

Morphological and ultrastructural variations in *Schizaea pectinata* (Schizaeaceae: Pteridophyta)

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ABSTRACT

Schizaea pectinata (L.) Sw. was collected from the extreme ends of its geographical range in South Africa for a study of sporangial development, sporogenesis and tapetal organisation. Differences were noted in the gross morphology, in sporangium size, spore size and in the patterning of the outer exospore from the two sites. Coiled structures were associated with the development of the inner perispore in spores collected from the Transvaal, whereas dense, heterogeneous bodies were associated with the formation of this layer in spores from the Cape. Differences were also noted in the organisation of the tapetum. A cellular, parietal tapetum and a plasmodial tapetum were present in the Cape material when the spores had developed the sculptured outer exospore. In sporangia from the Transvaal, however, only a plasmodial tapetum was present at the same stage of sporoderm development. A detailed study of *S. pectinata* throughout its distribution is required to determine the taxonomic importance of these findings.

UITTREKSEL

Schizaea pectinata (L.) Sw. is in die twee uithoeke van sy verspreidingsgebied in Suid-Afrika versamel vir 'n ondersoek van sporangiumontwikkeling, sporogenese en tapetumorganisasie. Verskille in algehele morfologie, sporangiumgrootte, spoorgrootte en in die versiering van die buitenste eksospoor, is in die twee versamelplekke aangeteken. By spore wat in die Transvaal versamel is, is gekrulde strukture met die ontwikkeling van die binneste perispoor geassosieer, terwyl digte, heterogene strukture met die vorming van hierdie laag by Kaapse spore, geassosieer is. Verskille in die organisasie van die tapetum is ook waargeneem. 'n Sellulêre, pariëtale tapetum en 'n plasmodiese tapetum het in die Kaapse materiaal voorgekom by spore waar die versierde buitenste eksospoor ontwikkel het. By sporangiums wat in die Transvaal versamel is, was daar teen dieselfde stadium van sporodermontwikkeling egter slegs 'n plasmodiese tapetum teenwoordig. 'n Breedvoerige ondersoek van *S. pectinata*, regdeur die organisme se verspreiding, word benodig om die taksonomiese belang van hierdie bevindings te bepaal.

INTRODUCTION

An investigation of sporangial development, sporogenesis and tapetal organisation in *Schizaea pectinata* (L.) Sw. was carried out on material collected from Ysterkroon in the Northern Transvaal (2429BB Zebediela) and from the Cape of Good Hope Nature Reserve (3418AD Simonstown). The Transvaal material was collected at an altitude of 2 046 m in an area of vegetation described as Northeastern Mountain Sourveld (Acocks 1988). The plants were small, approximately 80 mm high, and were found in well-protected sites at the bases of outcrops of Black Reef quartzite. The plants were obscured by large tussocks of *Themeda triandra* Forssk. and other coarse grasses. Plants from the Western Cape were collected from several sites, at altitudes below 150 m, all of which had well-drained, highly leached, shallow sandy lithosols in areas of Mesic Mountain Fynbos (Macdonald, Clark & Taylor 1989). These plants regenerated quickly after fire, becoming fertile after about four months of growth in open situations. They gradually became overgrown and shaded as other fynbos plants regenerated. In the second and subsequent years following fire they produced few new fronds. Voucher specimens (*Cape J067273*, *Transvaal J067274*) are housed in the C.E. Moss Herbarium

(J). In studying the ontogeny of the sporoderm, several features were noted which differed in the specimens from the two sites. These included variations in the sculpturing of the outer exospore and in structures involved with the development of the inner perispore. This led to a closer study of the material to ascertain whether the differences were constant features.

MATERIALS AND METHODS

Material was fixed in the field in 2% paraformaldehyde and 3% glutaraldehyde in 0.08 M Pipes buffer at pH 8.0 (Colhoun & Steer 1981), washed in ice-cold buffer and post-fixed on ice using 2% OsO₄ followed by washing and dehydration through a graded acetone series. Specimens were transferred into propylene oxide, infiltrated with resin using mixtures of propylene oxide and resin and finally embedded in Epon (Luft 1961).

Sections 1 µm thick were stained in toluidine blue for light microscopy and 60 nm ultra-thin sections were stained in uranyl acetate and lead citrate for viewing in a Jeol 100S transmission electron microscope at 80 Kv. Specimens for scanning electron microscopy were selected from material in primary fixative but were not post-fixed. They were washed in buffer, passed through a graded ethanol series, transferred to propylene oxide and dried by the critical point method in a Balzers Union CPD model 020. The specimens were mounted onto aluminium

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stubs coated with pressure sensitive adhesive and coated with gold-palladium prior to viewing in a Jeol JSM-840 microscope at 15 Kv.

Sporal features were observed on fixed and processed material from the two sites and a quantitative survey was also carried out on fixed material.

Measurements of the sporangia and the spores from each of the collection sites was based on a sample size of 50 specimens. Sporangium length was measured from the base of the capsule to the top of the annulus and the width was measured at the widest point. The length and width of spores complete with perispore were recorded.

RESULTS AND DISCUSSION

Gross morphology

Plants from the Transvaal were small, approximately 100 mm high with only a few fronds which had regenerated after fire (Figure 1A). Between seven and nine pairs of modified pinnae were supported on a straight rachis held at an angle of about 100° from the photosynthetic stipe.

Plants collected from the Cape (Figure 1B) were robust, about 200 mm high and formed extensive clumps with large numbers of fronds (± 50) which had regenerated after fire. The pinnae pairs numbered between 14 and 18. The rachis passed through a straight phase during the unfolding of the crozier but was distinctly curved at maturity.

Variations in the gross morphology of the plants from the two sites which are approximately 2 000 km apart, were initially thought to indicate ecotypes of *S. pectinata*. Variation in size of specimens collected at different altitudes had been commented on by Roux (1979). There are known cases of exceptional morphological variability within populations in other ferns from disjunct parts of their range, as in *Thelypteris palustris* Schott (Tryon & Lugardon 1991). Tryon & Tryon (1982) stated that the systematics of the tropical American *Schizaea* Sm. are not adequately known, largely due to a lack of field studies necessary to establish the extent of variation within the species and that there may be greater morphological diversity than is currently recognised. The latter comment probably also applies to the southern African members of the genus, as confirmed by the anomalous results obtained in this material.

Spore and sporangium size

Minimum and maximum dimensions of spores and sporangia are presented in Table 1 and sample mean values and standard deviations are presented in Table 2. All measurements were statistically analysed by the Student's two sample t test (Parker 1979) and the differences were found to be statistically significant.

In the Cape sample, 76% of the spores were found in a narrow range between $84 \mu\text{m}$ and $88.5 \mu\text{m}$ in length

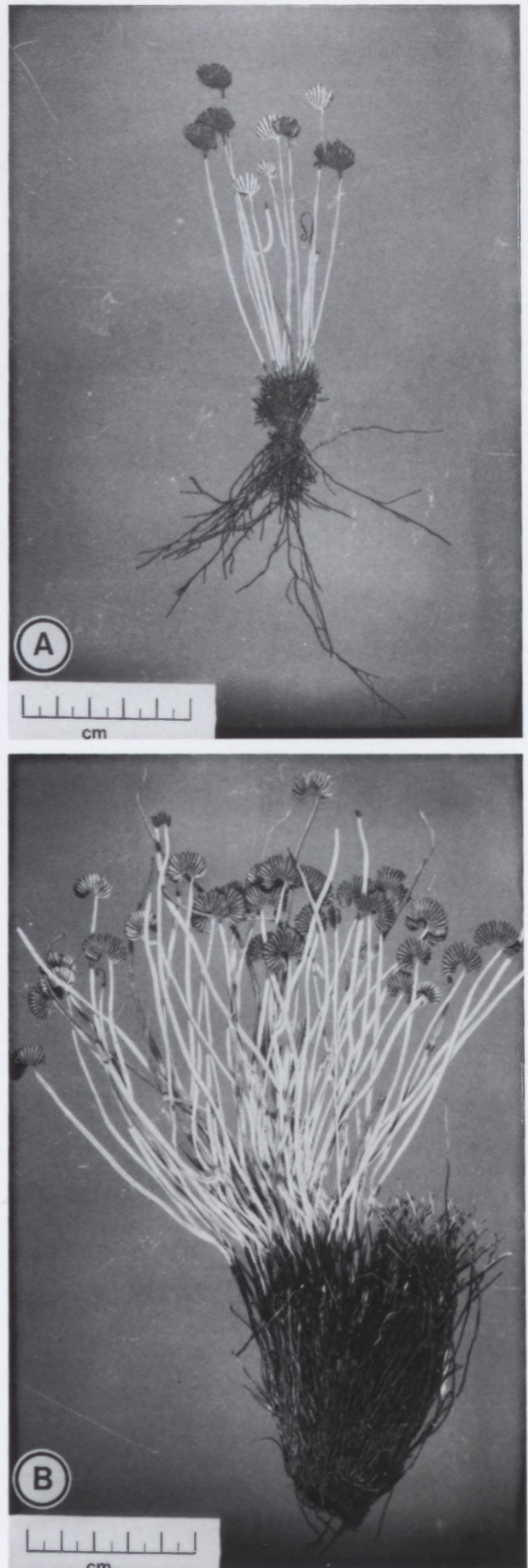


FIGURE 1.—Gross morphology of plants of *Schizaea pectinata*. A, J067274 from Ysterkroon in the Wolkberg, Transvaal; B, J067273 from the Cape of Good Hope Nature Reserve.

TABLE 1.—Spore and sporangial size in *S. pectinata* (µm)

	Spores				Sporangia			
	Min.		Max.		Min.		Max.	
	length	width	length	width	length	width	length	width
Cape	79	48.0	93.4	64.8	534	186	674	488
Transvaal	98	55.2	132.0	79.2	628	302	790	534

while 80% of the Transvaal spores lay in a range between 105.5 µm and 115 µm.

A narrow range of variation in width was also found in the Cape spores where 72% measured between 55.2 µm and 60 µm and 66% of the spores from the Transvaal population measured between 60 µm and 67.2 µm. When individual measurements of spore length and width were plotted, the scattergram (Figure 2A) showed that there was no overlap between the two populations with respect to spore size.

A similar trend was seen in dimensions of sporangia from the Cape sample where 84% of the sporangia lay within a relatively narrow range between 580 µm and 650 µm in length, and in the Transvaal sample 76% of the sporangia were found in the range between 698 µm and 744 µm.

Sporangial width showed a greater degree of variation than sporangial length with 60% of the sporangia from the Cape measuring between 372 µm and 418 µm and 78% of the sporangia from the Transvaal sample ranged from 442 µm to 512 µm wide.

When length and width of individual sporangia were plotted (Figure 2B) the scattergram revealed only a minimal amount of overlap between the two populations.

The systematic potential of spores with respect to size and spore morphology has long been recognised (Wood 1973). Differences in spore size in the two *Schizaea* samples could indicate a difference in ploidy between the two populations. Tryon & Lugardon (1991) found spores of diploid *Polystichum* Roth species to have smaller spores than tetraploids but they also found that spores from disjunct populations of *Thelypteris palustris* showed significant variation in size. It will be necessary to investigate the ploidy of the two populations and also to sample populations from the entire distribution range before the full significance of the present findings may be assessed.

TABLE 2.—Statistical analysis of spore and sporangial size in *S. pectinata* (µm). For pairs of data within columns $p < 0.01\%$ by two sample t test

	Spores		Sporangia	
	length	width	length	width
Cape	87.2 ± 3.1	58.1 ± 3.7	616.1 ± 35.5	366.9 ± 61.7
Transvaal	110.1 ± 5.7	65.5 ± 6.1	714.4 ± 31.5	461.4 ± 46.2

Outer exospore sculpturing

The exospore in *S. pectinata* consists of two layers (Parkinson 1991). The inner exospore is a smooth layer approximately 85 nm wide. The outer exospore which forms the bulk of the exospore is sculptured and reaches a maximum thickness of between 4 µm and 5 µm. The sculpturing differs in the Transvaal and Cape material (Figures 3 & 4). Spores from the Transvaal site (Figures 3A, C; 4A) have indentations (punctae) which are approximately 1 µm in diameter with ridges of the same dimension separating them. The ridges have an uneven but continuous surface and the inner surface of the punctae is smooth. Spores from the Cape site (Figures 3B, D; 4B) are also punctate. The punctae however, are 1.5 µm to 2.0 µm in diameter with uneven, 1 µm to 2 µm wide ridges separating them. The whole surface is uneven, consisting of irregular, juxtaposed granules.

The differences in exospore structure could be the result of the differences in the habitats of the two populations sampled. It has been suggested that the surface complexity of spores of *Pyrrhosia* Mirb. and *Asplenium* L. may

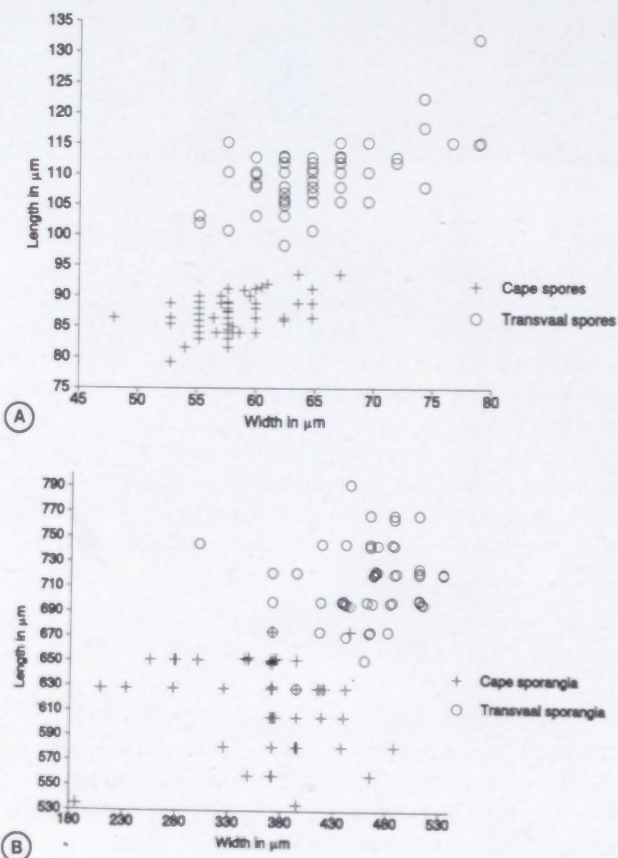


FIGURE 2.—Scattergrams derived from measurements of *S. pectinata*. A, spore size; B, sporangial size.

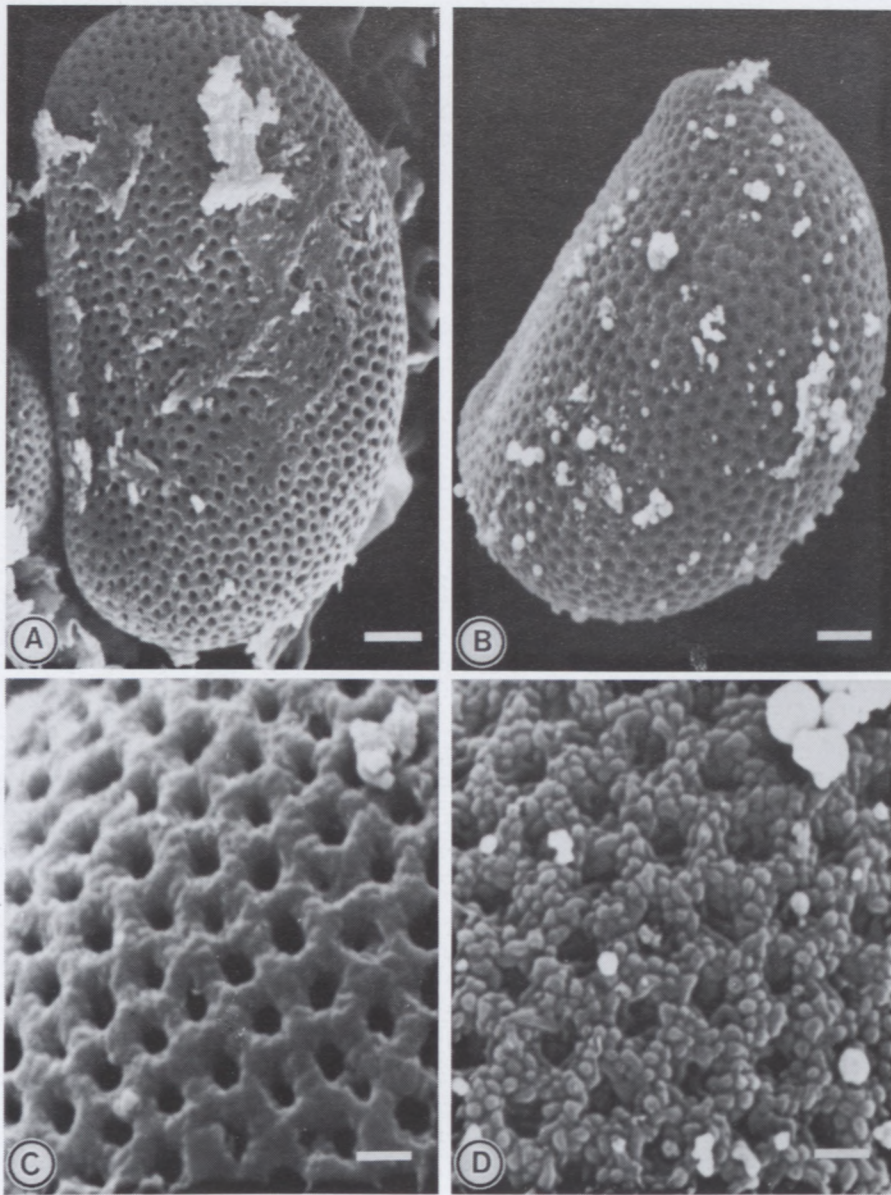


FIGURE 3.—Comparison of spore size and features of outer exospore sculpturing in *S. pectinata*. A, C, spore surface of Transvaal material; some of surface debris is a preparation artefact. B, D, spore surface of Cape material; surface debris consists of spherical particles which may be associated with early development of inner perispore. Scale bars: A, B, 10 μ m; C, D, 2 μ m.

be related to ecological specialisations, i.e. whether the plants are epiphytic, lithophytic etc. (Tryon 1990). Plants of *S. pectinata* at both sites were terrestrial. Correlations between spore morphology and overall morphology in the Thelypteridaceae have been shown to follow the taxonomic groupings of other authors based on purely morphological grounds (Wood 1973). However, since there have been few studies on the ultrastructure of the exospore in this genus or in the ferns as a whole, it is difficult to assess the taxonomic significance of the variation in exospore structure in *S. pectinata* without further study.

Formation of the inner perispore

In the spores from both the Cape and the Transvaal, the inner perispore consisted of a darkly staining, discontinuous and heterogeneous layer which was laid down on the ornamented surface of the outer exospore. In the material from the Cape (Figures 4B; 5A) the deposition of the inner perispore was associated with dense, heterogeneous spherical bodies. In the spores from the Transvaal at the same stage of development (Figure 4A)

coiled structures were found in close proximity to the developing inner perispore. There is some evidence (Parkinson 1991) that these bodies are derived, at least in part, from the plasmodial tapetum which disappears at about this time. They are thought to contain some sporopollenin as the perispore has been shown to be acetolysis resistant (Parkinson 1991). These structures also differ structurally and functionally from the spheroids in *Psilotum nudum* (L.) P. Beauv. (Parkinson 1988) and spherical structures in *S. pectinata* (Figure 5B) termed composite bodies (Parkinson 1990) which are present during the formation of the outer perispore. I suggest that the coiled and dense bodies are vehicles for the transport of materials, particularly sporopollenin or its precursors, from the plasmodial tapetum to the developing inner perispore.

Outer perispore

The junction between the inner and outer perispore layers was clearly demarcated in section, by an electron lucent area. The inner perispore was a narrow, darkly staining, heterogeneous layer, whereas the outer perispore

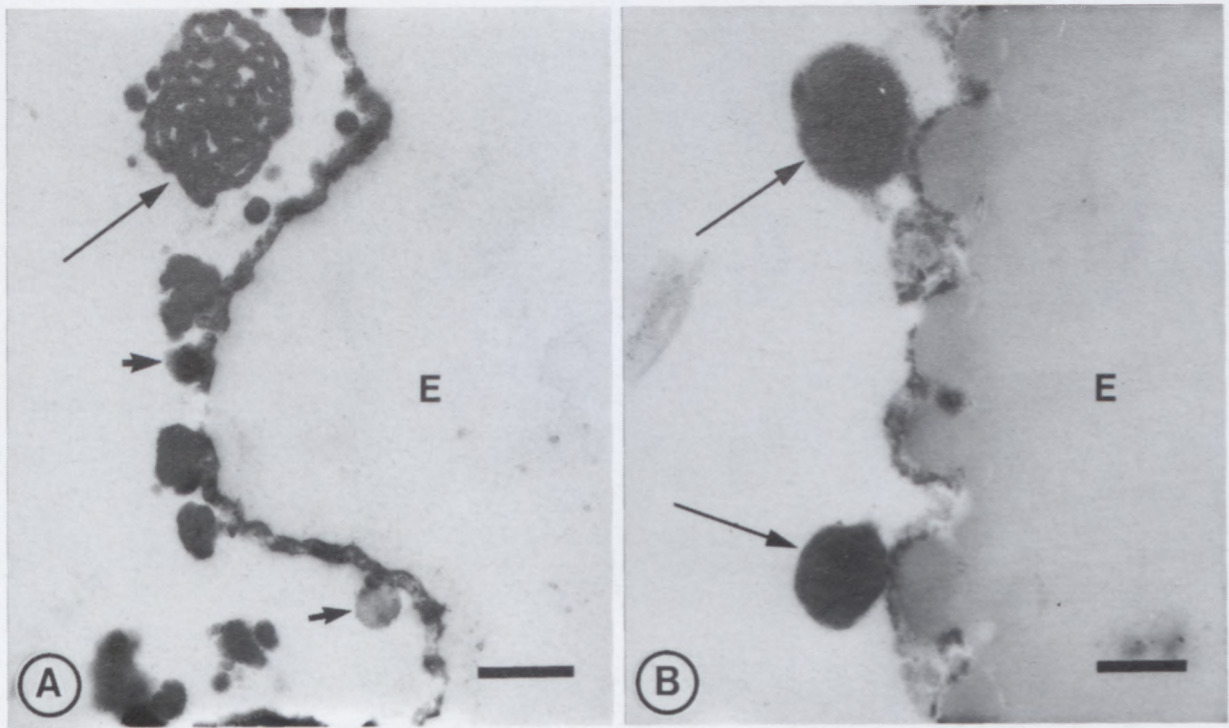


FIGURE 4.—Structures associated with the formation of inner perispore in *S. pectinata*. A, section through part of spore wall of Transvaal material. Sculptured outer exospore (E) with deep indentations, covered by narrow, heterogeneous layer of greater electron density, the inner perispore. Large bodies (arrowed) have convoluted or coiled appearance. Smaller globules (arrow-heads) found attached to or close to developing spore wall. B, section through part of spore wall of Cape material. Sculpturing of outer exospore (E) with shallowly rounded projections covered by narrow, heterogeneous layer of greater electron density, the developing inner perispore. Spherical bodies (arrowed) dense, but show some substructure or heterogeneity and are attached to developing spore wall. Scale bars: A, B, 0.5 μm .

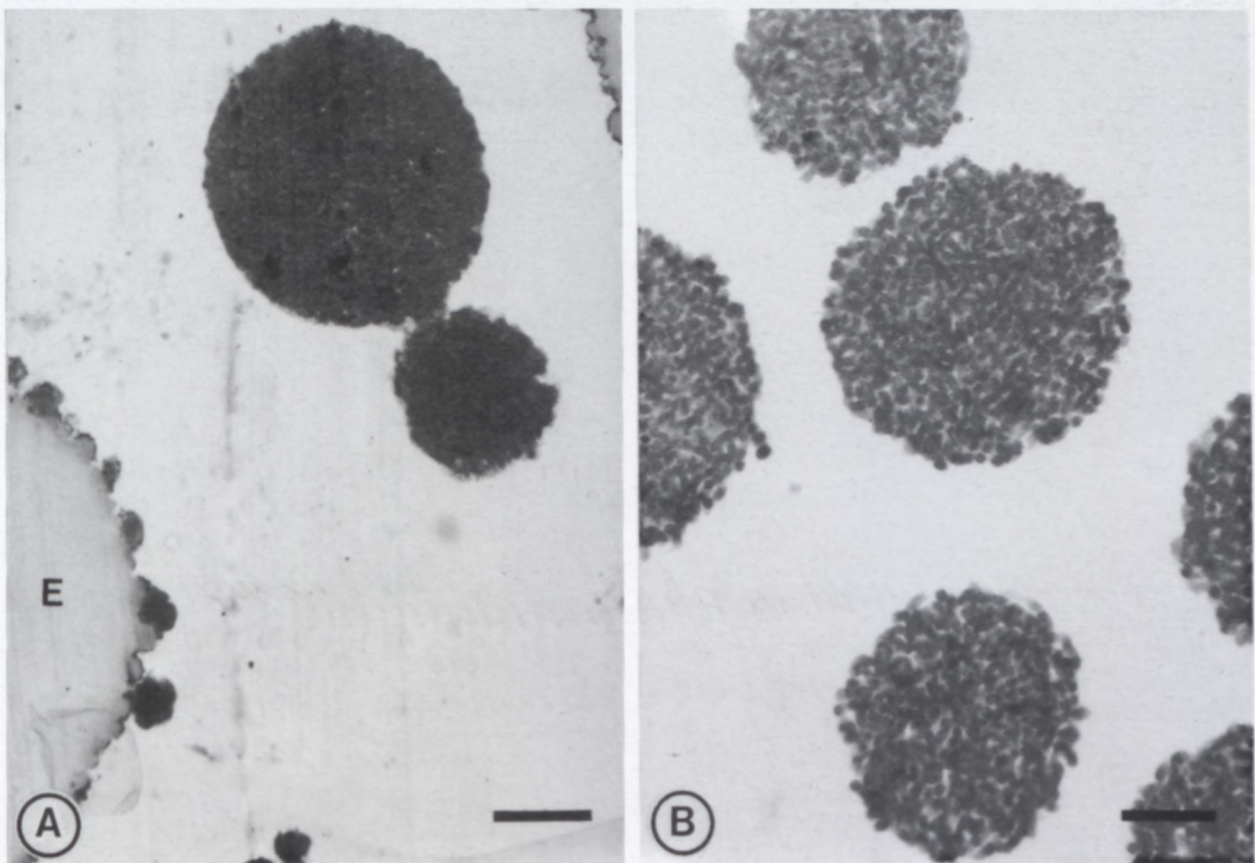


FIGURE 5.—A, rounded protrusions of outer exospore (E) of spore from Cape material during development of inner perispore. Note large size of dense bodies in sporangial loculus associated with this development. B, composite bodies from sporangial loculus from Transvaal material occur during final stages of spore wall development when outer perispore is being deposited. Similar structures also occur in the Cape material. Scale bars: A, 2 μm ; B, 0.5 μm .

was not so darkly stained and often had a striated appearance in section (Parkinson 1991). Composite bodies were associated with its development in both the Cape and the Transvaal material (Figure 5B). The outer perispore and the composite bodies were shown to contain silicon (Parkinson 1990) and phenolics (Parkinson 1992). The outer perispore was frequently displaced from the underlying layers during handling of the material. There were no observable differences between the outer perispore of spores from the Transvaal and the Cape.

The structure of the perispore in ferns and fern allies has been discussed by Hennipman (1970), Lugardon (1974), Schraudolf (1984) and Uehara & Kurita (1989a & b) but the ontogeny of the perispore layer is poorly known. The dense bodies and the coiled structures reported here are not the globules scattered on the spore surface in species with a thin perispore (Lugardon 1981) and which were regarded as the counterpart of orbicules or Ubisch bodies in pollen. Fern spores used to be described as perinous or non-perinous but work at the electron microscope level (Lugardon 1971, 1974) has shown that whereas the perine/perispore may be difficult to detect with light microscopy, genera which were originally described as being without a perispore have now been shown to possess this layer. Tryon & Tryon (1982) called for a re-examination of distinctions between

genera based on the presence or absence of a perispore and characteristics of its formation and final structure, as it may be taxonomically important.

Tapetal organisation

Tapetal organisation has been well studied in the angiosperms and Goebel (1905) recognised two types of tapeta, secretory and plasmodial, between which transition forms occur (Foster & Gifford 1959). The range of tapetal types and structural variations in the Embryophyta is large (Pacini, Franchi & Hesse 1985) but there have been few studies dealing with tapetal ontogeny and structure in the lower vascular plants.

A detailed, ontogenetic study of the tapetum has been carried out on *S. pectinata* from the Cape (Parkinson 1991) and this work will form the basis of a separate publication (in preparation). Essentially, what has been determined is that a central, tetrahedral, archesporial initial cell is responsible for the formation of a tapetal initial layer. These cells then divide periclinally initiating the formation of an inner and an outer tapetal layer. The inner layer differentiates into a periplasmodial tapetum associated with the developing archesporial tissue and the outer layer differentiates into a cellular, parietal tapetal layer (Parkinson 1991). A complete ontogenetic study of

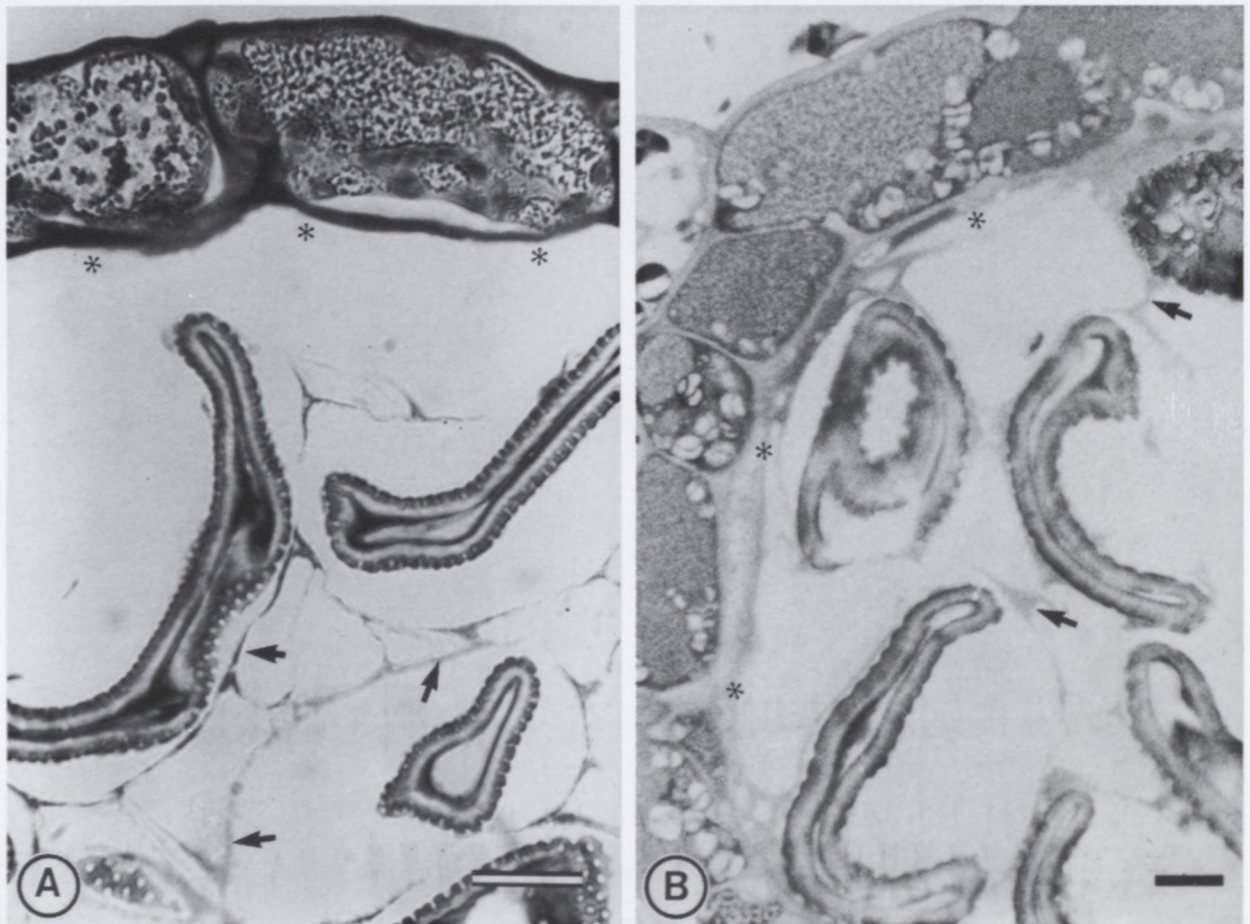


FIGURE 6.—Sections through sporangia: A, showing sculptured nature of outer exospore in *S. pectinata* from Transvaal material. Spores associated with last remnants of periplasmodial tapetum (arrow-heads). No evidence of cellular, parietal tapetum which would be situated at position marked by asterisks (*) adjacent to cells of sporangium wall. B, from Cape material. Sculptured outer exospore has developed and spores associated with periplasmodial tapetum (arrow-heads). Attenuated, cellular, parietal, tapetal layer (*) adjacent to cells of sporangium wall. Scale bars: A, B, 10 μ m.

the tapetum from material from the Transvaal has not been completed. In sections through sporangia from the Transvaal material, at the stage when the outer exospore was fully developed but perispore development was not yet complete (Figure 6A), only a periplasmodial tapetum was present. In sections of the Cape material at the same stage of development (Figure 6B) both a cellular, parietal tapetal layer and a periplasmodial tapetum were present.

The presence of a cellular, parietal tapetum and a plasmodial tapetum existing concurrently in *S. pectinata* from the Cape is similar in some respects to the condition previously reported in *Psilotum nudum* (Parkinson 1987). The condition also exists in other members of the Schizaeaceae, namely *Anemia phyllitidis* (L.) Sw. (Schraudolf 1984) and *Lygodium* Sw. (Binford 1907), as I have deduced from illustrations in these papers. It almost certainly exists in *Schizaea tenella* Kaulf. (Parkinson 1991).

There are examples in the literature where a parietal tapetum breaks down and becomes invasive during later stages of pollen development as in *Pinus banksiana* Lamb. (Dickinson & Bell 1972, 1976) and *Beta vulgaris* L. (Hoefert 1971). There are also descriptions of an invasive but non-syncytial type of plasmodium in *Canna* L. (Tiwari & Gunning 1986a, b) which is regarded by these authors as being intermediate between the secretory and plasmodial forms. The tapetal condition in *S. pectinata* from the Cape is interpreted as being dimorphic (Parkinson 1991) and the present author considers it to be a more truly transitional form between the secretory and plasmodial condition than the one described in *Canna*.

The significance of the differences noted in the condition existing in the material from the Cape and from the Transvaal, at the stage when outer exospore development has been completed, lies in the fact that in studies carried out on the angiosperms, the timing of events in the tapetum at specific stages of microspore development is noted as reflecting family- or genus-specific differences. The change from one type to another within families or genera was interpreted as being a significant evolutionary trend (Pacini, Franchi & Hesse 1985).

CONCLUSIONS

The statistically significant differences in sporangium and spore size, the differences in the outer exospore ornamentation, the differences in the formation of the inner perispore and the variation in tapetal organisation indicate that what has always been considered to be a single species may constitute more than one element. *S. pectinata* and *S. tenella* are the only species currently recognised in southern Africa (Sim 1915; Schelpe 1970; Roux 1979; Jacobsen 1983; Schelpe & Anthony 1986; Burrows 1990).

Tapetal origin and form was a basis for suggesting phylogenetic relationships within members of the Embryophyta which have been studied more completely, particularly the angiosperms and gymnosperms (Pacini, Franchi & Hesse 1985). Tapetal organisation in the ferns and fern allies may supply information which could be applied to phylogenetic problems within these groups. It

will be interesting to see whether work by Roux (1992) on certain Schizaeaceae, particularly *Mohria*, will support the present findings based on ultrastructural details.

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