

Session b1

BRYOPHYTES AND PTERYDOPHYTES

Sporoderm pattern in some Indian liverworts: An SEM study

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Bryophytes in general and liverworts (Hepaticae) in particular possess a considerable range of sporoderm ornamentation, varying from minutely tuberculate to spinulate and lamellate to reticulate as well as mesh-like. These sporoderm patterns are specific for a particular taxon and can be satisfactorily used as diagnostic property. An SEM study carried out on spores from some members of the Calobryales, Jungermanniales, Metzgeriales and Marchantiales fairly demonstrated the densely vermiculate ridged exine in *Trichocolea tenera* Udar et Singh while it is verrucose to pseudoreticulate in *Schiffneria hyalina* Steph. Similarly *Calobryum indicum* Udar et Chandra and *Calobryum dentatum* Kumar et Udar are distinctive in possessing dense round headed papillae in the former and a folded exine with minute tubercles in the latter. *Fossombronia cristula* Aust., *F. pusilla* (L.) Dum. and *Petalophyllum indicum* Kash. exhibited distinctive membranous reticulations, high lamellae and striated reticulations respectively. Sporoderm architecture is easily distinguishable in *Conocephalum conicum* (L.) Lindb. by an intermixed micro- and megagranulate pattern; a conspicuously lamellate (winged) pattern is present in *Cryptomitrium himalayaense* Kash., a reticulate (rather punctate) one in *Asterella mussooriensis* (Kash.) Kachroo; the exine is thin and minutely mesh-like in *A. reticulata* (Kash.) Kachroo, while intermixed warty and minute projections occur in *Sauteria spongiosa* (Kash.) Hatt., and a reticulate pattern in *Athalamia pinguis* Falc. A detailed micromorphological account of the sporoderm patterns investigated with SEM is provided.

The morphology and ultrastructure of end-permian lycopsid spore tetrads

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In widely separated Permian-Triassic transitional sequences, palynological assemblages are characterized by large amounts of unseparated tetrads of lycopsid microspores, and occasional megaspore tetrads. These spores are assignable to the form-genera *Densoisporites*, *Lundbladispore*, *Uvaesporites* and *Otynisporites*. Spore tetrads from Italy and Greenland were subjected to morphological and ultrastructural analyses. The results were compared with data from extant and fossil relatives and evaluated in the context of the process of sporogenesis in extant relatives. TEM-analysis of preserved sporoderm ultrastructure demonstrates that individual spores are morphologically completely mature. The spores are connected to each other by interlocking of the para-exospores at either the interradial contact areas or equatorial regions. This connection is likely the result of a reduction of the rate or duration of polysaccharide spore coat deposition by the gametophyte membrane. The sporoderm of *Densoisporites* and *Lundbladispore* consists of two walls, a homogeneous bi-layered exospore, a thick spongy para-exospore, proximal apertural thinnings and multilaminated zones. This wall organization is characteristic for microspores of extant and extinct Isoetales genera *Pleuromeia* and *Isoetes*. The ultrastructure of the latter microspores and *Otynisporites*, confirms an isoetalean (Pleuromeiaceae) affinity.

The wall structure of *Uvaesporites* is far more complex than so far recognized in extant and extinct Selaginellales and Isoetales. It might represent a distinct Paleozoic rhizomorphic lycopsid lineage, with an intermediate position between rhizomorphic lycopsids and the Selaginellales.

Morphology and ultrastructure of lycophyte spores from the permian deposits of the Ural mountains (Russia)

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The fossil spores studied came from the Polazna locality of the Ufimian (Roadian) age, situated near Perm city, Russia. The dispersed spores were extracted from a clayey matrix containing also stems and phylloids of the lepidophyte *Viatcheslavia vorcutensis* Zalessky, a characteristic lepidophyte of the Solikamskian horizon (Ufimian stage) of the Ural Mountains and Russian Platform. Although the palynological assemblage contains rare pollen grains of *Vitatina* and *Cladaitina* and spores of *Calamospora*, it is overwhelmingly dominated by the specimens of *Densoisporites*. Analogously, apart from *Viatcheslavia*, only a few members of the Peltaspermales, *Rufloria*, and Sphenophytes occur as plant macrofossils. We believe the most abundant sporotype to be correlated with the predominant type of plant macrofossils and assigned to one parent plant, precisising the systematic position of the dispersed spores. This assumption is corroborated by the morphological characteristics of the fossil spores and stems proving their relation to the same lepidophyte family Pleuromeiaceae. The spores were studied with LM, SEM, and TEM. They are trilete, round to subtriangular, with a distinct tetrad scar, nearly reaching the equator. With LM, the sporoderm appears punctate and two-layered. With SEM, the proximal surface is occasionally granulate, with an elevated tetrad scar. The distal surface bears numerous granules varying in size and shape. Rare perforations are present both proximally and distally. TEM confirms the two-layered structure of the sporoderm. The outer layer consists of numerous lamellae, interlacing in a network. The repeatedly thickened and thinned and perforated structure of outermost of these lamellae corresponds to the granulate and occasionally pitted morphology of the spore surface seen with SEM. The inner layer is a single lamella, splitting at a limited distance near the proximal pole into "laminated zones" (terminology according to Lugardon et al. 1999). A narrow cavity (supporting the cavate nature of the sporotype described) exists in the bottom of the outer sporoderm layer, better developed distally but also presenting proximally. The comparison with the available data on the spore morphology of the Pleuromeiaceae (Yaroshenko, 1975, 1985, 1988; Lugardon et al., 1999; Grauvogel-Stamm and Lugardon, 2001) shows the significant common features: two-layered sporoderm, presence of cavity, the comparable ultrastructure of the upper sporoderm layer and the presence of "laminated zones". The differences concern a more distinct sculpture, only rare surficial perforations, and a relatively weak development of the cavity.

GRAUVOGEL-STAMM, L. AND LUGARDON, B. 2001. The Triassic lycopsids *Pleuromeia* and *Annalepis*: relationships, evolution and origin. *Amer. Fern. J.*, 91: 115-149.LUGARDON, B., GRAUVOGEL-STAMM, L., AND DOBRUSKINA, I. 1999. The microspores of *Pleuromeia rossica* Neuburg (Lycopsida; Triassic): comparative ultrastructure and phylogenetic implications. *C.R. Acad. Sci. Paris, Sciences de la terre et des planetes*, 1999, 329, 435-44.YAROSHENKO, O.P., 1975. Morphology of spores of *Pleuromeia rossica* and *Densoisporites neburgii*. *Paleontol. Zh.* 3: 101-106.YAROSHENKO, O.P., 1985. Cavate spores from the Lower Triassic and their relation to Lycophytes. *Paleontol. Zh.* 1: 113-119 (in Russian).YAROSHENKO, O.P., 1988. Microspores from sporangium of the lycophyte *Tomostrobus radiatus* Neuburg (Lower Triassic, Kuznetsk Basin). *Palynology in the USSR. Novosibirsk: Nauka*, P. 77-79 (in Russian).

Microspore wall organization and ultrastructure in two species of *Selaginella* (Lycophyta) producing permanent tetrads

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The aim of this study is to bring out the features of the permanent microspore tetrads of *Selaginella convoluta* (Walk. Arn.) Spring and *S. lepidophylla* (Hook. & Grev.) Spring.

The sporoderm of *Selaginella* microspores generally has a compact exospore consisting of two layers that form a protruding apertural fold of which both sides are more or less fused together. This exospore includes a system of perforations consisting of either three tangentially cleft small areas arranged between the rays of the trilete aperture, or scattered radial channels. In many species, the elements of the ornamentation are formed by the exospore outer layer and, in most of these species, a perispore consisting of material clearly distinct from the exospore sporopollenin composes a surface layer that is usually stuck to, and much thinner than, the exospore. In other, less numerous species, the exospore is covered with a wide, more or less thick and complex envelope that composes the ornamentation. This envelope is generally connected to the exospore only between the apertural and equatorial regions, and forms in the apertural area two lips that extend beyond or merge with the apertural fold of the exospore. It consists of sporopollenin similar to that of the exospore outer layer (and has developed at the same time as this layer), which has led to name it "para-exospore". Usually the microspores of *Selaginella* are released individually, while in a number of species, they may remain grouped in tetrads thanks to different sorts of connections between exospores or para-exospores.

In *S. lepidophylla* tetrads, the four microspores are assembled within a common tetrad envelope, but they appear separate from one another and from the tetrad envelope. Their respective exospore shows the usual bilayered structure, with some particularities lying in the outer layer that: (i) includes, in its inner part, very abundant microcavities with opaque contents; (ii) has usually a nearly uniform thickness, but shows strongly reduced or expanded areas seeming to result from small ontogenetical disruptions; (iii) is appreciably thicker on both sides of the apertural fold than in other wall regions. Radial channels traversing completely the exospore are rather abundant in the apertural area, rarer elsewhere. The tetrad envelope appears as a wide common coat which, depending on the regions, consists of a simple layer or forms a more or less intricate network. This envelope is made up of the same sporopollenin as the exospore outer layer and shows, in its median zone, microcavities with opaque contents quite similar to those of that exospore layer.

As shown in the initial LM and SEM study of *S. convoluta* (1) microspore tetrads, the four members of each tetrad are linked together through a common envelope forming an unbroken complex system of alveoli that connects the equatorial and distal regions of the microspores. In TEM sections, the proximal exospore shows a light inner layer with irregular thinnings or even interruptions, and a darker outer layer forming dense, multiform small surface projections. Radial channels traverse the whole exospore at the level of the thinnest parts of the inner layer. Discontinuities are visible in the proximal exospore of the investigated microspores, but not any differentiation comparable to an apertural fold was observed. In distal and equatorial areas, the exospore is closely joined with the alveolate tetrad envelope through an intermediate zone including small, often radially elongated cavities more or less filled with opaque material. The exospore outer layer, intermediate zone and alveolate envelope are composed of the same sporopollenin, and there is no trace of splitting between them.

These two different types of tetrads emphasize the outstanding structural diversity of the microspore sporoderm in living *Selaginellaceae*, thus stressing the relevance of further ultrastructural and ontogenetical studies on the spores of this complex group of lycopsids.

(1) MORBELLI, M.A., ROWLEY, J.R. & CLAUGHER, D. 2001. Spore wall structure in *Selaginella* (Lycophyta) species growing in Argentina. *Bol. Soc. Argent. Bot.* 36: 315-368.

New insights on an overlooked feature: the perispore of fossil and extant pteridophytes

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Ever since Russow coined the term *perispore* in 1872, scientists have studied this sporoderm layer in great detail, dedicating their time to many different aspects such as definition, development, function, evolution and chemical composition. However, to the present no clear overall picture of this structure has been drawn. First, a very brief review on the occurrence of the perispore in fossil spores is given, ranging from the Silurian to the Tertiary. Second, the main emphasis will be on the chemical composition of the perispore in extant pteridophytes. Mass-spectrometry, usually used to characterise bio-macromolecules or bio-polymers, has been applied to elucidate the chemical composition of the perispore. Additionally, it is shown that silica is a major spore wall component, which has so far been underestimated in *Selaginella* and probably also is an important key in perispore structure in pteridophytes. The role of silica and sporopollenin in spore walls is discussed on the basis of novel results with hydrofluoric acid treatment.

Do micro and megasporangia produce the same amount of sporopollenin?

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For many heterosporous pteridophytes, the mechanisms that govern the differentiation of microsporangia and megasporangia are uncertain, although there is good evidence in that nutrient supply is an important factor. Studies of model systems seems to suggest that the mechanism of spore wall development is similar in these two types of sporangia even though they produce apparently different structures. So, apart from the size of the spores they contain, how different are micro and megasporangia? If there is little real difference in sporangia, might they both produce the same volume of sporopollenin? This hypothesis is investigated using TEM sections of spores from species of *Selaginella* and *Platzzoma*.

Perispore ornamentation of *Cyathea* (Cyatheaceae) and *Dicksonia* (Dicksoniaceae) from high altitudes in New Guinea

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The island of New Guinea is a major centre for diversity of the tree fern genera *Cyathea* s.l. and *Dicksonia*. The present study of the high altitude species in New Guinea arose from problems associated with the identification of collections made by the author during five expeditions to the Mount Jaya region of Papua. Over 100 collections of *Cyathea* were made from that area, many at elevations between 2600 and 4200 m. A detailed study of the spores of the tree fern species of other mountain areas in New Guinea was conducted to assist in their identification.

The sori of several high altitude species of *Cyathea* show a distinct arrangement of the sporangia in the sorus. Perhaps uniquely the sporangia are arranged so that their stalks form a tightly closed surface to the sorus, possibly providing protection during the development of the spores. The sporangia become unrolled to release the mature spores.

Dicksonia is represented by seven species in the Malesia region (HOLTUM 1963), of which five species occur in New Guinea, four endemic. This genus is easily recognised in the field by the densely hairy

nature of the stipe, in contrast to *Cyathea* where abundant scales occur. There are considerable taxonomic problems at the species level in *Dicksonia*. These are briefly described and the possible significance of perispore ornamentation interpretation is discussed.

Cyathea has been treated either as a large genus of some 720 species (HOLTUM 1963), or it can be divided into a series of smaller genera for example STEIN et al (1997). Over 130 species of *Cyathea* s.l. occur in New Guinea of which some 42 species occur at high altitudes. These represent several of the smaller genera (CONANT et al 1995).

Several questions were addressed in the present study of *Cyathea*:

- 1.- Does the perispore ornamentation support the recognition of several genera or a single genus?
- 2.- Is the perispore ornamentation consistent at the species level or do species show a marked variation between populations?
- 3.- Does the variation in perispore ornamentation provide valid support for species delimitation in *Cyathea*?
- 4.- Does the data support the patterns of regional evolution of *Cyathea* along the mountain chain of New Guinea or are the species widespread throughout the island?

CONANT, D.S., Raubenson, L.A., Attwood, D.K. & Stein, D.B. (1995). The Relationships of Papuan Cyatheaceae to New World Tree Ferns. *Amer. Fern Journ.* 85: 328-340.

HOLTUM, R.E. 1963. Cyatheaceae. *Flora Malesiana* ser. II, 1: 65-176.

STEIN, D.B., CONANT, D.S. & VALINSKA, A.E.C. (1997). The implications of chloroplast DNA restriction site variation on the classification and phylogeny of Cyatheaceae. In Johns, R.J. (ed). *Holtum Memorial Vol. pp. 235-254*, Royal Botanic Gardens, Kew.

Phylogeny of Ophioglossales inferred from spore morphology and *rbcl* sequences

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Ophioglossales which is a rather primitive group in the Pteridophyta is difficult to classify because of phenomena such as plant morphology, hemicryptophytes prothallium, etc. Recently, the taxonomical treatment of the Ophioglossales was based on molecular data.

In our latest observations of the spore morphology with SEM, we concluded that the existing genera and the new genus *Mankya* were treated according to a new key, inferred from the immature and mature surfaces and general shape and ultrastructure of the spores. The Ophioglossales were divided into two families, such as Ophioglossaceae s. str. and Botrychiaceae. Then the former classified into three genera (*Ophioglossum*, *Ophioderma*, *Cheiroglossa*), and the latter into five ones (*Botrychium*, *Botrypus*, *Sceptridium*, *Japanobotrychium*, *Helminthostachys*). However, the new genus *Mankya* was included into *Helminthostachys* at least from the SEM observations.

From our TEM observations, we found two layers in the perispore (P_1 , P_2) in *Sceptridium ternatum*, but only one layer (P_1) was recognized in *Ophioglossum thermale*. Furthermore, at the tetrad stages lamellate structures were recognized on the proximal face in *Sceptridium ternatum*, while they occur all over the surface of the spore in *Ophioglossum thermale*.

We also determined 1,194bp nucleotide sequences of *rbcl* gene for 37 plant samples of Ophioglossales. According to the obtained molecular tree, two clades were recognized. One clade consisted of the members of the Ophioglossaceae. The other consisted of those of the Botrychiaceae and *Mankya* which was recently described from Cheju Island, Korea. It was clearly shown that *Mankya* is genetically well differentiated from the other genera of Botrychiaceae, and its status as a new genus was supported by the molecular data. In the former clade, *Ophioderma* was distantly related to *Ophioglossum* sensu stricto and its status as independent genus was also supported.

Combining evidence from fossils and extant taxa to reconstruct the evolution of heterospory in ferns

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Throughout the evolutionary history of land plants there have been repeated re-invasions of aquatic environments by terrestrial plants from distantly related groups. In order to survive these very different physical conditions, these plants have had to dramatically modify their vegetative, reproductive, and dispersal systems. Few living ferns are aquatic, most are terrestrial and homosporous. The exceptions are heterosporous ferns, with morphologically distinct spores producing unisexual gametophytes.

Heterosporous ferns are monophyletic and comprise two extant families, the semi-aquatic Marsileaceae and the aquatic Salvinaceae. Except for very recent interest in the relationships among the five extant heterosporous genera, these ferns have been largely ignored in systematic studies and infrageneric relationships are virtually unknown. Heterosporous ferns are relative newcomers that diversified during the Cretaceous, at the same time as flowering plants. The fossil record has preserved a rich history of these ferns, mostly in the form of microfossils with a remarkable diversity of ornamented spores.

The primary goal of our collaborative research study is to reconstruct a comprehensive phylogeny for the heterosporous aquatic ferns (Marsileaceae and Salvinaceae), including living and fossil members, incorporating data from multiple genes, morphology, and developmental studies for the living taxa, and integrating morphological data from fossil taxa. We will present preliminary results from this research.

Understanding the history of character evolution in these ferns requires integration of fossil taxa, necessarily using morphology pertinent to the spores. Our study incorporates spore ultrastructural characters observed with light and electron microscopy and investigation of spore developmental characters. Understanding the development and structure of the spore wall, primarily through electron microscopy, is critical to this project because the character-rich spore wall is well preserved in fossil taxa and will permit fossils to be integrated into a comprehensive phylogeny.

Our emphasis is to investigate the morphological character state transitions associated with the ecological specialization of these ferns to aquatic habitats. Our phylogenetic hypothesis will be used to build a classification for all living and extinct heterosporous ferns, and as an explicit framework to investigate morphological character evolution. Numerous morphological trends have been proposed on the basis of the relative order of appearance in the fossil record. A well-resolved phylogeny will minimize sampling issues inherent in the fossil record and allow the evaluation of previous hypotheses about evolution in these ferns.

Developmental and functional aspects of heterospory inferred in extant heterosporous ferns using electron-microscopy and light-microscopy

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Heterospory evolved independently in several lineages of vascular plants such as seed plants, heterosporous lycophytes, and heterosporous ferns. Despite the phylogenetic independence, heterosporous plant lineages share various developmental and structural similarities that might be caused by similar developmental and functional constraints. This hypothesis suggests that observation of extant taxa can help to reconstruct the evolution of similar structures in extinct taxa. Under this assumption, heterosporous ferns are of particular interest because they are the only extant lineage showing extreme heterospory. The megasporangia develop only a

single megaspore but these spores are not enclosed in integuments. In this presentation we will focus on two critical aspects. First, functional aspects of the spore wall structure will be discussed with special reference to reproductive biology of the five extant genera of these ferns. Second, the development of mega- and microspores are compared in detail for these five genera: *Azolla*, *Marsilea*, *Pilularia*, *Regnellidium*, and *Salvinia*.

In this study we integrate observation of scanning electron microscopy (SEM), transmission electron microscopy (TEM), and light-microscopy (LM). By combining the observations obtained by SEM and TEM with the results of light-microscopic observations, in particular epifluorescence illumination, we are able to achieve an improved understanding of the development and structure of spores. Electron microscopic methods require in general a more or less complete dehydration of the studied material, whereas light microscopic methods allow observations of the hydrated material. Light-microscopy is today much more powerful than 20 years ago, because recent advances in image manipulation including computer controlled focus drives improved the quality of the obtained observations. This is critical if studied structures are strongly modified during the dehydration procedure.

The outer spore wall, perine, is strongly modified in the five extant genera. The three genera of the family Marsileaceae, the genera *Marsilea*, *Pilularia*, and *Regnellidium*, possess a gelatinous outer perine which is a critical adaptation to the reproduction of a free-sporing amphibious plant. The two genera of Salvinaceae, *Azolla* and *Salvinia*, have very different spore wall structures in comparison to Marsileaceae as well as in comparison to each other. Their spore walls are adaptation of free-sporing plants with a floating habit.

Very little is known about the development of the megaspores in heterosporous ferns despite earlier studies that provided critical data. We can now show that the microsporangia and megasporangia of heterosporous ferns follow a similar early developmental pathway, but after the meiosis, the developmental pathway of the megasporangium diverges significantly, culminating in the abortion of all but one megaspore tetrad. A putative mechanism of the tetrad selection is proposed which is found in all extant heterosporous ferns. The observation of the extant heterosporous ferns are compared with hypothetical explanations of similar processes in extinct ancestors of the seed plants

Session b2.1

POLLEN MORPHOLOGY AND PLANT SYSTEMATICS

Evolution of *Amorphophallus* pollen (Araceae)

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Two main Araceae pollen types exist: 1. aperturate and 2. inaperturate. The aperturate type is plesiomorphic in the family. Pollen of *Amorphophallus* (c. 200 spp.) belongs to the inaperturate type. Its exine ultrastructure (presence, size, shape and distribution of dark granules) and ornamentation are much diverse. Ten main ornamentation types are present: psilate, striate, striate with psilate caps, fossulate, areolate, verrucate, echinate, reticulate, scabrate and striate/scabrate. Two of the three echinate subtypes are much like the echinate pollen of the four closest outgroups used in molecular work on the genus (48 spp., 4 genes): *Arisaema*, *Filarum*, *Hapaline* and *Typhonium*. However, echinate *Amorphophallus* pollen is not basal in the molecular tree, which suggests that the outgroups are only distantly related to the ingroup. No pollen traits indicating the monophyly of *Amorphophallus* or any of the main clades in the molecular tree were found. The main clades are dominated either by psilate or striate pollen, which occur basally as well as distally. The less common types are found more distally and mostly in single species. This suggests that either psilate or striate ornamentation is plesiomorphic. Outgroup comparison does not allow a choice, but thin psilate exines occur more or less basally in three of the four main clades. The generally thicker striate type is ultrastructurally heterogeneous, and seems to have evolved several times. Within both striate main clades, reversals to the psilate type occur, which are mostly characterised by greater exine thickness, compared with the basal psilate type. Within the African clade, thick psilate exines seem

to have evolved directly from thin ones. Considering this, psilate pollen might be plesiomorphic in *Amorphophallus*. The rather common fossulate type is heterogeneous, and shows transitions to the psilate as well as the striate type. Also ultrastructurally, it proved to be diverse, and its diffuse distribution in the molecular tree confirms that it is polyphyletic. Striate pollen with psilate caps, which is nested in a striate clade (continental SE Asia), is a synapomorphy of *Pseudodracontium* (Indochina). In view of its typical exine ultrastructure, the psilate caps might have evolved in an *albispatus/longituberosus*-like ancestor. Reticulate (*A. ochroleucus*, Vietnam), scabrate (*A. galbra*, Queensland) and striate/scabrate ornamentation (*A. smithsonianus*, India) are considered as autapomorphies derived from psilate or striate forms. The other types, areolate, echinate and verrucate, occur in single species in the molecular tree, but have also representatives among the species not included in the analysis. Areolate pollen (6 spp., continental SE Asia) shows two alliances, one in a psilate main clade and another in a striate main clade. Echinate pollen (5 spp., Africa and Madagascar) is heterogeneous, showing three subtypes, one of which can be easily derived from a psilate type. Verrucate pollen (Africa: 5 spp. and India: 1 sp.) is clearly diverse. Derivation from psilate forms seems most likely. Several species appeared to be distinctly polymorphic: *A. abyssinicus* (verrucate, fossulate to coarsely striate), *A. beccarii* (psilate, striate-areolate) and *A. baumannii* (fossulate, coarsely striate, psilate, with or without distinct dark granules). These observations indicate that exine features are not always rigidly fixed, which might provide possibilities for rapid selection and evolution.

Pollen morphology in the Aristolochiaceae and its implications on systematics of the family

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Aristolochiaceae consist of 4 genera and ca. 500 spp. of temperate herbs and tropical or subtropical lianas, shrubs, and subshrubs. The family has received much recent attention because of its crucial placements a basal angiosperm, either as part of the order Piperales or as sister to the monocots. An overall study of the pollen morphology of ca 120 spp. representing all genera and infrageneric taxa was carried out by LM, SEM, and TEM techniques. Pollen in the Aristolochiaceae is more or less spherical, with a diameter ranging from about 20 µm in *Saruma* to 73 µm in *Aristolochia grandiflora*. *Saruma* pollen is semitectate, per-reticulate, monosulcate, with muri sometimes discontinuous, and lumina >2 µm in width with scattered free baculae. *Asarum* pollen is porate (less often inaperturate or colpate), verrucate or gemmate, microreticulate with continuous muri and small lumina. *Thottea* pollen is inaperturate, with circular to irregular, dissected exine subunits that are punctate or imperforate, and well-developed columellae. *Aristolochia* pollen is inaperturate and highly diverse: in subgen. *Siphisia* it is fossulate, the exine is densely punctate and strongly dissected; irregular warts and/or free clavae were found in some spp. In subgen. *Pararistolochia* it is fossulate and strongly dissected, the exine is punctate, forming nearly circular insulae and a long exine ridge, the infratectum is columellate, and free clavae are present. In subgen. *Aristolochia* two types of sculpture are found, which are more or less consistent to the two sections traditionally recognized: In sect. *Diplobolus* pollen is psilate or finely fossulate and has a complete or incomplete, scarcely punctate or imperforate tectum; the exine is thinner than in the remaining *Aristolochia* spp., the infratectum is columellate, and the nexine is thin. In the greatly diversified sect. *Gymnolobus*, exine is usually strongly dissected and fossulate; the exine units are massive, irregular, "cerebriform"; in *A. grandiflora* exine subunits are flake-shaped, punctate are missing or very scarce, and the infratectum is columellate; pollen of *A. pilosa* is tectate as in some species of section *Diplobolus*, but with small and large puncta. Exine subunits in *Aristolochia* subsect. *Pentandrae* are many tightly packed, flake-like, and densely punctate, with conspicuous supracteal warts. Exine in the South American monotypic *Euglypha* and *Holostylis* is strongly dissected in irregular subunits that are scarcely punctate, and a columellate infratectum is present.

Monosulcate pollen in *Saruma* has revealed a key character that connects the Aristolochiaceae with other basal angiosperms. Apertures are not strongly differentiated in the exine of *Asarum* and *Saruma*. Inaperturate pollen of the remaining genera appears to be omniaperturate as the intine and the exine are uniform (GONZÁLEZ ET AL. 2001). Orbicules are common in Aristolochiaceae. All these results were included in phylogenetic analyses by GONZÁLEZ & STEVENSON (2002) and KELLY & GONZÁLEZ (2003), which revealed that: (1) pollen characters support the uniqueness of *Saruma* (with semitectate, per-reticulate, monosulcate pollen) and the monophyly of *Asarum* (with apomorphic porate pollen with supracteal warts), *Thottea* (with apomorphic