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Systematic studies in the genus *Mohria* (Anemiaceae: Pteridophyta). IV. Comparative gametophyte morphology in *Mohria* and *Anemia*

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ABSTRACT

The sporophyte morphology in *Mohria* and *Anemia* (Anemiaceae) is dissimilar. However, similarities in their anatomy, trichomes, spores and chromosome numbers show clearly that these genera are related. The contribution of the gametophyte to the classification and phylogeny of the Pteridophyta is largely neglected. The gametophyte of Anemiaceae is primitive in many features when compared with that of other leptosporangiate ferns. The prothallus of *Mohria* is considered more advanced than that of *Anemia* in the germination pattern of the spores, the presence of mycorrhiza in the cushion, the permanently lateral position of the meristematic region and the simpler trichome types.

UITTREKSEL

Die sporofiet van *Mohria* en *Anemia* (Anemiaceae) verskil morfologies van mekaar. Ooreenkomste in hul anatomie, trigome, spore en chromosoomgetalle toon egter duidelik dat hierdie genusse verwant is. Die gametofiet van Anemiaceae is in baie opsigte primitief wanneer dit met dié van ander leptosporangiate varings vergelyk word. Die protallus van *Mohria* word as meer gevorderd beskou as dié van *Anemia* in die ontkiemingspatroon van die spore, die teenwoordigheid van mikorisa in die protalluskussing, die meristematiese streek wat permanent in 'n laterale posisie bly en die meer eenvoudige trigoomtipes.

INTRODUCTION

Mohria Swartz, together with *Anemia* Swartz, *Schizaea* J. Smith, *Actinostachys* Wall. and *Lygodium* Swartz are commonly placed in a single family, the Schizaeaceae (Engler & Prantl 1898–1902; Bower 1923; Christensen 1938; Copeland 1947; Tryon & Tryon 1982; Tryon & Lugardon 1990), because of their exindusiate, monosporangiate sori and characteristic apical ring of annulus cells. Eggert & Delevoryas (1967) have shown, however, that 'schizaeaceous' sporangia evolved independently in coenopteridid derivatives that are quite unrelated to the schizaeoid ferns. *Senftenbergia* Corda, formerly included in the Schizaeales (Reed 1947) because of its apical annulus, has been noted to occur on zygopteridid foliage (Mickel 1974). This sporangium type therefore appears to be polyphyletic in origin. The subdivision of the schizaeoid assemblage into distinct families, Anemiaceae (*Anemia* and *Mohria*), Lygodiaceae (*Lygodium*) and Schizaeaceae (*Schizaea* and *Actinostachys*) has recently gained wider acceptance (Nayar 1970; Bierhorst 1971; Löve *et al.* 1977; Clifford & Constantine 1980; De la Sota & Morbelli 1987).

Although *Anemia* and *Mohria* are dissimilar in morphology they are evidently related in view of similarities in their anatomy (Prantl 1881; Bower 1918; Roux *et al.* 1992), trichomes (Mickel 1962; Roux 1992a), spores (Mickel 1962; Hill 1977, 1979; Dettman & Clifford 1991; Roux 1992b) and chromosome numbers (n = 38, 76).

Bower (1923) discouraged the use of gametophyte morphology in pteridophyte classification. However, studies in that field were continued and results have proven useful as taxonomic and phylogenetic tools. The purpose of the present study is to summarize previously published work, and to extend the information on the anatomy and morphology of the prothallus. All the information relevant to the prothallus of *Mohria* is synthesized and analysed.

MATERIAL AND METHODS

Anatomical studies were carried out on prothalli collected in the wild. Wax embedding was done using standard techniques (Johansen 1940). Serial sections 8–10 µm thick were taken with a rotary microtome and stained with fast green and safranin. Photography was done with a Zeiss 'Axoscop' fitted with a M35W camera. Ilford PanF film was used throughout.

SEM studies were done on spores cultivated on clay pots. The prothalli were critical-point dried using CO_2 as a transitional fluid. Specimens were affixed to aluminium stubs using glue and sputter coated with Au/Pd and were viewed in a Cambridge S200 SEM at 5 kV.

Specimens examined

Mohria caffrorum (L.) Desv., 3318 (Cape Town): Stellenbosch, (–DD), *Roux 2363*.

M. marginalis (Sav.) J.P. Roux, 2829 (Harrismith): Cathedral Peak, (–CC), *Roux 2297*.

M. vestita Bak., 2430 (Pilgrim's Rest): Graskop-Sabie rd, (–DD), *Roux 2236*. 2630 (Carolina): Swaziland, (–DB), *Roux 2261*.

Voucher specimens are all deposited in the Compton Herbarium (NBG).

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RESULTS AND DISCUSSION

Spore germination

Atkinson (1960, 1962), based her observations on spore germination in *Mohria* and *Anemia* on whole mounts and found the pattern in both these genera to be identical. Atkinson (1960, 1962) recorded the first division of the spore protoplast to be in an equatorial plane, forming an apical cell at the proximal pole, this being the pole bearing the triradiate scar, and a distal cell at the distal pole. With the second division the apical cell divides at a right angle to the first to form a larger green prothallial cell and a smaller, almost colourless, rhizoidal cell (Figure 1). Nayar & Kaur (1968, 1971) categorised this germination pattern as polar and more specifically as the *Anemia*-type.

Observations made by Raghavan & Huckaby (1980) on sectioned material are in conflict with those of Atkinson (1960, 1962). They convincingly showed that in *Mohria* the first division is in an equatorial plane resulting in a larger distal cell at the distal pole and a smaller rhizoidal cell at the proximal pole. With the second division, however, it is the distal cell that divides to form a larger distal cell and a smaller protonemal cell (Figure 1). This pattern corresponds with the *Osmunda*-type of polar germination as defined by Nayar & Kaur (1968, 1971).

Raghavan & Huckaby (1980) found *Mohria* and *Anemia* to be dissimilar. In *Anemia*, as in *Mohria*, the first division is in an equatorial plane giving rise to a larger distal cell at the distal pole. However, at the proximal pole a smaller protonemal cell is formed. With the second cellular division the distal cell divides to form a larger distal cell and a smaller rhizoidal cell (Figure 1). This pattern of spore germination does not fit into the scheme of Nayar & Kaur (1971).

The distinct modes of germination in *Mohria* and *Anemia* probably indicate different trends of specialization which is also evident in the sporophyte morphology. The origin of the rhizoid and protonemal cell in *Mohria* by a route similar to that seen in genera considered advanced, confirm its specialized status.

Prothallial development

Prothallial development in *Mohria* (Bauke 1878; Atkinson 1960) and *Anemia* (Momose 1949; Twiss 1910; Kaur 1961; Atkinson 1962) is known in some detail and shows no differences which may be of any significance. Following the formation of the prothallial cell it continues to grow and divide by transverse divisions producing a filament five to six cells long. Longitudinal divisions are soon evident in cells behind the apex and these continue both in an anterior and posterior direction. Vertical, longitudinal and oblique divisions take place and the position of the localized dividing area in relation to the rest of the expanding but less rapidly growing gametophytes determines the shape of the early cellular plate. The margin of the prothalli may be variously lobed. I often found unicellular trichomes to be associated with the sinuses. A true apical initial is evidently never formed. A large wing is eventually formed with the more rapidly dividing meristem in a lateral position which, in *Mohria*, is maintained throughout the life of the plant. Circumstances contributing to the lateral position of the meristem can be ascribed to the anticlinal divisions which are more numerous than periclinal divisions. Also no wing is formed on the proximal side of the meristem but only hairs and gametangia. Divisions in a third plane give rise to a cushion directly behind the meristem. The gametophyte grows at first more or less parallel to the substrate but as the cushion continues to develop the thallus assumes an oblique position supported by long rhizoids. In *Mohria* the prothallus eventually becomes upright.

Mature prothallus

In the mature prothallus of *Mohria* the lateral meristem remains hidden by the continued development of the voluminous spiralling wing (Figure 2A). The older basal parts of the wing eventually turn brown as the thallus grows upwards. Marginal areas of all but the last formed parts of the wing are irregular in outline. The wings are one cell layer thick except close to the cushion where it may be two cells thick. As growth continues the massive cushion grows upwards and appears as a column. Small flaps of sterile tissue often appear on the cushion. These flaps of tissue may become winglike and aid in photosyn-

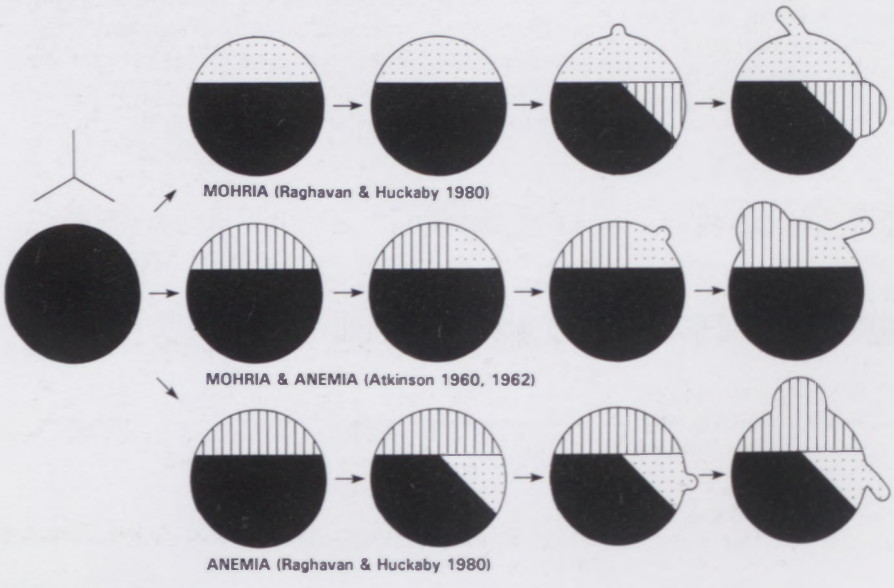

FIGURE 1.—Spore germination patterns in *Mohria* and *Anemia* as observed by Atkinson (1960, 1962) and Raghavan & Huckaby (1980). Dark-coloured = spore or distal cell; vertical lines = protonemal cell; dots = rhizoidal cell.

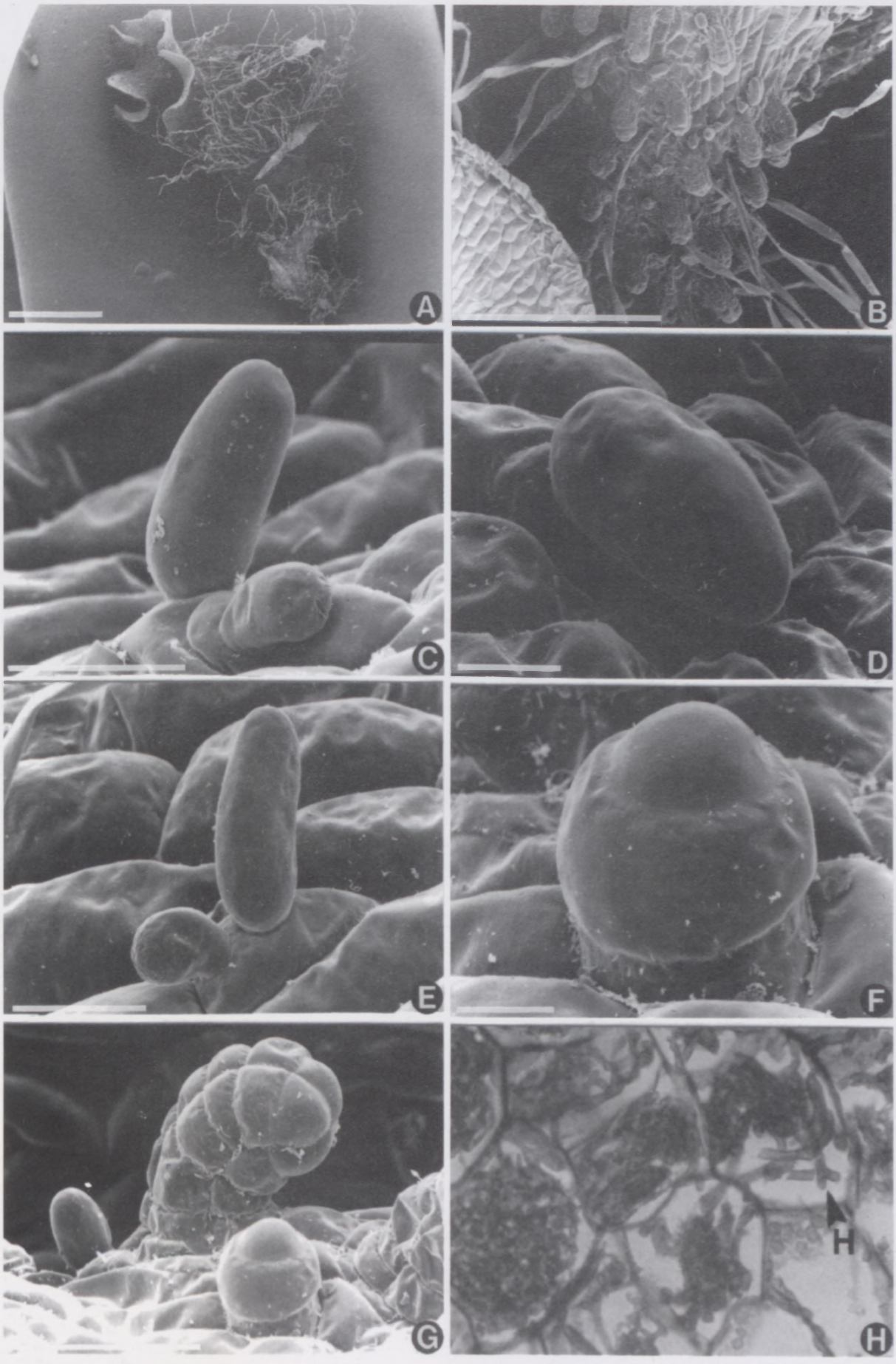

FIGURE 2.—Prothallus and prothallial structures in *Mohria vestita, Roux 2261*. A, mature prothallus showing spirally formed wing; B, prothallus cushion showing interspersed antheridia, archegonia and trichomes; C, three-celled hair on prothallus wing; D, unicellular hair on prothallus wing; E, two-celled hair on prothallus wing; F, mature antheridium on abaxial surface of prothallus cushion; G, archegonium on abaxial surface of prothallus cushion; H, cushion of *M. vestita, Roux 2236*, prothallus showing endofungal hyphae (H). Scale bars: A, 2 mm; B, 500 µm; C, E, 50 µm; D, F, 20 µm; G, 100 µm; H, × 1000.

thesis. Antheridia, although mostly confined to the posterior regions of the thalli may occur interspersed with archegonia (Figure 2B & G) which are formed later than the antheridia. As the columnar cushion increases in height, archegonia and hairs continue to be formed in great numbers. Abundant rhizoids are formed on the cushion. Older rhizoids are brown, long and stiff and continue to be produced along the columnar cushion. Mature thalli of *Mohria* are chlorophyllous and mycorrhizal (Figure 2H) with the mycorrhiza restricted to the ventral side of the cushion. The endophytic fungus evidently plays only a minor role in the nutrition of the thallus, as in cultivated material, where the fungus is absent, the thalli show no adverse effects.

In *Anemia*, cells on the proximal side of the meristem grow outwards until a small wing is formed. As this wing becomes larger, the meristem is carried into a vertical position and the only indication of an earlier lateral meristem is the inequality of the wings. This change in position of the meristem is accompanied by the development of a thick cushion bulging towards the ventral side, and by uplifting and recurving of the wings over the dorsal surface of the cushion (Atkinson 1962). Unlike *Mohria* the near vertical position of the prothallus is not maintained. In *Anemia* the thalli are elongate-cordate in form, more or less prostrate in position, green at the anterior end and dying off behind. Rhizoids and archegonia are produced in great numbers on the ventral surface of the cushion.

Nayar & Kaur (1971) suggested the more primitive thallus to be dorsiventral with a massive median midrib and heavy wings several cells thick near the midrib but progressively becoming one cell layer thick towards the margins. In this respect the prothalli of *Mohria* and *Anemia* can be considered primitive. A step in advancement in *Mohria* and *Anemia*, however, is the elimination of the apical initial and the development of a multicellular meristematic region. Nayar & Kaur (1971) furthermore suggested that among those genera with a thalloid-cordate type gametophyte, the most primitive is the symmetrical type. In *Anemia* the prothallus is temporarily asymmetrical but later becomes symmetrical. The gametophyte of *Mohria* remains permanently asymmetrical and is therefore considered more advanced.

Prothallial trichomes

The thallus of *Mohria* bears small one- to three-celled hairs (Figure 2C) on the wing and cushion surfaces among the antheridia and archegonia. Atkinson (1960) found most hairs in older thalli to occur mainly on the surface of the cushion. My observations, however, showed that the cushion is not significantly more hairy than the wings. Unicellular trichomes, up to 60 µm long, similar to the naviculate trichomes found on the fronds of the sporophyte (Roux 1992a), are the most common type in *Mohria* and occur on the wings as well as on the cushion (Figure 2D). Two-celled hairs common on young thalli, have previously been described by Bauke (1878) and Stokey (1960). The outermost colourless cell is at least twice as long as the basal cell. A chlorophyllous basal cell very frequently bears two colourless cells (Figure 2E). This was also reported by Bauke (1878) and Atkinson (1960). These cells are usually of unequal size. Atkinson (1960) also reported branched hairs and hairs up to three cells long from the wings.

One- to four-celled hairs also occur on mature prothalli in *Anemia*. At first, the hairs are marginal, developing from a cell adjacent to the meristem. As in *Mohria*, the basal cell of two-celled hairs is chlorophyllous and the outer cell colourless. Marginal multicellular hairs have been reported in some *Anemia* (*A. adiantifolia*, *A. aurita*) species (Atkinson 1962).

Prothallial trichomes have been considered of little value in taxonomic and phylogenetic studies (Stokey 1951, 1960; Atkinson & Stokey 1964) because similar trichome types occur in apparently unrelated groups of ferns, a situation that may be ascribed to parallel evolution. Nevertheless Nayar & Kaur (1971) suggest that the restricted distribution of hairy prothalli among the various phyletic groups may be of value in comparative studies. Naked prothalli appear to be the more primitive condition among the homosporous ferns. Unicellular hairs are more common in advanced families such as Polypodiaceae, Davalliaceae, Lomariopsidaceae and Grammitidaceae and are usually secretory (Nayar & Kaur 1971). The prothallus of Schizaeaceae is either subterranean or terrestrial and is devoid of any trichomes. In *Lygodium* the prothallus has been described as naked (Bauke 1878; Twiss 1910) but a few clavate trichomes have been reported for *L. flexuosum* (L.) Swartz (Nayar & Kaur 1971, *contra* Mahabale & Kulkarni 1949). I have observed clavate trichomes in a marginal position in cultured prothalli of *L. japonicum* (Thunb.) Swartz. In the Anemiaceae, however, non-secretory, often multicellular trichomes occur.

Gametangia

Antheridium

Antheridia are formed on the margins and ventral surfaces of the thallus in the region of the meristem. They are produced in great numbers on the cushion and may extend to the wing. Antheridia in *Mohria* and *Anemia* are similar in ontogeny and morphology. Atkinson (1960, 1962) described the antheridium as developing from a superficial initial. A thin disc-shaped cell is cut off from the antheridial initial to form a proximal ring cell and a distal terminal cell. This is followed by the formation of a dome-shaped wall which divides the terminal cell into an outer wall or ring cell and an inner primary spermatogenous cell. A division of the ring cell gives rise to the cover cell of the antheridium. The antheridium structure is thus typical of the leptosporangiate ferns (Figure 2F). Successive division of the spermatogenous cell gives rise to a small number of sperm. In *Mohria* and *Anemia* each spermatozoid is contained within a cell wall at the time of release (Atkinson 1960; Nester 1985). At dehiscence the cover cell is shed explosively and the spermatocytes emerge one by one through a pore.

Archegonium

My observations in the ontogeny of the archegonium in *Mohria* conform with those of Atkinson (1960). Archegonia are borne on the abaxial side of the cushion only. In mature archegonia I found the archegonial neck, which

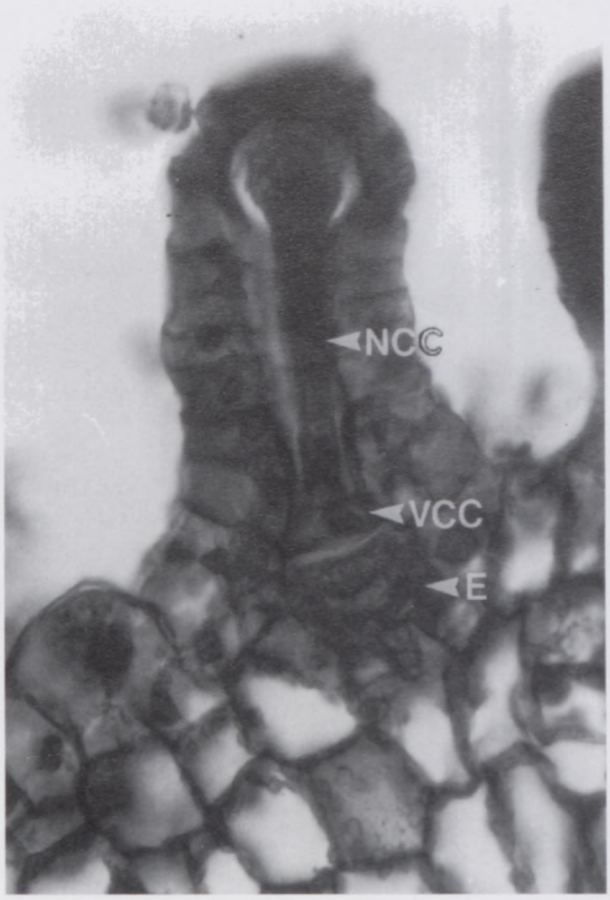

FIGURE 3.—Section through archegonium of *Mohria marginalis, Roux 2297*, × 400. E, egg cell; NCC, neck canal cell; VCC, ventral canal cell.

consists of four cells and up to eight tiers high (Figure 2G), to curve in the direction of the substrate (Figure 2B), a system which would be advantageous to the fertilization process. Prior to the opening of the archegonium the distal part of the neck becomes bulbous and the neck canal cell contorts to form a globular mass containing two to four nuclei (Figure 3) confirming Atkinson's (1960) observations. The venter is well embedded in the cushion and is surrounded by a layer of small cells containing a dense protoplasm. Ontogenetically and structurally the archegonia in *Mohria* and *Anemia* are similar.

Nayar & Kaur (1971) described an advanced archegonium as having a neck consisting of three to four tiers of cells high, which curve away from the apex and possess an undivided neck canal cell. Archegonia in Anemiaceae thus conform largely with the primitive type.

Embryogenesis

The division of the zygote has not been observed by me or by Atkinson (1960, 1962). De la Sota & Morbelli (1987), however, claim it to be of the 'leptosporangiate' type, in which the first division is longitudinal or parallel to the main axis of the archegonium.

The young embryo of *Mohria* is protected by a well-developed calyptra (Figure 4A). Initially only the foot and the stem can be identified. The foot is well embedded in the ground tissue of the prothallus cushion. The young embryos I examined showed the frond to differentiate before the root. The stem soon developed an apical initial with three cutting faces. The primary root developed endogenously and contained a large apical initial and a well-defined rootcap even before breaking through the cortical tissue of the stem (Figure 4B).

Like Atkinson (1960) I also found prothalli attached to young sporophytes containing up to five fronds. Sections through these prothalli show that the cell walls separating the foot from the prothallus tissue thicken and form an abscission layer. Mycorrhiza were also observed in tissue

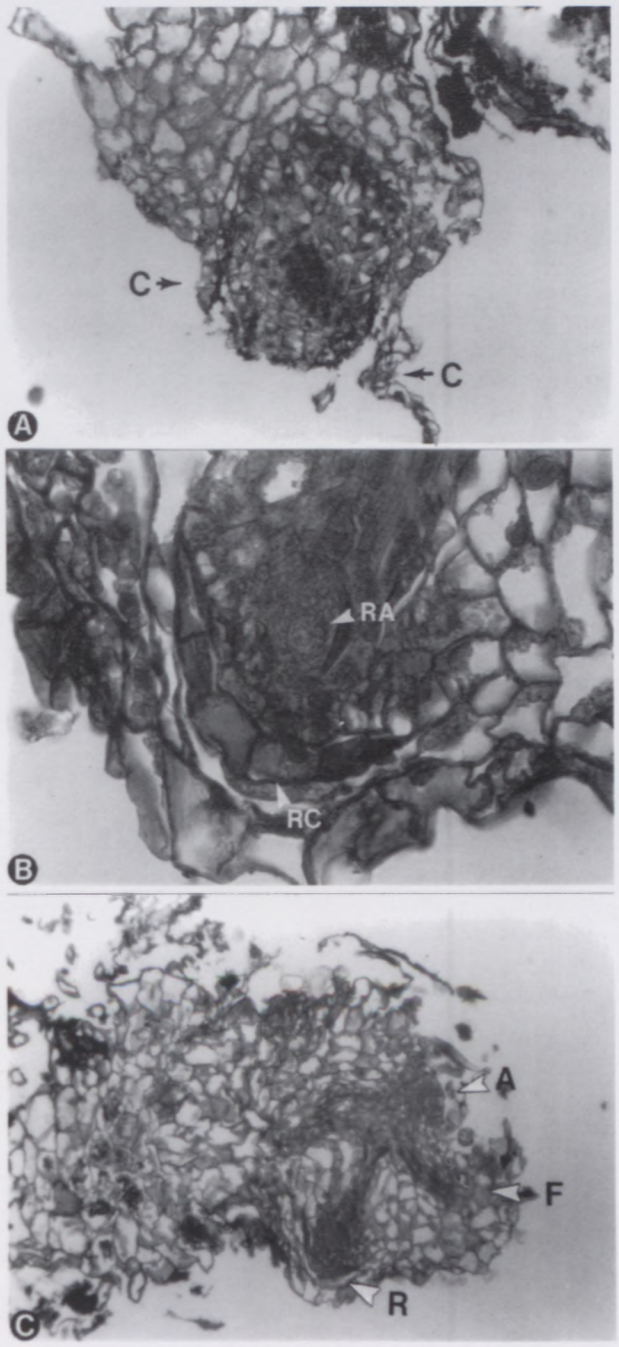

FIGURE 4.—Transverse sections of embryo in *Mohria vestita, Roux 2236*. A, young embryo showing calyptra (C), indicated by arrows, × 68; B, first root before breaking through prothallus tissue showing root cap and large apical initial, × 270; C, prothallus of a developing young plant with first root, frond and shoot apex, × 68. A, apical initial; C, calyptra; F, frond; R, root; RA, root apex; RC, root cap.

of the young sporophyte, especially the stem and roots, as well as in the tissues of the gametophyte.

Vascular tissue in the root, stem and frond is formed at an early stage (Figure 4C). Initially the tracheids show spirally arranged secondary thickenings but later pitting is of the scalariform or reticulate scalariform type.

CONCLUSIONS

Classification and phylogeny of the Pteridophyta is largely based on the dominant sporophyte generation. The small, usually overlooked, gametophyte generation or prothallus may, however, also contribute to the understanding of the evolutionary processes and phylogeny of the ferns. Bower (1923) and Holttum (1949), cautious of the effects of external influences during the development of the prothallus, realised the meagre but important contribution it can make with a view to classification.

Stokey (1951) and Nayar & Kaur (1971) suggested possible evolutionary trends in the structure of the prothallus and gametangia. Features that are considered advanced in the prothallus of the leptosporangiate ferns are: a small, short-lived, autotrophic, symmetric, cordate thallus with a centrally situated meristematic region and poorly developed midrib. Trichomes are present but are simple, unicellular and secretory. The antheridium is a three-cellular structure, consisting of a basal, ring and cap cell. The cap cell dehisces in its entirety and the antheridium has a small sperm output. The archegonia are small, form later than the antheridia, and are situated closer to the meristematic region. The mature archegonium has a neck that curves away from the meristem and consists of up to four tiers of cells. The neck canal cell is undivided.

Considering these changes, the prothallus of Anemiaceae is in many respects phylogenetically primitive, a feature which is also expressed in many morphological features of the sporophyte. On grounds of the sporophyte, *Mohria* is considered phylogenetically more advanced than *Anemia* (Bower 1923; Mickel 1962). The permanently laterally placed meristematic region and the absence of multicellular hairs from the prothallus are supportive of such an assessment.

It is thus evident that the prothallus can make an important contribution to an understanding of the phylogeny of the Pteridophyta at the family as well as at the generic level.

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