

Notes on the genus *Amorphophallus* (Araceae) – 13. Evolution of pollen ornamentation and ultrastructure in *Amorphophallus* and *Pseudodracontium*

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A strict consensus tree based on chloroplast and nuclear sequences (rbcL, matK, trnL, FLint2) from 46 Amorphophallus species, two Pseudodracontium species and six outgroups is used to develop a hypothesis for the evolution of ornamentation and ectexine ultrastructure in the pollen of Amorphophallus. There are four main clades: an exclusively African, largely psilate clade ('African clade'), an Asian, largely psilate clade ('Asian psilate clade') and an Asian, largely striate clade consisting of a mainly continental SE Asian clade ('continental SE Asian striate clade') and one centred in Malesia ('Malesian striate clade'). Ultrastructure provides a valuable contribution towards understanding pollen ornamentation in Amorphophallus. Pollen with a thin psilate ectexine without dark granules might be plesiomorphic in Amorphophallus. Then the diverse striate type would be derived. Within both striate clades, reversals to the psilate type occur. Striate pollen with psilate caps, which is nested in the continental SE Asian striate clade, is a synapomorphy of Pseudodracontium. The fossulate type is also diverse, and its distribution in the tree indicates a polyphyletic origin. Areolate, echinate and verrucate ornamentation, occur in single species in the tree, but are found also in species not included in the molecular analysis. All three are heterogeneous and probably polyphyletic too. Reticulate, scabrate and striate/scabrate ornamentation are autapomorphies, of which the reticulate type and the striate/scabrate type may derive from psilate and striate ornamentation, respectively. Of the four main clades, the Asian psilate and African clade seem to be basal, while both striate clades might have evolved from the Asian psilate clade via a species like A. rhizomatosus. Dark granules evolved more than once, which might explain their diverse size, shape and distribution.

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Amorphophallus Bl. is a genus of palaeotropical aroids (Araceae) occurring in Africa, Madagascar, India, continental SE Asia, Malesia and NE Australia (Mayo et al. 1997). The main centre of diversity is continental SE Asia and W Malesia. Many species are narrow endemics, while only a few are widespread. Of these, the edible A. konjac and A. paeoniifolius have been introduced to many countries. The last comprehensive treatment of the genus was by Engler (1911), who distinguished 78 species in 11 sections. Since then many new species have been described. Substantial recent contributions include papers by Hetterscheid (1994), Ittenbach & Lobin (1997), Hetterscheid et al. (1999), Hetterscheid & Van der Ham (2001) and Ittenbach (2003). Broadly confined to include Pseudodracontium N. E. Br. (Grob et al.: 2004), Amorphophallus comprises about 200 species. The genus is currently undergoing a revision (Hetterscheid: in prep.), which will include pollen morphology (Van der Ham et al. 1998, 2000; Van der Ham & Van Heuven 2001, Hetterscheid & Van der Ham 2001), odour biochemistry and pollination biology (Kite & Hetterscheid 1997, Kite et al. 1998) and molecular data (Grob et al. 2002, 2004).

Grob et al. (2004, Fig. 8) presented a phylogeny of *Amorphophallus* based on chloroplast and nuclear sequences (*rbcL*, *matK*, *trnL*, *FLint2*) from 46 *Amorphophallus* species, two *Pseudodracontium* species and six outgroups. In comparison to the result found by Grob et al. (2002, Fig. 4), which was based on *matK* en *trnL* sequences of the same species, the resolution in the strict consensus tree and the number of well-supported clades increased, but the basal polytomy as well as some of the end clades remained unresolved. In the present paper, the strict consensus tree presented by Grob et al. (2004) is used to develop a hypothesis for pollen evolution in *Amorphophallus*.

MATERIAL AND METHODS

The present paper focuses on the *Amorphophallus* species sampled by Grob et al. (2004) for the molecular work, but often reference is made to species that appeared to be more or less strongly associated with them in preliminary total evidence analyses (Hetterscheid: in prep.), in which molecular and morphological data of all species are analysed together. Pollen size, shape and ornamentation data (LM, SEM) of all 48 ingroup taxa except A. pingbianensis, and of all related species referred to in the present paper were available. The ultrastructural (TEM) data set offered by Van der Ham et al. (1998, 2000) has been expanded to contain all ingroup species except A. canaliculatus, A. commutatus, A. hirsutus, A. hottae, A. pingbianensis, A. pygmaeus and A. zenkeri, and most of the related species referred to. The unavailable A. pingbianensis was proposed to be conspecific with A. coaetaneus (Hetterscheid & Van der Ham 2001). Table I gives the material and values/states of selected pollen characters of Amorphophallus and Pseudodracontium species used, with references to the SEM and TEM illustrations in the present paper as well as those in Van der Ham et al. (1998). Pollen data of the outgroups were taken from the literature (Grayum 1992, Oh et al. 1990, Ohashi et al. 1983, Hesse et al. 2001, Weber et al. 1998).

Pollen of *Amorphophallus* usually does not resist acetolysis (Van der Ham et al. 1998, 2000). Therefore, the material (mostly dried) was boiled in water (10 minutes). Then the pollen grains could be freed by breaking the anthers and transferring, via alcohol, to a glycerin/alcohol 96% mixture (2/1). The pollen was mounted in glycerin jelly for LM observation. For SEM the pollen was mounted on stubs and sputter-coated with gold. Preparation for TEM included rehydration and fixing in 0.1% glutaraldehyde (1 week), followed by fixation with 1% OsO₄ (1 hour), prestaining with 1% uranyl acetate during dehydration, embedding in 3/7 Epon (Luft), post-staining with 5% uranyl acetate (10 minutes) and Reynolds' lead citrate (10 minutes).

Pollen grains of *Amorphophallus* are inaperturate, without any indication of the orientation of the polar axis. Therefore, size is given as the largest diameter (L). The ratio L/B (largest/smallest diameter) is given as an indication of pollen grain shape. The terminology for the ornamentation types follows Punt et al. (1994). As in many other Araceae (Weber et al. 1998), the outer wall layer in the pollen of *Amorphophallus* and *Pseudodracontium* is clearly less resistant to acetolysis than the endexine, being completely removed in most cases. For an extensive justification regarding the use in the present study of the term ectexine for this outer layer, the reader is referred to Van der Ham et al. (1998, p. 111).

The phylogeny presented by Grob et al. (2004, Fig. 8) and used in the present paper (optimised using McClade) is a strict consensus tree (RC=0.6001) of 6476 most parsimonious trees of length 1232 based on the combined analysis of chloroplast (*rbcL*, *matK*, *trnL*) and nuclear data (*FLint2*).

RESULTS

Table I presents the expanded data set of *Amorphophallus* and *Pseudodracontium* pollen used in the present study in combination with the results obtained by Grob et al. (2004). Our illustration of the phylogeny (Fig. 1) shows the ornamentation types (Fig. 2) plotted on the strict consensus tree provided by Grob et al. (2004, Fig. 8), as well as the presence/ absence of dark granules in the ectexine (Fig. 3). The 'long product' and 'short product' branches of *A. napiger* are combined into a single *A. napiger* branch, while of the six outgroups only *Arisaema tortuosum* is shown. The numbers in the tree are bootstrap values. Below, the combination of the results of the present study and those of the molecular study by Grob et al. (2004), as presented in Fig. 1 is discussed. On this basis, a hypothesis for pollen evolution in *Amorphophallus* and *Pseudodracontium* is proposed.

DISCUSSION

Outgroups

Amorphophallus, including Pseudodracontium, appears to be a monophyletic group. The closests of the six outgroups of Amorphophallus used by Grob et al. (2004) are species of Arisaema (tribe Arisaemateae), Filarum (tribe Zomicarpeae), Hapaline (tribe Caladieae) and Typhonium (= Sauromatum, tribe Areae), which have relatively small $(21-40 \ \mu m)$, inaperturate, echinate pollen (Grayum 1992). Echinate pollen is common in the Araceae, and dominant in the tribes to which Arisaema, Filarum and Typhonium belong. Of the six genera included in Caladieae (Mayo et al. 1997), only Hapaline and Syngonium have echinate pollen. In Amorphophallus the occurrence of echinate pollen is restricted to five African species (Van der Ham et al. 2000) of which A. dracontioides was included in the molecular analysis. The pollen of A. dracontioides belongs to the subtype with short, unstoreyed, basally connected spines (subtype a; Fig. 2L), which also includes A. aphyllus and A. elliottii. Subtype b (A. doryphorus) has more elongate, unstoreyed, isolated spines, while subtype c (A. antsingyensis) has elongate, storeyed spines (storeyed spine: 1-5 superposed parts that decrease in diameter towards the top of the spine). Few TEM studies of echinate Araceae pollen are available but, so far, subtype c seems to be unique within the Araceae. Subtype a resembles the pollen of Arisaema (compare Van der Ham et al. 2000, Figs 9, 10 with Oh et al. 1990, Figs 52-55 and Ohashi et al. 1983, Fig. 1), while subtype b is similar to the pollen of Typhonium (compare Van der Ham et al. 2000, Figs 9, 10 with Weber et al. 1998, Fig. 3). The ultrastructure of the spines of Filarum and Hapaline pollen is unknown. See section on possible pollen evolution.

Ingroup

The ingroup shows three large clades (Fig. 1): an exclusively African, largely psilate clade ('African clade'), an Asian, largely psilate clade ('Asian psilate clade') and an Asian, largely striate clade. The third consists of a mainly continental SE Asian clade ('continental SE Asian striate clade') and a clade centered in Malesia ('Malesian striate clade').

Pollen size and shape

There are only small differences between the four main clades with regard to pollen grain size and shape. Average size (L) varies from 53.7 to 60.5 μ m (minima: 32.1–38.1 μ m, maxima: 69.8–87.8 μ m), and shape (L/B) from 1.17 to 1.35 (minima: 1.01–1.06, maxima: 1.33–2.06). Furthermore, there are no large differences within the main clades, except for the Asian psilate clade where *A. napiger*, *A. margaritifer*, *A. hirtus* and *A. henryi* have almost spheroidal psilate pollen (L/B 1.06–1.08), and *A. muelleri*, *A. brevispathus* and *A. symonianus* have more elongate psilate pollen (L/B 1.44–2.06).

Table I. Species, material, and values and states of selected pollen characters of Amorphophallus and Pseudodracontium.

'ectexine' – ectexine thickness in μm. The <u>underlined</u> numbers in the column 'figures' refer to illustrations in the present paper, while the numbers that are not underlined refer to illustrations in Van der Ham et al. (1998).

Taxon		material	ornamentation	ectexine	dark granules	figures
Amorphophallus						
species included in molecular analysis						
abyssinicus ssp. abyssinicus (Rich.) N. E. Br.		H.AM.066B, Nigeria (L)	verrucate	0.8	?	<u>2J</u> , <u>30</u>
angolensis (Welw. ex Schott) N. E. Br.		H.AM.015, Gabon (L)	psilate	0.25	-	
ankarana Hett., Ittenb. & Bogner		H.AM.048, Madagascar (L)	psilate (locally undulate)	0.6	+ (small)	<u>3D</u>
baumannii (Engl.) N. E. Br.	А	Johnson s.n., Ghana (K)	foss. to coarsely str. to psil	0.55	+(numerous, crowded, very small)	<u>3A</u>
	В	H.AM.665, Ghana (L)	coarsely striate	1.3	+(few, loose, large)	125, <u>3J</u>
<i>beccarii</i> Engl.		H.AM.192A, Sumatra (L)	psilate	0.6	_	134. 3B
brevispathus Gagn.		H.AM.674. Thailand (L)	psilate	0.06	_	135
canaliculatus Ittenb.,		H.AM.014, Gabon (L)	psilate	TEM not		140
<i>cirrifer</i> Stapf		H.AM.462, Thailand (L)	areolate	0.6	+ (few large in basal part, many small in outer part)	<u>2G</u> , <u>3M</u>
<i>coaetaneus</i> Liu & Wei (incl. <i>4. arnautovii</i>) Hett.)		H.AM.338, China (L)	striate	0.4		52, <u>3E</u>
commutatus (Schott) Engl.		H.AM.218, India (L)	striate	TEM not available		
corrugatus N. E. Br.		H.AM.229, Thailand (L)	striate	1.55	+(crowded)	
decus-silvae Backer & Alderw.		H.AM.189, Java (L)	fossulate (to psilate)	1.3	_	108, 129
dracontioides (Engl.) N. E. Br.		Geerlings et al. 2214, Ivory C. (WAG)	echinate (subtype a)	0.2-2.85	+(small, in basal part)	3, <u>3P</u>
eburneus Bogner		H.AM.311, Sarawak (L)	striate	0.5	+(loose)	65
eichleri (Engl.) Hook. f.		H.AM.051, central Africa (L)	psilate	0.2	+ (indistinct)	
galbra F. M.Bail.		H.AM.174, Queensland (L)	scabrate	0.4	+ (few, large)	2N, 3Q
henryi N. E. Br.		H.AM.097, Taiwan (L)	psilate	TEM not available		<u>2A</u>
		H.AM.234, Taiwan (L)	psilate	0.15	_	
hirsutus Teijsm. & Binnend.		Trice s.n., Sumatra (MO)	psilate	TEM not available		
hirtus N. E. Br.		H.AM.132A, Taiwan (L)	psilate	0.15	_	
hottae Bogner & Hett.		H.AM.915, Sabah (L)	striate	TEM not available		
konjac K. Koch		H.AM.168, locality unknown (L)	fossulate (to coars striate)	1.0	+(in lower third to half)	118, 130
<i>krausei</i> Engl.		H.AM.092. Thailand (L)	striate	0.75	+(crowded)	
lambii Mavo & Widi		H.AM.071. Sabah (L)	psilate	0.75	_	
lewallei Malaisse & Bamps		H.AM.021, Burundi (L)	psilate	0.9	+(in inner part)	

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Table I. Continued.

Taxon	material	ornamentation	ectexine	dark granules	figures
longituberosus (Engl.) Engl. & Gehrm.	H.AM.068A, Thailand (L)	striate	0.8	+(crowded)	77, <u>3H</u>
margaritifer (Roxb.) Kunth	H.AM.422, India (L)	psilate	0.3	_	
maxwellii Hett.	H.AM.287, Thailand (L)	fossulate (to psilate)	1.2	+(in middle third)	3K
muelleri Bl.	H.AM.288, Thailand (L)	psilate	0.3	_	
napalensis (Wall.) Bogner & Mayo	H.AM.227, Nepal (L)	striate	0.6	+(crowded)	
napiger Gagn.	H.AM.709. Thailand (L)	psilate	0.1	_	3C
ochroleucus Hett. & V. D. Nguyen	H.AM. 927. North Vietnam (L)	reticulate	0.8	_	$\frac{1}{2M}$, 3G
<i>paeoniifolius</i> (Dennst.) Nicols.	H.AM.054. locality unknown (L)	psilate	0.1	_	151
palawanensis Bogner & Hett. pingbianensis H. Li &	H.AM.124B, Palawan (L)	striate	1.25	+(loose)	83
C. L. Long $(=A. coaetaneus)$					
pusillus Hett. & Serebr.	H.AM.247, South Vietnam (L)	striate	0.65	+(loose)	86
pygmaeus Hett.	H.AM.104, Thailand (L)	striate	TEM not available		
rhizomatosus Hett.	Sizemore 97-084, Vietnam (L)	striate	0.4	-	<u>3F</u>
sagittarius Steen.	H.AM.128, Java (L)	striate	0.8	+(loose)	3L
smithsonianus Sivad.	H.AM.216, India (L)	striate/scabrate	1.9	-	<u>20</u> , <u>3R</u>
sumawongii (Bogner) Bogner	H.AM.714, Thailand (L)	striate	1.3	+(loose)	
symonianus Hett. & M.Sizemore	Sizemore 97-168, Thailand (L)	psilate	0.2	_	
taurostigma Ittenb., Hett. & Bogner	Bogner 166, Madagascar (K)	fossulate	TEM not available		<u>2E</u>
	H.AM.038, Madagascar (L)	fossulate	0.55	_	
titanum (Becc.) Becc. ex Arcang.	HLB 27198A, Sumatra (L)	psilate	0.4	_	147
variabilis Engl.	HLB 27375, Java (L)	striate	0.6	+(loose)	101
yunnanensis Engl.	H.AM.157, Thailand (L)	striate	0.95	_	
zenkeri (Engl.) N.E.Br.	Fischer 1436, Cameroon (BONN)	psilate	TEM not available		148
species not included in molecular analysis					
aberrans Hett.	H.AM.741, Thailand (L)	areolate	TEM not available		<u>2H</u>
	Murata et al. 16977, Thailand (KYO)	areolate	2.2	+(few large in basal part, many small in outer part)	41, 42
abyssinicus ssp. unyikae (Engl. & Gehrm.) Ittenb.	Agnus 636, Zimbabwe (K)	fossulate to coarsely striate	TEM not available	· /	103
albispathus Hett.	H.AM.019, Thailand (L)	striate	0.7	+(crowded)	50, 95
annulifer Hett.	H.AM.089, Java (L)	fossulate	0.6	+(crowded)	2F
antsingvensis Bogner,	H.AM.099, Madagascar (L)	echinate (subtype c)	1.6 - 6.5	+(one per spine,	8, 12
Hett. & Ittenb.	,			large)	- 7
aphyllus (Hook.) Hutch.	Koenen s.n., Senegal (BONN)	echinate (subtype a)	0.1 - 1.8	+(granular basal sublayer)	1

Pollen ornamentation and ultrastructure in Amorphophallus and Pseudodracontium

Table	L	Continued.

Taxon		material	ornamentation	ectexine	dark granules	figures
brachyphyllus Hett.	А	H.AM.031A, Sarawak (L)	fossulate to coarse striate	TEM not available		106
	В	H.AM.032C, Sarawak (L)	striate	0.9	+(in basal two thirds)	55
coudercii (Bogner) Bogner		H.AM.241, central Vietnam (L)	striate	1.2	+(crowded)	2B
cruddasianus Prain ex Engl.		H.AM.773, Thailand/Myanmar (L)	striate	0.6	+ (few in inner part, many in outer part)	
doryphorus Ridl.		Eggers s.n., Senegal (K)	echinate (subtype b)	< 0.05 - 4.3	_	2L
elliottii Hook. f.		Elliott 4640, Sierra Leone (K)	echinate (subtype a)	TEM not available		4
gallaensis (Engl.) N.E.Br.	А	Langridge 11056, Kenya (K)	striate	0.6	+(crowded)	70, 127
	В	Riva 410, Ethiopia (ZT)	fossulate to coarse striate	1.0	+(crowded)	109, 128
gigas Teijsm. & Binnend.		Hetterscheid s.n., Sumatra (L)	psilate	0.5	_	143
gomboczianus Pic. Serm.		H.AM.003, Ethiopia (L)	striate	1.1	+(crowded)	71, 97
hayi Hett.		H.AM.278, North Vietnam (L)	psilate	0.8	_	
hohenackeri (Schott) Engl. et Gehrm.		H.AM.011, India (L)	fossulate	2.9	_	<u>2D</u>
linearis Gagn.		Kerr 19495, Thailand (K)	psilate	TEM not available		
maximus (Engler) N.E.Brown		H.AM.279, east Africa (L)	verrucate	1.1	+(one per verruca, large)	20, 21
mildbraedii K.Krause		Mildbraed 9287, Cameroon (K)	verrucate	1.0	+(few, irregular, in basal part)	25, 27
mossambicensis (Schott) N.E.Br.	А	Richards s.n., Tanzania (K)	fossulate	0.4	+(indistinct)	126, 132
(was A. swynnertonii Rendle)	В	Swynnerton 717A, Mozambique (BM)	psilate	TEM not available		
mullendersii Malaisse & Bamps		Quarré 2040, Zaïre (BR)	fossulate	1.6	_	
pilosus Hett.		H.AM.839, Vietnam (L)	areolate	1.4	+(indistinct, in outer part)	
richardsiae Ittenb.		H.AM.352, Zambia (L)	coarsely striate to + areolate	0.6	_	
saraburiensis Gagn.		Kerr 7037, Thailand (K)	areolate	TEM not available		38
scutatus Hett. & T.C. Chapman		H.AM.590, Thailand (L)	areolate	2.2	+(crowded, in middle part)	<u>3N</u>
sylvaticus (Roxb.) Kunth		Barnes 880, India (K)	verrucate	0.7	+(granular basal sublayer)	36, <u>2K</u>
tenuistylis Hett.		Beusekom et al. 492, Thailand (L)	areolate	2.8	+(crowded, in middle part)	<u>2I</u> , 48
verticillatus Hett.		H.AM.884, Vietnam (L)	striate	0.7		
yuloensis H.Li		H.AM.231, China (L)	striate	1.0	-	91, 133

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Table I. Continued

Taxon	material	ornamentation	ectexine	dark granules	figures
Pseudodracontium N.E.Br. species included in molecular analysis hormandii Enol	H AM 147 Thailand (I)	striate with neilate cans	0 0	⊥(in lower	
anmanan Lugi.		surary, with panary caps	0.0	corners of muri)	
lanceolatum Serebr. species not included in molecular analysis	H.AM.179, South Vietnam (L)	striate, with psilate caps	1.3	+(crowded)	164, <u>31</u>
allax Serebr.	H.AM.162, South Vietnam (L)	striate, with psilate caps	1.1	+(crowded)	158, 159
kuznetsovii Serebr.	H.AM. 165, South Vietnam (L)	striate, with psilate caps	0.0	+(crowded)	
lacourii (Lind. & André) N.E.Br.	H.AM.016, Thailand (L)	striate, with psilate caps	TEM not available		2C
	H.AM.074, South Vietnam (L)	striate, with psilate caps	0.0	+(crowded)	
latifolium Serebr.	H.AM.167, Thailand (L)	striate, with \pm psilate caps	0.7	+(crowded)	165
nacrophyllum Gagn. ex Serebr	H.AM.178, South Vietnam (L)	striate, with psilate caps	0.0	+(crowded)	167

Ornamentation

Ten main ornamentation types were distinguished within *Amorphophallus* (Fig. 2): 1. psilate, 2. striate, 3. striate with psilate caps, 4. fossulate, 5. areolate, 6. verrucate, 7. echinate, 8. reticulate, 9. scabrate and 10. striate/scabrate. The main clades are dominated by either psilate (Fig. 2A) or striate (Fig. 2B) pollen (together occurring in 80% of all *Amorphophallus* species), but each clade contains several other main ornamentation types. Psilate and striate pollen occur throughout the tree, basally as well as distally. The less common types (Fig. 2C–O), all represented in the tree, are found more distally.

Psilate and striate pollen occur throughout the distribution area of the genus. Striate pollen with psilate caps is restricted to Pseudodracontium (Cambodia, Laos, Thailand, and Vietnam). Reticulate (A. ochroleucus, Vietnam), scabrate (A. galbra, New Guinea, Queensland) and striate/ scabrate ornamentation (A. smithsonianus, India) occur within single species. Three other types occurring within single species in the molecular tree (areolate, verrucate, echinate) are also represented by some of the species not included in the analysis. Areolate pollen (six species) is restricted to continental SE Asia, echinate pollen (five species) to Africa and Madagascar and verrucate pollen to Africa (five species) and India (one species). The fossulate type is found in three of the four main clades (throughout distribution area of the genus). All three types, but especially fossulate (Fig. 2D-F), are very diverse.

Ultrastructure

Subdividing the ultrastructural diversity of *Amorphophallus* pollen appeared to be more difficult than classifying ornamentation (Van der Ham et al. 1998). However, if the ultrastructural features (Fig. 3) are compared with the ornamentation types plotted on the molecular tree, some patterns emerge that make sense but would be difficult to sort out on pollen morphological criteria alone.

Ectexine thickness

Ectexine thickness is between 0.06 and 1.55 μ m in most species in the molecular analysis. Pollen of *A. dracontioides* (echinate: Fig. 3P) and *A. smithsonianus* (striate/scabrate: Fig. 3R) have very thick ectexines (2.85 and 1.9 μ m, respectively) due to their high ornamentation elements. In general, psilate ectexines are thinner than ornamented ectexines although, in most ornamented ectexines, the thickness between the ornamentation elements is within the range of psilate ectexine thickness. From a developmental point of view, ornamented ectexines might be considered as differentially thickened psilate ectexines.

Excluding A. dracontioides and A. smithsonianus, there are only minor differences between the ranges of ectexine thickness in the main clades. The psilate species in the Asian psilate clade species have relatively thin ectexines $(0.06-0.3 \ \mu\text{m})$, compared with the psilate species in the African clade and Malesian striate clade $(0.1-1.3 \ \mu\text{m})$. This is even more distinct in the separate *FLint2* analysis by Grob et al. (2004), where the psilate *hirsutus-paeoniifolius* subclade



POLLEN ORNAMENTATION



DARK GRANULES IN ECTEXINE

+	present
	abaant

- absent
- ? unknown

is placed in the Asian psilate clade instead of in the Malesian striate clade. This then makes the thickness range for the latter $0.2-1.3 \mu m$ while *A. paeoniifolius* (ectexine 0.1 μm thick) fits conveniently into the thickness range for the Asian psilate clade. TEM data of *A. hirsutus* are unavailable but LM observation also suggests a very thin ectexine.

Dark granules

With particular emphasis on presence of dark granules in the ectexine, Van der Ham et al. (1998) divided the 29 Amorphophallus species studied with TEM into three groups: 1. dark granules absent or indistinct (e.g. Fig. 3B), 2. dark granules distinct (e.g. Fig. 3J), and 3. ectexine ultrastructure deviating from group 1. and group 2. At the time it appeared that psilate pollen was restricted to group 1. Distinct granules were observed in nearly all striate species and only one species with striate pollen, A. yuloensis, was placed in group 1. Now, TEM data of more than 100 Amorphophallus species are available which allows much more detailed correlations. An integrated account of the distribution of ornamentation types and the ultrastructural diversity in the main clades is given below in order to develop a scenario for the evolution of Amorphophallus pollen.

Asian psilate clade

This clade contains psilate (dominant), striate, areolate (A. cirrifer: Fig. 2G) and reticulate (A. ochroleucus: Fig. 2M) pollen. Thin $(0.06-0.3 \ \mu\text{m})$ psilate ectexines dominate and it seems that striate ornamentation (A. coaetaneus, A. pygmaeus, A. yunnanensis) evolved several times, mostly from psilate ancestors.

Dark granules are lacking in the ectexines of all species except A. cirrifer. This species, the only species in the tree with areolate pollen, and the related A. cruddasianus (not in tree) with striate pollen have different ornamentation types, but show similar ectexine ultrastructure: in both the elements show an outer ectexine with granules and a homogeneous basal part that locally contains a single large dark granule (Fig. 3M). Often pollen grains of A. cirrifer show patches of finely fossulate to irregularly striate ornamentation, which might be viewed as transitional between areolate and striate. The ectexine ultrastructure of A. cirrifer pollen is like the areolate pollen of A. aberrans (Fig. 2H) and A. pilosus but unlike that of the other Amorphophallus species with areolate pollen for which TEM data are available (A. scutatus and A. tenuistylis: Fig. 3N). Areolate ornamentation is restricted to continental SE Asia.

Ultrastructurally, the reticulate ectexine of *A. ochroleucus* (Fig. 3G) resembles the other ectexines without dark granules in the clade, for example, those of *A. symonianus*

(psilate) and the related *A. yuloensis* (striate — not in tree), and *A. coaetaneus* (striate: Fig. 3E).

Continental SE Asian striate clade

This clade contains striate (dominant) and fossulate pollen, striate pollen with psilate caps (*Pseudodracontium*: Fig. 2C) and striate/scabrate pollen (*A. smithsonianus*: Fig. 2O).

This clade is diverse with respect to the presence and distribution of dark granules. They do not occur in the basal A. rhizomatosus (striate: Fig. 3F), the related A. verticillatus (striate — not in tree) and A. havi (psilate — not in tree), or A. smithsonianus (striate/scabrate: Fig. 3R). The ectexine of A. hayi is distinctly thicker than the psilate ectexines in the Asian psilate clade (0.8 vs. $0.1-0.3 \mu m$) and even thicker than some of the striate ones (e.g. in A. rhizomatosus: 0.4 µm). A. smithsonianus has a unique striate/scabrate ornamentation type (Fig. 2O), but ultrastructurally (Fig. 3R) the ectexine does not differ from that of species with striate pollen such as A. rhizomatosus (Fig. 3F) apart from the muri which are indistinctly subdivided by irregular narrow fissures. The group consisting of A. longituberosus (Fig. 3H), the related A. albispathus (not in tree), Pseudodracontium lanceolatum (Fig. 3I), P. fallax, P. kuznetsovii, P. lacourii, P. latifolium and P. macrophyllum (the last five species not in tree) show ectexines with fine crowded granules, while in P. harmandii the granules are few and restricted to the basal parts of the muri. Dark granules occur in all other species in the main clade. In A. napalensis, A. krausei and A. corrugatus, all with striate pollen; dark granules fill the ectexine except for a thin, outer sublayer, just as in the longituberosus subclade. In the basal most sublayer they occur more loosely and tend to be larger and/or connected (Fig. 3H). Often the outer sublayer is continuous, though depressed, between the muri (Fig. 3H, I).

In the two fossulate species the dark granules show deviating distributions. In *A. konjac* they are restricted to the lower third to half of the ectexine, while in *A. maxwellii* (Fig. 3K) they occur in the middle third. These distinctive distributions are unknown in the striate members of the clade.

Malesian striate clade

This clade contains striate (dominant), psilate, fossulate (*A. decus-silvae*) and scabrate (*A. galbra*: Fig. 2N) pollen. There are several species with psilate pollen, of which those with a thin ectexine in the basal *hirsutus-paeoniifolius* subclade might belong to the Asian psilate clade (see section on Ultrastructure — Ectexine thickness).

All species with striate pollen have ectexines with granules, although these are less distinct, and less densely distributed, than in the continental SE Asian striate clade.

Fig. 1. Strict consensus tree based on chloroplast and nuclear sequences (*rbcL*, *matK*, *trnL*, *FLint2*) from 46 *Amorphophallus* species, two *Pseudracontium* species and six outgroups, of which only *Arisaema tortuosum* is shown here, according to Grob et al. (2004). The numbers in the tree are bootstrap values. A – 'African clade', B – 'Asian psilate clade', C – 'continental SE Asian striate clade', D – 'Malesian striate clade'. The colors shown refer to the pollen ornamentation types (optimised using McClade), and +, – and ? to the presence of dark granules in the ectexine.



Fig. 2. Amorphophallus and *Pseudodracontium* pollen, scanning electron micrographs (SEM). A. *A. henryi*, psilate ornamentation. B. *A. coudercii*, striate ornamentation. C. *P. lacourii* striate pollen with psilate caps. D. *A. hohenackeri*, fossulate ornamentation. E. *A. tauros-tigma*, fossulate ornamentation. F. *A. annulifer*, fossulate ornamentation. G. *A. cirrifer*, areolate ornamentation. H. *A. aberrans*, areolate ornamentation. L. *A. tenuistylis*, areolate ornamentation. M. *A. ochroleucus*, reticulate ornamentation. N. *A. galbra*, scabrate ornamentation. O. *A. smithsonianus*, striate/scabrate ornamentation. Scale bars – 10 µm (A–D, F–M) or 5 µm (E, N, O).

None of the species with psilate pollen or the species with fossulate pollen (*A. decus-silvae*) has dark granules in the pollen ectexine. Ultrastructurally, the thick ectexine of *A. decus-silvae* pollen resembles that of the pollen of the related *A. gigas* (not in tree) and ectexines of pollen in other species of the *beccarii-titanum* subclade (Fig. 3B). Thus, in the Malesian striate clade fossulate ornamentation appears to be connected with the psilate ectexine type, and not, as in the continental SE Asian striate clade, with the striate ectexine type.

Pollen of *A. galbra*, the easternmost species (New Guinea, Queensland) within the natural distribution area of *Amorphophallus* (Hetterscheid & Ittenbach 1996), possesses a unique scabrate ornamentation type (Fig. 2N) which is hard to connect with any of the other types. The ultrastructure (Fig. 3Q) is reminiscent of the ectexine ultrastructure of species with psilate pollen, where the surface is sometimes very finely scabrate (e.g. Fig. 3C). However, in *A. galbra* the surface is more coarsely scabrate, and a single large dark granule may be present in a scabra.

African clade

The exclusively African clade is the most diverse of the four main clades. It contains psilate (dominant), fossulate, echinate (*A. dracontioides*: Fig. 2L) and verrucate (*A. abyssinicus*: Fig. 2J) pollen. Preliminary total evidence analyses indicate that African *Amorphophallus* might represent a monophyletic group. Furthermore, if the African species not present in the molecular tree are considered, striate pollen would also be included, although it is relatively rare in African species. The other forms and subtypes of fossulate, echinate and verrucate ornamentation make the picture still more diverse. In addition, several African species are distinctly polymorphic (see below). Species with echinate and verrucate pollen are restricted to Africa and Madagascar, except for *A. sylvaticus* from India which has verrucate pollen (not in tree; Fig. 2K).

Psilate ornamentation, which is dominant in the African clade, is more diverse in character here than in the other main clades. Ectexine thickness varies from 0.2 to 0.6 (0.9) μ m. *A. ankarana* pollen, however, is distinguished by a locally undulate surface of the ectexine and the presence of small dark granules (Fig. 3D), similar to those observed in the verrucate pollen of the probably unrelated *A. mildbraedii* (not in tree). The pollen of *A. lewallei* deviates by its thick ectexine (0.9 μ m) with dark granules in the inner part and a spongy structure in the outer part. It is reminiscent of the echinate pollen ectexine of *A. dracontioides* and the related *A. aphyllus* (not in tree), and the verrucate pollen ectexine of the Indian *A. sylvaticus* (not in tree; Fig. 2K), where the spines/verrucae also stand on a granular basal sublayer.

The fossulate African species are diverse in ornamentation and ultrastructure. Several of them, for example, *A. baumannii*, *A. gallaensis* (not in tree) and *A. mossambicensis* (not in tree), are polymorphic. *A. baumannii* A has fossulate to coarsely striate or psilate pollen, and the ectexine ultrastructure shows numerous very small granules (Fig. 3A), while *A. baumannii* B has coarsely striate or fossulate pollen with few large dark granules which are largest in the basal part of the ectexine (Fig. 3J). The ultrastructure of the ectexine in the fossulate to coarsely striate pollen of *A. gallaensis* B (not in tree) is more like that of the striate pollen of *A. gallaensis* A (not in tree) and *A. gomboczianus* (not in tree) with many crowded small dark granules.

Echinate ornamentation can be subdivided into three subtypes (Van der Ham et al. 1998, 2000). The pollen of *A. dracontioides*, the only echinate species in the tree, represents subtype a, with short, unstoreyed, basally connected spines (Fig. 2L, 3P). *A. aphyllus* and *A. elliottii* are also included in subtype a. Ectexine ultrastructure in *A. aphyllus* and *A. dracontioides* pollen reminds of that of the psilate pollen of *A. lewallei*. Subtype b (*A. doryphorus*) has more elongate, unstoreyed, isolated spines, while subtype c (*A. antsingyensis*) has elongate, storeyed spines.

With regard to ornamentation and ultrastructure the verrucate species make up a heterogeneous group. The pollen of A. abyssinicus is polymorphic: subspecies abyssinicus has verrucate pollen (Fig. 2J), and is the only verrucate taxon in the tree. Subspecies unyikae (not in tree) has fossulate to coarsely striate pollen. The pollen of subspecies akeassii is unknown. It is difficult to ascertain if each verruca in the verrucate ectexine of subspecies abyssinicus pollen (Fig. 3O) contains a single large dark granule (as in the spines of A. antsingvensis pollen), or many crowded fine granules, or no granules at all. A. maximus has one large, clearly demarcated granule per verruca which is not in contact with the endexine. The verrucate pollen of A. mildbraedii has irregular small granules in the basal part of each verruca; this is also observed in the pollen ultrastructure of A. ankarana where the pollen is psilate with a locally undulate surface (Fig. 3D). The pollen of A. preussii has several distinct, diversely sized granules per verruca. The only non-African vertucate species is A. sylvaticus (Fig. 2K) from India, the pollen of which has an ectexine ultrastructure more closely resembling that of the echinate pollen of A. aphyllus and A. dracontioides and the psilate pollen of A. lewallei than that of any of the verrucate species.

Possible pollen evolution

Outgroup comparison

The molecular tree shows that the echinate pollen of *Amorphophallus dracontioides* is not homologous with the echinate pollen of the closest outgroups *Arisaema*, *Filarum*, *Hapaline* and *Typhonium* (represented in Fig. 1 by *Arisaema*), because it is embedded midway in the African clade. In preliminary total evidence analyses including all five echinate *Amorphophallus* species, only *A. aphyllus* and *A. dracontioides* form a robust subclade, which supports the homology of the particular type of echinate ornamentation in the pollen of these two species. *A. elliottii* is usually excluded from this subclade, possibly because it is a poorly known species represented by the type specimen only. *A. doryphorus* (echinate subtype b) and *A. antsingyensis* (echinate subtype c) are isolated from each other in another, predominantly fossulate African clade, and without



connection to the echinate outgroups. This seems to support the independent, polyphyletic origin of echinate ornamentation in African *Amorphophallus* (Van der Ham et al. 2000). Of the echinate outgroups, only the pantropical *Arisaema*, together with the Asian genus *Typhonium* at the base of the echinate outgroups, occurs in Africa. Thus, it appears that the echinate outgroups do not have echinate descendants within *Amorphophallus*.

Further, the echinate outgroups have small pollen grains $(21-40 \ \mu\text{m})$. In *Amorphophallus*, the averages of the main clades vary from 53.7 to 60.5 μm , while species with relatively small pollen $(22-38 \ \mu\text{m}: A. \ cirrifer, A. \ dracontioides, A. galbra, A. sagittarius, A. smithsonianus) are not basal in their clades. These disparities suggest that the outgroups are only distantly related to$ *Amorphophallus*.

Ingroup

Although *Amorphophallus*, including *Pseudodracontium*, was demonstrated to be monophyletic, no pollen morphological traits were found to support this, nor could any of the four main clades be shown as a monophyletic group by pollen characters.

Each of the four main clades of the ingroup is dominated by either psilate (Fig. 2A) or striate (Fig. 2B) pollen. Although psilate and striate ornamentation occur together in several groups of related species (e.g. A. havi and A. symonianus), the relationship between these two types does not seem to be very close; polymorphic species combine psilate with fossulate (e.g. A. mossambicensis), and striate with coarsely striate and/or fossulate (e.g. A. brachyphyllus), but not psilate with striate. Psilate and striate ornamentation occur throughout the distribution area of Amorphophallus and are found basally as well as distally in the molecular tree. The less common types are found more distally, often in single species. This suggests that either psilate or striate ornamentation is plesiomorphic. The molecular analysis did not resolve this. Thin (up to 0.3 µm) psilate ectexines without dark granules occur in three of the four main clades. The striate type is diverse: without granules in the Asian psilate clade, without (basal species) or with crowded dark granules in the continental SE Asian striate clade, with less distinct and less densely distributed granules in the Malesian striate clade or with many crowded small dark granules in the few striate African species. Considering this, pollen with a thin psilate ectexine without granules might be plesiomorphic in Amorphophallus. This would imply that the dark granules observed in the

outgroups *Arisaema* (Ohashi et al. 1983) and *Typhonium* (Weber et al. 1998) are not homologous with those found in many *Amorphophallus* species. Within both striate main clades, reversals to the psilate type occur, which differ from the supposed plesiomorphic psilate pollen by the greater ectexine thickness. In the Malesian striate clade the reversals are accompanied by the loss of the dark granules. Within the African clade, thick psilate ectexines and psilate ectexines with granules may have evolved directly from thin psilate ectexines without dark granules.

Striate pollen with psilate caps (Fig. 2C), which is nested in the continental SE Asian striate clade, is a synapomorphy of *Pseudodracontium* (Cambodia, Laos, Thailand, Vietnam). The ultrastructure of the ectexine is the same as that of *A. longituberosus* and related species (Thailand, northern W Malaysia). The psilate caps might have evolved in an *albispathus/longituberosus*-like ancestor.

After psilate and striate ornamentation, fossulate (Fig. 2D-F) is the next commonest type in *Amorphophallus*. In the tree it is found in five species in three main clades. It has been described as a heterogeneous type, with more or less gradual transitions to the psilate and striate types (Van der Ham et al. 1998). Also ultrastructurally, it proved to be diverse, and its diffuse distribution in the molecular tree indicates it as a polyphyletic 'box of convenience'. Its diversity might well be explained by its diverse roots (psilate and diversely striate).

Reticulate (*A. ochroleucus*, Vietnam: Fig. 2M), scabrate (*A. galbra*, New Guinea, Queensland: Fig. 2N) and striate/ scabrate ornamentation (*A. smithsonianus*, India: Fig. 2O) are autapomorphies. The reticulate ectexine of *A. ochroleucus* can indeed be easily derived from a psilate ectexine without dark granules in the Asian psilate clade, and the striate/ scabrate ectexine of *A. smithsonianus* from a striate one without dark granules in the continental SE Asian striate clade. Derivation of the pollen of *A. galbra* is more difficult. In the tree it is nested in striate ornamentation, but it is hard to imagine an 'easy transition' between the two types.

Three other types, areolate (Fig. 2G-I), echinate (Fig. 2L) and verrucate (Fig. 2J, K) ornamentation, occur in single species in the tree, but these types occur also in related and unrelated species not included in the molecular analysis. Areolate pollen, found in six species restricted to continental SE Asia, shows two alliances: one around *A. cirrifer* in the Asian psilate clade (Fig. 2G, H) and another consisting of *A. saraburiensis*, *A. scutatus* and *A. tenuistylis* (Fig. 2I), which might belong in one of the striate main clades. Pollen of the *cirrifer* group might be derived from the

Fig. 3. Amorphophallus and Pseudodracontium pollen, transmission electron micrographs (TEM). A. A. baumannii A (Johnson s.n.), fossulate to psilate ectexine with numerous crowded very small dark granules. B. A. beccarii, psilate ectexine (with pollenkitt) without dark granules. C. A. napiger, psilate ectexine without dark granules. D. A. ankarana, psilate (locally undulate) ectexine with small dark granules. E. A. coaetaneus, striate ectexine without dark granules. F. A. rhizomatosus, striate ectexine without dark granules. G. A. ochroleucus, reticulate ectexine without dark granules. H. A. longituberosus, striate ectexine with crowded dark granules. I. P. lanceolatum, striate ectexine with crowded dark granules. J. A. baumannii B (H.AM 665), coarsely striate or fossulate ectexine with few loose large dark granules. K. A. maxwellii, fossulate ectexine (with pollenkitt) with dark granules in middle part. L. A. sagittarius, striate ectexine with loose dark granules. M. A. cirrifer, areolate ectexine with single large dark granule in basal part. N. A. scutatus, areolate ectexine with crowded dark granules in middle part. O. A. abyssinicus, verrucate ectexine (with pollenkitt) without (?) dark granules. P. A. dracontioides, echinate ectexine with small dark granules in basal part. Q. A. galbra, scabrate ectexine with few large dark granules. R. A. smithsonianus, striate/scabrate ectexine without dark granules. Scale bars -1 µm; ec – ectexine, en – endexine, p – pollenkitt.

striate type of a *cruddasianus*-like ancestor. The origin of the other (not in tree) is uncertain. Maybe it derives from a psilate type like that of the related *A. linearis* (not in tree).

Echinate pollen, found in five species restricted to Africa and Madagascar, is heterogeneous and has three subtypes (Van der Ham et al. 2000). Subtype a which includes *A. dracontioides* (Fig. 2L) and related species, can indeed be derived from *A lewallei*-like psilate type. The other two subtypes (not in tree) probably have other origins. In preliminary total evidence analyses, which includes all five echinate species, *A. doryphorus* (subtype b) and *A. antsingyensis* (subtype c) are separated from each other within a different, predominantly fossulate, African subclade which supports the polyphyly of echinate ornamentation in African *Amorphophallus* species.

Verrucate pollen too, five species in Africa, one in India, is clearly diverse (Fig. 2J, K), and the pollen of *A. sylvaticus* (Fig. 2K) is the most differentiated. Derivation from diverse psilate ancestors, as illustrated by the tree for *A. abyssinicus*, seems most likely.

CONCLUSION

Ultrastructure, though very diverse and difficult to subdivide, appears to provide a valuable contribution towards better understanding pollen of ornamentation in Amorphophallus. Consistent with the general tree topology, it designates most ornamentation types (psilate, striate, areolate, verrucate, echinate) as polyphyletic, while unexpected connections are revealed between superficially different types (e.g. striate/areolate, psilate/echinate). Although none of the main clades can be shown to be monophyletic from pollen morphology, each clade has its own suite of pollen features hardly present, or not present, elsewhere. The Asian psilate clade lacks dark granules (except for A. cirrifer) and fossulate pollen, and accommodates reticulate pollen and the areolate *cirrifer* type. The continental SE Asian striate clade lacks thin psilate ectexines, possesses granules, mostly crowded, except in the basal species, and includes striate/scabrate pollen and striate pollen with psilate caps. The Malesian striate clade shows granules, mostly loose, in all nonpsilate ectexines, and includes scabrate pollen. The African clade is the most heterogeneous: it lacks granules in most psilate species and some fossulate species, and includes all echinate pollen, and nearly all verrucate pollen. The Asian psilate and the African clades seem to be basal, while both striate clades might have evolved from the Asian psilate clade via a basal rhizomatosus-like species. Dark granules evolved more than once, which resulted in different make-ups regarding their size, shape and distribution. It is not clear whether Amorphophallus and, according to our scenario, psilate ornamentation originated in Africa or Asia.

Several distinctly polymorphic species occur, for example *A. abyssinicus* (verrucate *vs.* fossulate to coarsely striate) and *A. baumannii* (fossulate to coarsely striate or psilate with numerous very small granules *vs.* coarsely striate or fossulate with few large granules). These observations indicate that ectexine features are not necessarily fixed in *Amorphophallus*, and this might provide possibilities for selection and

evolution, and might also explain the remarkably diverse pollen ornamentation in *Amorphophallus* compared to other big genera in the family.

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