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ULTRASTRUCTURAL INTERPRETATION OF THE LATE CRETACEOUS MEGASPORE *GLOMERISPORITES PUPUS* AND ITS ASSOCIATED MICROSPORES[1]

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The ultrastructure of the Late Cretaceous (Santonian–?early Campanian) megaspore *Glomerisporites pupus* and its associated microspores has been examined in an attempt to resolve a number of problems concerning the interpretation of their morphology. The new observations presented are based on an analysis of entire, fragmentary, and thin-sectioned specimens under scanning and transmission electron microscopes. These add to, and partly correct, previous observations on this taxon. They include the following: (1) The exine of the megaspore consists of thin, homogeneous, outer undulating and inner electron dense layers, with a thicker zone of spongy structure in-between. (2) The perispore (or perine) of the megaspore comprises four layers, in order towards the exterior: loose filamentous, dense filamentous, vacuolate, and columnar. (3) This is completely enclosed by a thick mat of hairs, which appears to be attached to the underlying perisporal layers by means of connections with a few of the ''spines'' that originate from the dense filamentous zone, and with some elements of the columnar perine. (4) The tripartite neck (acrolamella) of the spore, which is hidden beneath the mat of hairs, is predominantly an extension of the dense filamentous and vacuolate layers, but also involves the columnar layer, especially in the lower part. (5) Some of the numerous small floats that are embedded in the mat have hairs originating from them. (6) Both long tangled and circinate hairs surround the perispore of the microspores. (7) The exine of the microspore was at least partly attached to the perispore when the organ was viable. (8) It comprises four zones that vary in structure and electron density. These facts and comparisons made with other megaspores and their associated microspores confirm evolutionary links between *G. pupus* and several taxa included within the Salviniaceae (*Azolla, Parazolla, Salvinia*) and possible ancestors of this group (the parent plants of *Ariadnaesporites* and *Capulisporites*).

Key words: Cretaceous; evolution; megaspores; microspores; morphology; ultrastructure; water ferns.

The genus *Glomerisporites* was erected by Potonié (1956) to accommodate a single species of megaspore described by Dijkstra (1949) as *Triletes pupus* from Upper Cretaceous deposits in south Limburg, The Netherlands. The productive, and so far only, succession to have yielded these microfossils is the Aachen Formation, which comprises sediments that were laid down in conditions that varied from fresh to nearshore marine (Batten et al., 1987; Batten, Dupagne-Kievits, and Lister, 1988). Originally dated as (middle) Senonian, the more precise determination of Santonian to possibly early Campanian is now generally accepted (Batten, 1988; Batten, Dupagne-Kievits, and Lister, 1988; Bless and Streel, 1988).

In many places the formation rests directly on much more ancient clayey deposits. Both have yielded assemblages of megaspores, but of very different character, the older clearly indicating a Carboniferous age (Dijkstra and van Vierssen Trip, 1946). This is fortunate because the basal beds of the Aachen Formation often contain clay from the partly weathered uppermost Carboniferous, rendering precise identification of the boundary on lithological grounds difficult despite the unconformable relationship.

Dijkstra (1949) recovered megaspores in greatest abundance from sandy clay and clayey sand samples. Some came from borehole cores taken underground in coal mines. The holes were drilled upwards to determine the thickness of the Carboniferous succession and the character of the overlying deposits. About 50 productive samples from the Aachen Formation were collected in this way, but only one of these proved to contain *Glomerisporites pupus.* This came from the Maurits State Colliery in northwest Limburg (Fig. 1).

Almost all of the other samples that Dijkstra examined for megaspores were obtained from southeast Limburg (e.g., from Epen, Vaals, and near Simpelveld) where the Aachen Formation is close to the surface. Of these, ~60 from both short boreholes and quarry exposures were productive, a third yielding *G. pupus.* Batten and Kovach (1990) noted that there is only one other record of this species, namely a single damaged specimen recovered by Vangerow (1954), also from the Aachen Formation (recorded as ''*Triletes pupus* Dijkstra?'').

Dijkstra (1949, p. 25) provided the following diagnosis of the species: ''Spore body oval, a little elongated, inclusive of the neck 450–525 μ long (the mean being 482 μ, 7 spores measured), 350–525 μ broad (the mean being 376 μ). Tri-radiate ridges lacking, arcuate ridge not clearly distinct. Lagenicula formed of three blunt ending lips, 100–225 μ long, 100–200 μ broad. Exine slightly granulated, densely supplied with silk-like hairs, waven [sic]

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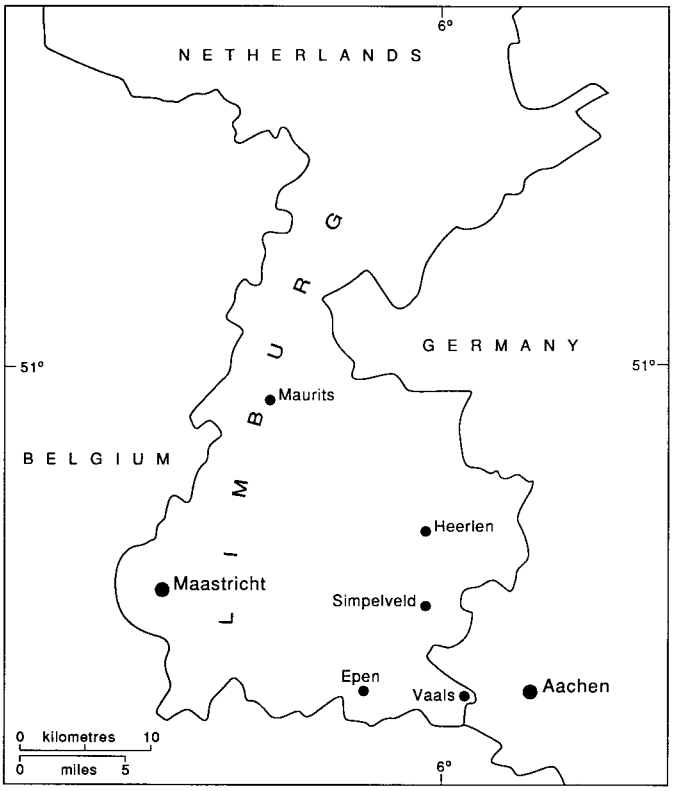


Fig. 1. Map of south Limburg and vicinity showing names of places mentioned in the text.

to a felt, surrounding the whole spore; hairs circa 1–2 μ thick. Exine of the neck not granulated, without hairs. Spore, including felt, 500–1000 μ long (the mean being 757 μ, 50 spores measured), 300–650 μ broad (the mean being 568 μ). Sporewall, composed of two layers, the outer layer circa 10 μ thick, ochre, inner layer circa 3 μ thick, dark brown.''

Dijkstra's use of the word ''Lagenicula'' for the tripartite neck, or acrolamella, of the spore is incorrect because it is not a descriptive term. He placed the species (Dijkstra, 1949, p. 24) in the ''Sectio LAGENICULA (Bennie et Kidson) Schopf,'' although the name had already been applied to a genus of megaspores (Zerndt, 1934; Schopf, 1938; Schopf, Wilson, and Bentall, 1944; type species designated by Potonié and Kremp, 1954).

More important than some unsatisfactory terminology is, however, the lack of detail in both Dijkstra's description and the accompanying illustrations, which are two very stylized drawings (1949, pl. 2, figs. 3, 8). These are not as helpful as they might have been in enabling recognition of the spore, let alone in fully appreciating and interpreting its unusual architecture.

Dijkstra (1949, p. 25) noted that most of the specimens he examined were surrounded by a ''hairfelt'' that obscured the ''real spore,'' but that occasionally this had partly come away. He likened the general aspect of a complete specimen to ''the cocoon of some Lepidoptera''; hence the specific epithet *pupus.* One ''had a lanceolate leaflet . . . closely against this hairfelt,'' with the leaf base being ''entirely surrounded by the t[h]reads of the felt.'' He suggested that it was a sporophyll. Unfortunately this specimen is no longer in his slide collection and is presumed lost. Another observation that Dijkstra made still holds true, namely that ''[c]onfusion of *Triletes pupus* with another spore is not possible.'' The closest to it in morphology is *Ariadnaesporites* Potonié 1956, emend. Tschudy 1966.

During the course of his studies on ancestral Salviniaceae, and with the benefit of access to both scanning and transmission electron microscopes (SEM and TEM), Hall (1974, 1975) re-examined *Glomerisporites pupus.* He provided detailed descriptions and illustrations that greatly increased understanding of the morphology of the species and led him to the conclusion that, although not a typical product of the family, its natural affinity lies with the Salviniaceae. Most of his morphological observations were confirmed by Batten (1988), who re-examined all of the Late Cretaceous megaspores in Dijkstra's collection.

For this paper we took the analysis of this intriguing microfossil a stage further because, despite the more recent studies, several questions have remained unanswered. We sought to determine: (1) the distribution of the floats; (2) the morphology of the associated microspore, and in particular the structure of its wall; (3) whether the massulae contain just one spore; and (4) whether the presence of massulae is diagnostic of a salvinialean affinity. During the course of our investigation we also encountered a number of other characters not previously recorded.

MATERIALS AND METHODS

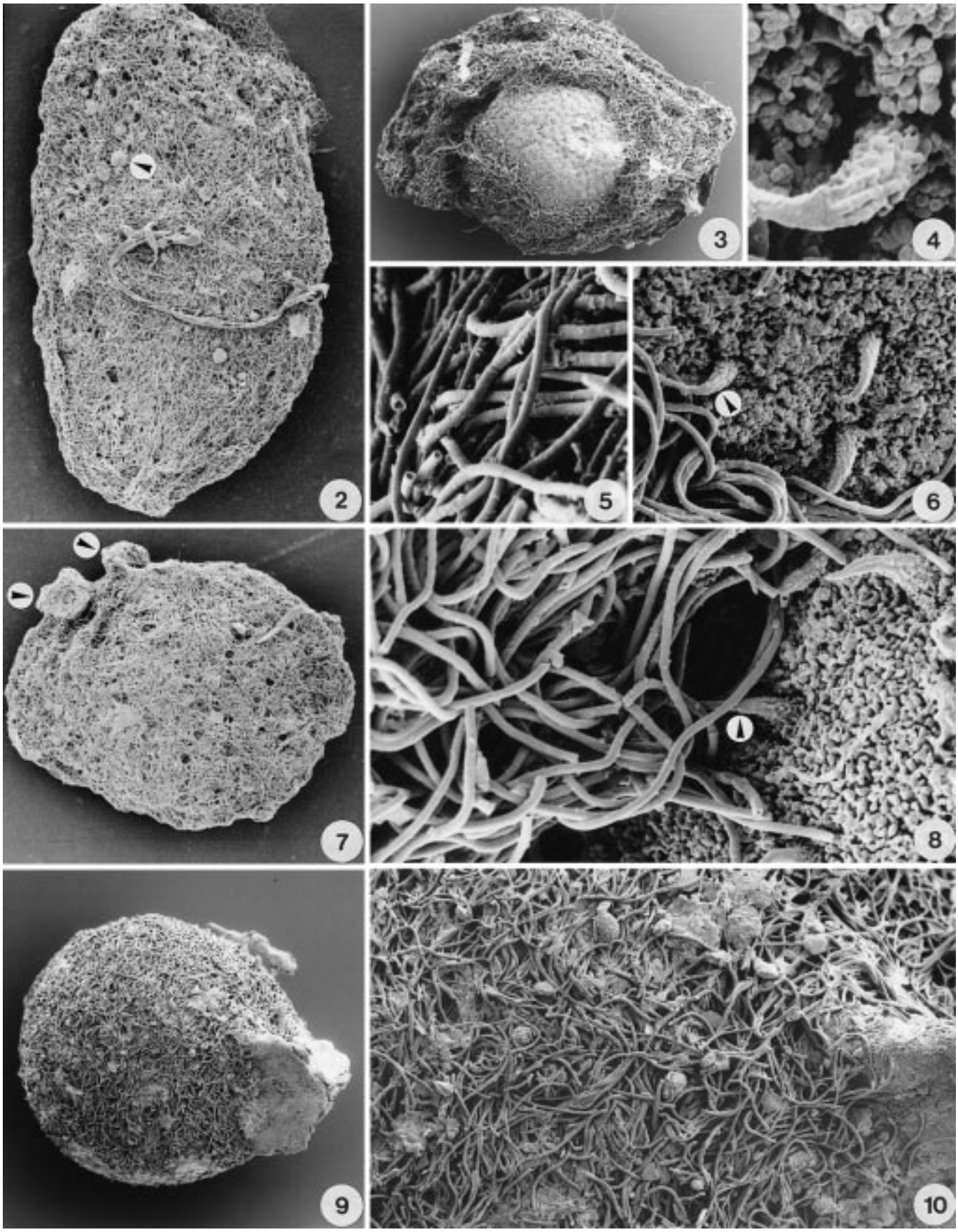
Four entire specimens, four that are partly or devoid of the mat of long perisporal hairs, and several badly damaged and fragmented remains were selected from an assemblage in Dijkstra's slide collection and examined under Hitachi and JEOL SEMs. A few were published in Batten (1988, pl. 15, fig. 11; pl. 16, figs. 2, 5, 6), but they are shown again here because most of the figures in his papezr were printed too pale and ''flat'' for all of the detail in them to be revealed.

The procedure adopted for obtaining transmission electron micrographs and the instruments used were as described by Collinson (1991, pp. 120–121). Sectioning proved difficult because of the presence of pyrite embedded in the specimens. Hence, there are score marks, gouges, and black lumps of pyrite on some micrographs and/or holes where this mineral dropped out during sectioning.

REVIEW OF MORPHOLOGY AND PREVIOUS INTERPRETATION

We review here, with reference to SEM micrographs, the characters of *Glomerisporites pupus* that have been described previously in some detail by Hall (1974, 1975), and also considered by Batten (1988) in the context of the total megaspore assemblage with which it is associated.

The wall of the megaspore comprises two main layers: exine (megaspore proper) and perispore (or perine). Specimens usually appear either as rounded to roughly egg-shaped, cocoon-like spongiose bodies (Figs. 2, 7) or partly to almost completely denuded (Figs. 3, 9, 11 respectively). When degraded in this way, the subspherical shape of the body of the spore and, commonly, its prominent ridged neck is revealed. In general, the entire microfossil, that is, with its ''hairfelt'' intact, is about twice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the size of the spore body of denuded specimens, which was recorded by Dijkstra (1949) and confirmed by Batten (1988) as being between 350 and 525 μm (cf. Figs. 9, 11). At low magnifications the exine of the megaspore appears to have more or less smooth to scabrate inner and outer surfaces (Fig. 13).

The perispore was divided into three zones by Hall (1975, p. 366, fig. 39). The inner zone was described as granular in structure but scanning electron micrographs obtained by Batten (1988, pl. 15, figs. 2, 3; Fig. 21 here) indicated a more ''fibrous'' meshwork of sporopollenin threads, and numerous small perforations in addition to larger holes that delineate the ''hollow'' bases of hairs (Hall, 1975, figs. 30, 39; Fig. 21). It is only loosely connected to the exine and may, therefore, become completely detached from it in damaged specimens (Fig. 20). The middle zone of the perispore is the thickest of the three, and of very open, vacuolate (pseudovaculate, vesicular) construction (Figs. 13, 17). According to Hall (1975, p. 366) the prominent neck (tripartite acrolamella) is continuous with the middle perisporal layer.

Capping the middle zone is a comparatively thin, open-textured, abundantly perforated layer (Figs. 4, 6, 8), the structure of which was described by Hall (1975) as tending to be columellate-tectate. It commonly has a granular to finely rugulate-reticulate aspect in surface view (e.g., Figs. 6, 8). In addition, extending from this layer are scattered bacula and spines. Hall suggested that the latter were possibly the points of attachment of the solid surface hairs. Hence, according to this interpretation, which has proved to be at least partly correct as we show here, the long hairs that form the dense mat over the perispore (e.g., Figs. 3, 5, 6, 8, 10, 25) arise from the inner zone of this layer as hollow structures that penetrate the middle and outer zones and extend a short distance above these as spinose elements (Figs. 4, 6, 8) before becoming solid for the remainder (the greater part) of their length.

Among the hairs are many microspore-sized floats (Figs. 9, 10, 24, 26, 27), which are apparently more numerous in the proximal region of the spore than elsewhere (Hall, 1975, figs. 26–28; cf. Figs. 9, 10). These bodies have an irregularly rugulate surface and, according to Hall (1975, fig. 38), an irregular, branched baculate, columellate-tectate wall structure.

Microspores may be attached to the megaspore (Figs. 7, 22). These are commonly difficult to examine because they are also covered by hairs, but not to the same extent as the megaspores. The tips of the hairs are usually coiled (Figs. 22, 23). These small spores are similar in shape to the megaspore without its surrounding ''hairfelt,'' and also bear some resemblance to the megaspore *Ariadnaesporites varius* Hall and Peake 1968.

As in the megaspore, there are two main components to the wall of the microspore of *G. pupus*: exine (microspore proper) and perispore. Again the perispore is divisible into three parts: inner comparatively dense and granular-filamentous, outer homogeneous, and a very open vacuolate (alveolate) structure in-between. The hairs extend from the vacuolate and outer homogeneous layers (cf. Fig. 23). Despite having the general appearance of a small megaspore, the ''body'' of the microspore consists only of perispore. The microspore proper is situated in the ''neck'' surrounded by this material. Hall (1975, p. 366) described the exine as ''psilate or granular, free from perispore.''

NEW OBSERVATIONS

In this section we add to the above description mainly with reference to a detailed re-examination of two undamaged specimens of *Glomerisporites pupus* from which many thin sections were taken and photographed under a TEM, but also following additional scanning electron microscopy. Our observations are supported by both SEM and TEM micrographs.

As previously, the megaspore wall is regarded as comprising an exine overlain by a complex perispore. The exine is the more difficult of the two to subdivide on structural differences. The previously recognized smooth to scabrate outer and inner surfaces are associated with thin homogeneous undulating and electron-dense layers, respectively. Sandwiched between them is a thicker layer composed of often very closely packed filaments, which we prefer to describe as forming a spongy rather than a granular structure (Figs. 28, 31).

We distinguish four components of the perispore. These are, from the innermost part outwards: loose filamentous, dense filamentous, vacuolate, and largely columnar (Figs. 28, 29, 32). The last of these gives rise to hairs (Fig. 33), some of which are clearly hollow (Figs. 5, 26), at least initially. The dense filamentous zone forms the surface of the spines, and the loosely filamentous part, which underlies it, is also present within them (Figs. 28–30). All four zones commonly merge with one another, and are not clearly separated by a pronounced break (Figs. 28, 30, 31). On the other hand, the connection between the inner and outer filamentous zones is weak (Fig. 16). As a result, in fragmentary material the outer surface of the exine usually seems to be covered by a thin filamentous to finely reticulate meshwork of

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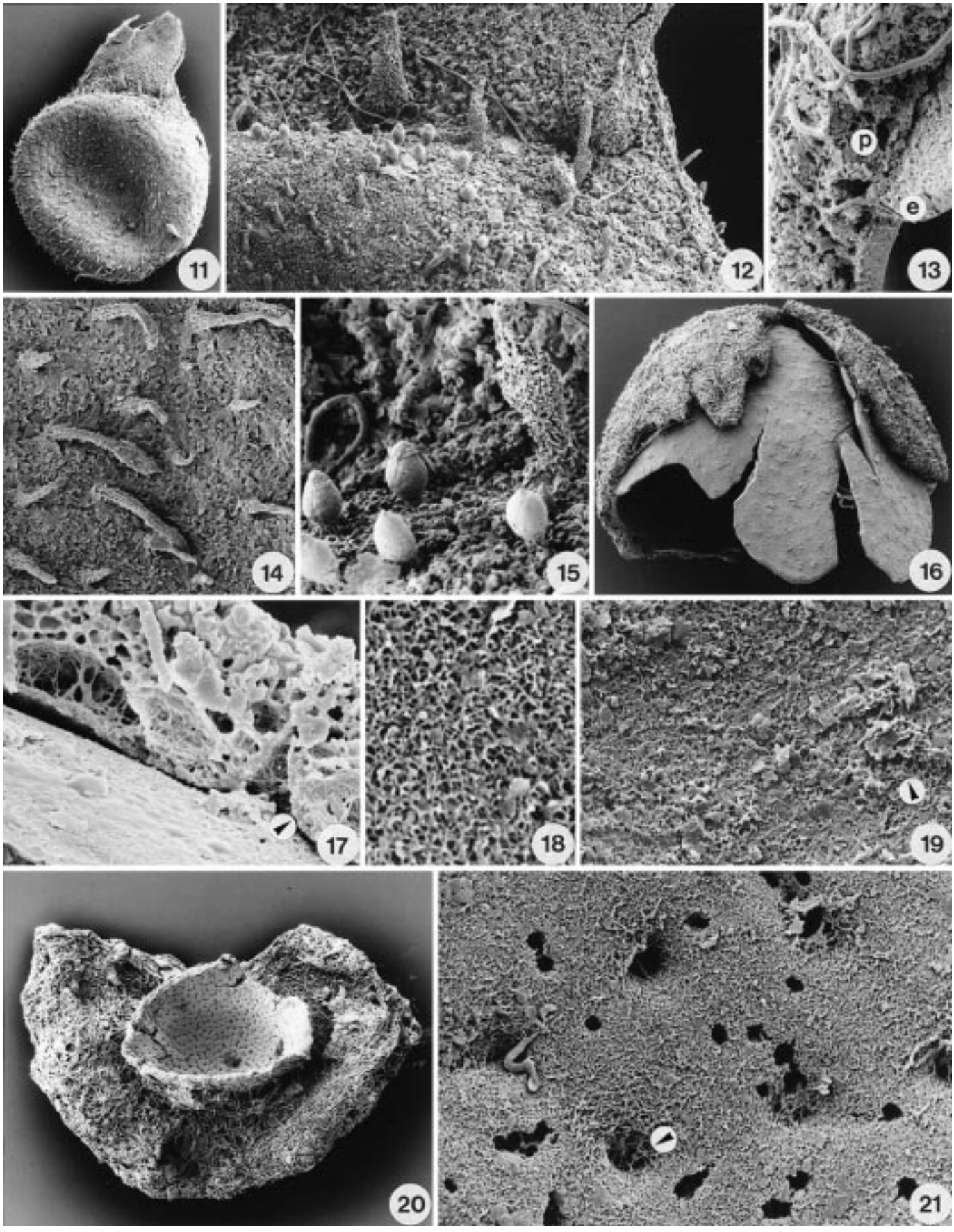
Figs. 2–10. *Glomerisporites pupus*; all SEM micrographs. **2.** Oval, cocoon-like specimen 1; morphology of megaspore completely obscured by a thick mat of hairs within which scattered floats (arrow) are enmeshed; extraneous strip of organic detritus adhering to middle region, ×100. **3.** Part of hair mat removed showing spines extending from surface of columnar perispore layer (columnar perine), specimen 2, ×100. **4.** Surface of columnar perine and a spine showing ribbed base and extension to smooth-walled hair, specimen 2, ×5000. **5.** Hairs extending horizontally from right-hand side may be derived from columnar layer (this could not be definitely determined); those broken on the left are hollow, specimen 2, ×2500. **6.** Surface of columnar perine showing several spines including that in Fig. 4, which is prolongated into a hair (arrow), ×1500. **7.** Example of a globular megaspore (specimen 3) with two microspores (arrows) attached to upper surface by hairs (see Figs. 22 and 23 for microspores on another specimen at higher magnification), ×100. **8.** Another spinose element in middle of photograph (arrow) which appears to be connected to mat of overlying hairs, specimen 2, ×2000. **9.** Although much of hair mat has been removed, a thin layer with numerous floats remains; tripartite neck (acrolamella) exposed, specimen 4, ×50. **10.** Close-up of numerous broken hairs and floats; base of neck on right-hand side, specimen 4, ×500.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sporopollenin threads (Fig. 18), which may be raised up into scattered ''mounds'' (Figs. 16, 19); these presumably indicate the locations of spines. A zone of lesser weakness is present between the hair mass and columnar zone, the hairs tending to become detached (e.g., Figs. 3, 8, 11). Occasionally there may be breakages between the outer filamentous and vacuolate components, especially if the basal vacuoles are large; as a result, these two layers may be partly separated from each other.

The acrolamella is seen in thin section to be composed of the dense filamentous and vacuolate layers, and also of columnar perine near the base (Figs. 35–37). It becomes difficult to differentiate these layers towards the apex. The floats were found not only to be enmeshed in the perisporal hairs but also to have hairs originating from them (Fig. 26), and to be particularly concentrated in the neck region (Figs. 34, 35).

One of the thin-sectioned megaspores had four microspores adhering to it, which, under the SEM, were seen to be smothered by, and closely attached to, the megaspore by numerous hairs. Some of these are short and/or have circinate tips, but others arising from its surface are long and tangled (Figs. 22, 23). The other sectioned specimen did not clearly show any microspores but usefully revealed many floats surrounded by hairs. The irregular surface of these bodies, and the fact that hairs also arise from them, are apparent at high magnifications in both this specimen and others (Figs. 24, 26, 27, 38).

The exine of the microspores proved to be surprisingly complex (Figs. 41, 42) in comprising a thin outer layer with an irregular surface, underlain by thicker granular and homogeneous zones, the latter becoming more electron dense towards the interior. The innermost layer is very thin, homogeneous, and electron dense (Fig. 42). By contrast, the bulk of the perispore consists of a very open vacuolate (alveolate) structure, which is under- and overlain by filamentous and thin homogeneous layers, respectively (Figs. 39–41, 43–46).

In most thin sections of the microspores, the perispore is usually separated from the exine. The irregular surface of the latter suggests, however, that when the spores were viable, the two layers were connected. Such an intimate association has been demonstrated in small parts of a couple of our thin sections (Figs. 39, 40). This is in marked contrast to the exine of the microspores of *Azolla, Parazolla,* and *Salvinia,* all of which have a smooth surface. It suggests that the exine shrank away from the perispore after germination or during fossilization, as is commonly seen in megaspores of *Azolla,* but there is no apparent physical manifestation of shrinking and no sign of crumpling. Its homogeneous appearance perhaps reflects this phenomenon. The flanges of the perispore are, therefore, interpreted to indicate the location of the acrolamella (Fig. 41). In Fig. 43 they match the two interruptions of the exine, which delineate portions of the triradiate suture that have been sliced obliquely.

INTERPRETATION

Our examination has demonstrated several advances beyond Hall (1974, 1975) and partly also Batten (1988). Hall's sections of the megaspore wall indicated its basic structure but did not reveal the detail we have uncovered. He described only three perisporal layers, and partly misinterpreted their roles and adhesion to one another. He assumed that the ''hollow,'' broad spines, which emanate from the filamentous zone and penetrate the outer perisporal layers, became the solid hairs that envelop the spore and enmesh the floats. A further search has revealed a somewhat greater variability in the size and shape of the spines than previously observed (Figs. 12, 14, 15), but this does not account for the irregularly dispersed small perforations on the inner surface of the perispore, and convincing links between the spines and the mat of overlying hairs were extremely hard to find. It is unlikely that the bulbous spines (Fig. 15) were extended into hairs. A few of the more conical structures do, however, seem to be connected (e.g., Figs. 4, 6, 8). The difficulty of finding proof of connection is perhaps because the point at which the hairs join the spines seems to have been a weak link, the hairs tending to break away (cf. Figs. 11, 14). The fact that some hairs appear to arise directly from the columnar outer perispore (Fig. 33) was not realized hitherto.

Our sections show that the bases of the robust spinose elements that penetrate the perispore are not hollow, as previously described, but contain very loosely packed filaments from the innermost perisporal layer. They also confirm that the inner perispore is made up of interconnecting filaments (described as a fibrous layer by Batten [1988]). Hall thought that all of the hairs forming the dense mat over the perispore were solid, and most of our TEM micrographs also suggest this, but those arising from the columnar layer appear to have been hollow, at least initially (Fig. 33). Several of our scanning micrographs show hollow hairs (Figs. 5, 8). Particularly con-

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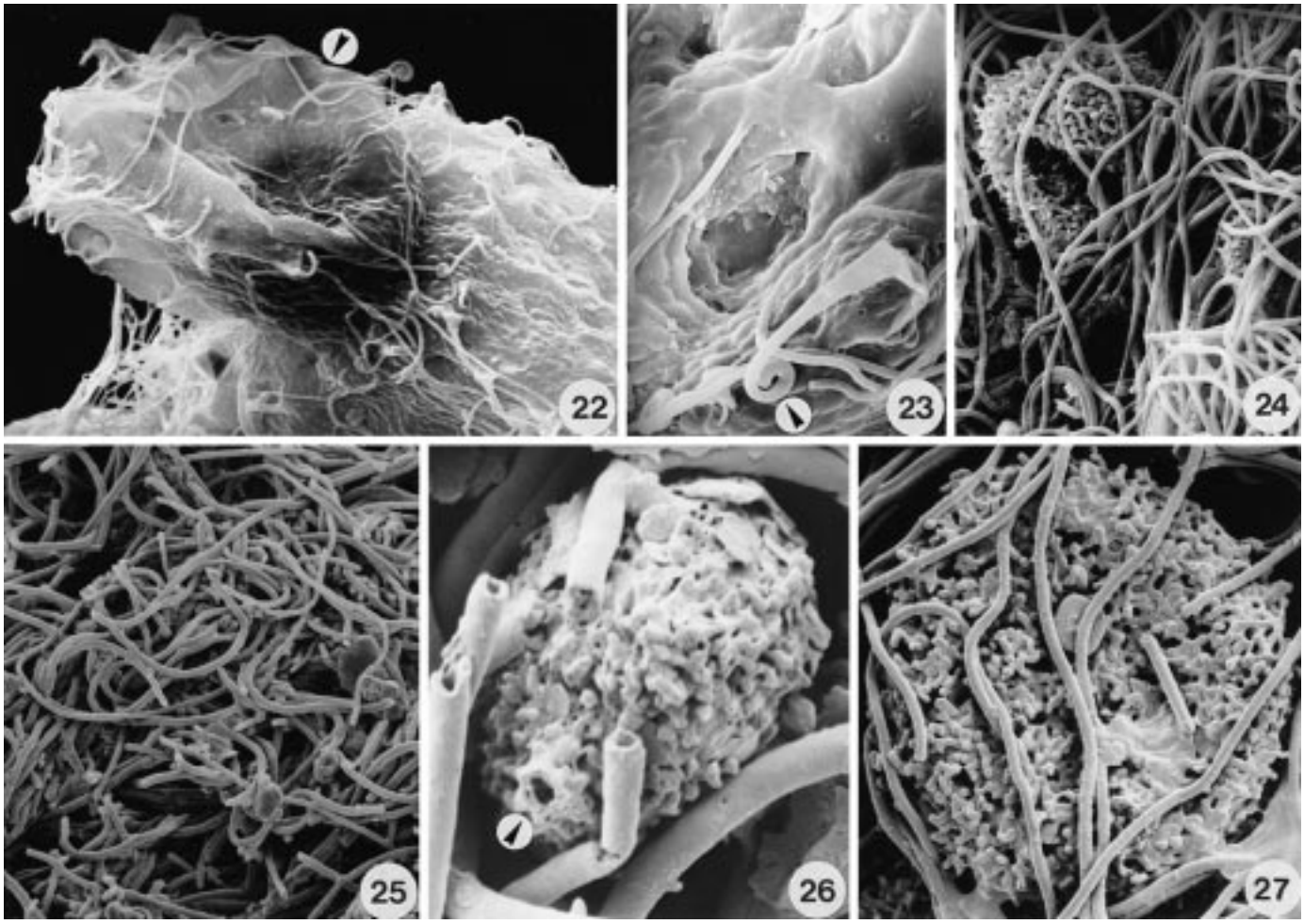
Figs. 11–21. *Glomerisporites pupus*; all SEM micrographs. **11.** Megaspore (specimen 5) with almost all hair mat removed, revealing neck, surface of columnar perine, and spines; same specimen as that in Batten (1988, pl. 15, fig. 5) but opposite side, ×100. **12.** Surface near base of neck of specimen 5 showing spines of various shapes and sizes; gently tapering, bulbous, and irregularly shaped forms with a reticulate-apiculate surface that are larger than the others, ×500. **13.** Both exine (e) and perispore (p) of incomplete, broken specimen 6 (see left-hand side of Fig. 16), ×1500. **14.** Close-up of mostly ribbed and apiculate spines on middle part of body of specimen 5, ×1000. **15.** Close-up of one irregularly shaped, and several bulbous spines near base of neck of specimen 5; see Fig. 12, ×1500. **16.** Specimen 6, showing clear separation of exine and part of innermost perisporal layer (with ''swellings''; see Fig. 19) from rest of perispore, ×200. **17.** Cross-section of a fragment of wall (specimen 7) showing vacuolate zone and spine arising from dense filamentous layer of perispore (arrow), ×2500. **18.** Detail of surface of exine of specimen 6, which appears to be covered by a reticulate meshwork of sporopollenin threads derived from attached innermost perisporal layer, ×5000. **19.** Close-up of irregular surface of exine of specimen 6 (see Fig. 16); ''swellings'' (one arrowed) appear to be composed of innermost perispore and probably coincide with bases of spines, ×2500. **20.** Broken specimen 8; perforated inner surface of perispore and outer mass of hairs with a few floats, ×100. **21.** Close-up of innermost part of perispore of specimen 8; loose filamentous layer, some of the threads from which enter the larger perforations (arrow), ×2500.



Figs. 22–27. *Glomerisporites pupus*; all SEM micrographs. **22.** Microspore adhering to perine of megaspore (arrow); covered with hairs, some of which are long and meandering, others have circinate tips, specimen 9 (one of the two thin-sectioned specimens), ×500. **23.** Close-up of surface of microspore on specimen 9, two hairs with circinate tips (one arrowed) in lower half of figure, ×1500. **24.** Floats enmeshed in hairs, specimen 9, ×1000. **25.** Perisporal hairs (many broken) of specimen 10, ×1000. **26.** Detail of small float showing perisporal hair attachment point (arrow); opening is same diameter as that of adjacent hollow hairs, specimen 4, ×5000. **27.** Close-up of larger float enmeshed in hairs, showing reticulate surface, specimen 1, ×2000.

vincing is Fig. 26 in which their diameter matches that of the opening in a float where a hair has clearly been attached.

Hall did not appreciate that more than one layer of perispore is involved in the construction of the acrolamellae of the megaspore, and he did not provide a view of the proximal pole in longitudinal section. His scanning electron micrographs do not show details of the floats, and neither these nor his TEM micrographs reveal hairs originating from them. His sections of the floats do not show continuity of the basal layer, nor do they demonstrate their general distribution.

The microspores he indicated as being attached to a megaspore are not like his isolated specimen (Hall, 1975, figs. 33 and 29, respectively); they appear to be floats. By contrast, both our scanning and transmission

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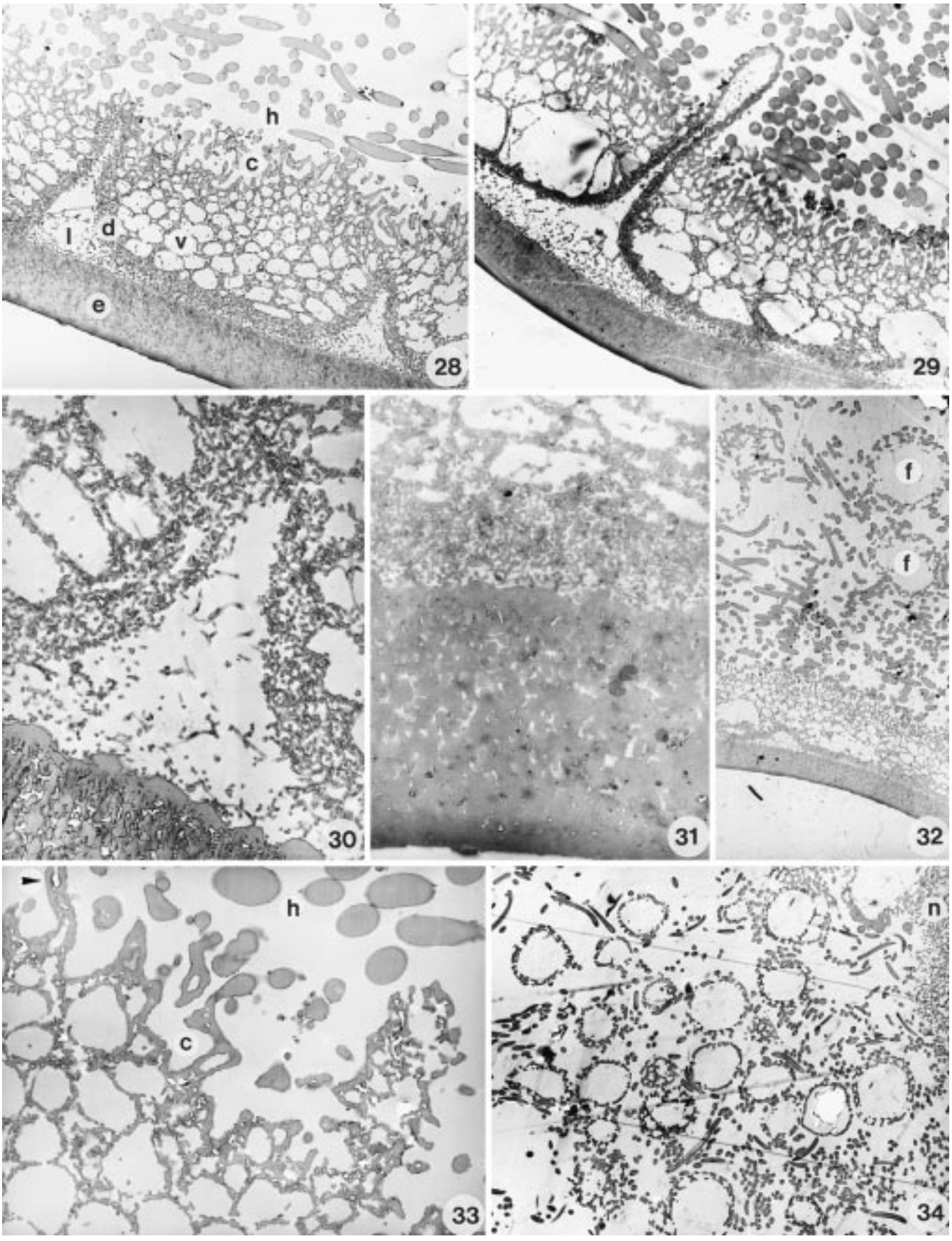
Figs. 28–34. *Glomerisporites pupus*; all TEM micrographs of thin sections of megaspore. **28.** Cross section through both exine and perispore: exine (e) consists of a thin, more or less homogeneous upper layer with an uneven surface underlain by thicker, spongy, and thin, homogeneous, electron-dense layers; perispore comprises, in order towards exterior: loose filamentous (l), dense filamentous (d), vacuolate (v), and columnar (c) layers overlain by a mat of hairs (h), ×2500. **29.** Similar to Fig. 28 but with lower part of vacuolate layer of more open construction (a result of degradation of the wall?), and showing a spine (base of long hair?; no sign of a connection) extending from the dense filamentous zone up through the vacuolate and columnar parts, and infilled with scattered threads of the loose filamentous layer, ×2000. **30.** Detail of construction of upper part of exine and loose filamentous, dense filamentous and vacuolate layers of perispore in vicinity of base of spine, ×10 000. **31.** Detail of exine and perispore in between spines; dense filamentous layer merges with both loose filamentous and vacuolate components of perispore, ×10 000. **32.** Cross section through whole specimen showing not only exine and layers of perine as in Figs. 28 and 29 but also floats (f), ×1000. **33.** Outer vacuolate layer giving way to columnar layer (c) and mat of hairs (h), some of which are hollow initially (arrow), ×7500. **34.** Section adjacent to neck (n) of megaspore showing numerous floats within hairs (see Fig. 35), ×1000.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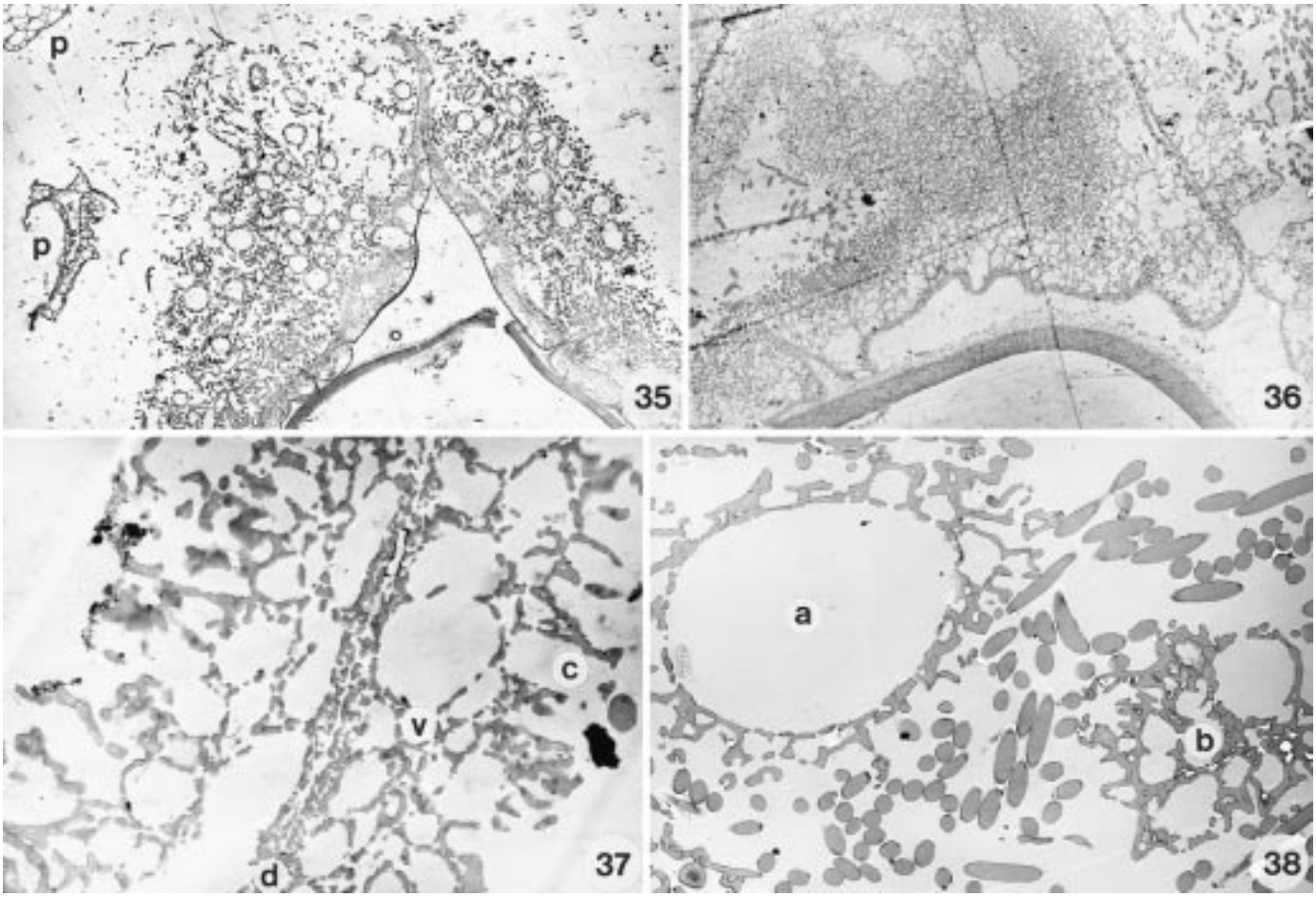


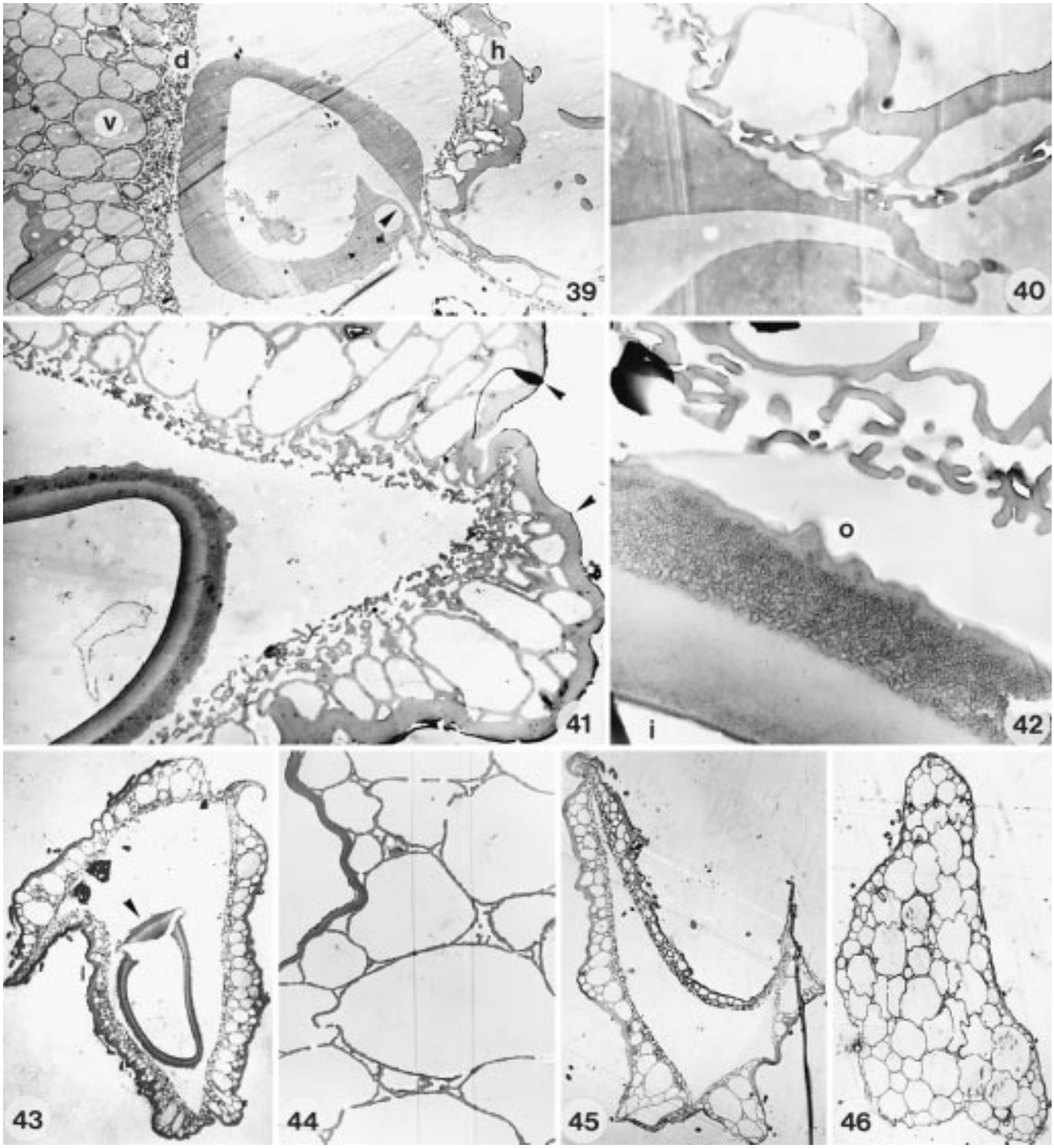
Figs. 35–38. *Glomerisporites pupus*; all TEM micrographs of thin sections. **35.** Section through neck (acrolamella) of megaspore, adjacent hairs and floats, and on left-hand side and in top left-hand corner, the perine of microspores (p: see Figs. 39–46), ×200. **36.** Neck is seen to be developed from dense filamentous, vacuolate, and columnar components of perispore, although the last of these is not clearly differentiated from the vacuolar layer towards tip, ×750. **37.** Detail of upper part of neck of specimen depicted in Fig. 35, showing dense filamentous (d), vacuolate (v), and poorly delineated columnar (c) layers, ×6000. **38.** Detail of two floats showing irregular columellate-reticulate aspect of their walls; section has cut through central part of specimen (a) on left-hand side, but on the right (b) it has passed through only some of the reticulate meshwork, ×3000.

electron micrographs clearly show microspores enmeshed by perisporal hairs (Figs. 22, 35). Hall noted only short circinate hairs on the microspore, whereas we recognize two types as described above. We also show that the exine and perispore of the microspores may be found connected, and that the usual separation of the two layers probably reflects shrinkage of the exine following germination or as a result of the processes of fossilization.

Despite these additional findings we remain unsure of a couple of points. (1) Although the ''large'' holes on the inner surface of the perispore can be related to the spines that penetrate the outer perisporal layers, the function of the smaller holes remains unclear. (2) It has been impossible to determine what proportion of the spines served as bases to which hairs are (or were) attached, as opposed to those emanating from the columnar layer. Clearly, the outer mass of hairs and floats can be separated fairly easily from the rest of the perispore, but the break is by no means always clean (cf. Figs. 3, 9, 11). This ties in with the fact that our TEM micrographs do not regularly show any breakage adjacent to the spines. By far the most commonly encountered zone of weakness is between the exine and the dense filamentous part of the perispore, that is, within the loose filamentous layer.

DISCUSSION

The small floats and microspores are invisible on dry specimens of *Glomerisporites pupus* at low magnifications in reflected light. It is not surprising that Dijkstra (1949) did not record their presence because he examined his material only by this method. While admitting that neither the megaspore nor the microspore is typical, Hall (1974, 1975) argued that their characters indicate derivation from a salviniaceous plant. The prominent tripartite neck of the megaspore is a feature it has in common with both the acrolamella of *Ariadnaesporites,* which Hall regarded as an ancestral representative of the Salviniaceae, and the ''columella'' of the megaspore of *Azolla,* an extant member of this ''water fern'' family with a fossil record extending back to the Late Cretaceous (Kovach and Batten, 1989). The neck of *Ariadnaesporites* is, however, generally prominently exposed instead of being



Figs. 39–46. *Glomerisporites pupus*; all TEM micrographs of thin sections of microspores. **39.** Oblique section of microspore within a perispore showing an inner, comparatively dense zone (d) overlain by very open vacuolate (v) and irregular, homogeneous (h) layers, respectively; irregular surface of microspore connected to perispore adjacent to trilete suture (arrow: see Fig. 40), ×2500. **40.** Detail of connection between exine and perispore from section in Fig. 39, ×10 000. **41.** Although there is no incision in the exine to mark the position of one of the arms of the trilete laesurae, the lobes of the perispore (arrows) suggest that this section passed close to a suture (cf. Fig. 43); layers of exine clearly delineated (see Fig. 42), ×4000. **42.** Exine is divided into four layers (those forming the upper and lower surfaces being much thinner than the other two) as follows, from interior (i) outwards (o): dark, homogeneous, electron dense; homogeneous, becoming less electron dense upwards; densely granular, and homogeneous but with an undulating to irregular (granulate-rugulate) outer surface, ×15 000. **43.** Entire microspore (arrow) showing exine and surrounding perispore; sutures in ?shrunken exine match position of projections (lips/flanges) in perispore, ×750. **44.** Detail of perispore showing construction of vacuolate layer, ×2500. **45.** Perispore only, ×750. **46.** Tangential section of perispore, ×750.

completely obscured by hairs. In *Azolla* it is composed of perisporal layers hidden under the floats of the ''swimming apparatus.''

Hall (1975) regarded the neck to be particularly important from the viewpoint of indicating a salvinialean origin. Although its tripartite character suggests an extension of the margin of the triradiate suture as in many megaspores, its perisporal construction renders it fundamentally different.

The floats of *Azolla* are more obvious than those of *G. pupus,* and there are fewer of them (typically 3–9, but up to 24 in some fossil species). The very small (~12–30 μm) spore-sized floats of *G. pupus* are intimately associated with the perisporal hairs and composed of perisporal material. Hall (1975, p. 367) discussed the mechanism by which they were probably formed and suggested a chronological trend from genera with many small to fewer, larger floats in the order: *Glomerisporites, Azollopsis, Azolla* Sect. *Krematospora* (Jain and Hall, 1969), *Azolla* Sect. *Rhizosperma,* and *Azolla* Sect. *Azolla.* Floats have been reported in *Ariadnaesporites varius*, but there are not many of them, and they are unknown in other species attributable to the genus, which does not, therefore, fit neatly into this group. It may, however, be a precursor of megaspores with floats, as indeed the hairs around their microspores may be ancestral to true massulae.

The massulae of *Azolla* are developed in the same way as the floats of the megaspore. In Sect. *Azolla* there are anchor-shaped glochidia, which are homologous to the hairs of the perispore of the megaspore (Hall, 1975, p. 368). The spores they contain are of very simple construction: subspherical, trilete, and mostly unsculptured. There is no individual perispore around them.

The microspores of *G. pupus* are similarly enveloped in perisporal matter. Hall (1974, p. 361) noted that it is possible to interpret them as simple massulae because they bear coiled hairs like those of massulae of *Azolla circinata* Oltz and Hall in Hall [1968], and have a ''ps[e]udovacuolate structure throughout.'' He later considered them not to be true massulae because the spores are borne singly in clearly defined perisporal masses (Hall, 1975, p. 368). In *Ariadnaesporites varius* there is a less complex perispore around the microspores, which may be clustered together, each with a long distal hair caught up with the perisporal hairs of the megaspore.

The megaspore of extant *Azolla* comprises an exine overlain by a perispore, which has the same derivation as the floats. Hall (1975) noted that the structure of the exine of both *Ariadnaesporites* and *G. pupus* is similar. He also commented (1975, p. 368) that the exine of spores of extant *Salvinia* is ''more dense internally'' than that of *Azolla.* Typical of salvinialean perispores is an outer hairy zone beneath which are columellae (exoperine) arising from a basal layer (endoperine: Collinson [1980] and others). Again, the construction of the perispore of *G. pupus* is similar, but that of *Ariadnaesporites* differs in comprising only a hairy layer. Hence, overall Hall (1974, 1975) made a good case for an evolutionary progression within the Salviniaceae from the ''primitive'' and geologically oldest genus *Ariadnaesporites* (see Kovach and Batten, 1989; Batten and Kovach, 1990) via *Glomerisporites* to *Azollopsis* and *Azolla.*

The evolution of the Salviniaceae was also considered by Martin (1976) shortly after the appearance of Hall's papers but, although including *Azinia* Baluyeva 1964 in his evolutionary synthesis, he did not take into account either *Ariadnaesporites* or *Glomerisporites.*

In her review of the diversification of modern heterosporous pteridophytes, Collinson (1991) briefly referred to all of the genera, illustrating their general morphology and main structural characters with scanning and transmission electron micrographs. With respect to *Glomerisporites,* she noted in particular: (1) the general similarity between it and *Azollopsis* but the much greater complexity of its perispore; (2) the vacuolate floats enmeshed in perisporal hairs; and (3) attached ''massulae'' containing single microspores. She considered that *Glomerisporites* and *Azollopsis* should probably be included with *Azolla* in the Azollaceae, whereas *Parazolla* was thought possibly to represent an extinct salvinialean family, and *Ariadnaesporites* perhaps an extinct order of heterosporous plants. This may also apply to *Capulisporites* Potonié 1956, another Late Cretaceous ''water fern'' megaspore (Batten, Collinson, and Knobloch, 1994).

The structure of the exine and inner perisporal layers of *Glomerisporites pupus* is clearly similar to that of the intexine and exoexine of *Ariadnaesporites pilifer* Batten, Collinson and Knobloch and *Capulisporites klikovensis* Batten, Collinson and Knobloch (see Batten, Collinson, and Knobloch, 1994), as are the roles of the perisporal layers and exoexine in the formation of the acrolamella, spines, and other body appendages. The fact that the microspores of *G. pupus* incorporate a perispore in their structure rather than being smooth to scabrate bodies within a vacuolate massula does not affect its position in the inferred evolutionary progression from *Ariadnaesporites* that lack massulae to massulate *Azollopsis* and *Azolla.*

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