'MISSING LINKS' IN THE EVOLUTION OF VASCULAR PLANTS

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INTRODUCTION

COLONISATION of land surface by plant life which originated and developed in an aquatic environment is perhaps the most important milestone in the evolutionary history of vegetable kingdom. The origin and evolution of a land flora is in essence the early history of vascular plants and it is interrelated with dominance of the sporophytic generation. On the basis of strong morphological evidence it is currently accepted that vascular plants have originated from Chlorophyceae. It is sometimes suggested that interpolation of a sporophytic generation between pre-existent gametophytic generations played a key role in colonisation of land, and evolution of a land flora is essentially the story of evolution of a vascular sporophyte[1]. However, the more accepted view is that colonisation of land and evolution of sporophyte are not necessarily interrelated but may only be coincidental. Church[2], Eams[3] and Fritsch[4][5] have elaborated arguments to show that in the algae which were parental to vascular plants, there exist a homologous alternation of generations, and the sporophyte and gametophyte are morphologically similar. Based on his extensive study of algal life histories, Fritsch[5] concluded that heterotrichous Chaetophorales are the group from which vascular plants evolved, and the earliest vascular plants are pteridophytes.

However, morphological differences between the Chaetophorales and the simplest of vascular sporophytes are so great that it is impossible to interconnect the groups except through highly conjectural intermediate forms. It was hoped that palaeobotany would fill the gap and one day the intermediary forms would be discovered, but search for them during the past half a century proved futile and, most palaeobotanists are today convinced that such forms would never be discovered. In this context it should be mentioned that the hypothetical life forms, proposed are those which would be intermediate between heterotrichous Chaetophorales and the simplest form of vascular plant sporophyte known, *viz.* Rhyniales. No attention was paid to the gametophytic generation, though colonisation of land was achieved by both the generations. This is possibly because pteridophyte gametophytes are little known and the dominant generation among land plants is undoubtedly the sporophyte, which in fact is the only vascularised plant.

THE MISSING LINKS

Fritsch[5] hypothesised that vascular plants evolved from heterotrichous Chaetophoralean ancestors like *Fritschiella* (figure 6) through, (a) parenchymatous development of the upright filamentous component of the thallus, as occur in many algae though most conspicuously in Rhodophyceae, (b) acquisition of an apical growing point and a dichotomous branching pattern, (c) reduction and ultimate loss of the prostrate system of branches, (d) development of vascular tissues in the upright system, and (e) development of a cuticle and consequent evolution of stomata. However, no living or fossil plants are known which give an indication of the morphological changes envisaged, and thus the plants in which they might have occurred remain today as the 'missing links' in evolution. A possibility exists that the evolutionary process as envisaged by Fritsch and widely accepted today may prove to be incorrect. This gains some support from the pattern of occurrence of oogamy among Chaetophorales; oogamy is not found in any which show specialisation in the upright system of branches, indicating that perhaps it was forms which exhibit elaboration of the prostrate

system and oogamy which are ancestral to vascular plants. It is difficult to conceive that during evolution of vascular plants which are heterogamous, there occurred a reduction and loss of the prostrate basal system of branches of the ancestral heterotrichous plant body, and elaboration of the upright system as conceived by Fritsch. While discussing the evolution of pteridophyte gametophytes, I have pointed out[6],[7] that far more than the sporophyte, the gametophyte of pteridophytes are morphologically closer to the heterotrichous Chaetophorales and that they exhibit step by step evolution leading to the typical cormophytic morphology of vascular plants, by progressive modification of the entire heterotrichous plant body. Pteridophyte gametophytes are, thus, the 'missing links' in the evolution of vascular plants from green algae.

EQUISETUM AND LYCOPODIUM

Among vascular plants the nearest approach to heterotrichous Chaetophorales like *Fritschiella* is found in the gametophyte of *Equisetum* (figures 7 and 8). It has a serporectus (having a parenchymatous basal creeping system and an upright system of photosynthetic branches) body composed of a cushion-like parenchymatous

sub-circular basal region bearing several leaf-like thin flat lamellae on the upper surface. There is no organised meristem but eventually cells in the central region cease to divide; and growth is only by cell divisions in the marginal region. Sex organs are produced marginally and an amorphous crescent-shaped meristem is differentiated subtending each gametangium towards its lower surface. When gametangia become crowded these discrete meristem pads merge together forming a lateral ring-like meristem on the basal cushion of the gametophyte, adding tissues on its basal upper and inner sides, the upper tissue developing fresh gametangia and lamellae. In older gametophytes some regions of the meristem become more active and then horizontal branches are developed marginally on the cushion. Thus, *Equisetum* gametophyte differs little from Chaetophoralean algae like *Fritschiella*. However, it has a more extensively developed basal cushion-like region, flattened multiseriate upright branches and a secondarily developed amorphous lateral meristem which girdles the basal cushion.

Nearly similar serporectus gametophytes are found in the Cernuum-group of species of *Lycopodium*. The gametophyte (figures 2, 9 and 10) has a parenchymatous cushion-like base
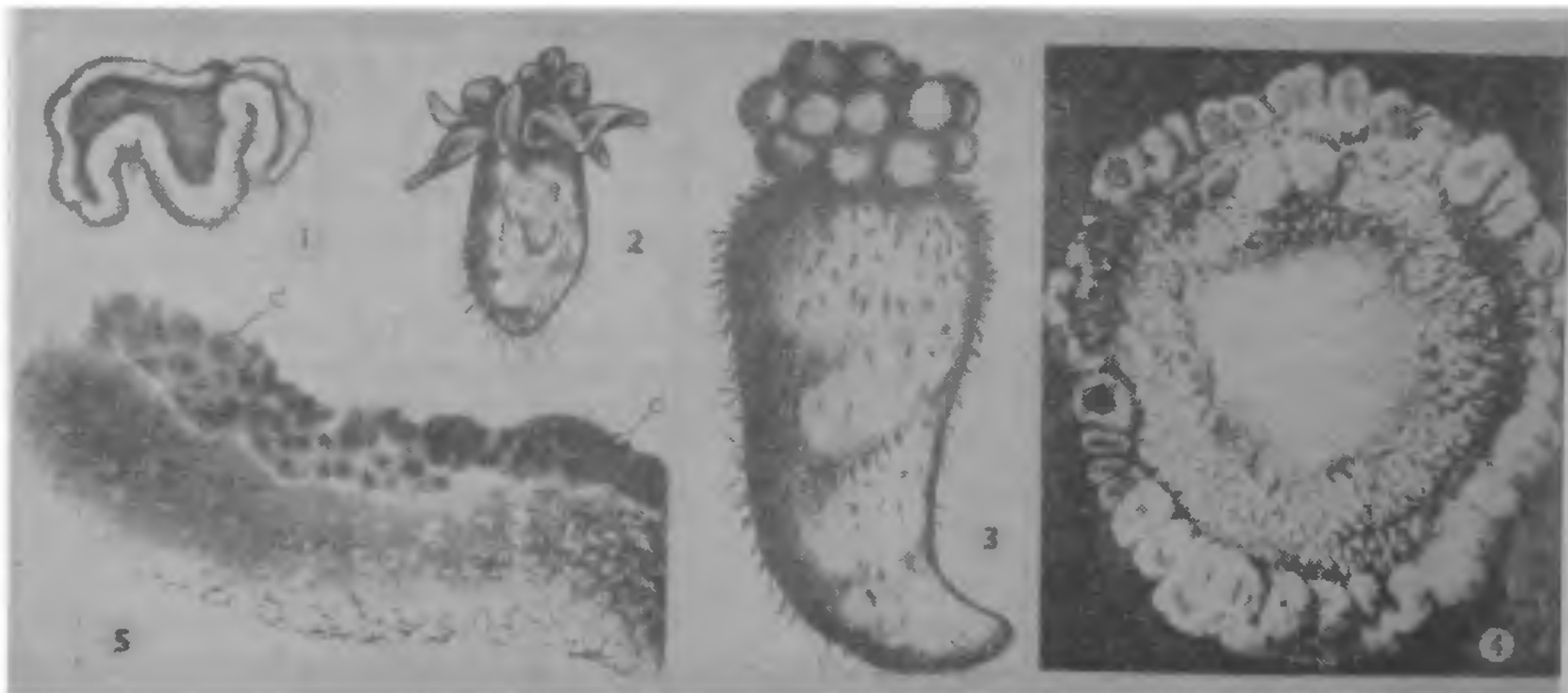
Figures 1–3. Lateral view of gametophytes of (*Lycopodium clavatum*), 1,(*L. cernuum*), 2, (*L. complanatum*),3. **4.** Dorsal view of the upper end of the gametophyte of *L. obscurum* showing marginal ridge with lobed crest. **5.** Lateral view of the gametophyte of *Botrychium* sp. showing dorsal median ridge (d).

bearing crowded leaf-like upright lobes on the upper surface and a lateral ring-like meristem separating the upper and lower regions. Most species are mycorrhizic with fungal association leading to tuberous development of the basal region (figures 2 and 10) which consequently becomes non-chlorophyllous and subterranean. In *L. salakense* which lacks mycorrhizic association, no tuberous development occurs. Others can be cultured on nutrient media without fungal association and then do not develop the basal tuberous region. The gametophytes of the Clavatum-group of *Lycopodium* are similar to those of the Cernuum-group, except that they have more pronounced mycorrhizic association, a more active lateral ring-like meristem and suppression of the upright system of photosynthetic branches; the gametophytes are subterranean, non-chlorophyllous and either top-like or disk-like (figures 1 and 3). The lateral ring-like meristem of some like *L. obscurum* (figure 4) develops annular ridges on the upper surface, and the ridges possess lobed crests. Apparently these lobes represent the reduced highly parenchymatised upright branches. Some like *L. selago* exhibit the capacity to produce a flared, irregular, chlorophyllous crown recalling the aerial branches of *L. cernuum*, when the apex of the gametophyte happens to become exposed above the substratum. Also, its ring-like lateral meristem produces elongated horizontal branches as in *Equisetum*, the branches bearing sex organs and uniseriate paraphyses on the upper surface; the paraphyses apparently are equivalent to the photosynthetic upright lobes of *L. cernuum*.

Lycopodium gametophytes exhibit an evolutionary advancement of profound significance and which makes them a step ahead of *Equisetum*. They show the beginning of an apical (terminal) meristem; an apical meristematic cell is differentiated in all species, early during development and it is central on the dorsal surface of the cushion-like basal part. In the Cernuum-group, (which is obviously the more primitive) this meristem is ephemeral and contributes little to the growth of the gametophyte. In the Clavatum-group it remains active a little longer, but its contribution to growth is still insignifi-

cant. However, in the Phlegmaria-group the apical meristem remains active throughout the life of the gametophyte and results in upward growth of the cylindrical gametophytic thallus. As a consequence of upward growth, the lateral ring-like meristem gets displaced and there is a continuous, nearly spiral lateral meristem formed. It is, however, active only at certain isolated points and develops into lateral horizontal branches as in *L. selago*. Thus, *Lycopodium* gametophytes represent the second step in evolution, *viz.* acquisition of an apical meristem and breaking up of the ring-like lateral meristem into a few active regions.

OPHIOGLOSSACEAE

Gametophytes of Ophioglossaceae exhibit a further advancement along the line of specialisation of *Lycopodium*. *Ophioglossum* has a mycorrhizic subterranean erect-growing cylindrical gametophyte. At spore germination a subglobose mass of cells is formed consisting of a less actively dividing basal tier and a more actively dividing upper tier[8]. The upper tier expands laterally but no organised lateral marginal meristem is differentiated; instead, an apical meristematic cell facing upwards is established medianly (figures 11–20). By its activity, the gametophyte grows upward as a cylindrical tuberous thallus which acquires a mycorrhizic association. Irregular meristematic activity in groups of lateral peripheral cells ('secondary thickening') occurs, which probably is a relic of the marginal meristem. In *Botrychium* mycorrhizic association is not as pronounced as in *Ophioglossum*, and the gametophyte is chlorphyllous and surface-growing. It acquires a creeping habit early in life so that the apical meristem is on one side. The thallus is dorsiventral and cushion-like (figures 5 and 21). It develops a series of hump-like, parenchymatous protrusions, medianly on the upper surface, forming a dorsal median ridge; archegonia are borne laterally on either side of the ridge. The ridge consists of a series of branches of limited growth and these become highly parenchymatised[9], and are similar to the lobed ring-like ridges on the gametophyte of *Lycopodium obs-*
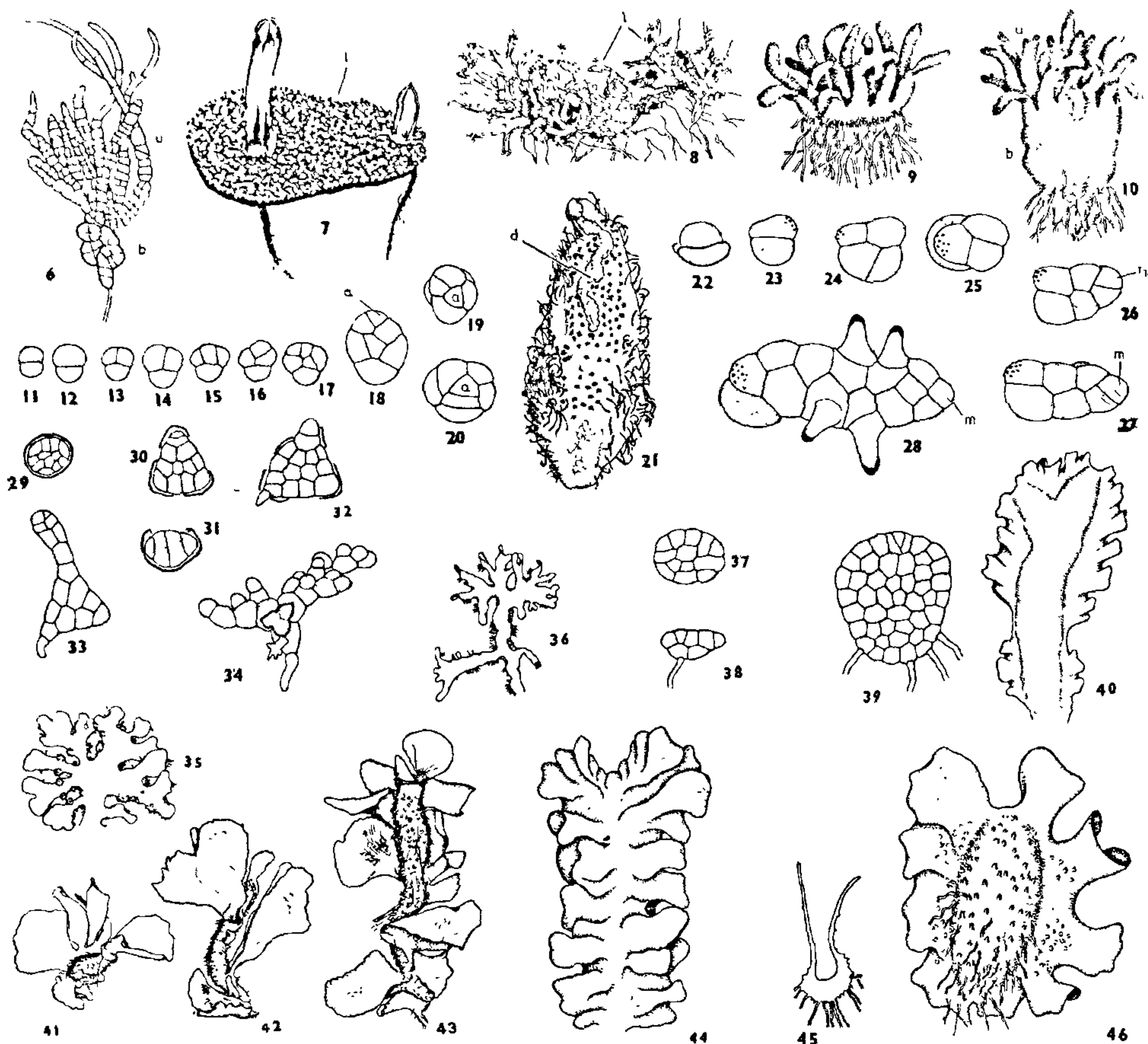
Figures 6–46. Gametophytes of *Fritschiella* and pteridophytes (*a*, apical meristematic cell; *b*, basal tuberous region; *d*, dorsal median ridge; *l*, lamellae; *m*, lateral meristematic cell; *u*, upright system of photosynthetic branches—figures **6, 8–18, 22, 23, 28, 31, 38, 41–43** & **45** are lateral views while others are dorsal views.) **6.** *Fritschiella tuberosa.* **7.** *Equisetum laevigatum* with two sporophytes attached[14]. **8.** *E. scirpoides* showing serporectus habit[15]. **9, 10.** *Lycopodium cernuum.* **11–20.** Early stages in development of Ophioglossaceae[10]. **21.** *Botrychium virginianum*[9]. **23–34.** Early stages in development of *Psilotum nudum* (**22–28**[8]) and *Mecodium* (**29–34**[16]). **35, 36.** Portions of adult prothallus of *Mecodium flabellatum* (**35**[18]) and *Hymenophyllum blumeanum* (**36**[19]). **37–40.** *Marattia sambucina*, early stages in development (**37–39**) and adult gametophyte (**40**[19]). **41–43.** *Mohria caffrorum*[17]. **44.** *Dipteris conjugata*[20] **45, 46.** *Matonia pectinata* (**46**) and vertical section of same showing uplifted nature of wings (**45**).

curum which represent stunted upright lobes equivalent to the lamellae of *Equisetum* and upright photosynthetic lobes of *L. cernuum*. In addition to the median ridge, the gametophyte of *Botrychium* has a lateral ring-like meristem similar to the ring-like meristem of *Lycopodium*. Thus, the gametophyte of *Botrychium* is intermediate between *Lycopodium* and *Ophioglossum*.

PSILOTALES AND FILICOPSIDA

Gametophytes of Psilotales and Filicopsida represent a line of evolution, in which an apical meristem is never developed. Spore germination in *Psilotum* gives rise to a subcircular plate of cells, usually having a less actively dividing lower tier and more active upper tier as in *Ophioglossum*. But in contrast to *Ophioglossum* no apical meristem is formed. Instead, one of the marginal cells of the upper tier establishes a lateral meristematic cell facing sideways[10] (figures 22–28). By the activity of this cell a horizontal cylindrical gametophyte is developed which, thus, is morphologically equivalent to one of the lateral branches developed by the marginal ring-like meristem of *Lycopodium* or *Equisetum*. Upright system of branches of the serporectus ancestral form is totally suppressed, possibly as a consequence of mycorrhizic nutrition. Similarly in some of the primitive ferns like Marattiaceae[11], Matoniaceae and Hymenophyllaceae, spore germination results in a subcircular plate of cells which is one cell thick in Hymenophyllaceae but two cells thick in the others (figures 29–31, 37, 38). The plate expands marginally, and in Hymenophyllaceae three growing points are established at the margin equidistant from each other. One or sometimes two of these grow out horizontally as rhizoid while the others establish a distinct lateral meristematic cell facing sideways (figures 32-34); a flat ribbon-like branched creeping prothallus is produced by the activity of each meristematic cell (figures 35, 36). In Marattiaceae and Matoniaceae in which the primary plate formed at spore germination is two cells thick as in *Psilotum*, expansion of the plate occurs primarily by division of the upper tier of cells. Gradually cells on one side of the circular plate cease to divide and the opposite margin alone then expands. A meristematic cell is established sooner or later in one of the median marginal cells on the expanded side and by its activity a cordate-thalloid gametophyte is developed (figures 39, 40). Thus the entire gametophyte (other than primary plate) is the product of the activity of a lateral marginal meristem. Whereas the Hymenophyllaceae lack a cylindrical midrib and flat wings characteristic of fern gametophytes, both Marattiaceae and Matoniaceae possess massive cylindrical midribs and narrow uplifted ruffled thin wings (figures 40, 45, 46).

Comparison with the other primitive ferns, clearly indicates that the wings of fern gametophytes are modified upright branches of a serporectus plant body and the midrib represents the basal prostrate system. In *Mohria* (Anemiaceae) the gametophyte consists essentially of a thick cylindrical creeping midrib bearing an irregular row of small leaf-like lobes on the dorsal surface[12] (figures 41 and 42). Occasionally the midrib may become erect-growing, and then bears many scattered leaf-like lobes (figure 43). In many cases the lobes merge together by their margins and form a large erect wing. Such margin-to-margin fusion of upright photosynthetic lamellae occur even in *Equisetum*[13]. The leaf-like lobes on the midrib of *Mohria* are evidently the upright system of branches of a serporectus plant body and are equivalent to the lamellae of *Equisetum*. It seems obvious that the wings of the common cordate-thalloid gametophyte of ferns are formed as in *Mohria* by regular edge-to-edge fusion of two dorsal rows of lamellae. This is supported by the fact that in many ferns, the wings are obliquely erect and not spread out (figure 45). Also, in many of the comparatively primitive ferns like *Marattia*, *Matonia* and *Dipteris* the wings of the gametophyte are narrow, obliquely erect and very prominently ruffled (figures 40, 44 and 46). The ruffled nature of the wings betray their origin by lateral fusion of broad lamellae-like lobes. If an irregular longitudinal row of lamellae like those of *Equisetum* (plate-like, often with a broad anterior end and narrower basal end) were to merge by the margins, the result would have been a prominently undulated flap, like the wings of *Marattia*, *Matonia* and *Dipteris*.

EVOLUTION OF SPOROPHYTE

Extending the analogy of the gametophyte to the sporophytic generation, it is possible that the sporophyte also followed a similar course in evolution. The embryo of pteridophytes is a

globose or spindle-shaped mass of cells similar to the young gametophyte of *Lycopodium* and *Ophioglossum*. In some of the primitive pterido-phytes like Psilotales and *Stromatopteris*,the globose embryo develops two (*Psilotum*) or many (*Stromatopteris*) lateral meristems which grow out as horizontal branches and develop into individual plants. These lateral meristems appear to be equivalent to the ring-like meristem of *Lycopodium* gametophyte. No apical (terminal, erect-growing) meristem is developed (as also in the gametophyte of *Psilotum*). But in the majority of pteridophytes an apical (terminal) meristem is established early in the embryo as also a lateral ring-like meristem. The lateral meristem in *Equisetum* forms a lateral annular ridge in which three growing points are established and these develop into the three cotyledonary leaves. Also, it gives rise to one or two roots on the ventral side close to the base of the annular ridge (sheath which interconnects leaf bases). Successive ring-like meristems are produced due to vigorous upward growth of the embryo and successive whorls of leaves result.

In ferns,it seems that the activity of the ring-like lateral meristem is restricted to a single point on one side as in the fern gametophyte. This region grows out as also the first leaf and is associated at its base with a root. The fleshy, stipule-like outgrowths at leaf base, found in primitive ferns like Ophioglossaceae and Marattiaceae,possibly are the result of a more extensive lateral meristem. Along with the leaf base, the stipules encircle the axis and recall the annular ridge (leaf sheath) of *Equisetum*. Whereas three growing points are established in *Equisetum*,only one is formed in Ophioglossaceae and Marattiaceae and this results in a solitary leaf with its base

expanded as stipules. The main axis of the leaf of ferns thus seems to be the elaborated lateral branch of the basal tuberous region of a serporectus plant body. The leaf lamina, in all likelihood, is developed like the wings of the gametophyte, *i.e.* by lateral fusion of a series of upright lobes borne on the lateral branch of the basal cushion-like region of the serporectus plant body.

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