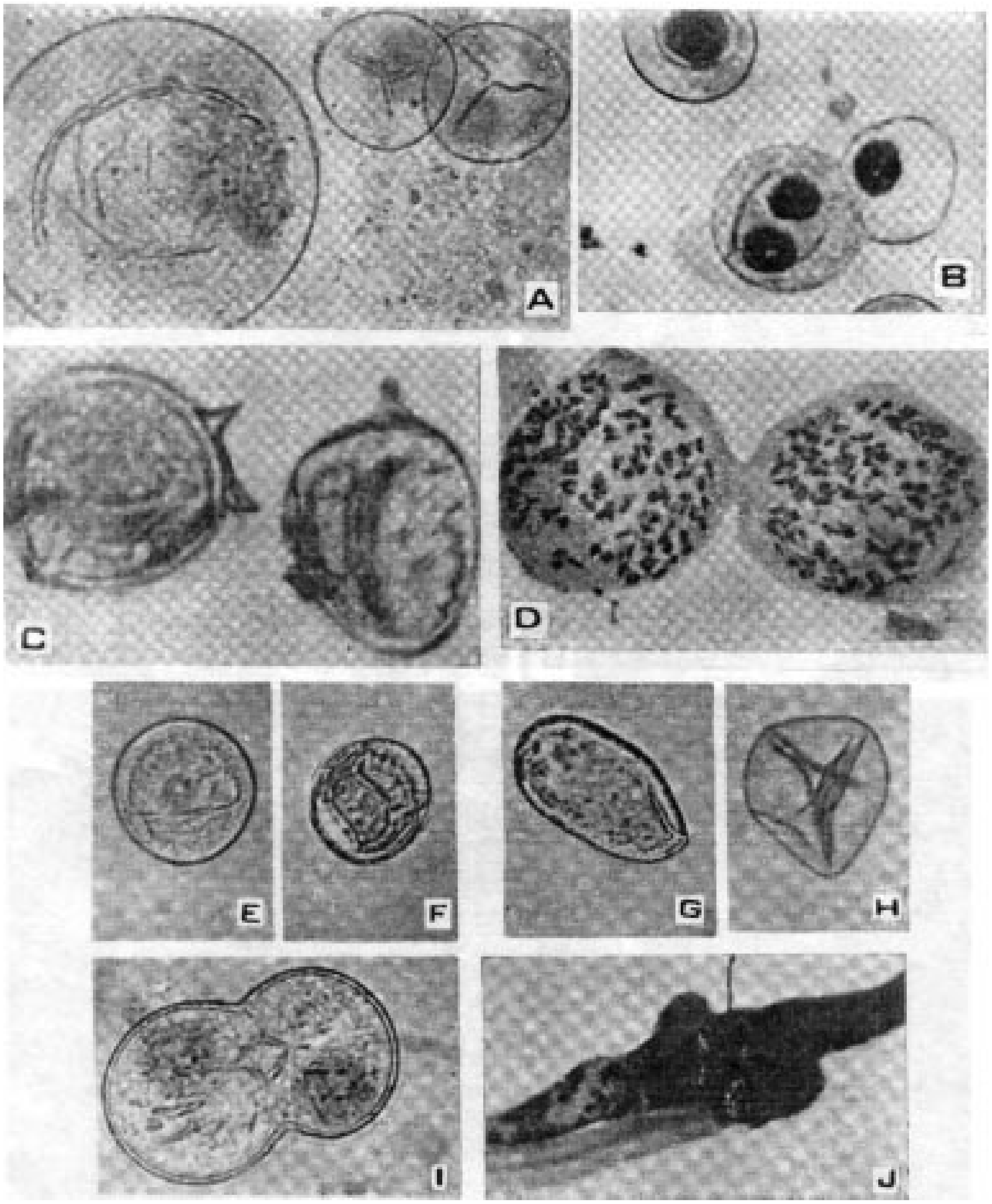


Fig. 7 — Abnormal spores in *O. eliminatum* (A-H; x 1700). A. A large and two small spores, the large spore may be an unreduced sporocyte developed into spore with irregular laesurae; the two trilete spores. B. Three spores with four nuclei stained with aceto-orcein, the binucleate spore explains the large size. It is possible that the large spore in A could be a binucleate spore. C. Spores with spiny outgrowths on their coat, monolete laesural have thickened area. D. Mitotically cleared sporocytes showing joining and simultaneous meiotic division; bivalents in earl diakinesis in both cells count Ca 90+3. E. A monolete spore (F) irregular bilete spore.OG. Irregular oblong spore. H. Abortive spore with double trilete laesural. I. A Siamese twin spore in *O. Costatum*, each spore has a nucleus. J. Gametophyte of *O. eliminatum* showing dark areas of sex organs and a broad bulbous base.

Riccia and *Sphaerocarpus*. Inseparable pollen grains are sometimes observed in few angiosperms (see Erdtman, 1952). We have been unable to observe them in *Botrychium ternatum* and *Helminthostachys zeylanica*.

(3) *Abortive and irregular spores:* Misshapen, ill formed and anucleate spores would be nonviable. The record of such spores, particularly in higher frequency in the plants which possess certain abnormalities of spike or sporangia (Fig. 5) emphasizes the involvement of some genetic mechanism resulting in the formation of abortive spores. A fact that abortive spores do not possess sexine layers has further strengthened such a possibility (Goswami and Khandelwal, 1973b).

2.5 Significance of teratological features

All spike abnormalities (Figs. 3 and 5) viz: ill developed spike originating from the flat leafy petiole, spike showing three sided sporangia, one side circinately coiled spike, spike showing one sided sterility, and spike showing one sided sporangia suggest genetic inefficiency of the individuals to reproduce by sexual means. Probably, during recombinations accompanied with mutations there has been a display of certain highly unusual features (especially one sided circinately coiled spike, and spores having tubular spore coat). Such teratological features should decidedly have evolutionary significance and indicate that the characters, either should have been possessed by some ancestors or might be present in future generation if the trait survived the cost of natural selection.

The evolutionary significance of teratology was held in high esteem in the times of Goethe, De Candolle, Moquin Tandon and Braün. While there are contradictory statements as well (Carlquist, 1969), many morphologists uphold even today the high significance of teratology (see Guedes and Dupuy, 1974). Sahni (1933) recorded abnormal funnel shaped leaves in Ginkgo and compared them with several Mesozoic fossil leaves exhibiting Gink gaolean features and also with cupules of some Pteridosperms. Posthumus in 1728 (see Sahni, 1933) had shown that certain fossil fern leaves viz: *Dictyophyllum* and *Camptopteris* resemble those of some living Dipteridineal in a peculiar twist in the base of the lamina.Heslop-Harrison (1952) has pointed out that, apart from terata representing multiple distortions which may be useful in analysing normal growth, examples existed where two or more well defined avenues of development were opened simultaneously to a structure. These teratologies could not reveal the actual ancestors of any present structure, they at least indicate existing homologies between them. The coiled spikes exhibit gradual sterility and one side bending is due to the weight of sporangia resulting in one side curvature. It can be mentioned, referring to the belief of Troll and Chrysler, that enumeration of leafy spikes, a feebly developed spike from the leafy petiole and 'circinately coiled spikes' is like a demonstration of pinna nature of fertile spike. Few spikes demonstrate a tendency of flattening and veins are seen shattered on the widened structure. Again, a demonstration of leafy face becoms apparent (Figs. 2, 3, and 5).

2.6 SEM Studies

Spore morphology has not revealed highly distinctive features under scaning electron microscope. Nevertheless attempts are being made and as shown in Fig. 8A

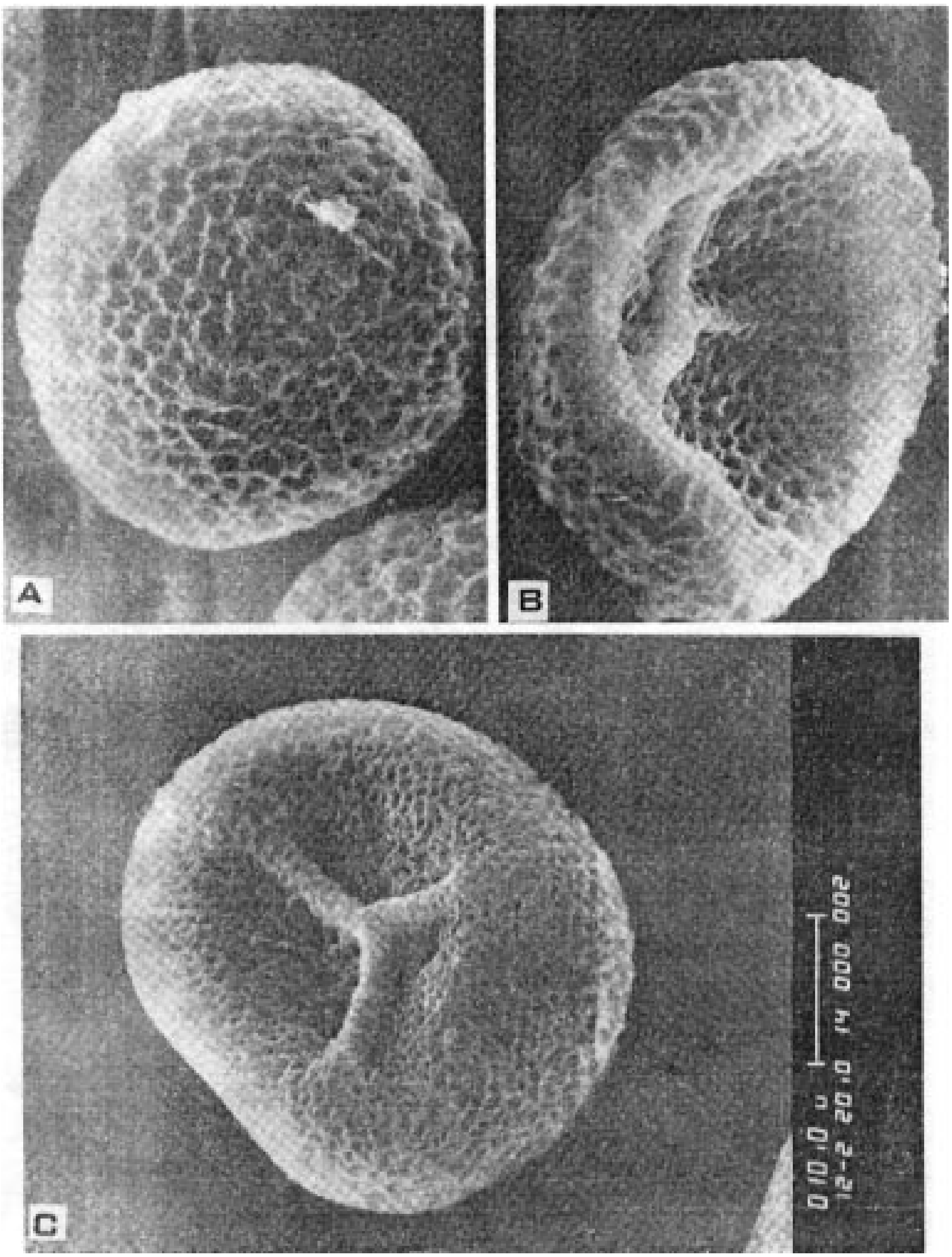


Fig. 8 — Scanning electromicrographs of spores. A. Distal face of a round spore of *O. eliminatum* showing webbedmesh surrounding pentagonal cavities in the exine. B. Proximal face showing depression, the triradiate spore appears round bowl shaped structure. C. Proximal view of a triradiate spore of *O. costatum* depression on the proximal face is not much pronounced.

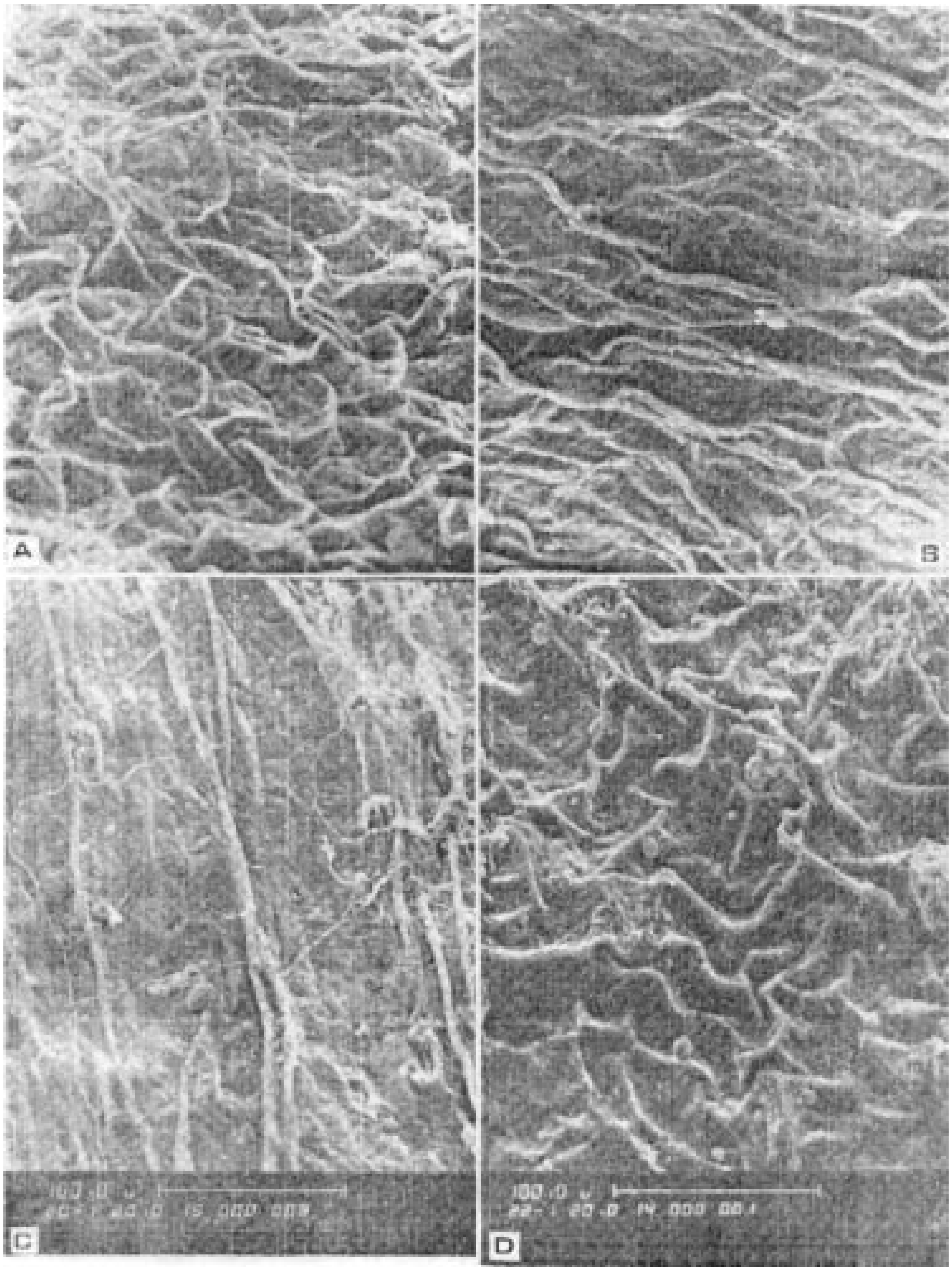


Fig. 9 — A. *O. nudicaule* showing broken interwoven undulating epidermal mesh forming short cover areas. B. *O. vulgalum* showing half parallel running undulating epidermal mesh forming long irregular cover areas. C. *O. gramineum* showing thick interwound, mesh fibres running parallel, forming long rectangular cover areas, note the striped smooth epidermal surface. D. *O. caslalum* showing undulating broken epidermal mesh with uneven distribution of globose epidermal hairs.

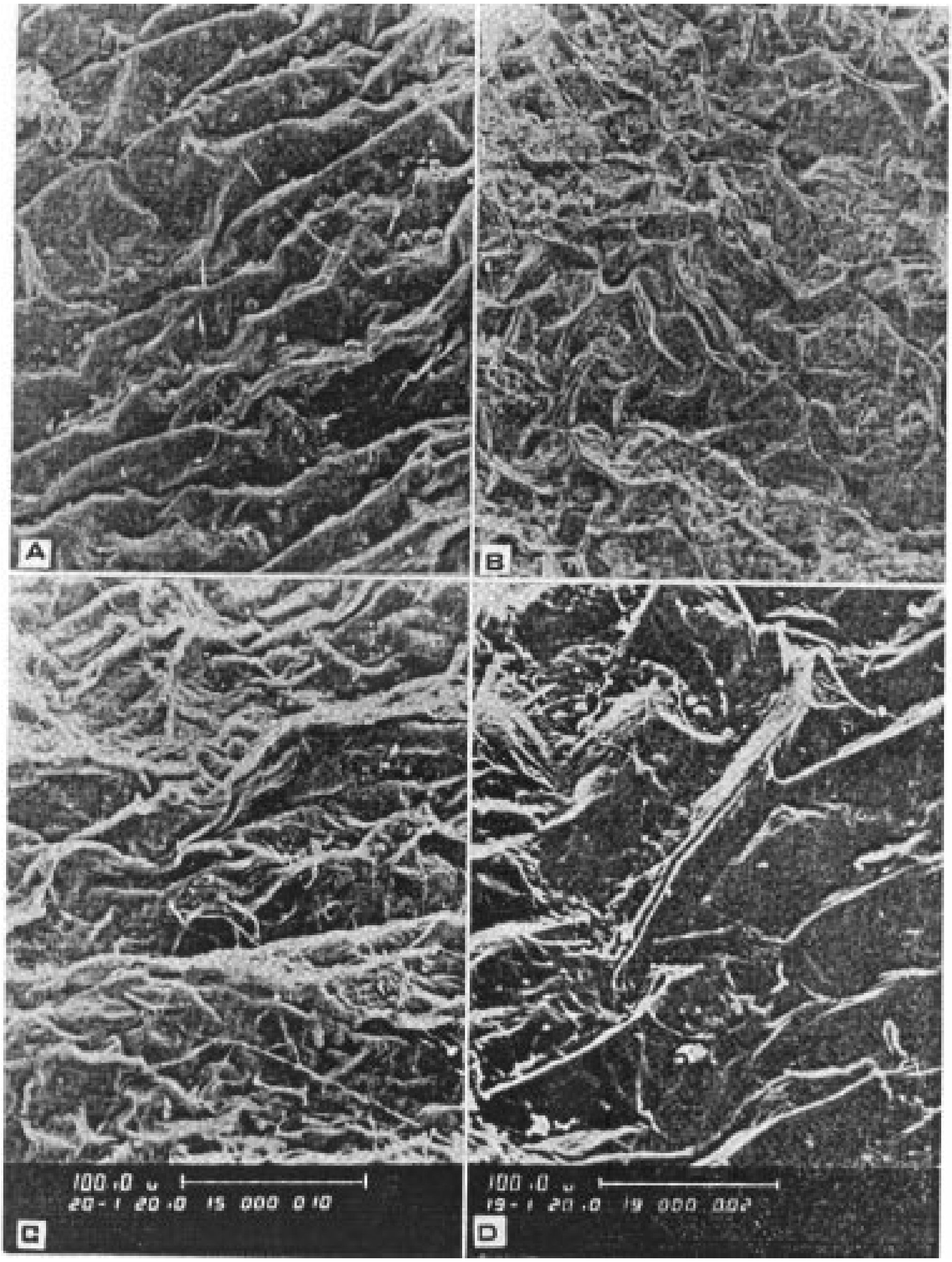
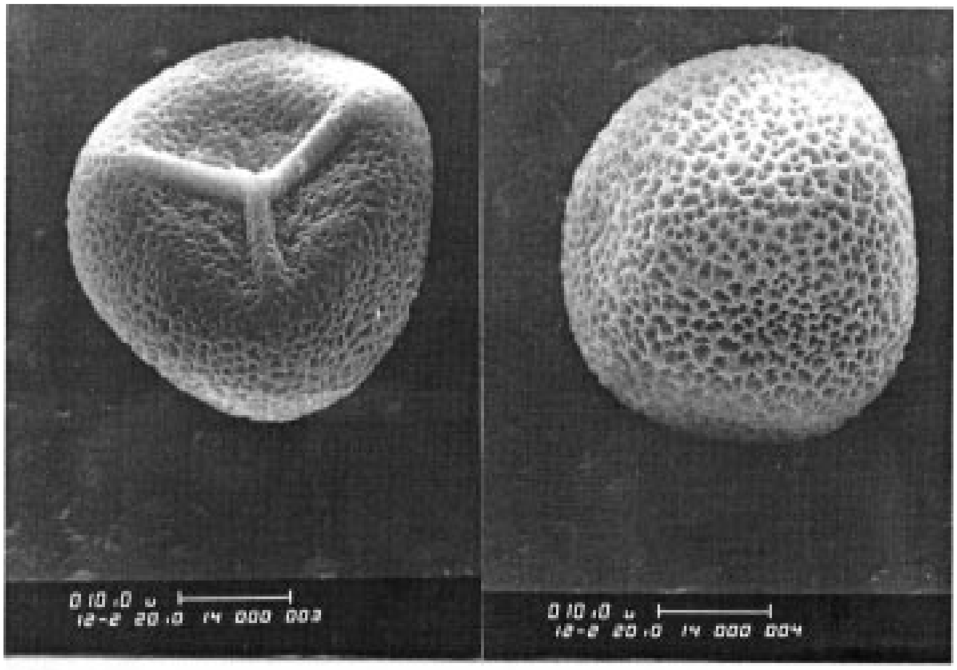
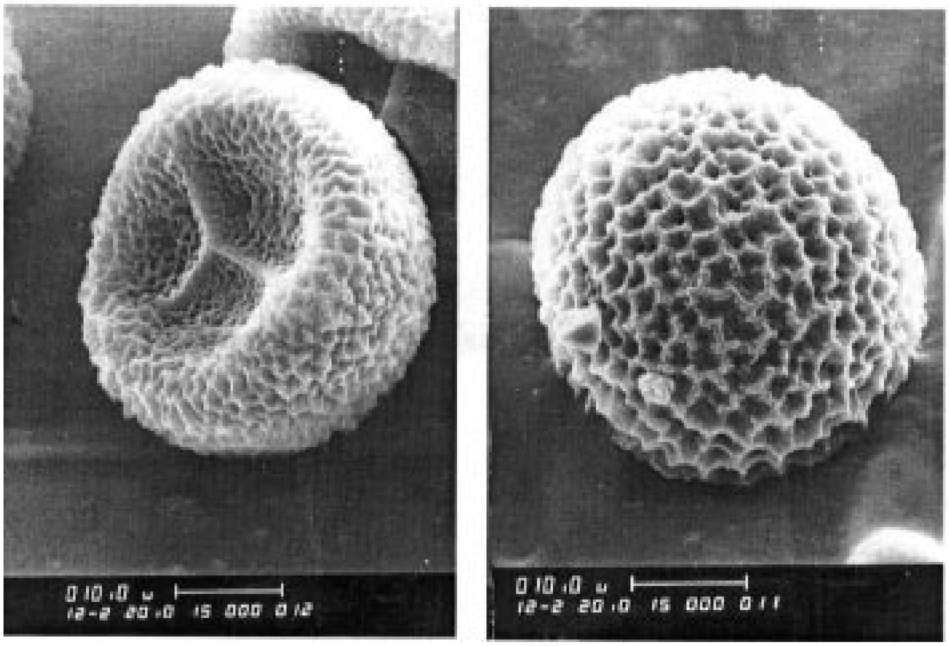


Fig. 10 — A. *O. polyphyllum* showing epidermal mesh aggregations to from ridges and furrows, uneven epidermal hairs arising in furrows. B. *O. lusitanicum* showing regularly branched epidermal mesh with open dichotomies, epidermal surface not smooth. C. *O. petiolatum* showing irregular mesh, epidermal surface uneven. D. *O. palmatum* showing short epidermal mesh fibres of variable thickness forming ridges and furrows, epidermal surface itself undulating, epidermal hairs elongated but too few in number.





Proximal and distal faces of spores under SEM :

Fig. 32 — *O. costatum* Proximal face and distal face

Fig. 33 — *O. petiolatum* Proximal face and distal face

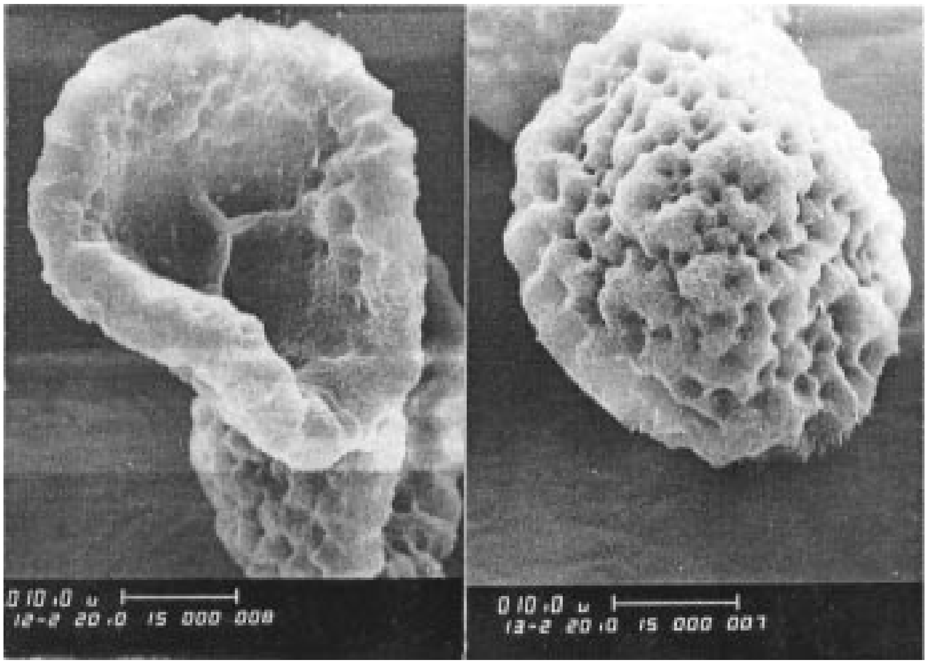


Fig. 34 — *O. gramineum*

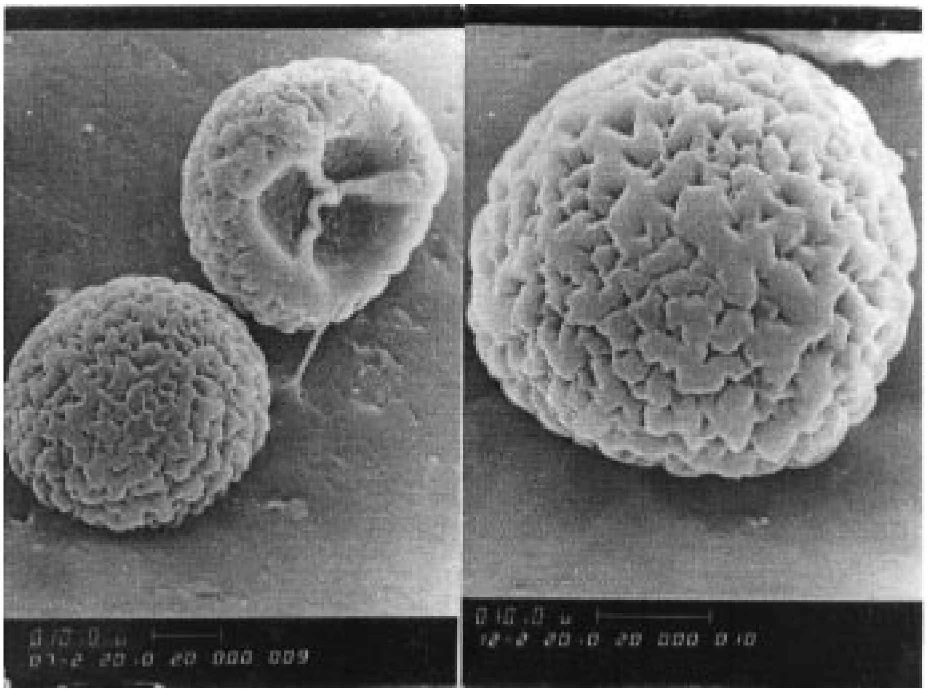


Fig. 35 — *O. polyphyllum*

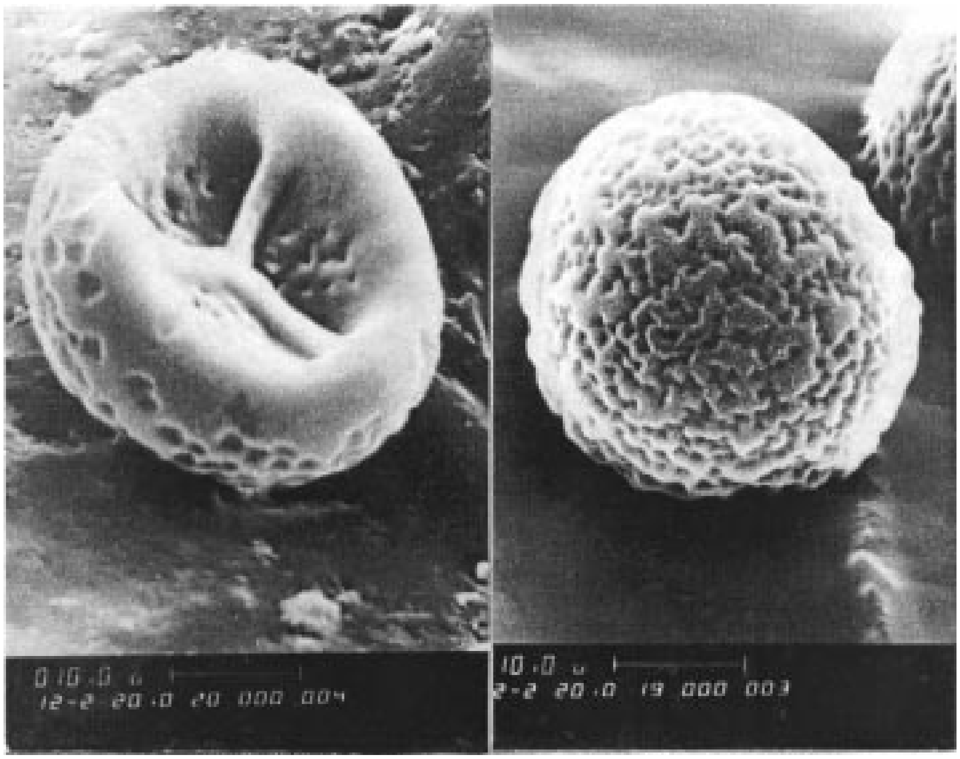


Fig. 36 — *O. vulgatum*

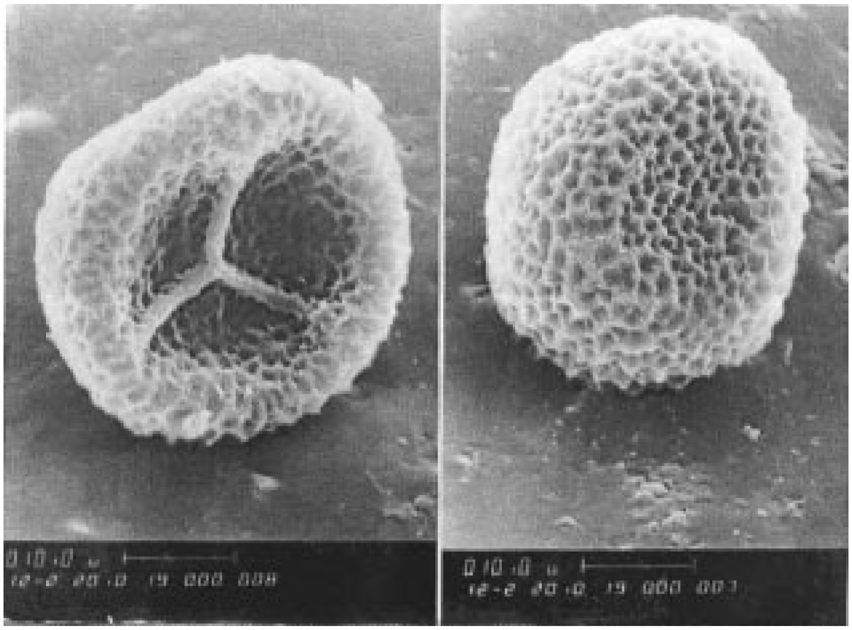


Fig. 37 — *O. eliminatum*

spores of *O. eliminatum* differs from the spore of *O. costatum* (Fig. 8C) in having deeper and broader webbed mesh around pentagonal depressions on exine. Triradiate marks also show minor differences especially in thickness but as is known, spores of the same sporangium can show such differences. Therefore it is difficult to base diagnosis exclusively on S E M studies (Table 10) although spores of *O.gramineum, O. polyphyllum* and *O. vulgatum* are distinct from *O. costatum* and *O. eliminatum.* Exine surface has more flat and wider areas (Figs. 34, 35 & 36).

For some species of *Ophioglossum* one can depend more on epidermal features. Figs. 9 and 10 are the scanning electron micrographs of upper surface of tropophylls of eight species of *Ophioglossum* which at a glance, appear as surfaces of "dried leaves". Differences in the epidermal mesh alignment and hairs are the two major features worthy of consideration.

Epidermal surfaces of *O.nudicaule*, *O. costatum* (Figs. 9, A,D) *O. polyphyllum* and *O. lusitanicum* (Fig. 10, A, B) exhibit broken and unulating epidermal mesh while, surfaces of *O. vulgatum, O. garmineum, O. petiolatum* and *O. palmatum* reveal essentially parallel nature of epidermal mesh forming fibrous net. The placement of epidermal hairs is not uniformly consistent in all species.

In fact, this brief description of SEM observations should only help us in making more elaborate studies on other genera and species. Certainly one would be able to offer a better insight into the surface marking of spores and leaf surfaces.

3. Anatomy

Young plants are protostelic with a large pith. The pith is intrastelar in origin, which in the developing phases shows mixed occurrence of tracheids with pith cells. In *Botrychium* and *Helminthostachys* the stele is simple but it is complex in *Ophioglossum*. The leaf traces are large single strands and form prominent gaps in the stele but in *Ophioglossum*, the stele is broken up into many strands. Often, leaf trace consists of several bundles. Sclerenchymatous tissue, is absent, hence the plant body is 50ft in all the three genera. Some specific features are presented below.

3.1 ROOT

Roots are succulent, sparsely branched, and devoid of hairs. They are micorrhizal and probably the fungus provides most of their absorbing surface.

In cross section, roots show massive cortex (3G and 6B) which is delimited from the central stele by an endodermis possessing characteristic thickening on the radial walls.

The stele is reported to be hexarch or heptarch in *H. zeylanica* but tetrarch condition is also observed. In *B. virginianum* and *B. ternatum*, the stele is tetrarch or triarch. But most species of *Botrychium* and Ophioglossum possess monarch or diarch stele.

3.2 RHIZOME

Ophioglossaceous stems are subterranean, but they are vertical in *Botrychium* and horizontal in *Helminthostachys* and *Ophioglossum*. These are soft, fleshy and mycorrhizal and like other parts of the plants, devoid of sclerenchyma. In cross sections (Fig. 6, A, C, E) all Ophioglossaceous stems have a definite stele in the basal region of the axis delimited by a rudimentary endodermis. The stele is an ectophloic siphonostele surrounding prominent leaf gaps (Bower, 1926). In many species of *Ophioglossum* the gaps are very extensive and overlapping, thus resulting in a distinct dictyostele or rudimentary type of dictyostele. Primary xylem maturation is mesarch in *Helminthostachys* and endarch in other genera.

3.3 Periderm

Periderm is a characteristic of stems, branches and roots of gymnosperms and most dicotylednous plants. During recent years the periderm has acclaimed wide evolutionary significance on account of its being present in progymnosperms (Khandelwal and Goswami, 1978). Among pteridophytes it is observed in woody lycopods, sphenopsids and *Botrychium* (Bower, 1926; Eames, 1936; Sporne, 1970; Bierhorst, 1971), but is absent in the Filicales. None of the earlier workers have authentically reported periderm in roots of *Botrychium* and in rhizomes of *Helminthostachys* and *Ophioglossum* although Boodle in 1899 (see Smith, 1955) had described small amount of secondary thickening in the stem of *O. vulgatum* and Maheswari and Singh (1934) mentioned it in *O. costatum*.

There has been in fact no demonstration of functional cork-cambium because as mentioned by Fahn (1967) periderm accompanies the development of secondary vascular tissue which was not described by earlier workers. However, difference states of activity of cambium and definite presence of periderm in some roots and rhizomes of certain plants of *Botrychium*, *Helminthostachys* and *Ophioglossum* under natural field conditions (Figs. 6, A, C,E) (Khandelwal and Goswami, 1978; Goswami and khandelwal, 1980b) have been now described.

Periderm in root

Secondary thickening in roots is completely unknown except that experimentally induced by Peterson (1971) in *O. petiolatum*. He observed a periderm like tissue in roots by the effect of Benzyladenine and 2, 4-dichlorophenoxyacetic acid. Incidentally we have observed secondary thickening in the extrastelar region in roots of *B. ternatum* under field conditions. Periderm is formed by the activity of phellogen (cork cambium) which originates in outer cortex and produces phellum (cork) externally. The chemical diagnosis by staining with Sudan IV. Further confirmed that the tissue in question is periderm (periderm consists of suberized cells which stain red with Sudan IV). Natural occurrence of periderm in roots of *Ophioglossum* continues to be unknown.

Periderm in rhizome

The genus *Botrychium* is known to possess secondary vascular tissues. Goswami and Khandelwal (1980) have observed secondary thickening in the stelar and extrastelar regions in *B. ternatum* and also encounter periderm in the rhizomes of *H. zeylanica*, *O. costatum, O. vulgatum, O. petiolatum, O. nudicaule, O. polyphyllum, O. gramineum* and *O. lusitanicum*. It must be indicated that not all plants of the same population reveal periderm although each species produces some plants with periderm. Such variability also explains that plants do reproduce sexually besides of course the production of clones by vegetative means. This also explains why the earlier workers missed this tissue and assigned it to be typical mainly of *Botrychium*.

Significance of periderm

Triloboxylon halli (Arnold) Scheckler and Banks is the oldest plant in which periderm has been found to be present. In younger plants from lower upper Devonian strata, the periderm has been reported in *Triloboxylon ashlandium, Tetraxylopteris schmidii* and *Proteokalon petryi* all belonging to Aneurophytales of Progymnosperms. Periderm in *Botrychium* has often lead to speculation that Ophioglossales might have arisen from certain progymnosperms or from coenopterids (Bierhorst 1971).

Our observation of periderm in three genera, *Ophioglossum, Botrychium* and *Helminthostachys* suggests that the periderm production is a typical feature inherent in Ophioglossales. Obviously, this group is anatomically different from Filicales, besides possessing many dubious relationships already known (see Bierhorst, 1971). With a view to find out the frequency of *Ophioglossum* plants possessing periderm a series of hand cut and microtome sections were stained with Sudan IV. Observations on 400 plants corresponding to eight species of *Ophioglossum* had indicated that all plants do not possess periderm. In other words, each population possesses certain plants which produce periderm (nearly 2022%).

Recently the role of environmental factors (such as day-lenght period, mechanical injury etc.) has also been studied in periderm production in certain angiosperm genera (Waisel *et al*, 1967; Borger and Kozlowski, 1972, a, b, c). Borger and Kozlowski have also stressed on physiological control over the development of secondary xylem and periderm production.

The plants of *O. costatum, O. nudicaule, O. gramineum, O. petiolatum, O. lusitanicum* have been collected from open areas measuring 3-5 square feet mainly from isolated hill tops. We thus believe that presence or absence periderm in *Ophioglossum* plants cannot be accounted due to environmental influence but they indicate internal (and physological) differences. This is also true for rhizome of *Helminthostachys* and rhizome and roots of *Botrychium* and we opine that the trait of production of periderm exhibits genetic segregation. All three genera and polyploids exhibit variability in expression suggesting common genes among them.

Mode of inheritance can be conclusively proved only by breeding experiments, but it is possile to conclude that periderm production is inherent within the gene pool, even by population studies.

3.4 SPIKE ANATOMY

Attempts in the past have also been made to bring about the origin of the vascular supply in fertile spike with a view to support certain theories. Mode of origin of vascular supply for sterile and fertile regions in the spike of *Botrychium virginanum* goes to support the theory of Roeper that the fertile spike represents the two basal pinnae of the fern-like leaf which have been fused. In *Ophioglossum lusitanicum* three petiolar strands are found disposed in a semicircle (Nishida, 1957). The extreme right and left go to the fertile spike while the other extend supply to the sterile ones. The section *Ophioderma* and *Cheiroglossa* of *Ophioglossum* show similar situation which can be regarded as derived from Botrychium. Yound plants of monotypic genus Helminthostachys, according to Lang (1912), show the presence of two leaf traces resembling the condition found in *Botrychium*.

Nishida (1957) mentioned that the vascular behaviour in all species of *Ophioglossum* seems to be similar in principle to that in *O. vulagatum* and the vestige of the second dichotomy was found in all of them except *O. costatum* group. The occurrence of two leaf traces in *O. aitchisnii*, (= O. polyhyllum) *O. costatum* and *O. englemanii* was interpreted as the result of second dichotomy. With this type of exhaustive anatomical background Nishida designated petiole as 'phyllomophore' and defined it as "an organ in Ophioglossales bearing the fertile and sterile fronds which are derived by anterio-posterior dichotomy from telomes and mesomes of the archaic pteridophyta" (Nishida, 1957, p. 202). He further emphasized that phyllomophore was not a mere petiole but stem like organ, homologous to rhizome derived from mesomes. Thus in principle, Nishida (1957) agreed with Maekawa (1948) on that the rhizome, fertile stalk and sterile stalk resemble respectively to the short branch, peduncle and petiole of *Ginkgo*.

4. Morphology of the Gametophyte

It is said that gametophytes of Ophioglossaceae are collected after difficulty. Not many workers have been able to locate them because of their geophilous nature, small size and superficial resemblance to pieces of roots and rhizomes (Cambell, 1907, 1911, Mahabale, 1933a, b).

A comprehensive monograph on gametophytes of Ophioglossaceae has been published (see Pant, Nautiyal and Misra, 1984) which exhaustively deals on all aspects of gametophytes. Hereunder I propose to mention only briefly the comparative observations on three genera with a view to enlighten the reader, to look for details in the above mentioned indispensable monograph.

4.1 MATURE GAMETOPHYTE AND SEXUALITY (Fig. 4b)

The gametophytes of all three genera, *Ophioglossum, Botrychium* and *Helminthostachys* are geophillous saprophytic and fleshy. These are found under ground. In *Ophioglossum* the gametophyte is essentially cylindrical which may be equally or unequally branched. Their length varies from 0.5 mm to 11.6 mm and 0.25 to 2.5 mm in diameter. The longest prothalli occur in *O. vulgatum* and the thickest in *O. costatum*. Some detailed diferences are exhibited by a few species of *Ophioglossum*. Essentially two types of antheridia are described in *Ophioglossum; viz*. small with unistratose and partially bistratose jacket while the other category is of large antheridia with multistratose jacket. Pant *et al*. (1984) consider more than four types. Peculiarly enough *O. reticulatum* and *O. vulgatum* show the small type but the antheridia of *O. costatum, O. gramineum* and *O. nudicaule* may produce more antherozoids.

The prothalli of monotypic *Helminthostachys* are cylindrical at the upper end. Lower end is often irregularly lobed. These are upto 9 mm long and 3.5 mm in breadth, may be monoecious or dioecious, antheridia are large and archegonia as in *Ophioglossum*, can be identified by their four celled tops.

In *Botrychium*, the prothalli are short, tuberous more or less flattened and typically dorsiventral. These are quite variable among the species of the genus but are often monoecious and dorsiventral. The ventral surface is sterile while dorsal face bears large

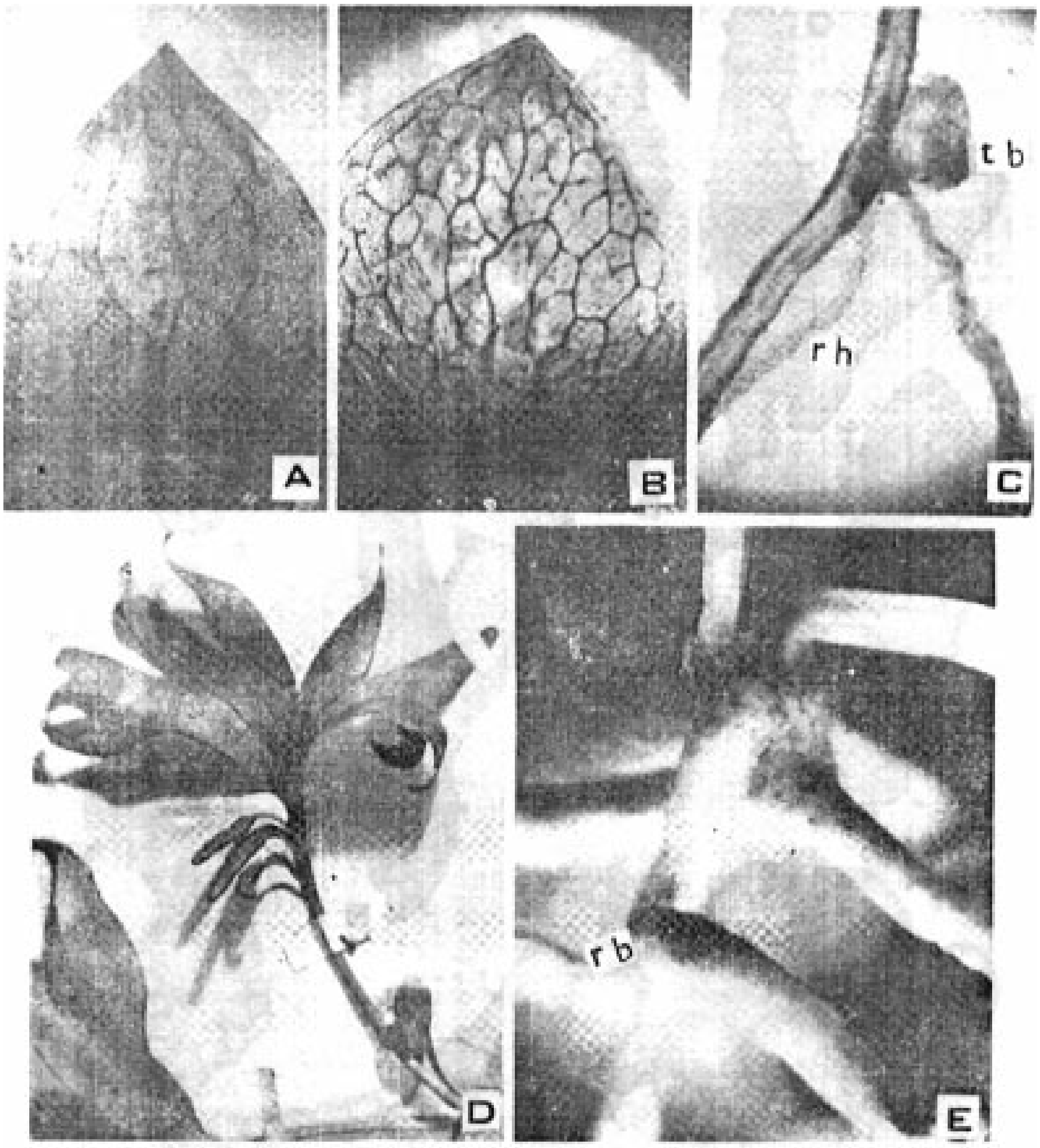


Fig. 4a — A Safranin stained leaf of *O. costatum* made translucent to show venation pattern. B. Similar leaf of *O nudicaule* veinlets are half and broken. C. A. spongy mass of cells forming tuberon the root of *O. eliminatum.* Unicellular rhizoids emerge from the tuber. D. A. plant of *O. palmatum* showing palmately branched frond and numerous spikes (young ones detached originating from the petiole; × ¼). E. Root bud formation in *O. eliminatum* (× 4)

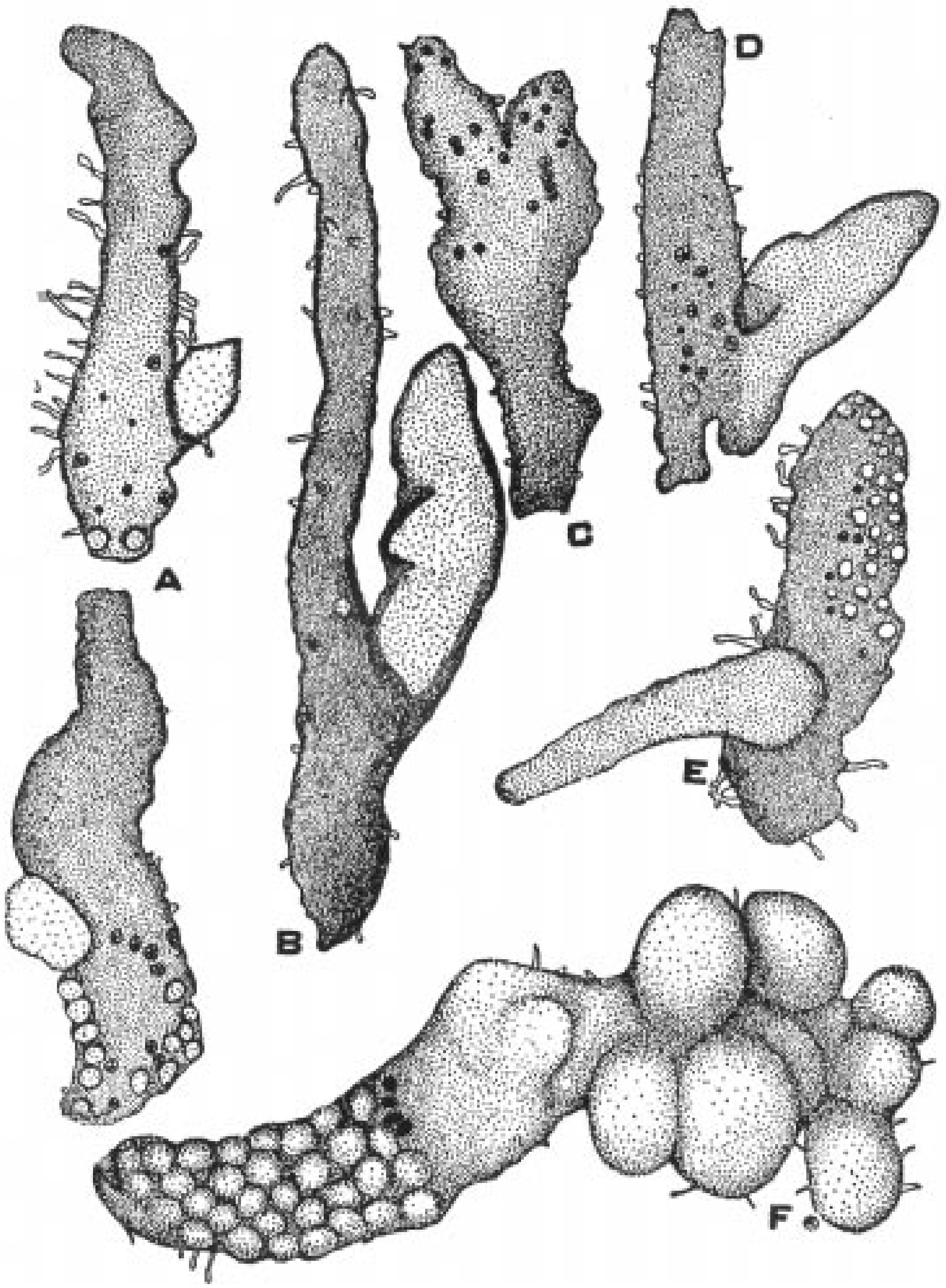


Fig. 4b — A Geophillous prothalli of *O. Costatum with young* sporophytes. B. Geophillous elongated gametophyte of *O. Petiolatum* showing young sporophyte and sex organs. C Branched gametophyte with forked apices in *O. nudicaule.* D. Gametophyte of *O. gramineum* showing young sporophyte. E. Gametophyte of *O. costatum* showing unilateral distibution of sex organs. F. Monoecious (archegonia) gametophyet of *H. zeylanice* shows a number of lobes at base. All × 25 (After Pant *et al.*).

antheridia. Both archegonia and antheridia resemble with those of *Ophioglossum* and *Helminthostachys.*

4.2 FERTILIZATION

One or many sperms may enter the archegonium through the neck canal, but ultimately one fuses with the egg and results in the beginning of a diploid phase.

4.3 EMBRYO

For detailed divisions and developments of the embryos the reader may consult Pant *et al* (1984). However, brief description may be necessary as there are certain remarkable differnces. For example, the first division in zygote of *Ophioglossum* is transverse or oblique followed. by another division at right angles. This quadrant divides in all directions which lead to the formation of a multicelled embryo. The embryos are exoscopic and the shoot develops from a single initial which divides by parallel walls to form a group of cells. The triangular or pyramidal leaf initial develops by the side of the shoot. Embryo development in *O. nucicaule* is different. In this species unlike other species of *Ophioglossum* the fourcelled stage leads to the formation of an Upper suspensor cell which divides to form suspensor. The suspensor bearing embryos of *O. nudicaule* become edoscopic on account of tilting of the developing embryo and formation of leaf and rhizome on opposite sides of the archegonial neck. While all developmental stages are exactly similar in species of *Ophioglossum* and Botrychium, the embryos are usually exoscopic suspensorless in *Ophioglossum* excepting *O. nudicaule* which has endoscopic embryo, with suspensor like *Helminthostachys zeylanica*.

4.4 EVOLUTIONARY CONSIDERATIONS

Part of embryogeny is very remarkable in Ophioglossaceae. As first investigated by Lang (1902, 1904, 1910) the embryos of *Helminthostachys* are suspensor bearing and endoscopic. In *Ophioglossum*, and *Botrychium* we now know that both types of embryos viz. exoscopic and endoscopic (with exceptions of suspensor) are found. The occurrence of two kinds of polarity within *Ophioglossum* and *Botrychium* is unique and this feature is also recorded in another eusporangiate family Marattiaceae but in separate genera, e.g. *Angiopteris, Danaea* and *Macroglossum*, have endoscopic embryos with suspensor while *Marattia* and other genera have exoscopic suspensorless embryos (Bower, 1935).

Such a variable situation in embryogeny has been of considerable evolutionary significance MacMillan in 1898 had suggested that exoscopic embryos without suspensors could be more primitive because such a situation occurs in all bryophytes and in other primitive forms e.g. *Psilotum* and *Tmesipteris* (see Foster and Gifford, 1973). Contrary to this, Lang and Bower indicated that an endoscopic embryo with a suspensor represents the "primitive spindle" from which the suspensorless type have taken their origin (see Pant *et al*, 1984).

5. Vegetative Propagation

In the genue *Ophioglossum*, especially *O. vulgatum* (Bower, 1926; Chakravarty, 1951; Ganguly and Roy, 1960; Peterson, 1969; Pant and Khare, 1971), *O. pollyphyllum* (Balkrishnan *et al*. 1960), *O. nudicaule* (Mahabale, 1962; Pant and Khare, 1971) *O. reticulatum* and *O. gramineum* (Pant and Khare, 1971) roots serve as the propagating organ. New plants arise from terminal buds on underground stolons. Efforts to obtain such a situation in some other species of the genus viz: *O. lusitanicum* and in *O.costatum* have also now succeeded (Fig. 25). Peterson (1969) (a) has however, reported bud formation on root segments of *O. petiolatum* by the effect of applicating cytokinins and auxin environment. Besides stolons, tuber production has also been observed in natural populations of *O. eliminatum* and *O. polyphyllum* (Fig. 4a, C and E). Except a few sporadic observations, nothing special is known about vegetative propagation in *Botrychium* and *Helminthostachys*.

6. Experimental Study

Among the three genera, often, *Ophioglossum* has been subjected to some experimental studies.

(a) *Production of buds and periderm:* Peterson (1969a, b) reported that the Pattern of bud formation in *Ophioglossum* under the influence of added cytokinins is strictly dependent upon the site of application. The marked root tissue proliferation is the result of extensive and protracted cell division at the locus of bud formation. However, root segments, unlike most root fragments of higher plants do not show a polarity in the regeneration of shoots and roots. Perhaps the hormonal control of polarity implicated in the regeneration of most higher plant root fragments is not expressed in the root segments of *Ophioglossum*. This may be associated in some way with the primitive nature of this group.

Peterson (1971) has also studied the effect of Benzyl adenine on the excised roots of *Ophioglossum petiolatum*. He found that the substance initiated periclinal divisions in the outer cortical cells producing a periderm like tissue. This tissue stains with Sudan-IV and the walls are resistant to sulphuric acid.

(b) *Spike elongation* : Peterson and Cutter (1969a,(b) carried out marking experiments on peduncles and spikes of *Ophioglossum petiolatum* with the help of historadioautography of spikes treated with H^3- thymidine. This study of spike elongation reveals that the growth of the spike proceeds by activity of an intercalary meristem located in the most distal region of the peduncle subtending the sporangial area. They found that surgical removal of the sterile segment of the frond has no effect on peduncle elongation. However when the entire sporangial area was removed from fertile spikes at any stage of elongation of the peduncle there was immediate cessation in elongation of the subtending peduncle proving thereby that peduncle elongation is correlated with the presence of the sporangial area. In all probability, which was subsequently proved by Peterson and Cutter by stimulating effects of auxins particularly IAA and NAA on removed sporangial areas, it appeared that the sporangial area synthesized substances which influence both the activity of the intercalary meristem and elongation of cells produced by the intercalary meristem.

Regarding aborted spikes Peterson and Cutter (1969b) opined that the rhizomes of *O. petiolatum* produce three types of fronds: sterile sterile fronds, bearing a normal fertile spike and fronds with an aborted spike. Since aborted spikes lack intercalary meristem and sporangia, they fail to elongate.

Thus it is of considerable interest that intercalary meristems of similar structure and function in the primitive fern genus *Ophioglossum* and in scapes of so distinct and many advanced angiosperm genera. Obviously this is of phylogenetic interest.

(c) *Spore germination:* Germination of spores to form gametophytes has so far failed in vitro conditions. In natural conditions the spores may germinate within a few days or may undergo dormancy period for more than two years. Period of germination may in fact be not only variable among the three genera but also may vary from one species to the other. In either case, the young gametophyte does not develop beyond the three or four celled stage unless it becomes infected with the mycorrizal fungus which enters through the lower cell. Young gametophytes are globose without rhizoids but at maturity the gametophytes become oblong, irregular, and even, in some species, branched.

Some germination attempted in various culture media (e.g. potato dextrose agar, Knop's and Myer's media both with and without soil) did not yield spores beyond the rupture of triradiate mark. Subsequently, hyphae of *Mucor* and *Rhizopus* were seen growing in the dishes. The same dishes were kept for 3-4 months but no further development was observed. Needless to mention, *in vitro* production of gametophytes has to be taken up seriously.

(d) *Oil and protein contents: O. vulgatum* is edible as vegetable in Ambikapur area of Chhatisgarh. Medicinal properties of *Botrychium, Helminthostachys* and *Ophioglossum* are also mentioned in the literature (The Wealth of India, 1959). Considering these factors the extraction of oil and estimation of proteins was attempted as per procedure described earlier (Khandelwal *et al*, 1980; see Table 2). These plants in fact need wider attention from such angles because iol content and analysis of other organic compound may reveal the antimicrobial activity thus making these weeds of great medicinal value. Oil extracted from an unidentified species of *Ophioglossum* has a protective action against a skin disease reserhbling *psoriasis*. Numerous such trials will be of much wider use.

Estimations of nitrogen and protein content in different species suggest that the amount is variable. When this was statistically compared with the nitrogen available in the soil it was further revealed that species present at the same spot, within the area of 20 square em assimilated nitrogen quite differently (Khandelwal *et al*, 1980). Also plants of same species showed different oil and nitrogen percentage at different localities. Obviously most ideally suited edaphic conditions have to be known for the species particularly which can yield more oil and protein contents and exploited for medicinal uses.

TABLE 2 — OIL AND NITROGEN PERCENTAGE IN DIFFERENT SPECIES OF POHIOGLOSSALES IN RHIZOME WITH ROOTS BY SOXHLET APPARATUS

Sl. No.	Name of the species	Oil percentage	Nitrogen* percentage
1.	*O. costaum*	27.91	0.90
		24.54	2.00
		26.91	6.72
2.	*0. nudicaule*	13.11	4.62
		39.53	1.40
3.	*O. lusitanicum*	7.60	1.60
4.	*O. polyphyllum*	26.77	
5.	*O. eliminatum*	19.32	3.00
6.	*H. zeylanica*	15.60	0.70
7.	*Botrychium sp.*	9.72	1.20

* Protein percentage can be obtained by multiplying with 6.25.

(e) *Amino acid differences and their significance* : There have been few hypotheses (Fowden, 1965) that certain non-protein amino acids confer protective or survival value upon the plant producing them. Since these compounds have nitrogen contents considerably in excess of that of protein molecules. Fowden considered them as storgae products possessing readily mobilised nitrogen atoms. S-substituted cysteins can be accumulated especially in

Fig. 11 — Camera lucida sketch of chromosomes of *O. eliminalum* n. sp. showing 90 ± 2 bivalents at diakinesis (Enlarged. x 3000).

storage organs (Fowden, 1962, 1964). These compounds probably act as readily available sources of sulphur at the time of new growth. We became interested in studying possible role of these amino acids in conferring advantage for survival of *Ophioglossum* plants in particular *O. eliminatum* (sp. novo Khandelwal and Goswami, 1984) which has demonstrated abnormal features already discussed.The problem we realized was how to compare the free pool of amino acids of one species with the other, because we know the amino acids present at one stage of development may be absent at the other phase (Fowden, 1965) To overcome this, a simple experiment was designed and we studied, by two dimensional paper chromatography, the free pool of amino acids content at the time of spike initiation. No sooner a spike appeared on the adaxial face of the sporophyll a dot mark was made by Indian ink and on 4th day (after 90-96 hrs) the plant parts were processed and chromatograms developed (Goswami and Khandelwal, 1976; and Table 3). The tabulated data based on repeated observations confirm our hypothesis that different species of the genus may require variable free pool of amino acids for an identical physiological function thereby suggesting genic differences. This becomes important because proline, a non-protein amino acid (imino acid) is found only in *O. eliminatum* collected and studied since 1974 from Kanharjhir forest near Gwalior and *O. gramineum* studied from Bhopal. Both these species show prolific vegetative propagation and also exhibit numerous spikesporangial abnormalities. Thus our, observations are in conformity with Fowden's (1965) belief that non-protein amino acids confer protective (hence adaptive) advantage (Khandelwal and Goswami, 1976).

TABLE 3 — AMINO ACID DISTRIBUTION IN TROPOPHYLLS OF EIGHT SPECIES OF OPHIOGLOSSUM AND HELMINTHOSTACHYS ZEYLANICA AT THE TIME OF SPIKE INITIATION

Distribution	Amino acids	Remarks
Amino acids common to species *O. lusitanicum, O. costatum* *O. undicaule, O. gramineum* *O. polyphyllum, O. vulgatum* *O. petiolatum, H. zeylanica*	Alanine, diaminobutyric acid, arginine, glutamic acid, serine, threonine, lysine	Hyoscine is absent in *O.eliminatum* (all other present)
Amino acids absent in all species	Aspartic acid, cysteine dihydroxy-phenylalanine, glycine, histidine, hydroxyproline, leucine, omithine, proline, tryptophan	Histidine and proline are present in *O. eliminatum* and proline in *H. zeylanica*
Amino acids confined to one or two species	Norleucine in phenylalamine	*O. lusitanicum and O. elminatum*
	Phenylalanine in	*O. vulgatum and O. eliminatum*
	Tyrosine in	*O. costatum and O. eliminatum*
	Valine in	*O. costatum and H. zeylanica* *O. zeylanica*
Amino, acids confined to three or more	Isoleucine in	*O. nudicaule, O. vulgatum and O. eliminatum*
	Methionine in	*O. nudicaule, O. lusitanicum, O. polyphyllum, O. vulgatum and O. eliminatum*

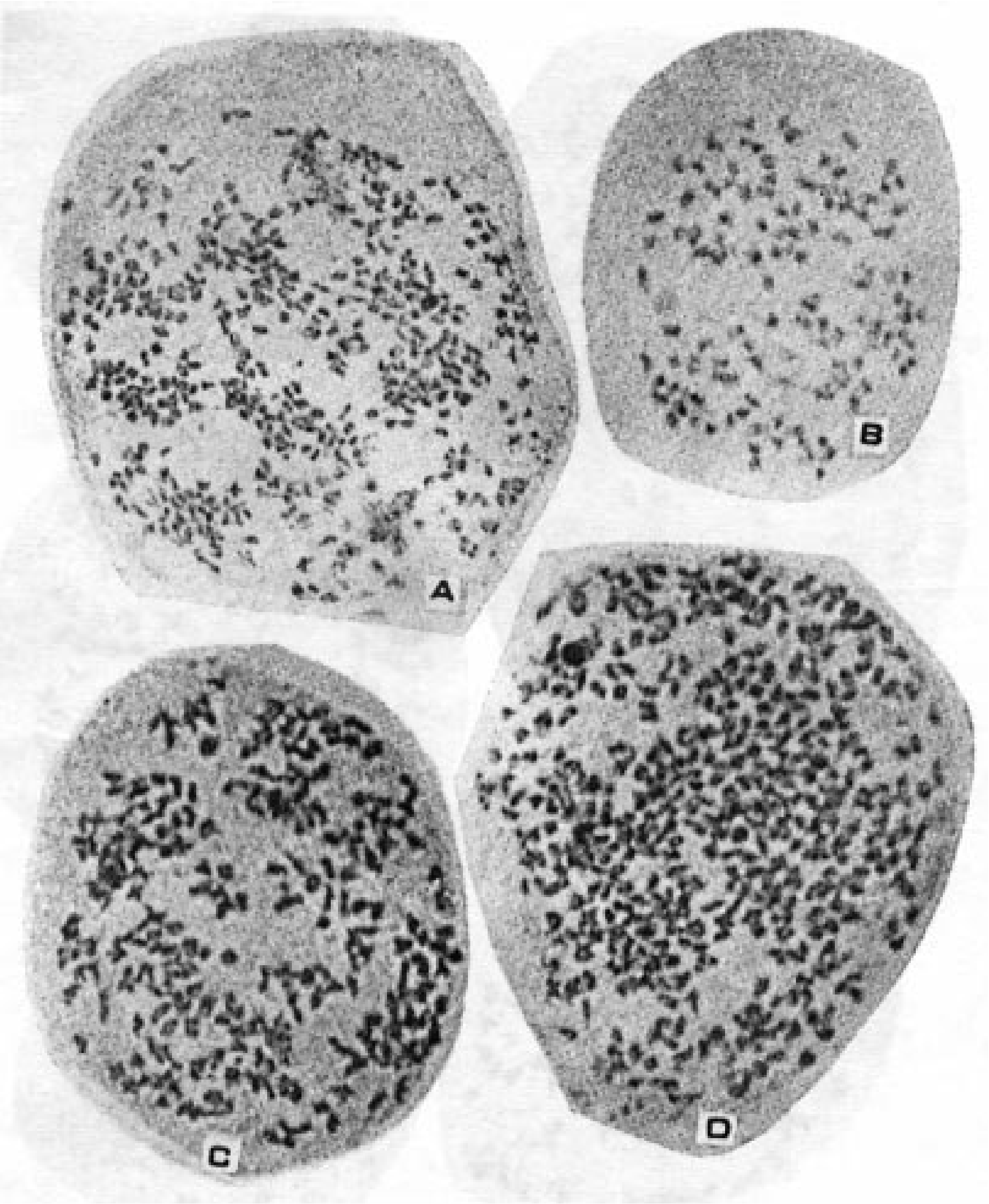


Fig. 12 — Diakinesis in *Ophioglossum* (x 2400). A Spore mother cell showing Ca 360 bivalents in *O. thermale*. B. 90 bivalents with two microchromosomes in *O. eliminatum*. C. *O. petiolatum* showing Ca 240 bivalents with 2-3 univalents. D. *O. lusitanicum* showing Ca 360 bivalents.

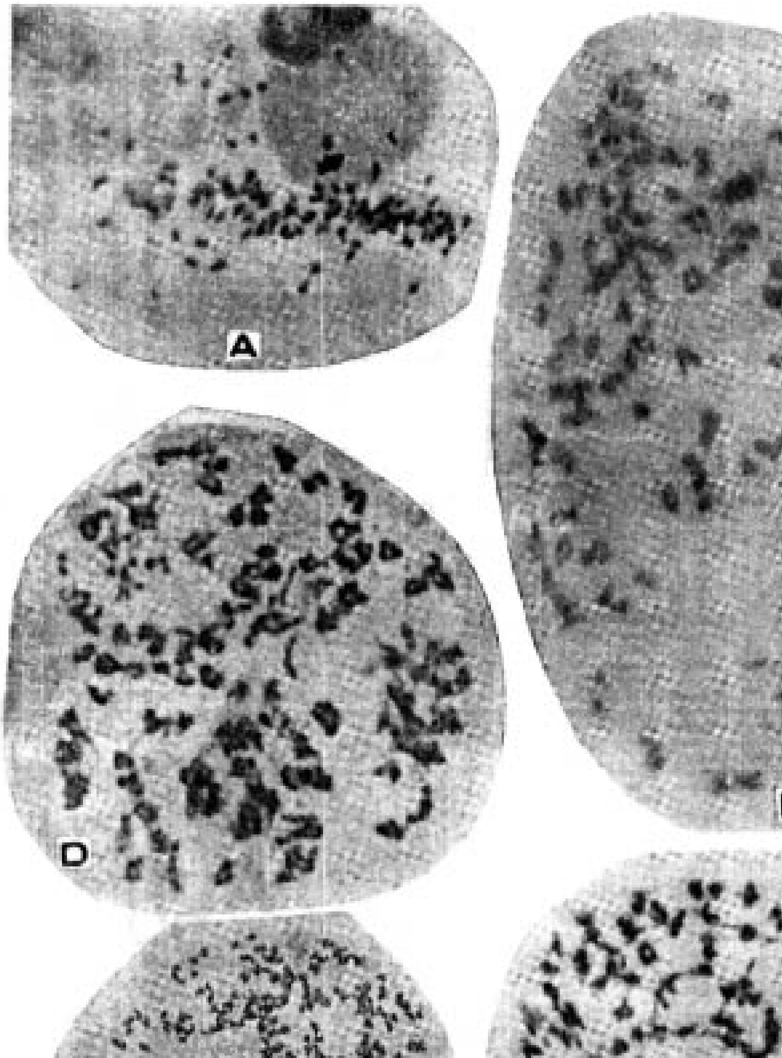


Fig. 13 — A. Metaphase I showing lagging bivalents a
diplotene showing 90 bivalents in *O. eliminatum* certai
are also seen (enlarged x 3000). C. Ca 90 bivalen
a phenomenon of rare occurrence and suggestive of
O. eliminatum partial pairing (homeologous) and enc
E. *O. thermale* showing

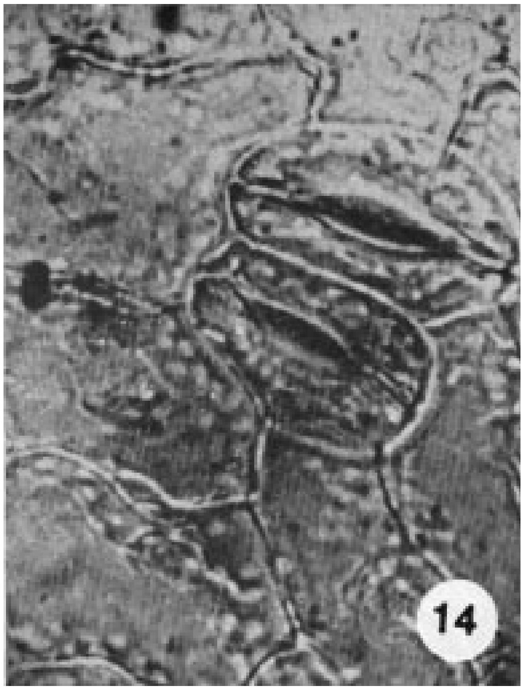


Fig. 14 — Twin stomata on the adaxial face of the tropophyll of *Ophioglossum elimination* (× 60).

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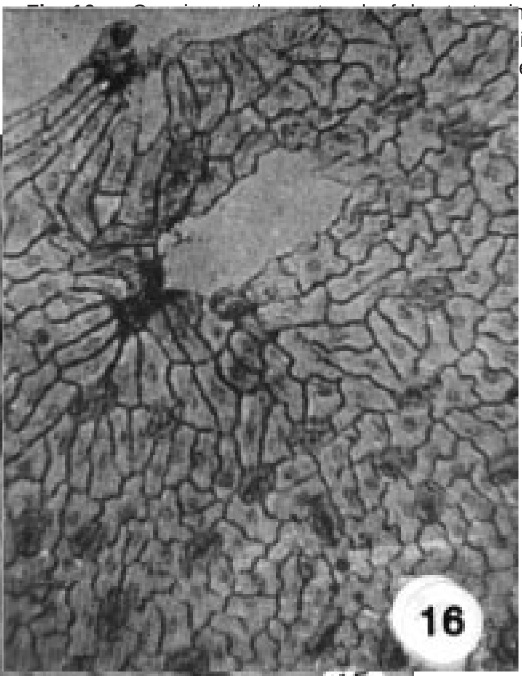

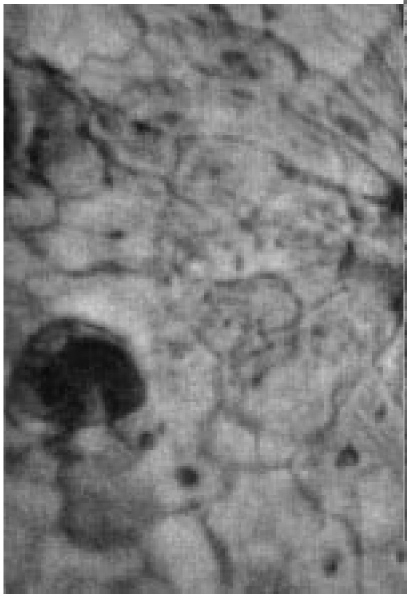

Fig. 15 — Unusual structures on a young leaf (tropolhyll) functioning probably as "hyadothodes" (× 75).

7. Cytology

Chromosome studies on *Ophioglossum* have made the entire group more popular in the biological system because *O. reticulatum* shows 2 n = 1260 highest number possessed by any plant or animal species (Ninan, 1958; Muntzing, 1967; Stebbins, 1971). Later, even higher number, n = 720 (n = 1440) in populations of *O. reticulatum* have been reported.

The first report of chromosome count in the genus *Ophioglossum* appears to be by Yuassa in 1934 (Mahabale and Nair, 1972) for *O. ellipticum* (= *O. petiolatum*; n = ca 172). O. *vulgatum* received next attention (see Manton, 1950) and within few years cytology of 36 species of Ophioglossales was known in the literature (Love *et al*, 1977). Some major points of cytogenetic interest, extensively worked out on *Ophioglossum* species are presented below:

7.1 Chromosome numbers and ploidy

(A) *Inter- and intraspecific chromosomal variations:* This became immediately clear that extraordinary diversity of chromosome number exists, especially in species of *Ophioglossum*. Kurita and Nishida (1956) reported intraspecific genomic variability in Japanese material of *O. petiolatum* and *O. thermale* var *nipponicum* which was subsequently confirmed for the Indian material of *O. vulgatum*. Numerous complications and similar studies (Mahabale and Nair, 1972; Goswami and Khandelwal, 1980a, (b) on a large number of species of *Ophioglossum* and also on *Botrychium* and *Helminthostachys* suggest that the same species may posses different chromosome number at the same and different places of occurrence. I would even presume that almost every species of *Ophioglossum* will turn out to have cytological races showing very high graded polyploidy (Figs. 12 and 13) and it would be no use to group the species to belong to one kind of evolutionary pattern as suggested by Mahabale and Nair. To cite examples; *O. lusitanicum* (n = 120; n = 240; n = 360); *O. nudicaule* (n = 120; n = 240); *O. thermale* (n = 360; n = 480) are characterised by intraspecific polyploidy (Goswami and Khandelwal, 1980a, Table 4) and even *O. reticulatum* which shows highest chromosome number exhibits middle n= 420) of variability. Faseena & Thoppil (2007) have recorded new polyploid in *O. nudicaute.*

7.2 Basic number and evolution

The primary basic chromosome number of the ophioglossales is suggested to be 15 by Ninan for a reason that the derived basic chromosome numbers 45 and 120 in *Botrychium* and *Ophioglossum* respectively, are multiples of 15. The haploid number in *Helminthostachys* (n = 92-94) is also explicable. This has been validated by almost all workers and everyone agrees that high graded polyploid plants have been left for survival, plants with lower numbers have been eliminated. The lowest chromosome number in *Ophioglossales* has been reported in *Botrychium* (n = 45 in Japanobotrychium, a subgenus synonym to *Osmundopteris*) and we have recently recorded n = 90 in *O. eliminatum* being the lowest count in the genus *Ophioglossum* Khandelwal opines n = 30 to be the lowest chromosome number in *O. eliminatum* and assumes it to be palaeobasic (Khandelwal. 1990). Sporne (1970) mentions n = 47 for *Helminthostachys* which may probably be an error as *H. zeylanica* has always shown 92-94 bivalents at meiosis I (Mahabale and Nair, 1972, Goswami and Khandelwal, 1980b). Nevertheless it is not impossible that we discover a cytotype in the monotypic genus as well.

Unfortunately, cytology has not been of much help in establishing taxonomy an relationships of the group. Ninan (1958) has emphasized on the phylogenetic relationship of Ophioglossaceae with Schizaeaceae mainly on the ground that the basic chromosome numbers in the former is 15 and *Lygodium* shows n = 30 chromosomes. Additionally, the occurrence of very high chromosome numbers in both groups n = 240, 280, 630 etc in *Ophioglossum* and n = 270. 350-370, 540 etc in *Schizaea* indicate parallelism in evolutionary histories. Mehra (1961) and Kurita and Nishida (1965) however consider n = 10 as the basic number of Schlzaeaceae and explain n = 30 in *Lygodium* and n = 38 in Anemia as the convenient outcome of polyploidy. Kurita and Nishida have stressed that high chromosome numbers in both groups may not necessarily indicate parallelism in evolution. They have extended an excellent idea that high chromosome numbers in *Ophioglossum*, *Schizaca*, *Tmesipteris* (2n = 400 etc) *Psilotum* (n = Ca 210) and *Phylloglossum* (2n = 502-510) might have arisen under the infuence of endophytic fungus during the course of evolution.

7.3 Comparative meiosis

Botrychium species and *Helminthostachys zeylanica* could not be repeatedly studied cytologically. However, it has been possible to study comparative meiosis of six species of *Ophioglossum* from the natural populations over three decades. Table includes chromosome counts during diplotene, dial kinesis and metaphase I and also chromosomal segregations at anaphase and telophase (Tables 4-6).

At a glance, every species appears to show slight variations in chromosomal content but meiotically significant variations leading to lagging uni and or bivalents at metaphase I are as a rule found only in *O. eliminatum*. This is of considerable interest because this species has demonstrated a large number of spike, sporangia and spore abnormalities.

Plants of this new species of *Ophioglossum*, which briefly resembles *O. nudicaule* can be grouped in two categories. One type exhibits gross morphological and meiotic irregularities and the other type apearing healthy, shows neither morphological nor meiotic irregularities. It has been carefully found that the latter plants are characterized by 90 bivalents with one or two microchromosomes (Fig 13).

Table 5 is based on fixations of maturing spikes which contained one or two mature sporangia. Spikes of every species are categorized as variable and stable, indicating the occurrence and nature of chromosome counts. In no case except in *O. eliminatum* and *O. gramineum* differences in chromosome counts have been observed in the same sporangium. Maturing sporangia of such spikes always demonstrate differences in metaphase I and spore types. Abnormal spores are observed in all spices which indicated some variations in meiotic counts. It may be assumed that abnormal spores must correspond to the non-viable complement. Slight fluctuation in chromosome number in *O. costatum, O. nudicaule* and *O. thermale* do not affect their phenotypes but affect tentatively calculated reproductive indix.

The highest spore output is in *O. lusitanicum*. The data on *O. lusitanicum* are contradictory to *O. thermale* and others wherein "stable count’ demonstrates reduced reproductive index. Thus it appears that production of spores by and large, is under the influence of respective genotypes.

7.4 CHROMOSOMAL ASSOCIATION WITH MORPHOLOGICAL ABNORMALITIES

Abnormalities involving spike and sporangia have been detected in natural populations of almost all species of *Ophioglossum*. It has been invariably found that spores produced by such plants include greater proportions of anucleate, deshaped and malformed spores. Considering that these spores would be abortive or nonviable, it has been hypothesized that these abnormalities must be expressing some underlying genetic mechanism (Goswami and Khandelwal, 1973a). Conversely, maximum number of spores have been detected from the sporangia of spikes exhibiting (Fig. 5, D, E) deformity. Chromosome preparations have been carefully made from at least one sporangium of each spike. Mature sporangia from such spikes have always yielded certain number of deformed or deshaped spores, large, anucleate or binucleate spores and also spores with variable laesurae. Certain spores have become of unusual (7, C, H) significance as briefly indicated in the discussion. A plant which exhibited varying number of bivalents and or, irregular meiosis is classified as "variable" and almost always, such a plant exhibits gross morphological abnormality (Cost of selection; Haldame, 1957).

7.5 SUPERNUMERARY CHROMOSOMES

Occurrence of few extra chromosomes is also long known in *Ophioglossum*. These either have been referred to as fragments or B chromsome (Manton, 1950; Ninan, 1958; kurita and Nishida, 1965; Stebbins, 1971). *O. reticulatum* possesses 630 bivalents and 10 fragments. It is observed that not all species inhabiting the same area possess them, and the individuals of the same species show variable number during meiosis. Thus the cytological entity of these chromosomes corresponds well with the definition of accessory or B chromosomes (Muntzing, 1967) and on account of their being comparatively smaller in size (av. 1.5 µ) these are termed as micro chromosomes. Among lower plants, such small chromosomes are recorded in mosses.

7.6 MULTIVALENT ASSOCIATIONS

Multivalent associations of chromosomes have been observed in *O. nudicaule* and it is no surprise that other species also show such occasional pairing. Because, by and large, high chromosome numbers in different species of *Ophioglossum* must have arisen by both auto and alloplypoloidy (Manton, 1950; Kurita and Nishida, 1965).Verma (1956) also reported multivalent associations of chromosomes in *O. vulgatum*.

7.7 INTEERCHROMOSOMAL CONNECTIONS

These have never been known for any member of Ophioglossales so far. This peculiar phenomenon of linear attachment of a few bivalents or many bivalents by fine threads or connections in certain spore mother cells exhibiting diplotene - diakinesis has been (Fig. 13 B, C, D) observed in *O. eliminatum*. During 1975 this observation was considered to be an artefact but recently Klasterska (personal communication) drew my attention regarding their nature. Klasterska (1976), and Klasterska *et al* (1977) have demonstrated