

MEGASPORES AND MASSULAE OF *AZOLLA PRISCA* FROM THE OLIGOCENE OF THE ISLE OF WIGHT

by K. FOWLER

ABSTRACT. Scanning and transmission electron microscopy, ultra-thin sectioning and light microscopy are employed in this investigation of *Azolla prisca*, which is placed in Section *Trisepta* sect. nov. of the genus. The columella and structural modifications of the proximal megaspore wall of *A. prisca* are compared with other species, both fossil and modern, and the phylogenetic interrelationship of these structures are discussed. The complex megaspore wall reveals an exine and two-layered perine, the outer perine layer being further subdivided into four zones. The inner perine layer thickens to form the proximal wall and associated labra. The wall structure of most *Azolla* species appears to be of this same basic pattern. Massulae, found dispersed and attached to megaspore apparatuses, reveal funnel-shaped cavities connecting the microspores to the exterior. Although previously unrecorded in *Azolla* such structure is present in both fossil and modern species, and is thought to form an escape mechanism for spermatozoids. A list of the better-known pre-Miocene *Azolla* species is presented, which includes stratigraphic range, and characteristics expressed as a formula. The evolutionary trends in *Azolla* are briefly reviewed.

THE genus *Azolla* belongs to the Salviniaceae, a family of heterosporous ferns. Plants are free-floating, occurring in freshwater habitats mainly in warm temperate to tropical zones. Some forty-eight species are recorded, six of which are extant. The fossil record of *Azolla*, dating back to the late Cretaceous of North America (Hall 1969b), is based mainly on its highly distinctive reproductive structures, the megaspore apparatus, and massula. In modern species, sporocarps are borne in pairs which arise from the ventral lobe of the first leaf of a branch. These paired sporocarps may be male and female, termed microsporocarp and megasporocarp respectively, or they may be of the same sex. The microsporocarp contains microsporangia holding a number of massulae; the megasporocarp contains a megasporangium within which is a single megaspore apparatus. The latter consists of a megaspore with an elaborate wall, and a unique complex swimming apparatus. The term 'Schwimmapparat', first applied by Strasburger (1873), is a misnomer in that the structure does not endow buoyancy, mature megaspores sinking following liberation (Sculthorpe 1967). The massula is a frothy pseudocellular structure in which microspores are embedded. The six modern species, together with their present geographical distribution are as follows (data from Mahabálé 1963; Sculthorpe 1967; and Svenson 1944):

A. filiculoides Lam. From Alaska to Guatemala in North America, and in Andean and southern South America. Introduced into eastern U.S.A., Hawaii, and Europe. *A. filiculoides* var. *rubra* (R. Br.) Strasburger, originally described from Australia, has since been found scattered throughout America.

A. caroliniana Willd. Essentially warm temperate. In eastern U.S.A. from Massachusetts to Florida, extending to the West Indies and Brazil. Introduced in western, central, and southern Europe.

A. pinnata R. Br. Australasia, Indomalaya, and Africa, including Madagascar. Introduced into southern Europe (Sculthorpe 1967).

A. microphylla Kaulfuss. Mainly South America, especially lowlands of Brazil and British Guiana.

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Scattered distribution in western South America and northward to central America, extending into California and the West Indies.

A. mexicana Presl. Mainly Mexico, with scattered occurrences northward through the Pacific States to British Columbia, and eastward to Lake Michigan. Extending southward in lowlands to French Guiana and Bolivia.

A. nilotica Dcne. African species with a less extensive latitudinal span than *A. pinnata*, occurring from Senegal and Ethiopia south to northern Transvaal.

As early as 1847 Mettenius reported seven species of *Azolla*, together with an already confused synonymy, and interpretation of the development of reproductive structures had commenced (Meyen 1836; Griffith 1844). The most significant work on morphology and development of reproductive structures in *Azolla* concentrates on *A. filiculoides* (Strasburger 1873, 1889; Campbell 1893; Hannig 1911; Duncan 1940; and Bonnet 1957). *A. caroliniana* was studied by Berggren (1880) and Pfeiffer (1907), *A. pinnata* by Rao (1935), and *A. nilotica* by Demalsy (1954). Initial developmental stages of megasporangium and microsporangium are identical, until the production of thirty-two spores embedded in a multinucleate mass derived from a periplasmodial tapetum. Subsequent megasporangial development involves abortion of all but a single spore, the degenerating nuclei becoming aggregated into three large vacuoles which later form floats of the swimming apparatus above the developing megaspore. Thickening of the megaspore wall occurs with deposition of an elaborate perine derived from surrounding cytoplasm. The hairs which then develop on the perine surface are considered homologous with glochidia which form on the massula. Glochidia serve as a means of attaching the massula to the megaspore apparatus. During microsporangial development, sixty-four spores are produced, eight to twelve of which become distributed toward the periphery of each of five to eight vacuoles. Each vacuole develops into the pseudocellular massula which bears glochidia at the surface. A float is regarded as the homologue of a massula.

Although the fundamental development and organization of reproductive structures in *Azolla* have been understood since 1889, little detailed comparative work of taxonomic importance has been attempted on modern species. This seems especially surprising in that probably no other group of embryophyte plants possess such complex spores.

The genus ranges from the late Cretaceous to the present. Taxonomic diversity within the genus, coupled with short stratigraphic ranges of individual species, renders *Azolla* a potentially valuable stratigraphic indicator. The stratigraphic ranges and characteristic features of the more important, and best-known, *Azolla* species from the late Cretaceous and Palaeogene are presented in Table 1. This list excludes certain species considered synonymous with others, and those established on such limited information as (i) only vegetative remains recorded, (ii) only massulae known, and (iii) megaspore apparatus inadequately described. Certain species established mainly on the basis of megaspore wall structure are included, but should be regarded as temporary, awaiting further information.

Taxonomically, *Azolla* species are placed in six sections of the genus, based on features of the megaspore apparatus and massulae. The essential characteristics of each section are given below, together with a list of suggested members which includes extant species, and those from the late Cretaceous and Palaeogene.

TABLE 1. Stratigraphic range and characteristic features of the reproductive structures of late Cretaceous and Palaeogene species of *Azolla*.

Important literature related to each species is listed in the second column. Characteristics are expressed as a formula in the third column. The formula is in three sections, referring to swimming apparatus, proximal pole of megaspore, and massula. Swimming apparatus: prefixed by C for columella, considered the basic component. Followed by columellar type (d = dome- or cone-shaped; t = triseptate), nature of floats (F = smooth, pseudovacuolate; f = hairy, or poorly known float-like structures), float number (N = numerous) and number of float tiers (in brackets). Proximal pole: prefixed by P; c = collar; l = labra, or similar structures. Massula: prefixed by M (+M = massulae found attached to megaspores). In brackets, g = glochidia recorded; -g = eglochidiate; a = anchor-shaped glochidia; d = anchor-shaped with distal dilation; s = simple hair-like or coiled glochidia. In some instances the presence of certain features is indicated only as a possibility.

			CRETACEOUS	PALAEOCENE	EOCENE	OLIGOCENE	MIOCENE
<i>A. simplex</i>	Hall (1969b)	Cd 1/P — / M (gad)	█				
<i>A. barbata</i>	Snead (1969) Hall & Bergad (1971)	Cd FN(2)/P — / +M (gs)	█				
<i>A. extinota</i>	Jain (1971)	Cd FN /P (?) / +M (-g)	█				
<i>A. geneeana</i>	Hills & Weiner (1965)	Cd l /P — / M (ga)	█				
<i>A. lauta</i>	Snead (1969)	Cd FN /P — / M —	█				
<i>A. distincta</i>	Snead (1969) Hall & Bergad (1971)	Cd FN(3) / P(?) / +M (ga)	█				
<i>A. schopfi</i>	Dijkstra (1961) Snead (1969)	Cd f18(3)/P (1) / M (ga)	█				
<i>A. montana</i>	Hall & Swanson (1968) Jain & Hall (1969)	Cd f15-20/P (1) / +M (ga)	█				
<i>A. bulbosa</i>	Snead (1969)	Cd f18(3)/P — / M —		█			
<i>A. fragilis</i>	Jain & Hall (1969)	Cd FN/P (1) +M —		█			
<i>A. stanleyi</i>	Jain & Hall (1969)	Cd F15+/P — / +M (ga)		█			
<i>A. velus</i>	Jain & Hall (1969)	Cd FN/P (1) / +M (ga)		█			
<i>A. teschiana</i>	Flörschutz (1945) Dijkstra (1961)	Cd F24(3)/P — / +M (ga)		█			
<i>A. intertrappea</i>	Sahni & Rao (1943) Hall (1969a) Trivedi & Verma (1971)	C? F3/P(c + 1) / +M (gad)			█		
<i>A. indica</i>	Trivedi & Verma (1971)	C? F3/P(c + 1) / M (gad)			█		
<i>A. primaeva</i>	Arnold (1955a) Hills & Weiner (1965) Hills & Gopal (1967) Hall (1969a)	Cd F1/P — / M (gad)			█		
<i>A. antiqua</i>	Dorofeev (1959)	Ct F6-9(2)/P (c+1) / M —				█	
<i>A. prisca</i>	Reid & Chandler (1926)	Ct F9 (2)/P (c+1) / +M (gad)				█	
<i>A. nana</i>	Dorofeev (1959)	Ct F9 (2)/P (c+1) / M (-g)				█	
<i>A. turgaiica</i>	Dorofeev (1959)	Ct F9 (2)/P (c+1) / M (-g)				█	
<i>A. sibirica</i>	Dorofeev (1959)	Ct F9 (2)/P (c+1) / M —				█	
<i>A. ventricosa</i>	Nikitin (1955) Dorofeev (1959)	Ct F9 (2)/P (c+1) / M (-g)				█	█
<i>A. nikitinii</i>	Dorofeev (1955)	Ct F9 (2)/P (c+1) / M —				█	█
<i>A. aspera</i>	Dorofeev (1963)	Ct F9 (2)/P (c+1) / M —				█	█

Section SIMPLICISPORa Hall, 1970. Float-like columella or single float; anchor-shaped glochidia. Fossil species *A. geneseana*, *A. primaeva*, *A. simplex*.

Section KREMASTOSPORa Jain and Hall, 1969. Megaspore apparatus with more than nine floats; anchor-shaped glochidia. Fossil species *A. distincta*, *A. montana*, *A. schopfi*, *A. stanleyi*, *A. teschiana*, *A. velus*.

Section FILIFERA Hall, 1968. Megaspore apparatus with more than nine floats; hair-like or coiled glochidia. Fossil species *A. barbata*.

Section ANTIQUA Dorofeev, 1959. Megaspore apparatus with six to nine floats in two tiers; massulae undescribed. Fossil species *A. antiqua*, *A. aspera*, *A. nikitinii*, *A. sibirica*.

Section RHIZOSPERMA Meyen, 1836. Megaspore apparatus with nine floats in two tiers; glochidia absent, or simple, straight or branched structures. Fossil species *A. nana*, *A. turgaica*, *A. ventricosa*. Modern species *A. nilotica*, *A. pinnata*.

Section AZOLLA Meyen, 1836. Megaspore apparatus with three large floats; glochidia simple, hooked or anchor-shaped. Fossil species *A. indica*, *A. intertrappea*. Modern species *A. caroliniana*, *A. filiculoides*, *A. mexicana*, *A. microphylla*.

Important contributions to our knowledge of the morphology of the megaspore apparatus and massula of fossil species of *Azolla* and their phylogenetic significance, have been made by Hills and Gopal (1967), Hall and Swanson (1968), Jain and Hall (1969), and Jain (1971). In some instances lack of suitable material has caused misinterpretation of structure. This has resulted in some terminological confusion, especially with regard to the application of the term columella. Apart from variation in form of the glochidia, little has been written concerning the structure of the massula. It now seems clear that the multifloated swimming apparatus is more primitive than both the nine-floated and three-floated type. Massulae with anchor-shaped glochidia appear to be more primitive than those with hair-like glochidia, or those in which the glochidia are absent. Although the megaspore wall structure of several fossil species has now been examined, comparatively few have received detailed attention. The most important of such contributions is that of Kempf (1969*a* and *b*) who examined, by means of light and transmission electron microscopy, the megaspore wall of *A. teschiana*, *A. nana*, *A. cf. aspera*, *A. tomentosa*, and *A. tegeliensis*. Structural details of the megaspore wall of modern species of *Azolla* are equally lacking, with the exception of *A. filiculoides* (Bonnet 1957), *A. nilotica* (Demalsy 1954), and *A. pinnata* (Rao 1935; Sweet and Hills 1971), and surprisingly little progress has been made in tracing the developmental history of the wall layers. Some confusion has resulted from the number of different terms which have been applied to these wall layers in both fossil and modern species. Structural variation of the megaspore wall between fossil *Azolla* species has been demonstrated, and attention drawn to this potentially useful method of identifying wall fragments (Kempf 1969*a*; Snead 1969, 1970; Hall and Bergad 1971).

A. prisca has the distinction of being the earliest fossil species of *Azolla* in which both vegetative and fertile remains were described (Reid and Chandler 1926). The only record prior to this was that of *Azollophyllum primaevum* Penhallow based on vegetative material (Dawson 1890), later to be known as *Azolla primaeva*. Increasing interest shown by many workers in Upper Cretaceous and Palaeogene species of *Azolla* stimulated this reappraisal of *A. prisca*, using ultra-thin sectioning, and both scanning and transmission electron microscopy. *A. prisca* is consistently described in the literature as an enigmatic species which cannot be placed in any existing

section of the genus due to the presence of anchor-shaped glochidia (Arnold 1955; Hills and Gopal 1967; Trivedi and Verma 1971). In this present work, the establishment of a new section to accommodate this species is considered both desirable and necessary. *Azolla* species with nine floats in the swimming apparatus are particularly characteristic of the Oligocene (see Table 1), a number of species having been recorded from Britain and the U.S.S.R. (Reid and Chandler 1926; Dorofeev 1959). Its occurrence in the lowest Oligocene makes *A. prisca* one of the oldest nine-floated species to be recorded, this type of swimming apparatus being rare in pre-Oligocene rocks. This species affords the opportunity of investigating what may possibly be an important evolutionary link between the Eocene and Oligocene representatives. Critical study, coupled with adequate description and illustration, has never previously been attempted for any Oligocene species of *Azolla*.

LOCALITY AND STRATIGRAPHY

A. prisca was described from the Insect Limestone of the Isle of Wight as part of the rich Bembridge flora (Reid and Chandler 1926). The Insect Limestone is a fine-grained blue-grey argillaceous limestone, varying in thickness up to 0.3 m and located just above the base of the Bembridge Marls which reaches a maximum thickness of some 33 m. The Bembridge Marls rest on the freshwater Bembridge Limestone. Much of the Insect Limestone is barren and, according to Reid and Chandler (1926), the plant collection was made from small pockets over a twenty-five year period. Numerous insect remains are reported from this horizon (Woodward 1879). The Insect Limestone is seen in the cliff-section of Gurnard Bay (SZ 467 943), but to the west in Thorness Bay it reaches shore level.

During the Palaeogene, Hampshire occupied a marginal position between sea to the east and land to the west. The Bembridge Marls accumulated during a regressive phase when non-marine conditions were re-established after an initial transgressive phase of short duration. According to Daley (1973), the depositional environment of the Insect Limestone is not well understood.

Most workers consider the Bembridge Marls to be of Oligocene age, though an Upper Eocene age has been suggested (Blondeau, Cavelier, Feugueur and Pomerol, 1965). Machin (1971), using palynological evidence, placed the Eocene-Oligocene boundary at the base of the Lower Hamstead Beds, at the same time suggesting that the base of the Bembridge Marls, lower in the succession, might be considered a possible alternative. Preliminary palynological investigation of the Insect Limestone by the author suggests that a lowermost Oligocene age might be appropriate for the base of the Bembridge Marls.

Rock specimens used in this investigation had well-preserved massulae and megaspore apparatuses scattered over the surface (Specimens V. 17729 and counterpart, British Museum (Natural History)). This material was originally collected by J. E. E. A'Court Smith during the latter half of the nineteenth century. Repeated attempts by the author to find suitable material of *A. prisca* from the type locality were unsuccessful.

METHODS

Megaspore apparatuses and massulae were excavated from the rock surface by means of fine needles, cleaned of adhering particles of matrix in 40% hydrofluoric acid, then washed thoroughly in distilled water. Megaspore apparatuses were examined and photographed, in dry condition and in water, using a Wild-Heerbrugg M7 Stereomicroscope and Photoautomat camera. Massulae were examined and photographed by means of a Wild-Heerbrugg M20 light microscope with Photoautomat. Air-dried specimens for scanning electron microscopy, using a Cambridge Instruments Company Stereoscan, were attached to double-sided sellotape on stubs, and coated with gold using a Polaron E5000 Sputter Coater. Ultra-thin sections of the megaspore apparatus were cut with a LKB Ultratome and glass-knife, after embedding in Taab resin. Sections of thickness 1.5 μm stained with basic fuchsin and methylene blue were used for light microscopical examination, and of thickness 600 Å stained with uranyl acetate and lead citrate for examination with the transmission electron microscope (Philips EM 300).

SYSTEMATIC DESCRIPTION

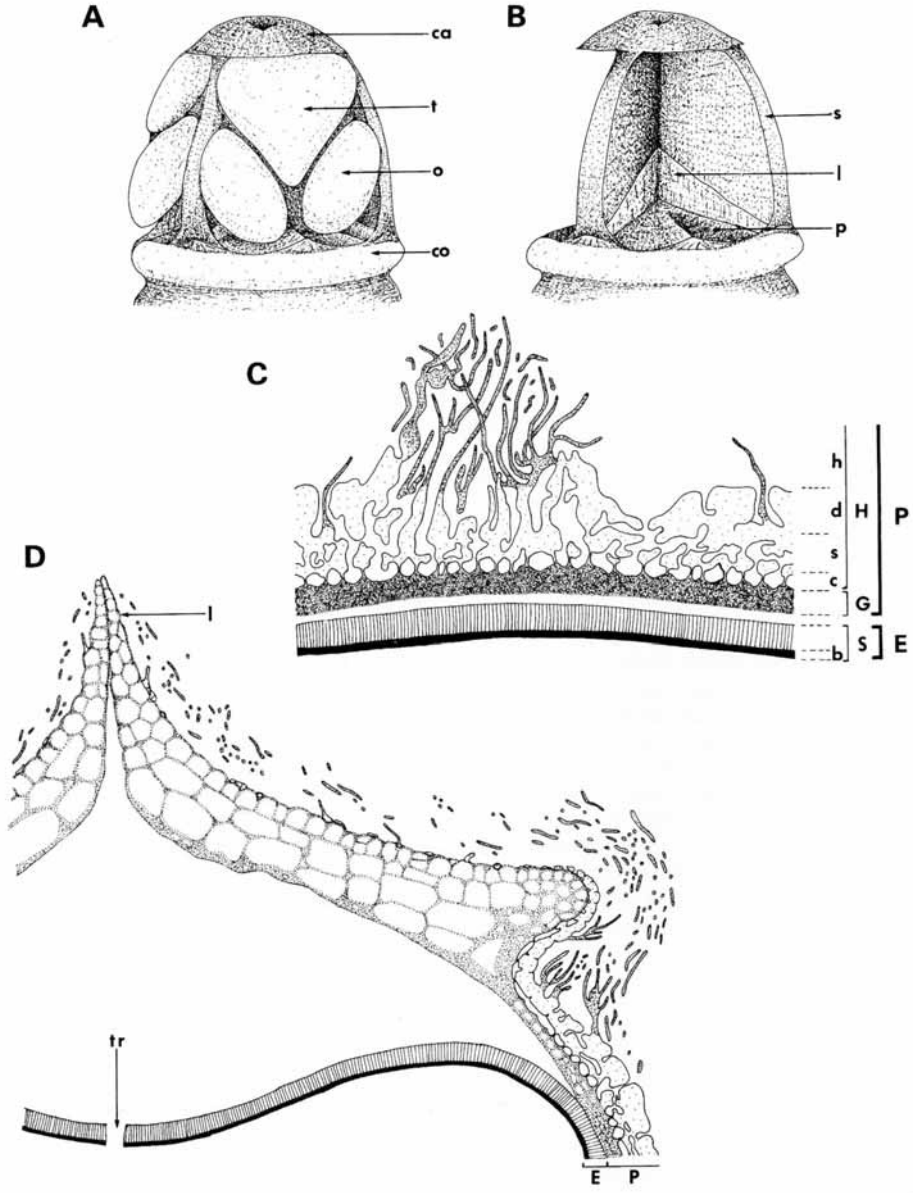
Order SALVINIALES
 Family SALVINIACEAE
 Genus AZOLLA Lamarck, 1783
 Section TRISEPTA sect. nov.
Azolla prisca Reid and Chandler, 1926

The new section *Trisepta* is erected to include species of *Azolla* with the following characteristics: megaspore apparatus clearly differentiated into megaspore and swimming apparatus; swimming apparatus consisting of a hairy peltate columella forming three compartments, each separated by a septum of columellar material; attached to the columella are nine floats arranged in two tiers, three triangular floats above and six oval floats beneath; each compartment of the columella accommodates one triangular and two oval floats; the massula bears non-septate anchor-shaped glochidia each with a distal dilation immediately beneath the anchor-shaped tip.

The name *Trisepta* refers to the three septa of the columella which divide the swimming apparatus into compartments housing the floats.

Details of vegetative features, structure of the sporocarp and sporangial wall of *A. prisca*, are not included in this investigation which is concerned solely with the dispersed megaspore apparatus and massula. According to Reid and Chandler (1926) the megasporocarps and microsporocarps occur together in pairs with at least twelve massulae in the microsporangium.

TEXT-FIG. 1. *Azolla prisca* Reid and Chandler. A and B, swimming apparatus, $\times 160$. A, intact. B, floats removed showing columella and labra on proximal surface of megaspore. C and D, section of megaspore wall. C, with tubercle, and showing stratification, $\times 1750$. D, at proximal pole, showing structural modification and labra, $\times 700$. b = base of striated layer; c = columellate zone; ca = tomentose cap; co = collar; d = dense zone; E = exine of megaspore proper; G = granular layer, much thickened at proximal pole; h = hairy zone; H = homogeneous layer; l = labrum; o = oval float; p = proximal surface of megaspore; P = perine; s = tomentose septum; sp = spongy zone; S = striated layer; t = triangular float; tr = position of triradiate suture in exine.



DESCRIPTION OF MEGASPORE APPARATUS

The megaspore is rounded, thick-walled, and bears several large rounded to vermiform tubercles toward the distal surface. Above the proximal surface of the megaspore lies the swimming apparatus, the basic component of which, the columella, is composed of long, unbranched and intertwined hair-like filaments. This columella is peltate, with a small dome-shaped cap at the apex, and a hollow central strand with wing-like longitudinal extensions dividing the swimming apparatus into three compartments. Attached to the columella are nine floats, an upper tier of three triangular floats and a lower tier of six smaller, oval floats (text-fig. 1A, B; Pl. 59, figs. 1, 2). Most of the hairs forming the columella originate from a thickened area of megaspore wall which forms a prominent collar around the megaspore delimiting the periphery of the proximal face. The columellar structure of *A. prisca* may best be understood if it is regarded as resulting from the invagination of a dome-shaped tomentose columella to form three compartments, each compartment being lined by hairs. As a result, adjoining compartments are separated by a thick double-layer of tomentose material which forms the septum. The thinner tomentose layer covering the base of each compartment is supplemented by hairs from the proximal surface of the megaspore. Each compartment accommodates a single upper triangular float and two oval floats beneath. Hair-like filaments of the columella are continuous with similar structures on the megaspore wall which, though scattered over the surface, are particularly abundant near tubercles. The cap covering the uppermost area of the triangular floats (Pl. 59, figs. 1-3) has an inner layer of closely interwoven hairs, continuous with the columella, and an outer darkly pigmented membrane layer. Length of megaspore apparatus 446 μm to 475 μm , average 455 μm ; width of megaspore apparatus 237 μm to 270 μm , average 255 μm (twelve specimens measured).

The floats are vacuolate pseudocellular masses, loosely attached to the columella by relatively few hairs limited to the inner faces of the float (Pl. 60, fig. 1). The central region of the float is occupied by pseudocellular cavities approaching 25 μm in diameter, whilst a well-delimited narrow zone of smaller cavities, average diameter 2.5 μm , occurs at the surface (Pl. 60, fig. 3). Hairs on the floats are tubular extensions of the peripheral cells (Pl. 60, fig. 2) with a uniform diameter of approximately 2 μm , often exceeding 75 μm in length and with blunt ends. A few closely spaced septa may occasionally be found toward the hair base. The knot-like structure described by various authors (Strasburger 1873; Campbell 1893; Hannig 1911; Kempf 1969b) can often be seen within the float occupying a more or less central position (Pl. 61, fig. 1).

EXPLANATION OF PLATE 59

Figs. 1-5. Scanning electron micrographs of *Azolla prisca* Reid and Chandler from the Insect Limestone (Bembridge Marls, Oligocene), Gurnard Bay, Isle of Wight. 1-2, intact megaspore apparatuses in different views. 1, swimming apparatus showing triangular float and two oval floats above hairy collar, and apical cap. Distal surface of megaspore shows tubercles, $\times 175$. 2, tomentose septum between two smooth oval floats, $\times 175$. 3, megaspore apparatus dissected revealing megaspore exine within perine. Vacuolate floats and apical cap clearly seen, $\times 170$. 4-5, perine surface. 4, tubercles formed of anastomosing cylindrical elements. The spherical bodies are fungal spore contaminants, $\times 750$. 5, surface regulate-
verrucate, with foveae in which hair bases lie, $\times 1900$.



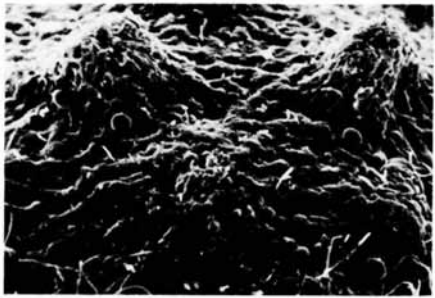
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2



3



4



5

FOWLER, *Azolla prisca*

The substance forming this structure is regarded as rudimentary exine by Hannig (1911) and Kempf (1969b).

Removal of the columella shows a triradiate wall which attains a height of some 75 μm at the centre of the proximal surface of the megaspore, decreasing in height toward the periphery (text-fig. 1B). In median longitudinal section the triradiate wall is seen to be formed by the close association of two vertically orientated labra bordering a suture (text-fig. 1D; Pl. 61, fig. 1). Pronounced sculptural and structural differences occur between the wall of the proximal face and that of the rest of the megaspore. This was initially indicated by the darker coloration of the latter, later to be confirmed by examination of the wall in thin section. The three compartments of the columella lie directly above the inter-labral areas, the septa thus coinciding with the labra.

The megaspore wall is composed of two principal parts, the exine of the megaspore proper to the inside, and perine to the outside. The perine surface is rugulate-verrucate, with foveae to a depth of some 2 μm . Hairs scattered over the surface are 0.5–1 μm in diameter, their bases appearing to originate in the depressions of the foveae (Pl. 59, figs. 4, 5).

Dissection of the megaspore apparatus, via the proximal surface, reveals the detached exine of the megaspore proper with the trilete mark uppermost (Pl. 59, fig. 3). This simply bordered trilete mark is small, with laesurae extending little more than a third of the way to the equator; its position is coincident with the trilete suture on the proximal face of the megaspore. The diameter of the megaspore proper is approximately 190 μm ; the surface is finely pitted to reticulate (Pl. 60, figs. 4–7).

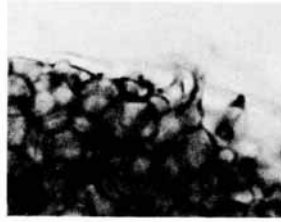
The megaspore wall is composed of three main layers, an exine and two-layered perine, structural details of which can be seen in text-fig. 1C and Plate 61, figs. 3, 4. In optical and thin section the exine is seen to have a thin basal zone above which it is striated, the total thickness approaching 3 μm (Pl. 60, fig. 5; Pl. 61, fig. 3). As seen with the transmission electron microscope, the exine has an essentially granular structure, with the basal zone formed by fusion of elements (Pl. 61, fig. 4). Above the basement zone the elements, though fused to form a spongy network, are orientated in such a way as to provide numerous narrow, radially arranged sinuous cavities of varying length. Lateral fusion of these radially arranged elements in the uppermost part of this layer provides a relatively smooth exinous surface. Numerous small granules on this outer surface allow for a certain degree of interlock with the layer above, yet rendering the exine readily detachable. The radially orientated elements

EXPLANATION OF PLATE 60

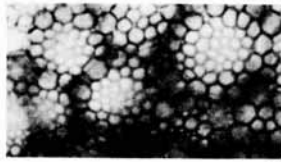
Figs. 1–12. *Azolla prisca*, megaspore apparatus and massula. 1–3, float structure. 1, oval float, biconvex in side view, with hairs near apex of inner face, $\times 375$. 2, hair base, $\times 1550$. 3, surface showing small pseudocellular cavities, with larger cavities beneath, $\times 480$. 4–7, megaspore proper. 4, proximal surface showing trilete mark, $\times 180$. 5, exine in optical section, $\times 1875$. 6, exine surface at high, and 7, low level of focus, $\times 1875$. 8–11, massula. 8, massulae attached to megaspore apparatus, just below collar, $\times 75$. 9, massula with anchor-shaped glochidia, $\times 250$. 10, anchor-tip showing two recurved prongs and dilation beneath, $\times 1250$. 11, massula with microspores and associated funnel-shaped cavities, $\times 940$. 12, microspore-containing cavity and associated funnel-shaped cavity opening by a pore to the exterior, $\times 940$.



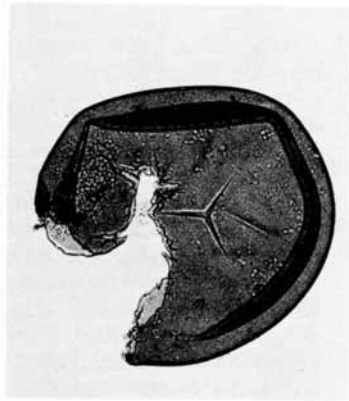
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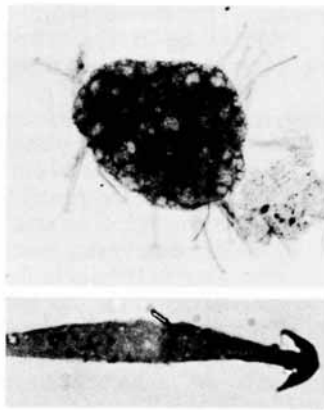
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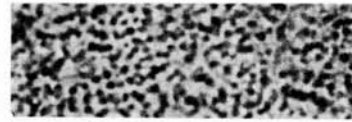
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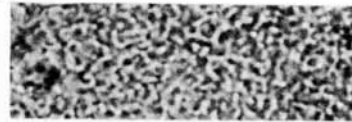
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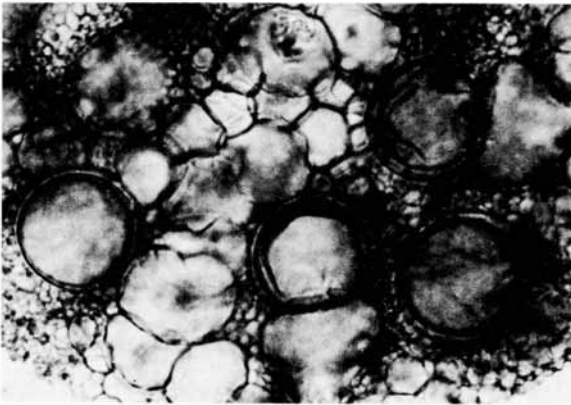
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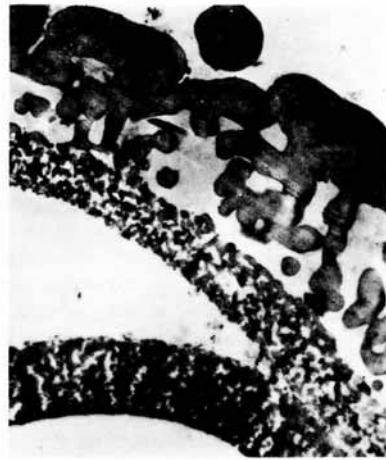
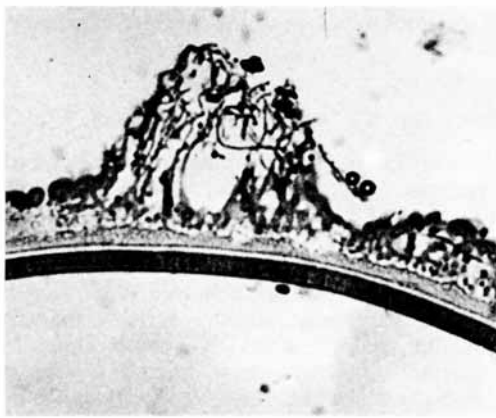
give the characteristic striated structure at lower magnification, and is consistent with the sculpture of the exine.

The perine is divisible into an inner granular and outer homogeneous layer, the latter stratified into four zones. Its thickness, though variable due to protuberances and elaboration at the proximal surface, is approximately $10.5\ \mu\text{m}$, excluding the outer hairy zone. The granular layer, which is approximately $3\ \mu\text{m}$ thick, has a smooth base and undulating upper surface (Pl. 61, fig. 3). Transmission electron microscopy shows that the structural elements within the striated and granular layers are similar, though more haphazardly arranged in the latter (Pl. 61, fig. 4). The homogeneous layer is supported above the granular layer by small solid columellae, $0.5\text{--}1\ \mu\text{m}$ in height, forming the columellate zone. This passes up into a spongy zone, approximately $4.5\ \mu\text{m}$ thick, composed of an irregular network of large elements which fuse to form a solid outer covering about $2.5\ \mu\text{m}$ in thickness. The surface of this outer dense zone is dissected by grooves and pierced by foveae to give the sculpture already mentioned. The outermost zone of the perine is composed of hair-like filaments originating from the dense or spongy zones at the bases of the foveae, or from the reduced spongy zone within the tubercles (text-fig. 1C; Pl. 61, fig. 3). The wall of the tubercle is composed of an anastomosing network of cylindrical elements developed from the dense zone (Pl. 59, fig. 4).

At the proximal pole of the megaspore, the perine structure is much modified from that described above, this transformation taking place in the collar region (text-fig. 1D; Pl. 61, figs. 1, 2). Here, the wall approaches $50\ \mu\text{m}$ in thickness (excluding the hairy zone), most of this being composed of the greatly thickened and vacuolated granular layer. Correspondingly, the homogeneous layer is much reduced in this region, being represented only by the dense and hairy zones. The proximal wall, with an average thickness of $25\ \mu\text{m}$ increasing to $100\ \mu\text{m}$ in the formation of labra, is composed almost entirely of the granular layer. On this proximal surface of the megaspore the homogeneous layer almost completely disappears, remaining only as scattered granules from which hairs may arise. The structure of the proximal wall, with pseudocellular cavities as large as $21\ \mu\text{m}$ in diameter, is reminiscent of that of both float and massula. A single layer of small cavities, approximately $4\ \mu\text{m}$ in diameter, form the outer surface of the proximal wall.

EXPLANATION OF PLATE 61

Figs. 1-4. *Azolla prisca*, megaspore apparatus in thin section. 1, median section through megaspore apparatus, showing two triangular floats covered by cap, oval float beneath with 'knot' at centre, partially detached exine, thick perine with tubercles, and vacuolate proximal wall with labra. Megaspore wall has fractured in the collar region, $\times 200$. 2, wall in collar region, showing thickening and vacuolation of granular layer and reduced homogeneous layer represented only by dense zone. Hairs occur on dense zone. Detached exine at bottom of figure, $\times 1400$. 3, wall and tubercle. Outside the detached striated exine, the granular layer supports the homogeneous layer divisible into columellate, spongy and dense zones. Tubercle composed of anastomosing cylindrical elements formed by the dense zone. Within the tubercle the spongy zone appears reduced, giving rise to hair-like structures, $\times 950$. 4, transmission electron micrograph showing partially detached exine, granular layer, spongy and dense zones of homogeneous layer, $\times 4000$.



FOWLER, *Azolla prisca*

DESCRIPTION OF MASSULA

Numerous massulae of the same type were found associated with, and attached to, megaspore apparatuses of *A. prisca*. There seems little reason to doubt that both types of reproductive structure belong to the same parent species. Invariably, the massulae are found attached by their anchor-shaped glochidia at, or near, the hairy collar region of the megaspore apparatus (Pl. 60, fig. 8). The massulae are vacuolate pseudocellular discoid bodies ranging in maximum diameter, excluding glochidia, from 98.8 to 167.2 μm , averaging 136.7 μm (fifty specimens) (Pl. 60, fig. 9). As in floats, the pseudocellular cavities of the massula range in diameter from approximately 5 μm at the periphery, to about 25 μm toward the centre. Occasionally, in both floats and massulae, large cavities may extend to the periphery. Glochidia are rarely seen projecting from the massula, being mainly restricted, and closely adpressed, to the flattened surfaces. The nonseptate glochidia have an average length of 70 μm ; the stalk, approximately 5.5 μm wide in the median region, tapers to about 2.3 μm at the proximal and distal ends. A slight dilation of the stalk near its distal extremity becomes abruptly constricted again at the junction with the anchor-shaped tip. Average width of the anchor-shaped tip is 8.4 μm , and the two prongs of the anchor are recurved (Pl. 60, fig. 10). An inverted V-shaped diaphragm, often seen 5–6 μm beneath the distal dilation, separates what appears to be a tubular stalk from a solid tip.

Microspores, averaging 20.8 μm in diameter (range 15–25 μm , 150 specimens measured), occupy large pseudocellular cavities within the massula. Six microspores per massula appears usual, though the number ranges from three to nine. Microspore walls are laevigate, with a thickness less than 1 μm . Each microspore-containing cavity is closely associated with the bulbous base of a funnel-shaped cavity, the neck of which extends to the periphery, opening by a pore to the exterior (Pl. 60, figs. 11, 12). The diameter of this pore approximates to that of the small cavities to the outside of the massula. The germinal area of the microspores, represented by both closed and open triradiate sutures, consistently occurs in a position adjacent to the base of the funnel-shaped cavity (Pl. 60, fig. 11). An incomplete partition appears to connect these two adjoining cavities.

DISCUSSION

Most *Azolla* species with nine floats in two tiers in the swimming apparatus can be included in sections Rhizosperma or Antiqua. Section Antiqua is established on the basis of *A. antiqua*, a fossil species from the late Eocene and early Oligocene of the U.S.S.R. in which the massulae are unknown (Dorofeev 1959). The nine-floated species *A. nana*, *A. turgaica*, and *A. ventricosa*, described by Dorofeev (1959), and in which the massulae are reported as eglochidiate (Hall and Swanson 1968; Trivedi and Verma 1971), can be included in the Rhizosperma, together with the modern species *A. nilotica* and *A. pinnata*. It would seem expedient to include the fossil species *A. aspera*, *A. nikitinii*, and *A. sibirica*, together with *A. antiqua*, in the section Antiqua until further information is available on the massulae. Since *A. prisca* cannot be included in either of these sections of the genus, due to its anchor-shaped glochidia,

it seems appropriate to establish a new section, Section Trisepta, to accommodate this, and similar species.

The columella in fossil and modern Azolla. Most pre-Eocene *Azolla* species were described between 1969 and 1971, resulting in some synonymy, and confusion in the descriptive morphology of the reproductive structures. The columella, as first recognized by Meyen (1836), is well defined by the time Campbell (1893) describes it as a short stalk from which microsporangia develop laterally. Since then, the term has taken on dual usage, being also applied to that part of the swimming apparatus bearing floats. Misinterpretation of the structure of the megaspore apparatus in both fossil and modern species has resulted in a confused and inaccurate definition of the term columella. One of the first applications of the term to the megaspore apparatus is made by Eames (1936) in an account of the reproductive structures of modern *Azolla* species. Here it is regarded as a conical pad of tissue situated, as the name suggests, in a central position between the floats. Hall and Swanson (1968) illustrate this interpretation of the columella with reference to the vacuolate pseudocellular peg-like structure between the floats of the modern *A. mexicana*. However, in the same work the authors describe the columella of the fossil species *A. montana* as a hairy, hollow, thimble-shaped structure with attached floats. Further complications arise with the definition by Jain and Hall (1969), given as 'a peg-like or cone-shaped structure, distally continuous with the perispore and commonly hairy or highly vacuolate'. Even when applied to the megaspore apparatus, the term columella is apparently being used to describe two different structures, features of both having become incorporated within the definition. This study of *A. prisca* indicates that the vacuolate peg-like structure is best regarded as an elaboration of the proximal wall of the megaspore, and not as part of the hairy superstructure primarily concerned with holding the floats. On the bases of priority and aptness, the term columella should be applied to the peg-like structure. However, as alternative terms may be found for modification of the proximal wall of the megaspore, and since the term has now been widely adopted for the tomentose superstructure in fossil species, it is proposed to retain the term for that particular purpose. A reappraisal of the structure of the columella would seem pertinent, especially as it is the major component of the swimming apparatus in Palaeogene species of *Azolla*.

The columella is essentially a hairy superstructure over the proximal surface of the megaspore, formed from hair-like filaments of the megaspore wall. The swimming apparatus can be composed solely of this one component, forming a structure called a columellate float, as in *A. simplex* (Hall 1969b). Commonly, a second component, the float, is developed. It is generally accepted that true floats are distinguished from the columella by their vacuolate pseudocellular structure and lack of surface hairs. Such distinction should be preserved, to the extent that a term such as columellate float should be abandoned, this type of structure simply being regarded as an undifferentiated columella. Similarly, in species such as *A. montana*, where structures described as floats are hardly distinguishable from the columella, it would seem more appropriate to refer to the structure as a segmented columella. The form of the columella varies in different species, such variation depending on the volume of the swimming apparatus given over to float production, and the size, shape, and number

of floats. At its simplest, the columella appears to be dome- or cone-shaped, as in *A. simplex* and many multi-floated species. In *A. prisca*, and possibly all nine- and three-floated species, modification of the dome-shaped type of columella by float production in three sectors has restricted its development to a thin layer in the form of a triseptate structure. A transverse section of this type of columella, as seen in the Lower Pleistocene species *A. tegeliensis*, is well illustrated by Kempf (1969b, fig. 8). Here, two layers of tomentose material are seen to form each septum dividing float compartments. The suggestion that the basic form of the columella is dome-shaped is an oversimplification. In some species there is a tendency for only the central part of the columella to be hollow, often with a pore-like opening at the apex. *A. montana* shows such a pore (Jain and Hall 1969), and Hall (1969b), commenting on *A. simplex*, states 'in many specimens there is a canal in the columellate float, extending from the apex of the megaspore body to the tip of the swimming apparatus'. The canal, though narrow, is present in *A. prisca*, passing up through the central strand of the columella, the position of the pore being marked at the apex by a small indentation in the apical cap (text-fig. 1A; Pl. 59, fig. 3).

There is some doubt as to whether the apical cap, so characteristic of *A. prisca* and other nine-floated species in the Oligocene, is truly part of the columella. According to Rao (1935), describing a similar structure in the modern nine-floated species *A. pinnata*, it is a remnant of the inner part of the megasporangial wall. However, as can be seen in *A. prisca*, both the columella and the outer membraneous megasporangial wall would appear to be represented (Pl. 59, fig. 1). Scanning electron microscopical examination of megaspore apparatuses of modern nine- and three-floated species, after removal of the enveloping megasporocarp and megasporangial walls, shows that the columella forms a tomentose peltate structure lining the inside of the megasporangial wall at the apex of the swimming apparatus. The columella within the swimming apparatus of the modern *A. filiculoides*, *A. pinnata*, and *A. nilotica* has been described by various authors (Bonnet 1957; Campbell 1893; Demalsy 1954; Rao 1935) but its apparent insignificance precluded a term being applied. In these species, representing both the three-floated and nine-floated condition, the peltate part of the columella, which becomes inverted on removal of the megasporocarp and megasporangial wall, is described simply as an abundance of hairs at the apex of the swimming apparatus. The present work indicates that the columella of all nine- and three-floated species, fossil and modern, is both peltate and triseptate. Furthermore, the small tomentose columellar cap seen in fossil species such as *A. prisca*, was probably more extensively developed in life, having been lost after release from the parent plant. The tomentose cap is not recorded with any degree of certainty before the Oligocene, though Hall (1969a) suggests that this feature may be present in *A. intertrappea*, an Eocene species from India. The author is not aware of a similar structure having been recorded for any megaspore apparatus other than the nine- and three-floated type.

There is variation in the extent to which the swimming apparatus, and hence the columella, covers the megaspore in fossil species. Jain and Hall (1969), suggests that the swimming apparatus completely envelops the megaspore in ancestral types similar to *Azollopsis*, becoming progressively more restricted to the proximal pole of the megaspore in the course of evolution.

A. prisca appears to be the first species, chronologically and stratigraphically, in which the triseptate columella is considered of structural and evolutionary significance, hence the use of this feature in naming the section Trisepta.

Wall structure. Modification of the proximal pole of the megaspore can be seen in fossil and modern species of *Azolla*. The earliest example of such modification can be found in the late Cretaceous to Palaeocene species *A. schopfi*, described by Dijkstra (1961) as having triradiate ridges, 60 μm in height, which are clearly visible when the swimming apparatus is absent. *A. distincta*, with a similar stratigraphic range, is described as having columella and floats with the 'same foamy texture' (Hall and Bergad 1971). Possibly this foamy columella in *A. distincta* is really the proximal face of the megaspore with vacuolate pseudocellular structure like that of the floats, as in *A. prisca*. Study of the literature suggests that apart from the above-mentioned species similar modification may occur in *A. extincta*, *A. montana*, *A. fragilis*, *A. velus*, *A. intertrappea*, and *A. indica*, and may be a common feature of pre-Oligocene species. In *A. prisca* structural modification of the proximal surface takes the form of lips bordering the triradiate suture. A transition zone of this sort between sutures and the remainder of the proximal surface, due to increased wall thickness, sculptural modification, or both, is termed a labrum (Couper and Grebe 1961). The term gula, applied by Kempf (1969b) to a similar structure in *A. tegeliensis*, is best retained for a more marked extension of the labra than that seen in *A. prisca*.

Wall stratification in *Azolla* megaspores is complex and a number of terminologies has been applied. Details of terminologies used, together with suggestions as to various authors' interpretations of the megaspore wall structure, is not within the scope of this work which is not primarily concerned with developmental history of the wall layers. However, it would be appropriate simply to outline the main terminologies used for both modern and fossil species. Campbell (1893) recognized two principal layers in the megaspore wall of *A. filiculoides*, an inner exospore, and an outer episporium which could be sub-divided into two zones. Later workers adopted the same terminology in describing the wall of modern *Azolla*, though Demalsy (1954) and Bonnet (1957) delimited an innermost endospore from the exospore. By this time, perispore was being used as an equivalent term to episporium. Kempf (1969a and b) uses the terms exine, instead of endospore and exospore, and perine, instead of episporium and perispore, for both fossil and modern species. In earlier work on fossil species the exine was termed endospore and most of the perine was called exospore, the term perispore being reserved only for the outer hairy zone of the perine (Hall and Swanson 1968; Jain and Hall 1969). Jain (1971), though using the same basic delimitation of layers, termed the hairy zone as perine, and applied the terms endexine and ectexine to the exine and remainder of perine respectively. The terminology adopted for *A. prisca*, and for fossil and modern species mentioned in this work, is that used by Kempf (1969a and b). Sweet and Hills (1971), describing the megaspore wall of *A. pinnata*, indirectly make use of the term perine by introducing the terms inperine and experine. Such introduction of new terms is, in the author's opinion, both unjustifiable and unnecessary.

Wall structure of modern species has received little detailed attention, and the development of the wall layers is imperfectly understood. Such information available

indicates that, as in *A. prisca*, the exine is composed of a basement zone and radially striated zone. The inner layer of the perine (equivalent to the granular layer of *A. prisca*) is described as granulate, reticulate, or foamy, in *A. pinnata*, *A. nilotica*, and *A. filiculoides* respectively, such differences possibly reflecting the degree of vacuolation of this layer in each species. In *A. filiculoides* it is called the 'couche écumeuse', is characteristically vacuolated and extensively developed, forming eruptions toward the surface (Bonnet 1957). Demalsy (1954), describing structural variation in the megaspore wall of *A. nilotica*, points out that this inner layer of the perine appears thicker and heterogeneous in some megaspores. However, similar structural differences may be observed in oblique sections of the wall of *A. prisca*. The outer homogeneous layer of the perine is composed primarily of radially elongated elements in *A. pinnata* and *A. nilotica*, that of *A. filiculoides* appearing to have a denser structure. As in *A. prisca*, the outermost zone of the homogeneous layer is composed of hairs. Hannig (1911) suggests that in *A. filiculoides* hairs originate from eruptions into the dense zone of the vacuolate granular layer, a suggestion later accepted by Bonnet (1957). Photographs of the ultrastructure of the megaspore wall of *A. filiculoides*, loaned to the author (Pettitt 1974, pers. comm.), would appear to substantiate this. However, it is possible that a very thin layer of homogeneous material is deposited over the granular layer, at the site of the eruption, before hairs form. Hairs certainly appear to originate directly from the surface of the dense zone in the intervening areas, as illustrated by Bonnet (1957, fig. 35). In *A. prisca*, hairs appear to be composed of the same material as that of the homogeneous layer, and they can be seen to arise from the spongy zone within the tubercle and from the dense zone in the collar region. Hairs which originate in foveae, away from the tubercle, appear to originate from the dense zone, though a connection between the hair base and spongy zone beneath is not discounted.

Details of wall structure are now known for several Palaeogene species, with *A. prisca* furnishing the best example from the Oligocene. Although there is distinct variation in thickness and structure of individual layers or zones, a similar pattern to that seen in modern species is shared by the majority of fossil types. This basic pattern in fossil species can be summarized as follows: An exine, 3–4 μm thick, is covered by a two-layered perine. The inner layer of the perine is smooth, granular, or laminated, and between 3–8 μm in thickness. It is the outer homogeneous layer which shows most interspecific variation, and it is divided into at least three zones by most authors. There is often a columellate zone supporting a reticulate or clavate zone, the elements of which become fused toward the surface from which hairs arise.

As Pettitt (1966) points out, the term perine, and equivalent terms, are indiscriminately used for a variety of exinous and extra-exinous layers of the spore or pollen wall. Erdtman (1952) defines the perine as an outer extra-exinous layer formed by the activity of a tapetal plasmodium. It is acknowledged that use of such terms as exine and perine for *A. prisca* has developmental implications. However, structural similarities between the vacuolated granular layer, massulae, and floats of *A. prisca* cannot be disregarded, and suggest that these structures may be homologous. This similarity is even more marked in modern *A. filiculoides* where the vacuolated granular layer is extensively developed in the megaspore wall, not merely limited to the proximal pole as in *A. prisca*. As massulae and floats are usually accepted as perinous,

there seems reasonable justification in regarding the granular and homogeneous layers of *A. prisca* as perine. Only intensive study of the development of the megaspore wall of modern *Azolla* will reveal whether a particular layer is exinous or perinous. Structural resemblances between the wall of modern and fossil species suggest the possibility that conclusions reached from study of modern species may also be applied to fossil species.

Kempf (1969*a* and *b*, 1973), equates the perine of megaspore walls with the ektexine of angiosperm pollen grains, emphasizing the presence of foot layer, columellae, and tectum in both. At the same time the spore exine is equated with the endexine of the pollen grain. This would seem unacceptable, as the term perine can only correctly be applied to plants with a periplasmodial tapetum, and which is known to contribute sporopollenin to the pollen grain wall. Though Kempf's (1969*a*) terminology is used, the author does not hold the view, expressed by Kempf (1973), that all spore walls have perine to the outside, the exine beneath playing no part in surface ornamentation. The terms adopted here are intended only for species of *Azolla*.

Snead's (1969) contention that wall structure is sufficiently variable in fossil *Azolla* to allow species identification from wall fragments would seem to be correct, though it should be applied with caution. More intensive study using ultra-thin sectioning would seem necessary before individual species can be identified with any degree of confidence. The extent of variation within a single megaspore wall, and between spores of the same species must be evaluated. Interpretation of the wall of fossil megaspores found attached to the parent plant may result in description of immature structure, since megaspores become detached at maturity.

Development of labra, or similar structures, appears to be linked with a thickening of the megaspore wall to form a collar around the proximal face of the megaspore. The Eocene species *A. intertrappea* not only provides the earliest example of this association, but is also the earliest species to demonstrate the significant part played by the granular layer in the formation of the proximal wall and labra (Sahni 1941). Such development of the proximal pole is readily seen in *A. prisca*, where the collar and labra are prominent features of the megaspore. Here, the collar region and proximal wall with labra are composed almost entirely of the thickened vacuolated granular layer, the homogeneous layer becoming reduced to a thin covering of scattered granules which may be associated with hair development. This association of labra and collar, with a prominent granular layer in the proximal wall, probably occurs in other nine-floated Oligocene species. After the Oligocene, it can again be seen in the Lower Pleistocene species *A. tegeliensis* (Kempf 1969*b*) and *A. pyrenaica* (Florschütz and Menéndez Amor 1960), representing the nine-floated and three-floated condition respectively. The apparent size difference between the gula of *A. nana*, as illustrated by Kempf (1969*a*, pl. 13, fig. 8), and the labrum of *A. prisca*, may possibly be accounted for by differences in preparation technique. In *A. nana*, contraction of the megaspore wall in the collar region, with associated infolding of the exine, has also resulted in the extrusion of the vacuolate proximal wall upward between the floats, so giving an exaggerated size to the structure which has been termed a gula. It is conceivable that the apparent size of the gula is partly responsible for the importance attached to this structure by Kempf (1969*b*) with regard to anchorage of floats, the tomentose columella being considered of minor significance.

Maximum development of the proximal pole of the megaspore occurs in modern species. Concerning the role of the granular layer, Rao (1935) states that this layer forms the spongy conical structure at the top of the megaspore in *A. pinnata*. The author's own observations, together with published description and illustration, indicate that modern species have a much enlarged collar, a central conical structure trisected by sutures, and a similar role for the granular layer as that seen in *A. prisca* (Rao 1935, pl. 19, fig. 45; Demalsy 1954, pl. 10, figs. 201-202; Bonnet 1957, text-fig. 3; Hall and Swanson 1968, fig. 14).

Relevance of funnel-shaped cavities. Funnel-shaped cavities occurring in association with microspore-containing cavities is a consistent feature of all massulae of *A. prisca* examined. The germinal area of the microspore, represented by a triradiate mark, consistently occurs in a position adjacent to the base of the funnel-shaped cavity, which often appears as a thin incomplete partition. The exterior pore and the opening in the base of the funnel-shaped cavity are found associated with microspores having both closed and open sutures. The occurrence of uniform funnel-shaped cavities associated with closed sutures, together with the intact nature of the funnel, not appearing to have formed by breakdown of pseudocellular material, would seem to support the suggestion that these structures are features of the primary development of the massula, and not formed as a result of post-germination prothallial activity. Such organization could conceivably have formed an escape mechanism for spermatozoids, and to the author's knowledge, no directly comparable structure has previously been recorded for *Azolla* species.

Eames (1936), describing microspore germination in modern *Azolla*, states that a papilla protrudes through the opened sutures, then differentiates to form a small prothallus on which develops an antheridium producing eight spermatozoids. The gametophyte remains embedded in the massula, spermatozoids being freed by the eventual breakdown of the outer part of the massula. Most accounts of microspore germination in *Azolla* are similar, there being no mention of funnel-shaped cavities. It seems unlikely that such organization, which would seem to impart distinct biological advantage, was no longer a feature of the massulae of modern species. Preliminary examination of massulae of modern *A. filiculoides* by the author reveals a similar organization to that described for *A. prisca*. It seems possible, therefore, that such organization may occur in the massulae of all *Azolla* species, fossil and modern.

Evolutionary trends. Pioneering work by Hills and Gopal (1967) led to the conclusion that the three-floated condition in the swimming apparatus of *Azolla* is ancestral to the nine-floated condition. This conclusion was based largely on the discovery of *A. geneseana*, a late Cretaceous species purported to possess three floats from study of a few poorly preserved specimens (Hills and Weiner 1965). Other workers have since failed to establish the presence of three-floated specimens at the type locality (Snead 1969; Jain 1971). Together with *A. simplex*, the oldest species yet described (Hall 1969b), *A. geneseana* is best considered as possessing a tomentose columella without float differentiation. *A. primaeva*, an Eocene species once erroneously reported as having three floats (Hills and Weiner 1965; Hills and Gopal 1967), is now believed to have a single large vacuolate pseudocellular float-

like structure on which surface hairs may be present (Hall 1969a). Apart from species just mentioned, the majority of late Cretaceous and Palaeocene species are multifloated, bearing up to twenty-four floats which may, or may not, have structure different from that of the columella. Readily distinguishable floats probably made their appearance before the close of the Cretaceous, as in *A. barbata* (Snead 1969; Hall and Bergad 1971). Many multifloated species, however, have little more than a segmented columella, as in *A. montana*, in which the so-called floats are hardly differentiated from the hairy columella. The float-like structures described in *A. distincta*, *A. fragilis*, *A. stanleyi*, and *A. velus* may represent an evolutionary advancement in float development, in that they lack hairs and are readily removed from the dome-shaped columella (Hall and Bergad 1971; Jain and Hall 1969). Megaspores in which the floats, proximal wall, and collar region are distinctly vacuolate make their first appearance in the Eocene of India, with *A. intertrappea* and *A. indica*. These two species are the earliest recorded as showing the three-floated condition, though this is still a matter for speculation. Hall (1969a), in a revision of *A. intertrappea*, accepts the presence of three floats, and describes this species as having a well-developed peltate columella bearing small floats approximately half the size of those of extant three-floated species. Megaspores with three floats, similar to those of extant species do not appear until the Oligo-Miocene (Láncucka-Srodoniowa 1958). Sahni (1941) points out that the exact number of floats in *A. intertrappea* is difficult to ascertain in longitudinal section, due mainly to the thickness of the sectioned material, the floats being seen at different levels of focus. From such material, interpretation of *A. intertrappea* as having one or nine floats would appear equally possible, and on stratigraphic evidence, would seem more plausible. *A. indica*, described by Trivedi and Verma (1971) as probably having three floats, furnishes no better evidence for the existence of the three-floated condition in the Eocene. Until further information is available, *A. intertrappea* and *A. indica* are accepted as having three floats in the swimming apparatus. These two species are very similar, and may eventually be regarded as con-specific. Main differences reported are number of microsporangia, size of microsporangia and massulae, and structure of the glochidia, which is described as septate in *A. indicia* and non-septate in *A. intertrappea* (Trivedi and Verma 1971). Presence or absence of a septum provides the only difference of possible value, though it seems likely that the diaphragm separating the solid glochidial head from the stalk, as seen in *A. prisca*, may have been interpreted as a septum.

Evolutionary development of the swimming apparatus of *Azolla* seems reasonably clear, and may be summarized as follows. The multifloated condition appears more ancient than the nine-floated and three-floated condition, but due to the presence of *A. simplex* and *A. geneseana* in the Cretaceous, it is not known if the most ancient types were multifloated or were without floats. Considering the tomentose columella as the basic component of the swimming apparatus, it seems likely that the dome-shaped columella without floats is the most primitive type. From this type of structure the columella becomes progressively organized into separate areas to form float-like structures of the multifloated condition. At first the columella becomes segmented, as in *A. montana*, the segments hardly distinguishable from the hairy columella. Some species, like *A. distincta*, are reported as having foamy floats, implying that true floats appear before the close of the Cretaceous. A number of Cretaceous

species probably possess floats which, though distinguishable from the columella, do not have the vacuolate structure of later species. Numerous vacuolate float-like structures, readily distinguished and easily removed from the columella, is a feature of some Palaeocene species. Eocene records are scanty and not particularly reliable, though it is evident that the number of floats is reduced, the columella becomes less extensive and floats take up a correspondingly larger volume of the swimming apparatus. Eocene species are known to possess true floats showing smooth surface with vacuolate pseudocellular structure, and the proximal wall of the megaspore is modified to form a collar region and labra, as in *A. intertrappea*. In this species, the columella is still a significant feature of the swimming apparatus, the floats are much smaller than those of extant species and undifferentiated columella remains in the form of a dome at the apex. In Oligocene species such as *A. prisca*, perhaps we see maximum elaboration in the form of the triseptate columella, resulting from float development in three sectors of the swimming apparatus. The columella, though much reduced in volume, is still a significant structure supporting nine floats. The three floats of the upper tier are held together by the cap-like development of the columella at the apex, whilst those of the lower tier are supported in position by the contours of the modified proximal pole and held together by the triseptate columella. Species having three large floats like those of living species first occur, with certainty, in the late Oligocene or Miocene. The structure and role of the columella appears to have remained largely unaltered since the close of the Eocene, despite the development of the three-floated condition. However, with the development of three large floats, the significance of the columella has possibly been slightly diminished as a result of increased elaboration of the proximal pole, more support for the floats being provided by the development of the collar, labra, gulae, etc. How a species such as *A. primaeva*, with a single pseudocellular float, could fit into this basic evolutionary pattern is not known. Its late appearance, in the Eocene, suggests that it might have developed from an ancestor similar to *A. simplex*, as a result of vacuolation.

Evolutionary tendencies are not clear regarding the massulae. High numbers of microspores per massula is a feature of multifloated species, with fifty microspores recorded in the massula of *A. extincta*, considered by Jain (1971) to represent the contents of a single microsporangium. Less than eight microspores per massula occur in the Eocene species *A. indica* and the Oligocene species *A. prisca*, this low number being retained as a feature of post-Oligocene species, both fossil and modern. Mahabalé (1963) proposed an evolutionary scheme for *Azolla* based on structure of glochidia, on the assumption that section *Azolla*, with septate anchor-shaped glochidia, is primitive. He suggested that these primitive glochidia became reduced, losing both septa and anchor-tip, to become filamentous as in modern *A. pinnata* and eventually eglochidiate as in modern *A. nilotica*. Section *Azolla* is no longer considered primitive, but anchor-shaped glochidia, though found occurring with every known type of megaspore apparatus throughout the stratigraphical range of *Azolla*, are consistently associated with multifloated megaspores (Jain and Hall 1969). According to Hills and Gopal (1967), septation is a recently acquired character, not an ancestral one, the earliest record of septate glochidia occurring in the Pleistocene. As previously mentioned, there is some doubt concerning the reported presence of septa in the glochidia of the Eocene species *A. indica*.

It was Kempf (1969*a* and *b*) who provided the basis of our understanding of the structural morphology of the megaspore apparatus of *Azolla* species, with particular emphasis being placed on the ultrastructure of the megaspore wall. This present investigation of *A. prisca*, using modern techniques, further extends this knowledge. Ultra-thin sectioning and transmission electron microscopy of the megaspore wall supports Kempf's (1969*a*) suggestion that wall structure is sufficiently variable as to provide a useful means of taxonomic separation and identification of both modern and fossil types, and provides further information on the origin of the hair-like structures. Furthermore, the significance of the innermost layer of the perine in the formation of the proximal wall of the megaspore and associated labra, gulae, etc., is given more attention than in previous work. Scanning electron microscopy employed in the study of the megaspore apparatus of *A. prisca* and some modern species, has led to a better understanding of the structure and significance of the columella, the sculpture of the megaspore wall, and the nature of the apical cap. Critical study of the massulae of *A. prisca* and modern species, has revealed structural organization previously unrecorded. As a result of this investigation on the reproductive structures of *A. prisca*, this species becomes one of the best known of all species of *Azolla*, both fossil and modern.

Our knowledge concerning fossil Salviniaceae has expanded considerably in recent years, indicating great diversity in reproductive morphology and megaspore wall structure. At one time, the Salviniaceae was regarded as having two living genera, *Salvinia* and *Azolla*, the latter divided into two sections, *Azolla* and *Rhizosperma*. We are now aware of the importance of the genus *Azolla* in the Upper Cretaceous and Lower Tertiary, from which over thirty species have been recorded and placed in seven sections of the genus. In addition, two new genera, *Azollopsis* (Hall 1968) and *Parazolla* (Hall 1969*b*), have been established within the Salviniaceae.

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