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POLLEN WALL ULTRASTRUCTURE OF ARACEAE AND LEMNACEAE IN RELATION TO MOLECULAR CLASSIFICATIONS

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ABSTRACT

A comparative ultrastructural study of Araceae pollen walls was conducted for the first time. The results are based mainly on investigations by transmission electron microscopy of 101 species in 70 genera (out of a total of 105 genera), while pollen of 83 genera with 219 species were studied by scanning electron microscopy, generally without acetolysis. Special attention was given to Lemnaceae (including *Limnobiophyllum*) considered to be closely related to Araceae (especially to *Pistia*). The ultrastructural pollen wall characters are mostly in accordance with and strongly support the morphological classifications and the arrangement of genera within recent molecular trees. For example, pollen ultrastructural characters favor the placement of Lemnaceae (as Lemnoideae) between the two subfamilies Orontoideae and Pothoideae, and this novel view is strongly supported by molecular data. Within the large subfamily Aroideae, especially, the tribal arrangement based on molecular and morphological data is supported by palynological evidence with respect to the ektexine, which is mostly an unusual outer exine layer devoid of sporopollenin. In the morphologically basal tribes and in the corresponding “lower” clades of the molecular trees, a highly reduced or specifically modified smooth outer exine layer is typical. In contrast, a spiny outer exine layer is representative of the morphologically derived tribes, corresponding to the “higher” clades in the molecular trees.

Key words: Araceae, Aroideae, classification, Lemnaceae, Lemnoideae, *Limnobiophyllum*, palynology, pollen ornamentation, pollen wall, sporopollenin, ultrastructure.

INTRODUCTION

In classifications before Mayo et al. (1997), the great diversity in Araceae pollen ornamentation and pollen wall configuration appeared to be distributed without logical order and at random. The subfamilies in the classifications by Thanikaimoni (1969) and Grayum (1992), based on earlier systematic treatments, illogically emerged as eurypalynous. In addition, the treatment disagreed with the placement of Lemnaceae, hitherto considered to be closely related to Araceae, especially to *Pistia* L. (Aroideae) (Mayo et al. 1997; Stockey et al. 1997; see both also for reviews), based on morphological and embryological characters. In light of recent molecular data (Barabé et al. 2002; Cabrera et al. 2003) it seemed timely to conduct a comparative ultrastructural study of the pollen wall. We wondered if these results would support or contradict Araceae classification by Mayo et al. (1997) and the new classifications based on molecular data. All suprageneric taxa cited are those of Mayo et al. (1997), except where indicated otherwise.

MATERIALS AND METHODS

The results are based on investigations by transmission electron microscopy (TEM) of 101 species in 70 genera (out of a total of 105 genera in Araceae, as recognized by Mayo et al. 1997), and 83 genera with 219 species were studied by scanning electron microscopy (SEM). At least one member of each tribe was studied by SEM and TEM. Additionally, pollen material of *Lemna trisulca* L., *Wolffiella caudata* Landolt (Lemnaceae) and the fossil monocot *Limnobiophyl-*

lum sp. (“*Pandaniidites*” pollen, courtesy of Dr. R. Zetter, Institute of Palaeontology, University of Vienna) was investigated. For TEM investigations the pollen material was prepared according to Weber et al. (1999). For SEM investigations pollen material was prepared using 2,2-dimethoxypropane (DMP) and Critical-Point Drying according to Halbritter (1998). Acetolysed pollen was dehydrated in acetone and air-dried (Hesse et al. 2001).

The original pollen observations derive from and depend upon the respective embedding blocks for TEM ultrathin sections housed in the Department of Ultrastructure Research and Palynology, Institute of Botany, with the acquisition numbers: *Anthurium utleyorum* Croat & R. A. Baker (Bogner 2811, our negative number 02081999 for the SEM); *Anthurium digitatum* (Jacquin) D. Don (Bogner 2812, our embedding block 1574/1/1534 for the TEM), *Anthurium wendlingeri* G. M. Barroso (Bogner 2684, our embedding block 1582/1/1542 for the TEM), *Pistia stratiotes* L. (HBV ARA 040111, our embedding block 926/1/727 for the TEM), *Wolffiella caudata* Landolt (Landolt Nr. 9214, our embedding block 1481/1/1448 for the TEM), and *Limnobiophyllum* sp. (material from R. Zetter, our embedding block 1671/3/1605 for the TEM). Vouchers of Araceae and Lemnaceae are housed in (M) and (WU), respectively. Vouchers of the *Limnobiophyllum* material are housed in the Institute of Palaeontology, Department of Palaeobotany (Dr. R. Zetter).

RESULTS AND DISCUSSION

Two quite different pollen wall configurations are typical for Araceae. The first is the common tectate-columellate

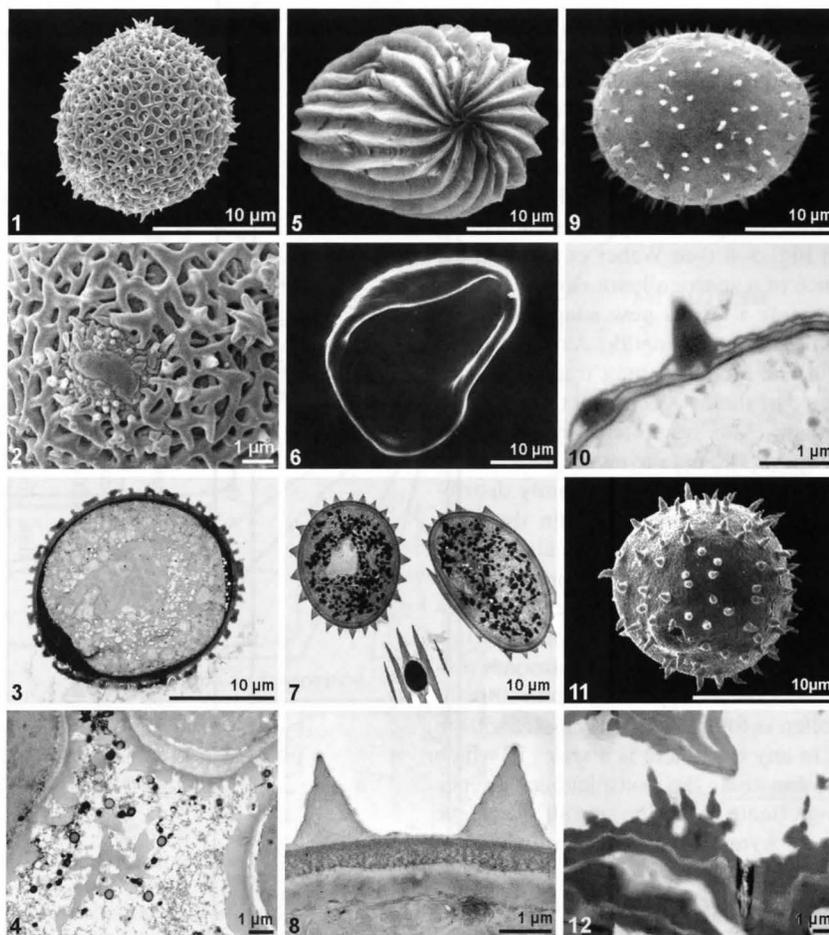


Fig. 1–12.—Pollen wall details of *Anthurium*, *Lemna*, *Limnobiophyllum*, *Pistia*, and *Wolffella*.—1–4. *Anthurium*.—1–2. *A. utleyorum*; pore indicated in Fig. 2.—3. *A. digitatum*. Note the pore in cross section on the left.—4. *A. wendlinger*.—5–8. *Pistia stratiotes*.—5. Before acetolysis.—6. After acetolysis.—7–8. Not acetolyzed, stained with uranyl-acetate.—9–10. Lemnaceae/Lemnoideae.—9. *Lemna trisulca*. Note the pore at the upper left (picture with permission from Grayum [1992, Fig. 493]).—10. *Wolffella caudata*.—11–12. *Limnobiophyllum* sp.—11. Note the annulate pore at the lower left (picture with permission from R. Zetter [Vienna, Austria]).

condition, forming an elaborated sporopollenin ectexine, and the second an atectate condition, where a highly reduced ectexine or even a novel nonsporopollenin outer exine layer is present (Hesse et al. 1999; Weber et al. 1999; see both for reviews). The first condition is common for all Araceae subfamilies (exemplified in Fig. 1–4) except Aroideae, while the second condition (exemplified in Fig. 5–8) is restricted to Aroideae (except Zamicolcadeae). Ultrastructural pollen wall characters support the arrangement of tribes and genera in the molecular trees and strongly support the recent morphological classification by Mayo et al. (1997). The reverse is also true: for instance, molecular data not only support the palynologically evident separation of the present tribe Zamicolcadeae from the bulk of subfamily Aroideae (the tribe will be proposed as a new subfamily, Zamicolcadoideae, in a separate paper: Bogner and Hesse 2005), but also the monophyly of Lasioideae and of (true) Aroideae, both with unique combinations of pollen characters (Weber et al. 1998, 1999; Hesse et al. 2001; Hesse 2002a). For the first time, results from pollen characters, morphological features, and molecular data are not contradictory, although this is primarily due to the extensive use of the TEM (details will be shown in a forthcoming paper, Hesse et al. in prep.).

A similar picture emerges if the external pollen morphology of Araceae is considered. The apertural condition and the pollen ornamentation are diverse and highly variable. However, smooth or spiny surface conditions predominate, while other conditions as for instance the reticulate ornamentation are rare, as shown perfectly by Grayum (1992). But using SEM results only, surface characters will give an incorrect picture of character classification within the family. If TEM is used to study pollen wall characters, a wealth of information is added to the SEM and LM results, as demonstrated for instance for the inaperturate monocot pollen by Furness and Rudall (1999), and for the only superficially similar polylicate condition appearing at least ten times within Araceae (Hesse et al. 2000).

Greater simplicity is now achieved in the classification of the subfamilies from Gymnostachydoideae to Lasioideae, where the distribution of pollen stratification and aperture configuration based on Thanikaimoni (1969) and Grayum (1992) was at odds with recent morphological classifications and the molecular trees. By far the largest subfamily, Aroideae, deserves a closer look. Pollen characters support the arrangement of tribes and genera in the molecular trees. Only the most basal genus, the zono-aperturate, sporopol-

leninous tectate-columellate *Zamioculcas* Schott (incl. *Gonatopus* Hook. f. ex Engl.) conforms to the tectate-columellate stratification (cf. Fig. 1–4). Hence, palynological and molecular (Cabrera et al. 2003) evidence agree in calling for a modified treatment of this genus (Bogner and Hesse 2005).

The remaining (inaperturate) Aroideae share a thick, spongy endexine, covered either by a highly reduced ectexine (a sporopollenin lamella) or by a non-sporopollenin outer exine layer as shown in Fig. 5–8 (see Weber et al. 1998 for terminology). The absence of a sporopollenin ectexine in the pollen of Aroideae represents a totally new adaptation, perhaps playing a beneficial role in pollination ecology. Polysaccharide or protein surface elements may transport volatiles (odors) better because of their less compact nature than the compact sporopollenin ectexine. Interestingly, pollen with a highly reduced sporopollenin layer or pollen with nonsporopollenin outer exine layers is not randomly distributed (scattered) over the Aroideae tribes. Within the morphological classification of Aroideae (Mayo et al. 1997) a clear boundary-line separates the tribes and genera of Aroideae with a more or less smooth pollen surface from Aroideae with distinct spiny pollen (Fig. 13). This boundary line is mirrored in the molecular trees: the typically smooth pollen is restricted to the tribes from Cryptocoryneae to Spathicarpeae, while spiny pollen is found nearly exclusively from Thomsonieae to Areae. In any case, here is a series in which form obviously follows function: the conspicuous absence of an elaborated sporopollenin ectexine in all Aroideae might be related to the idiosyncratic pollination biology of Aroideae.

The pollen wall studies also reveal a surprising placement for Lemnaceae (as Lemnoideae; Keating 2002). Lemnaceae were often linked to Aroideae, especially to *Pistia*, not least because of their floating aquatic habit (Mayo et al. 1997; Rothwell et al. 2004; see both also for review). Results discussed by Rothwell et al. (2004) support the new concept of two independent origins of the floating aquatic habit in extant members of the aroid/lemnoid lineage: *Pistia* and Lemnaceae/Lemnoideae are placed in two separate clades. Already, a preliminary molecular study by French et al. (1995) had placed Lemnaceae within the subfamily Aroideae, though not close to *Pistia*. From the standpoint of palynology, both interpretations (on the one hand the placement close to *Pistia*, and on the other hand within Aroideae) are very unlikely. Aroideae lack the classical tectate-columellate ectexine, which is typical for all other Araceae subfamilies and also for Lemnaceae (Hesse 2001, 2002b; Fig. 13). Pollen of all Lemnaceae/Lemnoideae and of the fossil *Limnobiophyllum* (pollen in situ belongs to the morpho-genus *Pandaniidites* Elsik, found in flowers of *Limnobiophyllum* [Stockey et al. 1997; Collinson et al. 2001]) is monoporate (i.e., ulcerate), spherical, and tectate-columellate (Fig. 9–12). Pollen of *Pistia* and of all other Aroideae is omniaperturate (inaperturate), and not tectate-columellate. The usual pollen wall stratification, with a sporopollenin tectum, columellae, and a foot-layer upon a thin endexine, is lacking in *Pistia* as in most Aroideae. Instead, the exine consists mostly of a (polysaccharide) outer layer upon a thick, spongy endexine, which is typical for Aroideae. Moreover, *Pistia* pollen is not spherical, but ellipsoid and polyplicate. Hence, palynologically Lemnaceae/Lemnoideae cannot be related to Aroideae,

Table 1. Pollen characters of Lemnaceae (Lemnoideae), *Limnobiophyllum*, *Anthurium*, *Orontium*, and *Pistia* (Araceae).

	Lemnoideae	<i>Limnobiophyllum</i>	<i>Anthurium</i>	<i>Orontium</i>	<i>Pistia</i>
Aperture	ulcerate (porate)	ulcerate (porate)	2- to 4-porate	sulcate	omniaperturate
Sculpture and shape	spiny, spherical	spiny, spherical	reticulate to spiny, spherical	micro-perforate	striate-plicate, ellipsoid
Before acetolysis	tectate-columellate	tectate-columellate	tectate-columellate	tectate-columellate	ribs upon endexine
After acetolysis	unmodified ectexine	unmodified (fossil) ectexine	unmodified ectexine	unmodified ectexine	smooth, outer layer dissolved
Endexine	thin, compact	(compact?)	thin, compact	thin, compact	thick, spongy

Pollen of Araceae sensu MAYO et al. (1997) and BOGNER (pers. comm.)

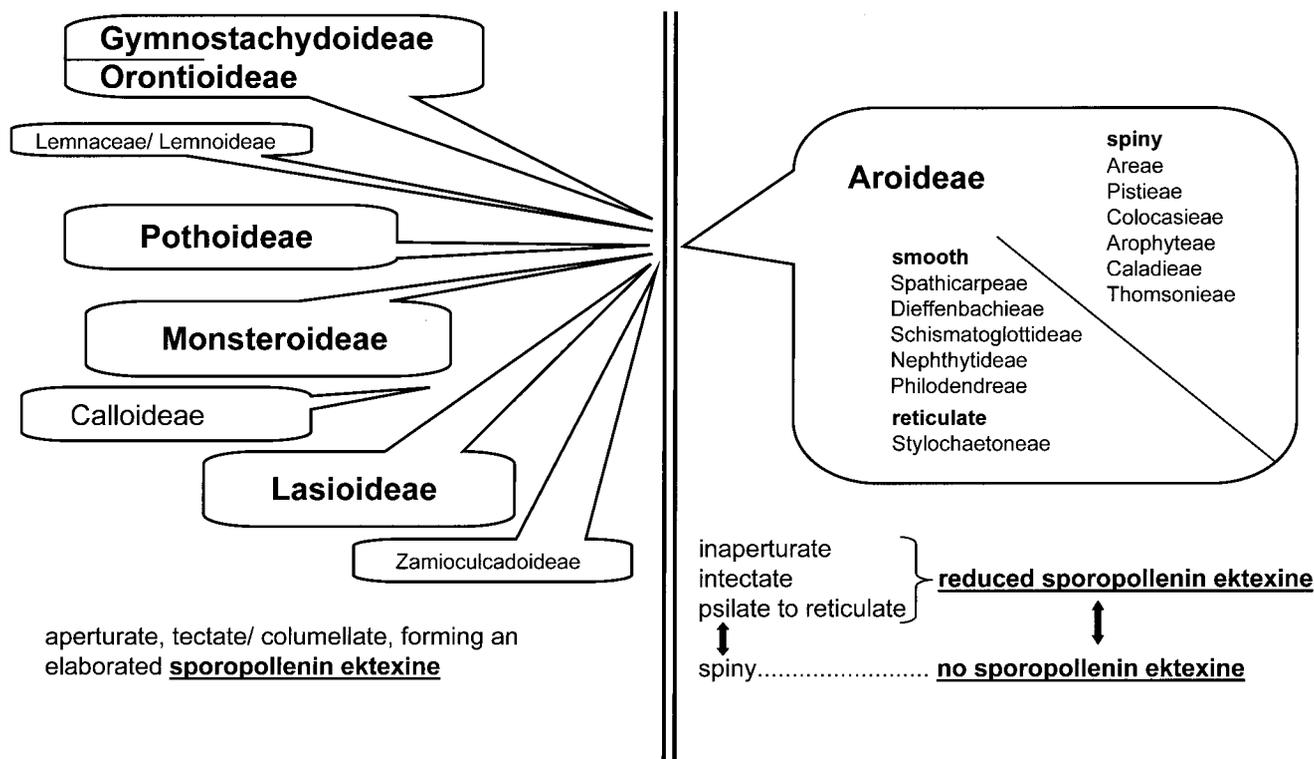


Fig. 13.—The diagram refers to the sporoderm configuration within Araceae sensu Mayo et al. (1997). The diagram shows the strict border line between all Araceae subfamilies with a more or less elaborated sporopollenin ektexine (left-hand side), including *Zamioculcas* and *Gonatopus* (representing *Zamioculcadoideae* as a new subfamily [Bogner and Hesse 2005]), and the bulk of Aroideae (right-hand side) divided into a group of tribes with mostly smooth pollen grains (and reticulate *Stylochaetoneae*), as well as another group of tribes with mostly spiny pollen. Within the tribes of Aroideae those with spiny pollen grains usually lack a sporopollenin outer exine layer, while those with smooth or reticulate pollen show mostly a highly reduced—or even absent—sporopollenin ektexine.

only to another subfamily of Araceae. All pollen wall details fit best with a placement of Lemnaceae (as a new subfamily Lemnoideae) and of *Limnobiophyllum* (Kvacek 2003: Limnobiophylloideae) between Orontioideae and Pothoideae, perhaps even near *Anthurium* Schott; its pollen is spherical, tectate-columellate, and, interestingly, within Araceae sensu Mayo et al. (1997), the only porate genus (Grayum 1992; Hesse 2002b). *Orontium* L., the other Orontioideae, and all Pothoideae are exclusively sulcate, and reticulate to perforate (Table 1). This point of view is greatly supported and confirmed by the molecular trees of Barabé et al. (2002) and Cabrera et al. (2003, and in prep.), where Lemnaceae is placed near Orontioideae and Pothoideae.

Detailed ultrastructural studies of pollen characters also support the assignment of zona-aperturate and polyplcate taxa, respectively, to two distinct subfamilies, as emerging from the studies by Mayo et al. (1997), as well as the published molecular trees. The distinctive zona-aperturate pollen condition appears in Monstereae and *Zamioculcadeae*, in which the superficially very similar pollen differs in the extent of apertural stratification (Hesse et al. 2001). Ten genera with the otherwise rare polyplcate condition are found in Araceae. Curiously, eight genera with polyplcate pollen are

located in the “spiny group” of Aroideae and form four “pairs” in the Mayo et al. (1997) classification (Hesse et al. 2000). The presence of four isolated/unrelated twin genera is exactly mirrored in the molecular tree. Significantly, the polyplcate condition is not found in the “smooth pollen” tribal series of Aroideae as they might be unable to form prominent ornamentation elements, such as ribs upon the endexine. The remaining two (likewise paired) polyplcate genera in Monsteroideae (*Holochlamys* Engl. and *Spathiphyllum* Schott) are only superficially similar to the polyplcate genera of Aroideae, differing markedly at the ultrastructural level. The fact that seemingly identical characters are only superficially similar and, instead, a result of parallel evolution has important implications for cladistic studies and serves as a reminder to be extremely careful when character coding.

CONCLUSION

In contrast to previous classifications by Thanikaimoni (1969) and Grayum (1992) based on palynological characters, the subfamilies sensu Mayo et al. (1997) appear to be widely or even completely stenopalynous. This is true for

the small lower subfamilies, especially for Lasioideae, and also for the largest subfamily Aroideae. For the first time, there is congruence between pollen characters, morphological features, and molecular data, not only in the lower subfamilies, but also especially in Aroideae. A critically evaluated pollen ultrastructural character can be applied as a practical tool in systematics as it acts like a compass needle (sensu Gunnar Erdtman, Herbert Straka, or Steve Blackmore) with a significant diagnostic value, supporting the results of molecular studies. The predictive value of pollen morphology is therefore extremely high at the tribal or even subfamily level in Aroideae and at the tribal level and in the molecular classification of the other aroid subfamilies.

A much-extended version of this contribution focusing on Aroideae tribes, with full details on pollen ultrastructure and possible relationships to ecology, is in preparation.

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