

NOTES ON THE SPORE MORPHOLOGY OF OPHIOGLOSSACEAE AND THE OCCURRENCE OF *OPHIOGLOSSUM* AND ITS GAMETOPHYTES IN THE GANGETIC VALLEY

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ABSTRACT

Six species of *Ophioglossum*, viz., *O. costatum*, *O. gramineum*, *O. nudicaule*, *O. petiolatum*, *O. reticulatum* and *O. vulgatum* are described from numerous localities in the Gangetic valley. Their geophilous gametophytes are being described and illustrated for the first time from India. The morphology of the spores of the above species as well as those of *Botrychium lanuginosum*, *B. virginianum*, *B. lunaria* and *Helminthostachys zeylanica* is also described.

INTRODUCTION

HOOKE and BAKER (1874) described five species of *Ophioglossum*, viz., *O. lusitanicum*, *O. nudicaule*, *O. vulgatum*, *O. reticulatum* and *O. pendulum* from Peninsular India, Himalayas and Assam. BEDDOME (1892) also described five species but he mentioned *O. fibrosum* in place of *O. lusitanicum* among the Indian species. He also expressed doubts about the occurrence of *O. pendulum* which was reported from Assam. In the supplement of his book he described one more species, i.e., *O. gramineum* from South India and Calcutta and suspected *O. nudicaule* to be a form of this species. Beddome believed that all specimens of *O. vulgatum* from the Himalayas were referable to *O. reticulatum* since they had a similar venation pattern. D'ALMEIDA (1922) reported the occurrence of six species of *Ophioglossum* from Assam, Bombay Presidency and South India. He reported *O. aitchisonii* for the first time, left out *O. gramineum* and the remaining five of his species were the same as those reported by previous workers. CHAKRAVARTY (1951) reported seven species by making the following alterations in D'Almeida's list: (i) segregation of *O. reticulatum* from *O. vulgatum*, (ii) addition of *O. capense* and *O. gramineum* and (iii) elimination of *O. aitchisonii* and *O. lusitanicum*. MAHABALE (1962) raised the number of Indian species to 10 by excluding *O. capense* and adding *O. aitchisonii*, *O. japonicum*, *O. lusitanicum* and *O. pedunculatum* to the list given by CHAKRAVARTY (1951). In their review of the occurrence of *Ophioglossum* in India PANIGRAHI and DIXIT (1969) confirmed the occurrence of the same 10 species but pointed out that *O. fibrosum*, *O. japonicum*, *O. aitchisonii* and *O. pedunculatum* were synonyms of *O. costatum*, *O. thermale*, *O. polyphyllum* and *O. petiolatum*, respectively. They restated doubts expressed earlier by BEDDOME (1892) about the occurrence of *O. pendulum* in India and pointed out that no one, after Mann, had collected any material of the species from any part of the country. It is important to note that the above authors reported species of *Ophioglossum* chiefly from localities in the Himalayas, Assam, Bombay Presidency and South India or other hilly areas of the country.

OBSERVATIONS

Occurrence in the Gangetic Valley

A careful scrutiny of the Indian occurrences of *Ophioglossum* shows that the only reports of the genus from the Gangetic valley are: (i) *O. gramineum* from Lucknow tank, Calcutta

(BEDDOME, 1892), (ii) *O. fibrosum* from village Lohgarha in Allahabad district (SAKSENA & MATHUR, 1925), (iii) *O. vulgatum* and *O. capense* respectively from Kusmi forest in Gorakhpur district and the campus of Banaras Hindu University, Varanasi (GANGULI & ROY, 1960), (iv) *O. fibrosum*, *O. nudicaule*, *O. reticulatum*, *O. pedunculatum* and *O. vulgatum* from isolated spots within a radius of 3 kilometers at Gyanpur in Varanasi district (SAKSENA & SINGH, 1968) and (v) *O. reticulatum* from Bahraich, Saharanpur, Hazaribagh and Parasnath Hill (see PANIGRAHI & DIXIT, 1969). All these reports are thus from isolated and restricted localities and even among these, Lohgarha and Parasnath hill do not lie strictly in the alluvial plain of the Ganga since they are situated in the rocky areas adjoining the hills of Vindhya Pradesh and the Deccan Plateau. However, during the last three years we have extensively collected plants referable to six species, viz., *Ophioglossum costatum* R. Br., *O. gramineum* Willd., *O. nudicaule* L., *O. petiolatum* Hook., *O. reticulatum* L. and *O. vulgatum* L. of which *O. costatum*, *O. gramineum* and *O. nudicaule* were collected from the low hills of Chunar, Mirzapur and Shankargarh, while others and also *O. nudicaule* from rather widespread localities in the alluvial plain of the Ganga, e.g., in Naini, Bamrauli, Manauri, Phaphamau and other suburbs of Allahabad and in numerous localities in the districts of Allahabad, Fatehpur, Pratapgarh, Rae Bareilly, Lucknow, Kanpur, Unnao, Mirzapur, Varanasi and Gorakhpur. Herbarium sheets of these from the various localities are kept in the Herbarium of the Botany Department of the University of Allahabad.

Habitat of different species

Plants of *O. costatum*, *O. gramineum* and *O. nudicaule* were found growing in coarse sandy soil at various spots from Rewa in Madhya Pradesh to the district of Mirzapur in Uttar Pradesh. These plants generally grow mixed with grasses under the shade of spiny bushes like *Zizyphus* and *Carissa* or under trees like *Butea monosperma* and others, receiving a considerable amount of direct sunlight. Plants of other species occur in the fine clayey alluvium under the dense shade of mixed or pure grooves of *Mangifera indica* and *Madhuca indica* trees mixed with grasses and weeds like *Corchorus*, *Commelina*, *Justicia*, *Evolvulus*, *Cynodon*, *Paspalum*, *Medicago*, etc. All the species of *Ophioglossum* are annuals, as a rule coming up soon after the first rains in July and usually persisting during the whole of the rainy season upto October, after which all the aerial parts die down.

Perennation and vegetative reproduction

Older plants of all the species described in this paper perennate by means of their roots and rhizomes while their aerial parts die out during winter. With the onset of the rains, they produce new roots, leaves and spikes. In all species, except *O. costatum*, new plants are formed from terminal buds on underground stolons which appear to be somewhat thicker than roots (Pl. 1, fig. 1). Sections of these stolons confirm that they are roots. Similar buds have been reported earlier in *O. vulgatum* by BOWER (1926), EAMES (1936), CHAKRAVARTY (1915) and GANGULI & ROY (1960); in *O. polyphyllum* (= *vulgatum*) by BALAKRISHNAN *et al.* (1969) and in *O. pedunculatum* (= *petiolatum*) by MAHABALE (1962).

Branched spikes

A few abnormal branched spikes were observed in *O. reticulatum* and *O. gramineum*. In three such spikes of *O. reticulatum* the fertile region of the spike is terminally forked but in four plants the stalk itself is forked, and each fork ends in a spike (Pl. 1, fig. 2). In two plants of *O. gramineum* the stalk was found alternately branched and each branch ends in a small

spike (Pl. 1, figs. 3-4). Similar abnormalities were reported earlier, by BOWER (1926) in *O. vulgatum* and *O. pendulum*.

Spores of different species

Microscopic examination of the spores of all presently investigated species of *Ophioglossum* and also those of *Botrychium lanuginosum*, *B. virginianum*, *B. lunaria* and *Helminthostachys zeylanica* was made in the wet mounted condition as well as in the dry state. Common spore characteristics of all species are: spores free, anisopolar, radiosymmetrical and containing chloroplasts (the last mentioned characteristic could not be observed in species of *Botrychium* and *H. zeylanica* since these were observed only in previously fixed or pressed material), nexine is smooth and attached proximally to the sexine. However, all species have their own characteristic size (in the descriptions given below, the size is measured in glycerine jelly mounts and the averages are mentioned within parenthesis) and exine sculpturing. Brief descriptions of the characteristic features of the spores of different species are mentioned below:

Ophioglossum reticulatum: Spores alete or sometimes monolete; 43 (50) 56 μ in size; laesura if present half equatorial diameter of spore; contact faces concave; amb rounded; exine verrucate, verrucae blunt and closely arranged, generally anastomosing; area of contact faintly ornamented (Pl. 2, figs. 15-17; Pl. 3, figs. 27-28; Pl. 4, figs. 43-44).

O. vulgatum: Spores trilete; 37 (43) 52 μ in size; laesurae generally sinuous, about one third equatorial diameter of spore, sometimes forked at the tips and the forked ends join to form an orbicular ridge; contact faces concave; amb rounded; exine verrucate, verrucae fine and blunt, generally anastomosing; area of contact faintly ornamented (Pl. 2, fig. 18; Pl. 3, figs. 29-30; Pl. 4, figs. 47-48).

O. costatum: Spores trilete; 34(37) 41 μ in size; laesurae generally sinuous, about one third equatorial diameter of spore, occasionally forked at the tips and the forked ends join to form an orbicular ridge; contact faces slightly concave; amb rounded; exine verrucate, verrucae fine and blunt like *O. vulgatum*, generally anastomosing; area of contact smooth or faintly ornamented (Pl. 2, fig. 19; Pl. 3, figs. 31-32; Pl. 4, figs. 49-50).

O. nudicaule: Spores trilete; 34(45) 51 μ in size; laesurae occasionally sinuous, about half equatorial diameter of spore; contact faces flattened or slightly concave; amb rounded or slightly trianguloid; exine verrucate, verrucae blunt and sometimes anastomosing; area of contact faintly ornamented (Pl. 2, fig. 21; Pl. 3, figs. 33-34; Pl. 4, figs. 51-52).

O. petiolatum: Spores trilete; 34(37) 43 μ in size; laesurae straight, about half equatorial diameter of spore; contact faces flattened or slightly concave; amb rounded or slightly trianguloid; exine verrucate, verrucae blunt and sometimes anastomosing like that of *O. nudicaule*; area of contact faintly ornamented (Pl. 2, fig. 20; Pl. 3, figs. 35-36; Pl. 4, figs. 53-54).

O. gramineum: Spores trilete; 34 (38) 43 μ in size; laesurae occasionally sinuous, about one fourth equatorial diameter of spore, sometimes forked at the tips and the forked ends join to form an orbicular ridge; contact faces concave; amb rounded; exine reticulate, ridges with blunt projections; area of contact smooth (Pl. 2, fig. 22; Pl. 3, figs. 37-38; Pl. 4, figs. 45-46).

Botrychium lanuginosum: Spores trilete; 28 (32) 38 μ in size; laesurae narrow, reaching upto the margin; contact faces flattened; amb trianguloid; exine rugulate, rugulae blunt; area of contact faintly ornamented (Pl. 2, fig. 24).

B. virginianum: Spores trilete; 30 (35) 38 μ in size; laesurae narrow, reaching upto the margin; contact faces flattened; amb trianguloid; exine rugulate, rugulae blunt, appearing

as reticulum, anastomoses incomplete; area of contact faintly ornamented (Pl. 2, fig. 25; Pl. 3, figs. 41-42; Pl. 4, figs. 57-58).

B. lunaria: Spores trilete; 34 (41)47 μ in size; laesurae narrow, reaching upto the margin, contact faces slightly concave or flattened; amb trianguloid; exine rugulate, rugulae blunt; appearing as reticulum, anastomoses incomplete; area of contact faintly ornamented or smooth (Pl. 2, fig. 26; Pl. 4, figs. 59-60).

Helminthostachys zeylanica: Spores trilete; 32 (37) 38 μ in size; laesurae narrow, about half equatorial diameter of spore; contact faces flattened or slightly concave; amb rounded or slightly trianguloid; exine verrucate, verrucae blunt, frequently anastomosing, anastomoses incomplete; area of contact equally ornamented (Pl. 2, fig. 23; Pl. 3, figs. 39-40; Pl. 4, figs. 55-56).

Gametophytes

Careful examination of the soil from spots where *O. costatum* was the only species growing in Lohgarha and Shankargarh in Allahabad district and Sindhoraghat in Mirzapur district revealed a few underground gametophytes of that species. They are of the usual cylindrical and mycorrhizal type with clearly marked sex organs (Pl. 1, figs. 5-6). Sections of the gametophytes have confirmed the existence of sex organs. Young sporophytic plants attached with the gametophytes were also observed (Pl. 1, figs. 7-14). Similar looking gametophytes have also been found in the soil from various localities of *O. reticulatum*, *O. vulgatum*, and *O. petiolatum* in the alluvial plain of the Ganga.

DISCUSSION

In view of the presently reported common and widespread occurrence of six species of *Ophioglossum* in the Gangetic plain and also previous reports from areas in eastern U.P. and Calcutta, we feel certain that the genus ought to occur throughout the Gangetic valley and also elsewhere in India. Indeed, it may appear surprising that the occurrence of the plants of *Ophioglossum* is not reported from this region by most of the earlier collectors and systematists. Perhaps, it was due to the fact that most field collections were undertaken by non-botanists while botanists confined themselves to their city laboratories and also because the plants of all the above species have an insignificant size and grass or weed-like form particularly when they are without spikes and when they grow intermixed with grasses and other weeds.

We hope that the publication of the present report will not only stimulate botanists to look for these plants and collect them from nearby convenient localities around villages throughout the Gangetic valley and other areas but it will also make the material of the genus readily available for class work. We are sure that these collections will very soon turn *Ophioglossum* into one of the cosmopolitan genera of Indian ferns.

During our collections we came across plants of *O. reticulatum* and *O. vulgatum* whose leaves showed such a gradation of intermediate forms that they seem to merge one species imperceptibly with the other (see also D'ALMEIDA, 1922). In fact we found that such plants of these species which develop two leaves in one year may sometimes bear one leaf of *reticulatum* type and the other of *vulgatum* type. Earlier authors, e.g. BEDDOME (1892), D'ALMEIDA (1922), BLATTER & D'ALMEIDA (1922), MAHABALE (1962) and PANIGRAHI & DIXIT (1969) have also pointed out that the differences between *O. reticulatum* and *O. vulgatum*, which are based mainly on their leaf form, are not always reliable. However, we found that the spores are consistently alete in all plants which are referable to *O. reticulatum* on account of their having typically cordate bases, whereas, they are trilete in plants of *O. vulgatum*, having

leaves with gradually tapering bases. This distinction can be applied even to plants with intermediate forms of leaves, and we suggest that those of them which have alete spores be included under *O. reticulatum* while those having trilete spores should come under *O. vulgatum*.

The alete spores of *O. reticulatum* not only distinguish it from *O. vulgatum* but also from all other Indian species of the genus. In this connection it is interesting to point out that MAHABALE (1962) gave sketches of spores of various Indian species, although he did not describe any details, some of his figures, including that of a spore of *O. reticulatum*, do not show a trilete while others are drawn trilete. On the contrary, PANIGRAHI & DIXIT (1969) figured and described the spores of all Indian species and found them to be uniformly trilete and reticulate. However, as described earlier, our observations show that the spore morphology of various Indian species of *Ophioglossum* as well as those of *Botrychium* and a *Helminthostachys* described here presents characteristic features which are at variance with the figures given by MAHABALE (1962) and also with the accounts given by PANIGRAHI & DIXIT (1969). Presence of chloroplasts in the mature spores of the presently investigated species of *Ophioglossum* is being described for the first time. It may, however, be mentioned that EAMES (1936) had already reported their presence in spores of the genus, although CAMPBELL (1911, 1918) mentioned that they are absent in the adult spores.

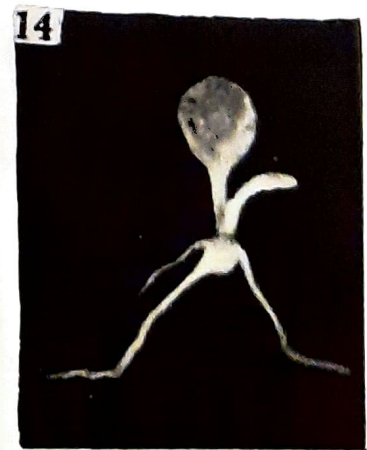
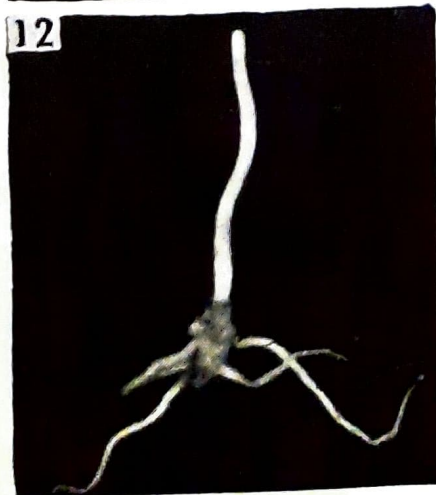
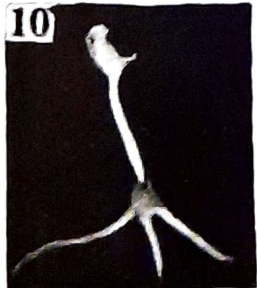
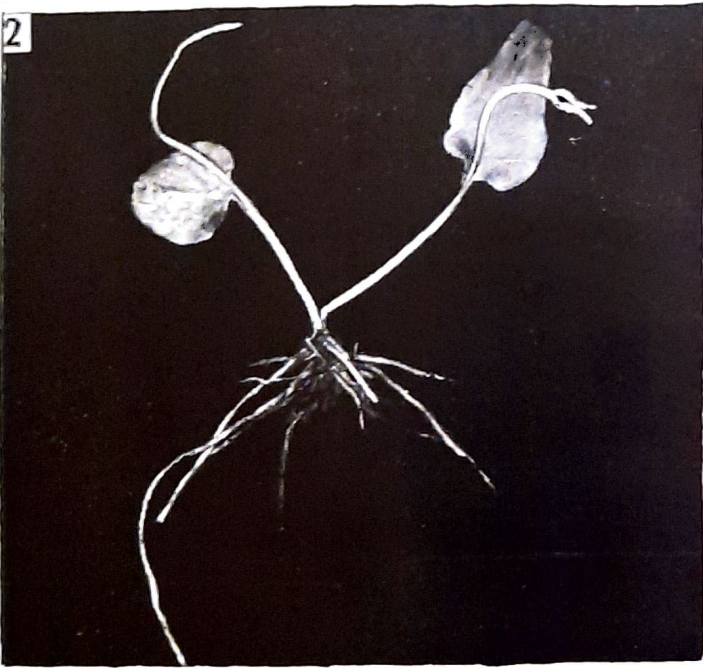
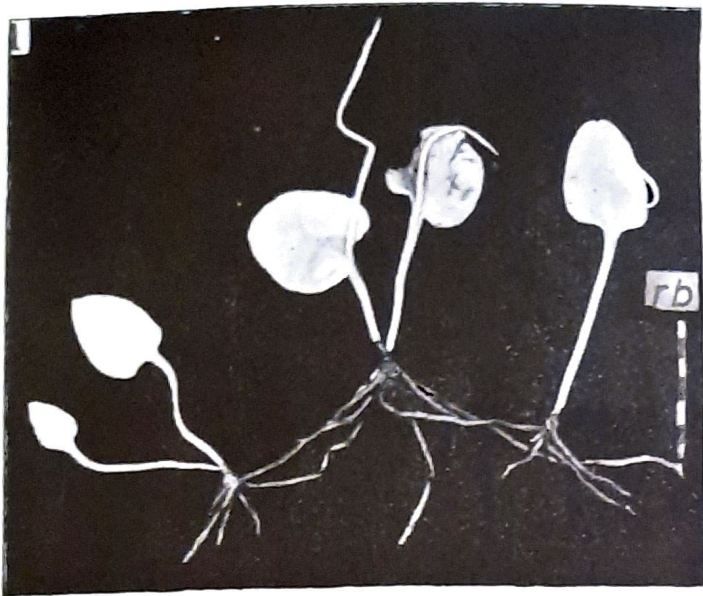
Except for the reported occurrence of some gametophytes of *Ophioglossum* by MAHABALE (1933a, 1933b), and of *O. fibrosum* (= *costatum*) by MAHESHWARI & SINGH (1934) at Machgawan (about 85 miles south west of Allahabad), details of which were never published, ours is the first illustrated report of the gametophytes of *Ophioglossum* from India. Recently, GANGULI & ROY (1960) and SAKSENA & SINGH (1968) have stated that they failed to find any gametophytes in the soil where plants of *O. vulgatum*, *O. fibrosum*, *O. nudicaule*, *O. pedunculatum* and *O. reticulatum* were growing. The occurrence of gametophytes with developing embryos clearly points out that the plants of *Ophioglossum* in the presently worked out localities not only reproduce vegetatively, by means of root buds, but also by sexual means. A detailed report of these gametophytes will be published elsewhere.

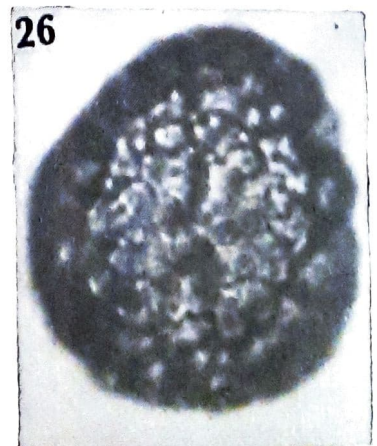
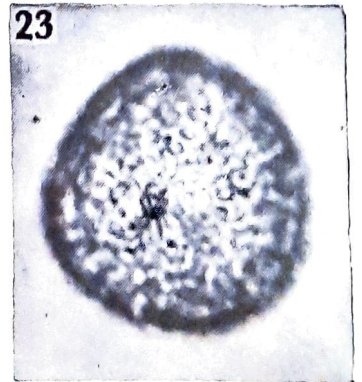
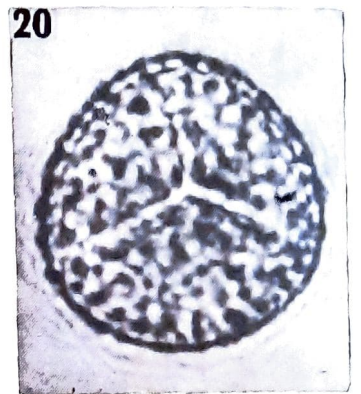
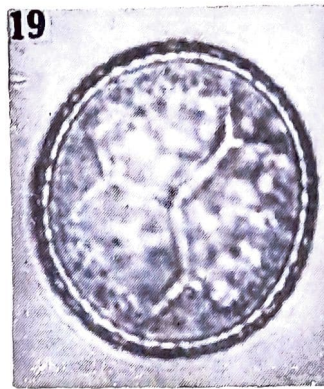
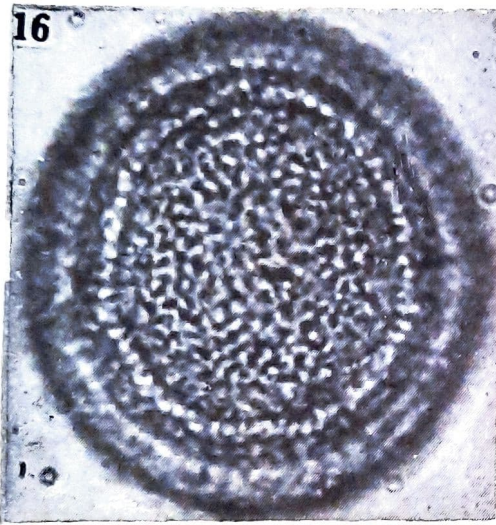
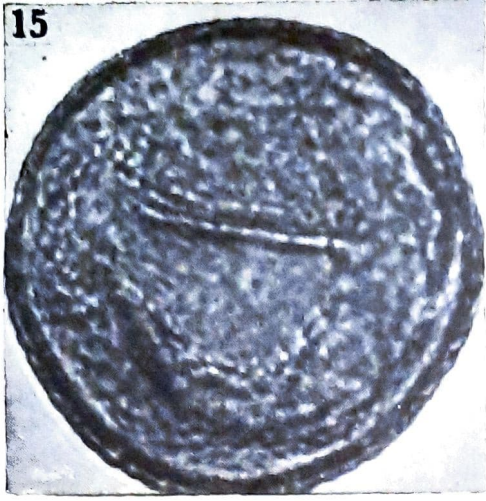
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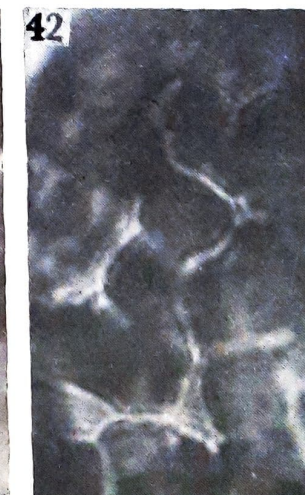
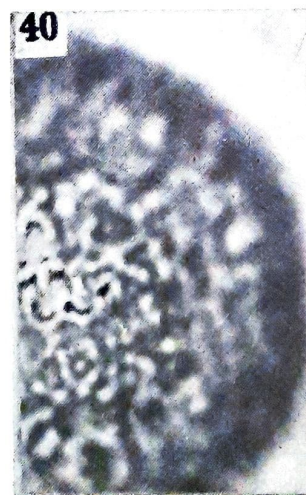
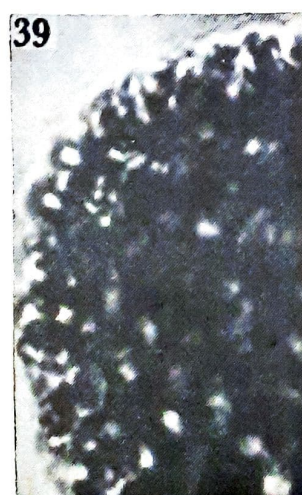
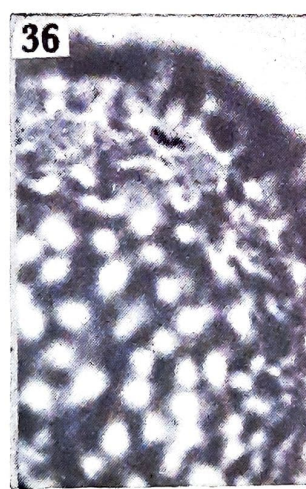
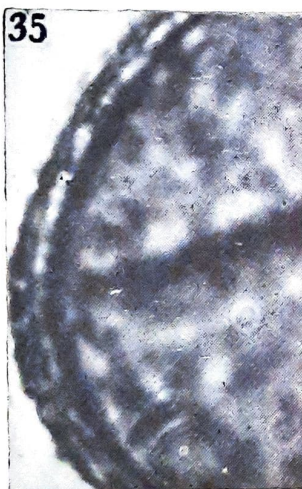
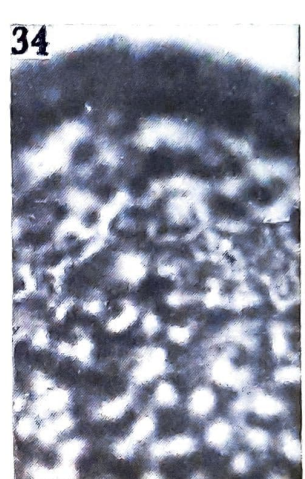
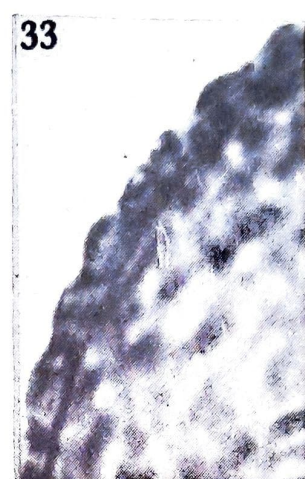
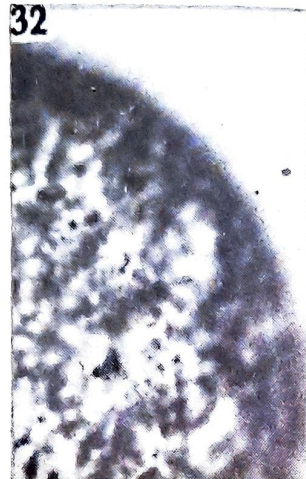
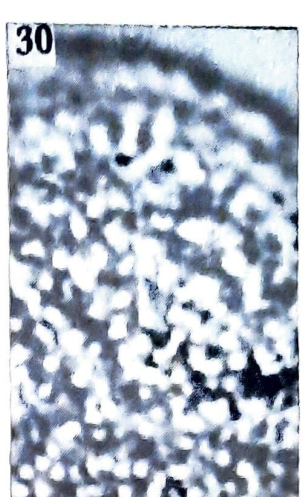
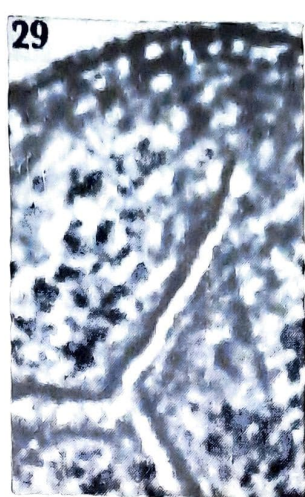
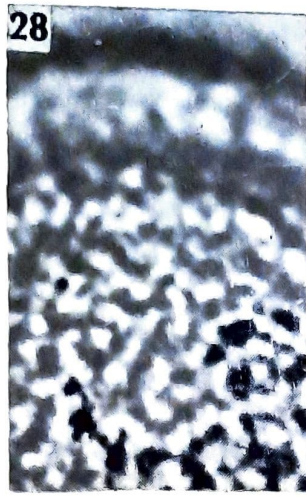
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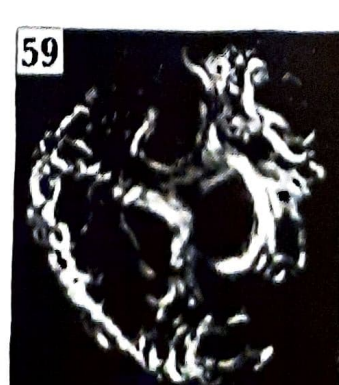
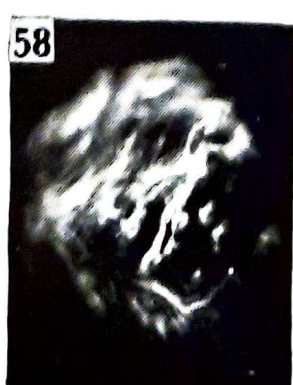
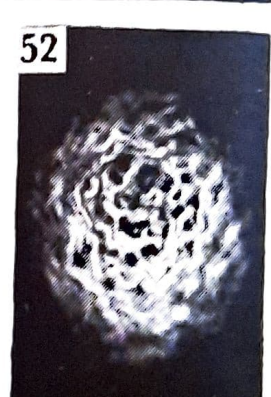
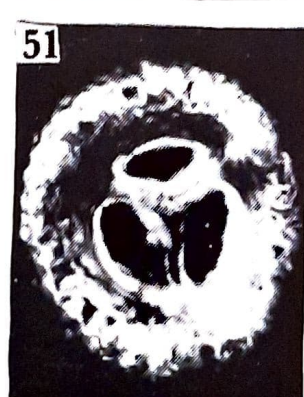
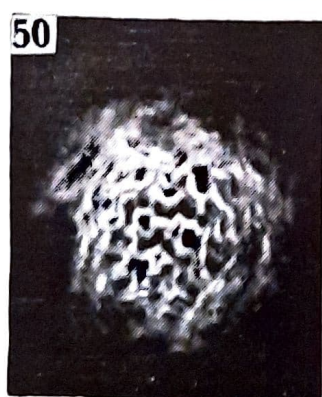
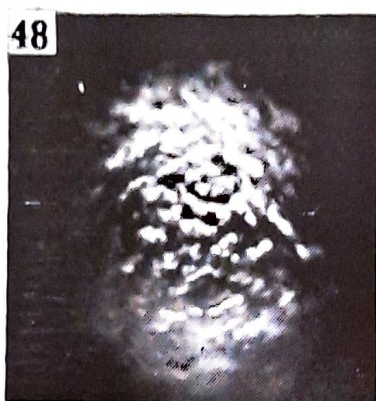
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EXPLANATION OF PLATES

Plate 1. 1. *Ophioglossum reticulatum* plant in centre showing two plants (on left and right) produced from root buds. A root of the plant on the right has a small root bud at the end (*rb*). x.25. 2. *O. reticulatum* plant having two leaves one of them with an unforked and the other with a forked spike. x.25. 3-4. *O. gramineum* plants showing branched spikes. Each spike has alternately produced two smaller lateral branches. x.75-5-6. *O. costatum* subterranean gametophytes showing sex organs (appearing as black spots on the surface). 5, x 13.5; 6, x 18. 7-14. *O. costatum* gametophytes with young sporophytic plants. x 2.

Plate 2 Spores of different species. 11, x 800; all others x 1000. 15-17. *O. reticulatum* monolete, alete and an alete folded spore, respectively. 18. *O. vulgatum*. 19. *O. costatum*. 20. *O. petiolatum*. 21. *O. nudicaule*. 22. *O. gramineum*. 23. *Helminthostachys zeylanica*. 24. *Botrychium lanuginosum*. 25. *B. virginianum*. 26. *B. lunaria*.

Plate 3. Photographs of acetolysed spores to show exine sculpturing with marginal and central region in sharp focus respectively. All x 2000. 27-28. *O. reticulatum*. 29-30. *O. vulgatum*. 31-32. *O. costatum*. 33-34. *O. nudicaule*. 35-36. *O. petiolatum*. 37-38. *O. gramineum*. 39-40. *H. zeylanica*. 41-42. *B. virginianum*.

Plate 4. Proximal and distal faces respectively of dry spores showing exine sculpturing in incident light. All x 1000. 43-44. *O. reticulatum*. 45-46. *O. gramineum*. 47-48. *O. vulgatum*. 49-50. *O. costatum*. 51-52. *O. nudicaule*. 53-54. *O. petiolatum*. 55-56. *H. zeylanica*. 57-58. *B. virginianum*. 59-60. *B. lunaria*.