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Pollen morphology and ultrastructure of selected Araceae species

New examples for the significance of pollen characters in systematics and for the spathe as an osmophore

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1. ABSTRACT

This PhD-thesis gives insight into pollen morphology and ultrastructure of selected Araceae-species. New examples are provided for the significance of pollen characters in systematics as well as for the spathe as an osmophore. The present thesis demonstrates that the application of different preparation and staining methods as well as a combined analysis with light microscopy, scanning- and transmission electron microscopy are essential for the interpretation of pollen characters as well as for the study of floral organs related to pollination. Furthermore, this work shows that morphological studies of pollen grains are indispensable for the understanding of evolutionary processes and systematics. Four published studies are included and discussed. Six congress as well as seminar contributions are added as supplements. The thesis deals with the following three main topics:

1. Pollen morphology and ultrastructure of selected Araceae-species. — The genera of Araceae, also known as aroids, display a high morphological diversity, which extends to pollen wall morphology and exine sculpturing. In contrast to all other subfamilies of Araceae the pollen wall of Aroideae pollen lacks the common sporopollenin tectate-columellate exine. Instead, the outermost pollen wall layer consists of polysaccharides, which is a unique feature of some Aroideae pollen. The aim of this thesis is to give new insights into the pollen morphology and ultrastructure of selected Araceae species and to clarify contradicting literature reports. For this purpose, a total of 136 species (including 120 Aroideae) out of 69 genera (including 58 Aroideae) were investigated. The results of the investigated genus *Amorphophllus* and the schismatoglottid genera *Apoballis* and *Schismattoglottis* show that the pollen wall is exclusively made of polysaccharides. Moreover an additional thin surface layer, either polysaccharide or cuticula-like was detected. The investigation of *Calla palustris* tetrads in different developmental stages revealed that pollen is basically disulcate or with a ring-like aperture. *Calla* pollen is therefore extraordinary within the entire Araceae.

2. The significance of pollen characters in Araceae systematics. — One of the most important characters is the pollen wall, with its structural and

ornamental features. In comparative pollen morphology and plant systematics pollen characters are at least as important as any other morphological character. The pollen characters of the investigated genera *Apoballis*, *Schismattoglottis* and *Calla* accord well with recent DNA-based phylogenies. Pollen characters, especially the aperture condition of *Calla* combined with other morphological and anatomical characters gives direction for a good placement of the controversial genus *Calla* within the Araceae.The results show that they are an excellent example for the significance of pollen characters in Araceae systematics.

3. Ultrastructure of osmophoric epidermal cells. — Many Araceae have floral traps to catch their pollinators. Several organs of the aroid inflorescence are involved in trapping. To attract pollinators, epidermal cells of the spathe or even of anthers are known to produce nectar or wax. To test the hypothesis of the spathe as an osmophore also the ultrastructure of secretory epidermal cells of different *Colocasia* species was investigated. Osmophoric epidermal cells in spathes are indicated by the prescence of numerous mitochondria, smooth endoplamatic reticulum, ribosomes, polyribosomes and vesicles that are transported through the cuticle. To proof the hypothesis, adaxial and abaxial parts of the spathe were investigated by transmissionelectronmicroscopy to find ultrastructural evidence for the synthesis of odors.The ultrastructural results as well as the morpho-anatomical results of the spathe epidermis indicate that it is an elaborate osmophore and serves for the emission of odours only.

2. ZUSAMMENFASSUNG

Die vorliegende Dissertation gibt einen Einblick in die Pollenmorphologie und Ultrastruktur ausgewählter Araceae-Arten. Weiters liefert die Arbeit neue Beispiele für die Bedeutung der Pollenmerkmale in der Systematik sowie für die Spatha als Osmophor. Die vorliegende Doktorarbeit zeigt auch, wie wichtig die Anwendung verschiedener Präparationsmethoden, sowie die Kombination aus Licht- und Elelektronenmikroskopie für die Interpretation von Pollenmerkmalen als auch für die Studie von Blütenorganen im Zusammenhang mit der Bestäubung ist. Weiters zeigt diese Arbeit, dass morphologische Untersuchungen von Pollenkörnern für das Verständnis evolutionärer Prozesse und der Systematik unverzichtbar sind. Insgesamt sind vier Publikationen in dieser Arbeit inkludiert und diskutiert. Weiters sind sechs Kongress- sowie Seminarbeiträge als Anhang hinzugefügt. Die folgenden drei Hauptthemen werden behandelt:

1. Pollenmorphologie und Ultrastruktur ausgewählter Araceen Arten. — Die Gattungen der Araceae (Aronstabgewächse) zeigen eine große morphologische Vielfalt, die sich auch in der Struktur und Skulptur der Pollenwand zeigt. Im Gegensatz zu allen anderen Unterfamilien der Araceae, besitzt die Pollenwand der Aroideae kein Sporopollenin in der äußersten Wandschicht, sondern besteht aus Polysacchariden. Dieses Merkmal macht sie einzigartig. Das Ziel dieser Dissertation ist es, neue Erkenntnisse über die Pollenmorphologie und Ultrastruktur ausgewählter Arten der Araceae zu gewinnen und widersprüchliche Literaturangaben zu klären. Dazu wurden insgesamt 136 Arten (davon 120 Aroideae) von 69 Gattungen (davon 58 Aroideae) untersucht. Die Ergebnisse der untersuchten Gattungen *Amorphophallus*, *Apoballis* und *Schismatoglottis* zeigen, dass die äußere Pollenwand ausschließlich aus Polysacchariden besteht. Darüber hinaus wurde eine aufgelagerte dünne Schicht nachgewiesen, die entweder aus Polysacchariden besteht oder wachsartig ist (ähnlich einer Kutikula). Die Untersuchung von Pollentetraden bei *Calla palustris* zeigt, dass der Pollen im wesentlichen disulcate ist. Dieses Merkmal macht *Calla*-Pollen einzigartig innerhalb der gesamten Familie der Araceae.

2. Die Bedeutung von Pollenmerkmalen in der Araceen Systematik. — Bestimmte Merkmale, wie beispielsweise die Lage und Form der Keimöffnungen, die Oberflächenskulptur und Variationen im Aufbau der Pollenwand sind für die Taxonomie von großer Bedeutung, da sie evolutionäre und phylogenetische Zusammenhänge aufzeigen können. Die vorliegenden Ergebnisse zeigen, dass die Pollenmerkmale der Gattungen *Apoballis*, *Schismattoglottis* und *Calla* gut mit den aktuellen molekulargenetischen Studien übereinstimmen. Die Variationsbreite der Keimöffnungen bei *Calla* Pollen von disulcate zu ringförmig ermöglicht in Kombination mit anderen morphologischen Merkmalen eine gute Platzierung der bisher umstrittenen Gattung innerhalb der Araceen. Diese Ergebnisse sind exzellente Beispiele für die Wichtigkeit der Pollenmerkmale in der Araceae-Systematik.

3. Ultrastruktur und sekretorische Epidermiszellen. — Viele Araceae besitzen Kesselfallenblumen um ihre Bestäuber einzufangen. Für diesen Zweck können mehrere Bereiche des Blütenstandes am Bau der Falle beteiligt sein. Zur Anlockung der Bestäuber können die Epidermiszellen der Spatha oder auch die der Staubblättern Nektar oder Wachs produzieren. Duftproduzierende Epidermiszellen der Spatha sind characterisiert durch die Anwesenheit zahlreicher Mitochondrien, glattem Endoplamatischem Reticulum, Ribosomen, Polyribosomen und Vesikeln, die durch die Kutikula transportiert werden.Um die Hypothese zu testen, ob die Spatha bei der Gattung *Colocasia* Düfte produziert, wurde auch die Ultrastruktur sekretorischer Epidermiszellen unterschiedlicher *Colocasia* Arten untersucht. Die ultrastrukturellen Ergebnisse in Kombination mit anderen morphologisch-anatomischen Ergebnissen zeigen, dass die Spatha als Osmophor fungiert um Bestäuber anzulocken.

3. GENERAL INTRODUCTION

This PhD-thesis deals with pollen morphology and ultrastructure of the family Araceae, in particular of subfamily Aroideae, as well as with the spathe as an osmophore. The aim of the present thesis is to give new insights into the pollen morphology and ultrastructure of selected species and to clarify contradicting literature. This work presents four new examples on Araceae pollen, focused on the application of different preparation and staining methods, as well as a combined analysis with light microscopy, scanning- and transmission electron microscopy. A total of 136 species (including 120 Aroideae) out of 69 genera (including 58 Aroideae) were investigated.

3.1. THE ARACEAE FAMILY

3.1.1. Araceae systematics

Araceae are known as the ‘arum family’ or ‘aroids’ (Mayo et al. 1997). The family (including the Lemnoideae) is monophyletic and basal to the rest of the Alismatales (Stevens 2001; Keating 2002, 2004; Cabrera et al. 2008; Chartier 2011; Cusimano et al. 2011; Nauheimer et al. 2012; Chartier et al. 2013). It is the fouth largest family of monocots and comprises about 118 (to 132) genera and approximately 6000 species, with about 3500 published species (Boyce & Wong 2012; Boyce & Croat 2014). Moreover, new species are regularly discovered e.g. *Amorphophallus* in Madagascar (Hetterscheid & Claudel 2014) or many aroids of Malesia including many indigenous genera (Boyce & Wong 2012).

One of the oldest fossil records among angiosperms is from the Araceae (Nauheimer et al. 2012). Fossil pollen has been found in deposits in Portugal and dated to the Early Cretaceous (120-110 million years) (Friis et al. 2004; Hesse & Zetter 2007). Modern studies dated the crown group Araceae (the living representatives and their ancestors) to approximately 122 million years (Nauheimer et al. 2012). According to Nauheimer et al. (2012), the aroids began to diversify in the Early Cretaceous and all subfamilies

evolved before the Cretaceous-Tertiary Mass Extinction event (Friis et al. 2010; Nauheimer et al. 2012). An origin in wet habitats is assumed based on fossil records (Nauheimer et al. 2012), as well as on the ecological habitat of the early-diverging aroid clades, e.g. Orontioideae (Bogner et al. 2007).

The current generic framework is essentially that of Mayo et al. (1997) which bases on morpho-anatomical studies. At present eight subfamilies of Araceae are accepted: (1) Gymnostachydoideae, (2) Orontioideae, (3) Lemnoideae, (4) Pothoideae Engler, (5) Monsteroideae Schott, (6) Lasioideae; (7) Zamioculcadoideae and (8) Aroideae (Stevens 2001). The phylogeny proposed by French et al. (1995) and Mayo et al. (1997) is in most parts supported by all recent molecular analyses (Cabrera et al. 2008; Cusimano et al. 2011; Chartier et al. 2011, 2013; Nauheimer et al. 2012). Moreover they also support the subfamily Lemnoideae (former Lemnaceae) to be included within the Araceae (sister to Pothoideae).

The subfamily Calloideae, with the monospecific genus *Calla,* has attracted attention for many years and has recently become again controversial in the light of molecular phylogenies (Keating 2002, 2004; Cabrera et al. 2008; Chartier 2011, Cusimano et al. 2011; Nauheimer et al. 2012; Chartier 2013). In all morpho-anatomical-based studies *Calla* was placed in a subfamily of its own, the Calloideae (Grayum 1990, 1992; Bogner & Nicolson 1991; Mayo et al. 1997, 1998; Keating 2004). According to French & al. (1995) and Keating (2004), *Calla* is placed between Lasioideae and Aroideae. In recent molecular classifications, *Calla* emerged on quite different positions, mainly basal to or within the Aroideae (Cabrera et al. 2008; Chartier 2011; Cusimano et al. 2011; Nauheimer et al. 2012; Chartier et al. 2013). Hence, *Calla* (which has bisexual flowers) is placed together with the genera *Montrichardia* and *Anubias*, embedded among more derived Aroideae (which have unisexual flowers). According to Chartier et al (2013) neither placement of *Calla* has statistical support.

The Aroideae (unisexual flower clade) is the youngest and largest subfamily, which comprises about 72 genera and about 2670 species (Stevens 2001; Boyce et al. 2012). In the sense of Cabrera et al. (2008) and Cusimano et al. (2011) the subfamily Aroideae is composed of five main

supported clades, the *Zantedeschia* clade, Rheophytes clade, *Amorphophallus* clade, *Colletogyne* clade, *Pistia* clade and the three isolated genera *Anubias*, *Montrichardia* and *Callopsis*. The subfamily includes all unisexual-flowered taxa except for *Zamioculcas*, *Gonatopus*, *Stylochaeton*, which have been grouped as “expanded Zamioculcadoideae” (including *Stylochaeton*) and named *Stylochaeton* clade (Cabrera et al. 2008; Cusimano et al. 2011). In contrast, the Aroideae sensu Mayo et al. (1997) include all taxa with unisexual flowers (“unisexual flowers clade” by Cusimano et al. 2011), comprising the Zamioculcadoideae (*Gonatopus* and *Zamioculcas*) and *Stylochaeton* at its base.

3.1.2. Distribution and life forms

The family of Araceae is predominantly tropical in distribution and the majority of species (90% of genera and 95% of species) are restricted to the tropics (Boyce & Wong 2012). They are most diverse in the humid tropics (Neotropics, tropical Asia and the Malesian archipelago), but they are also distributed in the subtropics (Africa, Asia) and in habitats ranging from swamps to deserts (Madagascar, the Mediterranean region and Australia) (Mayo et al. 1997; Boyce & Croat 2014; Boyce & Wong 2012). Only few genera are distributed in temperate regions and those are either helophytes (e.g. *Lysichiton, Symplocarpus* and in boreal regions *Calla*) or geophytes (e.g. *Arum*, *Biarum*, *Dracunculus*) (Mayo et al. 1997). The aroids display a high morphological diversity and the majority of the genera are endemic to the major regions (Mayo et al. 1997).

Leaf blade size and shape is notably diverse and they may be simple or variously compound and highly divided (Mayo et al. 1997; Boyce et al. 2012). The Araceae are perennial herbs and regarding life-forms they are probably the most diverse family of flowering plants (Boyce & Wong 2012). Life forms include e.g. submerged plants (stems and leaves entirely underwater, e.g. *Cryptocoryne*), free-floating aquatics (Lemnoideae, *Pistia*), rheophytes (flood-resistant plants; majority of genera of tribe Schismatoglottideae), helophytes (various stem-types e.g. arborescent in *Montrichardia*, rhizomatous in *Calla*), terrestrials (most Aroideae), epiphyts

(e.g. *Remusatia*), hemiepiphyts (most genera of subfamilies Pothoideae and Monsteroideae), mesophytic herbaceous phanerophytes (e.g. some *Apoballis* and *Schismatoglottis*), or lithophytes or geophytes (e.g. *Amorphophallus*, *Thyphonium*) (Mayo et al. 1997; Boyce et al. 2012; Boye & Wong 2012).

3.1.3. Inflorescence and flower morphology

The Araceae can be easily defined by characters of the inflorescence. The typical aroid inflorescence is an unbranched spadix, bearing numerous small inconspicuous aperigoniate or perigoniate flowers, surrounded by a modified bract called the spathe (Mayo et al. 1997; Boyce et al. 2012; Boyce & Wong 2012). Moreover, even taxonomic groups can be well defined by the sex of the individual flowers and their arrangement on the spadix. The Aroideae are well delimited by unisexual flowers from all other aroid subfamilies (Mayo et al. 1997; Cusimano et al. 2011; Boyce et al. 2012; Boyce & Wong 2012). In the unisexual flowered genera the pistillate (female) flowers are usually arranged at the base of the spadix, which are occasionally separated by sterile flowers from the staminate (male) flowers above (Boyce & Wong 2012). All other aroid subfamilies (earlier diverging taxa) are characterised by bisexual flowers, which are mostly uniformly and densely arranged over the spadix and with a simple undifferentiated spathe (Mayo et al. 1997; Boyce & Wong 2012).

From this construction many different modifications can be found in the araceous genera, representing an evolutionary trend towards the formation of a pseudanthium (Mayo et al. 1997). Linked to the formation of the pseudanthium is a modification of the flowers into aperigoniate flowers, a specialization of the flowers into a female zone in the lower spathe tube and a male zone in the upper expanded blade, often with sterile flowers inbetween (Mayo et al. 1997; Boyce et al. 2012). This inflorescence type is typical for the Aroideae. Terminal appendices of the spadix are known to produce odours to attract pollinators (osmophore, Vogel 1963, 1990) and occurs in many tribes of the Aroideae (e.g. Areae, Thomsonieae, Schismatoglottideae). The appendix either is smooth and without flowers

(e.g. *Thyphonium*) or consists entirely of staminodes (e.g. *Amorphophallus*) (Boyce & Wong 2012).

The staminode flowers also show a high diversity (Boyce & Wong 2012). In perigoniate flowers and aperigoniate bisexual flowers of most Monsteroideae the stamens comprise a distinct filament, basifixed anther and a thin connective. Contrary, the stamens in unisexual flowers of most Aroideae are with short or without a filament and with a thick, fleshy connective which might acts as an osmophore. Furthermore, stamens can be fused into synandria (e.g. Colocasieae, Spathicarpeae) (Boyce & Wong 2012). Anthers are usually terminal, facing outward from the axis (extrorse), composed of two thecae each with two microsporangia. Theca dehiscence can be by apical, or subapical pores or short slits (many Aroideae) which is frequently correlated with the extrusion of pollen in strands, or by a longitudinal rarely transverse short slit as found in most bisexual-flowered genera and some unisexual-flowered genera (Mayo et al. 1997; Boyce & Wong 2012; Hetterscheid 2012).

In summary, from the typical aroid inflorescence a wide range of morphological variations exists. In particular the spathe, the spadix and the flowers are subject to various modifications, including the formation of the pseudanthium within the Aroideae subfamily.

3.1.4. Pollination biology in the Araceae

All aroid genera are protogynous (Mayo et al. 1997; Boyce et al. 2012). In the unisexual flowered taxa the anthesis is clearly separated into a pistillate and staminate phase (Boyce et al. 2012), usually involving trapping mechanisms in which pollen is received on the first day and exported on the second (Chartier et al. 2013). Araceae pollen is usually produced in huge amounts and extruded in strands or powdery, finally deposited on trapped or picked up by escaping insects (Gibernau 2003; Punekar & Kumaran 2010; Bröderbauer 2012). Aroids with unisexual flowers (e.g. *Amorphophallus*, *Montrichardia*) are known to have a short flowering cycle (two days) and pollen is viable for a few days only (Gibernau et al. 2003; Barabé et al. 2008). For example, pollen of *Montrichardia arborescens* is not viable after 24 hours and no pollen

tube formation occurs after 36 hours (Barabé et al. 2008). For two *Arum* species, it is documented that the flowering cycle and the pollen viability takes two (to three) days only (Gibernau et al. 2003). The hypothesis that the lack of a sporopollenin outer pollen wall - as characteristic for Aroideae - is correlated with short pollen viability and rapid germination was assumed by Hesse (2006a, 2006b).

Depending on the pollination mode the outer pollen wall may be either highly ornamented, often with plenty of pollen coatings (mainly pollenkitt), or with a more or less smooth pollen surface. The pollen wall of zoophilous plants, as well as autogamous plants, is usually highly ornamented and the thick exine consists of high amounts of sporopollenin (Fægri & Iversen 1989). Whereas pollen of anemophilous plants are known to have less ornamentation and less sporopollenin (Hesse 1980, 2006a; Friedman & Barrett 2009). Usually psilate pollen of temperate and boreal zones is indicative for anemophily (Fægri & Iversen 1989), whereas in the tropics it is rather indicative for zoophily (Furness & Rudall 1999). For example, in Aroideae (e.g. *Montrichardia*, *Dieffenbachia*, *Philodendron*, *Gearum*) psilate pollen, together with its sticky surface, is adapted for entomophily (Weber & Halbritter 2007).

The Araceae mainly include three types of pollinating insects: bee, beetle, and fly. Quite often the inflorescence becomes part of the reproductive cycle of the pollinator (Gibernau 2003). A correlation between pollinator type and pollen ornamentation is suggested by some authors (Grayum 1992; Sannier et al. 2009; Gibernau et al. 2010). Usually beetle pollination is correlated with psilate pollen and fly pollination with echinate pollen (Grayum 1992). In their study, Sannier et al. (2009) suggest that the correlation between psilate pollen and pollination by beetles seems specific to the Araceae, given that pollen of insect-pollinated species is usually highly ornamented for a better storage of pollen coatings. In the Araceae however, pollen grains are depicted as poor in pollenkitt. It was suggested that sticky secretions on the stigma and/or the inner spathe surface may play the same role as pollenkitt, accounting for the lack of pollen ornamentation in beetle pollinated species (Hesse 1980; Hesse 2006a; Sannier et al. 2009). In more

recent studies (Chartier et al. 2013), correlations between pollinator type and pollen ornamentation are not found, probably due to the different and more numerous genera included in their study. Information on Araceae pollinators and their behaviour on the inflorescences are still rare. In many aroid species, flowers are visited by various insects (often more than 50 species), although only a single or a small number effectively act as pollinators (Sannier et al. 2009; Bröderbauer et al. 2013). A mixed pollinated system (generalist species), is suggested for e.g. *Calla palustris* (Gibernau et al. 2010).

3.1.5. Trap pollination

The aroid inflorescence acts as a single large flower to attract pollinators (Mayo et al. 1997; Boyce et al. 2012; Bröderbauer 2012). Many Araceae have floral traps to catch pollinators. Having unisexual flowers seems to be the morphological precondition for the evolution of traps in Araceae (Chartier et al. 2013). A recent analysis by Bröderbauer et al. (2012) inferred that trapping inflorescences (some offering liquid substances for the trapped insects) evolved at least 10 times in 27 genera (out of 120–130 genera of the family). Such floral traps, called trap blossoms or kettle traps (Kesselfallenblumen) according to Vogel (1965), are found in many Araceae genera (Bröderbauer 2012). The Araceae include taxa with rewarding and deceptive (e.g. mimicking a brood site) trap pollination systems (Bröderbauer 2012; Chartier et al. 2013). The family contains the largest number of deceptively pollinated species, aside from the orchids (Renner 2006; Chartier et al. 2013).

Several organs of the aroid inflorescence are involved in trapping. Elaborated modifications of the spathe and spadix are often mechanisms to trap pollinators, such as: the terminal appendix of the spadix, separate chambers formed by the division of the spathe, emission of odours (osmophore function) of either the spathe and/or the spadix, colour patterns as well as the persistence of the spathe and papillate epidermis cells of the spathe (e.g. Vogel 1963; 1990; Mayo et al. 1997; Bröderbauer 2012). Moreover for sterile flowers it is assumed that they may have diversified from

osmophores to trapping devices in the Areae clade (Bröderbauer 2012). Papillate cells on the adaxial side of the spathe are known form slippery surfaces to trap pollinating insects (Poppinga et al. 2010). In some species the papillate epidermis cells do not point downwards, as found e.g. in some *Colocasia* species (Bröderbauer et al. 2014). Moreover, epidermal cells of the spathe or even of anthers are also known to produce nectar (e.g. *Spathicarpa*, unpublished data) or wax (Mayo et al. 1997; Boyce et al. 2012). The occurrence of odour-emitting glands, called osmophores, was first described by Vogel (1963). Odour production in Araceae is generally associated with the spadix or with the sterile terminal appendix as typical for later diverging clades (Vogel 1963). For example, in *Sauromatum*, sterile flowers situated below the staminate flowers produce odours (Hadacek & Weber 2002). The emission of odours is often correlated with thermogenesis, as it intensifies odour emission and also serves as a heat reward for departing beetles (Barthlott et al. 2009; Seymour et al. 2003, 2009). However, odour production by the spathe and/or spadix has been recorded in several taxa, e.g. *Sauromatum, Arum* (Kite et al. 1998; Hadacek & Weber 2002). The main energy supply for odour synthesis is starch, which is stored in epidermal and parenchyma cells. In other angiosperms, lipids are known to be an important resource for odour production (Hadacek & Weber 2002; Wiemer et al. 2009). The odour producing cells usually show a dense cytoplasm and high intracellular activity, indicated by numerous organelles that are characteristic for the synthesis of various compounds e.g. vesicles that are transported through the cuticle (Hadacek & Weber 2002; Bröderbauer et al. 2014). Such adaptations are reported for several species, but still poorly investigated, especially by transmission electron microscopy.

Table 1. Araceae taxa/clades in morpho-anatomical and recent molecular studies including some important pollen and flower characters.

Araceae subfamilies sensu Mayo et al. (1997) and Cabrerea et al. (2008); in part sensu Cusimano et al. (2011)	**Aroid clades in recent phylogenies** with well supported clades in bold (Cusimano & al. 2011; Nauheimer et al. 2012; Chartier & al 2013)	**Aperture type**	**Ornamentation types**	**Pollen wall ultrastructure** (pollen wall type according to Weber & al. 1999)	**Flower sexuality** Mayo & al. (1997)	**Perigone** Mayo & al. (1997)	**Flowering behavior** Mayo & al. (1997)
Gymnostachydoideae Bogner et Nicolson	Proto-Araceae	sulcate	reticulate	Type 1a	bisexual	present	acropetal
Orontioideae Mayo, Bogner et Boyce	Proto-Araceae	sulcate	reticulate	Type 1a	bisexual	present	acropetal
Lemnoideae Engler	Spirodela clade	ulcerate	echinate, micro-echinate	Type 1a	bisexual	absent	acropetal
Pothoideae Engler	Spirodela clade	sulcate to (rarely) porate	reticulate	Type 1a	bisexual	present	acropetal
Monsteroideae Schott	Bisexual climbers clade	ring-like	psilate, perforate	Type 1a	bisexual	present or absent	acropetal
Lasioideae Engler	Podolasia clade	sulcate	reticulate to microreticulate	Type 1a	bisexual	present or absent	basipetal
Calloideae Endl.	*Calla* (within Aroideae)	disulcate or ring-like	psilate, perforate	Type 1a*	bisexual	absent	acropetal
Zamioculcadoideae Bogner et Hesse	*Stylochaeton* clade *(Gonatopus, Zamioculcas)*	ring-like	psilate, perforate	Type 1a	unisexual	present	simultaneously
Stylochaetoneae Schott	*Stylochaeton* clade *(Stylochaeton)*	inaperturate	foveolate, microreticulate	Type 1a	unisexual	present	simultaneously
Aroideae Arnott	***Callopsis, Montrichardia, Anubias*** *Anchomanes clade, Philodendron* clade, *Homalomena* clade, ***Zandedeschia* clade**, **Rheophytes *clade***, *Philonotion clade,* ***Amorphophallus* clade**, *Dracunculus* clade, ***Colletogyne* clade**, *Ambrosina* clade, *Colocasia* clade, ***Pistia* clade**, *Alocasia* clade	inaperturate (omniaperturate)	psilate, verrucate, plicate, striate, gemmate, or echinate	Type 1b (rare) Type 2a (most) Type 2b (*Zantedeschia*)	unisexual	absent	simultaneously

*In pollen wall type 1a (acoording to Weber & al. 1999) the intine is bi-layered over the whole pollen wall. In pollen of *Calla*, the intine was bi-layered in the aperture only.

3.2. POLLEN OF ARACEAE

The morphology of monocot pollen, especially of Araceae, has been studied iteratively since the pioneering work of Thanikaimoni (1969), Zavada (1983) and Grayum (1992). In his comprehensive study of about 380 species, representing 99 genera, Grayum (1992) gives a detailed description of the main pollen characters, including pollen type, shape, pollen unit, size, and exine sculpturing.

Pollen shape and aperture type directly relate to pollen polarity, which is determined by the orientation of the microspores within the tetrad (e.g. Albert et al. 2011). The Araceae family has a wide range of aperture types, of which inaperturate is the most frequent type (Furness & Rudall 1999; Boyce et al. 2012). All bisexual-flowered aroid genera are characterized by aperturate pollen grains, whereas the unisexual-flowered genera (Aroideae) have inaperturate pollen. The bisexual-flowered genera are subdivided in subfamilies by means of the aperture type: sulcate (Gymnostachydoideae, Orontioideae), ulcerate (Lemnoideae), sulcate to (rarely) porate (Pothoideae), ring-like aperture (Monsteroideae) or diaperturate (*Calla*, Calloideae). Some exceptions are found in tribe Spathiphylleae (inaperturate pollen, bisexual-flowered), *Anadendrum* (bisexual-flowered, inaperturate pollen), and tribe Zamioculcadeae (unisexual-flowered, ring-like aperture). During germination, usually a single pollen tube is formed at one of the germination sites or in inaperturate pollen grains from anywhere of the pollen surface (omniaperturate) (Hesse et al. 2009).

3.2.1. Pollen wall ornamentation and stratification

The high morphological diversity of the genera of Araceae also extends to pollen wall morphology and exine sculpturing (Grayum, 1992; Mayo et al.1997; Hesse, 2006a, 2006b). Pollen ornamentation of subfamily Aroideae is mostly psilate (smooth) or echinate (spiny). One outcome of the current palynological research is the almost absolute presence of psilate or verrucate pollen in all earlier-diverging clades of Aroideae, including Schismatoglottideae (Cusimano et al. 2011). Until recently the monospecific

genus *Callopsis* was the only example with echinate pollen within the earlier-diverging clades. Further exceptions are also found in Schismatoglottideae, as pollen of the resurrected genus *Apoballis* revealed to be echinate (Ulrich et al. 2012). Echinate pollen is typical for all more derived clades of Aroideae, except for the genus *Amorphophallus*, where many different ornamentation types occur within a single genus (e.g. Grayum 1992; Van der Ham et al.1998, 2005; Ulrich et al. 2015).

During pollination pollen may be transported over a long distance. For this purpose the genetic material is well protected by the pollen wall (Fægri & Iversen 1989; Hesse et al. 2009). The pollen wall of seed plants usually consists of two main layers: (1) the outer exine, with ektexine and endexine, which mainly comprises the acetolysis-resistant biopolymer sporopollenin (e.g. Steemans et al. 2010, Jardine et al. 2015), (2) the inner intine, which comprises polysaccharides (Hesse et al. 2009). Various studies on angiosperm pollen, demonstrated the high diversity in pollen wall morphology. Variations from the “standard pollen wall type” exist in that all layers of the wall may be partly or totally reduced. The sporopollenin ektexine is lacking e.g. in some genera of Monimiaceae and Lauraceae (Walker 1976), in the aquatic Ceratophyllaceae (Takahashi 1995) and in many genera of Aroideae subfamily. To date, information concerning the wall structure of Araceae pollen is still rare (e.g. Ohashi et al. 1983; Lugardon et al. 1988; Weber et al. 1998; Hesse et al. 2001; Hesse 2006a, 2006b; Ulrich et al. 2012, 2013, Van der Ham et al. 1998, 2005).

In contrast to all other subfamilies the pollen wall of Aroideae pollen lacks the common sporopollenin tectate-columellate exine. Instead, the outermost pollen wall layer consists of polysaccharides. This polysaccharide wall ornamentation is a unique feature of Aroideae pollen, first documented in *Arum italicum* (Pacini & Juniper 1983), and later in *Sauromatum venosum* (Weber et al. 1998). Furthermore, Weber et al. (1999) defined two basic pollen wall types in Araceae, each type including 2 subtypes.

Table 2: Araceae pollen wall types sensu Weber et al. 1999:

Type 1	**Pollen walls with ektexine** Sporopollenin ektexine (acetolysis-resistant), continuous spongy bilayered endexine, usually bilayered or monolayered intine
Subtype 1a	**Sporopollenin, thick structured tectate-columellate ektexine** e.g. *Anthurium*, *Calla*, *Spathiphyllum*
Subtype 1b	**Sporopollenin, thin unstructured ektexine** *Arisaema, Caladium*, *Callopsis*, *Remusatia*, *Spathicarpa* and *Synandrospadix* and the special case of *Montrichardia* lacking an endexine
Type 2	**Pollen walls without ektexine** Polysaccharide outer pollen wall (not acetolysis resistant), continuous spongy bilayered endexine, usually bilayered or monolayered intine
Subtype 2a	**Polysaccharide outer wall layer or polysaccharide ornamenation elements**, and some species **with additional polysaccharide surface layer** e.g. *Arum*, *Pistia, Sauromatum, Alocasia*
Subtype 2b	**Endexine, forms the outermost pollen wall layer** unique for *Zantedeschia*

Type 1 includes pollen with an acetolysis resistant ektexine, a continuous spongy bilayered endexine and a usually bilayered or monolayered intine. The two subtypes characterise pollen with either a sporopollenin, thick structured tectate-columellate ektexine (subtype 1a, e.g. *Anthurium*, *Calla*, *Spathiphyllum*) or pollen with *a* sporopollenin, thin unstructured ektexine (subtype 1b, e.g. *Spathicarpa*, *Callopsis*). Pollen of type 2 is characterised by the absence of a spopollenin tectate-columellate ektexine. Instead, the outer pollen wall comprises polysaccharides, which are not acetoylsis resistant. The subtypes are characterised by the presence (type 2a) or absence (type 2b) of polysaccharide ornamentation elements covering the endexine. Subtype 2a constitutes either a polysaccharide outermost pollen wall layer or polysaccharide ornamentation elements (echini, verrucae, plicae, striae) covering the endexine. Moreover, an additional polysaccharide layer may cover the pollen surface (e.g. *Arum*,

Pistia). This surface layer can be very thin (e.g. *Sauromatum, Alocasia*) and might therefore be easily overlooked. The pollen wall of subtype 2b constitutes a thick intine and thick endexine, which forms the outermost pollen wall layer. To date, this subtype is unique for the genus *Zantedeschia*. More recently a further type (type 1b, lacking an endexine) was found in *Montrichardia*, where the pollen wall constitutes a thick intine and a thin sporopollenin ektexine, without an endexine (Weber & Halbritter 2007). Pollen of *Montrichardia* is special case in many ways as pollen germination begins with a massive expansion of the thick intine, resulting in an explosive opening of the pollen wall (Weber & Halbritter 2007).

In contrast to all other subfamilies of Araceae the pollen wall in Aroideae lacks the common sporopollenin tectate-columellate exine, excluding *Calla*. Within the Aroideae the outer pollen wall of most species comprises polysaccharides. To date, only few genera are known to have thin unstructured sporopollenin ektexines, e.g. *Arisaema, Caladium*, *Callopsis*, *Montrichardia, Remusatia*, *Spathicarpa* and *Synandrospadix* (pollen wall type 1b, Weber et al. 1999). For some aroid taxa there is still need of knowledge. For example, the assumed the presence of sporopollenin in the outer pollen wall of *Amorphophallus* (Punekar & Kumaran 2010; Van der Ham et al. 2000) could be refuted by our results (Ulrich et al. 2015).

3.2.2. Ontogenetic aspects (microspores, pollen tetrads and mature pollen)

Pollen is the place of origin and transport vehicle for the male gametes (sperm cells) of seed plants. Pollen development includes microsporogenesis (diploid microspore mother cells to tetrad stage) and microgametogenesis (formation of the sperm cells) (Hesse et al. 2009). At the beginning of microsporogenesis, the diploid microspore mother cell is enclosed by a thick callose wall and undergoes meiosis, forming a tetrad of four haploid microspores. The callose wall is also responsible for the separation of the microspores, as well as from the surrounding tapetal cells. The formation of the pollen wall usually begins within the tetrad, while still enclosed by callose (Hesse et al. 2009).

The presence of a callose and of a primexine, which are both polysaccharides, is common for almost all angiosperm pollen, aside from Aroideae (Hesse 2006b). The primexine is a layer formed during an early developmental stage, wherein the later exine structures are preformed (Hesse et al. 2009). The fact that a callose wall can be absent in the microspore ontogeny of Aroideae was first documented for *Arum alpinum* (Anger & Weber 2006). Compared to all other angiosperms, the ontogenetic timetable is different in Aroideae as the endexine is formed in the tetrad stage before the formation of the outer pollen wall layer and the intine, which are formed simultaneously (Hesse 2006b). In angiosperms a tectate-columellate ektexine is usually formed before the endexine (Ubera Jiménez et al. 1996). The outer pollen wall layer in Aroideae - as known from *Sauromatum* (Weber et al. 1998) and *Arum* (Anger & Weber 2006) - is produced exclusively by the amoeboid tapetum shortly after dissociation of the tetrad. By contrast, ektexines are usually produced by the microspore and tapetum during the early microspore stage (Heslop-Harrison 1971; Pacini & Juniper 1983; Hesse 2006b). This mode of exine formation is also typical for Araceae, except for the Aroideae (Hesse 2006b). As known from the pollen wall formation of *Arum alpinum*, the amoeboid tapetum is responsible for the separation of the microspores from the tetrad arrangement (Anger & Weber 2006). Moreover, in late tetrad stage, the tapetum forms the polysaccharide spines (Anger & Weber 2006). As far as known, the amoeboid tapetum is the common type within the Araceae (Pacini & Juniper 1983; Pacini et al. 1985; Grayum 1991; Weber et al. 1998; Anger & Weber 2006; Hesse 2006b). In Aroideae, observations of pollen tetrads are very rare (e.g. Anger & Weber 2006; Ulrich et al. 2013, 2015). In Aroideae, tetrads are difficult to find as the male flowers are usually flowering simultaneously and for one or two days only. Tetrads can be found in closed anthers about three days before the female flowers become receptive. With the exception of Lasioideae (basipetally flowering), all Araceae with bisexual flowers, including *Calla*, are flowering in acropetal (from base to top) sequence (see also table 1).

Mature pollen is usually shed as single pollen grains (monads) or in tetrads, as documented for two genera only (*Xanthosoma, Chlorospatha*) (Mayo et al. 1997; Bogner & Hannon 2007). As far as known, only in 25% of flowering plants pollen is shed in a three-celled (tri-nuclear) stage, whereas in the vast majority of flowering plants pollen is two-celled (bi-nuclear). In the latter case, the division of the generative cells into the two sperm cells starts within the pollen tube. The cell number is a character that is highly conservative at the generic level and thus do not vary within a genus (Grayum 1986). Despite, two- and three-cellular species exist within one genus e.g. *Schismatoglottis,* (Ulrich et al. 2013). Grayum (1986) studied the nuclear cell number of 74 aroid genera and found tri-nucleate pollen to be typical for the higher aroid taxa, while bi-nucleate pollen is regarded as the primitive type (Grayum 1986). Moreover the author did not find a correlation between the pollen size, starch content and pollinator type. To date, the nuclear cell number of many taxa remains unknown, whereas for some taxa deviating literature exists (e.g. Hyndman 2001).

According to Mayo et al. (1997), most aroid pollen (73%) contains starch as reserve (e.g. *Amorphophallus*), which may also vary within a single genus (e.g. in *Schismatoglottis*). Starch-less pollen is mainly found in sulcate species and therefore considered to be the primitive type in Araceae (Mayo et al. 1997). Furthermore, pollen collected by dipterans and hymenopterans - especially by bees - are generally starch-less (e.g. Endress 1994; Mayo et al. 1997). According to Baker and Baker (1979) the type of reserves is related to pollen size and pollinator type. Small pollen is reported to contain lipids rather than starch, which is confirmed by the present work (Ulrich et al. 2012, 2015).

3.2.3. Method diversity in pollen wall investigations and the problem of misinterpretations

Although the considerable diversity of the pollen morphology in *Amorphophallus* has been demonstrated in previous studies (e.g. Thanikaimoni 1969; Tarasevich 1988; Grayum 1992; Van der Ham et al. 1998, 2005), contradicting literature exists about the chemical nature of the

pollen wall, as well as on the resistance of *Amorphophallus* pollen to acetolysis. Whether pollen is resistant to acetolysis or not, is correlated with the presence or absence of sporopollenin in the pollen wall (Hesse 2006a). According to Thanikaimoni (1969), Tarasevich (1988, 1992), Weber et al. (1999), Hesse et al. (2000) and Van der Ham et al. (2005) in *Amorphophallus* the pollen ornamentation does not resist acetolysis. The hypothesis, that a slow dissolution of the ornamenting elements may be due to incorporation of sporopollenin, was put forward by Van der Ham et al. (2000). Only Grayum (1992) and more recently Punekar and Kumaran (2010) reported that pollen of *Amorphophallus* species retained an intact patterned surface after acetolysis. Given that some *Amorphophallus* species showed resistance to acetolysis the presence of sporopollenin in the outer pollen wall (ektexine) was assumed (Punekar & Kumaran 2010; Van der Ham et al. 2000). Furthermore, Van der Ham et al. (1998) supposed a correlation between the low acetolysis resistance and the presence of electron-dense (dark) granules in the outer pollen wall. Despite the various studies on the pollen morphology and ultrastructure, the chemical nature of the pollen wall remains unclear. In the present thesis, the application of different histochemical staining methods (TEM) proved whether pollen wall layers are made of polysaccharides, sporopollenin or lipids.

There are a number of studies reporting difficulties in discerning endexines, a distinct layer between ektexine and intine (e.g. Zavada 1983; Grayum 1992). The first ultrastructural studies on Araceae pollen focused on the presence or absence of an endexine, given that many species of Araceae and other monocots were reported to lack this layer (Zavada 1983). Later, Grayum (1992) detected an endexine in five genera of Araceae. Weber et al. (1998) used different techniques and histochemical staining methods, especially the lipid test, to demonstrate the existence of an endexine in many species of Araceae. In a comprehensive study based on 60 Araceae species, representing 47 genera, a bi-layered spongy endexine was found in all investigated Araceae (Weber et al. 1999).

Given that the endexine manly comprises of lipids, it usually stains electron-dense with the lipid test. However, even after this staining

technique, the endexine is not always visible. The staining behaviour is very heterogeneous and often leads to misinterpretations. Even within the same plant family or the same genus, the staining properties can be very different (e.g. Weber & Ulrich 2010; Ulrich et al. 2015). If the endexine remains unstained after the lipid-test, staining with potassium permanganate can contribute to clarification (Ulrich 2006, Weber & Ulrich 2010; Ulrich et al. 2015).

3.3. POLLEN AND SYSTEMATICS

In comparative pollen morphology and plant systematics pollen is at least as important as any other morphological character. One of the most important characters is the pollen wall, with its structural and ornamental features. (e.g. Hesse 2006c; Hesse et al. 2001; Hesse 2009; Hesse & Blackmore 2013; Ulrich et al. 2012, 2013).

Pollen characters of Araceae accord well with recent phylogenies and phylogeny-supported taxonomic accounts (Mayo et al. 1997, Hay & Yuzammi 2000, Keating 2002, 2004, Cabrera et al. 2008, Cusimano et al. 2011). Comparative studies of aroid pollen structure by Thanikaimoni (1969) and Grayum (1992) showed that palynological characters are important for the suprageneric taxonomy. For example, all bisexual-flowered aroid genera are characterized by aperturate pollen. Moreover, they are subdivided in subfamilies by means of the aperture type (Mayo et al. 1997) e.g. within the sulcate Lasioideae, monophyly is strongly supported by their unique aperture characters (Hesse 2002). In contrast the unisexual-flowered genera (Aroideae) have exclusively inaperturate pollen (Mayo et al. 1997).

They can be mainly assigned to two groups: (1) smooth pollen with sporopollenin ektexine (type 1b) as is typical for the early-diverging tribes or (2) ornamented pollen with polysaccharide outer wall layer (type 2a) as is typical for the later-diverging tribes. Moreover, Aroideae genera with mainly echinate pollen (despite *Amorphophallus*) and an polysaccharide outer pollen wall (type 2a) are typical for Thomsonieae up to Areae (e.g. Weber et al. 1998, 1999; Cabrera et al. 2008; Ulrich et al. 2015).

Nowadays the molecular data are well supported by pollen characters gained by a combined use of LM, SEM and TEM studies (e.g. Weber 1998, 1999; Hesse 2006a, 2006b, 2006c; Anger & Weber 2006, Weber & Halbritter 2007). In the Araceae systematics the position of some taxa are again controversial due to recent molecular studies, such as the puzzling case of the genus *Calla* (e.g. Cabrera et al. 2008, Cusimano et al. 2011; Nauheimer et al. 2012; Chartier et al. 2013).

3.4. AIMS OF THIS STUDY

This thesis aims to contribute to two main topics: (1) Pollen morphology and ultrastructure of selected Araceae-species and the significance of pollen characters in Araceae systematics (Chapters 3A, 3B, 3C) and (2) Ultrastructure of osmophoric epidermal cells (Chapter 3D). Moreover, this thesis focuses on the application of different preparation and staining methods as well as a combined analysis with light microscopy, scanning- and transmission electron microscopy.

1) Pollen morphology and ultrastructure of selected Araceae-species and the significance of pollen characters in Araceae systematics

The first part of the thesis (Chapter 3A) is a case study on pollen of the genus *Schismatoglottis* and *Apoballis*. The aim of the investigation was to use pollen as an additional character for generic delimitation of the two closely related genera. *Schismatoglottis* pollen, as so far analysed, was reported to be psilate, typical for all Schismatoglottideae. During the study pollen of one species, at that time determined as *Schismatoglottis lancifolia*, reveals to be echinate. At the same time, it turned out, that this species was

transferred to the resurrected genus *Apoballis*. Subsequently, 12 former *Schismatoglottis* species were also transferred. The genus *Apoballis* is well defined by palynological and molecular characters and is sister to all other Schismatoglottideae.

The second part of the thesis (Chapter 3B) deals with pollen of the monospecific genus *Calla*, which is unique in the entire family of Araceae. The aim was to provide new insights into pollen morphology and the ultrastructure with special regard to its controversial systematic position. The placement of *Calla* within Araceae has attracted attention for many years and has recently become again controversial in the light of molecular phylogenies. In all morpho-anatomical-based studies *Calla* was placed in a subfamily of its own, the Calloideae. In recent molecular classifications, *Calla* emerged on quite different positions, mainly basal to or within the Aroideae. The intention was to briefly review the current status of systematic research with molecular and morpho-anatomical methods. Therefore we (1) clarified the aperture condition, (2) compared the pollen characters of *Calla* with pollen characters of the most closely related taxa in the various molecular phylogenies and (3) mapped the selected flower characters and palynological characters on the cladograms to place *Calla* in a distinct position within the Araceae.

The third part of the thesis (Chapter 3C) is an extensive study on the pollen morphology and ultrastructure of the genus *Amorphophallus*. The considerable pollen diversity has been demonstrated in previous studies. However, some questions remained ambiguous such as the chemical nature of the outer pollen wall (‘ektexine’) and of the granules associated within it. The polarity of *Amorphophallus* pollen has also been unclear as observations of pollen tetrads are very rare. The aim of the present study was to clarify contradicting reports on pollen characters and to test conclusions based on different preparation techniques. We specifically addressed the following questions: (1) Can we specify shape and polarity of the inaperturate ellipsoidal pollen by observing pollen tetrads? (2) Is the outer pollen wall made of polysaccharides or sporopollenin? (3) Are electron-dense (dark) granules in the outer pollen wall of some species made of sporopollenin or

polysaccharides? (4) If and to what extent does pollen may retain its ornamentation after acetolysis and which parameters influence the results? (5) To what extent are the results influenced by the preparation methods and staining techniques?

2) *Ultrasturucture of secretory epidermal cells of Colocasia*

The fourth part of the thesis (Chapter 3D), deals with secretory epidermal cells of the genus *Colocasia*. The aim was to investigate the role of adaptations for brood-site pollination in inflorescences of *Colocasia* that resemble adaptations for trap pollination present in other taxa of Araceae. Many aroids have floral traps to catch their pollinators. Several organs of the inflorescence are involved in trapping. To attract pollinators, epidermal cells of the spathe or even of anthers are known to produce nectar or wax. For different *Colocasia* species, the hypothesis was put forward for the spathe to act as an osmophore. Osmophoric epidermal cells in spathes are indicated by the presence of numerous mitochondria, smooth endoplasmic reticulum, ribosomes, polyribosomes and vesicles that are transported through the cuticle. To proof the hypothesis we investigated the ultrastructure of secretory epidermal cells of different species to find ultrastructural evidence for the synthesis of odours.

The results of the four papers (chapters) are reviewed in the general discussion (Chapter 5).

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4. PUBLICATIONS

A. *Schismatoglottis* and *Apoballis* (Araceae: Schismatoglottideae): A new example for the significance of pollen morphology in Araceae systematics

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SYSTEMATICS AND PHYLOGENY

Schismatoglottis and *Apoballis* (Araceae: Schismatoglottideae): A new example for the significance of pollen morphology in Araceae systematics

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Abstract Pollen characters in Araceae accord well with recent DNA-based phylogenies, and here we provide a new example of "compass needle" quality in Araceae on the basis of two closely related genera, *Schismatoglottis* and *Apoballis*. All investigated *Schismatoglottis* pollen is psilate (smooth pollen surface) with calcium crystals covering the pollen surface. By contrast, pollen of species transferred to recently resurrected *Apoballis* (*Apoballis acuminatissima* and *A. mutata*) is distinctively echinate (spiny). A unique layer covers the endexine of *Schismatoglottis*, and the whole pollen surface of *Apoballis*. Our findings strongly suggest that "*Schismatoglottis*" species with echinate pollen fall into the genus *Apoballis*. Moreover, all schismatoglottid taxa perform spathe movements during anthesis to control the movement of pollinators. The spathe movements of *Apoballis acuminatissima* clearly differ from those known in *Schismatoglottis* species, and indeed are so far unique for the entire family. This, together with differences in floral odour is strongly suggestive of differences in pollination ecology between the genera *Schismatoglottis* and *Apoballis*.

Keywords *Apoballis*; Araceae; pollen; *Schismatoglottis*; systematics; tropical Asia

■ INTRODUCTION

The genera of Araceae display a high morphological diversity, which extends to pollen wall morphology and exine sculpturing (Grayum, 1992; Mayo & al., 1997; Hesse, 2006). Tribe Schismatoglottideae is a well circumscribed basal clade within subfamily Aroideae (French & al., 1995; Hay, 1996; Mayo & al., 1997; Hay & Yuzammi, 2000; Keating, 2002, 2004; Cabrera & al., 2008; Cusimano & al., 2011). Schismatoglottideae is the most speciose and diverse aroid taxon in Borneo, with a very high percentage of endemic species (Wong & Boyce, 2010a). *Schismatoglottis* Zoll. & Moritzi is the largest genus of the tribe, with probably in excess of 250 species restricted to perhumid and everwet tropical Asia (Boyce & Wong, 2007). Recent taxonomic and systematic treatments for the genus include an alpha taxonomy (Hay & Yuzammi, 2000), and various additional novel taxa (e.g., Wong & Boyce, 2010a, b, c; Wong & al., 2010). One outcome of the partial phylogenetic treatment was the resurrection of the genus *Apoballis* Schott, and the transfer of 12 former *Schismatoglottis* species to *Apoballis* (Table 1). The genus *Apoballis* is well defined by morphological and molecular characters (Wong & Boyce, 2010a) and is sister to all other Schismatoglottideae.

The morphology of monocot pollen, especially of Araceae, has been studied iteratively since the pioneering work of Thanikaimoni (1969) and Zavada (1983). Pollen ornamentation of subfamily Aroideae (sensu Cabrera & al., 2008; Cusimano & al., 2011) is mostly psilate (smooth pollen surface) or echinate (spiny), but, disregarding *Calla* L., never reticulate. In contrast to all other subfamilies the pollen wall in Aroideae (including Schismatoglottideae and excluding the puzzling case of *Calla*) lacks the common sporopollenin tectate-columellate exine. Instead, a non-sporopollenin, polysaccharidic outermost pollen wall layer (Weber & al., 1998, 1999), or polysaccharidic echini (Pacini & Juniper, 1983; Weber & al., 1998) cover the pollen wall (endexine). This polysaccharidic wall ornamentation is a unique feature of some Aroideae pollen, first documented in *Arum italicum* Mill. (Pacini & Juniper, 1983), and later in *Sauromatum venosum* (Ait.) Schott (Weber & al., 1998). It was also reported for *Pistia stratiotes* L., in which there are polysaccharidic plicae (ribs), and an additional thin polysaccharidic layer (Weber & al., 1999).

During our studies of the pollen ultrastructure of Araceae, the pollen of a *Schismatoglottis* species (at that time determined as *Schismatoglottis lancifolia* Hallier f. & Engl.) was revealed to be echinate. This, together with the occurrence of a thin outer acetolysis-resistant wall layer, was a novel finding for this tribe. Compared to all other investigated *Schismatoglottis* species and related genera with smooth pollen, this seemed to be, at first sight, a result of a possible taxon mix-up, for example with a spiny genus such as *Callopsis* Engl. (Weber, 2004). At that time no *Schismatoglottis* species was known to be spiny and the *Apoballis* resurrection was not yet published (Wong & Boyce, 2010a). *Schismatoglottis* pollen, as so far analysed, was reported to be psilate, typical for all Schismatoglottideae (Thanikaimoni, 1969; Grayum, 1992). A possible correlation between pollen ornamentation and pollinator type in Araceae was first postulated by Grayum (1986, 1992). Grayum (1986)

Table 1. The resurrected genus *Apoballis* and the 12 transferred *Schismatoglottis* species (Wong & Boyce, 2010a).

Species of the resurrected genus *Apoballis*	Basionym in *Schismatoglottis*
A. acuminatissima (Schott) S.Y. Wong & P.C. Boyce	*S. acuminatissima* Schott
A. belophylla (Alderw.) S.Y. Wong & P.C. Boyce	*S. belophylla* Alderw.
A. brevipes (Hook. f.) S.Y. Wong & P.C. Boyce	*S. brevipes* Hook. f.
A. grandiflora (Alderw.) S.Y. Wong & P.C. Boyce	*S. grandiflora* Alderw.
A. hastifolia (Hallier f. ex Engl.) S.Y. Wong & P.C. Boyce	*S. hastifolia* Hallier f. ex Engl.
A. javanica (Engl.) S.Y. Wong & P.C. Boyce	*S. javanica* Engl.
A. longicaulis (Ridl.) S.Y. Wong & P.C. Boyce	*S. longicaulis* Ridl.
A. mutata (Hook. f.) S.Y. Wong & P.C. Boyce	*S. mutata* Hook. f.
A. okadae (M. Hotta) S.Y. Wong & P.C. Boyce	*S. okadae* M. Hotta
A. ovata (Schott) S.Y. Wong & P.C. Boyce	*S. ovata* Schott
A. rupestris (Zoll. & Moritzi ex Zoll.) S.Y. Wong & P.C. Boyce	*S. rupestris* Zoll. & Moritzi ex Zoll.
A. sagittifolia (Alderw.) S.Y. Wong & P.C. Boyce	*S. sagittifolia* Alderw.

and Sannier & al. (2009) found a correlation between echinate pollen and fly pollination and psilate pollen with beetle pollination in Araceae. Regarding the differences in pollen ornamentation of *Schismatoglottis* and *Apoballis*, we studied movements of the inflorescence, which are indicative for pollination mode (Vogel, 1965), in order to check whether the differences in pollen ornamentation could be linked to differences in the pollinator type. Movements of the spathe are found throughout Araceae (Mayo & al., 1997), and are known to play an important role in controlling pollinator movements (Young, 1986; Ørgaard & Jacobsen, 1998; Vogel & Martens, 2000). In Schismatoglottideae all species so far observed display spathe movements (Boyce & Wong, 2007; Wong & Boyce, 2010b).

In this publication we present the first description of spathe movements in *Apoballis acuminatissima* (Schott) S.Y. Wong & P.C. Boyce, which are unique for the tribe and clearly differ from those observed in *Schismatoglottis*, and we use pollen as an additional character for generic delimitation of *Apoballis* and *Schismatoglottis*.

■ MATERIALS AND METHODS

Plant material. — Plant material was collected in Sarawak, Malaysian Borneo, the Munich Botanical Garden, and the Botanical Garden of the University of Vienna, studied fresh or stored in silica gel or in alcohol. The choice of species sampled in each genus was guided primarily by the availability of suitable material. A list of all voucher specimens is provided in the Appendix.

Preparation. — For light microscopy (LM), fresh and silica gel–dried material was rehydrated in water. Pollen was acetolysed for 5 minutes at 100°C (Erdtman, 1960; Hesse & Waha, 1989).

For scanning electron microscopy (SEM), pollen was rehydrated in water, dehydrated with 2,2-dimethoxypropane, acetone and critical point–dried (Halbritter, 1998), and sputter coated with gold. Silica-dried pollen and pollen fixed in alcohol were only sputter coated with gold.

For transmission electron microscopy (TEM), anthers were rehydrated and fixed in 3% glutaraldehyde (GA), postfixed with 1% osmiumtetroxide (OsO_4) and 0.8% potassium hexacyanoferrate ($K_4Fe(CN)_6 \cdot 3H_2O$). Fixed material was dehydrated in 2,2-dimethoxypropane and then embedded in Agar's low viscosity resin (LV-Resin) and in Spurr's low-viscosity epoxy resin (Spurr, 1969; Agar Scientific, 2004). Sections (60–90 nm thick) were cut with a diamond knife on a Reichert Ultracut microtome. For common contrast, sections were stained with the modified Thiéry-test (Rowley & Dahl, 1977). All samples where stained with uranyl acetate followed by lead citrate (pictures not presented in this paper). The occurrence of polysaccharides was detected with the Thiéry-test (Thiéry, 1967). The detection of lipids followed the procedure of Rowley & Dahl (1977). For the detection of the endexine, sections were treated with 1% aqueous potassium permanganate solution ($KMnO_4$) (Hayat, 2000; Ulrich, 2006).

The course of anthesis in *Apoballis acuminatissima* was studied on several inflorescences of one plant in the greenhouses of the Botanical Garden of the University of Vienna. Movements of spathe and spadix were observed and documented in two inflorescences with a camera (Nikon Coolpix P 5000), which automatically took a picture every ten minutes. In addition, three further inflorescences were observed during daily visits.

■ RESULTS

Pollen analyses. — Pollen of *Apoballis* (Fig. 1; Table 2) and S*chismatoglottis* (Fig. 2; Table 2) is small and inaperturate (omniaperturate), but there are differences in pollen wall ultrastructure and sculpturing.

External morphology. — The most eye-catching difference between the pollen of the two genera is the external morphology. Pollen of all investigated species of *Apoballis* is echinate (spiny; Figs. 1, 3A–D) whereas the pollen of all investigated species of *Schismatoglottis* is psilate (smooth; Figs. 2, 3E–H). The echini (spines) of *Apoballis* consist of polysaccharids (Fig. 3D) and are resistant to acetolysis (Fig. 1G–J). Under the light microscope the pollen surface of *Schismatoglottis celebica* Engl. and *Schismatoglottis calyptrata* (Roxb.) Zoll. & Moritzi appears to be echinate (Fig. 2C, G). Scanning electron microscopy revealed that irregularly distributed calcium oxalate crystals of different size, not echini, cover the whole pollen surface (Fig. 2A–B, D–F). In contrast, the psilate pollen grains of *Schismatoglottis multiflora* Ridl. (Fig. 2H, K) are clumped together by large calcium crystals (Fig. 2I–J).

Internal structure. — The pollen wall of both genera consists of an intine (Fig. 3A–H; Table 2), a continuous, compact to spongy endexine (Fig. 3A–H; Table 2), and a thin layer

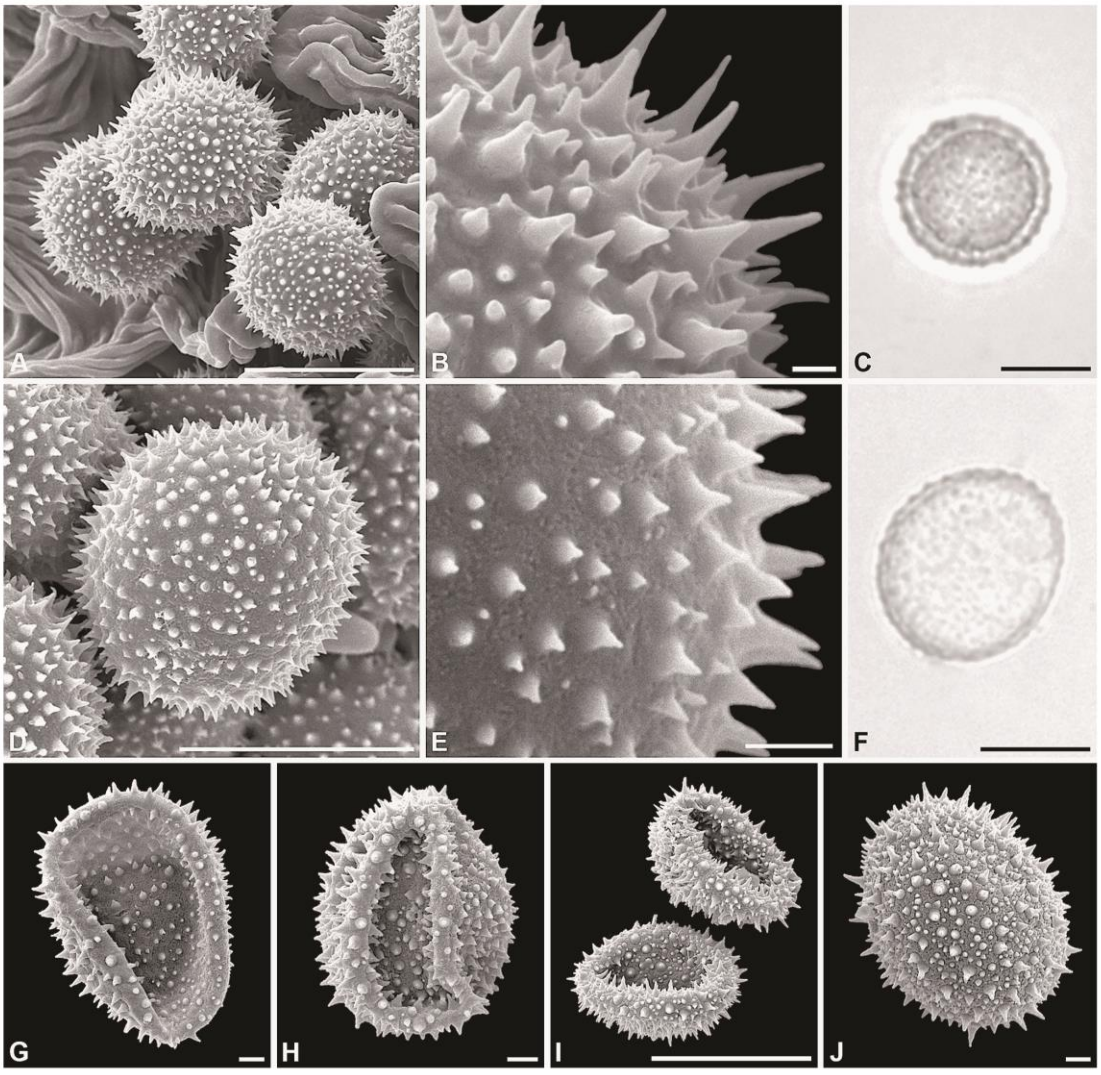

Fig. 1. Echinate pollen typical for *Apoballis*. **A–C,** *Apoballis acuminatissima*: **A,** pollen grains under SEM, air-dried; **B,** detail of pollen surface, air-dried; **C,** hydrated pollen grain in LM. **D–F,** *Apoballis mutata*: **D,** hydrated pollen grains under SEM, critical point–dried; **E,** detail of pollen surface; **F,** hydrated pollen grain under LM. **G–H,** acetolyzed pollen of *Apoballis mutata*; note that echini are acetolysis-resistant. **I–J,** acetolyzed pollen of *Apoballis acuminatissima*; note that echini are acetolysis-resistant. — Scale bars = 10 µm (A, C, D, F, G–J), 1 µm (B, E).

Table 2. Summary of the relevant pollen characters of all investigated species of Schismatoglottideae (n.i. = not investigated).

Genus / number of species	Species investigated	Size	Shape hydrated	Aperture	Ornamentation in LM-view
Apoballis Schott / 20	*A. acuminatissima* (Schott) S.Y. Wong & P.C. Boyce	Small	Spheroidal to elliptic	Inaperturate	Echinate
	A. mutata (Hook. f.) S.Y. Wong & P.C. Boyce	Small	Spheroidal to elliptic	Inaperturate	Echinate (Thanikaimoni, 1969)
	A. longicaulis (Ridl.) S.Y. Wong & P.C. Boyce	Small	n.i.	Inaperturate	Echinate (Thanikaimoni, 1969)
Bucephalandra Schott / 3	*B. motleyana* Schott	Small	Elliptic	Inaperturate	Psilate
Hestia S.Y. Wong & P.C. Boyce / 1	*H. longifolia* (Ridl) S.Y. Wong & P.C. Boyce	Small	Elliptic	Inaperturate	Psilate
Ooia S.Y. Wong & P.C. Boyce / 2	*O. grabowskii* (Engl.) S.Y. Wong & P.C. Boyce	Small	Elliptic	Inaperturate	Scabrate
Phymatarum M. Hotta / 1	*P. borneense* M. Hotta	Small	Elliptic	Inaperturate	Scabrate
Piptospatha N.E. Br. / 10	*P. viridistigma* P.C. Boyce, S.Y. Wong & Bogner	Small	Elliptic	Inaperturate	Scabrate
	P. ridleyi N.E. Br ex Hook. f.	Small	Elliptic	Inaperturate	Scabrate
Schismatoglottis Zoll. & Moritzi / 100	*S. calyptrata* (Roxb.) Zoll. & Moritzi	Small	Elliptic	Inaperturate	Scabrate
	S. celebica Engl.	Small	Elliptic	Inaperturate	Scabrate
	S. conoidea Engl.	Small	Elliptic	Inaperturate	Scabrate
	S. ifugaoensis S.Y. Wong, Bogner & P.C. Boyce	Small	Elliptic	Inaperturate	Scabrate
	S. matangensis S.Y. Wong	Small	Elliptic	Inaperturate	Scabrate
	S. modesta Schott	Small	Elliptic	Inaperturate	Scabrate
	S. motleyana (Schott) Engl.	Small	Elliptic	Inaperturate	Scabrate
	S. multiflora Ridl.	Small	Elliptic	Inaperturate	Scabrate
	S. roseospatha Bogner	Small	Elliptic	Inaperturate	Scabrate
	S. tecturata (Schott) Engl.	Small	Elliptic	Inaperturate	Scabrate
	S. viridissima A. Hay	Small	Elliptic	Inaperturate	Scabrate
Schottariella P.C. Boyce & S.Y. Wong / 1	*Schottariella mirifica* P.C. Boyce & S.Y. Wong	Small	Elliptic	Inaperturate	Scabrate

Cellular condition	Raphids	Crystals	Ornamentation in SEM-View	Intine	Endexine	Peculiarities	Illustrated
2 (& 3)	Raphids	Absent	Echinate	Bi-layered	Continuous, spongy	Thin outer layer, acetolysis resistant, polysaccharidic echini	Figs. 1, 3
2	Raphids	Absent	Echinate	Bi-layered	Continuous, spongy	Thin outer layer, acetolysis resistant, polysaccharidic echini	Fig. 1
n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	
2 (& 3)	Raphids	Absent	Verrucate	Bi-layered	Continuous, compact	Discontinous outer ektexine (verrucate)	
2	Raphids	Small	Psilate, with crystals	n.i.	n.i.	n.i.	
3 (& 2)	Raphids	Absent	Psilate	Bi-layered	Continuous, compact	No outer ektexine layer	
2	Raphids	Absent	Psilate	Bi-layered			
3 (& 2)	Raphids	Absent	Psilate	n.i.	n.i.	n.i.	
n.i.	n.i.	Absent	n.i.	n.i.	n.i.	n.i.	
2	Raphids	Small	Psilate, with crystals	Bi-layered	Continuous, spongy	Thin outer layer, acetolysis-resistant	Fig. 2
2	Raphids	Small & large	Psilate, with crystals	Bi-layered	Continuous, spongy	Thin outer layer, acetolysis-resistant	Figs. 2, 3
2	Raphids	Small	Psilate, with crystals	n.i.	n.i.	n.i.	
2	Raphids	Small	Psilate	n.i.	n.i.	n.i.	
2 (& 3)	Raphids	Small	Psilate, with crystals	n.i.	n.i.	n.i.	
2 (&3)	Raphids	Small	n.i.	n.i.	n.i.	n.i.	
2	Raphids	Small & large	Psilate, with crystals	n.i.	n.i.	n.i.	
2 (& 3)	n.i.	Large	Psilate with crystals	Bi-layered	Continuous, spongy	Thin outer layer, acetolysis-resistant	Fig. 2
n.i.	n.i.	Absent	Psilate (Halbritter, unpub. data)	n.i.	n.i.	n.i.	
2 (& 3)	n.i.	Small	Psilate (Grayum, 1992)	n.i.	n.i.	n.i.	
2 (& 3)	Raphids	Small	Psilate with crystals	n.i.	n.i.	n.i.	
2 (& 3)	n.i.	Absent	Psilate	Bi-layered	Continuous, spongy	Thin outer layer (ektexine); holes between ektexine and endexine	

covering the whole pollen surface (Fig. 3A–H; Table 2). The intine always stains electron-lucent (Fig. 3A–C, E–G) except with the Thiéry-test (Fig. 3D, H). The compact to spongy endexine of the investigated species appeared electron-dense (Fig. 3A, C–H) or electron-lucent (Fig. 3B), depending on the staining method. The outer pollen wall layer of *Apoballis acuminatissima* pollen was only clearly visible after the Lipid-test (Fig. 3C). In contrast to this, the outer pollen wall layer of *Schismatoglottis celebica* pollen stained differently, depending on the staining method. After the Thiéry-Test (Fig. 3H), the

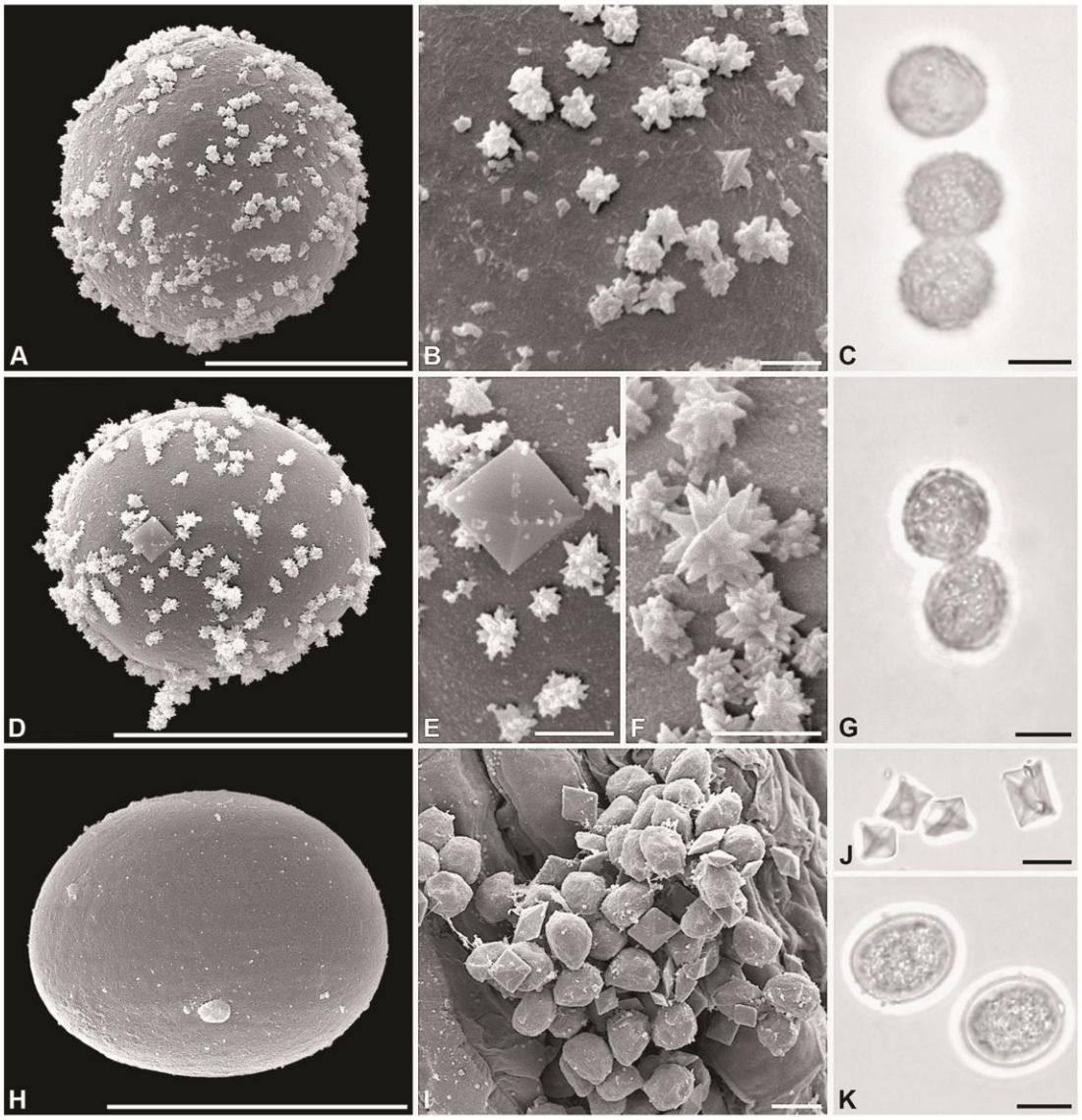

Fig. 2. Psilate pollen typical for *Schismatoglottis*. **A–C,** *Schismatoglottis celebica*: **A,** hydrated pollen grains under SEM, critical point–dried; **B,** detail of pollen surface; note the crystals covering the pollen surface; **C,** hydrated pollen grains under LM. **D–G,** *Schismatoglottis calyptrata*: **D,** hydrated pollen grains under SEM, critical point–dried; **E–F,** small and large crystals covering the pollen surface; **G,** hydrated pollen grains under LM; note crystals on the pollen surface. **H–K,** *Schismatoglottis multiflora*: **H,** hydrated pollen grains under SEM, critical point–dried: **I,** hydrated pollen grains under SEM, showing smooth pollen with large crystals attached, critical point–dried; **J,** crystals under LM; **K,** hydrated pollen grains under LM. — Scale bars = 10 µm (A, C, D, G, H–K), 1 µm (B, E, F).

layer stained electron-dense, but after treatment with potassium permanganate (Fig. 3E) and after the Lipid-test (Fig. 3G) it stained electron-lucent.

Spathe movements of *Apoballis acuminatissima*. — The inflorescence of *Apoballis acuminatissima* consists of a fertile spadix surrounded by a spathe. The inflorescence is monoecious, with pistillate flowers at the base of the spadix, an intermediate sterile zone, staminate flowers above, and a terminal sterile zone, the appendix. In common with all Araceae *Apoballis* is protogynous. In *Apoballis* the pistillate flowers are receptive during the first day of anthesis, and staminate flowers release pollen on the second day. During anthesis the inflorescence performs a series of movements (Fig. 4). Before onset of anthesis the spathe clings tightly to the spadix (Fig. 4A). Around 00:00 h of the first day the spadix bends forwards and the spathe limb starts to unfurl ventrally, finally completely exposing the sterile and staminate section of the spadix, and giving access to the pistillate flowers contained in the lower part of the spathe. Meanwhile, the tip of the spathe limb remains furled around the distal part of the spadix. The

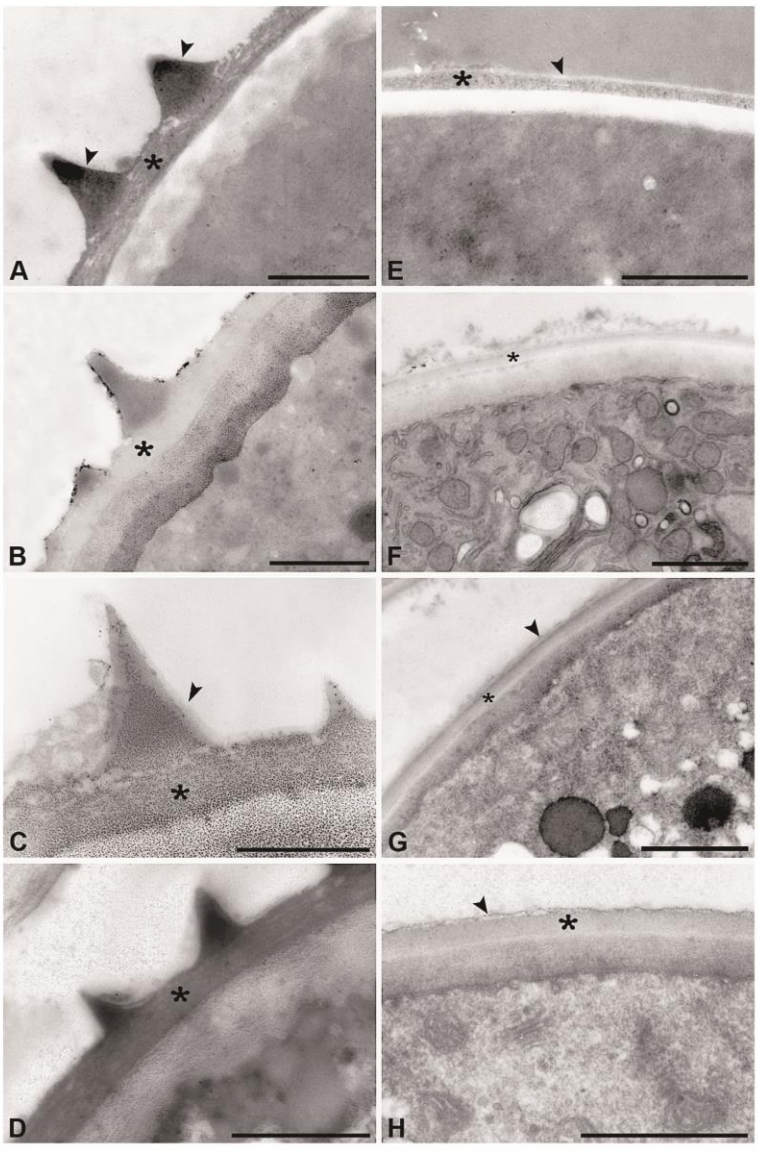

Fig. 3. Cross-sections of pollen walls of *Apoballis* and *Schismatoglottis* using different staining methods. **A–D,** *Apoballis acuminatissima*; **E–H,** *Schismatoglottis celebica*. **A, E,** pollen wall after potassium permanganate staining; **B, F,** pollen wall after modified Thiéry-test; **C, G,** pollen wall after Lipid-test; **D, H,** pollen wall after Thiéry-test. — Arrowheads point to a thin continuous layer, covering the pollen surface; asterisks indicate the endexine. Below the endexine a bi-layered intine is found. — Scale bars = 1 µm.

opening persists during the first day, throughout which the pistillate flowers are receptive. Maximum spathe limb opening is reached around 12:00 h of the first day (Fig. 4B). After 15:00 h the spadix bends back again and the spathe limb starts to close around the ventral part of the intermediate (sterile) zone of the spadix. By 04:00 h on the second day of anthesis the closing motion ends and the spathe tube enclosing the pistillate flowers is closed ventrally. The ventral side of the staminate zone of the spadix remains exposed while the dorsal side is enclosed by the spathe margins (Fig. 4C). After 14:30 h of the second day pollen is extruded from the staminate flowers (Fig. 4D). The staminate flowers on the dorsal side of the staminate zone extruded only a few pollen grains, while on the spathe-enclosed dorsal side more pollen was produced, which then fell into the spathe tube below. The moment of reopening of the spathe limb after the staminate phase differed in the observed plants. In two plants the spathe limb reopened on the same level with the staminate spadix zone two days after staminate anthesis (Fig. 4E) whereas in a third plant the spathe limb remained closed until the inflorescence started to decay. As the upper part of the spathe limb remained furled throughout anthesis, the appendix was never exposed. After anthesis the spathe limb is marcescent (Fig. 4F).

■ DISCUSSION

Pollen characters of Araceae (ornamentation, ultrastructure) accord well with recent phylogenies and phylogeny-supported taxonomic accounts (Hay, 1996; Mayo & al., 1997; Hay & Yuzammi, 2000; Keating, 2002, 2004; Cabrera & al., 2008; Cusimano & al., 2011). One outcome of our current palynological research in Araceae is the almost absolute presence of psilate or verrucate pollen in all the earlier-diverging clades of Aroideae, including Schismatoglottideae (Cusimano & al., 2011). Until recently the monospecific genus *Callopsis* was the only example with echinate pollen within the earlier-diverging clades. Echinate pollen is typical for all more derived clades

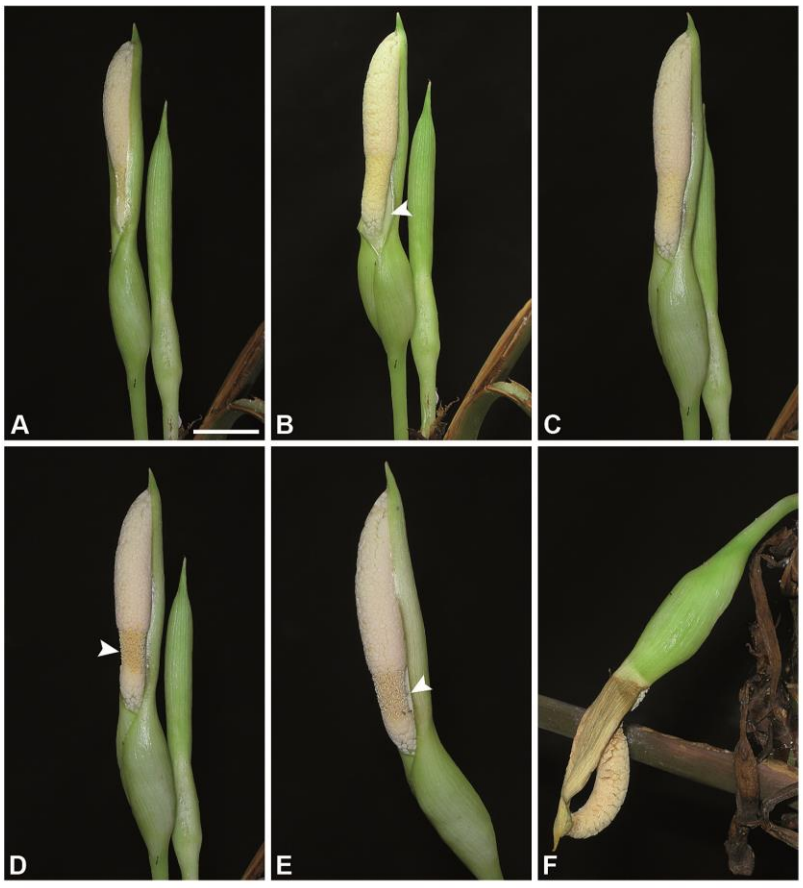

Fig. 4. Spathe movements of *Apoballis acuminatissima*. **A,** pre-anthesis (1 day before anthesis); **B,** pistillate phase (day 1 of anthesis, 12:10 h), arrowhead indicates spathe opening; **C,** pre-staminate phase (day 2, 10:38 h); **D,** pollen shedding (day 2, 16:58 h), arrowheads indicate anthers releasing pollen; **E,** post-staminate phase (day 4, 11:23 h), arrowhead indicates spathe opening; **F,** withered inflorescence (day 9). — Scale bar = 2 cm.

of Aroideae subfamily (Hesse, 2006; Halbritter, unpub. data), except for the genus *Amorphophallus* Blume ex Decne., where many different ornamentation types occur within a single genus (Van der Ham & al., 1998). Pollen of all *Schismatoglottis* species and species within the recently resurrected New World genus *Philonotion* Schott (Wong & al., 2010), so far studied by us (Appendix), is psilate, in accordance with literature reports (Grayum, 1992; Wong & al., 2011). Curiously Thanikaimoni (1969) reported 14 *Schismatoglottis* species with echinate (spiny) pollen, but only illustrated *Schismatoglottis kurzii* Hook. f. (= *Apoballis mutata* (Hook. f.) S.Y. Wong & P.C. Boyce), and *Schismatoglottis forbesii* Engl. (= *Apoballis longicaulis* (Ridl.) S.Y. Wong & P.C. Boyce). Unfortunately, Thanikaimoni's report was overlooked and even suspected as a misinterpretation of fungal spores (Grayum, 1992). The puzzling presence of a spiny-pollen *Schismatoglottis* species (the original *Schismatoglottis lancifolia*) in our collections, and the desire to verify or finally refute the largely ignored findings of Thanikaimoni (1969), were the reasons to undertake a close look at potentially spiny-pollen *Schismatoglottis* species.

Calcium crystals. — Under the light microscope, pollen of *Schismatoglottis celebica* and *Schismatoglottis calyptrata* appear to be echinate, but this is a misinterpretation. The scanning electron microscope reveals that irregularly distributed crystals of different size, not echini, cover the whole pollen surface. The smooth pollen surface of *Schismatoglottis multiflora* has no small crystals attached, but the pollen grains are clumped together with large crystals. Many aroids produce large amounts of oxalic acid and most of it is deposited as crystals of calcium (Mayo & al., 1997). A common feature of *Schismatoglottis* and some other Araceae (*Caladium* Vent., *Gearum* N.E. Br., *Scaphispatha* Brongn. ex Schott) is the occurrence of small and large calcium oxalate crystals attached to the pollen surface (Grayum, 1992; D'Arcy & al., 1996; Barabé & al., 2004).

Pollen analyses. — Pollen analyses under scanning and transmission electron microscope reveal that pollen of *Apoballis acuminatissima*, *A. longicaulis*, and *A. mutata*, is distinctively echinate. Because all species of *Apoballis* so far investigated have spiny pollen, a study of species of *Schismatoglottis* with *Apoballis*-like macromorphology should include pollen analyses. If their pollen is spiny and their morphology is as found in *Apoballis* then they should be transferred to *Apoballis*. If echinate pollen turns out to be common to all *Apoballis* species, it would be another fine example for the "compass needle" quality of pollen characters (Erdtman, 1952; Blackmore, 2000). In Schismatoglottideae, echinate pollen so far is restricted to *Apoballis*, the basalmost genus of the tribe (Wong & Boyce, 2010c).

Pollen wall. — The pollen wall of *Apoballis* and *Schismatoglottis* consists mainly of a thick, continuous spongy endexine overlaying a thick intine. A thin outermost layer is covering the endexine. The echini of *Apoballis* mainly consist of polysaccharides, which is a common feature of spiny pollen in Aroideae, and so far known only for Araceae (Weber & al., 1998, 1999). Although sporopollenin is absent, the spines of *Apoballis* are resistant to acetolysis. The use of different staining methods revealed a thin outer pollen wall layer, covering the whole pollen surface. The echini are protected by this outer wall layer and therefore resistant to chemical attack. This is similar to *Callopsis volkensii* Engl., where the outer pollen wall layer was interpreted as a cuticula (Weber, 2004). Surprisingly, this outer wall layer stained electron-lucent or electron-dense depending on the staining method. This staining behaviour of a pollen wall layer is so far only known from the endexine. The results of the cytochemical reactions (Thiéry-test, Lipid-test, potassium permanganate) are in accordance with those reported in Weber & al. (1998) and as demonstrated for the staining behaviour of the endexine in Weber & Ulrich (2010). The staining results indicate that the chemical compounds of the outer wall layer might be similar to those of the endexine, which mainly consists of lipidic compounds, sporopollenin and proteins (Heslop-Harrison, 1968a, b; Heslop-Harrison & al., 1973). According to Weber (2004) the staining properties of the outer pollen wall layer of *Schismattoglottis* and *Apoballis* indicates lipidic compounds rather than sporopollenin and definitely no polysaccharides. Based on the staining results and the resistance to acetolysis, it seems more likely that this ektexine-like layer is a type of cuticula, This layer is unique for the tribe Schismattoglottideae, and for the Araceae so far only documented for *Callopsis* (Weber, 2004).

Pollen and pollinator. — Ornamented pollen (e.g., reticulate, echinate pollen) is significant for zoophily (Punt, 1986; Fægri & Iversen, 1989). Usually the ornamenting elements consist of sporopollenin, like the rest of the ektexine (Hesse, 2006). It is not understood if and how the non-sporopollenin (polysaccharidic) echini in *Apoballis*, and in many other members of Aroideae, are related to the mode of pollination.

Usually psilate pollen of temperate and boreal zones is indicative for anemophily (Fægri & Iversen, 1989), whereas in the tropics it is not indicative for anemophily, but for zoophily (Furness & Rudall, 1999). In Aroideae (e.g., *Montrichardia* Crueg., *Dieffenbachia* Schott, *Philodendron* Schott, *Gearum* N.E. Br.) psilate pollen, together with its sticky surface, is adapted for entomophily (Weber & Halbritter, 2007; our unpub. data). In Araceae, a correlation between pollinator type and pollen ornamentation is strongly suggested: beetle pollination is correlated with psilate pollen, fly pollination with echinate pollen (Grayum, 1992; Sannier & al., 2009). However, without pollinator observations for *Apoballis* it remains unclear whether there exists such a correlation in this genus, i.e., whether flies are the pollinators of *Apoballis*. According to the scarce literature (Toda & Lakim, 2011; Wong, Boyce & co-workers, pers. obs. & in prep.), at least some species of *Schismatoglottis* are pollinated by flies of the genus *Colocasiomyia* (Drosophilidae). This conflicts with the presence of smooth pollen grains which are interpreted as adaptation to beetle pollination. Moreover, the appearance of echinate pollen grains only in the derived clades of Aroideae (Cusimano & al., 2011) indicates a phylogenetic signal rather than an ecological trigger such as pollinator type.

Interestingly, all *Apoballis* so far investigated produce a floral odour reminiscent of benzaldehyde (almond oil; Boyce, pers. obs.) which contrasts with the floral odour of

Schismatoglottis (mainly methyl esterase-like—model airplane glue). This, together with the differences in spathe mechanics (Boyce & Wong, 2007), strongly suggests pollinator differences.

Spathe movements. — Variously complex spathe movements occur in all Schismatoglottideae species so far observed (Boyce & Wong, 2007; Wong & Boyce, 2010b), but to date no studies on the function of the movements have been published, although much data has been accumulated. In most genera, including *Schismatoglottis*, the spathe limb is caducous during or at the end of anthesis. This is not the case in *Apoballis*. In tribe Areae movements similar to those of *Apoballis* have been observed and published for *Typhonium* Schott, *Sauromatum* Schott, and *Theriophonum* Blume (Vogel, 1965; Armstrong, 1979; Dakwale & Bhatnagar, 1997). In these genera, spathe movements serve as trapping mechanisms for flies as well as beetles that would otherwise escape from the lower spathe tube before pollen is extruded. In these taxa insects are arrested in the lower spathe tube containing the pistillate flowers until pollen is extruded from the staminate flowers above the secluded chamber and deposited onto the constriction that separates the lower spathe and the spathe limb. When the constriction loosens insects escape with pollen attached to their bodies. The crucial event in *Apoballis acuminatissima* is the locking of the spathe tube during the pistillate phase; we hypothesize that the primary purpose of these spathe movements is to arrest pollinators in order to exploit them as pollen vectors during the staminate phase. In contrast to *Typhonium*, *Sauromatum* and *Theriophonum*, part of the staminate section is situated inside the secluded chamber and thus pollen directly falls into the lower spathe tube. Two scenarios seem possible: trapped insects take up pollen during their arrestment within the spathe tube, or when they leave the spathe tube through the narrow opening on a level with the staminate flowers. In effect spathe movements, and changes in spadix morphology during anthesis function as "pollinator management systems". Such a mechanism can greatly increase reproductive success (Lack & Diaz, 1991). The observation that traps are more often found among fly-pollinated Araceae (Bown, 2000) would indicate flies as pollinators in *Apoballis* rather than beetles. Whether or not differences in spathe movements between *Apoballis* and *Schismatoglottis* are owing to different types of pollinators needs further investigation.

Compared to the trapping species of Areae, where insects are released immediately after pollen production, the two days delay before the reopening of the spathe in *Apoballis* might seem atypical. However, exceptions to the rule exist. For example, in *Arum hygrophilum* Boiss. times of arrestment of up to 10 days have been recorded (Koach, 1985). The fact that the moment of reopening differed in inflorescences of the same plant indicates certain variability. However, more observations on different plants, ideally in their natural habitat with pollinators present, are necessary to understand the function of the delayed opening. The reversible bending of the spadix as part of the spathe movements reveals a high degree of synorganisation of the inflorescence. It is a unique feature of *Apoballis* which has not been observed yet in any other taxon of Araceae.

■ CONCLUSION

In this paper, we provide another compelling example for the "compass needle" quality of pollen characters: it indicates that spiny pollen in the genus *Apoballis* is plesiomorphic for Schismatoglottideae, while pollen in *Schismatoglottis* (and indeed all other studied Schismatoglottideae) is psilate. The echinate pollen of *Apoballis* may indicate different types of pollinators. A specialized relationship between plant and pollinator is indicated by the spathe movements in *Apoballis*, which clearly differ from those in *Schismatoglottis*. The observed traits would indicate flies as pollinators. To clarify this issue field studies are needed. Moreover, we recommend further pollen studies of *Schismatoglottis* species with *Apoballis*-like macromorphology.

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Appendix. Species sampled. Specimens where collected in Malaysia, from Munich Botanical Garden, and from the Botanical Garden of the University of Vienna.

Species, locality, collector (herbarium/voucher).

Apoballis Schott: ***A. acuminatissima*** (Schott) S.Y. Wong & P.C. Boyce, cult. Botanical Garden of the University of Vienna, *J. Bogner 1797, Anon. s.n.*, (090609-1/2); ***A. mutata*** (Scort. ex Hook. f.) S.Y. Wong & P.C. Boyce, Malaysia, Perak, Hulu Perak, Tasik Banding, cult. USM Penang, ex *Baharuddin S. s.n.* sub. *P.C. Boyce & S.Y. Wong AR-2616* (SAR, USM). ***Bucephalandra*** Schott: ***B. motleyana*** Schott, cult. Munich Botanical Garden, *J. Bogner 2974* (M). ***Hestia*** S.Y. Wong & P.C. Boyce: ***H. longifolia*** (Ridl.) S.Y. Wong & P.C. Boyce, Malaysia, Sarawak, Kuching, Bau, Kampung Grogo, *Jeland ak. Kisai AR-233* (SAR). ***Schismatoglottis*** Zoll. & Moritzi: ***S. calyptrata*** (Roxb.) Zoll. & Moritzi, Malaysia, Perak Hulu, Perak, Tasik Banding, cult. USM Penang, *Baharuddin S. s.n.* sub. *P.C. Boyce & S.Y. Wong AR-2617* (SAR, USM); ***S. calyptrata*,** cult. Botanical Garden of the University of Vienna [ARA090165] ex *J. Bogner s.n.* (090402-1/1); ***S. celebica*** Engl., Indonesia, Sulawesi, cult. Botanical Garden of University Vienna [ARA090160], ex *Chr. Kasselmann s.n.*; ***S. conoidea*** Engl., Malaysia, Sarawak, Kuching, Matang, Kubah N.P., Waterfall Trail, 01°35′40.2″ N, 110°10′45.9″ E, 190 m asl, *P.C. Boyce & S.Y. Wong AR-2113* (SAR); ***S. ifugaoensis*** S.Y. Wong, Bogner & P.C. Boyce, Philippines, Luzon, Ifugao Province, near Banaue, ca. 1500 m asl, *J. Bogner 1630* (M); ***S. matangensis*** S.Y. Wong, Malaysia, Sarawak, Kuching, Matang, Kubah N.P., Waterfall Trail, 01°35′40.2″ N, 110°10′45.9″ E, 190 m asl, *P.C. Boyce & Wong Sin Yeng, AR-1864* (SAR); ***S. modesta*** Schott, Indonesia, Kalimantan Barat, Sanggau, Kampung Penyeladi between Sekadau and Sanggau, 00°05′00.1″ N, 110°39′54.8″ E, *P.C. Boyce & S.Y. Wong AR-2547* (BO, SAR); ***S. motleyana*** (Schott) Engl., Malaysia, Sarawak, Kuching, Matang, Kubah N.P., Waterfall Trail, 01°35′40.2″ N, 110°10′45.9″ E, 190 m asl, *P.C. Boyce, Wong Sin Yeng & S. Maclean AR-2116* (SAR); ***S. multiflora*** Ridl., Malaysia, Sarawak, Kuching, Matang, Kubah N.P. boundary, Sungai Cina, cult. Botanical Garden of the University of Vienna, [ARA090167], *J. Bogner 1453*, (091027-1/1); ***S. roseospatha*** Bogner, Malaysia, Sarawak, Kapit, Gaat ('Gaad') River, *J. Knüppel & H. Link s.n.*, cult. Munich Botanical Garden sub. *J. Bogner 1472* (M); ***S. tecturata*** (Schott) Engl., Malaysia, Sarawak, Kapit, Kapit town, Taman Rekreasi Sebabai, 01°56′45.6″ N, 112°54′16.8″ E, ca. 50 m asl, *P.C. Boyce, Wong Sin Yeng & Jeland ak Kisai AR-1797* (SAR); ***S. viridissima*** A. Hay, Malaysia, Sarawak, Kuching, Matang, Kubah N.P., Waterfall Trail, 01°35′40.2″ N, 110°10′45.9″ E, 190 m asl, *P.C. Boyce, Wong Sin Yeng & S. Maclean AR-2126* (SAR). ***Ooia*** S.Y. Wong & P.C. Boyce: ***O. grabowskii*** (Engl.) S.Y. Wong & P.C. Boyce, Malaysia, Sarawak, Kapit, Kapit town, Taman Rekreasi Sebabai, 01°56′45.6″ N, 112°54′16.8″ E, ca. 50 m asl, *P.C. Boyce & Wong Sin Yeng AR-2430* (SAR). ***Philonotion*** Schott: ***P. spruceanum*** Schott, Venezuela, Amazonas, 1°53′N, 67°02′E, cult. Munich Botanical Garden, *J. Bogner, G. Davidse, J.S. Miller 26477* (M). ***Phymatarum*** M. Hotta: ***P. borneense*** M. Hotta, Malaysia, Sarawak, Miri, Marudi, Long Lama, Mulu N.P., trail to Deer Cave, 04°02′23.8″ N, 114°48′54.6″ E, ca. 60 m asl, *Low Shook Ling 3* (SAR). ***Piptospatha*** N.E. Br.: ***P. ridleyi*** N.E.Br. ex Hook. f., cult. Munich Botanical Garden, *J. Bogner 1270* (M); ***P. viridistigma*** S.Y. Wong, P.C. Boyce & Bogner, Malaysia, Sarawak, Samarahan, Serian, Taman Rekreasi Ranchan, 01°08′34.9″ N, 110°35′02.4″ E, ca. 55 m asl, *P.C. Boyce & Wong Sin Yeng AR-2432* (SAR). ***Schottariella*** P.C. Boyce & S.Y. Wong: ***S. mirifica*** P.C. Boyce & S.Y. Wong, Malaysia, Sarawak, Sarikei, Maradong, Sungai Matob, 01°52′06.1″ N, 111°55′30.7″ E, ca. 55 m asl, *P.C. Boyce & al. AR-1615* (SAR).

B. *Calla palustris* (Araceae): New palynological insights with special regard to its controversal systematic position and to closely related genera

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Text: All text about pollen (100%) together with M. Hesse and revised by M. Weber. Text about the flowering behaviour was provided by Josef Bogner. Simplified cladograms showing the position of *Calla* and related taxa in the different molecular trees by D. Bröderbauer.

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Calla palustris (Araceae): New palynological insights with special regard to its controversial systematic position and to closely related genera

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Abstract Almost all systematic treatments agree that *Calla* is a puzzling case, being a highly autapomorphic taxon with obscure relationships. In molecular-based classifications the variable placements of *Calla* within Aroideae conflict strongly with those in morphologically and anatomically based systematic classifications, which treat the genus as a subfamily (Calloideae) of its own. We studied the pollen morphology and ultrastructure of *Calla* by light and electron microscopy, and mapped the relevant pollen characters as well as some flower characters to the proposed placements of *Calla* within the Araceae as indicated in the various molecular phylogenies. *Calla* pollen is extraordinary within the entire Araceae. Pollen grains are small, and basically disulcate or with a ring-like aperture. The ornamentation is psilate to perforate, and the pollen wall consists of a sporopolleninous tectate-columellate exine. These pollen characters are shared with those of several earlier-diverging aroid taxa, especially with those of subfamily Zamioculcadoideae, whereas pollen characters in members of subfamily Aroideae deviate significantly. These findings are in accordance with other floral characters. Therefore, we propose that *Calla* is best placed in a transition zone between either subfamily Zamioculcadoideae (*Stylochaeton* clade) and subfamily Aroideae (Aroideae clade) or between subfamily Zamioculcadoideae (*Stylochaeton* clade) and subfamily Lasioideae.

Keywords Araceae; flowering behaviour; pollen; sulcus; systematics; ultrastructure

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■ INTRODUCTION

Pollen represents an extra generation in seed plants, the highly reduced male gametophyte, which is the hidden haploid counterpart to the more dominating plant, the diploid generation. This tiny, male haploid generation has only a limited number of variable characters. One of the most important characters is the pollen wall, with its structural and ornamental features. In comparative pollen morphology and plant systematics pollen characters are at least as important as any other morphological character of the diploid generation (Hesse & al., 2003, 2009). For the Araceae, there exist excellent examples for the diagnostic quality of pollen characters for classifications. For example, within the Schismatoglottideae the resurrected genus *Apoballis* Schott is separated by its echinate pollen from all other members of this tribe, which are exclusively psilate (Ulrich & al., 2012). Another example is the sulcate Lasioideae, where monophyly is strongly supported by their unique aperture characters (Hesse, 2002).

Calla L. is a monospecific genus of Araceae, with the single species *Calla palustris* L. While the majority of aroid genera are distributed in the tropics, *Calla* is one of few genera of Araceae distributed in temperate regions, showing a unique circumboreal distribution in the Northern Hemisphere. The plant is a seasonally dormant herb with a more or less submersed, green, rhizomatous stem, rooting at nodes (helophyte), preferring acid habitats in shaded or partially shaded sites (Mayo & al., 1997). The solitary inflorescence has a white, fully expanded spathe and a more or less uniform spadix that includes spirally arranged bisexual, aperigoniate flowers (Fig. 1). Only a few distal flowers are always male (Scribailo & Tomlinson, 1992; Mayo & al., 1997; Bogner, 2009). The flowers are maturing from the base toward the apex. Pollinators for *C. palustris* have been reported to be syrphid flies (*Sphegina* spp.), common on inflorescences, and the occasionally visiting flower fly *Toxomerus geminatus* (Thomson, 2000). Other documentations by Krause (1908) and Knuth (1899, 1909) refer to small flies as only visitors.

Almost all systematic authorities concluded that *Calla* is a puzzling case, being a highly autapomorphic taxon with obscure relationships and with a peculiar combination of features, including bisexual flowers (Mayo & al., 1997). The placement of *Calla* within Araceae has attracted attention for many years and has recently become again controversial in the light of molecular phylogenies (Keating, 2002, 2004; Cabrera & al., 2008; Chartier, 2011; Cusimano & al., 2011; Nauheimer & al., 2012). In all morpho-anatomical-based studies *Calla* was placed in a subfamily of its own, the Calloideae (Grayum, 1990, 1992; Bogner & Nicolson, 1991; Mayo & al., 1997, 1998; Keating, 2004). In recent molecular classifications, *Calla* emerged on quite different positions, mainly basal to or within the Aroideae (Cabrera & al., 2008; Chartier, 2011; Cusimano & al., 2011; Nauheimer & al., 2012).

The aim of the present paper is to provide new insights into pollen morphology and the ultrastructure of *Calla* with special regard to its controversial systematic position. For the first time the ultrastructure of *Calla* pollen and the occurrence of a sometimes ring-like aperture are documented. Some pollen characters of *Calla* are unique for the entire family.

We briefly review the current status of systematic research with molecular and morpho-anatomical methods (French & al., 1995; Mayo & al., 1997; Keating, 2002, 2004; Cabrera & al., 2008; Chartier, 2011; Cusimano & al., 2011) with respect to the position of *Calla* within the Araceae. The results of our investigation of the pollen of *Calla* and the comparison with pollen characters of the most closely related taxa in the various molecular phylogenies will place *Calla* in a distinct position within the Araceae.

■ MATERIALS AND METHODS

Plant material. — *Calla palustris* was collected in the Botanical Garden of the University of Vienna (outdoor area, swamp basin, display group 46, *ARA120243*) and studied fresh,

Fig. 1. Inflorescence of *Calla palustris* consisting of the flower-bearing spadix and the expanded spathe.

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or stored in silica gel. A voucher specimen was deposited in the herbarium (WU 0069754).

Pollen preparation. — For light microscopy (LM) fresh and dry pollen was rehydrated in water. Pollen tetrads were taken from flower buds in different flowering stages and from different inflorescences and stained with acetocarmine and toluidine blue.

For scanning electron microscopy (SEM), pollen was rehydrated in water, dehydrated with 2,2-dimethoxypropane, acetone and critical-point dried (Halbritter, 1998). To verify the presence of sporopollenin, pollen was acetolysed for 5 minutes at 100°C (Erdtman, 1960; Hesse & Waha, 1989). Fresh, dry and acetolysed pollen were sputter-coated with gold and investigated with a JEOL JSM 6390 SEM at 10 kV.

For transmission electron microscopy (TEM), anthers were rehydrated and fixed in 3% glutaraldehyde (GA), postfixed with 1% osmiumtetroxide (OsO_4) and 0.8% potassium hexacyanoferrate ($K_4Fe(CN)_6 \cdot 3H_2O$). Fixed material was dehydrated in 2,2-dimethoxypropane and embedded in Agar's low viscosity resin (LV–Resin) (Agar Scientific Limited, 2004). Sections of 60–70 nm were cut with a diamond knife on a Reichert Ultracut microtome. All sections were examined in a Zeiss EM 109 or a ZEISS 900 TEM at 50 kV. For common contrast, sections were stained with uranyl acetate (U) followed by lead citrate (Pb) (Hayat, 2000).

Nomenclature. — In the present paper subfamily Aroideae is understood in the sense of Cabrera & al. (2008) and Cusimano & al. (2011) and is composed of five main supported clades, the *Zantedeschia* clade, Rheophytes clade, *Amorphophallus* clade, *Colletogyne* clade, *Pistia* clade and the three isolated genera *Anubias* Schott, *Montrichardia* Crueg. and *Callopsis* Engl. (Cabrera & al., 2008; Cusimano & al., 2011). The Aroideae include all unisexual-flowered taxa except for *Zamioculcas* Schott, *Gonatopus* Engl., *Stylochaeton* Lepr., which have been grouped as "expanded Zamioculcadoideae" (i.e., including *Stylochaeton*) and named *Stylochaeton* clade (Cabrera & al., 2008; Cusimano & al., 2011).

In contrast, the Aroideae sensu Mayo & al. (1997) include all taxa with unisexual flowers (renamed "Unisexual Flowers clade" clade by Cusimano & al., 2011), comprising the Zamioculcadoideae (*Gonatopus* and *Zamioculcas*) and *Stylochaeton* at its base.

Comparison of palynological characters among taxa closely related to *Calla* in recent phylogenies. — Pollen terminology follows Hesse & al. (2009). Information on Araceae pollen was taken from the literature and our unpublished data.

Pollen morphology of *Calla* was compared to pollen of the various taxa that appeared closely related to *Calla* in one morpho-anatomical classification (French & al., 1995) and recent molecular phylogenies (Cabrera & al., 2008; Chartier, 2011; Cusimano & al., 2011; Nauheimer & al., 2012) of Araceae. For the taxa considered related to *Calla* references for pollen morphology are presented in Table 1. For comparison, we used the classification of pollen wall types in Araceae by Weber & al. (1999). In addition, we also compared other floral characters such as sexuality, presence/absence of a perigone, and flowering behaviour according to Mayo & al. (1997).

■ RESULTS

Pollen of *Calla palustris*. — Under light microscopy the hydrated pollen grains are ovoid to spheroidal with scabrate ornamentation (Fig. 2A). The generative nucleus of the bi-cellular pollen is visible after staining with acetocarmine (Fig. 2B). Pollen is shed in huge amounts, falling to the bottom of the spathe, indicating that pollen is not sticky, which is affirmed by the absence of pollenkitt (Fig. 2C). The hydrated pollen is small, on average 20 µm, ovoid to spheroidal and usually diaperturate (Fig. 2D, arrowheads). Pollen ornamentation in the interapertural area is psilate to perforate (Fig. 2E) and the aperture membrane is ornamented with verrucae or rugulae (Fig. 2E, arrowheads). In the transition zone from the aperture to the interapertural area the ornamentation elements are dominated by rugulae (Fig. 2E, arrow).

In TEM cross-sections of the pollen grain the two apertures are visible (Fig. 2F, arrowheads). The vegetative cytoplasm contains many lipid droplets (Fig. 2F, arrow) and encloses the large generative cell with the generative nucleus (Fig. 2F, asterisk). The pollen wall consists of a compact sporopolleninous tectate-columellate ektexine (Fig. 2G–I). In the interapertural area the ektexine (ekt) shows a massive tectum (T), very short columellae (C) and a thin, discontinuous foot layer (arrow) (Fig. 2G). The ektexine (ekt) is tectate in the interapertural area only (Fig. 2H). The endexine (en) and the intine (i) are thick in the aperture area (arrowheads) and thin in the interapertural area (Fig. 2H). In the aperture area, the ektexine (ekt) is formed by mostly isolated ektexine elements (verrucae and rugulae), and a discontinuous, extremely thin foot layer (arrow) (Fig. 2I). Below the discontinuous ektexine, the continuous-compact endexine (en) and the intine (i) are present (Fig. 2I). The intine is bilayered in the aperture only.

Dry pollen is infolded, with rectangular or irregular shape, and the apertures of dry pollen are sunken (Fig. 2J–K, arrowheads). Acetolysed pollen is also infolded (Fig. 2L, arrowheads) and the acetolysis-resistant ektexine indicates the presence of sporopollenin.

Aperture peculiarities. — *Calla* pollen is usually disulcate and the two apertures are elongated and situated parallel to the equator (Fig. 3A, arrowheads). From this aperture type there are some interesting deviations found in pollen grains of the investigated flowers of different individuals. The apertures can be extremely shortened (Fig. 3B, arrowheads). The short sulcus can sometimes be inconspicuous, but due to the ornamentation of the aperture membrane, which is rugulate (Fig. 3C, arrow) and verrucate (Fig. 3C, arrowhead), the aperture is still visible. The aperture can be extremely extended (Fig. 3D–E), or sometimes completely encircling the whole pollen grain, forming a single ring-like aperture (Fig. 3F–I, arrowheads). Different developmental stages of pollen tetrads show that successive cytokinesis leads to different tetrad arrangements (Fig. 4). In early tetrad stages the tetrad is enclosed by callose (Fig. 4A, B, arrow) whereas late tetrad stages and free microspores are released from callose after the formation of the pollen wall (Fig. 4B, C, arrowheads). The apertures are clearly visible in free microspores (Fig. 4C, arrowheads) and in some late tetrads

Table 1. Araceae taxa/clades in morpho-anatomical and recent molecular studies with the most important pollen and flower characters.

Araceae subfamilies (sensu Mayo & al., 1997 and in part Cusimano & al., 2011)	Aroid clades in recent phylogenies (sensu Cusimano & al., 2011)	Aperture	Ornamentation	Ektexine ultrastructure (pollen wall type according to Weber & al., 1999)	Flowers (Mayo & al., 1997)	Perigone (Mayo & al., 1997)	Flowering behaviour (Mayo & al., 1997)
Gymnostachydoideae (Hesse, 2009)	Proto-Araceae	Sulcate	Reticulate	Sporopolleninous, thick tectate-columellate ektexine (type 1a)	Bisexual	Present	Acropetal
Orontioideae (Hesse, 2009)	Proto-Araceae	Sulcate	Reticulate	Sporopolleninous, thick tectate-columellate ektexine (type 1a)	Bisexual	Present	Acropetal
Lemnoideae (Hesse, 2009)	Spirodela clade	Ulcerate	Echinate, micro-echinate	Sporopolleninous, thick tectate-columellate ektexine (type 1a)	Bisexual	Absent	Acropetal
Pothoideae (Hesse, 2009)	Spirodela clade	Sulcate to (rarely) porate	Reticulate	Sporopolleninous, thick tectate-columellate ektexine (type 1a)	Bisexual	Present	Acropetal
Monsteroideae (Hesse, 2009)	Bisexual Climbers clade	Ring-like	Psilate, perforate	Sporopolleninous, thick tectate-columellate ektexine (type 1a)	Bisexual	Present or absent	Acropetal
Lasioideae (Hesse, 2006a, b, 2009)	Podolasia clade	Sulcate	Reticulate to micro-reticulate	Sporopolleninous, thick tectate-columellate ektexine (type 1a)	Bisexual	Present or absent	Basipetal
Calloideae (sensu Mayo, 1997; Hesse, 2009)	***Calla***	Disulcate or ring-like	Psilate, perforate	Sporopolleninous, thick tectate-columellate ektexine (type 1a*)	Bisexual	Absent	Acropetal
Zamioculcadoideae (Bogner & Hesse, 2005; Hesse, 2009)	***Stylochaeton* clade** (*Gonatopus*, *Zamioculcas*)	Ring-like	Psilate, perforate	Sporopolleninous, thick tectate-columellate ektexine (type 1a)	Unisexual	Present	Simultaneously
Stylochaetoneae (Hesse & al., 2001; Bogner & Hesse, 2005)	***Stylochaeton* clade** (*Stylochaeton*)	Inaperturate	Foveolate, microreticulate	Sporopolleninous, thick tectate-columellate ektexine (type 1a)	Unisexual	Present	Simultaneously
Aroideae (sensu Cusimano & al., 2011; Hesse, 2009)	***Callopsis*** (Weber, 2004), ***Montrichardia*** (Weber & Halbritter, 2007; Halbritter, unpub. data), ***Anubias*** (Pacini & Hesse, 2012), ***Zantedeschia* clade** **Rheophytes *clade*** (Ulrich & al., 2012) ***Amorphophallus* clade** (Van der Ham & al., 1998, 2005), *Colletogyne* clade, *Colocasia* clade, *Pistia* clade, *Alocasia* clade	Inaperturate (omniaperturate)	Psilate, verrucate, plicate, striate, gemmate, or echinate	Thin unstructured sporopolleninous ektexine (type 1b), polysaccharidic outer wall layer (type 2a) or no ektexine (type 2b)	Unisexual	Absent	Simultaneously

The closely related taxa/clades in the recent molecular studies are in bold.
* According to Weber & al. (1999) the intine of type 1a is bilayered over the whole pollen wall. In the current investigation this was not definite in all pollen grains of *Calla*.

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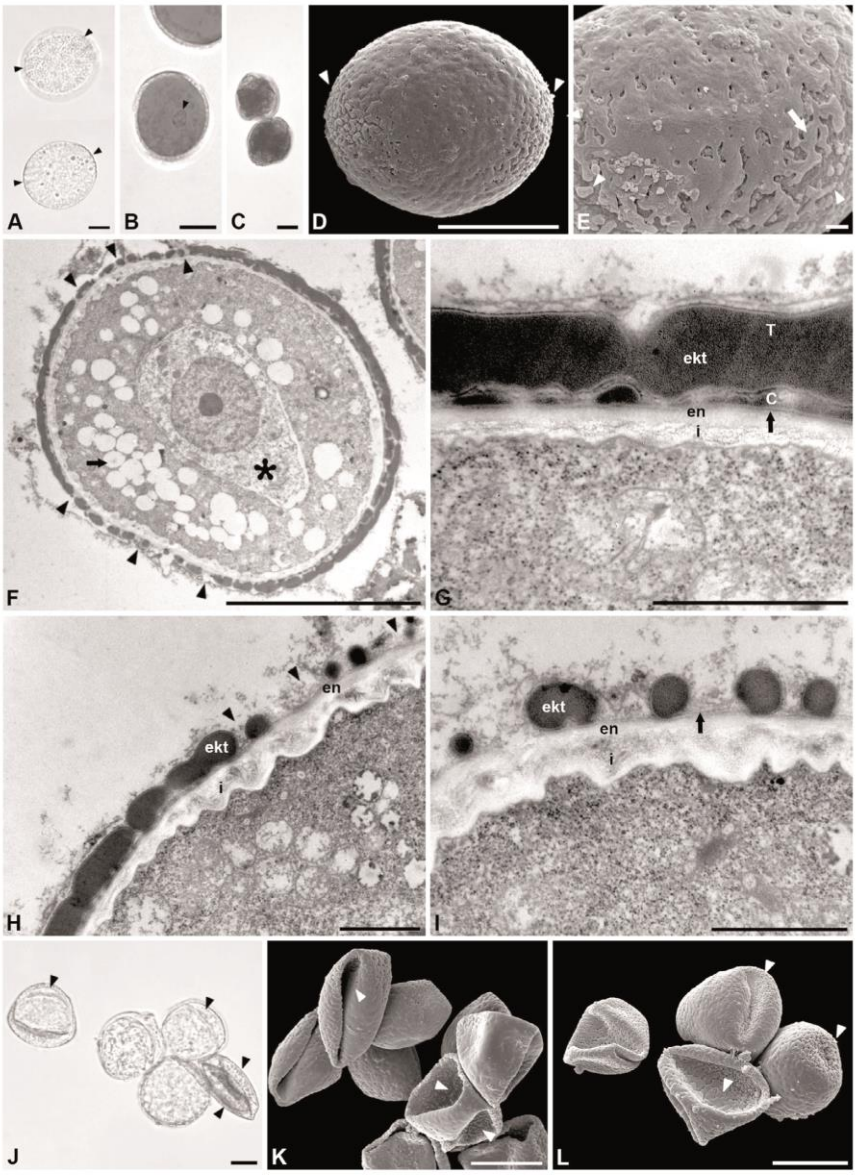

Fig. 2. LM, SEM and TEM micrographs of *Calla palustris* pollen. **A,** Hydrated pollen in upper focus and optical section, apertures visible (arrowheads). The aperture membrane is ornamented which is visible in upper focus. **B,** Generative nucleus (arrowhead) stains dark red with acetocarmine (bi-celled pollen). **C,** Pollen stained with iodine, indicating absence of starch and pollenkitt. **D,** Hydrated pollen grain with two apertures (sulci; arrowheads), critical-point dried. **E,** Detail of the exine surface with psilate and perforate ornamentation. The aperture membrane is ornamented with rugulae (arrowhead) and verrucae (arrow), critical-point dried. **F,** Cross section of a pollen grain with two sulci (arrowheads). Vegetative cytoplasm with lipid droplets (arrow) and generative cell (asterisk), U+Pb. **G,** Pollen wall in interapertural area. Pollen wall with a columellate-tectate ektexine, a thin discontinuous footlayer (arrow), a compact-discontinuous endexine and an intine, U+Pb. **H,** Pollen wall at transition of aperture (arrowheads) and interapertural area. The intine is bilayered in aperture only, U+Pb; **I,** Pollen wall in aperture area with ornamented aperture membrane with an extremely thin foot layer (arrow), U+Pb. **J,** Dry pollen in LM. Pollen infoldings due to sunken apertures (arrowheads). **K,** Dry pollen grains in SEM. In dry pollen the apertures are sunken (arrowheads) due to harmomegathy. **L,** Acetolysed pollen in SEM. The apertures are infolded due to acetolysis (arrowheads). — Abbreviations: C, columellae; ekt, ektexine; en, endexine; i, intine; T, tectum; U+Pb, uranylacetate and lead citrate. — Scale bars: A–D, F, J–L = 10 µm; E, G–I = 1 µm.

(Fig. 4C, arrows, Fig. 4D–L, arrowheads). Due to the position of the pollen grains in the tetrad (d = distal, p = proximal, arrows indicate the polar axis), the apertures are situated in the equatorial plane (Fig. 4D, white arrowheads) and are running equatorially (Fig. 4D–I), thus pollen is disulcate. The nucleus of the microspore stains with basic fuchsin (Fig. 4G–L, asterisk). Some of the free microspores appear to have a ring-like aperture, but a small remaining exine bridge of the almost anastomosing apertures is still visible (Fig. 4J–L, arrows).

Comparison of palynological characters among taxa closely related to *Calla* in recent phylogenies. — Pollen and flower characters of the major taxa of Araceae are summarized in Table 1. Simplified cladograms show the position of *Calla* and related taxa as reconstructed in the different molecular trees and morpho-anatomical classifications (Fig. 5). Selected morphological and palynological character states are mapped on the cladograms (Fig. 5A–D). According to French & al. (1995) and Keating (2004), *Calla* is placed between Lasioideae and Aroideae (Fig. 5A). In the other four papers, *Calla* is placed in subfamily Aroideae. In Cabrera & al. (2008: strict consensus of the combined parsimony analysis), *Calla* is sister to the Rheophytes clade (Fig. 5B). In Cusimano & al. (2011: maximum likelihood analysis), Cabrera & al. (2008: Bayesian analysis) and Nauheimer & al. (2012: maximum likelihood analysis), *Calla* emerges between the Rheophytes clade and the *Amorphophallus* clade (i.e., *Amorphophallus* and

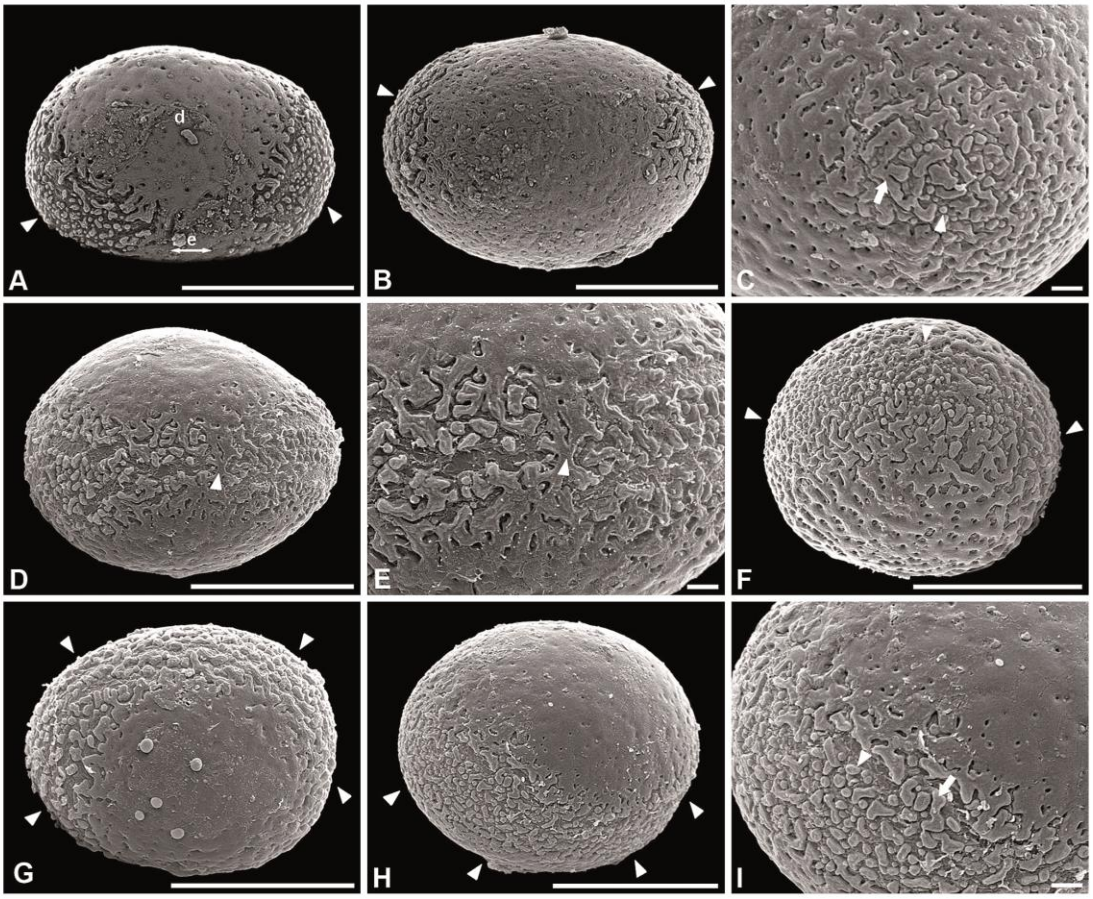

Fig. 3. SEM micrographs of *Calla palustris* pollen, critical-point dried. Notice is given to the special aperture condition, which varies from disulcate to ring-like. **A,** Pollen in oblique polar view (d = distal pole) with two elongated sulci (arrowheads) situated at the equator (e, arrow). **B,** Pollen in polar view with two short sulci (arrowheads). **C,** Detail of an extremely shortened sulcus. The aperture membrane is ornamented with rugulae (arrow) and verrucae (arrowhead). **D,** Pollen in equatorial view with an almost ring-like aperture (arrowhead). **E,** Detail of D showing the almost ring-like aperture, with a small remaining exine bridge between the sulci (arrowhead). **F–H,** Pollen in oblique equatorial view with a ring-like aperture (arrowheads). **I,** Detail of H, showing the ornamented aperture membrane with rugulae (arrow) and verrucae (arrowhead). — Scale bars: A, B, D, F–H = 10 µm; C, E, I = 1 µm.

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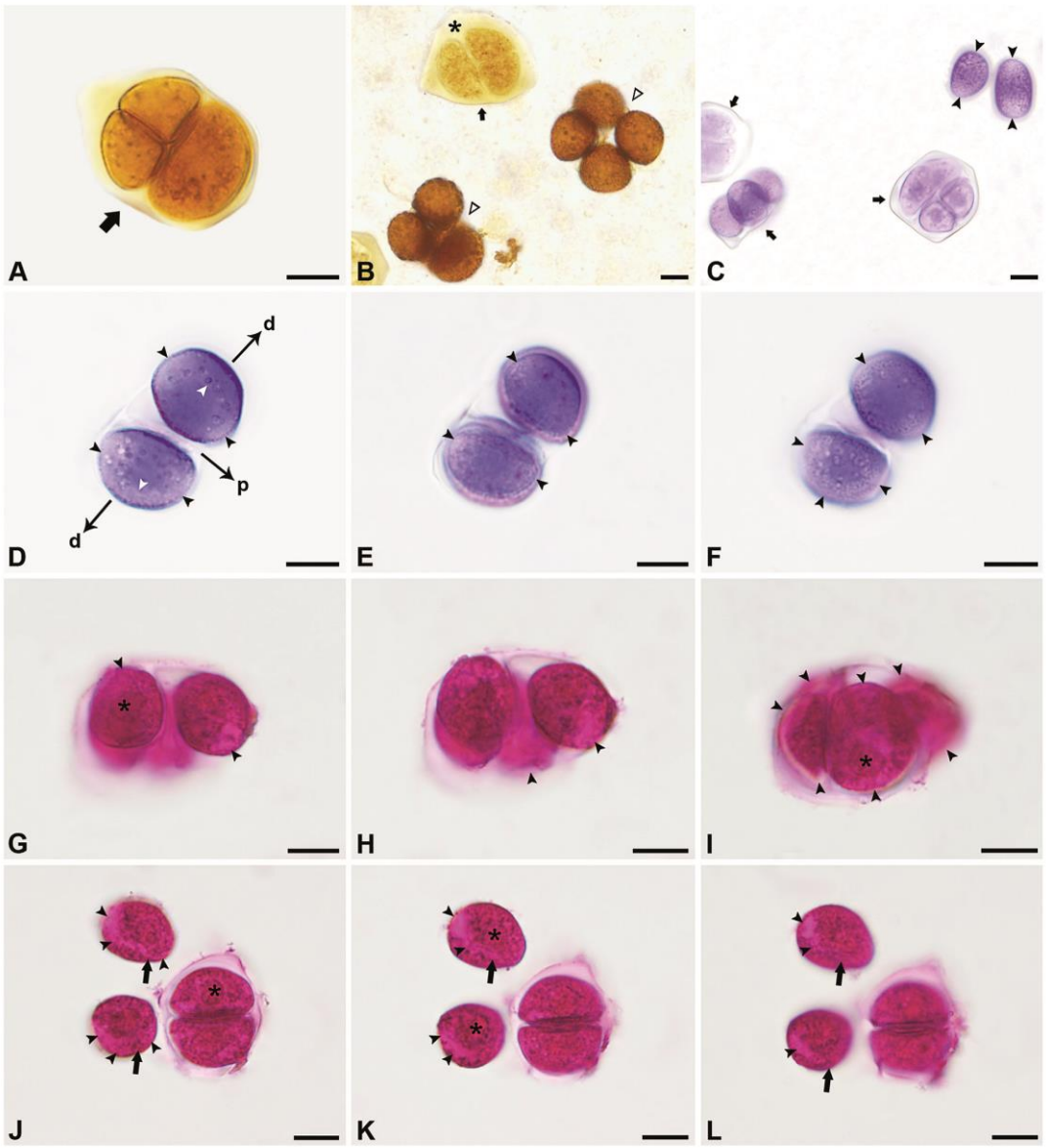
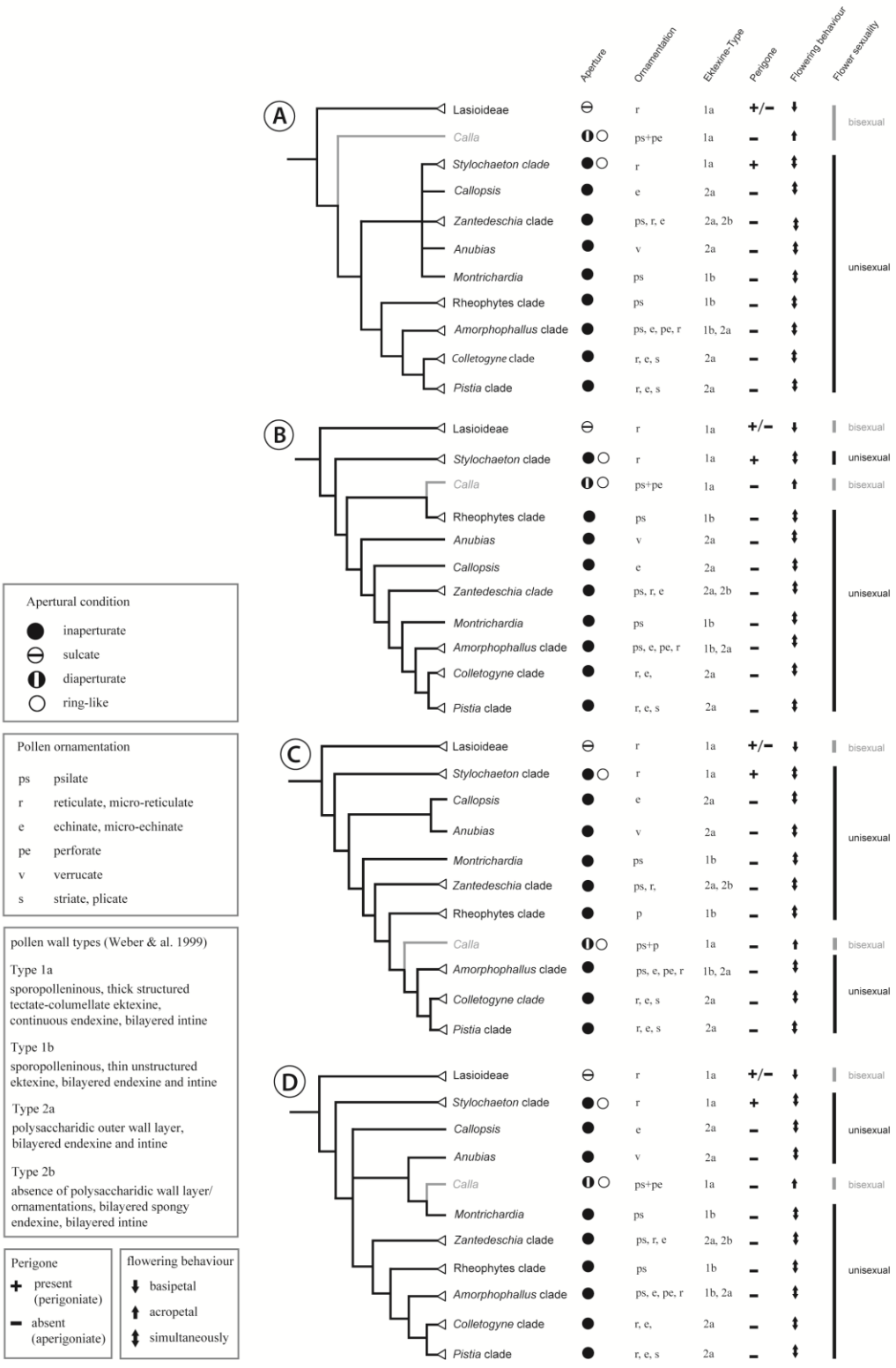


Fig. 4. LM micrographs of hydrated *Calla palustris* tetrads in different developmental stages. **A,** Early tetrad stage stained with potassium permanganate. The tetrad (arrow) is enclosed by callose. **B,** Early tetrad stage (arrow) and late tetrad stages (arrowheads) stained with potassium permanganate. In the early tetrad stage the microspores are enclosed by callose in the tetrad (asterisk) whereas in the late tetrad stages the microspores are released from callose (arrowheads). **C,** Tetrads in different stages stained with toluidine blue. Early tetrad stages with microspores enclosed by callose (arrows). The apertures in the free microspores are clearly visible (arrowheads). **D–F,** Late tetrad stage stained with toluidine blue in different optical sections showing the position of the apertures (black arrowheads, d = distal, p = proximal, arrows indicate the polar axis); the apertures are situated in the equatorial plane (white arrowheads) and are running equatorially. **G–I,** Late tetrad stage in different optical sections stained with basic fuchsin. In the tetrad the position of the apertures (sulci) is clearly visible (arrowheads). The nucleus of the microspores stains dark red (asterisks). **J–L,** Artificially destroyed tetrad in different optical sections stained with basic fuchsin. In the free microspores the sulci (arrowheads) and the nucleus (asterisks) are clearly visible. In the free microspores the aperture appears to be ring-like, but a small remaining exine bridge of the almost anastomosing apertures is still visible (arrows). — Scale bars: 10 µm.


Aperture
Ornamentation
Ektexine-Type
Perigone
Flowering behaviour
Flower sexuality
A
B
C
D
Lasioideae
Calla
Stylochaeton clade
Callopsis
Zantedeschia clade
Anubias
Montrichardia
Rheophytes clade
Amorphophallus clade
Colletogyne clade
Pistia clade
bisexual
unisexual
Apertural condition
inaperturate
sulcate
diaperturate
ring-like
Pollen ornamentation
ps psilate
r reticulate, micro-reticulate
e echinate, micro-echinate
pe perforate
v verrucate
s striate, plicate
pollen wall types (Weber & al. 1999)
Type 1a
sporopolleninous, thick structured tectate-columellate ektexine, continuous endexine, bilayered intine
Type 1b
sporopolleninous, thin unstructured ektexine, bilayered endexine and intine
Type 2a
polysaccharidic outer wall layer, bilayered endexine and intine
Type 2b
absence of polysaccharidic wall layer/ ornamentations, bilayered spongy endexine, bilayered intine
Perigone
present (perigoniate)
absent (aperigoniate)
flowering behaviour
basipetal
acropetal
simultaneously

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Pseudodracontium) (Fig. 5C). According to Chartier (2011: Bayesian analysis), *Calla* together with *Montrichardia* and *Anubias* forms a new and well supported clade, whereas *Callopsis* remains isolated (Fig. 5D).

■ DISCUSSION

Pollen characters of *Calla palustris*. — The most striking feature of *Calla* pollen is the aperture configuration, with usually two apertures, lying parallel to the equator, and not at the pole. Due to the position in the tetrad, *Calla* pollen can be described as disulcate.

Usually, a sulcus is defined as an elongated aperture situated distally (typically at the distal pole), as found in many monocot pollen or in many magnoliids. Apart from taxa with typical sulcate pollen, there also exist some taxa with disulcate pollen. The term disulcate characterizes two elongated apertures situated distally (but not directly at the distal pole), running parallel to or even in the equator. Examples for this are the monocots *Tofieldia calyculata* (L.) Wahlenb. (Erdtman, 1952; Huynh, 1976), *Uvularia grandiflora* Sm. and *Eichhornia crassipes* (Mart.) Solms (Hesse & al., 2009), some *Dioscorea* L. species (Schols & al., 2001), *Pontederia cordata* L. (Halbritter, 2012), and the magnoliid *Calycanthus floridus* L. (Huynh, 1976). In all these cases pollen has been described as disulcate (if the apertures are running meridionally, pollen would be dicolpate) (Halbritter & Hesse, 1993). A diaperturate condition is otherwise extremely rare in Araceae (Thanikaimoni, 1969; Grayum, 1992).

In the dry state, pollen is very characteristically infolded, forming a rectangle, where the sunken apertures are placed at the long or short sides of the rectangle, a feature otherwise unknown in aroids (Grayum, 1992; Halbritter, 2011).

In *Calla palustris* there is variation from disulcate to ring-like. This might be a modification resulting from changing environmental conditions, especially with respect to the harmomegathic effect (to accommodate the change of osmotic pressure in the cytoplasm during hydration or dehydration). Before the advent of the tricolpate eudicots, a ring-like aperture in monocots (Araceae, Arecaceae, Iridaceae, Lomandraceae, Rapateaceae) and early branching dicots (Nymphaeaceae, Eupomatiaceae, Degeneriaceae, Atherospermataceae, Monimiaceae) perhaps was the best means for a harmomegathic movement, to expand or close a large area for possible pollen tube formation during germination (Harley, 2004; Hesse & Zetter, 2005). The ring-like aperture with a thicker intine and endexine and a reduced ektexine is forming a highly elastic band around the pollen grain. In the dry state, the aperture is completely closed, whereas in a hydrated condition it is fully expanded, the ring opens widely, and pollen germination can take place anywhere around it (Hesse & Zetter, 2005).

The systematic position of *Calla*. — Regarding the pollen characters in combination with flower morphology and flowering behaviour, *Calla* can be clearly differentiated from the other aroid taxa/clades (Table 1; Fig. 5). Due to a combination of morphological and anatomical characters, which is unique in the entire family (e.g., bisexual aperigoniate flowers, acropetal flowering, disulcate spheroidal to ovoid pollen, simple laticifers, long-ligulate petiole sheath, unilocular ovary), *Calla* seems to be highly autapomorphic and its relationships remain obscure (Mayo & al., 1997).

The type of pollen wall structure of *C. palustris*—consisting of a sporopolleninous discontinuous, tectate-columellate ektexine, a compact-continuous endexine, an intine, and the presence of apertures—is typical for all early-diverging aroid subfamilies with bisexual flowers (Weber & al., 1998; Hesse & al., 2001; Cusimano & al., 2011). Within the Araceae this pollen wall type is characterized as type 1a, only the intine is bilayered in the aperture only (Weber & al., 1999). In contrast, all Aroideae lack this pollen wall type and are characterized by type 1b or 2a or 2b (Weber & al., 1999) (Table 1; Fig. 5). Pollen ornamentation and wall ultrastructure of related Aroideae genera (Table 1) have been published by Van der Ham & al. (1998, 2005), Weber & al. (1999), Weber & Halbritter (2007), Hesse (2009), Pacini & Hesse (2012) and Ulrich & al. (2012).

The newly described ring-like aperture condition of *Calla* deserves special attention. Within the Araceae this aperture type only occurs in Monsteroideae and Zamioculcadoideae (Hesse & al., 2001; Bogner & Hesse, 2005). Pollen of *Gonatopus* and *Zamioculcas* is heteropolar (different size of the halves) with a ring-like aperture ("hamburger-like") and with rugulate, psilate to perforate ornamentation (Hesse & al., 2001; Bogner & Hesse, 2005).

Psilate to perforate ornamentation, the aperturate condition and the pollen wall with a sporopolleninous tectate-columellate ektexine (type 1a) point at a close relationship of *Calla* to the Zamioculcadoideae (*Stylochaeton* clade) (Hesse & al., 2001; Bogner & Hesse, 2005) and Lasioideae (Mayo & al., 1997; Weber & al., 1999; Hesse, 2006a, b). Thus, *Calla* pollen is most similar to pollen of subfamily Zamioculcadoideae (*Gonatopus*, *Zamioculcas*), which are part of the *Stylochaeton* clade. In the molecular-based study by French & al. (1995) and the morpho-anatomical study by Keating (2004), *Calla* is placed isolated at the base of subfamily Aroideae (Fig. 5A). *Calla* appears to be intermediate between the *Stylochaeton*

Fig. 5. Simplified cladograms showing the position of *Calla* and related taxa in the different molecular trees and morpho-anatomical classifications. Selected morphological/palynological character states are mapped on the cladograms. The symbols and abbreviations are illustrated on the figure. **A,** French & al. (1995) and Keating (2004); **B,** Cabrera & al. (2008: strict consensus of the combined parsimony analysis); **C,** Cusimano & al. (2011: maximum likelihood analysis), Cabrera & al. (2008: Bayesian analysis) and Nauheimer & al. (2012: maximum likelihood analysis) (Note: while the placement of *Calla* is identical in both trees, there is a slight difference regarding the position of the *Stylochaeton* clade and Lasioideae. In the tree by Cusimano & al. (2011) the *Stylochaeton* clade diverges first, in the tree by Cabrera & al. (2008) the Lasioideae are diverging first.); **D,** Chartier (2011: Bayesian analysis).

clade and earlier-diverging taxa (Lasioideae). This placement is supported by our palynological results, where *Calla* is best placed in a transition zone between bisexual-flowered clades (i.e., after Lasioideae) and the unisexual-flowered clade, either diverging before or after the *Stylochaeton* clade (Fig. 5A).

Aroideae are characterized by four morphological synapomorphies (Table 1): unisexual flowers, simultaneous flowering, inaperturate pollen, no elaborated, highly reduced ektexine (type 1b) or polysaccharidic wall ornamentations (type 2a, 2b) (e.g., Mayo & al., 1997; Van der Ham & al., 1998; Weber & al., 1999; Keating, 2004; Weber, 2004; Van der Ham & al., 2005; Cusimano & al., 2011; Pacini & Hesse, 2012; Ulrich & al., 2012; Halbritter, unpub. data; our unpub. obs.).

A placement of *Calla* within the Aroideae as indicated by several molecular phylogenies is not supported by the pollen characters. Cabrera & al. (2008) suggested a placement of *Calla* as sister to the Rheophytes clade (i.e., tribe Schismatoglottideae) of the Aroideae (Fig. 5B). However, in contrast to *Calla*, pollen of this clade is inaperturate and the pollen wall (type 1b) consists of a thin unstructured sporopolleninous ektexine only (Table 1; Fig. 5).

In several studies (Cabrera & al., 2008: Bayesian analysis; Cusimano & al., 2011; Nauheimer & al., 2012) *Calla* is placed in the Aroideae between the Rheophytes clade and the *Amorphophallus* clade (Fig. 5C). This proposed close relationship to the Rheophytes clade and *Amorphophallus* clade is unique in the history of the classification of the genus and likewise not supported by our results. In the *Amorphophallus* clade (comprising the heterogeneous tribes Thomsonieae and Caladieae), the inaperturate pollen varies from echinate with sporopollenin-ous exines (*Zomicarpa*, *Zomicarpella*) to pollen with exines from striate to plicate or psilate, sometimes echinate and rarely reticulate (Van der Ham & al., 1998, 2005) with the pollen wall type 1b and 2a (Weber & al., 1999).

By adding a new marker to the alignment of Cusimano & al. (2011), Chartier (2011) for the first time placed *Calla* in a well-supported clade (Fig. 5D) at the base of the Aroideae (sensu Cusimano & al., 2011), sister to *Montrichardia*, and closely related to *Anubias*. The *Stylochaeton* clade, i.e., the expanded Zamioculcadoideae (*Stylochaeton*, *Zamioculcas*, *Gonatopus*) sensu Cusimano & al. (2011), diverges before this clade which includes *Anubias*, *Montrichardia* and *Calla*. Like in all phylogenetic studies, the genera *Anubias* Schott, *Montrichardia* Crueg. and *Callopsis* Engl. are isolated within the "Unisexual Flower clade" (subfamily Aroideae). Although their pollen varies in pollen ornamentation and pollen wall structure (Weber, 2004; Weber & Halbritter, 2007; Halbritter unpub. data, our unpub. obs.) they share the typical Aroideae pollen characters (inaperturate pollen with a usually not acetolysis resistant, polysaccharidic outer wall layer; Table 1). Based on our palynological results, we do not support the placement of *Calla* as sister to *Montrichardia* and closely related to *Anubias*, as suggested by Chartier (2011).

In summary, pollen characters point strictly against any position of *Calla* within the Aroideae clade. All characters of *Calla* discussed are inconsistent with the features of Aroideae pollen. Moreover, several additional flower characters are in accordance with our palynological results. *Calla* and all other ancestral subfamilies are characterised by bisexual flowers (Mayo & al., 1997). In contrast, the typical inflorescence of the "Unisexual Flower clade", the Aroideae, is a spadix with unisexual flowers (Mayo & al., 1997). The female flowers are located at the lower part and the male flowers in the upper part of the inflorescence (Mayo & al., 1997; Barabé & al., 2002). Moreover, the aperigoniate flowers open simultaneously, i.e., all female flowers mature first at the same time, and later all male flowers mature simultaneously. Moreover, although all Araceae have protogynous flowers, there is a significant difference in the flowering behaviour between bisexual- and unisexual-flowered genera. So far, not much attention has been paid to flowering behaviour, but there are several observations in the literature (e.g., Knuth, 1899, 1909; Krause, 1908; Engler & Krause, 1912, 1920; Engler, 1920a, b; De Wit, 1983; Mayo & al., 1997). With the exception of Lasioideae, all Araceae with bisexual flowers, including *Calla*, are flowering in acropetal sequence (Bogner pers. comm., our observations). Uniquely, the Lasioideae are basipetally flowering (from top to base). As most early diverging taxa are perigoniate (except the aperigoniate *Pycnospatha* Thorel ex Gagnep.), the absence of a perigone seems to link *Calla* to the Aroideae, but in many Monsteroideae a perigone is also absent (Mayo & al., 1997; Cabrera & al., 2008) (Table 1; Fig. 5). Another remarkable aspect of contrasting flowering behaviour is the duration of anthesis. In all bisexual genera, including *Calla*, the flowering period lasts over a long time (up to several weeks). However, in the unisexual genera except for *Arisaema* Mart., *Ambrosina* Bassi and *Arisarum* Mill. (Barriault & al., 2009) it usually lasts only for a very short time (two to four days).

As stated by Cusimano & al. (2011), it seems highly implausible that a distinct taxon, situated within a clade with exclusively derived characters (i.e., Aroideae), exhibits so many ancestral characters, as this would require the reversal of a whole "character package" (e.g., bisexual flowers, aperturate pollen with an elaborated sporopollenin ektexine). Thus, the relevant pollen characters, the contrasting flowering behaviour within the aroids together with all other morphological features, as described by Keating (2004), point strictly against a position of *Calla* within the Aroideae clade.

■ CONCLUSION

The pollen characters of *Calla* indicate a position in the transition zone either between Zamioculcadoideae (*Stylochaeton* clade) and Lasioideae or between Zamioculcadoideae (*Stylochaeton* clade) and Aroideae (Aroideae clade). This placement is also supported by flower morphology, in particular the bisexual flowers, the duration of anthesis and the flowering sequence. Such placement would not require a reversal of the pollen and other morphological and anatomical characters discussed above. Further studies using additional molecular markers are necessary to resolve the discrepancy in systematic position of *Calla* between the molecular phylogenies and the morphological classifications.

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C. *Amorphophallus* – New insights into pollen morphology and the chemical nature of the pollen wall.

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***Amorphophallus*: New insights into pollen morphology and the chemical nature of the pollen wall.**

Running title: 'Amorphophallus *pollen – New insights', Ulrich et al. 2015*

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Abstract

In pollen characters *Amorphophallus* is one of the most diverse genera in the Araceae. The present work is a critical survey of contradicting reports on the impact of acetolysis treatment of *Amorphophallus* pollen and on the chemical nature of the outer pollen wall layer and of electron-dense (dark) granules found within it. Furthermore, we wanted to clarify the pollen polarity and to test conclusions based on different preparation techniques. Pollen morphology of 25 species is investigated by light microscopy, scanning electron microscopy and transmission electron microscopy. Our results show that *Amorphophallus* pollen is not resistant to acetolysis treatment. The use of different transmission electron microscopy staining methods proved the polysaccharide nature of the outer pollen wall layer and of the granules within it. Moreover, an additional thin surface layer was found in all investigated species. Microspores in early and late tetrad stages show that the less convex side of the microspore is the proximal face and the more convex side the distal face. The extrusion of pollen in strands is illustrated for the first time by light- and scanning electron microscopy. Furthermore, observations of pollen in water showed that in some of the investigated species the pollen wall is shed immediately before pollen tube formation.

Keywords: Araceae, Thomsonieae, ultrastructure, staining methods, polysaccharides, granules, tetrads, pollen strands, pollen wall shedding.

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Introduction

The large genus *Amorphophallus* Blume ex Decne. (tribe Thomsonieae Blume) consists of more than 200 species (Hetterscheid 2012). It is one of the morphologically most diverse genera of subfamily Aroideae (Mayo et al. 1997). Based on molecular classifications the tribe Thomsonieae was recently recognised as monotypic, as the smaller genus *Pseudodracontium* N. E. Br., was reduced to *Amorphophallus* (Grob et al. 2002, 2004; Sedayu et al., 2010; Hetterscheid & Claudel 2012). *Amorphophallus* is distributed in paleotropic regions from West Africa to Polynesia (Hetterscheid & Ittenbach 1996). Many *Amorphophallus* species are geophytes usually with one leaf. The inflorescence of *Amorphophallus* is usually solitary and plants are usually flowering without leaves (Hetterscheid 2012). The spathe is variously coloured and boat-shaped or differentiated into lower tube and limb (Hetterscheid 2012). The flower bearing spadix is sessile or stipitate and either short or much longer than the spathe (Hetterscheid 2012). An appendix is usually present and very variable in shape and structure (Hetterscheid 2012). The unisexual flowers are lacking a perigone and the male and female zones are adjacent in most *Amorphophallus* species. In a smaller number of species there is a sterile zone between the male and female zones, often covered by staminodes (Mayo et al. 1997; Hetterscheid 2012). The male flowers are divers. The stamens may be short and the one to six stamens are either free or connate in basal flowers or in synandria (Hetterscheid 2012). Filaments are either absent or distinct with a broad connective that is sometimes as broad as thecae, rarely forming a synconnective (Hetterscheid 2012). Theca dehiscence is either by an apical, rarely lateral, pore or a transverse short slit often with pollen extruding in strands or powdery (Mayo et al. 1997; Hetterscheid 2012). The inflorescence acts as a single large flower to attract pollinators (Mayo et al. 1997; Punekar & Kumaran 2010; Bröderbauer 2012).

Pollinators are beetles, flies or bees (e.g. Gibernau 2003; Sannier et al. 2009), which in some species are trapped by ridges or warts on the inside of the spathe (Punekar & Kumaran 2010). Insects are reported to be trapped in some

species by the overhanging limb of the spathe and carrion beetles have been observed to use the closed spathe chamber for mating and for egg laying (Punekar & Kumaran 2010). Punekar and Kumaran (2010) described five stages of insect trapping for *Amorphophallus* based on their observations: first is a visual trap (e.g. spathe colour), followed by odour trap (odour emission by appendix), slippery trap (spathe surface), food trap (e.g. visual attraction of stigmatic fluid, sterile flowers, pollen) and finally by a reproductive trap (safe spathe chamber). According to Bröderbauer (2012), it seems unlikely for most *Amorphophallus* species that the sterile flowers play a role in trapping insects due to their shape and position. Instead straight papillate cells and epicuticular wax crystalloids on the spathe surface as well as a wax layer on the sterile appendix were found in several species of *Amorphophallus* (Bröderbauer 2012). Pollen is produced in huge amounts and extruded in strands or powdery, finally deposited on trapped insects or picked up by escaping insects (Mayo et al. 1997, Gibernau 2003, Punekar & Kumaran 2010). Although, well known and often described, pictures of pollen strands are rare and limited to few macroscopic pictures (e.g. Mayo et al. 1997; Gibernau et al. 1999; Chartier et al. 2014; Wong et al. 2013).

The considerable diversity in pollen morphology in *Amorphophallus* has been demonstrated in previous studies (e.g. Thanikaimoni 1969; Tarasevich 1988; Grayum 1992; Van der Ham et al. 1998, 2005). However, pollen of many species remains unexplored while new species of this large genus are regularly discovered (e.g. Hetterscheid & Claudel 2014). Pollen of most *Amorphophallus* species is distinctively sculptured in a variable manner and up to ten ornamentation types are described: psilate, striate, striate with psilate caps, areolate, verrucate, fossulate, echinate, reticulate, scabrate and striate/scabrate (Mayo et al. 1997, p.249; Van der Ham et al. 2005, p. 257). The comprehensive studies of Van der Ham et al. (1998, 2005) showed that psilate and striate ornamentation are the dominating types. According to literature (e.g. Grayum 1992; Mayo et al. 1997; Weber et al. 1999; Keating 2004; Weber 2004; Pacini & Hesse 2012; H. Halbritter unpubl. data) *Amorphophallus* pollen shares characters typical for Aroideae pollen: inaperturate with either a highly reduced sporopollenin thin ektexine (type 1b; Weber et al. 1999, p. 418), polysaccharide wall ornamentations (type 2a, Weber et al. 1999, p. 418) or without polysaccharide

outer pollen wall (type 2b; Weber et al. 1999, p. 420). Van der Ham et al. (1998, 2005) described two principal wall types for *Amorphophallus*; pollen with a thick spongy endexine and a non-sporopollenin elaborated outer pollen wall layer (Van der Ham et al. 1998, p. 98, 2005, p. 257, 'Van der Ham-Type 1') and pollen with a thin endexine and a probably sporopollenin thin, unstructured ektexine (Van der Ham at al. 1998, p. 98 2005, p. 257, 'Van der Ham-Type 2').

Contradicting information exists on the resistance of *Amorphophallus* pollen to acetolysis. Whether the outer pollen wall (ektexine) is resistant to acetolysis or not is correlated with the presence or absence of sporopollenin (Hesse 2006a). In general, the pollen wall of seed plants consists of two main layers: the outer exine, which consists mainly of an acetolysis- and decay-resistant biopolymer (sporopollenin) and the inner polysaccharide intine (Hesse et al. 2009). Sporopollenin is the major component of the exine (e.g. Dobritsa et al. 2009; Jardine et al. 2015). Recent studies suggest that sporopollenin may have two different types of chemical structures, oxygenated aromatic compounds and aliphatic compounds (e.g. Guilford et al. 1988; Dutta 2006; Gabarayeva et al. 2010; Steemans et al. 2010). According to Thanikaimoni (1969), Tarasevich (1988, 1992), Weber et al. (1999), Hesse et al. (2000) and Van der Ham et al. (2005) in *Amorphophallus* the pollen sculpturing does not resist acetolysis. Van der Ham et al. (1998) reported that among 24 *Amorphophallus* species only two species had pollen that retained the ornamentation to a variable degree. Although Van der Ham et al. (1998, 2005) also reported non-resistance of *Amorphophallus* pollen to acetolysis, the interpretation of the nature of the outer pollen wall was unclear. The hypothesis that a slow dissolution of ornamenting elements may be due to incorporation of sporopollenin was put forward by Van der Ham et al. (2000). Only Grayum (1992) and more recently Punekar and Kumaran (2010) reported that pollen of *Amorphophallus* species retained an intact patterned surface after acetolysis. Given that some *Amorphophallus* species showed resistance to acetolysis the presence of sporopollenin in the outer pollen wall (ektexine) was assumed (Punekar & Kumaran 2010; Van der Ham et al. 2000). Furthermore, Van der Ham et al. (1998, p. 113) assumed a correlation between the low acetolysis resistance and the presence of electron-dense (dark) granules in the outer pollen wall. In subsesquent studies by Van der Ham et al. (2000,

p. 241; 2005, p. 259) the granules are described to be osmiophilic occurring in the ektexines of all species with striate pollen, and absent or indistinct in psilate pollen.

Studies on *Amorphophallus* pollen have given information on pollen ornamentation and ultrastructure on many species (e.g. Grayum 1992; Mayo et al. 1997; Van der Ham et al. 1998, 2005). However, some questions remain ambiguous such as the chemical nature of the outer pollen wall ('ektexine') and of the granules associated with the pollen wall. The polarity of *Amorphophallus* pollen has also been unclear as observations of pollen tetrads are very rare. The only observation of a single pollen tetrad (decussate) and occasional tetrad fragments of *A. konjac* K. Koch by Van der Ham et al. (1998) suggests the less convex side to be proximal. Two 'shape types' have been described for *Amorphophallus* pollen by Van der Ham et al. (1998). The 'ellipsoidal pollen type' includes pollen with a long and a short axis and with one side more convex than the other (Van der Ham et al. 1998). The 'spheroidal pollen type' includes pollen with two identical axes with spheroidal shape and circular outline (Van der Ham et al. 1998).

The aim of the present study is to clarify contradicting reports on pollen characters and to test conclusions based on different preparation techniques. We used different histochemical staining methods to study the stratification and chemical nature of the pollen wall and to resolve the occurrence of electron-dense (dark) granules in the outer pollen wall ('ektexines') reported by Van der Ham et al. (1998, 2005). Pollen of 18 *Amorphophallus* species was acetolysed to investigate the chemical nature (polysaccharide or sporopollenin) of the outer pollen wall. Pollen of 25 *Amorphophallus* species was investigated by light microscopy, scanning- and transmission electron microscopy. New results on the pollen wall ultrastructure are presented for *A. asterostigmatus* Bogner et Hett., *A. bulbifer* Blume, *A. henryi N. E. Br., A. ongsakulii* Hett. et A. Galloway, *A. polyanthus* Hett. et Sizemore, *A. prainii* Hook. f., *A. operculatus* Hett. et Sizemore, *A. serrulatus* Hett. et A. Galloway, *A. stuhlmannii* (Engl.) Engl. et Gehrm. and *A. taurostigma* Ittenb.

We specifically addressed the following questions: (1) Can we specify shape and polarity of the inaperturate ellipsoidal pollen by observing pollen tetrads? (2) Is the outer pollen wall of the *Amorphophallus* pollen made of polysaccharides or sporopollenin? (3) Are electron-dense (dark) granules in the outer pollen wall of some *Amorphophallus* species made of sporopollenin or polysaccharides? (4) If and to what extent does *Amorphophallus* pollen may retain its ornamentation after acetolysis and which parameters influence the results? (5) To what extent are the results influenced by the preparation methods and staining techniques?

Material and Methods

Plant material

Pollen of 25 *Amorphophallus* species was investigated (including two former *Pseudodracontium* species) (Table I). The material was taken from the Botanical Garden of the University of Vienna (HBV) and from the Munich Botanical Garden (M). Mature anthers were examined by light microscopy, scanning- and transmission electron microscopy. Due to the low amount of the gained material or to fixation problems, no TEM studies were carried out for *A. atrorubens* Hett. et Sizemore*, A. interruptus* Engl. et Gehrm., *A. mangelsdorffii* Bogner*, A. myosuroides* Hett. et A. Galloway*, A. krausei* Engl., *A. paeoniifolius* (Dennst.) Nicolson*, A. rhizomatosus* Hett. *and A. sumawongii* (Bogner) Bogner et Mayo.

Pollen preparation

For light microscopy (LM) fresh and dry pollen was rehydrated in water. For the detection of starch the material was stained with potassium iodine (Gerlach 1984). For the detection of the cellular condition (bi- or trinucleate) pollen was stained with acetocarmine (Gerlach 1984). Pollen tetrads were taken from anthers of a single inflorescence before anthesis and investigated in glycerin or stained in water with toluidine blue (Siegel 1967). The material was observed with an Olympus BX50-F light microscope.

For scanning electron microscopy (SEM) of investigat hydrated pollen the material was rehydrated in water, dehydrated with 2,2–dimethoxypropane (DMP), and critical point dried (CDP) (Halbritter 1998). Critical point dried as well as air dried pollen was mounted with adhesive tape on aluminum stubs and sputter coated with gold. All samples were investigated with a JEOL JSM 6390 SEM at 10 kV.

For transmission electron microscopy (TEM) mature anthers or pollen in suspension were rehydrated and fixed in 3% glutaraldehyde (GA) in 0.1 M phosphate buffer (pH 7.4), postfixed with 1% osmiumtetroxide (OsO_4) and 0.8% potassium hexacyanoferrate ($K_4Fe(CN)_6 \bullet 3H_2O$) 2:1 at 4°C (Weber 1992). Fixed material was dehydrated in 2,2−dimethoxypropane and embedded in Agar's low viscosity resin (LV–Resin) (Agar Scientific Limited 2004). Sections of about 60–70 nm were cut with a diamond knife on a Reichert Ultracut microtome. All sections were examined in a Zeiss EM 109 or a ZEISS 900 TEM at 50 kV.

Conventional staining was done by staining sections with uranyl acetate (U: prefabricated solution Ultrostain 1, Leica) for 40 minutes, followed by lead citrate (Pb: prefabricated solution Ultrostain 2, Leica) for five minutes (Hayat 2000) and by staining with a modified Thiéry-test: 1% periodic acid for ten minutes, 0.2% thiocarbohydrazide for 15 minutes and with 1% silver proteinate for ten minutes (Weber & Frosch 1995). For the detection of neutral polysaccharides a Thiéry-test (Thiéry 1967) was carried out: staining with 1% periodic acid for 60 minutes instead of 30 minutes, to remove the osmiumtetroxide, 0.2% thiocarbohydrazid for 16 hours and 1% silver proteinate for 30 minutes. For the detection of unsaturated lipids (lipid test) a Thiéry-test without periodic acid oxidation according to Rowley and Dahl (1977) was carried out: staining with 0.2% thiocarbohydrazid for 16 hours, followed by 1% silver proteinate for 30 minutes. For detecting the endexine sections were stained with potassium permanganate (1% aqueous potassium permanganate solution, for seven minutes; Hayat 2000; Weber & Ulrich 2010).

For acetolysis fresh or air dried pollen was acetolysed by 100 degrees for at least five minutes in a mixture of nine parts acetic anhydride and one part concentrated sulphuric acid (Erdtman 1960; Hesse & Waha 1989). For light

microscopy one part of the acetolysed material was transferred in glycerine and observed in an Olympus BX50-F light microscope. For scanning electron microscopy, acetolysed pollen was transferred with a drop of acetone on a stub, sputter coated with gold and observed with a JEOL JSM 6390 SEM at 10 kV.

Pollen terminology follows Hesse et al. (2009).

Results

Selected pollen characters for species examined are summarized in Table I. The investigated *Amorphophallus* species appear in order of pollen ornamentation type.

Inflorescence, theca dehiscence and pollen strands

The typical inflorescence of *Amorphophallus,* as shown by *A. konjac,* consists of a spadix, with a sterile terminal appendix, and a modified leaf, called the spathe (Figure 1A). The size of the inflorescence of the investigated *Amorphophallus* species can be very big, e.g. *A. konjac*, about sixty centimeters (Figure 1A) or very small e.g. *A. serrulatus,* only a few centimeters (Figure 1B). The unisexual flowers are lacking a perigone (aperigoniate) and the male (♂) and female (♀) zones are adjacent in most *Amorphophallus* species (Figure 1C). Theca dehiscence of the investigated species may be by apical or subapical pores or short slits, frequently correlated with the extrusion of pollen in strands (Figure 1D-F, 2A-C). Three types of stamens are observed: 1. Anthers with three free stamens (♂, number can vary on a single spadix), elongated filaments, connate to free and globose thecae, dehiscing by a pore (Figure 1D); 2. Anthers lacking a filament, with subapical pores releasing pollen in strands (Figure 1E); 3. Anthers connate with thick connective (Figures 1F), lacking a filament and theca with transverse short slit extruding pollen in strands (Figures 1F, 2A). In dry condition (Figure 2B) as well as after rehydration and critical point drying (Figure 2C) pollen grains are still clumped together in strands. Inside an artificially broken anther the critical point dried pollen grains are not clumped, only loose together and some pollen grains are ruptured (Figure 2D).

Table I. Pollen characters for species examined in this study; only selected characters are shown (see text for further explanation). Species listed according to pollen ornamentation type.

Taxon	Shape and Size, hydrated and dry pollen (LM, SEM)							Ornamentation, hydrated and acetolyzed pollen (LM, SEM)			Wall stratification (TEM)				Figure(s)
***Amorphophallus* species investigated** (25)	**Shape** (equatorial view)	**Outline** (polar view)	**Polarity**	**Pollen size (µm)**	**Shape (dry)**	**Outline (dry)** (polar view)	**Infoldings (dry)**	**Ornamentation type** our results	**Ornamentation type** according to Van der Ham et al. 1998, 2000 and 2005	**Acetolyzed pollen**	**Pollen wall**	**Thin surface layer**	**Granules in the outer pollen wall** our results	**Granules** according to Van der Ham et al. 1998, 2005	Illustrated results
Striate pollen type															
A. interruptus	oblate	elliptic	heteropolar (or isopolar)	large (63 µm)	oblate	elliptic	not infolded	striate	striate (Van der Ham et al. 1998, p. 100)	n.i.	n.i.	n.i.	n.i.		2, 4,
A. sumawongii	oblate	elliptic	heteropolar	medium (34–40 µm)	oblate	elliptic	not infolded	striate	striate (Van der Ham et al. 1998, p. 100)	n.i.	n.i.	n.i.	n.i.	distinct, loose (Van der Ham et al. 2005, p. 255)	4
A. krausei	oblate to spheroidal	elliptic to circular	isopolar	medium to large (50–70 µm)	oblate	elliptic	not infolded	striate	striate (Van der Ham et al. 2005, p. 254)	psilate to striate	n.i.	n.i.	n.i.	distinct, crowded (Van der Ham et al. 2005, p. 254)	2, 3, 4, 15
A. yunnanensis	oblate to spheroidal	elliptic to circular	isopolar	medium to large (50–60 µm)	oblate	elliptic	not infolded	striate	striate (Van der Ham et al. 2000, p. 465)	psilate to striate	type 2a	present	without	without (Van der Ham et al. 2005, p. 255)	2, 3, 4, 11, 15
*A. atrorubens**	oblate	elliptic	heteropolar	large (80–90 µm)	oblate	elliptic	not infolded	striate (untypical)	n.i.	psilate	n.i.	n.i.	n.i.	n.i.	4, 15
Striate to reticulate pollen type (subtype 1)															
A. longituberosus	spheroidal to oblate	circular to elliptic	isopolar	medium to large (45–70 µm)	spheroidal to oblate	circular to elliptic	not infolded	striate to reticulate	striate, sometimes striate-reticulate (Van der Ham et al. 1998, p. 114)	n.i.	type 2a	present	distinct	distinct (Van der Ham et al. 1998, p. 114) indistinct, crowded (Van der Ham et al. 2005, p. 262)	5, 10, 15
A. asterostigmatus	oblate	elliptic	heteropolar	large (50–70 µm)	oblate	elliptic	not infolded	striate to reticulate	striato-reticulate (Van der Ham et al. 1998, p. 112)	psilate	type 2a	present	distinct	n.i.	5, 10, 15
Striate to plicate pollen type (subtype 2)															
*A. myosuroides**	oblate	elliptic	heteropolar	medium (50–53 µm)	oblate	elliptic	not infolded	striate to plicate	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	3, 5
*A. polyanthus**	spheroidal	circular	isopolar	medium (30–37µm)	oblate	elliptic	not infolded	striate to plicate	n.i.	psilate, with remains	type 2a	present	distinct	n.i.	5, 10, 15
*A. ongsakulii**	oblate	elliptic	isopolar	medium (40–50 µm)	oblate	elliptic	not infolded	striate to plicate	n.i.	psilate	type 2a	present	distinct	n.i.	5, 11, 15
Striate to plicate pollen type, with appandages (subtype 3)															
*A. serrulatus**	oblate	elliptic	heteropolar	medium to large (55 µm)	oblate	elliptic	not infolded	striate to plicate, with appendages	n.i.	psilate to striate	type 2a	present	indistinct	n.i.	1, 6, 11 15
*A. operculatus**	oblate	elliptic	heteropolar	medium (50µm)	oblate	elliptic	not infolded	striate to plicate, with appendages	n.i.	psilate, with remains	type 2a	present	indistinct	n.i.	1, 3, 6, 11, 15
Striate to plicate pollen type, with psilate apices (subtype 4)															
*A. lacourii**	oblate to spheroidal	elliptic to circular	heteropolar	large (58 µm)	oblate	elliptic	not infolded, sometimes flat	striate to plicate, with psilate apices	striate, with psilate caps (Van der Ham et al. 1998, p. 131; 2005 p. 260)	psilate, with remains	type 2a	present	distinct	distinct, crowded (Van der Ham et al. 2005, p. 257)	6, 11, 15
A. latifolius	oblate to spheroidal	elliptic to circular	heteropolar	large (55–65 µm)	oblate	elliptic	not infolded	striate to plicate, with psilate apices	striate, with psilate caps (Van der Ham et al. 1998, p. 131)	psilate, with remains	type 2a	present	distinct	distinct, crowded (Van der Ham et al. 2005, p. 257)	1, 2, 6, 11, 15
Irregularly striate pollen type (subtype 5)															
A. palawanensis	spheroidal to oblate	circular to elliptic	heteropolar	medium (40–48 µm)	oblate	elliptic	ornamentation free areas sunken	irregularly striate	striate (Van der Ham et al. 1998, p. 116)	psilate to fossulate	type 2a	present	distinct	distinct, loose (Van der Ham et al. 2005, p. 255)	7, 12, 15
A. rhizomatosus	spheroidal	circular	isopolar	medium (40–50 µm)	n.i.	n.i.	n.i.	irregularly striate	striate (Van der Ham et al. 1998, p. 100)	psilate	n.i.	n.i.	n.i.	without granules (Van der Ham et al. 2005, p. 262)	2, 7, 15
A. konjac	spheroidal to oblate	circular to elliptic	heteropolar to isopolar	medium (40 µm)	spheroidal to oblate	circular or elliptic	not infolded	irregularly striate	fossulate (Van der Ham et al. 1998, p. 123)	psilate to striate/fossulate	type 2a	present	indistinct, in lower part	indistinct, in lower third to half (Van der Ham et al. 1998, p. 125)	1, 7, 12, 15
*A. taurostigma**	oblate	elliptic	heteropolar	large (60–70 µm)	oblate	elliptic	not nfolded	irregularly striate	fossulate (Van der Ham et al. 1998, p. 121; 2005, p. 260)	psilate, with remains	type 2a	present	indistinct, in basal sublayer	n.i.	7, 12, 15
A. mossambicensis	spheroidal	circular	isopolar	large (60–70 µm)	spheroidal	irregular	irregularly infolded	irregularly striate, sometimes striate (to psilate)	fossulate (Van der Ham et al. 1998, p. 122-123)	psilate	type 2a	present	indistinct, in basal sublayer	indistinct, with darker basal sublayer (Van der Ham et al. 2005, p. 256)	7, 12, 15
Echinate pollen type															
*A. mangelsdorffii**	spheroidal	circular	isopolar	medium (40–54 µm)	spheroidal to ellipsoidal	circular	not infolded	echinate (micro-echinate)	n.i.	psilate to echinate	n.i.	n.i.	n.i.	n.i.	2, 8, 15
Verrucate pollen type															
A. stuhlmannii	spheroidal	circular	isopolar	large (80–90 µm)	spheroidal	circular	not infolded	verrucate	verrucate (Van der Ham et al. 1998, p. 109)	psilate, with remains	type 2a	present	distinct	n.i.	8, 13, 15
Psilate pollen type															
*A. bulbifer**	oblate	elliptic	heteropolar to isopolar	large (64–76 µm)	oblate	elliptic or irregular	not infolded or irregularly infolded	psilate	psilate (Van der Ham et al. 1998, p. 127)	psilate	type 2a	uncertain	indistinct, in upper part	n.i.	9, 14, 15
A. henryi	spheroidal	circular	isopolar	large (58–75 µm)	spheroidal	circular or irregular	not infolded or irregularly infolded	psilate	psilate (Van der Ham et al. 1998, p. 113; 2005, p. 260)	n.i.	type 2a	uncertain	indistinct, in basal sublayer	n.i.	2, 9, 14
A. paeoniifolius	spheroidal	circular	isopolar	large (76–93 µm)	spheroidal	circular	irregularly infolded	psilate	psilate (Van der Ham et al. 2000, p. 465)	psilate, with remains	n.i.	n.i.	n.i.	without granules (Van der Ham et al. 1998, p. 129)	9, 15
A. prainii	spheroidal	circular	isopolar	medium to large (50–68 µm)	spheroidal	irregular	irregularly infolded	psilate	psilate (Van der Ham et al. 1998, p.101)	psilate	type 2a	uncertain	indistinct, in basal sublayer	n.i.	9, 14, 15

Abbreviations and symbols: n.i., not investigated; * new palynological data

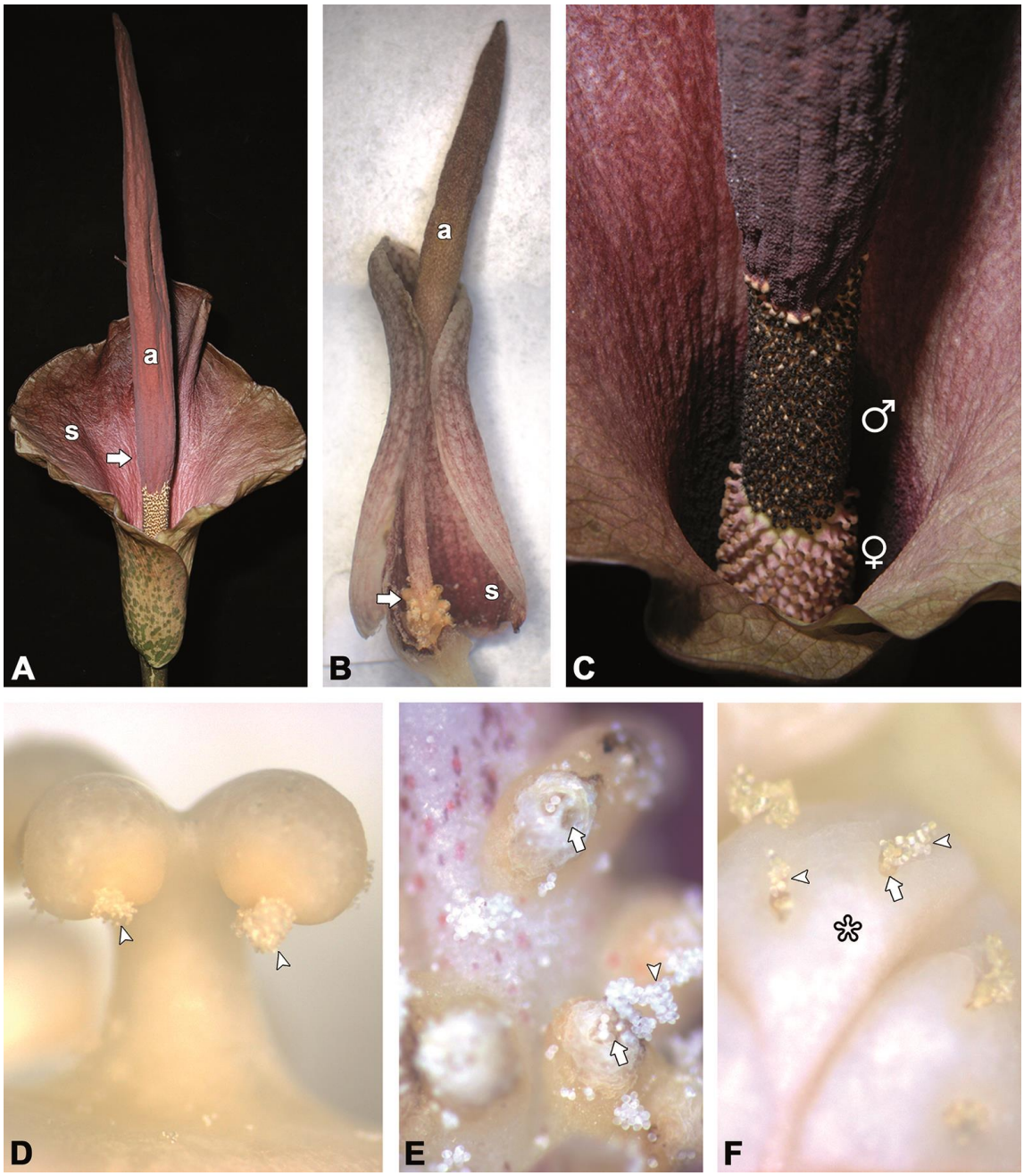


Figure 1. Inflorescence, floral morphology and anhers of selected *Amorphophallus* species, demonstrating the morphological diversity within the genus. **A.** *A. konjac,* inflorescence with flower bearing spadix (*arrow*), sterile terminal appendix (*a*) and spathe (*s*), inflorescence about 60 cm in size. **B.** *A. serrulatus,* spathe (*s*) partly removed to show spadix with flowers (*arrow*) inside the spathe chamber; sterile terminal appendix (*a*); inflorescence about 8 cm in size. **C.** *A. konjac,* inflorescence with unisexual aperigoniate flowers separated into a female (♀) and male zone (♂). **D.** *A. latifolius,* globose thecae, dehiscing by a pore and extruding pollen in strands (*arrowheads*). **E.** *A. serrulatus,* anthers with subapical stomial pores (*arrows*) releasing pollen strands (*arrowheads*). **F.** *A. operculatus*, anthers connate, with broad connective (*asterisk*), lacking a filament, thecae with transverse short slit (*arrow*), releasing pollen strands (*arrowheads*).

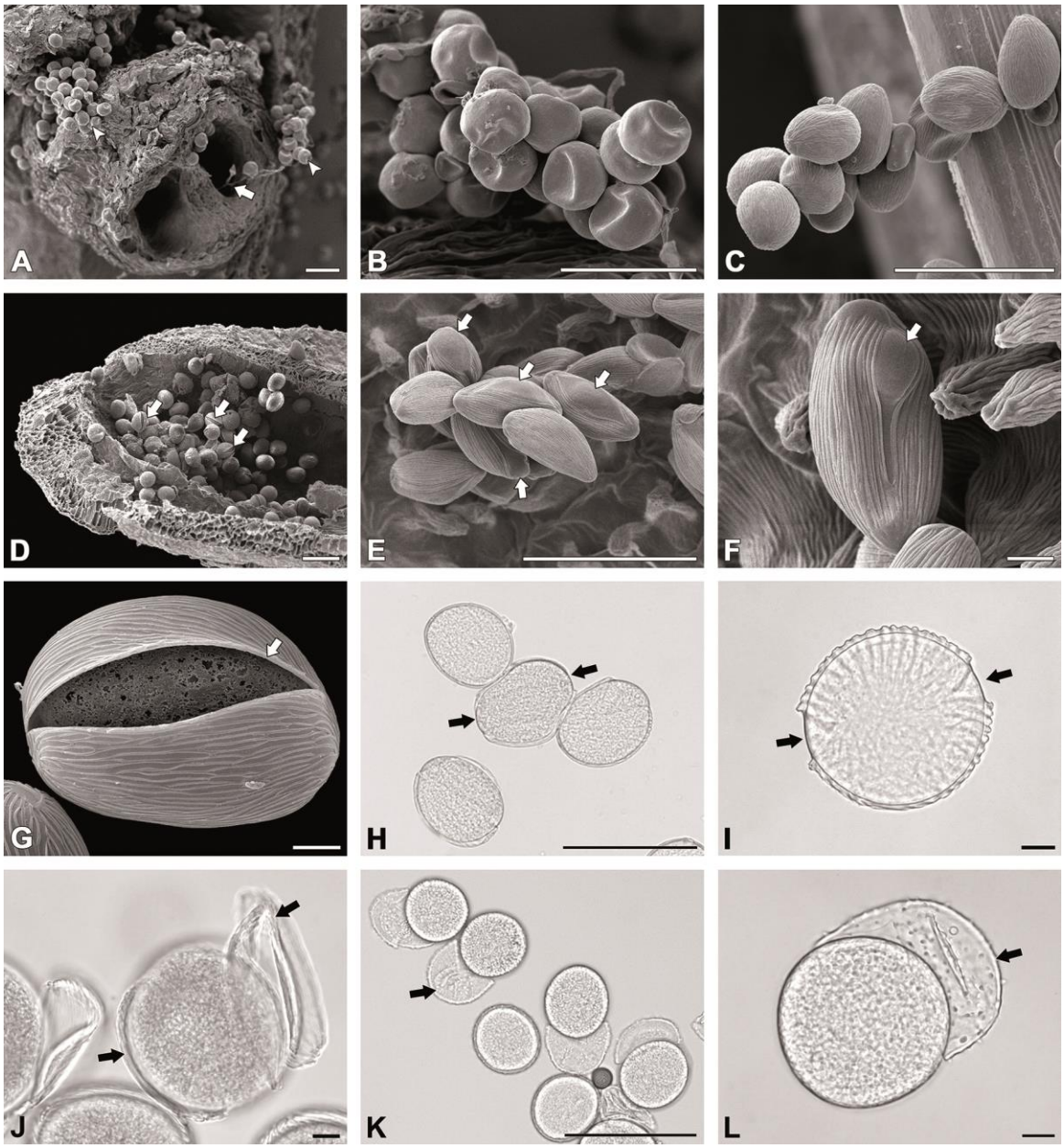


Figure 2. Pollen strands, pollen unit and pollen wall shedding. **A.** *A. henryi*, theca with short slits (*arrow*) extruding pollen in strands (*arrowheads*); SEM, dry. **B.** *A. henryi*, pollen strand with psilate pollen. SEM, dry. **C.** *A. krausei*, pollen strand with striate pollen. SEM, hydrated. **D.** *A. yunnanensis,* longitudinal section of pollen sac with pollen inside; some pollen ruptured (*arrows*); SEM, hydrated. **E.** *A. latifolius*, pollen starts to germinate (*arrows*). SEM, dry. **F.** Pollen of *A. latifolius* pollen tube formation (*arrowhead*); SEM, dry. **G.** Pollen of *A. interruptus,* exine splitting (*arrow*), note the protoplast inside; SEM, hydrated. **H.** Striate pollen of *A. yunnanensis*, with splitting pollen wall (*arrows*); LM, hydrated. **I.** Striate pollen of *A. krausei* starts to split (arrows); LM, hydrated. **J.** *A. rhizomatosus,* the pollen wall (arrow) is shed immediately in water; LM, hydrated. **K–L.** Echinate pollen of *A. mangelsdorffii*, the pollen wall is shed instantly in water; LM, hydrated. Scale bars: 10 µm (F–I, J, L), 100 µm (A–E, H, K).

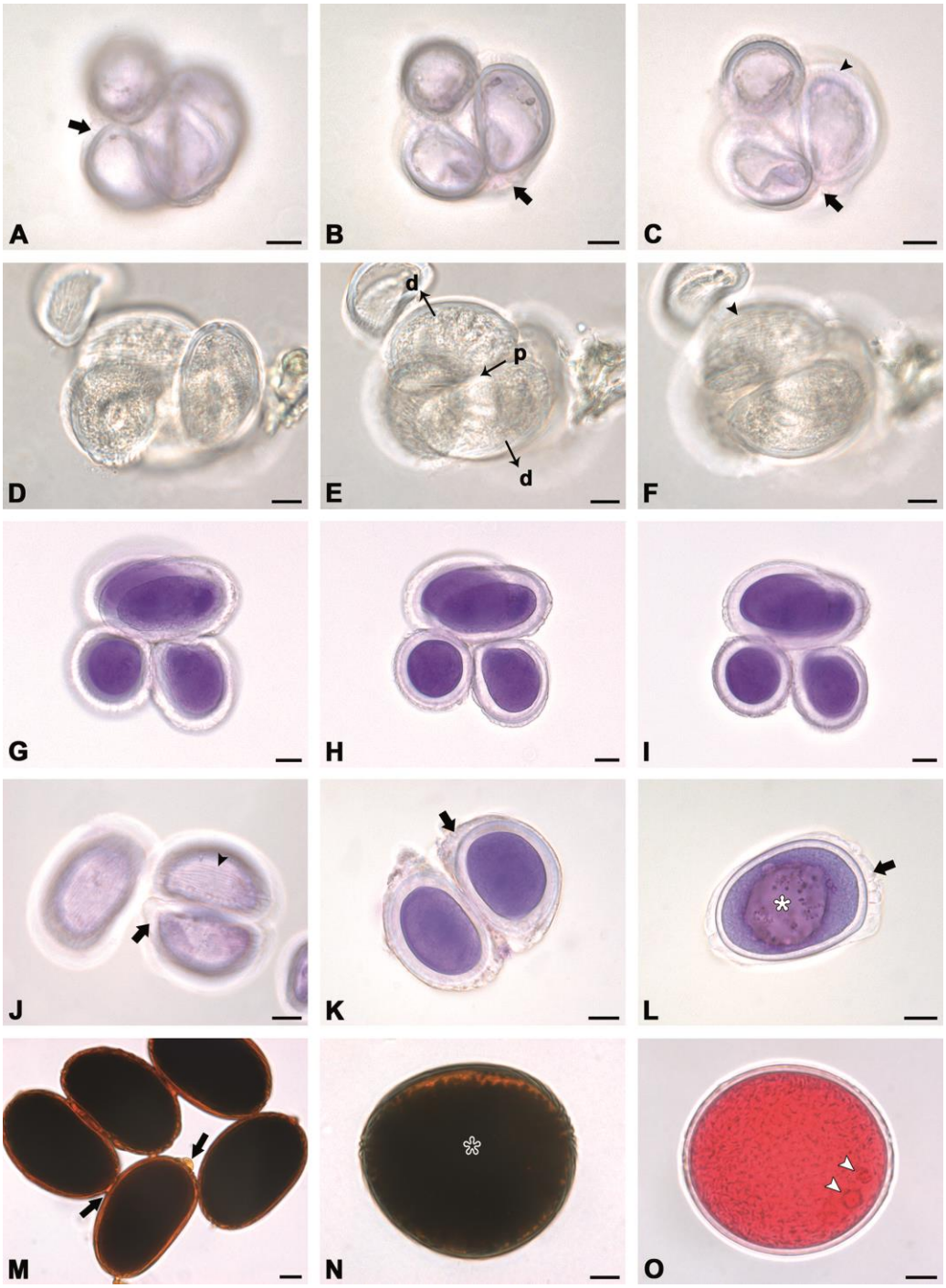


Figure 3. Different developmental stages (pollen tetrads, young microspores and mature pollen grains) of selected *Amorphophallus* species. Light micrographs. **A–L.** Pollen tetrads of *A. myosuroides*; note that each microspore is enclosed by a rather thick polysaccharide outer wall layer (*arrow*) and that a callose wall is absent; **A–C.** Early tetrad stage in different optical sections stained with toluidine blue. striate ornamentation visible (*arrowhead*); **D–F.** Late tetrad stage in different optical sections, distal face (*d*) and proximal face (p, *arrows* indicate the polar axis), striate ornamentation visible (*arrowhead*); **G–I.** Late tetrad stage in different optical sections stained with toluidine blue; **J, K.** Artificially destroyed tetrads, young microspores with rather thick polysaccharide outer wall layer (*arrow*), striate ornamentation visible (*arrowhead*) **L.** Artificially destroyed tetrad, young microspore with central vacuole (*asterisk*). **M.** *A. operculatus,* mature pollen with variable amounts of pollen coatings (*arrows*); potassium iodine. **N.** *A. yunnanensis*; mature pollen contain huge amounts of starch (*asterisk*); potassium iodine. **O.** *A. krausei*, trinucleate pollen, note the two sperm nuclei (*arrowheads*) stained dark red with acetocarmine. All scale bars: 10 µm.

Pollen strands, pollen unit and pollen wall shedding

In dry condition pollen is usually ellipsoidal and often asymmetric, with a less convex and a more convex side (Figure 2E). After release from the anther pollen may start to germinate instantly (Figure 2E-F) and sometimes the outer pollen wall just splits, releasing the protoplast (Figure 2G).

Observations of pollen in water show that germination usually begins within a few minutes up to one hour. In striate to plicate pollen, the pollen tube emerges either between the striae/plicae or near the apices (Figure 2E, F). In some species (*A. krausei, A. mangelsdorfii, A. rhizomatosus, A. yunnanensis* Engl.), the outer pollen wall is shed immediately after contact with water (Figure 2H–L). Subsequently, the naked protoplast is floating in water without indication of pollen tube growth. For the observed striate/plicate pollen (*A. krausei, A. rhizomatosus, A. yunnanensis*) the outer pollen wall often splits along the striae/plicae, respectively in the equatorial plane (Figure 2H–J). In echinate pollen of *A. mangelsdorfii* the outer pollen wall (Figure 2K) splits on one side of the spheroidal pollen grain, releasing the protoplast (Figure 2L).

Pollen tetrads, shape and polarity

Different developmental stages of pollen tetrads of *A. myosuroides* show microspores arranged tetragonal or decussate (Figure 3A–I). Pollen of *A. myosuroides* is ellipsoidal and heteropolar, with a less convex and a more convex side (Figure 3A–I). Within the tetrad the less convex side of the microspore is the proximal half and the more convex side the distal half (Figure 3E). Accordingly, the polar axis of the microspores is shorter than the equatorial diameter. Ellipsoidal pollen of *Amorphophallus* can thus be described as oblate with elliptic outline, with the apices (narrow ends) in the equatorial plane. Each microspore is enclosed by a rather thick outer pollen wall (Figure 3A-L). A callose wall was not found even in early tetrad stages. The striate ornamentation is already visible in early tetrad stages (Figure 3C, F) and as seen in young microspores from artificially destroyed tetrads (Figure 3J). At the apices the outer pollen wall is thick and often in touch with apices of the adjacent microspore

(Figure 3A-K). Microspores with a central vacuole, indicating the beginning of the gametogenesis, appear in the same sample (Figure 3L).

Pollen coatings, reserves and cell number

Mature pollen of all investigated *Amorphophallus* species is sticky due to the presence of pollenkitt (Figure 3M). While striate and echinate pollen usually has less pollenkitt, psilate and verrucate pollen (especially *A. stuhlmannii)* has plenty of pollenkitt. Pollen of all investigated species contains huge amounts of amyloplasts (starch) (Figure 3N) and is trinucleate/tricellular (Figure 3O).

Pollen morphology and ornamentation of hydrated and dry pollen for species studied in LM and SEM

Pollen morphology. — Pollen of all investigated species is inaperturate (Figures 4–9). Pollen in hydrated condition can be of oblate shape with elliptic outline (Figures 4–7) or of spheroidal shape with circular outline (Figures 8–9). The pollen shape may vary from oblate to spheroidal (Figures 4-9) and the pollen polarity from heteropolar to isopolar (Figures 4–9). The pollen size of the investigated species varies from medium (26–50 µm) to large (51–100 µm). The smallest pollen belongs to the striate type, e.g. *A. polyanthus*, *A. sumawongii* (30-50 µm), whereas the largest pollen (up to 90 µm) belongs to the psilate and verrucate type. Pollen in dry condition is usually unfolded or irregularly infolded (Figure 4–9). Pollen shape, outline, polarity, size and ornamentation types of the investigataed *Amorphophallus* species are summarized in table I.

Ornamentation types. — For the investigated *Amorphophallus* species four ornamentation types are distinguished: striate, echinate, verrucate and psilate. The dominating ornamentation type is the striate type, given that 19 out of the 25 species have striate pollen (Figures 4-7). Striate *Amorphophallus* pollen is highly diverse and the striae differ in their thickness, the space between them, their branching frequency as well as the way they are formed towards the apex. Besides the ypical striate type (Figure 4A-T) five subtypes are differentiated: subtype 1 striate to reticulate (Figure 5A-J), subtype 2 striate to plicate (Figure 5K-V), subtype 3 striate to plicate with appendages (Figure 6A-I), subtype 4

striate to plicate, with psilate apices (Figure 6J-Q) and subtype 5 irregularly striate (Figure 7A-Q).

Striate type. — *Amorphophallus* pollen with typical striate ornamentation (striae: elongated exine elements separated by grooves predominantly parallel arranged). Many narrow often multi-branching striae, twisted towards the center of the apex, are typical for *A. interruptus* (Figure 4A-D). The pollen surface of *A. sumawongii* (Figure 4E-H) is formed by broad and sometimes branched striae, which are fused in the middle of the apex. Many short, narrow, often multi-branching striae, sometimes twisted in the center of the apex (Figure 4K) are characteristic for *A. krausei* (Figure 4I-L). Predominantly long sometimes bifurcating striae are characteristic for pollen of *A. yunnanensis* (Figure 4M-P). The striae of *A. atrorubens* (Figure 4Q-T) are untypical as they are arranged transversal to the long axis. Moreover, the striae are fused laterally to the apices (Figure 4R).

Striate to reticulate type (subtype 1). — The ornamentation of *A. longituberosus* Engl. et Gehrm. (Figure 5A-E) and *A. asterostigmatus* (Figure 5F-J) varies from striate to reticulate (reticulum: network like pattern formed by exine elements (muri), where the lumina are wider than 1 µm). The ornamentation of hydrated pollen is formed by many narrow often branching striae with reticulate pattern in between (Figure 5C-D, H-I). In dry condition the ornamentation is striate only (Figure 5E, J). Dry pollen of *A. asterostigmatus* with striae running crisscross is found quite often in the sample (Figure 5E).

Striate to plicate type (subtype 2). — The striae of *A. myosuroides* (Figure 5K-N) are long, rarely branched and fused at the apices (Figure 5R), and the ornamentation is described as striate to plicate (plicae: circumferential, parallel ridge-like folds). The pollen surface of *A. polyanthus* (Figure 5O-R) is formed by broad sometimes branched striae or plicae, which are fused in the middle of the apex. Predominantly long sometimes bifurcating striae to plicae are characteristic for pollen of *A. ongsakulii* (Figure 5S-T). The striate ornamentation of hydrated pollen in *A. ongsakulii* is often deviating (Figure 5S-U), compared to pollen in dry condition (Figure 5V).

Striate to plicate type with appendages (subtype 3). — The ornamentation of *A. serrulatus* (Figure 6A-D) and *A. operculatus* (Figure 6E-I) is formed by striae or plicae. The striae or plicae are quite often fused in the center of the apices, ending up in, a single, distant ornamentation element of variable length – an appandage - (Figure 6A-B, D-H). Such appendages are also found on dry pollen of *A. serrulatus* (Figure 6D), but they are absent on pollen of *A. operculatus* (Figure 6I). Moreover, mesh-like marks are found on the pollen surface on dry pollen grains of *A. serrulatus* (Figure 6D).

Striate to plicate type with psilate apices (subtype 4). — The ornamentation of *A. lacourii* Linden et André (Figure 6J-M, Synonym: *Pseudodracontium lacourii* N. E. Br.) and *A. latifolius* (Serebryanyi) Hett. et Claudel (Figure 6N-Q, Synonym: *P. latifolium* Serebryanyi) is formed by long sometimes branching striae or plicae ending up in psilate apices (Figure 6J, K, O, Q). Dry pollen is flat in shape and often curled (Figure 6M).

Irregularly striate pollen (subtype 5). — The ornamentation of *A. palawanensis* Ittenb., Bogner et Hett. (Figure 7A-E), *A. rhizomatosus* (Figure 7F-I) and *A. konjac* (Figure 7J-M) is formed predominantly by many very short, discontinuous, often broad striae and only by a few long, bifurcating striae. Many pollen grains of *A. palawanensis* appear with a psilate (ornamentation-free) area (Figure 7C), which is often sunken in dry condition (Figure 7E). In *A. konjac* the ornamentation is formed either by elongated, rarely branched striae (Figure 7J, K) or predominantly short, rather broad, often curved or bifurcated striae (Figure 7L). Interesting deviations are also found on pollen of *A. taurostigma* (Figure 7N-Q) where pollen with either psilate or slightly striate (Figure 7N) or distinct striate ornamentation (Figure O, P) is found within the same sample. After critical point drying many pollen grains show a thin skin-like layer loosely attached to the underlying pollen surface (Figure 7N). The thin layer tends to rupture and sometimes detaches to a more or less extent from the pollen surface. Elongated distant ornamentation elements (appendages) are found on some pollen grains (Figure 7O, P). Aberrations are also found on the pollen surface of *A. mossambicensis* (Schott ex Garcke) N. E. Br. (Figure 7R-U). Depending on the preparation method, the pollen surface appears either striate (Figure 7R) or

psilate (Figure 7S-U) or with remains of a thin skin-like pollen wall layer (Figure 7S, T). Remains of the thin surface layer are also found on dry pollen grains (Figure 7U).

Echinate type. — The ornamentation of *A. mangelsdorffii* is formed by (micro)-echini (echinus: pointed ornamentation element longer and/or wider than 1 µm; pl. echini; micro: prefix for small; features smaller as 1 µm) (Figure 8A-D). Dry pollen is usually not infolded and spheroidal or sometimes irregular in shape (Figure 8D).

Verrucate type. — The ornamentation of *A. stuhlmannii* is formed by verrucae (verruca: wart-like element more than 1 µm broad, broader than high) of different size and shape, irregularly distributed over the whole pollen surface (Figure 8E-L). In LM, the verrucae appear translucent, and huge amounts of bright yellow pollenkitt is attached to the pollen wall (Figure 8E-F, H-K). Dry pollen is not infolded (Figure 8H-L).

Psilate type. — Pollen of *A. bulbifer* (Figure 9A-D), *A. henryi* (Figure 9E-H), *A. paeoniifolius* (Figure 9I-L) and *A. prainii* (Figure 9M-P) is characterised by a psilate (smooth) pollen surface. In LM the pollen surface appears either scabrate (term used for light microscopy only, describing minute sculpture elements of undefined shape and of a size close to the resolution limit of the light microscope) (Figure 9A, E) or psilate (Figure 9I, M).

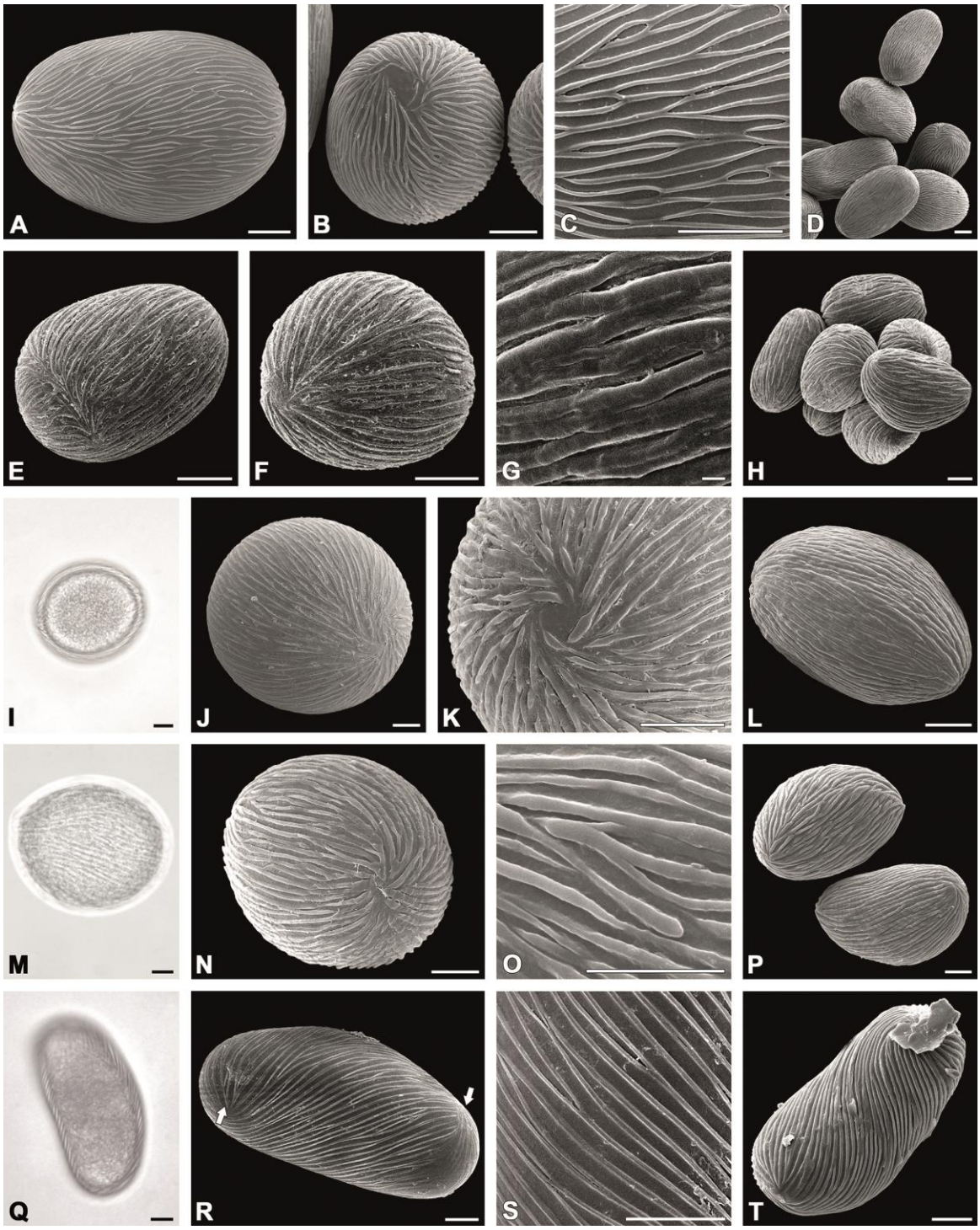


Figure 4. Striate type**. A–D.** *A. interruptus;* **A.** Hydrated pollen; SEM; **B.** Equatorial view; SEM, hydrated; **C.** Detail of pollen surface; SEM, hydrated; **D.** Dry pollen; SEM; **E–H.** *A. sumawongii;* **E.** Hydrated pollen, SEM; **F**. Oblique equatorial view; SEM, hydrated; **G.** Pollen surface; SEM, hydrated; **H.** Dry pollen; SEM. **I–L.** *A. krausei;* **I.** Hydrated pollen; LM; **J.** Oblique polar view; SEM, hydrated; **K.** Equatorial view; SEM, hydrated; **L.** Dry pollen; SEM. **M–P.** *A. yunnanensis.* **M.** Hydrated pollen; LM; **N.**Oblique equatorial view; SEM hydrated; **O.** Pollen surface; SEM hydrated; **P.** Dry pollen; SEM. **Q–T.** *A atrorubens;* **Q.** Striate ornamentation, polar view; LM, hydrated; **R.** Hydrated pollen; note striae are transverse to equatorial axis and fused laterally to the apices (*arrows*); SEM; **S**. Pollen surface; SEM, hydrated; **T.** Dry pollen; SEM. Scale bars: 1 µm (G), 10 µm (A–F, H–T).

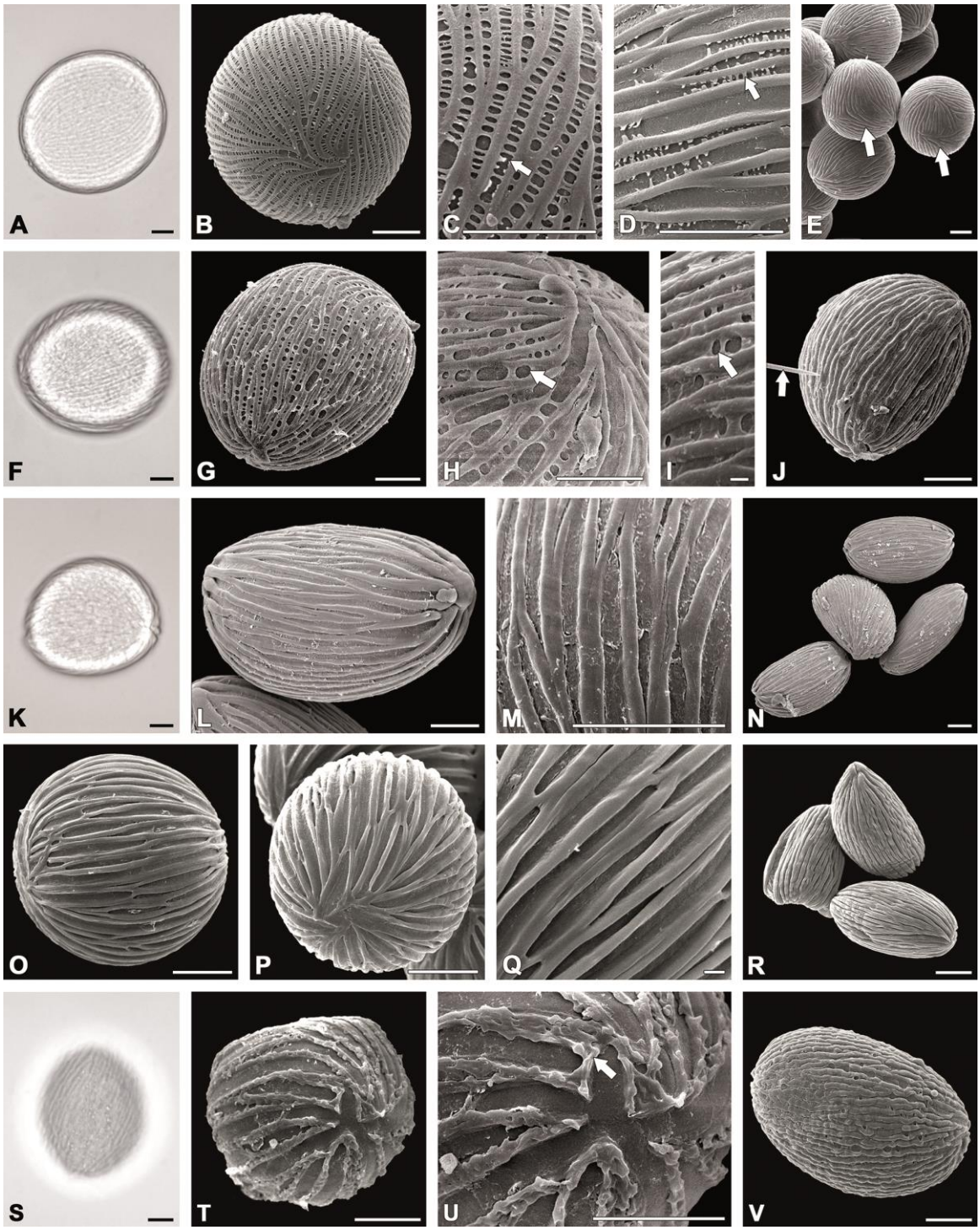


Figure 5. Striate type including subtype 1 'striate to reticulate' and subtype 2 'striate to plicate'**. A–E.** *A. longituberosus;* **A.** Polar view, .note striate ornamentation; LM, hydrated; **B.** Equatorial view, note striate to reticulate ornamentation (*arrow*); SEM, hydrated; **C.** Pollen surface, note striae with reticulate pattern in between (*arrow*); SEM, hydrated; **D.** Pollen surface, note remains (*arrow*) between the striae, SEM, hydrated; **E.** Dry pollen with striae running criss-cross (*arrows*); SEM. **F–J.** *A. asterostigmatus;* **F.** Polar view; Note striate ornamentation, LM, hydrated; **G.** Hydrated pollen, Note striae with reticulate pattern in between (*arrow*); SEM; **H.** Equatorial view; SEM, hydrated; **I.** Pollen surface; SEM, hydrated; **J.** Dry pollen with striate ornamentation and raphid attached to pollen wall (*arrow*), polar view; SEM. **K–N.** *A. myosuroides;* **K.** Striate ornamentation, polar view; LM, hydrated; **L.** Oblique polar view; SEM, hydrated; **M**. Pollen surface; SEM, hydrated; **N.** Dry pollen; SEM. **O–R.** *A. polyanthus;* **O.** Hydrated pollen; SEM; **P.** Equatorial view; SEM, hydrated; **Q**. Pollen surface; SEM, hydrated; **R.** Dry pollen; SEM.

S–V. *A. ongsakulii*; **S.** Hydrated pollen; LM; **T.** Oblique equatorial view; SEM, hydrated; **U.** Note that the striae on hydrated pollen is deviating compared to dry pollen (*arrow*); SEM, hydrated; **V.** Pollen surface; SEM, hydrated. Scale bars: 1 µm (I, Q), 10 µm (A–H, J–O, R–V).

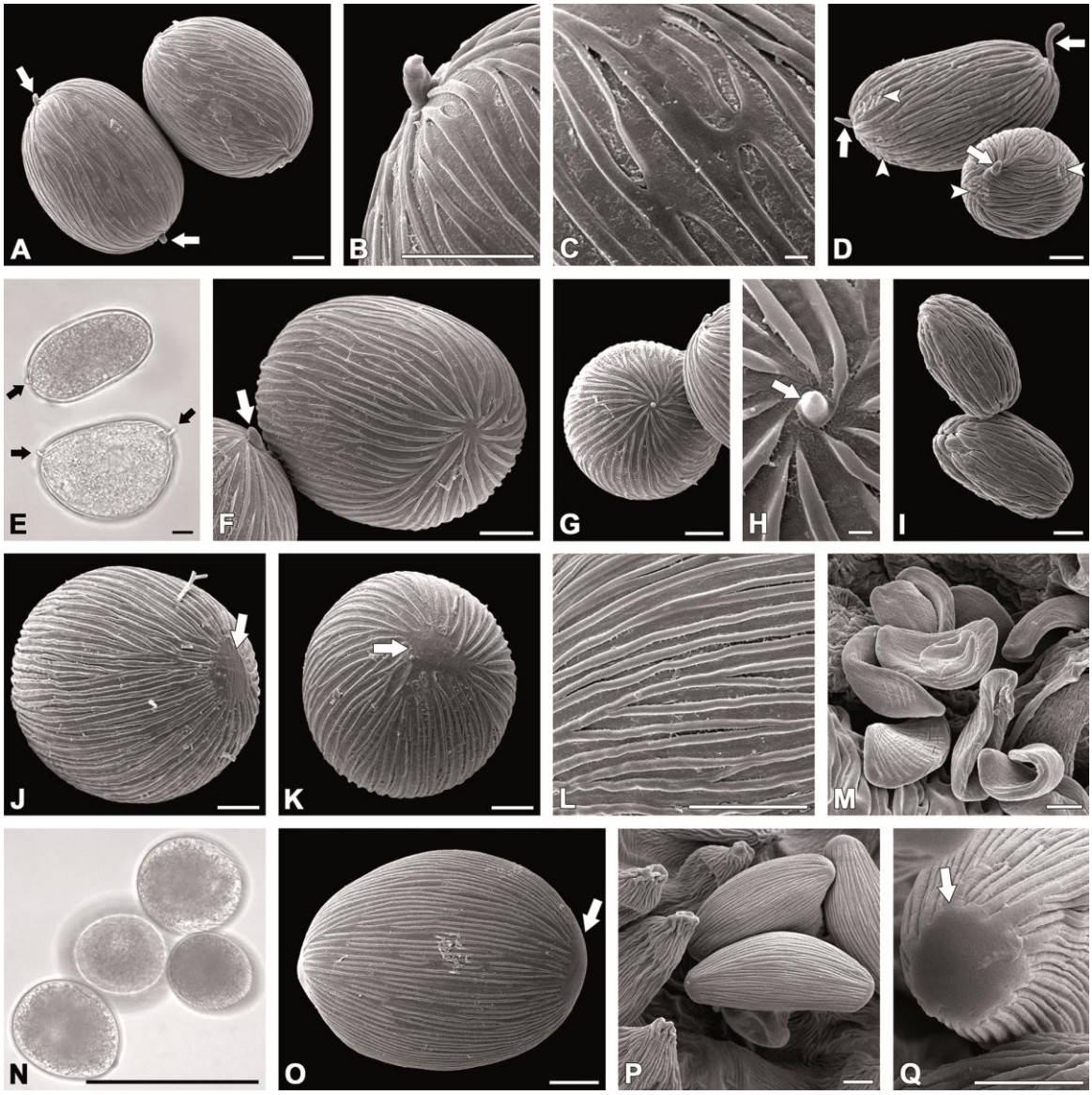


Figure 6. Striate type, including subtype 3 ‘striate to plicate, with appendages’ and subtype 4 ‘striate to plicate, with psilate apices’. **A–D.** *A. serrulatus*; **A.** Hydrated pollen, note apex with single distant ornamentation element (*arrows*); SEM; **B.** Detail of apex; SEM, hydrated; **C.** Pollen surface; SEM, hydrated; **D.** Dry pollen with appendages of variable length (*arrows*), note mesh-like marks on the pollen surface (*arrowheads*); SEM. **E–I.** *A. operculatus;* **E.** Hydrated pollen, note the appandages (*arrows*); LM. **F.** Oblique equatorial view, note the appandage (*arrow*); SEM, hydrated; **G.** Equatorial view; SEM, hydrated; **H.** Detail of apex with appandage (*arrow*); SEM, hydrated. **I.** Dry pollen; SEM. **J–M.** *A. lacourii;* **J.** Oblique polar view, note the psilate apex (*arrow*); SEM, hydrated; **K.** Oblique equatorial view, note the psilate apex (*arrow*); SEM, hydrated; **L.** Pollen surface; SEM, hydrated; **M.** Dry pollen appeared often flat and curled; SEM. **N–Q.** *A. latifolius;* **N.** Hydrated pollen; LM; **O.** Hydrated pollen with psilate apices (*arrow*); SEM; **P.** Dry pollen. SEM; **Q.** Dry pollen with psilate apex (*arrow*); SEM. Scale bars: 1 µm (C, H, Q), 10 µm (A, B, D–G, I–M, O–Q), 100 µm (N).

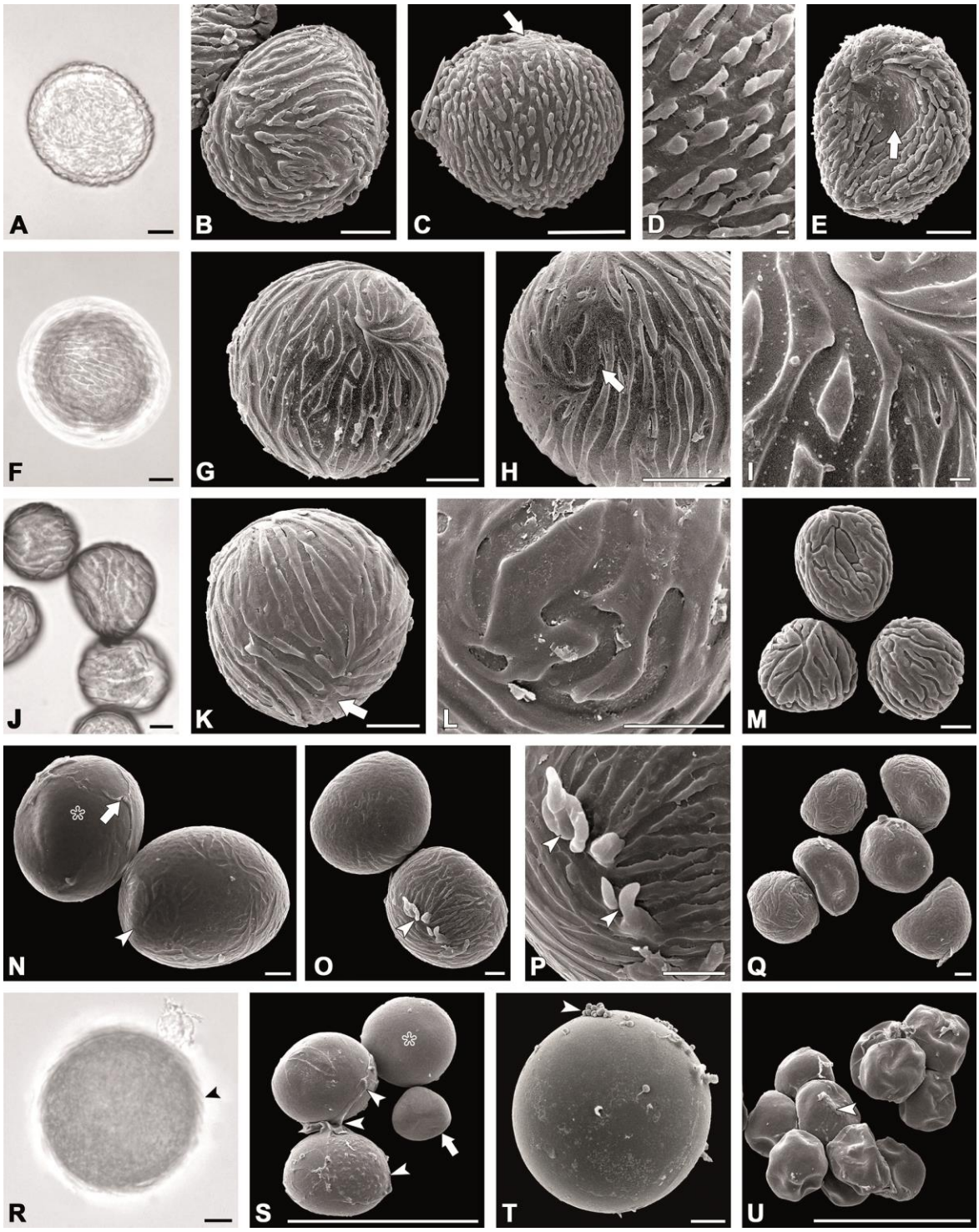


Figure 7. Striate type, including subtype 5 'irregularly striate'. **A–E.** Pollen of *A. palawanensis;* **A.** Striate ornamentation; LM, hydrated; **B, C.** Striae very short, note the psilate area (*arrow*); SEM, hydrated; **D.** Pollen surface, SEM, hydrated. **E.** Dry pollen with sunken area (*arrow*). SEM. **F–I.** *A. rhizomatosus*; **F.** Striate ornamentation; LM, hydrated; **G.** Striae broad, either short or long and bifurcated; SEM, hydrated; **H.** Striae fused in the center of the apex (*arrow*); SEM, hydrated; **I.** Pollen surface; SEM, hydrated. **J–M.** *A. konjac*; **J.** Striate to fossulate ornamentation; LM, hydrated; **K.** Striae broad, often bended, sometimes bifurcated and not fusing at the apex (*arrow*); SEM, hydrated; **L.** Detail of pollen surface; note the broad striae; SEM, hydrated; **M.** Dry pollen with striate to fossulate ornamentation; SEM. **N–Q.** *A. taurostigma.* **N.** Pollen with a thin skin-like layer (*arrow*) loosely attached to the underlying psilate (*asterisk*) pollen surface and with remains of the striate ornamentation (*arrowhead*); SEM, hydrated; **O.** Pollen, with psilate to slightly striate and distinct striate ornamentation within the same sample; SEM, hydrated; **P.** Pollen surface sometimes with

elongated distant ornamentation elements (*arrowheads*); SEM, hydrated; **Q.** Dry pollen; SEM. **R–U.** *A. mossambicensis*; **R.** Striate ornamentation (*arrowhead*) clearly visible on hydrated pollen; LM; **S.** Hydrated pollen of different size either with psilate ornamentation (*asterisk*), or with thin skin-like layer (*arrowheads*) loosely attached to the underlying psilate pollen surface. Note the small, psilate, sterile pollen (*arrow*); SEM, hydrated; **T.** Remains of ornamentation elements (*arrowhead*) on the psilate pollen surface; SEM, hydrated; **U.** Dry pollen with remains of ornamentation elements on the psilate surface (*arrowhead*). Scale bars: 1 µm (D, I, S), 10 µm (A-C, E–H, J–R, T), 100µm (S, U).

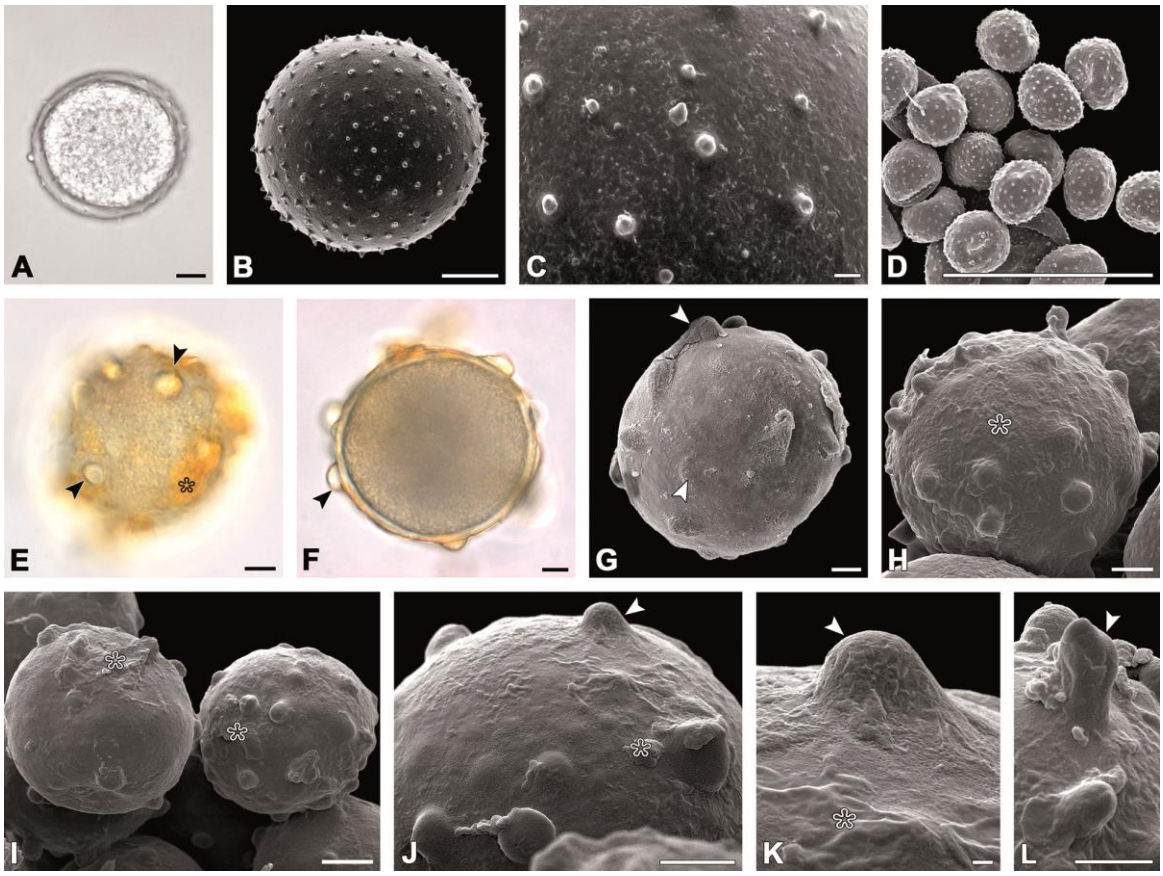


Figure 8. Echinate and verrucate type. **A–D.** *A. mangelsdorffii*; **A.** Echinate ornamentation; LM, hydrated; **B.** Hydrated pollen; SEM; **C.** Pollen surface; SEM, hydrated; **D.** Dry pollen; SEM. **E–L.** *A. stuhlmannii*; **E, F.** Pollen surface with irregularly distributed verrucae (*arrowheads*) and huge amounts of pollenkitt (*asterisk*); LM, hydrated; **G.** Hydrated pollen with irregularly distributed verrucae of different size (*arrowheads*); SEM; **H, I.** Pollen surface with pollenkitt attached (*asterisks*); SEM, dry; **J, K.** Pollen surface with verrucae (*arrowhead*) and huge amounts of pollenkitt attached (*asterisk*); SEM, dry; **L.** Pollen surface with verrucae of variable shape and size (*arrowhead*); SEM, dry. Scale bars: 1 µm (C, K), 10 µm (A, B, E–J, L), 100 µm (D).

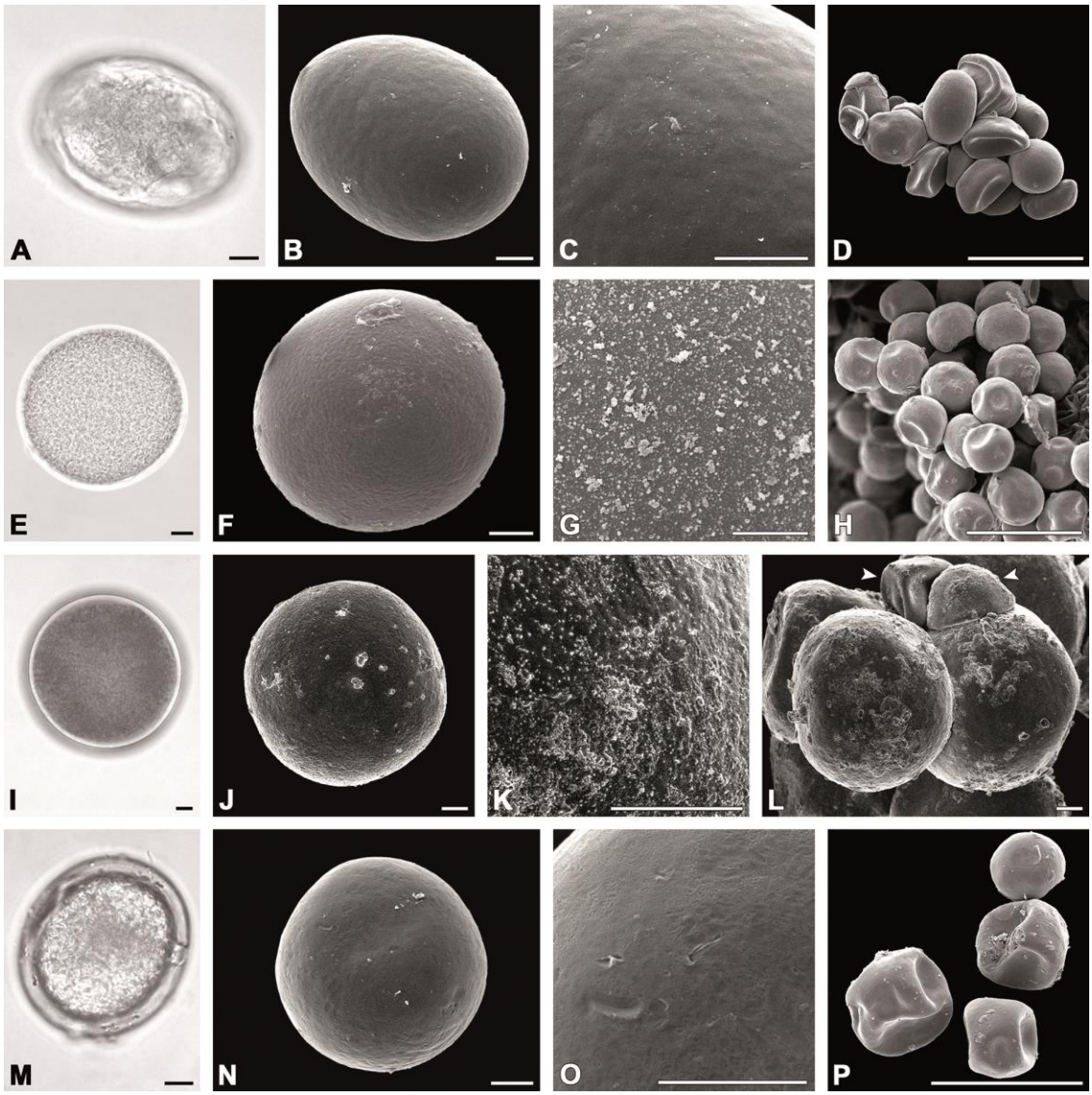


Figure 9. Psilate type. **A–D.** *A. bulbifer;* **A.** Hydrated pollen; LM; **B.** Oblique polar view; SEM, hydrated; **C.** Pollen surface; SEM, hydrated; **D.** Dry pollen, irregularly infolded; SEM. **E–H.** *A. henryi*; **E.** Hydrated pollen; LM; **F.** Hydrated pollen; SEM; **G.** Pollen surface; SEM, hydrated; **H.** Dry pollen; Irregularly infolded; SEM; **I–L.** *A. paeoniifolius;* **I.** Hydrated pollen; LM; **J.** Hydrated pollen, SEM; **K.** Pollen surface; SEM, hydrated; **L.** Dry pollen, note sterile pollen grains attached to mature pollen grains (*arrowheads*). SEM, dry. **M–P.** *A. prainii*; **M.** Hydrated pollen; LM; **N.** Hydrated pollen; SEM; **O.** Pollen surface; SEM, hydrated. **P.** Dry pollen, irregularly infolded; SEM. Scale bars: 10 µm (A–C, E–G, I–L, M–O), 100 µm (D, H, P).

Pollen wall ultrastructure in TEM

The ultrastructure of 17 *Amorphophallus* species was studied and TEM pictures are presented for the striate (Figures 10-12, with subtypes 1-5), verrucate (Figure 13) and psilate type (Figure 14). Different staining methods are used to verify the chemical nature of the pollen wall (Figures 10-14). For the detection of

unsaturated lipids - to verify the assumed oily nature of the granules - sections are stained with the lipid test. Sections are stained with the Thiéry-test to detect neutral polysaccharides, e.g. as assumed in the outer pollen wall. In case all staining techniques are illustrated for a species, the pictures are exceptionally arranged vertical in the figure (Figures 10, 14).

Investigated species of the striate type (including subtypes 1-5), with distinct, indistinct or without granules in the outer pollen wall. — The pollen wall of the investigated species is formed by a monolayered intine, a bilayered endexine (spongy inner layer and thin compact outer layer), an outer pollen wall and a thin mainly continuous surface layer (Figures 10-13). The distinct electron-dense granules are homogeneously distributed in the outer pollen wall of: *A. polyanthus*, *A. longituberosus, A. asterostigmatus, A. ongsakulii, A. lacourii, A. latifolius* and *A. palawanensis.* Indistinct electron-dense granules, located in a basal sublayer, are detected in *A. serrulatus*, *A. operculatus, A. konjac*, *A. taurostigma* and *A. mossambicensis.* In *A. yunnanensis* granules are absent.

Amorphophallus polyanthus (subtype 2, Figure 10A–E). — The distinct granules in the outer pollen wall are clearly visible with the modified Thiéry-test (Figure 10A), whereas they did not stain with uranyl acetate and lead citrate (Figure 10B). After staining with potassium permanganate the bilayered endexine and the thin surface layer as well as the distinct granules in the outer pollen wall appear electron-dense (Figure 10C). Whereas with the lipid test the granules, as well as the endexine are not clearly visible (Figure 10D). With the Thiéry-test, the granules (Figure 10E), the intine (Figure 10E, *in*) as well as amyloplasts (Figure 10E) stains electron-dense.

Amorphophallus longituberosus (subtype 1, Figure 10F–J). — The granules and the endexine stains electron-dense with the modified Thiéry-Test (Figure 10F*)*, as well as with uranyl acetate and lead citrate (Figure 10G) and with potassium permanganate (Figure 10H). With the lipid test, only the endexine strongly stains (Figure 10I). After staining with the Thiéry-test, all layers appears electron-dense (Figure 10J). The thin surface layer is clearly visible with all staining methods (Figure 10F–J).

Amorphophallus asterostigmatus (subtype 1, Figure 10K–O). — With the modified Thiéry-test, the granules are not detectable in the outer pollen wall (Figure 10K), whereas after staining with uranyl acetate and lead citrate, the granules appear electron-dense (Figur 10L). With potassium permanganate (Figure 10M), the lipid test (Figure 10N) and the Thiéry-test (Figure 10O) only the endexine distincly stains. The thin surface layer is visible with all staining methods (Figure 10K-O). In particular with uranyl acetate and lead citrate the surface layer produces a strong contrast (Figur 10L). Plenty of pollenkitt is located between the ornamentation elements (Figure 10L, N).

Amorphophallus ongsakulii (subtype 2, Figure 11A–C). — The distinct granules are distributed loosely in the outer pollen wall (Figure 11A, B). They appear electron-dense after staining with the modified Thiéry-test (Figure 11A), as well as with uranyl acetate and lead citrate (Figure 11B). Whereas with the lipid test the granules remain unstained (Figure 11 C). The thin surface layer stained electron-dense with the lipid test (Figure 11C).

Amorphophallus yunnanensis (striate type, Figure 11D). — With the Thiéry-test, the surface layer stains electron-dense (Figure 11D). Granules are absent in the outer pollen wall (Figure 11D). After staining with the Thiéry-test, the endexine stains electron-translucent and the intine faintly electron-dense (Figure 11D).

Amorphophallus serrulatus (subtype 3, Figure 11E–F). — A very thin surface layer is only visible after staining with uranyl acetate and lead citrate (Figure 11E). The indistinct granules in the outer pollen wall are only visible after staining with potassium permanganate (Figure 11F). The endexine appears electron-dense and the intine electron-translucent with both staining methods (Figure 11E-F).

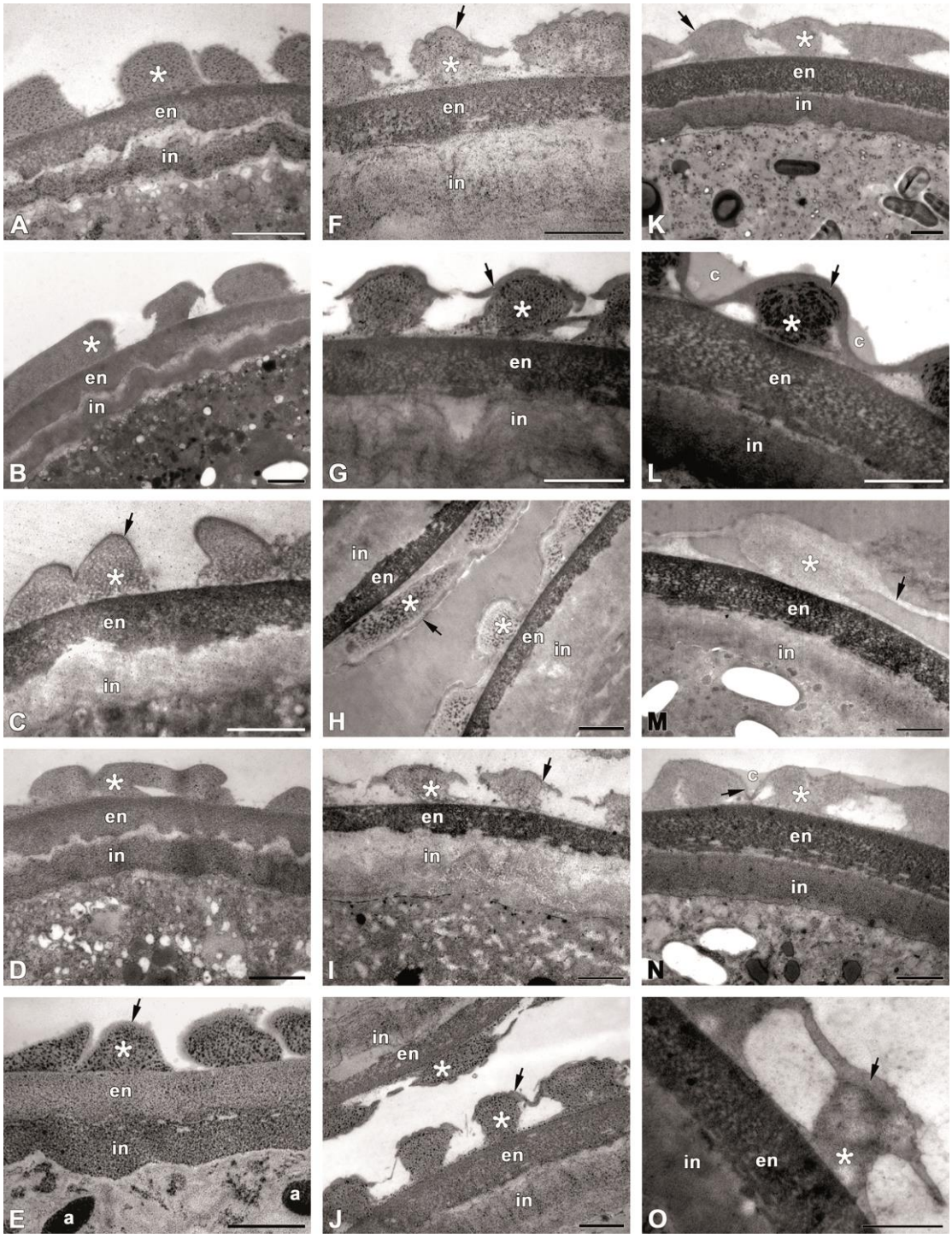


Figure 10. TEM images of the striate to plicate and striate to reticulate type with distinct granules in the outer pollen wall. Cross-section of pollen walls: Pollen wall formed by a monolayered intine (*in*), a bilayered endexine (*en*), an outer pollen wall (*asterisk*) and a thin surface layer (*arrow*). **A–E.** *A. polyanthus* (striate type); **A.** Modified Thiéry-test; **B.** Uranyl acetate and lead citrate; **C.** Potassium permanganate; **D.** Lipid test; **E.** Thiéry-test, the outer pollen wall, the intine and amyloplasts (*a*) stain electron-dense, indicating the presence of polysaccharides, note strongly stained distinct granules in the outer pollen wall (*asterisk*). **F–J.** *A. longituberosus* (striate to reticulate type)*;* **F.** Modified Thiéry-test; **G**. Uranyl acetate and lead citrate; **H.** Potassium permanganate; **I.** Lipid test; **J.** Thiéry-test. **K–O.** *A. asterostigmatus* (striate to reticulate type)*;* thin surface layer (*arrow*) visible with all staining methods; **K.** Modified Thiéry-test; **L.** Note pollenkitt (*c*) located between the striae; uranyl acetate and lead citrate, **M.** Potassium permanganate; **N.** Lipid test, note pollenkitt (*c*) located between the striae; **O.** Thiéry-test. All scale bars: 1µm.

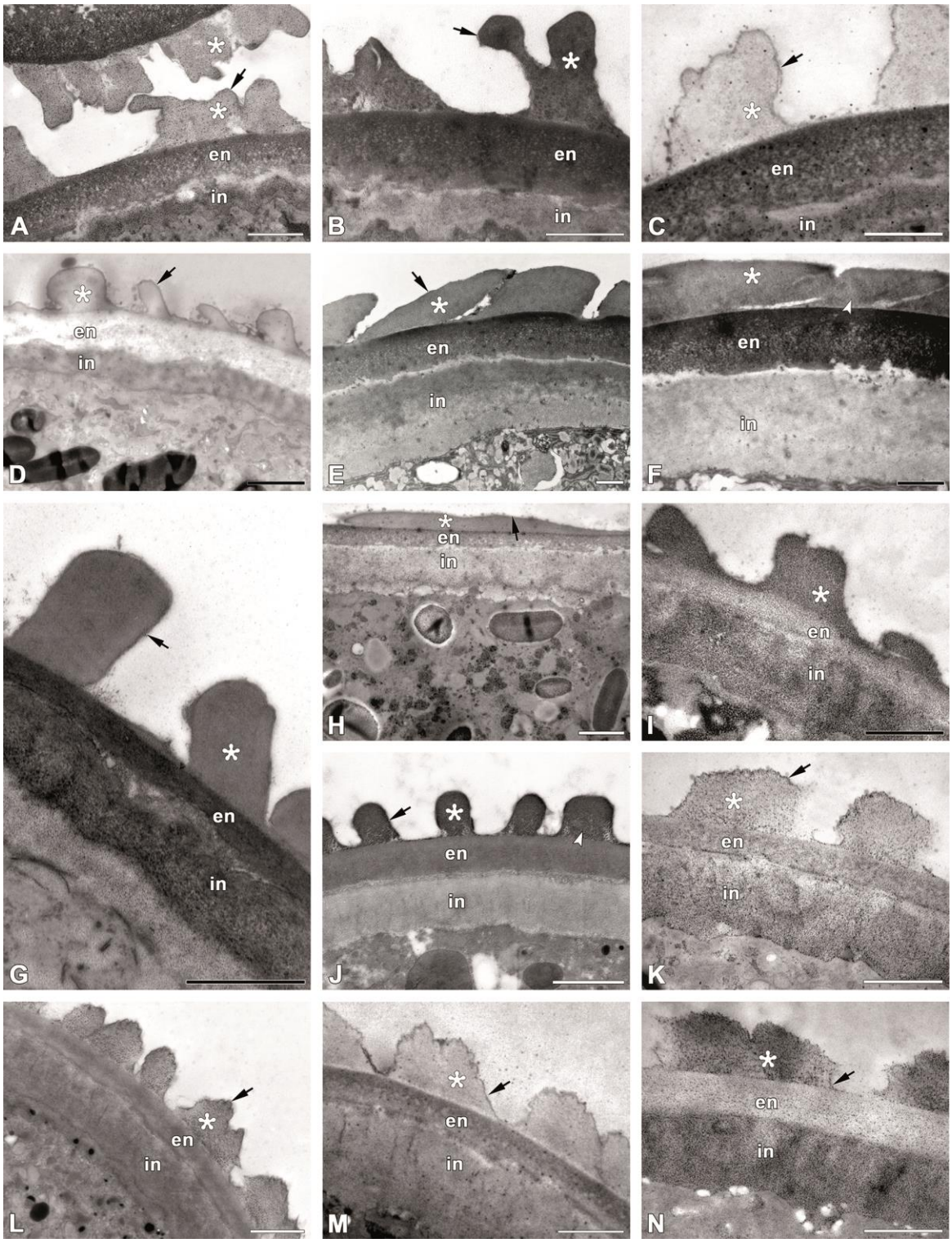


Figure 11. TEM images of the striate and striate to plicate type with distinct, indistinct or without granules in the outer pollen wall. Cross-section of pollen walls: Pollen wall formed by a monolayered intine (*in*), a bilayered endexine (*en*), an outer pollen wall (*asterisk*) and a thin surface layer (*arrow*). **A–C.** *A. ongsakulii* (striate to plicate), distinct granules in the outer pollen wall (*asterisk*); **A.** Modified Thiéry-test; **B.** Uranyl acetate and lead citrate; **C.** Lipid test. **D.** *A. yunnanensis* (striate type), granules absent in outer pollen wall (*asterisk*); Thiéry-test. **E–F.** *A. serrulatus* (striate to plicate type, with appendages); **E.** Uranyl acetate and lead citrate; **F.** Potassium permanganate. **G–I.** *A. operculatus* (striate to plicate type, with appendages); **G.** Uranyl acetate and lead citrate; **H.** Modified Thiéry-test, longitudinal section of pollen wall; **I.** Thiéry-test. **J.** *A. lacourii* (striate to plicate type, with psilate apices), uranyl acetate and lead citrate.

K–N. *A. latifolius* (striate to plicate type, with psilate apices), distinct granules in the outer pollen wall (*asterisk*); **K.** Modified Thiéry-test; **L.** Uranyl acetate and lead citrate; **M.** Lipid test; **N.** Thiéry-test. All scale bars: 1 µm.

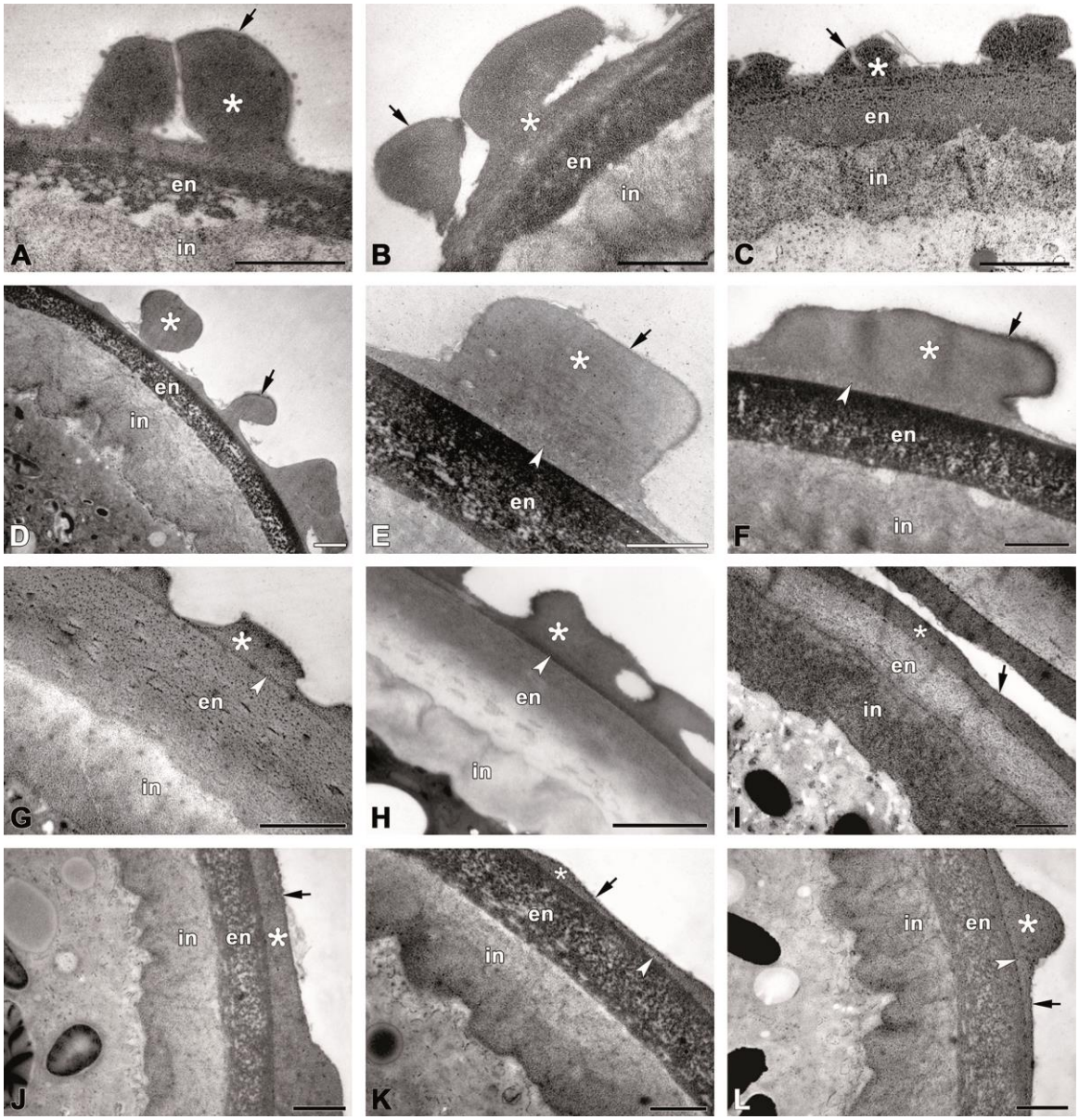


Figure 12. TEM images of the irregularly striate type with distinct or indistinct granules in the outer pollen wall. Cross-section of pollen walls: Pollen wall formed by a monolayered intine (*in*), a bilayered endexine (*en*), an outer pollen wall (*asterisk*) and a thin surface layer (*arrow*). **A–C.** *A. palawanensis*, **A.** Modified Thiéry-test; **B.** Lipid test; **C.** Distinct granules in the outer pollen wall (*asterisk*) clearly visible with the Thiéry-test. **D–F.** *A. konjac,* indistinct granules (*arrowhead*) located in the lower part of the outer pollen wall (*asterisk*); **D.** Modified Thiéry-test; **E.** Potassium permanganate; **F.** Lipid test. **G–I.** *A. taurostigma*, indistinct granules (*arrowhead*) visible in the lower part of the outer pollen wall (*asterisk*); **G.** Modified Thiéry-test; **H.** Uranyl acetate and lead citrate; **I.** Thiéry-test; longitudinal section of the pollen wall. **J–L.** *A. mossambicensis,* indistinct granules (*arrowhead*) located in the outer pollen wall (*asterisk*); **J.** Modified Thiéry-test; **K.** Lipid test; **L.** Thiéry-test. All scale bars: 1 µm.

Amorphophallus operculatus (subtype 3, Figure 11G–I). — The thin surface layer and the endexine appears electron-dense with uranyl acetate and lead citrate (Figure 11G). With the modified Thiéry-test the bilayered endexine and the monolayered intine are clearly visible, whereas the thin surface layer is only slightly visible (Figure 11H). After staining with the Thiéry-test, the intine, the outer pollen wall and indistinct, homogeneously distributed fine granules (Figure 11I) appear electron-dense.

Amorphophallus lacourii (subtype 4, Figure 11J). — After staining with uranyl acetate and lead citrate, the distinct granules in the outer pollen wall, the thin surface layer and the endexine are clearly visible (Figure 11 J).

Amorphophallus latifolius (subtype 4, Figure 11K–N). — After staining with the modified Thiéry-test (Figure11K) and with uranyl acetate and lead citrate (Figure 11L) the distinct granules in the outer pollen wall and a thin surface layer are cleary visible (Figure 11K-L). With the lipid test the surface layer and the endexine stains electron-dense (Figure 11M). The intine, the outer pollen wall and the granules stain electron-dense with the Thiéry-test, whereas the endexine appears electron-translucent (Figure 11N).

Amorphophallus palawanensis (subtype 5, Figure 12A–C). — The bilayered endexine is clearly visible with the modified Thiéry-test, whereas the thin surface layer is only slightly visible (Figure 12A). After staining with the lipid test the endexine and the surface layer appear electron-dense (Figure 12B). The intine and the distinct granules in the outer pollen wall stain electron-dense with the Thiéry-test (Figure 12C).

Amorphophallus konjac (Figure 12D–F). — After staining with the modified Thiéry-test the bilayered endexine is clearly visible, whereas the thin continuous surface layer is only slightly visible (Figure 12D). Indistinct granules are only slighly visible both after staining with potassium permanganate and the lipid test (Figure 12F). The granules are detected in the lower part of the outer pollen wall (Figure 12E–F). The thin continuous surface layer stains electron-dense with potassium permanganate (Figure 12E) and with the lipid test (Figure 12F).

Amorphophallus taurostigma (Figure 12G–I). — The indistinct granules in the outer pollen wall are only slightly visible both after staining with the modified Thiéry-test (Figure 12G) and with uranyl acetate and lead citrate (Figure 12H). The granules are detected in the outer pollen wall as a basal sublayer (Figure 12G-H). After staining with the Thiéry-test, the intine, the outer pollen wall and the thin surface layer appear electron-dense (Figure 12I).

Amorphophallus mossambicensis (Figure 12J–L). — The thin surface layer appears slightly electron-dense both with the modified Thiéry-test (Figure 12J) and the lipid test (Figure 12K). The indistinct granules are only slightly visible with the lipid test (Figure 12K). Whereas, with the Thiéry-test, the granules are detected in the outer pollen wall in a basal sublayer (Figure 12L). The bilalyered endexine is as thick as the intine (Figure 12J–L). The spongy nature of the inner layer of the endexine is visible with all staining methods (Figure 12J–L).

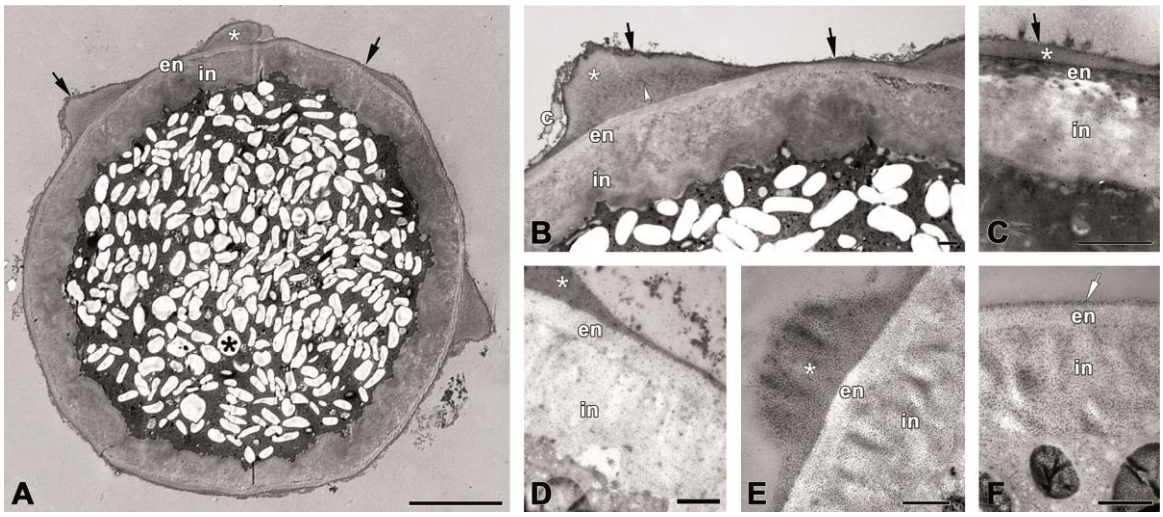


Figure 13. TEM images of the verrucate type with few distinct granules in the outer pollen wall. Cross-section of pollen walls: Pollen wall formed by a thick monolayered intine (*in*), a bilayered endexine (*en*), an outer pollen wall (*asterisk*) and a thin surface layer (*arrow*). Few distinct granules (*arrowhead*) in the outer pollen wall (*asterisk*). **A–F.** *A. stuhlmannii;* **A.** Cross section of a pollen grain, note the thick intine (*in*) and starch (*black asterisk*); uranyl acetate and lead citrate; **B**. Note pollenkitt (*c*) attached to pollen wall; uranyl acetate and lead citrate; **C.** Potassium permanganate; **D.** Lipid test; **E, F,** Thiéry-test. Scale bars: 1 µm (B–F), 10 µm (A).

Investigated species of the verrucate type, with few distinct granules in the outer pollen wall. — The pollen wall is formed by a thick intine, a thin bilayered endexine (spongy inner layer and thin compact outer layer), an outer pollen wall and a thin continuous surface layer. The few distinct granules are irregularly

distributed in the lower half of the verrucae, and homogeneously distributed in the thin outer pollen wall (Figure 13).

Amorphophallus stuhlmannii (Figure 13A–F). — After staining with uranyl acetate and lead citrate, the thin surface layer, huge amounts of starch and the monolayered intine are visible in cross section of the pollen grain (Figure 13A). The granules (Figure 13B) in the outer pollen wall, as well as the pollen coatings appear electron-dense with uranyl acetate and lead citrate (Figure 13B). The thin surface layer is clearly visible both after staining with uranyl acetate and lead citrate (Figure 13B) and potassium permanganate (Figure 13C). After staining with the lipid test the endexine appears electron-lucent and the outer pollen wall electron-dense (Figure 13D). After staining with the Thiéry-test the outer pollen wall (Figure 13E, F) appears electron-dense, whereas the granules and the surface layer remained unstained.

Investigated species of the psilate type with indistinct granules in the outer pollen wall. — The pollen wall is formed by a thick intine, a bilayered endexine (spongy inner layer and thin compact outer layer) and a thin outer pollen wall with indistinct granules located in an upper or basal sublayer. Remnants from the tapetum attached to the pollen wall made it difficult to detect a surface layer.

Amorphophallus bulbifer (Figure 14A–E). — The granules (Figure 14A) appear electron-dense after staining with the modified Thiéry-test, uranyl acetate and lead citrate (Figure 14B) and potassium permanganate (Figure 14C). After staining with the lipid test (Figure 14D) and the Thiéry-test (Figure 14E), the granules are only slightly visible, whereas a thin surface layer appears electron-dense (Figure 14DE). Moreover huge amount of pollen coating (Figure 14A–E) and/or remnants from the tapetum is detected on the pollen wall with all staining methods.

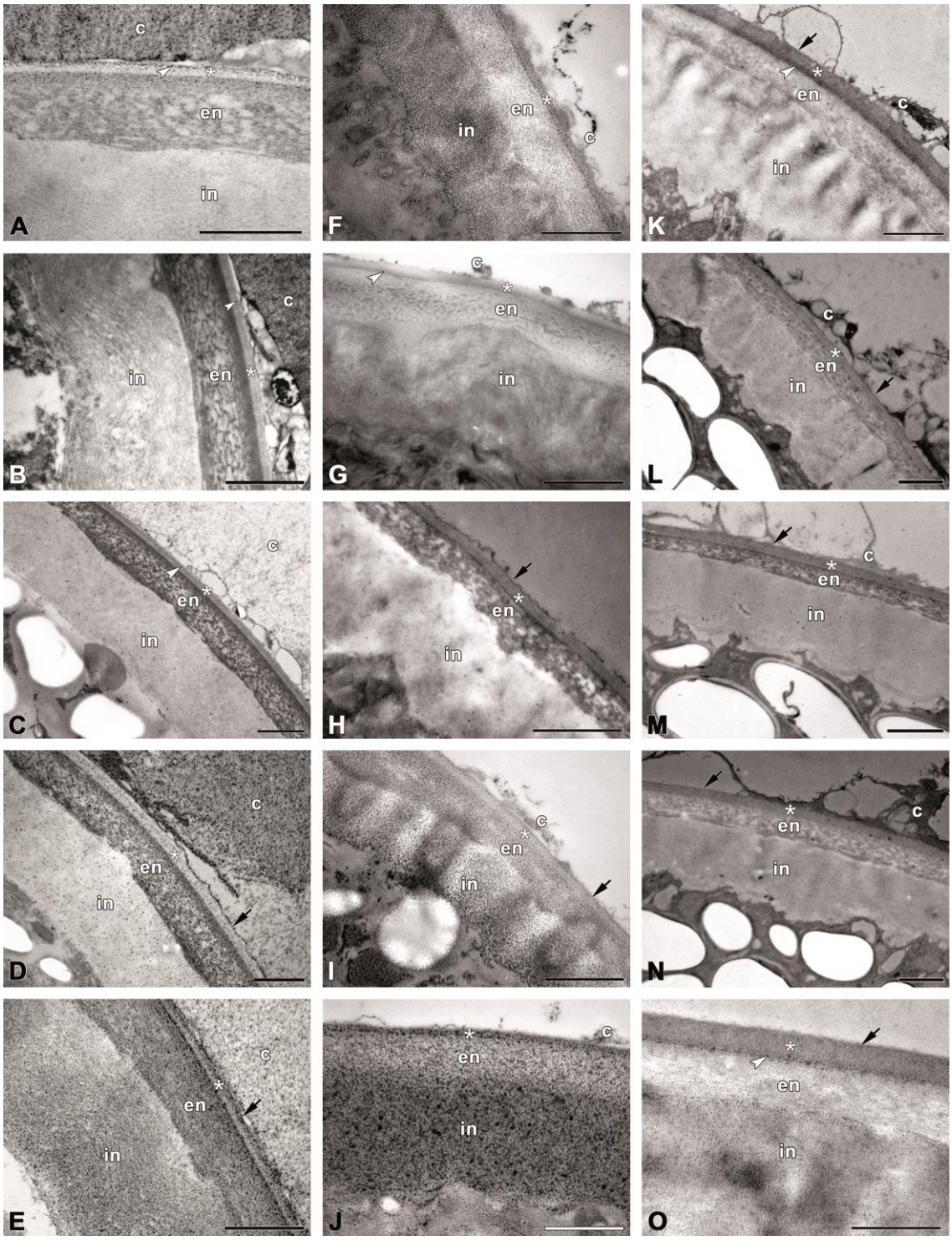


Figure 14. TEM images of the psilate type with indistinct granules in the outer pollen wall. Cross-section of pollen walls: Pollen wall formed by a thick monolayered intine (*in*), a bilayered endexine (*en*) and an outer pollen wall (*asterisk*) with indistinct granules located in a sublayer (*arrowhead*). A thin surface layer (*arrow*) and pollen coatings (*c*), like pollenkitt and remnants from the tapetum, are attached to the pollen wall. **A–E.** *A. bulbifer*; **A.** Modified Thiéry-test; **B.** Uranyl acetate and lead citrate; **C.** Potassium permanganate; **D.** Note the thin surface layer (*arrow*) stains electron-dense with ther lipid test. **E.** Thiéry-test. **F–J.** *A. henryi*; **F.** Modified Thiéry-test; **G.** Uranyl acetate and lead citrate; **H.** Potassium permanganate; **I.** Note the thin surface layer (*arrow*) stains electron-dense; Lipid test; **J.** Thiéry-test. **K–O.** *A. prainii;* **K.** Note that the outer pollen wall appears bilayered (*arrowhead*) with the modified Thiéry-test; **L.** Uranyl acetate and lead citrate; **M.** Potassium permanganate; **N.** Lipid test; **O.** Thiéry-test. All scale bars: 1 µm.

Amorphophallus henryi (Figure 14F–J). — With the modified Thiéry-test the outer pollen wall, the endexine and the intine are clearly visible (Figure 14F). A basal sublayer of the outer pollen wall stains electron-dense with uranyl acetate and lead citrate (Figure 14G). The spongy nature of the endexine (Figure 14H) is only visible after staining with potassium permanganate. A thin surface layer appears electron-dense both after staining with potassium permanganate (Figure 14H) and the lipid test (Figure 14I). After staining with the Thiéry-test the intine and the outer pollen wall appear electron-dense (Figure 14J).

Amorphophallus prainii (Figure 14K–O). — A basal sublayer of the outer wall appears electron-dense after staining with the modified Thiéry-test (Figure 14K), whereas it remains unstained with uranyl acetate and lead citrate (Figure 14L), potassium permanganate (Figure 14M) and the lipid test (Figure 14N). After staining with the Thiéry-test, the outer pollen wall appears bilayered (Figure 14O). A thin surface layer (Figure 14K–O) as well as pollen coatings (Figure 14K–O) attached to the pollen wall stains electron-dense with all staining methods.

Acetolysis resistance of the pollen wall

Pollen of 18 *Amorphophallus* species was acetolysed to verify the presense or absence of sporopollenin in the outer pollen wall (Figure 15, Table I). Pollen of all investigated species is usually collapsed. Although pollen is usually completely psilate after acetolysis, remains of the ornamentation elements are observed in some grains. Only few pollen grains may partly or completely resistent the acetolysis treatment.

Acetolysed striate pollen. — The pollen ornamentation of *A. krausei* (Figure 15A-B), *A. yunnaensis* (Figure 15C) and of *A. atrorubens* (Figure 15D) sometimes retain either partly or completely after acetolysis.

Acetolysed striate to reticulate pollen. — Pollen of *A. asterostigmatus* (Figure 15E) is completely psilate and irregularly infolded after acetolysis.

Acetolysed striate to plicate pollen. — Some pollen of *A. polyanthus* appear with remains of the striate ornamentation (Figure 15F), whereas pollen of

A. ongsakuli usually is psilate and pollen grains collapse after acetoylsis (Figure 15G).

Acetolysed striate to plicate pollen, with appendages. — In *A. serrulatus* (Figure 15H) and *A. operculatus* (Figure 15I) the striate ornamentation is partly retained after acetolysis and pollen is irregularly infolded and without appendages.

Acetolysed striate to plicate pollen, with psilate apices. — Pollen of *A. lacourii* (Figure 15J) and *A. latifolius* (Figure 15K) are irregularly infolded after acetolysis and rare striae are only slightly visible.

Acetolysed irregularly striate pollen. — Pollen of *A. rhizomatosus* appears completely psilate after acetolysis (Figure 15L). Remains of the striate ornamentation are observed in *A. palawanensis* (Figure 15M), *A. konjac* (Figure 15N-O), *A. taurostigma* (Figure 15P) and *A. mossambicensis* (Figure 15Q). Acetolysed pollen of *A. palawanensis* (Figure 15M) and *A. konjac* (Figure 15O) appears with distinct striate to fossulate (fossula: irregularly shaped groove in the surface of a pollen wall) ornamentation.

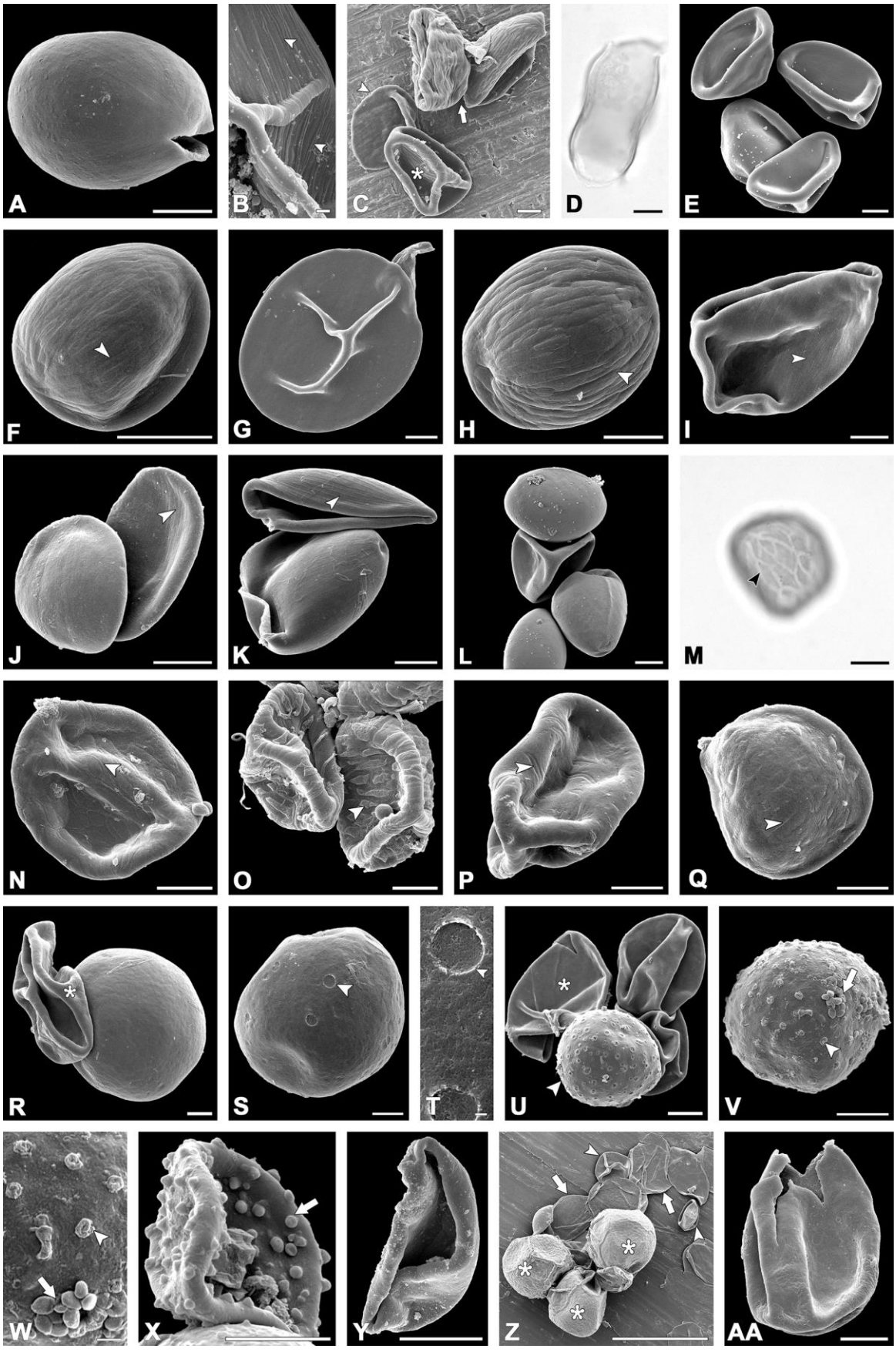
Acetolysed verrucate pollen. — Verrucate pollen of *A. stuhlmannii* is predominantly psilate after acetolysis (Figure 15R, S), but circular marks on the pollen surface, presumably remnants of the verrucae, are also found (Figure 15S, T).

Acetolysed echinate pollen. — Echinate pollen of *A. mangelsdorffii* (Figure 15U–X) usually appears irregularly infolded and psilate after acetolysis (Figure 15S, U). Pollen with psilate ornamentation and circular remnants on the pollen surface are also found (Figure 15U–W). The remaining echini are deformed and they are often only loosely attached to the pollen wall or clumped together (Figure 15V–W). However, only few pollen grains resist the acetoylsis treatment without being completely destroyed (Figure 15X).

Acetolysed psilate pollen. — Psilate pollen of *A. bulbifer* (Figure 15Y), *A. paeoniifolius* (Figure 15Z) and *A. prainii* (Figure 15AA) are either irregularly

infolded (Figure 15Y), plain (Figure 15Z), or completely destroyed (Figure 15AA). Only few pollen grains of *A. paeoniifolius* did not collapse completely after acetoylsis, being irregularly infolded and with a rough pollen surface (Figure 15Z).

Figure 15. Acetolysed pollen of selected *Amorphophallus* species. After acetolysis treatment pollen grains usually collapse (irregular or flat in shape) and pollen ornamentation is usually completely psilate. Sometimes the ornamentation elements remain after acetolysis. **A–B.** *A. krausei,* note remains of striae (*arrowheads*); SEM. **C.** *A. yunnaensis*, ornamentation psilate (*arrowhead*), partly striate (*asterisk*) or rarly completely striate (*arrow*). SEM. **D.** *A. atrorubens*; LM. **E.** *A. asterostigmatus*; SEM. **F.** *A. polyanthus*, note remains of striae (*arrowhead*); SEM. **G.** *A. ongsakulii*; SEM. **H.** *A. serrulatus,* striae not completely destroyed (*arrowhead*). SEM. **I.** *A. operculatus,* remains of striae (*arrowhead*); SEM. **J.** *A. lacourii,* pollen surface with remains of striae/plicae (*arrowhead*). **K.** *A. latifolius,* remains of striae/plicae rarley found (*arrowhead*); SEM. **L.** *A. rhizomatosus*; SEM. **M.** *A. palawanensis,* ornamentation appeares fossulate (*arrowhead*); LM. **N–O.** *A. konjac*; **N.** Remains of striae (*arrowhead*); SEM; **O.** Some pollen with striate ornamentation (*arrowhead*); SEM. **P.** *A. taurostigma*, remains of striae (*arrowhead*); SEM. **Q.** *A. mossambicensis,* remains of striae (*arrowhead*); SEM. **R–T.** *A. stuhlmannii*, pollen surface often with circular marks (**S**, **T**, *arrowhead*), presumably remnants of the verrucae, some pollen completely destroyed (**R**, *asterisk*); SEM. **U–X.** *A. mangelsdorffii;* **U.** Pollen surface psilate (*asterisk*); SEM; **U–W** Pollen surface often with circular remnants of the echini (*arrowhead*); SEM; **W–X**. Some pollen with remains of echini, which are deformed and loosely attached (*arrow*); SEM. **Y.** *A. bulbifer;* SEM. **Z.** *A. paeoniifolius,* pollen irregularly infolded and often completely flat in shape (*arrows*). Note pollen with rough pollen surface (*asterisks*) as well as few defect pollen (*arrowhead*) with psilate ornamentation (*arrowheads*); SEM. **AA.** *A. prainii,* pollen irregularly infolded or destroyed. Scale bars: 1 µm (D, T, W), 10 µm (A-C, E-S, U-V, X-T, AA), 100µm (Z).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Discussion

Pollen strands, aperture condition and pollen wall shedding

Pollen strands. —Pollen grains of *Amorphophallus* are typically produced in huge amounts and extruded in string-like structures, so-called pollen strands, which are deposited on trapped insects or picked up by escaping insects (beetles, flies, bees) (e.g. Mayo et al. 1997; Van der Ham et al. 1998; Punekar & Kumaran 2010). Our observations of pollen strands of *Amorphophallus* showed that the single pollen grains are clumped together by pollenkitt of variable amounts. Striate and echinate pollen is correlated with only little pollenkitt, whereas psilate and verrucate pollen is usually correlated with abundant pollenkitt. The pollen strands may also be sticky due to other secretions from the inflorescence, e.g. as known for the genus *Philodendron* Schott (Hesse 1980; Gibernau et al. 1999; Sannier et al. 2009). Moreover, this type of pollen dispersal is frequently correlated with theca dehiscence by a subapical rounded pore or a short slit (Mayo et al. 1997). *Amorphophallus* species with psilate or verrucate pollen are reported to be pollinated by beetles (Mayo et al. 1997, Ham et al. 1998; Sannier et al. 2009). According to Sannier et al. (2009) the correlation between psilate pollen and pollination by beetles seems specific to the Araceae, given that pollen of insect-pollinated species is usually highly ornamented for a better storage of pollenkitt.

Aperture condition and pollen germination. — Pollen of the investigated *Amorphophallus* species is inaperturate, respectively omniaperturate, as the whole pollen surface acts as an aperture (Thanikaimoni 1978; Weber et al. 1999). Inaperturate pollen is common in monocotyledons (e.g. Zavada 1983; Kress 1986; Furness & Rudall 1999) as well as many dicotyledons (Kress 1986). It is the most common aperture type in the Araceae (Grayum 1992; Furness & Rudall 1999) and typical for monoecious genera (Mayo et al. 1997). According to Furness and Rudall (1999) inaperturate pollen may result from mutations, which increase the germination efficiency. After release from the anther, pollen often starts to germinate immediately. Striate to plicate pollen has been observed to germinate from anywhere between the striae/plicae and near the narrow ends, which is in accordance with other reports (Furness & Rudall 1999).

Pollen wall shedding. — In some species (*A. krausei, A. mangelsdorfii, A. rhizomatosus, A. yunnanensis*) the pollen wall splits immediately in water and is shed soon afterwards. Subsequentely, the naked protoplast was floating in water without pollen tube growth. Within the scope of our project we also observed shedding of the pollen wall in other aroid taxa: in *Gearum brasiliense* N. E. Br., *Spathantheum fallax* Hett., Ibisch et E. G. Gonç*., Taccarum weddellianum* Brongn. ex Schott, *Alloschemone occidentalis* (Poepp.) Engl. et K. Krause *Arophyton crassifolium* (Buchet) Bogner and *Carlephyton glaucophyllum* Bogner (our unpubl. data). This effect of a shed pollen wall is also described for other angiosperm taxa e.g. in some Annonaceae (Hesse et al. 1985; Hesse et al. 2009), as well as some gymnosperm taxa, e.g. Cupressaceae, Taxodiaceae, *Cephalotaxus* Siebold et Zucc. ex Endl., *Gnetum* L. and *Ephedra* L., where it is denoted as exine shedding and as a first event in pollen germination (Bhatnagar & Moitra 1996; El-Ghazaly et al. 1998; Takaso & Owens 2008). For plicate pollen of *Ephedra* the naked protoplast starts to germinate at the equatorial plane near the narrow tips about one hour after the exine is shed (El-Ghazaly et al. 1998). This is very similar to our observations on pollen of *Amorphophallus*, as well as the aroid taxa with a shed pollen wall. Similar observations have also been made for the aroid genus *Montrichardia* L. (Weber & Halbritter 2007) where a massive expansion of the thick intine results in an explosive opening of the pollen wall, though without shedding of the wall. Similar to our observations on *Amorphophallus* pollen the protoplast of *Montrichardia* remains unprotected and pollen tube formation occurred within 60 minutes at the equatorial plane near the narrow tips. Weber and Halbritter (2007) hypothesised that this germination strategy is a substitute for pollenkitt in *Montrichardia*, making pollen grains clump together and sticky to pollinators. As the pollen wall shedding was only observed in striate and echinate *Amorphophallus* pollen with little pollenkitt, this effect might also play a role in pollination as a substitute to sticky pollen coatings. Further studies are necessary to document whether the shed pollen wall of *Amorphophallus* is associated with the germination process. Moreover, the observed *Amorphophallus* pollen with shed pollen wall as well as the above-named aroid taxa had exclusively medium- to large-sized pollen grains. Barabé et al. (2008) detected a correlation between the viability (how long (time in hours, days) pollen is able to germinate after pollen release) of pollen,

the flowering cycle and pollen grain size. In their study, the authors reported that pollen grains of species with a long flowering cycle and small pollen (e.g. *Anaphyllopsis americana* Engl.) are more viable than pollen grains of species with a short flowering cycle and large pollen, e.g. *Montrichardia arborescens* (L.) Schott (Barabé et al. 2008). Aroids with unisexual flowers (e.g. *Amorphophallus*, *Montrichardia*) are known to have a short flowering cycle (two days only) and pollen is viable for a few days only (Gibernau et al. 2003; Barabé et al 2008). For example, pollen of *Montrichardia arborescens* is not viable after 24 hours and no pollen tube formation occurs after 36 hours (Barabé et al. 2008). For two *Arum* L. species, it is documented that the flowering cycle and the pollen viability takes two (to three) days only (Gibernau et al. 2003). Moreover, our observations on *Amorphophallus* pollen support the hypothesis by Hesse (2006a, 2006b) that the lack of a sporopollenin outer pollen wall, as characteristic for Aroideae, is correlated with short pollen viability and rapid germination. As documented by Van der Ham et al. (1998, p. 105), *Amorphophallus* pollen has a wide size range. Pollen of our investigated *Amorphophallus* species had a size range from 30 to 93 µm, whereas most species had large pollen grains (see also table I). The largest pollen grains investigated are spheroidal in shape and with psilate or verrucate ornamentation, which is in accordance with Van der Ham et al. (1998). The pollen size depends on different parameters, such as the degree of hydration, the method used and natural variation (Hesse et al. 2009). Therefore, size categories according to Hesse et al. (2009) are used rather than exact measurements of single pollen grains.

Reserves and cell number

Pollen of all investigated species contained a huge amount of starch. According to Mayo et al. (1997, page 49), 73% of aroid pollen contains starch, which may also vary within a single genus, e.g. in *Schismatoglottis* Zoll. et Moritzi. Pollen of all investigated species was trinucleate, as is typical for the higher aroid taxa (Grayum 1986b). Deviating literature reports about the cell number in *Amorphophallus* pollen has only been found in a paper by Gibernau et al. (2003) that referred to binucleate pollen of *A. konjac,* as noted by Hyndman (2001) on the aroid website (www.aroid.org). According to Hyndman (2001), ‘binucleate

pollen of *A. konjac* is viable for many months, whereas trinucleate pollen of *A. paeonifolius* loses viability quickly'. Our results do not confirm this, given that the observed pollen of *A. konjac* was trinucleate throughout.

Pollen tetrads, shape and polarity

Based upon an occasionally-found single intact decussate tetrad and tetrad fragments of *A. konjac* Van der Ham et al. (1998, p. 97) hypothesised that the less convex side of the pollen grain might be the proximal face. However, thus far the pollen polarity and shape of *Amorphophallus* pollen remains unknown. Pollen tetrads of *Amorphophallus* are difficult to find as the male flowers flower simultaneously and for one or two days only. When the spathe opens the female flowers are receptive and the male flowers remain closed, although pollen is already mature. We finally found pollen tetrads of *A. myosuroides* in closed anthers about three days before the female flowers became receptive. Our results showed microspores of *A. myosuroides* arranged tetragonal or decussate in the tetrad, as is typical for pollen of monocots (Maheshwari 1949; Walker & Doyle 1975). Due to the position in the tetrad, ellipsoidal pollen of *Amorphophallus* can thus be described as being oblate with elliptic outline and heteropolar as assumed by Van der Ham et al. (1998). In spheroidal pollen the polar axis is more or less equal to the equatorial diameter and pollen can thus be described as isopolar, with a circular outline.

Ontogenetic aspects. — Microspores of *A. myosuroides* are enclosed by a rather thick polysaccharide outer wall layer, which is especially thickened at the apices. As seen in the tetrad the microspores are often in touch with adjacent microspores, seemingly at the apices only. A callose wall was not observed in tetrads of *A. myosuroides*, even in early tetrad stages. Usually the callose wall separates the microspores in the tetrad, as well as from the surrounding tapetal cells .The presence of callose and a primexine (a polysaccharide layer formed during an early developmental stage, wherein the later exine structures are preformed) is common for almost all angiosperm pollen, aside from Aroideae (Hesse 2006b). The fact that a callose wall can be absent in the microspore ontogeny of Aroideae was first documented for *Arum alpinum* Schott et Kotschy (Anger & Weber 2006). Compared to all other angiosperms, the ontogenetic

timetable is different in Aroideae as the endexine is formed in the tetrad stage before the formation of the outer pollen wall layer and the intine, which are subsequently formed simultaneously (Hesse 2006b). In angiosperms, a tectate-columellate ektexine is usually formed before endexine formation (Ubera Jiménez et al. 1996). The outer pollen wall layer in Aroideae, as known from *Sauromatum* Schott (Weber et al 1998) and *Arum* (Anger & Weber 2006), is produced exclusively by the amoeboid tapetum shortly after dissociation of the tetrad. By contrast, the ektexine is usually produced by the microspore and tapetum during early microspore stage as long as they are enclosed by the callose wall (Heslop-Harrison 1971; Pacini & Juniper 1983; Hesse 2006b). This mode of exine formation is also typical for Araceae, except for the Aroideae subfamily (Hesse 2006b). As known from the pollen wall formation of *Arum alpinum* the amoeboid tapetum is responsible for the separation of the microspores from the tetrad arrangement (Anger & Weber 2006). Moreover, in late tetrad stage the tapetum forms the polysaccharide spines in *Arum* (Anger & Weber 2006). This agree with our results, given that the striate ornamentation is already visible in late tetrad stages. As far as is known, the amoeboid tapetum is the common type within the Araceae (Pacini & Juniper 1983; Pacini et al. 1985; Grayum 1991; Weber et al. 1998; Anger & Weber 2006; Hesse 2006b).

Mesh-like marks have been observed on dry pollen grains of *A. serrulatus.* Such a pattern has also been documented by Van der Ham et al. (1998, p. 130) on pollen of *A. macrophyllus* (Gagnep. ex Serebryanyi) Hett. et Claudel (Synonym: *Pseudodracontium macrophyllum* Gagnepain ex Serebryanyi). We assume that this is most likely a result of pollen wall formation process. As previously mentioned, the outer pollen wall in Aroideae is produced by the tapetum, shortly after dissociation of the tetrad (Weber et al. 1998; Anger & Weber 2006). Pollen is reported to expand rapidly after release from the tetrad (Van der Ham et al. 1998; Takahashi & Skvarla 1991). During pollen expansion within the anther, pollen grains probably still adhere close to each other. The striate ornamentation of adjacent pollen grains is thus sometimes visible as striate marks on the pollen wall of mature pollen grains. Depending on the position of adjacent pollen this pattern is mostly mesh-like and often near the apices, where microspores in the tetrad are already in touch with each other.

Ornamentation types

The most common pollen ornamentation in the subfamily Aroideae (sensu Cabrera et al. 2008; Cusimano et al. 2011) is psilate or echinate and rarely striate or plicate (Grayum 1992; Cusimano et al. 2011). Pollen of *Amorphophallus* reveals a great diversity in ornamentation types as documented by our results as well as previous studies (e.g. Thanikaimoni 1969; Tarasevich 1988; Grayum 1992; Van der Ham et al. 1998, 2005). According to Thanikaimoni (1986) such diversity is influenced by various factors, primarily in their function in plant reproduction. Van der Ham et al. (1998) distinguished eight ornamentation types (echinate, verrucate, areolate, striate, stirate/scabrate, fossulate, psilate and scabrate). For the investigated *Amorphophallus* species the striate type was dominating (19 out of 25 species), which has many variations (striate subtypes 1-5).

Striate and striate to plicate type. — In species with striate pollen *(A. myosuroides, A. ongsakulii*, *A. operculatus*, *A. polyanthus,* and *A. serrulatus),* striae are quite long, running more or less circumferential and pollen is thus described as striate to plicate. By contrast *A. lacourii* and *A. latifolius* have plicate ornamentation elements (long ribs, running circumferentially) rather than striate. Various studies on *Amorphophallus* (including *Pseudodracontium*) refer to striate pollen (e.g. Van der Ham 1998, 2005), whereas plicate *Amorphophallus* pollen was so far only described by Hesse et al. (2000). Typical plicate pollen such as *Ephedra* (El-Ghazaly et al. 1998), *Spathiphyllum* Schott (Grayum 1992; Hesse et al. 2000; Halbritter 2009) or *Steudnera* K.Koch (Hesse et al. 2000; Halbritter & Weber 2005) usually has long, parallell-arranged, compact and unbranched plicae. The pollen ornamentation of *Amorphophallus* is not always clearly striate or plicate and it is often a subjective decision of the palynologist or the pollen terminology used. For *Amorphophallus* pollen, such transition type is thus described as striate to plicate. Pollen of the investigated former *Pseudodracontium* species (*A. lacourii*, *A. latifolius)* is well-defined from *Amorphophallus* by their unique pollen type, namely plicate with psilate apices (Van der Ham et al. 1998; Halbritter 2005; our results). This pollen type is similar

to the plicate pollen of the aroid genus *Spathiphyllum,* where the plicae also end in psilate apices (Grayum 1992; Halbritter & Weber 2005).

Striate to reticulate type. — The striate to reticulate orrnamentation of *A. longituberosus* and *A. asterostigmatus* proved to be a result of an expanding thin surface layer. During rehydration, the expansion of the thin layer itself forms a reticulum (Fig. 5B-C, G-I), which finally ruptures partly (Fig. 5C) or completely (Fig. 5D). This was also confirmed for pollen in dry condition, where the ornamentation was striate only. Similar observations on striate to reticulate pollen were made by Van der Ham et al. (1998, p. 121) for pollen of *A. albispathus* and *A. longituberosus.* Deviations were found in hydrated pollen of *A. atrorubens* with striae running transverse to the equatorial axis and in dry pollen of *A. longituberosus* with striae running criss-cross. Transversely, striate pollen is also documented by Van der Ham et al. (1998, p. 115, 117) for *A. elegans* Ridl. and *A. richardsiae* Ittenb.

Echinate type. — The echini (micro-echini) of *A. mangelsdorffii* are distributed over the pollen surface and vary in size and shape. Although a TEM study for *A. mangelsdorffii* is lacking acetolysed pollen was usually psilate, indicating a polysaccharide nature of the spines. Acetolysed pollen usually has a psilate pollen surface with circular remnants of the echini, although some completely echinate pollen also occurred in the sample. Further evidence of their polysaccharide nature is supplied by remaining echini, because they are deformed and only loosely attached to the pollen wall or clumped together after acetolysis. Van der Ham et al. (1998) distinguished three echinate subtypes for the echinate ornamentation type of *Amorphophallus*, with storeyed, short unstoreyed and elongate unstoreyed spines. The latter authors used either fresh anthers/pollen suspended in a mixture of glycerin and 96% alcohol or stamens from herbarium material boiled for 10 minutes in water (Van der Ham et al. 1998, p. 97, Van der Ham et al. 2005, p. 253). Our results clearly demonstrate that the echini are made of polysaccharides, susceptible to the various preparation methods (e.g. boiling in water, acetolysis) and thus they vary in shape and size or dissolve completely. We assume that the storeyed spines are a result of the preparation method used and that the polysaccharide spines are affected by

boiling in water and thus they vary in shape. By contrast, sporopollenin spines would resist both methods, boiling in water as well as acetolyis treatment, as documented e.g. for *Arum* L. (Pacini & Juniper 1983; Weber et al. 1999), *Sauromatum* (Weber et al. 1998) and *Pistia* (Weber et al. 1999).

Verrucate type. — Similar to the echini in *A. mangelsdorfii*, the verrucae in *A. stuhlmannii* vary in size and shape, e.g. some of the verrucae are more elongated with an acute top, which is in accordance with literature reports (Van der Ham et al. 1998). Based upon our TEM results and the non-resistance of pollen to acetolysis, we suggest a polysaccharide nature of the outer pollen wall and of the verrucae.

Psilate type. — Pollen of the investigated *Amorphophallus* species with psilate ornamentation is medium- to large-sized and with spheroidal to oblate shape. Pollen with psilate ornamentation occurs in about one-third of the genera of the Aroideae subfamily (Mayo et al. 1997). Aroid pollen with psilate ornamentation is usually small and ellipsoidal, e.g. *Schismatoglottis* (Ulrich et al. 2012). Only in a few aroid genera psilate pollen is medium- to large-sized and spheroidal, such as in *Amorphophallus*, *Montrichardia*, *Taccarum* Brongn. ex Schott, *Pseudohydrosme* Engl. (Mayo et al. 1997; Weber & Halbritter 2007).

Pollen wall stratification and chemical nature

Intine. — All investigated *Amorphophallus* pollen have an exclusivley monolayered more or less thick intine. This fact deviates from the Araceae pollen wall type 2a (Weber et al. 1999, p. 418), where the intine is defined as bilayered over the whole pollen wall. In the investigated striate pollen the intine is often as thick as the endexine, whereas in verrucate pollen and psilate pollen the intine is much thicker than the endexine. A uniformly thickened and channelled intine is characteristic for inaperturate pollen (Thanikaimoni 1978, 1986; Furness & Rudall 1999). Characteristic for inaperturate Araceae pollen lacking an ektexine is a continuous thick endexine and intine throughout the pollen grain (Weber et al. 1999). Various studies of the intine indicate that it comprises several different types of polysaccharides, mainly neutral polysaccharides (cellulose and pectin) and acidic polysaccharides (especially pectic polysaccharides) in the outer zone

(Heslop-Harrison & Heslop-Harrison 1982; Kress & Stone 1982). The intine usually stained electron-dense with the Thiéry-test (Thiéry 1967). With this method, polysaccharides can be classified as neutral polysachharides like glycogen, starch (amylose and amylopectin), pectins, chitin, callose and cellulose (Thiéry 1967; Clement & Audran 1999). As osmium-fixed material was used for the Thiéry-test (Thiéry 1967), the staining results of the intine differed in electron opaqueness and intensity.

Endexine. — The endexine of *Amorphophallus* pollen is bilayered and characterised by a spongy inner layer and a more compact thin surface layer, which is in accordance with literature reports (Van der Ham et al. 1998, Weber et al. 1999). With potassium permanganate the endexine always stained electron-dense. With the modified Thiéry-test (Weber & Frosch 1995), uranyl acetate and lead citrate and with the Thiéry-test (Thiéry 1967) the endexine in *Amorphophallus* stains either intensive electron-dense or electron-lucent. For the endexine it is well known that its electron opaqueness changes depending on the method used (Weber & Ulrich 2010). This layer comprises sporopollenin, lipids and proteins (Heslop-Harrison 1968a, b; Heslop-Harrison et al. 1973) and accordingly it stained electron-dense with the lipid test and produced a strong and distinct contrast with potassium permanganate (Weber & Ulrich 2010).

The outer pollen wall. — Our results showed that the outer pollen wall of *Amorphophallus* pollen is made of polysaccharides rather than sporopollenin. The outer pollen wall comprises either a continuous thin polysaccharide layer (psilate type) or polysaccharide ornamentation elements (striate, echinate and verrucate type). The presence of neutral polysaccharides in the outer pollen wall was indicated by the Thiéry-test (Thiéry 1967). With this method, polysaccharides stained electron-dense. This is equivalent to the Araceae pollen wall type 2a described by Weber et al. (1999). Moreover, the absence of sporopollenin was proved by the susceptibility of the pollen wall to acetolysis treatment. Although Van der Ham et al. (2000) and Hesse et al. (2000) reported remains of ornamentation elements after acetolysis (e.g. for *A. konjac* and *A. elatius* Hook. f.), they described the pollen wall as usually acetolysis-susceptible, which is in accordance with our results. In the investigated species with psilate pollen

(*A. prainii, A. henryi, A. bulbifer and A. paeonifolius*), the outer pollen wall layer is very thin and continuous. Van der Ham et al. (1998, 2005) describes psilate pollen with a thin endexine and a probably sporopollenin thin, unstructured ektexine. Our results showed that psilate pollen is completely destroyed after acetolysis treatment which proves the polysaccharde nature of the outer pollen wall.

In various studies on *Amorphophallus* (Van der Ham et al. 1998, 2000, 2005) the outer pollen wall layer is usually defined as a non-acetolysis-resistant ektexine. Given that ornamentation elements sometimes retained after acetolysis at least in some species the presence of sporopollenin in the outer pollen wall layer was assumed and thus homology with resistant ektexine (Van der Ham et al. 2000, p. 242). A polysaccharide outer pollen wall layer is not homologous to an ektexine (Hesse 2006b). According to Hesse et al. (2000, p. 236), an ektexine is lacking in the genus *Amorphophallus*, given that the outer pollen wall is a tapetum-borne, polysaccharide layer. In contrast to all other subfamilies of Araceae, the pollen wall in Aroideae (including *Amorphophallus*) lacks the common sporopollenin tectate-columellate exine: instead, a polysaccharide outermost pollen wall layer (Weber et al. 1998; 1999) or polysaccharide echini (Pacini & Juniper 1983; Weber et al. 1998) cover the endexine. This polysaccharide wall ornamentation is a unique feature of Aroideae pollen, first documented in *Arum italicum* Mill. (Pacini & Juniper 1983) and later in *Sauromatum venosum* (Ait.) Schott (Weber et al. 1998), where polysaccharide echini are covered by a thin polysaccharide surface layer (Weber et al. 1998). It was also reported for *Pistia stratiotes* L., where the outer pollen wall is formed by polysaccharide plicae and a thin polysaccharide surface layer (Weber et al. 1999).

Granules in the outer pollen wall. — The outer pollen wall of *Amorphophallus* pollen is reported to be ultrastructurally diverse through the presence or absence of variously sized, shaped and distributed dark granules (Van der Ham et al. 1998, p.95). Granules were first reported by Ohashi et al. (1983) in echinate pollen of *Arisaema* Mart. and later by Tarasevich (1988) in *Arisaema*, *Pistia* L. and *Amorphophallus konjac.* In the current study, we used

different staining methods to detect and clarify the chemical nature of the granules. Granules of different size, shape and distribution were found in the outer polysaccharide pollen wall of almost all investigated species, aside from *A. yunnanensis*. For the striate and verrucate type, the results conform to Van der Ham et al. (1998, 2005). Contrary to Van der Ham et al. (1998, 2005) we also found indistinct granules as an upper or basal sublayer in the outer pollen wall in psilate pollen of *A. bulbifer*, *A. henryi* and *A. prainii*. According to Van der Ham et al. (1998, 2000) the granules might be responsible for the low resistance of the outer pollen wall to acetolysis, which they denoted as an ektexine. Van der Ham et al. (1998, 2005) suggested an oily nature of the granules due to their osmiophilic staining, although sections were only stained with uranyl acetate and lead citrate and not specifically for lipids.

Our results demonstrated that the staining behaviour of the granules in the outer pollen wall varied with the method used. For instance, with the modified Thiéry-test and with the lipid test, the granules were not clearly visible in striate pollen of *A. polyanthus, A. longituberosus* and *A. asterostigmatus*, although they were clearly visible with uranyl acetate and lead citrate, except for *A. polyanthus*. With potassium permanganate the granules stained either electron-dense or electron-lucent and with the Thiéry-test the granules again strongly stained. Although the staining results of the granules were quite different, they stained electron-dense with the Thiéry-test in most cases, similar to the polysaccharide outer wall, thus indicating a polysaccharide nature. The only exception was the staining behaviour of the granules found in *A. stuhlmannii*, as they were clearly visible with uranyl acetate and lead citrate, but did not stain with the Thiéry-test. One reason for the deviating staining behaviour might be the use of osmium-fixed material for the Thiéry-test. Because osmiumtetroxide was removed from the sections by staining with periodic acid for one hour instead of 30 minutes (Thiéry-1967), the staining results might be ambiguous, especially in critical cases. Therefore, it is recommended to use osmium-free material (Thiéry 1967).

Surface layer. — An additional thin surface layer covers the pollen surface of all investigated species with striate and verrucate pollen. This layer is usually extremely thin and continuous. Sometimes the layer also appeared disconnected

between the ornamentation elements, which very likely is a result of the preparation/fixation process. Compared to other investigated species, this layer was thicker in striate pollen of *A. polyanthus*, *A. longituberosus* and *A. asterostigmatus*. For species with psilate pollen the presence of an additional surface layer remains unclear. Although the use of different staining techniques indicates an additional surface layer the presence of remnants from the tapetum or pollenkitt attached to the pollen wall on all investigated psilate pollen made it difficult to distinguish between surface layer and pollen coatings.

The staining behaviour of the thin surface layer was quite contradicting in all investigated species. With the Thiéry-test the layer often stained electron-dense indicating a polysaccharide nature. However, with the lipid test and with potassium permanganate it also often stained electron-dense, similar to the endexine, thus indicating a lipid nature. Although different staining methods were used the chemical nature of the surface layer remains unclear. For *Sauromatum venosum* and *Pistia stratiotes* Weber et al. (1998, 1999) clearly demonstrated that the polysaccharide echini are covered by a thin polysaccharide surface layer. Due to this and given that pollen of *Amorphophallus* is not resistant to acetolysis we also assume a polysaccharide nature of the surface layer.

Acetolysis resistance of the pollen wall

Resistance of *Amorphophallus* pollen to acetoylsis was first reported by Grayum (1992) and more recently by Punekar and Kumaran (2010, p. 328) who stated that pollen of different *Amorphophallus* species retained the ornamentation after acetolysis. Punekar and Kumaran (2010), who acetolysed pollen samples according to Erdtman (1952) at 70 degrees until the mixture turned brown, presumed that ‘the occurrence of sporopollenin in the ektexine of *Amorphophallus* pollen reduced the exine susceptibility to acetolysis’. Moreover, although Van der Ham et al. (1998, 2005) reported non-resistance of *Amorphophallus* pollen to acetolysis they also suggested a correlation between the occasional resistance to acetolysis and the presence or absence of granules. Further, the hypothesis that a slow dissolution of ornamenting elements may be due to the incorporation of sporopollenin was put forward by Van der Ham et al. (2000).

The acetolysed *Amorphophallus* pollen studied here usually collapsed and lost the ornamentation elements completely. This is in accordance with most previous observations (Thanikaimoni 1969; Tarasevich 1988, 1992; Van der Ham et al. 1998; Weber et al. 1999; Hesse et al. 2000; Van der Ham et al. 2005). Our results show that *Amorphophallus* pollen despite usually being psilate after acetoylsis may partly or completely resist the acetolysis treatment. Remains of the ornamentation elements were observed on almost all pollen types. *Amorphophallus* pollen within the same sample usually collapsed, while neighbouring grains more or less retained the ornamentation elements, e.g. as seen in *A. yunnaensis* and *A. konjac.* Our observations reveal a broad spectrum of damage to the ornamentation elements in all *Amorphophallus* species studied. Within a given sample some pollen grains appeared to be more resistant than others. We assume that dissolution or rupture of ornamentation elements proceeds in stages, as demonstrated by pollen with remaining ornamentation elements. Moreover, we assume that the rare undamaged pollen might be the result of random protection by small clumps of pollen grains and/or small gaseous bubbles surrounding these clumps, because in such cases the acetolysis mixture cannot sufficiently affect all pollen grains. Similarly, such a slow and inhomogeneous reaction to acetolysis was also found in *Arisarum* (H. Halbritter unpublished data), *Pistia* (Weber et al. 1999) and *Steudnera* with remnants of plicae, and even in *Sauromatum* with occasionally remaining polysaccharide spines (Weber et al. 1998).

Acetolysis is primarily applied to clean pollen surfaces rather than to to test any susceptibility reaction of the exine (Hesse & Waha 1989). The reaction depends on various parameters, primarily heating temperature and time. Aside from reports concerning the instability of Aroideae pollen walls (Thanikaimoni 1969; Pacini & Juniper 1983; Tarasevich 1988, 1992) or fragile exines (Hesse & Waha 1989) only few reports (Reitsma 1969; Jardine et al. 2015) deal experimentally with time or heating parameters. According to Reitsma (1969) the reaction caused by the acetolysis mixture primarily depends on the temperature. More recently Jardine et al. (2015) demonstrated that ‘standard palynological processing techniques (e.g. cold and hot acetolysis or nitric acid) not only affects the physical nature of pollen and spores, but that the chemical signature is

altered both through the isolation of the exine, and through changes to the chemical structure of sporopollenin.' For the acetoylsis treatment described by Erdtman (1943, 1952) the heating temperature is limited to 80 degrees and the heating is stopped immediately after the boiling point is reached. The heating temperature had to be limited as centrifuge tubes were not heat-resistant at that time. Later on, following Erdtman (1960), samples were heated up in the acetolysis mixture until the boiling point had been reached. Subsequently the heating was stopped and the samples were left in the hot water bath for about 15 minutes. For our study, *Amorphophallus* pollen was acetolysed in the acetolysis mixture according to Erdtman (1960) at 100 degrees, at least for five minutes. Although heating time and temperature slightly deviated from the traditional method (Erdtman 1960) both versions lead to the same result (Hesse & Waha 1989; Hesse et al. 2009). Our results demonstrated that the heating temperature and time as well as the amount of acetolysis mixture used for the investigated material strongly influence the results of the acetolysis treatment. Moreover, the acetolysis mixture should be prepared each time that it is used (Erdtman 1943, 1960).

The application of different methods and the problem of misinterpretations

As documented here the impact of the preparation method can strongly influence the results. Polysaccharide ornamentation elements are susceptible to acetolysis. Moreover, dehydration (DMP) and critical point drying may affect the pollen wall in *Amorphophallus* pollen as well as other Aroideae with polysaccharide wall elements. Some of the *Amorphophallus* pollen investigated with SEM clearly demonstrated problems of misinterpretations. For example, in the investigated species *A. longituberosus* and *A. asterostigmatus* the striate to reticulate ornamentation was only visible on critical point dried pollen grains (Halbritter 2005), whereas pollen in dry condition appeared striate only with LM as well as TEM. Consequently, the interpretation of this ornamentation type is strongly dependent on the method used. Similarly, pollen of *A. taurostigma* and *A. mossambicensis* showed up psilate with remains of a thin pollen wall layer with SEM, though distinct striate with TEM and LM. As seen in pollen of *A. ongsakulii,* the striate ornamentation on hydrated pollen deviated, compared to pollen in dry

condition. As the striae on dry pollen also appeared undulated we assume that these differences are due to natural variation in pollen wall patterning and not a consequence of the preparation method. Another interesting example of deviating results depending on the method used is echinate *Amorphophallus* pollen with 'storeyed or unstoreyed spines' as reported by Van der Ham et al. (1998, 2000). For their study, the authors used either fresh or dry pollen boiled in water. We suppose that the storeyed spines might be a result of the preparation method used and that the polysaccharide spines are affected by boiling in water. Our results demonstrated that the polysaccharide echini on pollen of *A. mangelsdorffii* vary in shape and size or dissolve completely depending on the method used.

For TEM studies different histochemical staining methods should be used to investigate whether pollen wall layers are made of polysaccharids, sporopollenin or lipids. In most studies sections are stained with uranyl acetate and lead citrate only (e.g. Van der Ham et al. 1998, 2005). Our results demonstrate that the application of different TEM staining methods leads to a better understanding of the chemical composition of the pollen wall layers. The presence of neutral polysaccharides in the outer pollen wall was indicated by the Thiéry-test. The hypothesis that a slow dissolution of ornamentation elements may be due to incorporation of sporopollenin (Punekar & Kumaran 2010; Van der Ham et al. 2000; Jardine et al. 2015) was also refuted by our results of acetolysed pollen. Our investigation demonstrated that the reaction to the acetolysis treatment depends on the acetolysis method used, particularly concerning the heating temperature and time.

Conclusion

Our study on pollen tetrads clarified shape and polarity in *Amorphophallus* pollen. Based on the position in the tetragonal or decussate tetrad ellipsoidal pollen of *Amorphophallus* can be described as oblate with elliptic outline with the apices in the equatorial plane. Observations of pollen hydrated in water demonstrated that some in species the pollen wall was shed immediately before pollen tube growth. This effect was only observed in *Amorphophallus* species with striate (*A. krausei, A. rhizomatosus, A. yunnanensis*) and echinate (*A. mangelsdorfii*) pollen. Four main ornamentation types were distinguished for *Amorphophallus*: striate,

echinate, verrucate and psilate type with striate ornamentation being the most common type (19 out of 25 species) with many variations that could be differentiated into five subtypes. Besides the high variation in pollen wall ornamentation our study demonstrated that the impact of the preparation method (e.g. critical point drying) can strongly influence the results. One of the main findings of this study is the clarification of the chemical nature of the pollen wall in *Amorphophallus* by the application of different histochemical staining methods. The presence of neutral polysaccharides in the outer pollen wall layer was primary indicated by staining with the Thiéry-test. In addition, the absence of sporopollenin in the pollen wall was approved by the susceptibility of pollen to acetolysis treatment. Granules of different size, shape and distribution were found in the outer pollen wall of almost all species except for *A. yunnanensis*. Although the staining results of the granules were quite different they stained electron-dense with the Thiéry-test in most cases similar to the polysaccharide outer wall, thus indicating a polysaccharide nature. An additional thin surface layer covers the whole pollen surface of the striate and verrucate type, but the existence of such a layer remains unclear for the psilate type. The histochemical staining results of the thin surface layer are ambiguous, but due to the non-resistance of *Amorphophallus* to acetolysis we suggest a polysaccharide nature. Although *Amorphophallus* pollen is usually not acetolysis-resistant, the pollen ornamentation sometimes remained partly or completely after acetolysis. We also demonstrated that the reaction to acetolysis depends on the acetolysis method used with temperature and timing being particularly important. It is further clear that the application of different preparation and staining methods as well as a combined LM, SEM and TEM analysis is important for the interpretation of pollen characters. Therefore the use of different staining methods for the same species is highly recommended for the detection of pollen wall stratification.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Specimens investigated

Amorphophallus asterostigmatus Bogner et Hett. Bogner J., HBV, cult. (ARA130292, M 2096).

Amorphophallus atrorubens Hett. et Sizemore. (Hetterscheid H.AM. 737).

Amorphophallus bulbifer Blume. HBV, cult. (ARA120233).

Amorphophallus henryi N. E. Br. HBV, cult. (ARA110214).

Amorphophallus interruptus Engl. et Gehrm. Hetterscheid W., HBV, cult. (ARA120234).

Amorphophallus konjac K. Koch, Koch R. HBV, cult. (ARA060119).

Amorphophallus krausei Engl. HBV, cult. (ARA090136).

Amorphophallus lacourii Linden et André. comb. nov. Basionym: *Pseudodracontium lacourii* N. E. Br., Bogner J. (M 2640).

Amorphophallus latifolius (Serebryanyi) Hett. et Claudel. comb. nov. Basionym: *Pseudodracontium latifolium* Serebryanyi, Hetterscheid W., HBV, cult. (ARA090177).

Amorphophallus longituberosus Engl. et Gehrm. Hetterscheid W. (Hetterscheid H.AM. 1252).

Amorphophallus mangelsdorffii Bogner. Mangelsdorff R. (M 550).

Amorphophallus mossambicensis (Schott ex Garcke) N. E. Br. Hetterscheid W. (Hetterscheid H.AM.0447).

Amorphophallus myosuroides Hett. et A. Galloway. Prehsler D., HBV, cult. (ARA120236)

Amorphophallus ongsakulii Hett. et A. Galloway. Prehsler D., HBV, cult. (ARA120237).

Amorphophallus operculatus Hett. et Sizemore. Prehsler D., HBV, cult. (ARA120262).

Amorphophallus paeoniifolius (Dennst.) Nicolson. Bogner J. (M 1280).

Amorphophallus palawanensis Bogner et Hett. Hetterscheid, W. (Hetterscheid HAM. 124).

Amorphophallus polyanthus Hett. et Sizemore. HBV, cult. (ARA110215).

Amorphophallus prainii Hook. f. Bogner J. (M 2661).

Amorphophallus rhizomatosus Hett. Bogner J. (M 2305).

Amorphophallus sumawongii (Bogner) Bogner et Mayo. Bogner J. (M 372).

Amorphophallus serrulatus Hett. et A. Galloway. Prehsler D., HBV, cult. (ARA130293).

Amorphophallus stuhlmannii (Engl.) Engl. et Gehrm. HBV, cult. (ARA110216).

Amorphophallus taurostigma Ittenb., Hett. et Bogner. Madagascar, north of Morondava, near Baobab alley, coastal forest, near the street, 20°12' 27''S, 44°22' 5''E, (M 4689), Sieder A., Knirsch W., Berg Ch. & Pinter M. HBV, cult. (WU).

Amorphophallus yunnanensis Engl. HBV, cult. (ARA 090135).

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D. Adaptations for insect-trapping in brood-site pollinated *Colocasia* (Araceae)

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RESEARCH PAPER

Adaptations for insect-trapping in brood-site pollinated *Colocasia* (Araceae)

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Keywords

Colocasiomyia; deceptive pollination; endoplasmatic reticulum; mutualism; nursery pollination; osmophore.

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ABSTRACT

The Araceae include both taxa with rewarding and deceptive trap pollination systems. Here we report on a genus in which rewarding and imprisonment of the pollinators co-occur. We studied the pollination of four species of *Colocasia* in Southwest China and investigated the morpho-anatomical adaptations of the spathe related to the attraction and capture of pollinators. All four species were pollinated by drosophilid flies of the genus *Colocasiomyia*. The flies are temporally arrested within the inflorescence and departure is only possible after pollen release. Trapping of the flies is accomplished by the closure of the spathe during anthesis. Moreover, in two species the spathe is covered with papillate epidermal cells known to form slippery surfaces in deceptive traps of Araceae. However, in *Colocasia* the papillae proved not slippery for the flies. The morpho-anatomical properties of the spathe epidermis indicate that it is an elaborate osmophore and serves for the emission of odours only. Despite its similarity to deceptive traps of other aroids, *Colocasia* and *Colocasiomyia* have a close symbiotic relationship, as the attracted flies use the inflorescence as a site for mating and breeding. The trap mechanism has presumably evolved independently in *Colocasia* and is supposed to facilitate more efficient pollen export.

INTRODUCTION

Brood-site pollination, with plants providing breeding sites as reward for pollinators, occurs in various angiosperm families (Sakai 2002; Armbruster 2012). Although the relationship between the plants and the pollinators is considered mutualistic, brood-site pollination is also associated with costs for the plant because of the behaviour of the pollinators and their offspring (Pellmyr 1989; Bronstein 2001). Therefore, brood-site offering flowers such as *Ficus* and *Yucca* have evolved adaptations to reduce the detrimental impact of the insects and increase reproductive success (Huth & Pellmyr 2000; Dufay & Anstett 2003).

In the Araceae, brood-site pollination is mainly restricted to taxa of tropical Asia that are associated with drosophilid flies of the genus *Colocasiomyia* (Carson & Okada 1980; Toda & Lakim 2011). These flies have been found breeding in various aroid taxa, but the process of pollination has so far only been studied in *Alocasia*, *Furtadoa* and *Steudnera* (Mori & Okada 2001; Miyake & Yafuso 2005; Takenaka *et al.* 2006; Takenaka Takano *et al.* 2012), while their role in the pollination of *Colocasia* is unknown. *Colocasia* is estimated to comprise about 20 species distributed throughout Southeast Asia, six of which occur in China (Li *et al.* 2010). These species possess a highly synorganised inflorescence consisting of an elaborate spathe and spadix. The spathe (a modified bract) forms a basal spathe tube separated from the expanded spathe blade by a constriction. It surrounds the flower-bearing spadix with pistillate flowers in the lower part, staminate flowers in the upper part, and in some cases sterile parts below and above the staminate flowers (Fig. 1A).

Although the presence of breeding *Colocasiomyia* flies indicates a mutualistic relationship, in some species of *Colocasia* spathe movements (Cleghorn 1913) and papillate epidermal cells on the spathe (Poppinga *et al.* 2010) resembling trapping devices of brood-site mimicking aroids (Bröderbauer *et al.* 2012) have been observed. These taxa attract saprophilous insects by mimicking a brood-site such as dung or carcass without offering rewards and successful breeding is prevented (Dafni 1984; Urru *et al.* 2011). Trapping of insects is caused by papillate surfaces of the spathe epidermis that are slippery and cause the insects to glide into the floral chamber (Knoll 1926; Vogel & Martens 2000). Moreover, in some taxa, spathe movements occlude the floral chamber (Armstrong 1979). Thus, insects are prevented from escape and stay trapped until pollen is released (Vogel 1965).

The aim of the present study is to investigate the role of adaptations for brood-site pollination in inflorescences of *Colocasia* (Araceae) that resemble adaptations for trap pollination present in other taxa of Araceae. We address the following questions: (i) do the breeding *Colocasiomyia* flies act as pollinators; (ii) what is the role of spathe movements and papillate cells forming the adaxial spathe epidermis; and (iii) is the relationship between *Colocasia* and its pollinators mutualistic or has it shifted from rewarding to deceptive trap pollination?

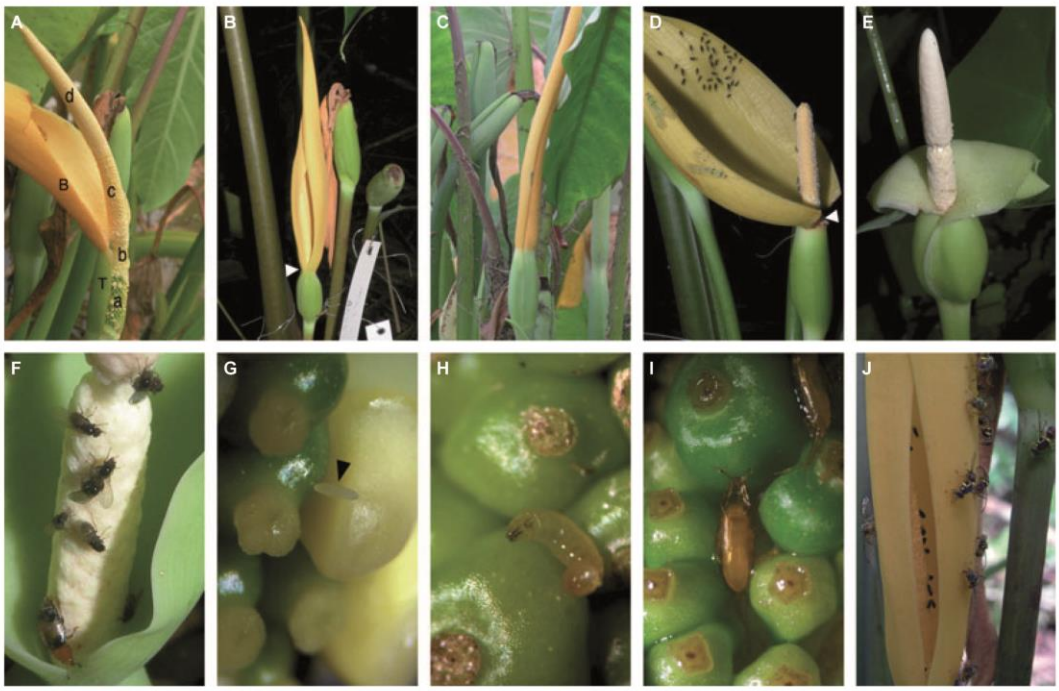

Fig. 1. Inflorescences and insect visitors of *Colocasia* spp. A: *C. esculenta*, spadix; a = pistillate and sterile flowers, b = intermediate sterile flowers, c = staminate flowers, d = sterile appendix. Note that parts of the spathe blade (B) and the spathe tube (T) were removed for better visibility of the spadix. B: *C. fontanesii*, inflorescence during pistillate phase of anthesis. Note that the yellow spathe blade is separated from the green spathe tube by a constriction (arrowhead). C: *C. esculenta*, spathe closure during anthesis. D: *C. lihengiae*, inflorescence during the staminate phase of anthesis visited by *Colocasiomyia* spp. (Drosophilidae) and a neuropterid species (Chloropidae). Note that the margins of the spathe blade break (arrowhead) and the blade bends back; the spadix lacks an appendix. E: *C. affinis*, inflorescence after anthesis with the spathe blade furled. F: *Colocasiomyia steudnerae* (Drosophilidae) and *Aethina humeralis* (Nitidulidae) on the spadix of *Colocasia affinis*. G: Egg (arrowhead) of *Colocasiomyia* sp. on the pistillate flowers of *Colocasia esculenta*. H: Larva of *Colocasiomyia* sp. on the pistillate flowers of *Colocasia lihengiae*. I: Pupa of *Colocasiomyia* sp. on the pistillate flowers of *Colocasia esculenta*. J: *Bactrocera* sp. (Tephritidae) and *Colocasiomyia* spp. on the inflorescence of *Colocasia fontanesii*.

MATERIAL AND METHODS

Pollination ecology

Study site

The reproductive biology of four species of *Colocasia* was studied in and around the Xishuangbanna Tropical Botanical Garden (XTBG; 21°41′ N, 101°25′ E, 570 m a.s.l.), Menglun, Yunnan Province, China. In this area the climate is seasonal, with most rainfall occurring between May and October. The mean annual precipitation is 1493 mm and the average temperature is 21.8 °C (Cao *et al.* 2006). The area of XTBG and its surroundings was previously covered with tropical seasonal rain forest and tropical montane rain forest, a major part of which has been converted to rubber plantations during the last decades (Zhang & Cao 1995).

Study species

Our study focuses on two species of *Colocasia*, *C. esculenta* and *C. fontanesii*. In addition, we report on two further species (*C. affinis*, *C. lihengiae*) that have been observed in the field: (i) *C. fontanesii* Schott (synonym *C. antiquorum* Schott). Study site: XTBG. The population in the garden originates from wild collections and grows in the garden in a secondary forest within the species' natural distribution range. (ii) *C. lihengiae* C.L.Long & K.M.Liu. This species has been considered conspecific with *C. fontanesii* by Li *et al.* (2010), but differs in lacking a sterile appendix above the staminate flowers. As the examined population of *C. lihengiae* was very uniform and the specimens never produced an appendix, we consider it to represent a distinct species. Study site: XTBG. The population in the garden originates from wild collections and grows in the garden in a secondary forest within the species' natural distribution range. (iii) *C. esculenta* (L.) Schott. This species is widely cultivated in the tropics as a food crop. It probably originates from Southeast Asia (Matthews & Naing 2005), but the natural distribution range is unknown. Study site: ponds at XTBG. (iv) *C. affinis* Schott. Study site: natural population growing at the forest margin along the old road between the cities of Menglun and Jinghong northwest of XTBG.

Vouchers of the four species studied in the field (*Colocasia affinis* Yinjiantao s.n., *Colocasia esculenta* Yin jiantao1726, *Colocasia fontanesii* C310005, *Colocasia lihengiae* Yin jiantao 1728) have been deposited in the herbarium of XTBG (HITBC).

Two of the four species studied in the field were available in the living collections of the Botanical Garden of Vienna and were used for the morpho-anatomical analyses. Vouchers of these species have been deposited in the herbarium of the University of Vienna: *Colocasia esculenta* WU0064967, *Colocasia fontanesii* WU0064969. Fieldwork was carried out from 30 June to 19 August 2010 in and around XTBG.

The course of anthesis
The course of anthesis in *C. esculenta* (number of specimens studied = 14), *C. fontanesii* (n = 5) and *C. lihengiae* (n = 4) was recorded on different occasions from 04:00 h until 19:00 h. In *C. affinis*, several inflorescences could be observed on 2 days in August for 1 h each, from 11:00 h to 12:00 h and from 15.30 h to 16.30 h, respectively.

Thermogenesis
Thermogenesis of the spadix was recorded with a combined thermometer and data logger (Scanntronik Thermofox Universal) in two inflorescences of *C. fontanesii* and three inflorescences of *C. esculenta*. Three thermocouples were inserted into the appendix, the staminate zone and the central sterile zone. A fourth thermocouple was put close to the inflorescence in order to measure the ambient temperature. Measurements were started before onset of anthesis and were stopped a couple of hours after pollen release. The temperature was recorded every 5 min.

Pollinators and visitors
Pollinators and visitors were collected from inflorescences with nets and stored in 70% ethanol. The collected insects were identified by specialists (see Acknowledgements).

Bagging experiments
Thirteen inflorescences of C. *esculenta* were covered with organza bags prior to anthesis in order to exclude pollinators. The bags were removed after the end of anthesis. Fruit set of these inflorescences was then compared to 17 open-pollinated inflorescences. In addition, fruit set was also checked for eight open-pollinated inflorescences each in *C. fontanesii* and *C. lihengiae*. For all inflorescences, the number of fertilised and unfertilised ovaries was counted as well as the number of fertilised ovules for ten fruits per inflorescence.

Morphology and anatomy of the spathe

The morphology and anatomy of the spathe was studied from specimens of *C. esculenta*, and *C. fontanesii* cultivated in the glasshouses of the Botanical Garden of Vienna.

Spathe movements
Spathe movements in specimens of *C. esculenta* and *C. fontanesii* were recorded with a Nikon Coolpix P 5000 camera (Nikon, Tokyo, Japan), automatically taking pictures every 10 min.

Odour emission
Inflorescences were submerged in neutral red (1:10000 neutral red:tap water) and checked for staining at 1-h intervals (Vogel 1963; Dobson *et al.* 2005). The sections from the spathe tube and the spathe blade, and the pistillate, staminate and sterile parts of the spadix were enclosed in small vials and checked for odour emission by nose at intervals of 30 min (Vogel 1963).

Scanning electron microscopy (SEM)
Surface morphology of the spathe epidermis was studied with SEM. Samples used for the assessment of cell shape were dehydrated in a graduated series of ethanol and then transferred to acetone. Subsequently, samples were critical point-dried and sputter-coated with gold and investigated with a JEOL JSM6390 SEM at 10 kV. Samples used for the examination of epicuticular wax crystalloids only were air-dried before sputter-coating, as the application of ethanol and heat would alter the crystal structure of the wax (Barthlott & Wollenweber 1981).

Light microscopy (LM)
For investigation under LM, spathes were fixed in FAA for at least 7 days and transferred to ethanol 70% afterwards. Subsequently, samples were dehydrated in a graduated series of ethanol, embedded in 2-hydroxyethyl methacrylate (Kulzer's Technovit 7100, Heraclaeus Kulzer, Hanau, Germany) and cut to 6 µm with a Thermo Scientific rotary microtome (Microm HM355S, Microm, Walldorf, Germany). The sections were stained with Ruthenium red and Toluidine blue. The presence of starch and lipids in fresh spathes of *C. fontanesii* was checked through staining with iodine tincture and Sudan IV.

Transmission electron microscopy (TEM)
Pieces of the spathe from living material of *C. esculenta* and *C. fontanesii* were fixed in 3% glutaraldehyde (GA), post-fixed with 1% osmium tetroxide (OsO_4) and 0.8% potassium hexacyanoferrate ($K_4Fe(CN)_6 \cdot 3H_2O$). Fixed material was dehydrated in 2,2-dimethoxypropane and then embedded in Agar's low viscosity resin (LV-Resin; Agar Scientific Limited 2004). Sections (60–90-nm thick) were cut with a diamond knife (Diatome Ultra 45°; 3.5 mm) on a Leica Ultracut EM UC6 microtome. For common contrast, the sections were stained with uranyl acetate (U: 1% methanolic solution) followed by lead citrate (Pb: 0.1% solution). The occurrence of polysaccharides was detected with the Thiéry test (Thiery 1967). Presence of lipids was investigated according to the procedure of Rowley & Dahl (1977). All sections were examined with a Zeiss EM 109 TEM at 50 kV.

RESULTS

Pollination ecology

Course of anthesis
In all four species, the spathe is divided into a basal tube and an apical blade, which are separated by a constriction (Fig. 1B). The spathe tube forms the lower floral chamber enclosing the pistillate flowers, while the spathe blade forms the upper floral chamber, enclosing the staminate flowers and the sterile appendix. All species are protogynous, with anthesis lasting for about 24 h. In *C. esculenta*, *C. fontanesii* and *C. lihengiae* the inflorescence opened before dawn concomitantly to the emission of an intense fruity odour with a musty component (Fig. 1B). As shown in the automatic camera recordings, opening started after midnight on the first day of anthesis (Figure S1). Around the same time, the stigmas became wet. During the day, odour emission decreased and the entire spathe gradually closed again until the spathe margins overlapped completely around 17:00 h (Fig. 1C). Between 17:00 and 20:00 h, the spathe constriction closed, thereby occluding the passage between the lower and

Table 1. Species distribution of *Colocasiomyia* spp. in inflorescences of *Colocasia* spp.

species (n)	*Colocasiom. steudnerae* n(f)	*Colocasiom. alocasiae* n(f)	*Colocasiom. xenalocasiae* n(f)	*Colocasiom. sp.3 aff. colocasiae* n(f)
Colocasia affinis affinis (3)	29 (10)	0	0	0
Colocasia esculenta (14)	3 (1)	20 (10)	255 (190)	17 (13)
Colocasia fontanesii (4)	1 (0)	6 (1)	67 (48)	36 (23)
Colocasia lihengiae (3)	0	2 (2)	38 (27)	3 (3)

n = number of specimens; f = number of female specimens.

upper floral chamber. The stigmatic surface of the pistillate flowers decayed and produced large aqueous droplets. During the next morning, between 06:30 and 07:30 h, the pollen was released; at the same time, the spathe blade reopened. In *C. esculenta* the blade only opened in the lower part, while in *C. fontanesii* and *C. lihengiae* the blade reflexed and curled completely within less than 30 min (Fig. 1D). The reflexing and curling of the spathe after pollen release was also observed in *C. affinis* (Fig. 1E).

Pollinators and visitors

The insects most commonly found in inflorescences of the four *Colocasia* species studied were flies of the drosophilid genus *Colocasiomyia* (Fig. 1D). Usually ten to 30 individuals (60 in one inflorescence of *C. fontanesii*) were found per inflorescence. Three drosophilid species (*Colocasiom. alocasiae* (Okada, 1975), *Colocasiom. xenalocasiae* (Okada, 1980), *Colocasiom.* sp. 3 aff. *colocasiae*) co-occurred in *C. fontanesii, C. lihengiae and C. esculenta* (Table 1). Another species, *Colocasiom. steudnerae* (Takenada & Toda, 2006), occurred only rarely in the above three taxa, but was regularly present in inflorescences of *C. affinis* (Fig. 1F). The flies arrived at the onset of anthesis, landed on the outside of the spathe blade and soon walked down into the lower floral chamber. The females oviposited mainly between the pistillate flowers (Fig. 1G). Male and female flies were frequently observed mating inside the inflorescence. Prior to closure of the spathe constriction, the flies moved upwards into the upper floral chamber and assembled on the staminate part of the spadix inside the occluded spathe blade. As the spathe margins overlapped tightly, the insects could not escape from the inflorescence at this stage of anthesis. After pollen extrusion and reopening of the spathe on the second day of anthesis, the drosophilids quickly left the inflorescence; sometimes they first aggregated on the reflexing spathe blade before departing. Fly larvae hatched within the next 24 h and developed between the pistillate and sterile flowers inside the lower floral chamber without damaging the developing fruits (Fig. 1H and I). In none of the species studied were larvae found to occur on the staminate flowers of the spadix.

Another flower visitor was *Aethina humeralis* (Grouvelle, 1890), a nitidulid beetle of the subfamily Nitidulinae (Fig. 1F); this species was, however, only rarely found. In inflorescences of both *C. esculenta* (n = 14) and *C. lihengiae* (n = 4) only a single beetle was found. In *C. fontanesii* (n = 5) one inflorescence contained three beetles, two inflorescences a single beetle and two inflorescences none. The beetles moved around within the inflorescence, but in contrast to the drosophilid flies, they were never observed mating or laying eggs. After pollen extrusion, they fed on pollen and then left the inflorescence. Based on our short observations, beetles seem to be present more regularly in *C. affinis*, but as in the other *Colocasia* species, also only in low numbers per inflorescence.

Regular visitors of the anthetic inflorescences of all *Colocasia* species were an unidentified species of the Chrysopidae (Neuroptera; Fig. 1D) and an unidentified fly of the genus *Bactrocera* (Tephritidae; Fig. 1J). These insects were apparently attracted by the intense odour and usually arrived before dawn. They assembled on the outside of the spathe blade, but never entered the inside of the spathe and thus did not contact the flowers.

Bagging experiments

The inflorescences of *C. esculenta* contained on average 184 ± 27 pistillate flowers (number of specimens studied = 17), of which 26% produced fruits after open-pollination. Pollinated ovaries contained 2.2 ± 2.5 fertilised ovules. In bagged inflorescences (n = 13), one inflorescence was aborted as a whole, and in the remaining specimens only 0.7% of the flowers produced fruits. In open-pollinated inflorescences of *C. fontanesii* (n = 8) 169 ± 28 pistillate flowers were present; of these, 85% produced fruits that contained 16.6 ± 12.1 fertilised ovules per ovary. *Colocasia lihengiae* (n = 8) contained 162 ± 25 pistillate flowers, of which 81% produced fruits after open pollination. Pollinated ovaries contained 11.2 ± 11.8 fertilised ovules on average.

Thermogenesis

Thermogenesis in *C. esculenta* and *C. fontanesii* occurred during the pistillate and the staminate phase of anthesis (Fig. 2). The thermogenic pattern was consistent in the different specimens of the respective species studied. In both species, peaks of heat production occurred in the appendix and the staminate flowers during the first and the second morning, reaching 6–8 °C above the ambient temperature. In the second morning, heat was mainly produced by the staminate flowers. *C. esculenta* differed from *C. fontanesii* by the presence of a third but weaker phase of heat production in the afternoon of the first day of anthesis (Fig. 2A).

Morphology and anatomy of the spathe

In both *C. esculenta* and *C. fontanesii*, the whole inside of the spathe (*i.e.* the adaxial epidermis) stained more or less uniformly red after embedding in neutral red. The outer (abaxial) epidermis of the spathe blade showed at least a weak staining reaction.

Odour emission

Odour emission by the spathe could be detected in *C. esculenta* and *C. fontanesii* through smelling stored samples in separate

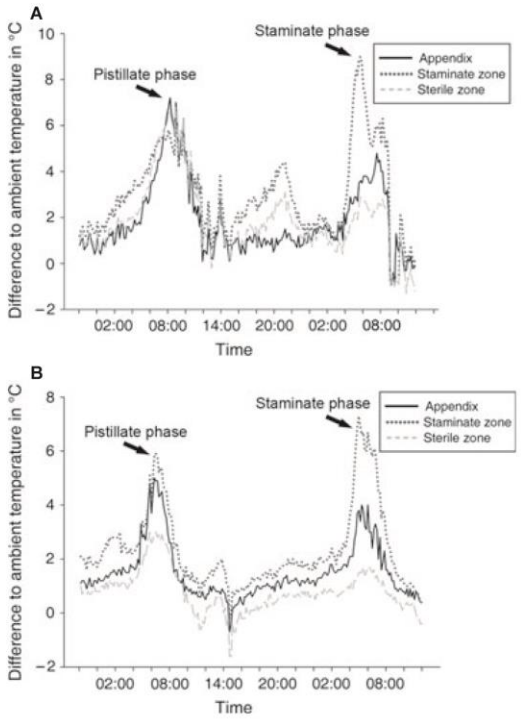

Fig. 2. Heat production during anthesis in different zones of the spadix relative to the ambient temperature A: *Colocasia esculenta*. B: *Colocasia fontanesii*.

glass vials. The spathe blade in particular produced a strong sweet to musty smell. In the spadix, the staminate flowers as well as the sterile appendix served as osmophores, emitting very intense odours. The appendix mainly emitted a strong musty to sweet odour, while the staminate flowers first smelled musty and in a later stage of anthesis often produced a foul smell.

Scanning electron microscopy
In both *C. esculenta* and *C. fontanesii* the adaxial (inner) epidermis of the spathe blade consisted of densely packed papillate cells (Fig. 3A) that collapsed after anthesis, while the epidermal cells of the tube were tabular to convex (Fig. 3B). The abaxial epidermal layer of the spathe blade consisted of papillate cells bearing cuticular folds, covered with wax platelets (Fig. 3C). These papillae where less densely packed than the papillae of the adaxial spathe epidermis. Moreover, they did not collapse after anthesis.

Light microscopy
After staining of fresh spathes of *C. fontanesii* with Sudan IV, lipids could be detected in the epidermis but not in the parenchyma cells. As indicated by staining with iodine tincture, starch was common in most cells of the spathe tube and very abundant in the spathe blade.

Cross-sections of embedded spathe blades showed that the papillate cells of the adaxial epidermis in *C. esculenta* and *C. fontanesii* contained dense cytoplasm. In *C. esculenta* dense cytoplasm was also present in parenchyma cells adjoining the abaxial epidermis of the spathe blade during the pistillate phase of anthesis (Fig. 3D). In both species, the mesophyll of the spathe blade contained lacunar tissue (Fig. 3D). In the spathe tube, lacunar tissue was less widespread and the lacunae were often filled with mucilage (Fig. 3E).

Transmission electron microscopy
In *C. esculenta* and *C. fontanesii*, epidermal papillae of the adaxial spathe tube contained a dense cytoplasm and high intracellular activity (Fig. 3F). Besides the presence of amyloplasts and lipids, the cells contained numerous mitochondria, dictyosomes, polyribosomes and smooth endoplasmic reticulum (sER; Fig. 3G). Moreover, vesicles were transported from the cell to the cuticle (Fig. 3H). Similar activity was also observed but to a lesser extent in epidermal cells of the abaxial spathe blade (Fig. 3I) and the adaxial spathe tube. Amyloplasts were particularly abundant in the parenchyma cells of the spathe blade (Fig. 3J). Moreover, in papillate cells of *C. fontanesii* unusual gorgon-head-shaped endoplasmic reticulum (ER), not documented so far to the best of our knowledge, was common (Fig. 3K). After anthesis, the number of organelles and the overall cytological activity within the papillae decreased significantly (Fig. 3L).

DISCUSSION

Course of anthesis

Protogyny is a consistent feature of Araceae (Mayo *et al.* 1997) and also occurs in *Colocasia*. Moreover, as in most taxa of the large subfamily Aroideae, anthesis lasts for 2 days only (Armstrong 1979; Chouteau *et al.* 2007a; Barabé *et al.* 2008; Takenaka Takano *et al.* 2012). In three of the four *Colocasia* species studied in the field (*i.e. C. esculenta*, *C. fontanesii*, *C. lihengiae*) the attraction of pollinators and pollen extrusion occur during the early morning hours, as is also the case in other *Colocasiomyia*-pollinated Araceae, whereas in aroids pollinated by scarab beetles the major events occur at dusk (Gibernau *et al.* 2000; Maia & Schlindwein 2006; Takenaka *et al.* 2006). As *C. affinis* appears to also be pollinated by drosophilid flies, the deviating timing of anthesis – pollen release and curling of the spathe blade take place during the afternoon - is very unusual, but the precise reason is unknown.

Thermogenesis
Thermogenesis is a common phenomenon in Araceae (Barthlott *et al.* 2009; Seymour *et al.* 2009). It enhances odour emission and serves as a heat reward for departing beetles, facilitating their warm-up before takeoff (Seymour *et al.* 2003a). In *C. esculenta* and *C. fontanesii* the peaks of heat production occur in the first and second morning of anthesis. In the first morning, when the spathe opens and a strong odour is emitted, both the appendix and the staminate flowers warm up to 6–8 °C above ambient temperatures. During the second morning, heat production is mostly from the staminate flowers. Odour emission is very weak in the staminate phase inflorescences and new insect visitors are not attracted. Thus, the second temperature peak might serve as a heat reward that attracts the insects retained within the spathe and guides them

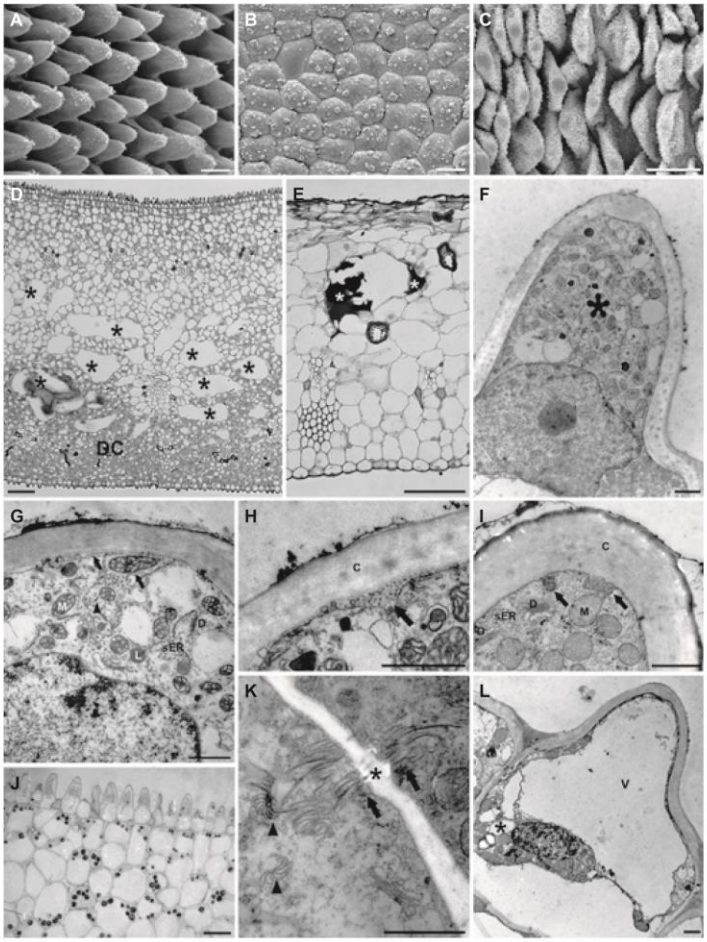

Fig. 3. Morphology and anatomy of the spathe in *Colocasia*. A: *C. esculenta*, adaxial epidermis of the spathe blade with densely packed papillate cells. SEM. B: *C. fontanesii*, adaxial epidermis of the spathe tube with tabular to convex cells. SEM. C: *C. fontanesii*, abaxial epidermis of the spathe blade. Note that the papillate cells bear cuticular folds and are covered with wax platelets. Also note that the cells have shrunk due to drying. SEM. D: *C. esculenta*, cross-section of the spathe blade during the pistillate phase of anthesis. Note the dense cytoplasm (DC) in cells of the abaxial part of the spathe and the lacunar tissue (asterisks) in the mesophyll. LM. E: *C. esculenta*, cross-section of the spathe tube in the pistillate phase of anthesis. Note the lacunar tissue in the mesophyll filled with mucilage (asterisks). LM. F: *C. fontanesii*, papilla of the adaxial spathe epidermis. Note the dense cytoplasm and high intracellular activity (asterisk). TEM, U + Pb. G: *C. fontanesii*, detail of a papilla of the adaxial spathe blade during the pistillate phase of anthesis. Abbrevations: lipid droplets (L), mitochondria (M), dictyosomes (D), polyribosomes (arrowhead), vesicles (arrow), and smooth endoplasmic reticulum (sER). TEM, U + Pb. H: Detail of G: vesicles (arrow) are transported from the cell to the cuticle (C). TEM, U + Pb. I: *C. fontanesii*, detail of a papilla of the abaxial spathe blade during the pistillate phase of anthesis. Vesicles are transported from the cell to the cuticle (C) via multivesicular bodies (arrow). Abbreviations: mitochondria (M), dictyosomes (D), and smooth endoplasmic reticulum (sER). J: *C. esculenta*, amyloplasts appear dark (electron dense) in the parenchyma cells of the spathe blade adjoining the adaxial epidermis. TEM, Thiery test. K: *C. fontanesii*, irregular shaped ER (arrowhead) in the adaxial epidermis of the spathe blade during the pistillate phase of anthesis. Note the transport of cell compounds through the cell wall (asterisk) *via* plasmodesmata (arrows). TEM, Lipid test. L: *C. fontanesii*, papilla on the adaxial spathe blade after anthesis. Note the large vacuole (V) and the low intracellular activity (asterisk). TEM, U + Pb. Scale bars = 100 μm (D, E), 10 μm (A–C, J), 1 μm (F–I, K, L).

to the staminate flowers extruding pollen. Simultaneously, the heat possibly stimulates the flies to leave inflorescence after their body temperature has reached optimum. However, such a heat reward has not yet been proved in flies (Seymour *et al.* 2003b) and its occurrence in *Colocasia* remains doubtful. In *C. esculenta*, an additional but weaker – thermogenic phase takes place in the afternoon of the first day of anthesis. This intermediate peak was not observed in inflorescences of *C. esculenta* cultivated on Vanuatu (Ivancic *et al.* 2004). However, the difference could be simply a consequence of the high genetic variance in *C. esculenta*, which is especially prominent between cultivars of the Asian and Pacific regions (Kreike *et al.* 2004). Although many Araceae have two phases of heat production during anthesis, thermogenesis with more than two

temperature peaks – as observed in the specimens of *C. esculenta* studied – is also known from other taxa, such as *Syngonium angustatum* Schott and *Arum* spp. (Gibernau *et al.* 2004; Chouteau *et al.* 2007a).

Spathe movements

Spathe movements are known from many aroids. In some taxa they serve as protection for developing fruits (Mayo *et al.* 1997), in others they enable the arrest of pollinators (Dakwale & Bhatnagar 1985; Bröderbauer *et al.* 2012). In the former case, the spathe constriction closes after anthesis, thereby secluding the spathe tube containing the pollinated pistillate flowers. In the latter case, *e.g.* in deceptive traps such as *Typhonium* and *Theriophonum*, the narrowing of the constriction occurs during the pistillate phase in order to arrest the insects inside the spathe tube (Armstrong 1979; Dakwale & Bhatnagar 1997). In contrast, in *Colocasia* it is not the constriction but the spathe blade that closes again during the pistillate phase of anthesis, thereby occluding the entire spadix and causing the trapping of pollinators. The constriction only closes in a later stage of anthesis. Such movements have so far only been observed in *Colocasia* and in taxa of the tribe Schismatoglottideae (Cleghorn 1913; Boyce & Wong 2007; Ulrich *et al.* 2012).

Except for *C. esculenta*, the spathe blades of the *Colocasia* species studied reflex very quickly after pollen release, similar to some schismatoglottids (Boyce & Wong 2007). The upper part of the spadix is thereby exposed within a few minutes. We hypothesise that this spathe movement serves to stimulate the pollinators to leave the inflorescence quickly, as their shelter – formed by the spathe blade – vanishes.

Pollinators and visitors

Pollinators

Flies of the genus *Colocasiomyia* were the most common insects present in each inflorescence of *Colocasia* during our study. All four species belonged to the *Colocasiom. cristata* species group. Three of the four species (*i.e. Colocasiom. alocasiae*, *Colocasiom. xenalocasiae*, *Colocasiom.* sp. 3 aff. *colocasiae*) visited inflorescences of all except *C. affinis*. *Colocasiom.* sp. 3 aff. *colocasiae* is so far known to visit *C. esculenta* in Vietnam (Toda, personal communication). *Colocasiom. alocasiae* and *Colocasiom. xenalocasiae* are known as pollinators of *Alocasia odora* and *A. cucullata* (Yafuso 1994). In our study, *Colocasiom. xenalocasiae* was the most common species and appears to be the main pollinator, at least in *C. esculenta* and *C. lihengiae*.

As far as we can conclude from our short observations, *C. affinis* was only visited by *Colocasiom. steudnerae*, which was otherwise only rarely found in *C. esculenta* and *C. fontanesii*; *Colocasiom. steudnerae* is known to be the main pollinator of *Steudnera colocasiifolia* K.Koch in XTBG (Takenaka *et al.* 2006). Contrary to the species of *Colocasia* studied in XTBG, *S. colocasiifolia* flowers from March to April. Thus, it is probable that *Colocasiom. steudnerae* switches its hosts during different times of the year (Toda, personal communication). In contrast to *Steudnera colocasiifolia* and other *Colocasia* species with pollen release occurring during the morning hours, in *C. affinis* pollen is released in the afternoon. The different timing possibly serves as a reproductive isolation mechanism from sympatric *Colocasia* species flowering at the same time of the year.

As in *A. odora*, drosophilids visiting the different species of *Colocasia* were observed to remain in the spathe tube at the beginning of anthesis, laying eggs mainly between the pistillate flowers (Miyake & Yafuso 2005). After the drosophilid flies have oviposited, larvae quickly hatched (usually within a day). The sterile flowers situated between and/or below the pistillate flowers decayed and formed a mucilaginous substrate for the larvae. In inflorescences of *C. esculenta* from which flies were excluded with organza bags, these sterile flowers remained intact, indicating that their decay is caused by activity of the larvae. The fertile pistillate flowers remained undamaged, unlike well-know examples of brood-site pollination such as in *Ficus* or *Yucca* (Armbruster 2012). The larvae did not leave the inflorescence before pupation; as in *Alocasia macrorrhizos*, they developed within the ripening infructescences (Takenaka Takano *et al.* 2012).

Unlike other aroids, oviposition of the different drosophilid species only took place in the pistillate zone of the spadix, and the larvae remained there during development. In contrast, several drosophilid species are known to form synhospitalic pairs using the same inflorescence as brood site, but one species breeds in the pistillate part of the spadix while the other uses the staminate part (Yafuso & Okada 1990). Such behaviour has also been observed in *Colocasiom. alocasiae* (staminate zone) and *Colocasiom. xenalocasiae* (pistillate zone) breeding on *A. odora* (Yafuso 1994). Despite the presence of *Colocasiom. alocasiae* we did not find eggs or larvae on the staminate part of the spadix of the *Colocasia* species studied. As *Colocasiom. alocasiae* has also been found breeding in the pistillate zone of *A. cucullata* (Miyake & Yafuso 2005), the switch to the staminate zone might be a facultative behaviour. Breeding of two synhospitalic species in the pistillate region of the spadix has also been recorded in *A. macrorrhizos* (Takenaka Takano *et al.* 2012). Therefore, the absence of resource partitioning in our species could indicate that this is facultative behaviour, which could depend on host identity or other environmental factors.

Bagging experiments

Because of their abundance and behaviour, flies of the genus *Colocasiomiya* appear to be the most important pollinators of *Colocasia*. Our bagging experiment with *C. esculenta* proves that inflorescences do not produce fruit unless visited by *Colocasiomyia* spp. Seed set in open-pollinated *C. fontanesii* and *C. lihengiae* was even higher than in *C. esculenta*. This might be due to the fact that many inflorescences of *C. esculenta* produced only low amounts of pollen, which is probably related to selection for vegetative traits affecting reproductive traits during human cultivation.

The presence of protogyny prevents self-fertilisation in *Colocasia*. Moreover, the spathe constriction above the pistillate flowers closes before pollen extrusion, thereby preventing pollen from falling onto stigmas. In general, autogamy is uncommon in Araceae while geitonogamy has been observed in some taxa (Mayo *et al.* 1997).

Mutualism versus antagonism

Colocasia and *Colocasiomyia* display a very intimate pollination mutualism in which the inflorescences of *Colocasia* serve as breeding and mating sites for the flies. Despite the fact that the flies obtain rewards for their pollination services, they are also

arrested in *C. esculenta*, *C. fontanesii* and *C. lihengiae*. The reason for trapping insects in *Colocasia* is not fully understood, but its resemblance to trap mechanisms in Araceae that mimic a brood site and deceive their pollinators is remarkable. According to ancestral state reconstructions, the trap mechanism has evolved independently in *Colocasia* (Bröderbauer *et al.* 2012). In general, the convergent evolution of trap pollination in different clades of Araceae has probably been facilitated by protogyny (Bröderbauer *et al.* 2012). Retention mechanisms (*i.e.* closure of the flower during anthesis) have also evolved in other protogynous lineages, mainly within the 'basal angiosperms' such as *Calycanthus* (Grant 1950) or *Magnolia* (Gottsberger *et al.* 2012). Trapping is necessary in protogynous species without rewards in order to retain pollinators until pollen is released in the staminate phase of anthesis. But why do rewarding protogynous species retain their pollinators? A possible explanation is that rewards may only be present for a restricted period of time. For example, in *Arum creticum* bees are rewarded with pollen during the staminate phase and can enter and leave the inflorescence unhindered while collecting pollen. However, they are arrested in the reward-free pistillate phase in order to secure fertilisation (*i.e.* female reproductive success; Diaz & Kite 2006). In contrast, in *Colocasia* the reward (*i.e.* the brood site) is only available during the pistillate phase. During this time the spathe remains open; however, later the spathe blade closes and the flies are trapped. Moreover, before the onset of the staminate phase, the spathe constriction narrows and occludes the lower floral chamber. Before this occlusion, flies must progress into the upper floral chamber containing the staminate flowers. We postulate that the trapping of the pollinators may be necessary in *Colocasia* in order to secure their presence until pollen is released. The combination of trapping and the rapid reflexing of the spathe blade increases the probability that flies will depart at the right time (*i.e.* during pollen release), whereby more efficient pollen transfer is enabled. Consequently, adaptations for the retention and release of pollinators in *Colocasia* have probably evolved in order to increase male rather than female reproductive success.

In other taxa pollinated by drosophilids, *e.g.* species of *Alocasia*, the spathe does not close during anthesis (Miyake & Yafuso 2005; Takenaka Takano *et al.* 2012). Nevertheless, the drosophilids remain inside the inflorescence. Trapping is thus probably not the sole factor controlling the successful pollen export by drosophilid flies in *Colocasia* and other aroids.

Visitors

While the regularly observed *Bactrocera* flies (Tephritidae) and lacewings (Chrysopidae) can be excluded as pollinators as they never enter the inflorescences, the situation is different with the nitidulid beetle *Aethina humeralis* (subfamily Nitidulinae). Nitidulidae are known as pollinators in various plant families, including Annonaceae (Corlett 2004; Teichert *et al.* 2011), Arecaceae (Nunez *et al.* 2005; Fava *et al.* 2011), Magnoliaceae (Ishida 1996) and Cycadales (Kono & Tobe 2007; Proches & Johnson 2009). As in *Colocasia*, many of these taxa have flowers/inflorescences forming a pollination chamber and/or producing a fruity odour and heat. Moreover, a close relative of *A. humeralis*, *A. concolor* (Macleay, 1872), has been observed to visit *Gossypium tomentosum* on Hawaii (Burraston *et al.* 2005). In Araceae, nitidulids have been found on inflorescences of several genera (*e.g. Amorphophallus*, *Cyrtosperma*, *Typhonium* and *Urospatha*; Gibernau 2003; Punekar & Kumaran 2010) but information on their behaviour remains scarce. Chouteau *et al.* (2007b) show that the nitidulid pollinator *Colopeterus amputatus* uses inflorescences of *Monstera obliqua* as mating sites and feeds on the pollen during the staminate phase of anthesis.

Aethina humeralis behaved similarly to the drosophilids in inflorescences of *Colocasia*. It entered the inflorescence on the first morning of anthesis and departed only after pollen release on the second morning. During the staminate phase of anthesis, the beetles were observed to feed on pollen; pollen grains were also found on the beetle's body during investigations with light microscopy. Thus, the beetle might successfully transfer pollen between inflorescences. We did not find the beetle's eggs or larvae in the inflorescence, but it is possible that it might oviposit in the inflorescences and the larvae hatch there (Kirejtshuk, personal communication). Nevertheless, an important role as pollinator of *Colocasia* spp. seems unlikely because of its low abundance, at least in the season in which our observations were recorded. However, further observations are needed to examine the activities of *A. humeralis* in the inflorescences of *Colocasia*.

Morphology and anatomy of the spathe

In the two species of *Colocasia* examined for odour emission, the spathe serves as an osmophore. Odour production in Araceae is generally associated with the spadix, in later diverging clades in particular, with its sterile appendix (Vogel 1963). Nevertheless, odour production by the spathe has been recorded in several taxa (Vogel 1978; Patt *et al.* 1995; Zhu & Croat 2004). The main energy supply for odour synthesis in spathes of *Colocasia* appears to be starch, which is stored in epidermal and parenchyma cells. Lipids, known to be an important resource for odour production in other angiosperms (Hadacek & Weber 2002; Wiemer *et al.* 2009; Pansarin & Pansarin 2011), were also present but less abundant. Unlike other osmophores (Vogel 1963), in spathes of *Colocasia* there are no specialised cell layers for the storage of starch, which is distributed in parenchyma and epidermal cells. The intense osmophore activity was most obvious in the papillate cells of the adaxial epidermis of the spathe blade in *C. esculenta* and *C. fontanesii*. These cells contain numerous mitochondria, sER, ribosomes, polyribosomes and vesicles that are transported through the cuticle. Especially in the papillate cells of *C. fontanesii* we also found unusual gorgon-head-shaped ER that appears to be associated with synthesis of odour compounds.

Papillate cells on the adaxial side of the spathe are known from several taxa of Araceae, as well as other angiosperms, where they form slippery surfaces that aid in the capture of pollinating insects (Poppinga *et al.* 2010). In contrast to these cells, papillae of *C. esculenta* and *C. fontanesii* do not point downwards. The drosophilid flies and the nitidulid beetles observed in the field were able to move along the adaxial epidermis. Therefore, we conclude that in *C. esculenta* and *C. fontanesii* the papillate cells serve only as osmophores. However, a common origin of papillate slippery surfaces and osmophoric epidermal cells in spathes of Araceae is possible, as slippery surfaces in several aroids (*e.g. Arum*, *Typhonium*) also produce odour (Bröderbauer *et al.* 2012).

The abaxial spathe blade also seemed to act as an osmophore, showing similar albeit weaker intracellular activity compared to the adaxial epidermal cells. Concordantly, osmophoric activity was only indicated as a weak staining with neutral red in both species. We conclude that the spathe emits odours in different parts, probably at varying intensity. Thus, arriving insects might be guided by an odour gradient from the outside to the inside of the inflorescence. Whether odour gradients might also be important in influencing the spatial distribution of insects within the inflorescence is unclear. It has been shown that the odour compounds produced by the spathe or specialised sterile organs can differ from those produced by the spadix (Hadacek & Weber 2002; Kakishima *et al.* 2011). These different odours might influence the behaviour and spatial distribution of pollinators and thereby cause more efficient pollen transfer. Possibly, such an odour gradient, in combination with the second thermogenic peak in the staminate flowers, stimulates flies in *Colocasia* to leave the spathe tube and move to the staminate flowers prior to the closure of the spathe constriction and pollen extrusion. Such an effect of odour on the behaviour of pollinators has already been found in the aroid *Peltandra virginica*, where flies either oviposit or feed on pollen, depending on the varying concentrations of odour compounds emitted by the spathe (Patt *et al.* 1995).

A prominent feature in the spathe blades of *C. esculenta* and *C. fontanesii* was the presence of aerenchyma-like lacunar tissue. Such tissue is known from the specialised osmophoric appendices in several members of Araceae (Vogel 1963). The intercellular spaces are thought to be important to provide oxygen for respiration during thermogenesis, thereby fuelling the odour emission (Seymour *et al.* 2009). The presence of such tissue in *Colocasia* indicates that in Araceae not only the spadix but also the spathe can be a highly elaborate osmophore.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Spathe movements of a *Colocasia fontanesii* inflorescence recorded in the glasshouses of the University of Vienna Botanic Garden.

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5. GENERAL DISCUSSION

5.1. NEW INSIGHTS INTO ARACEAE POLLEN MORPHOLOGY AND ULTRASTRUCTURE OF SELECTED SPECIES

The application of different methods on Aroideae pollen revealed interesting new insights on pollen morphology and ultrastructure. The relevant pollen and flower characters of the major taxa of Araceae are summarized in Table 1 (see 'General Introduction').

Apoballis and *Schismatoglottis*

Pollen of tribe Schismatoglottideae is expected to have the typical Aroideae pollen wall either of type 2a (polysaccharide outer wall layer) or 1b (with a thin outer sporopollenin ektexine) (Weber et al. 1999). Our results demonstrated that Schismatoglottideae pollen only constitutes a polysaccharide pollen wall of type 2a (Weber et al. 1999). Furthermore the ultrastructural studies revealed interesting new findings for the tribe: (1) echinate pollen in the basalmost genus *Apoballis*, with polysaccharide acetolysis-resistant echini (2) irregularly distributed calcium oxalate crystals of different size on the pollen surface in *Schismatoglottis,* (3) and the presence of a thin surface layer, in most *Schismatoglottis* and *Apoballis* species (Chapter 4A).

The unexpected resistance of *Apoballis* and *Schismatoglottis* pollen to acetolysis was the reason for an intensive TEM-investigation. The use of different histochemical staining methods revealed a thin surface layer. Based on our staining results (indicating lipid compounds rather than sporopollenin) and the resistance to acetolysis, it seems more likely that this ektexine-like layer is a type of cuticula. This layer is unique for the tribe Schismattoglottideae, and for the Araceae so far only documented for *Callopsis* (Weber 2004).

The occurrence of small and large calcium oxalate crystals attached to the pollen wall of most *Schismatoglottis* species revealed to be a common feature for the genus and is also known for other aroids e.g. *Caladium, Gearum,*

Scaphispatha (Grayum 1992; Barabé et al. 2004). Under the light microscope, pollen of *Schismatoglottis celebica* and *S. calyptrata* appeared to be echinate at first sight. Only the scanning electron microscope revealed that irregularly distributed crystals of different size, not echini, cover the whole pollen surface. More often, crystals are found mixed with aroid pollen as observed for e.g. *Anthurium*, *Calla*, *Zantedeschia* (D'Arcy et al. 1996). The presence of oxalate crystals together with pollen was suggested to play a defensive role against consumption by pollinators, in pollen liberation or as a visual signal to insect visitors (Franceschi & Horner 1980; D'Arcy et al. 1996; Coté & Gibernau 2012). This is supported by a more recent study on Schismatoglottideae pollen and pollinator observations by Low et al. (2015), who found some support for the role of oxalate crystals as protection against herbivory by chrysomelids, at least during pistillate anthesis.

Calla palustris

Regarding the pollen characters in combination with flower morphology and flowering behaviour, *Calla* can be clearly differentiated from all other aroid taxa/clades (Chapter 4B, see also table 1 and Introduction). Thus, *Calla* seems to be highly autapomorphic and its relationship remained so far obscure (Mayo et al. 1997). The disulcate to ring-like aperture condition is a new finding for the genus and is an important indicator for systematics (see also chapter 5.3). Disulcate pollen is unique in Araceae but is also found in other monocots, e.g. *Calycanthus floridus* (Albert et al. 2011). Moreover, the newly described ring-like aperture condition of *Calla* is highly interesting as this aperture type only occurs in Monsteroideae and Zamioculcadoideae (Hesse et al. 2001; Bogner & Hesse 2005). Within the Araceae, *Calla* pollen is most similar with pollen of *Gonatopus* and *Zamioculcas,* both having a ring-like aperture (hamburger-like) and rugulate, psilate to perforate ornamentation (Hesse et al. 2001; Bogner & Hesse 2005). Whereas the vast majority of Araceae is predominantly tropical in distribution (Boyce et al. 2012), the helophytic genus *Calla* is distributed in boreal regions. The variation from disulcate to ring- like, might be a modification resulting from changing environmental conditions, especially with respect to harmomegathy.

Amorphophallus

One of the main findings of this study is the clarification of the chemical nature of the pollen wall in *Amorphophallus* by the application of different histochemical staining methods (Chapter 4D). The results showed that the outer pollen wall, as well as the granules within it, are made of polysaccharides. Consequently, the hypothesis by Van der Ham et al. (1998, 2000), that the granules might be responsible for the low resistance of the outer pollen wall to acetolysis, which they denoted as an ektexine could be refuted by our results. Furthermore, the presence of sporopollenin as stated by Punekar and Kumaran (2010), could be disproved by the susceptibility of pollen to acetolysis treatment. Although the histochemical staining results of the thin surface layer were ambiguous, we assume a polysaccharide nature due to the non-resistance to acetolysis. This is also supported by studies of Weber et al. (1998, 1999), given that the polysaccharide echini in *Sauromatum venosum* and *Pistia stratiotes* are covered by a thin polysaccharide surface layer. However, this layer differs from the surface layer found in *Schismatoglottis* and *Apoballis,* where the staining results and the resistance to acetolysis indicated lipid compounds rather than sporopollenin.

Pollen of *Amorphophallus* reveals a great diversity in ornamentation types as documented by our results as well as previous studies (e.g. Thanikaimoni 1969; Tarasevich 1988; Grayum 1992; Van der Ham et al. 1998, 2005). The natural diversity of pollen ornamentation types, is sometimes artificially supported by descriptive types (terms) used for one and the same species. For example, the various papers on *Amorphophallus* (including *Pseudodracontium*) refer to striate pollen only (e.g. Van der Ham 1998, 2005), whereas plicate pollen was so far only described by Hesse et al. (2000). Our results demonstrated, that the ornamentation type is not always clearly striate or plicate (e.g. the former *Pseudodracontium* species). Such a transition type is thus described as striate to plicate. Moreover, the pollen ornamentation is often a subjective decision of the palynologist or the terminology used for pollen description. Besides the high variation in pollen wall ornamentation our study demonstrated that the impact of the preparation method (e.g. critical point drying) can strongly influence the

results. Dehydration (DMP) and critical point drying can affect the pollen wall in *Amorphophallus* pollen, which may also lead to misinterpretations.

Ontogenetic aspects

Pollen tetrads of *Amorphophallus* and *Calla* were studied for the first time. For *Amorphophallus* and *Calla* the pollen shape, polarity and aperture type could be determined by the orientation of the microspores within the tetrad (Chapter 3B, D). The presence of a callose wall, as observed in tetrads of *Calla palustris* is typical for pollen with sporopollenin ektexines (Heslop-Harrison 1971; Pacini & Juniper 1983; Hesse 2006b). The most striking difference between the two tetrad observations was the absence of a callose wall in tetrads *of Amorphophallus*, which is conform to previous studies on the Aroideae microsporogenesis (Anger & Weber 2006). Moreover, the striate ornamentation of *A. myosuroides* is only visible in late tetrad stages, which is conform to observations in *Arum* (Anger & Weber 2006) and *Sauromatum* (Weber et al. 1998). As known from *Sauromatum* and *Arum*, the outer pollen wall layer in Aroideae is produced exclusively by the amoeboid tapetum shortly after dissociation of the tetrad (Weber et al. 1998; Anger & Weber 2006). In late tetrad stage, the tapetum forms the polysaccharide echini in *Arum* and it is also responsible for the separation of the microspores from the tetrad arrangement (Anger & Weber 2006). As known from *Arum alpinum*, the four microspores are separated by a non-callosic space within the tetrad (Anger & Weber 2006). The questions raised how the microspores of *Amorphophallus* are separated without a callose wall? In tetrads of *Amorphophallus myosuroides,* each microspore is enclosed by a rather thick polysaccharide outer wall layer, which is especially thickened at the apices. As demonstrated by our study, the microspores are often in touch with adjacent microspores, seemingly at the apices only. Further studies are necessary to find out, whether microspores of *Amorphophallus* are also separated by a non-callosic space within the tetrad

In Aroideae, the lack of callose is suggested to be the reason for the uncommon pollen wall, where a sporopollenin ektexine is missing (Anger & Weber 2006). Based upon our studies on *Amorphophallus* it can be concluded,

that this “uncommon” pollen wall displays the same diversity in ornamentation types as documented for the sporopollenin counterpart.

The ultrastructure of odour producing papillate epidermis cells (osmophores)

Observations of pollinators (drosophilid flies) of *Colocasia* together with observations of spathe movements and of papillate epidermal cells on the spathe revealed that the spathe in *Colocasia* acts as an osmophore (Bröderbauer 2012; Bröderbauer et al. 2014). The hypothesis that the epidermal cells in spathes of *Colocasia* species are odour producing could be proved by an intensive investigation of the morphology an anatomy of the spathe by light microscopy, scanning- and transmission electron microscopy (Bröderbauer et al. 2014). My part of the work was to find ultrastructural evidence for the synthesis of odours in epidermal spathe of the investigated *Colocasia* species (Chapter 4D).

The papillae in the *Colocasia* species studied showed the typical osmophoric activity (Hadacek & Weber 2002; Wiemer et al. 2009; Pansarin & Pansarin 2011) and the shape of the papillae proved to be similar to the odour emitting surfaces of various other angiosperms (Vogel 1963; Garcia et al. 2007; Płachno et al. 2010). The intense osmophoric activity was most obvious in the papillate cells of the adaxial (inner) epidermis of the spathe blade in *C. esculenta* and *C. fontanesii*. The epidermal papillae of the adaxial spathe tube showed a dense cytoplasm and high intracellular activity (e.g. numerous mitochondria, amyloplasts and smooth endoplasmic reticulum). In the papillate cells of *C. fontanesii* an unusual type of endoplasmic reticulum was found (described as “unusual gorgon-head-shaped ER”). The main energy supply for odour synthesis in spathes of *Colocasia* appeared to be starch. Unlike other osmophores (Vogel 1963), in *Colocasia* there are no specialised cell layers for the storage of starch, which is found in epidermal as well as in parenchymatic cells. Lipids, known to be an important resource for odour production in other angiosperms (Hadacek & Weber 2002; Wiemer et al. 2009; Pansarin & Pansarin 2011) were also present but less abundant. Moreover, our study demonstrated that the ultrastructure of the epidermal spathe cells in *Colocasia* together with the presence of aerenchym-

like lacunar tissue in the spathe (as found in LM) indicates that in Araceae not only the spadix but also the spathe can be a highly elaborate osmophore.

5.2. METHOD DIVERSITY

Pollen wall investigations and the problem of misinterpretations

In most studies on pollen walls, sections are stained with uranyl acetate and lead citrate only (e.g. Van der Ham et al. 1998, 2005). In the past, the staining behaviour of the endexine often leads to misinterpretations, given that the staining behaviour is very heterogeneous depending on the technique used (Weber & Ulrich 2010). In the present thesis an endexine could be easily detected in all investigated species by the use of potassium permanganate. In particular the spongy inner layer of the endexine can be clearly seen with potassium permanganate.

Our results of *Amorphophallus* pollen demonstrated that only the application of different TEM staining methods lead to a better understanding of the chemical composition of the pollen wall layers (Chapter 4C). The presence of polysaccharides in the outer pollen wall and in the intine was primary indicated by the Thiéry-test (Thiéry 1967). Moreover, the absence of sporopollenin in the outer pollen wall was approved by the susceptibility of pollen to the acetolysis treatment.

For the granules in the outer wall layer, Van der Ham et al. (1998, 2005) suggested an oily nature due to their osmiophilic staining, although sections were only stained with uranyl acetate and lead citrate and not specifically for lipids. The results of my TEM study demonstrated, that the granules stained electron-dense with the Thiéry-test in most cases, similar to the polysaccharide outer wall, indicating a polysaccharide nature. Moreover, the staining behaviour of the granules in the outer pollen wall varied with the method used. For instance, they were not clearly visible after both, the modified Thiéry-test and with the lipid test (Rowley & Dahl 1977).

The staining behaviour of the thin surface layer was quite contradicting in all investigated species. Although different staining methods were used, the

chemical nature remained unclear. With the lipid test and with potassium permanganate, it also often stained electron-dense, similar to the endexine, thus indicating a lipid nature. However, with the Thiéry-test the layer often stained electron-dense, indicating a rather polysaccharide nature. In their study, Weber et al. (1998, 1999) clearly demonstrated that the polysaccharide echini in *Sauromatum venosum* and *Pistia stratiotes* are covered by a thin polysaccharide surface layer. Taking all factors into account, a polysaccharide nature of the surface layer is suggested. Sometimes the layer also appeared disconnected between the ornamentation elements, which is very likely a result of the preparation/fixation process. For the investigated *Amorphophallus* species with psilate pollen the presence of a surface layer remained unclear.

As documented in this study, the polysaccharide ornamentation elements are susceptible to acetolysis. Although, *Amorphophallus* pollen is usually not acetolysis-resistant, the pollen ornamentation sometimes remained partly or completely after the acetolysis. In the present work it could be demonstrated that the reaction to the acetolysis treatment depends particularly on the heating temperature and time.

Moreover, dehydration (DMP) and critical point drying may affect the pollen wall in Aroideae with polysaccharide wall elements. Some of the *Amorphophallus* pollen investigated with SEM clearly demonstrated changes in wall ornamentation, e.g. as seen in *A. longituberosus* and *A. asterostigmatus* with striate to reticulate ornamentation, or in *A. taurostigma* and *A. mossambicensis* with psilate to striate ornamentation (Chapter 4C).

The use of H2O

Observations on pollen hydrated in water can reveal interesting new aspects. For the Araceae, one of the best examples is *Montrichardia,* where a massive expansion of the thick intine results in an explosive opening of the pollen wall (Weber & Halbritter 2007). As previously mentioned, within the Aroideae *Montrichardia* constitutes a unique pollen wall type (type 1b, lacking an endexine). The observations obtained from hydrated pollen leads to a better understanding of the pollen wall stratification (with extremely thick intine for rapid germination) and might also explain the lack of an endexine. A rapid germination

seems necessary, as the pollen wall is missing and thus a protective coating around the pollen protoplast is absent. Aroids with unisexual flowers (e.g. *Amorphophallus*, *Montrichardia*) are known to have a short flowering cycle (two days) and pollen is viable for a few days only (Gibernau et al. 2003; Barabé et al. 2008). This supports the hypothesis by Hesse (2006a, 2006b), that the lack of sporopollenin is correlated with short pollen viability and fast germination. Even pollen grains with sporopollenin exines loose germination capacity the longer the pollen adheres to the pollinator (Pacini 2000). Moreover, Weber and Halbritter (2007) hypothesized that this germination strategy in *Montrichardia* is a substitute for pollenkitt, making pollen grains clump together and sticky to pollinators.

In the present thesis observation on pollen hydrated in water also revealed new interesting aspects, given that some species shed the pollen wall immediately before pollen tube growth. This effect was observed for different Araceae species (Chapter 4C). It is also described for other angiosperm taxa (Hesse et al. 1985; Hesse et al. 2009), as well as some gymnosperm taxa, where it is denoted as exine shedding and as a first event in pollen germination (Bhatnagar & Moitra 1996; El-Ghazaly et al. 1998; Takaso & Owens 2008). As the pollen wall shedding was only observed in striate and echinate *Amorphophallus* pollen with little pollenkitt, this effect might also play a role in pollination as a substitute to sticky pollen coatings. Further studies are necessary whether pollen wall shedding is associated with the germination process.

To conclude, the present thesis demonstrates that the application of different preparation and staining methods as well as a combined analysis with light microscopy, scanning- and transmission electron microscopy are essential for the interpretation of pollen characters as well as for the study of floral organs related to pollination. Furthermore, for the detection of pollen wall layers the use of different staining methods for one and the same species is highly recommended.

5.3. POLLEN AND SYSTEMATICS

The puzzling presence of echinate pollen in *Schismatoglottis.* What do the ignored findings of Thanikaimoni (1969) and the resurrection of the genus *Apoballis* have in common?

To date, the earlier-diverging clades of Aroideae, including Schismatoglottideae, are known to have exclusively psilate or verrucate pollen (Grayum 1992; Wong et al. 2010; Cusimano et al. 2011; Ulrich et al. 2012). Curiously Thanikaimoni (1969) was the first who reported 14 *Schismatoglottis* species with echinate pollen, but only illustrated *Schismatoglottis kurzii* (=*Apoballis mutata*) and *Schismatoglottis forbesii* (=*Apoballis longicaulis*). Unfortunately, Thanikaimoni's report was overlooked and even suspected as a misinterpretation of fungal spores (Grayum 1992). A reason for denying these findings might be that echinate pollen is known to be typical for all more derived clades of Aroideae subfamily (Hesse 2006c). The puzzling presence of echinate pollen in *Schismatoglottis* species (former *Schismatoglottis lancifolia*) and the desire to verify or finally refute the largely ignored findings of Thanikaimoni (1969), was the reason for a close look at potentially echinate pollen. Our study confirmed the presence of echinate pollen in some *Schismatoglottis* species (Chapter A) and was therefore conform to Thanikaimoni (1969). Before that, the monospecific genus *Callopsis* was the only example with echinate pollen within the earlier-diverging clades. In Schismatoglottideae echinate pollen so far is restricted to the genus *Apoballis*, the basalmost genus of the tribe (Wong & Boyce 2010). The results indicate that echinate pollen in *Apoballis* is plesiomorphic for Schismatoglottideae, while pollen in *Schismatoglottis* (and indeed all other studied Schismatoglottideae) is psilate. Such exceptions to the rule, especially if the results appears unlikely, are nowadays easy to verify by combining both, modern techniques for pollen investigation - and of course other relevant morphological characters - and new molecular phylogenies.

The study on *Schismatoglottis* revealed new insights into the pollen morphology and ultrastructure of tribe Schismatoglottideae but also raised new questions. For example, whether there exists a correlation between pollen

ornamentation and pollinator, e.g. whether flies are the pollinators of *Apoballis*. It is not understood if and how the polysaccharide echini in *Apoballis*, and in many other members of Aroideae, are related to the mode of pollination. However, without pollinator observations for *Apoballis* it remains unclear. According to the scarce literature (Toda & Lakim 2011; Low et al. 2015), at least some species of *Schismatoglottis* are pollinated by flies (*Colocasiomyia*). This conflicts with the presence of psilate pollen grains which are interpreted as adaptation to beetle pollination. Moreover, the appearance of echinate pollen grains only in the derived clades of Aroideae (Cusimano et al. 2011) indicates a phylogenetic signal rather than an ecological trigger such as pollinator type. Together with newly found differences in the floral odours between *Apoballis* and *Schismatoglottis* and differences in spathe movements (Boyce & Wong 2007; Bröderbauer 2012; Bröderbauer et al. 2014) the results strongly suggest pollinator differences.

The puzzling case of *Calla*

Almost all systematic authorities concluded that *Calla* is a puzzling case, being a highly autapomorphic taxon with obscure relationships and with a peculiar combination of features (Mayo et al. 1997). One of the questions was whether it is possible to place *Calla* into a distinct position within the Araceae based on the latest findings. Our results demonstrated, that the pollen characters of *Calla*, in particular the disulcate aperture condition and the sporopollenin tectate-columellate exine, are two big arguments against a placement within the Aroideae as suggested in most recent molecular classifications (Cabrera et al. 2008; Cusimano et al. 2011; Chartier 2011; Nauheimer et al. 2012a; Chartier et al. 2013). *Calla* appears to be intermediate between the *Stylochaeton* clade and earlier-diverging taxa (Lasioideae). The pollen characters (especially the disulcate to ring-like aperture), are indicating a position between either (1) Zamioculcadoideae (*Stylochaeton* clade) and Lasioideae or (2) between Zamioculcadoideae (*Stylochaeton* clade) and Aroideae (Aroideae clade). This placement is supported by the palynological results, where *Calla* is best placed in a transition zone between the bisexual-flowered clades (after Lasioideae) and the unisexual-flowered clade (Aroideae), either diverging before or after the *Stylochaeton* clade. This placement is also supported by flower morphology, in

particular the bisexual flowers, the duration of anthesis (up to several weeks) and the flowering sequence (acropetal) (Ulrich et al. 2013). As most early diverging taxa are perigoniate (despite the aperigoniate *Pycnospatha*), the absence of a perigone seems to link *Calla* to the Aroideae, but in many Monsteroideae a perigone is also absent (Mayo et al. 1997; Cabrera et al. 2008). Furthermore, this placement would not require a reversal of the pollen and other morphological and anatomical characters discussed above.

The position of *Calla*, as well as of the three isolated genera *Montrichardia*, *Anubias* and *Callopsis* deserves special attention. For the first time, Cusimano et al. (2011) and Chartier (2011) placed *Calla* in a well-supported clade at the base of the Aroideae, sister to *Montrichardia*, and closely related to *Anubias*. In all phylogenetic studies, the three genera *Anubias*, *Montrichardia* and *Callopsis* are isolated within the "Unisexual Flower clade" (subfamily Aroideae). Although they share the typical Aroideae pollen characters (Weber 2004; Weber & Halbritter 2007; present thesis), they are special in many ways. *Montrichardia* pollen is so far unique within the Aroideae, having a pollen wall of type 1b, lacking an endexine (Weber & Halbritter 2007). Within the earlier-diverging clades of Aroideae, the monospecific genus *Callopsis* is one of the few examples with echinate pollen, of type 1b (Weber et al. 1999). Within these four genera, *Anubias* is the only genus with a polysaccharide pollen wall of type 2a and verrucate ("blister-like") pollen (Hesse 2009). Based on these facts, we do not support the placement of *Calla* as sister to *Montrichardia* and closely related to *Anubias*, as suggested by Chartier (2011). To conclude, further studies using additional molecular markers are necessary to resolve the discrepancy in systematic position of *Calla,* as well as of the three isolated genera *Montrichardia*, *Callospsis* and *Anubias*, between the molecular phylogenies and the morphological classifications.

Colocasia affinis – Facing the difference

The genus *Colocasia* comprises about 20 species distributed throughout Southeast Asia, six of which occur in China (Li et al. 2010). Recent studies suggest that *Colocasia* might be polyphyletic (Nauheimer et al. 2012b). According to molecular studies of Nauheimer et al. (2012b), *Colocasia affinis* is more closely

related to *Steudnera* than to the species of *Colocasia*. This close relationship is supported by different facts: (1) *S. colocasiifolia* and *C. affinis* share the same pollinator, (2) the epicuticular wax layer on the adaxial spathe blade in *C. affinis* resembles that of *Steudnera* spp., (3) *C. affinis* differs from other species of *Colocasia* through the absence of papillate epidermal cells and the temporary closure of the spathe during anthesis (Bröderbauer et al. 2014). Based on these observations a placement of the species *C. affinis* as sister to the genus *Steudnera* is supported.

Regarding the pollen characters, both taxa (*Colocasia gigantea*, *Steudnera griffithii*) are reported to have a polysaccharide pollen wall of type 2a (Weber et al. 1999). This is confirmed by an ultrastructural study on pollen of *Steudnera henryana* (Ulrich, unpublished data). To date, the ultrastructure of only a few species has been investigated and whether the result applies to all species has to be proved. Regarding the pollen ornamentation, the genus *Colocasia* usually is echinate (Ulrich, unpublished data), except for *C. affinis,* which proved to be plicate. The fact that pollen of *C. affinis* and of investigated *Steudnera* spp. is plicate (Hesse et al. 2001; Ulrich, unpublished data) conforms the hypothesis by Nauheimer et al. (2012b). In this case the pollen characters are also useful for delimiting *C. affinis* from the other species of *Colocasia*. Thus, further studies are necessary to clarify the relationship within *Colocasia* and related genera.

Present status of palynology – An endangered science?

Despite a long tradition on palynology and its application on many fields (e.g. melissopalynology, forensic palynology, palaeobotany) it might be considered to why it is important and where it might be headed in the near future. One of the main research interests in palynology focuses on the often taxon-specific patterns of the pollen wall, how they developed and how they have evolved. Moreover, pollen provide phylogenetic evidence important in plant systematics (Hesse & Blackmore 2013). The reconstruction of phylogenies, a subarea of systematics, has continuously developed. The advances in modern phylogenies are resulting in constant changes in plant systematics. In the meanwhile even whole genomes are being used together with multiple analyses

of data for a better insights into relationships (Stuessy & Funk 2013). Moreover, new data are generated by use of information from a variety of other disciplines, including palynology. Critically evaluated pollen characters may be a useful tool for systematics with a significant diagnostic value, supporting or contradicting the results of molecular studies (“The palynological compass” sensu Blackmore 2000; Hesse 2006c; Hesse & Blackmore 2013). Palynological characters are very valuable in particular in delimiting taxa (Simpson 2006; Ulrich et al. 2012). Regarding multiple-gene trees studies with conflicting results, pollen data combined with other morphological data (e.g. floral characters) have more recently become an important indicator of which tree may be the best ones to use (Stuessy & Funk 2013; Ulrich et al. 2012, 2013). This work demonstrates that morphological studies of pollen grains are indispensable for the understanding of evolutionary processes and systematics.

5.4. CONCLUSIONS AND FUTURE PERSPECTIVES IN ARACEAE RESEARCH

Despite a long tradition of research on pollen in Araceae, pollen morphology, ultrastructure and systematics are still at the beginning and thus several questions remain unanswered. Especially information concerning the wall structure of Araceae pollen is still rare. Recent literature based on Araceae pollen provided a lot of new information but at the same time raised many questions that need to be answered by the use of modern techniques (especially by a combined analysis with LM, SEM and TEM). Regarding the chemical nature of the pollen wall only the application of different histochemical staining techniques will provide good results and accordingly satisfying answers (e.g. Weber & Ulrich 2010; Ulrich et al. 2012, 2013, 2015). My study on Araceae pollen provided new insights into the pollen morphology and ultrastructure on many species. The evaluated pollen characters proved to be a useful tool for systematics with a significant diagnostic value, supporting or contradicting the results of molecular studies, as well as in delimiting taxa. The investigated taxa which are not included in the present thesis are listed in the appendix. Future work will include remaining TEM-studies (see appendix: embedded material).

Furthermore, only a combination of all research fields will lead to a full understanding of this highly diverse family. The studies will probably need further centuries of studies, not only for the investigation on pollen, but also for observations on pollinators of the tropical or subtropical species and molecular studies. Moreover, of the expected 6000 species only half of them (about 3500) are published yet (Boyce & Wong 2012; Boyce et al. 2012; Boyce & Croat 2014). To date, in many ways, the Araceae are still a puzzling case and future research will contribute to find all pieces and to unravel all mysteries.

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6. CURRICULUM VITAE

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Name	Silvia ULRICH
Date of birth	25.03.1975
Place of birth	Vienna, Austria
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Adresse	Department of Structural and Functional Botany Rennweg 14 1140 Vienna, Austria
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	Education
2010 – 2015	Doctoral thesis at the University of Vienna, Department of Botany and Biodiversity Research, Department of Structural and Functional Botany, on: “Pollen morphology and ultrastructure of selected Araceae-species.New examples for the significance of pollen characters in systematics and for the spathe as an osmophore.”
2003 – 2006	Master in “Ecology and Evolution”, University of Vienna. Degree for Master of Science (Mag. rer. nat.) in May 2006. Diploma thesis in botany at the Institute of Botany, Department of Ultrastructure Research and Palynology: “Pollenmorphologie und Ultrastruktur ausgewählter Lamianae. Eine Datenerhebung für PalDat mit besonderer Berücksichtigung der Pollenmerkmale im Hinblick auf die aktuelle Systematik.“ (Pollen morphology and ultrastructure of selected Lamianae. A data collection for PalDat and with special regard to pollen characters compared with recent DNA-based phylogenies).
1995 – 2006	Study of “Ecology and conservation biology”, University of Vienna, Austria
1994 – 1996	Study of history of art and study of pharmacy, University of Vienna, Austria
1993 – 1994	Study of Philosophy and study of English and American Studies, University of Vienna, Austria
1985 – 1993	Secondary school (Bundesrealgymnasium and Bundesoberstufen-realgymnasium, Henriettenplatz, Vienna, Austria), finished with finals (Matura) in June, 1993
1981 – 1985	Primary School (Vienna, Austria)

	Professional Background & Academic Positions
2014/15	Revision of the database PalDat (Palynological Database) – Specification and webdesign of PalDat 3.0, Service contracts
Since 2010	Lecturer at the University of Vienna, Department of Botany and Biodiversity Research, Department of Structural and Functional Botany for the following courses: “Computer-assisted presentation techniques”, “Special methods of structural botany 2”, “Morphology and function of pollen and spores” and “Ultrastructure of cells”.
2009 – Mai 2012	Scientific project collaborator at the Centre of Biodiversity and Botany, Department of Structural and Functional Botany in the research project P 20666: “Pollination in Araceae: are pollen characters related to evolution, diversification and functional optimisation of kettle traps.” Granted by the Austrian Science Fund (FWF).
2006 – 2008	Data input in PalDat – the palynological Database (Service contracts)
2006 – 2008	Technical Assistant (half day) at at the University of Vienna, Institute of Botany, Department of Ultrastructure Research and Palynology.
2004 – 2007	Tutor at the University of Vienna, Institute of Botany, Austria, Department of Ultrastructure Research and Palynology.

	Main fields of research
Since 2009	Pollen of Araceae
Since 2008	Forensic Palynology – Forensic palynological work on several cases (murder, missing persons)
Since 2003	Ultrastructure research and palynology

	Cooperations
2009 – 2012	P. Boyce, Kuching, Sarawak, Malaysia

	Invited talks and Workshops
Juli 2014	Lecturer in a workshop on Forensic Palynology
Nov 2010, April 2011	Guest lectures on Forensic Palynology in the course of “KDFR-Seminar Tatort” for crime scene officers in Großarl (Austria)

7. PUBLICATIONS

a. Publications in Peer-Reviewed Journals

Ulrich S, Hesse M, Weber M, Halbritter H. 2015. *Amorphophallus* – New insights into pollen morphology and the chemical nature of the pollen wall. Grana, submitted.

Bröderbauer D, Ulrich S, Weber A. 2014. Adaptations for insect-trapping in brood-site pollinated *Colocasia* (Araceae). Plant Biology 16: 659–668.

Ulrich S, Hesse H, Bröderbauer D, Bogner J, Weber M, Halbritter H 2013. *Calla palustris* (Araceae): New palynological insights with special regard to its controversial systematic position and to closely related genera. Taxon *6:* 701–712. doi:10.12705/624.34.

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Weber M, Ulrich S. 2010. The endexine: a frequently overlooked pollen wall layer and a simple method for detection. Grana 49: 83–90.

b. Congress Contributions (Lectures and Posters)

Ulrich S, Weber M. 2012. Forensic Palynology: Sample collection and preparation for forensic investigantions. - EAFS 2012 – Towards Forensic Science 2.0., Den Haag, Niederlande; Poster

Ulrich S. 2010. Lamiales: pollen characters compared with recent DNA-based phylogenies. *In*: Jasprica N, Pandza M, Milovic M (Eds.) 3. Croatian Botanical Congress. Murter, Croatia. 24.-26.September 2010. Book of Abstracts.201.

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Hesse M, Ulrich S. 2010. The stunning diversity of aroid pollen. *In*: Albach D, Greimler J (Eds) 19th International Symposium “Biodiversity and Evolutionary Biology” of the German Botanical Society (DBG). Vienna, Austria. 16.-19. September 2010. Program & Abstracts.

Ulrich S, Bröderbauer D, Yeng WS, Boyce PC, Hesse M. 2010. *Apoballis*: A new example for the significance of pollen morphology in Araceae systematics. *In*: Albach D, Greimler J (Eds.) 19th International Symposium “Biodiversity and Evolutionary Biology” of the German Botanical Society (DBG). Vienna, Austria. 16.-19. September 2010. Program & Abstracts.

Ulrich S., Bröderbauer D, Yeng WS, Boyce PC., Hesse M. 2010. *Apoballis*: A new example for the significance of pollen morphology in Araceae systematics. Early

events in monocot evolution. *In:* Meeting of the Linnean Society of London and Royal Botanic Gardens. Kew. 20.-22. July 2010. Abstracts. p 20.

Bröderbauer D, Ulrich S, Hesse M, Weber A. 2009. Evolution of trapping inflorescences in the aroid family: gliding devices and pollen characters. Xth International Aroid Conference, Nancy, France. 8.-10.7.2009. Abstracts.

Ulrich S. 2006. Die Gliederung der Lamianae - palynologisch betrachtet. *In:* 12. Österreichisches Botanikertreffen. Kremsmünster, 21.-24. September 2006. Beitr. Naturk. Oberösterreichs 16: V20.

c. Publications in Edited Books

Weber M, Ulrich S. 2013. Forensic Palynology: How pollen in hey can link to a crime scene. Soil Forensics Proceedings, Springer. Accepted.

d. Other Publications

Hesse M, Ulrich S. 2012. Pollen – erstaunliche Schönheit – verblüffende Vielfalt. Biologie in unserer Zeit 2012/1: 34–41.

Hesse M, Halbritter H, Zetter R, Weber M, Buchner R, Frosch-Radivo A, Ulrich S. 2009. Pollen Terminology. An illustrated handbook. Springer, Wien. 264 pp.

Ulrich S. 2006. Pollenmorphologie und Ultrastruktur ausgewählter Lamianae. Eine Datenerhebung für PalDat mit besonderer Berücksichtigung der Pollenmerkmale im Hinblick auf die aktuelle Systematik. Diplomarbeit, Universität Wien.

Vienna, November 2015

SUPPLEMENTS A
POSTERS AND ABSTRACTS

Results of this thesis presented on scientific conferences and seminars

Xth Aroid Conference

Nancy, France

8 – 10 July, 2009

Abstracts, Early Events in Monocot Evolution. *In:* A joint meeting of the Linnean Society of London and Royal Botanic Gardens, Kew: *20.-22. July 2010.* p. 20-21*:*

EVOLUTION OF TRAPPING INFLORESCENCES IN THE *ARACEAE* FAMILY

D. Bröderbauer, Ulrich, M. Weber & M. Hesse

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Since the pioneering work of Fritz Knoll on the trap-mechanism of *Arum nigrum* the existence of "kettle traps" in *Araceae* has been accounted for several genera of *Araceae*. However, many aspects of the structure, function and evolution of insect-trapping inflorescences in *Araceae* are still poorly known or even simply unknown.

For example, how widespread is the occurrence of trapping devices and do different structures and mechanisms occur? How – and how many times- did trapping inflorescences (in a broader sense) develop during the evolution of *Araceae*? Are such trapping inflorescences restricted to the by far largest subfamily, the *Aroideae*? Are distinct features of trapping inflorescences typical for distinct tribes or even distinct aroid genera? Do trapping devices exist in taxa so far not suspected? Is there any correlation between pollen features and distinct trapping devices? And, what was the role of the different plant-pollinator interactions in the evolution of "kettle traps"?

Our poster presents the status of current knowledge (published and unpublished data) on trapping devices together with our own pollen data, using the most recent molecular tree of Cabrera et al. (2008) as a frame for our presentation.

Besides showing the distribution of trapping inflorescences along the phylogenetic tree, we provide information on the occurrence of morphological features such as downward-pointing epidermal papillae, oil or wax on the inner spathe surface, "light-windows" within the spathes, and details of the pollen ultrastructure and ornamentation, together with the floral ecology of all *Araceae* genera for which data are available.

To answer the questions mentioned above we are performing SEM and TEM studies of spathes and pollen of different genera and species and will study details of the pollination biology of distinct *Araceae* in the field during a three-year project (sponsored by the Austrian Science Fund FWF) "Evolution of kettle traps in *Araceae*".

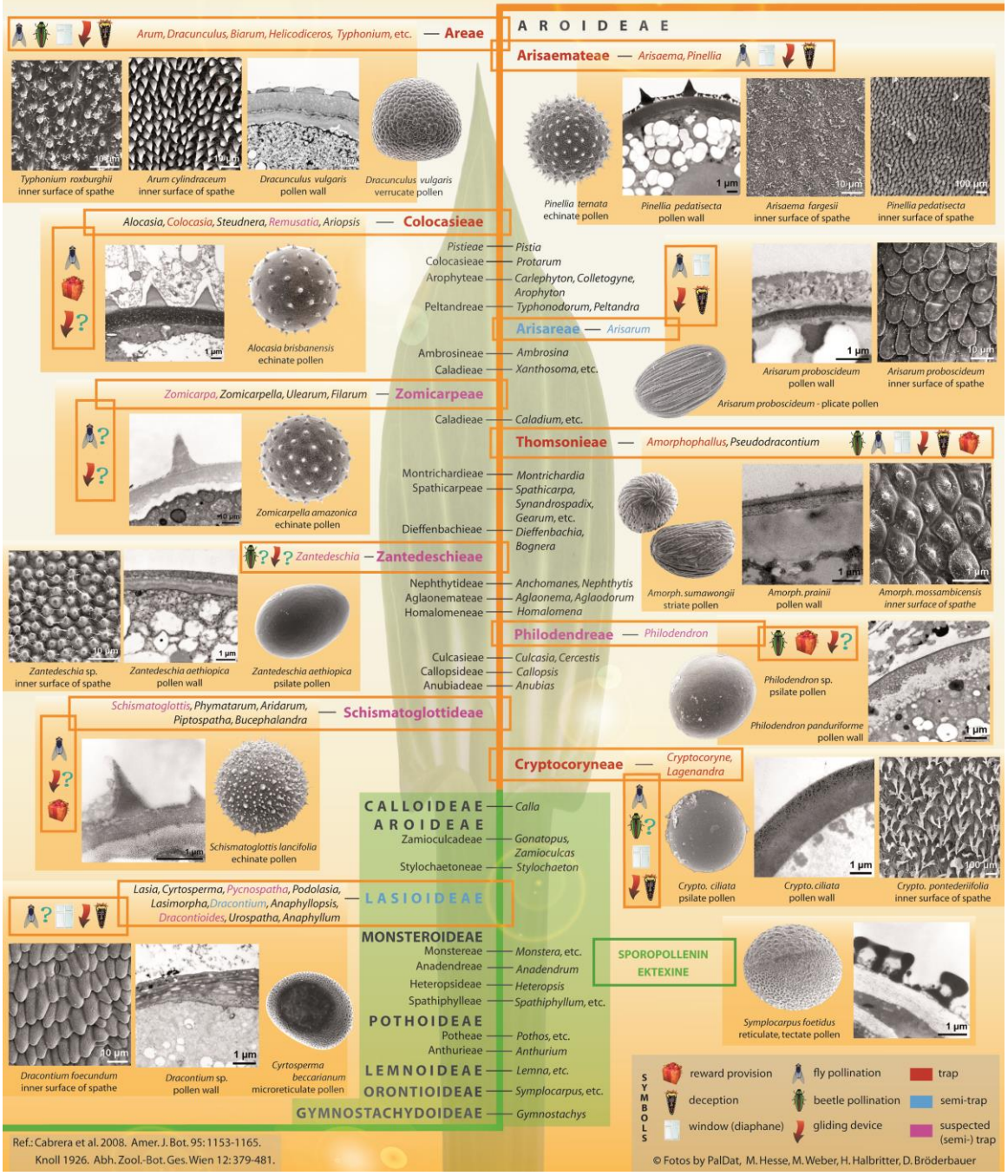
Bröderbauer D, Ulrich S, Hesse M, Weber A. 2009. Evolution of trapping inflorescences in the aroid family: gliding devices and pollen characters*.* Xth International Aroid Conference, Nancy, France. 8.-10.7.2009. Abstracts.

Vienna Araceae Project (D. Bröderbauer, S. Ulrich, M. Hesse, A. Weber - Faculty Centre of Biodiversity, University of Vienna)

Evolution of trapping inflorescences in the aroid family: gliding devices and pollen characters

The presence of kettle traps in the Araceae is well known. A perfect example is Arum nigrum, which has been paradigmatically studied by Fritz Knoll (1926). However, many aspects of the structure and function of the traps are still poorly known, and, of course, many aroids have no traps or only imperfect traps ('semi-traps'). Araceae thus provide a unique opportunity to study the evolution and functional elaboration of pollination traps in detail. In that context, pollen characters also play an important role. The work will be performed in a three-year research project sponsored by the Austrian Science Fund (FWF).

Our poster presents the status of current knowledge (published and unpublished data) on trapping devices (gliding surfaces) and pollen characteristics, using the most recent molecular tree of Cabrera et al. (2008, Fig. 1) as a systematic frame. Besides showing the distribution of trapping inflorescences along the phylogenetic tree, we provide information on the occurrence of "light-windows" within the spathes, and details of the pollen ultrastructure and ornamentation, together with the floral ecology of genera for which data are available.



Ref.: Cabrera et al. 2008. Amer. J. Bot. 95: 1153-1165.
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3rd Croatian Botanical Congress

The island of Murter, Croatia

24th – 26th September 2010

Book of Abstracts, 3. Croatian Botanical Congress. 2010. p. 202:

Treći hrvatski botanički kongres
Third Croatian Botanical Congress

THE RESURRECTION OF *APOBALLIS* (ARACEAE)

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Ulrich S, Bröderbauer D, Yeng WS, Boyce PC, Hesse M. 2010. The resurrection of *Apoballis* (Araceae). In: 3. Croatian Botanical Congress. Murter, Croatia. 24.-26.September 2010. Jasprica N., Pandza M., Milovic M. (Eds.) Book of Abstracts. 202.

The resurrection of *Apoballis* (Araceae)

Silvia Ulrich*, David Bröderbauer*, Wong Sin Yeng**, Peter C. Boyce***, Michael Hesse*

*Department of Structural and Functional Botany, University of Vienna, 1030 Wien, Rennweg 14, Austria, **Department of Plant Science and Environmental Ecology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Samarahan, Sarawak, Malaysia, ***Pusat Pengajian Sains Kajihayat [School of Biological Sciences], Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia.

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The character "spiny pollen" seems to be a synapomorphy for the newly resurrected genus *Apoballis*. Remaining *Schismatoglottis* species have exclusively smooth ("psilate") pollen.

Echinate (= spiny) pollen of the genus *Apoballis*

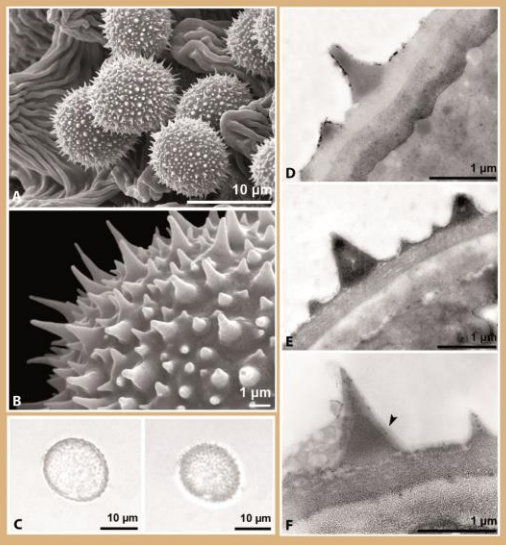

Figure 1. A-B. *Apoballis acuminatissima,* (SEM, critical point dried); C. *Apoballis mutata*, optical section and upper focus (LM); D-F. *Apoballis acuminatissima*, cross sections of pollen walls with different staining methods; D. pollen wall after modified Thiery Test; E. pollen wall after potassium permanganate; F. pollen wall after the Lipid-Test;

Smooth pollen of the genus *Schismatoglottis*

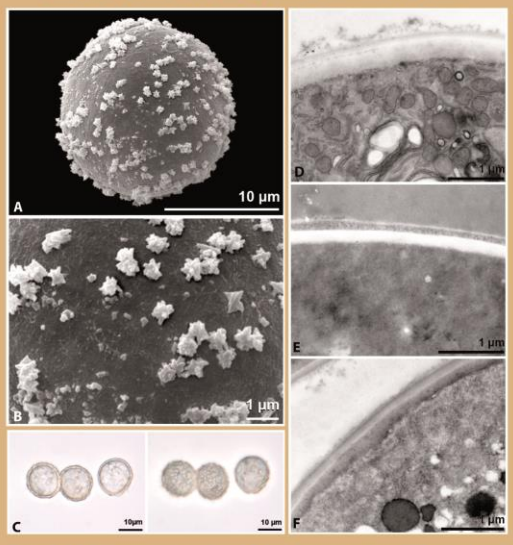

Figure 2. A-F. *Schismatoglottis celebica;* A-B. hydrated pollen grain (SEM, critical point dried); C. hydrated pollen grains, optical section and upper focus (LM); D-F. cross sections of pollen walls with different staining methods; D. pollen wall after modified Thiery Test; E. pollen wall after potassium permanganate; F. pollen wall after the Lipid-Test.

Apoballis acuminatissima - spathe movements in the course of anthesis

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Figure 3.
Apoballis acuminatissima

A. pre-anthesis (8 days before anthesis),
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C. female phase (day 1, 12:10 p.m.),
D. pre-male phase (day 2, 10:38 a.m.),
E. pollen shedding (day 2, 16:58 p.m.),
F. post-male phase (day 4, 11:23 a.m.),
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(copyright David Bröderbauer)

Early Events in Monocot Evolution

A joint meeting of the Royal Botanic Gardens, Kew and the Linnean Society of London

20th – 22nd July 2010

Abstracts, Early Events in Monocot Evolution. *In:* A joint meeting of the Linnean Society of London and Royal Botanic Gardens, Kew: *20.-22. July 2010. Abstracts.* p. 20-21*:*

Apoballis: A new example for the significance of pollen morphology in Araceae systematics

Silvia Ulrich[1], David Broederbauer[1], Wong Sin Yeng[2], Peter C. Boyce[3], Michael Hesse[1]
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Ulrich S, Bröderbauer D, Yeng WS, Boyce PC, Hesse M. 2010. *Apoballis*: A new example for the significance of pollen morphology in Araceae systematics. Early events in monocot evolution. In: Meeting of the Linnean Society of London and Royal Botanic Gardens. Kew. 20.-22. July 2010. Abstracts. p 20.

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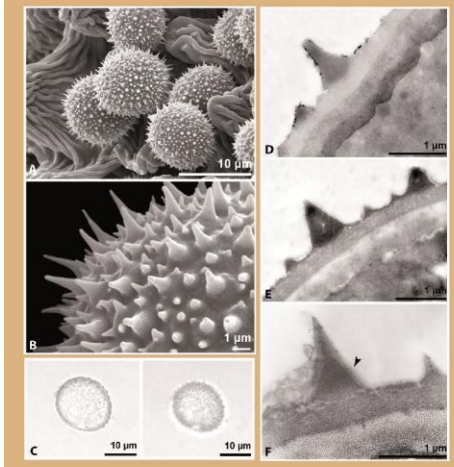

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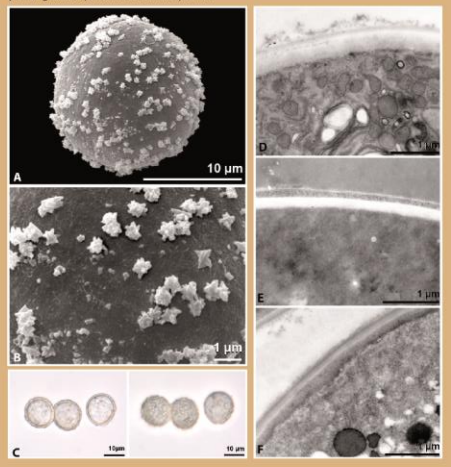
Pollen of the genus *Apoballis* (Figure 1) and *Schismatoglottis* (Figure 2) is small and inaperturate, but there are differences in the pollen wall sculpture. Pollen of all investigated species of *Apoballis* is echinate, whereas the pollen of all investigated species of *Schismatoglottis* is completely smooth. In the light microscope the pollen surface of the genus *Schismatoglottis* seems to be echinate (Figure 2C). But the scanning electron microscope reveals that crystals, not echini, cover the pollen surface (Figure 2A-B). The pollen wall of both genus consists of an intine, a compact to spongy endexine and a thin layer covering the whole pollen surface (Figure 1D-F, Figure 2D-F).

The pollen wall of both genera consists of an intine, a compact to spongy endexine and a thin layer covering the whole pollen surface (Figure 1D-F, Figure 2D-F). This thin outer pollen wall layer also covers the spines of the *Apoballis* pollen grains (Figure 1F) protecting them from chemical attack. The spines of the genus *Apoballis* do not consist of sporopollenin, which is a common feature of spiny pollen in Aroideae (Figure 1D-F). Protected by the outer pollen wall layer the spines are resistant to acetolysis.

Smooth pollen of the genus *Schismatoglottis*

Figure 2. A-F. *Schismatoglottis celebica*; A-B. hydrated pollen grain (SEM, critical point dried); C. hydrated pollen grains, optical section and upper focus (LM); D-F. cross sections of pollen walls with different staining methods; D. pollen wall after modified Thiery Test; E. pollen wall after potassium permanganate; F. pollen wall after the Lipid-Test.



Material & Methods:

For SEM, pollen was rehydrated in water, fixed with 2,2-dimethoxypropane and acetone and critical point dried in carbon dioxide. Dehydrated and air dried samples were sputter coated with gold. For TEM, anthers of the investigated species were fixed in 3% glutaraldehyde (GA) postfixed with 1% osmiumtetroxide and 0.8% potassium hexacyanoferrate. The fixed material was dehydrated in 2,2-dimethoxypropane. The material was embedded in LV-Resin.

Modified Thiéry-test: sections where stained with 1% periodic acid (PA) for ten minutes, 0.2% thiocarbohydrazid (TCH) for 15 minutes and with 1% silver proteinate (SP) for ten minutes.

Lipid-test: sections were stained with 0.2% thiocarbohydrazid (TCH) for 5–16 hours, followed by 1% silver proteinate (SP) for 30 minutes.

Potassium permanganate: sections were treated with 1% aqueous potassium permanganate solution ($KMnO_4$) for seven minutes.

Literature:

Armstrong, J.A. (1979). Biotic pollination mechanisms in the Australian flora – a review. New Zealand Journal of Botany 17: 467–508.

Boyce, P.C. & Wong, S.Y. (2007). Studies on Schismatoglottideae (Araceae) of Borneo IV: Preliminary observations of spathe senescence mechanics in *Schismatoglottis* Zoll. & Moritzi, in Sarawak, Malaysian Borneo. Aroideana 30: 56–70.

Cleghorn, M.L. (1913). Notes on the pollination of *Colocasia antiquorum*. Journal for the Proceedings of the Asiatic Society of Bengal 9: 313-315.

Thanikaimoni, G. (1969). Esquisse palynologique des Aracees. Inst. Fr. Pondichery, Trav. Sect. Sci. Tech. 5(5): 1–31.

Vogel, S. (1965). Kesselfallen-Blumen. Umschau 1: 12–17.

Wong, S.Y. & Boyce, P.C. (2010). Studies on Schismatoglottideae (Araceae) of Borneo IX: A new genus, *Hestia*, and resurrection of *Apoballis*. Botanical Studies 51. in press.

Apoballis acuminatissima - sphathe movements in the course of anthesis

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A: pre-anthesis (8 days before anthesis), B: pre-anthesis (1 day before anthesis), C: female phase (day 1, 12:10 p.m.), D: pre-male phase (day 2, 10:38 a.m.), E: pollen shedding (day 2, 16:58 p.m.), F: post-male phase (day 4, 11:23 a.m.), G: withered inflorescence (day 9)

SUPPLEMENTS B
LECTURES AND ABSTRACTS

Results of this thesis presented on scientific conferences and seminars

19th International Symposium "Biodiversity and Evolutionary Biology"of the German Botanical Society (DBG)

Vienna, Austria, 16. - 19. September 2010

Lecture on "The stunning diversity of aroid pollen" was held on 17th of July, .2010. Program & Abstracts:

The stunning diversity of aroid pollen

Michael Hesse & Silvia Ulrich

Department of Structural and Functional Botany, Faculty Centre of Biodiversity, University of Vienna, 1030 Wien, Rennweg 14, Austria
e-mail: michael.hesse@univie.ac.at

Pollen grains of Araceae exhibit a broad spectrum of different features in cellular organisation, in ornamentation as well as in pollen wall structure. Pollen features are a good tool for systematic classifications (Erdtman's compass needle metaphor) at the generic or even the subfamily level. The occurrence of two- or three-celled pollen coincides well with all classifications of Araceae. Pollen of all small subfamilies is characterized by apertures, a preferably reticulate ornamentation, and an elaborate tectate-columellate exine. Quite different features are typical for the Aroideae: the pollen is inaperturate, never tectate-columellate, smooth in basal tribes, spiny in derived tribes. The pollen wall ultrastructure is much more variable. The usual subdividing layers (ektexine, endexine) may be present or absent, conspicuously thick or extremely thin, with or without sporopollenin. These pollen wall modifications are not distributed by accident within Aroideae, but they are typical either for genera or for tribes, or even of use for the subfamily delimitation. For example, *Stylochaeton* is most basal in Aroideae from molecular and pollen characters. *Apoballis*, a genus recently splitted from *Schismatoglottis*, is the only spiny genus within the early-divergent clades, all with smooth pollen. The unique pollen of *Calla* supports the placement of *Calla* as an own subfamily in morpho-anatomical classifications, and simultaneously questions the embedding within the Aroideae in molecular trees.

Hesse M, Ulrich S. 2010. The stunning diversity of aroid pollen. In: Albach D, Greimler J (Eds) 19th International Symposium "Biodiversity and Evolutionary Biology" of the German Botanical Society (DBG). Vienna, Austria. 16.-19. September 2010. Program & Abstracts.

Seminar in Structural Botany and Palynology

Vienna, Austria

25th of November, 2009

Lecture on „Aronstab-Kesselfallen – Welche Rolle haben Pollenmerkmale?“ was held on 25th of November, 2009 by Hesse M. & Ulrich S.

We introduced our project (P 20666) “Pollination in Araceae: are pollen characters related to evolution, diversification and functional optimisation of kettle traps.”, granted by the Austrian Science Fund, to the scientific community at the Department of Structural and Functional Botany at the Centre of Botany. We presented the three main project goals of the project 1) Are distinct features of trapping inflorescences typical for distinct tribes or even distinct aroid genera? 2) Do trapping devices exist in taxa so far not suspected? 3) Is there any correlation between pollen features and distinct trapping devices?
Our talk presented the status of current knowledge (published and unpublished data) on trapping devices together with our own pollen data. We provided information on pollen morphology and ultrastructure and about a possible correlation between pollen characters and distinct trapping devices.

Hesse M, Ulrich S. 2009. Aronstab-Kesselfallen: Welche Rolle haben Pollenmerkmale? Seminar in Strukturelle Botanik und Palynologie (WS 2009/10). Fakultätszentrum für Biodiversität, Wien. 25.11.2009

Interdisziplinäres Seminar „Neues aus Licht - und Elektronenmikroskopie“ (New Concepts in Light – and Electron – Microscopy)
Vienna, Austria, 09th of December, 2013

Lecture on „Pollen morphology and systematic position of Calla (Araceae)“ was held on 9th of December, 2013 by Hesse M. & Ulrich S.

We presented the results of our publication in Taxon:

TAXON 62 (4) • August 2013: 701–712 Ulrich & al. • **Pollen morphology and systematic position of *Calla***

Calla palustris (Araceae): New palynological insights with special regard to its controversial systematic position and to closely related genera

Silvia Ulrich,[1] Michael Hesse,[1] David Bröderbauer,[1] Josef Bogner,[2] Martina Weber[1] & Heidemarie Halbritter[1]

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2 *Botanischer Garten, Menzinger Strasse 63 & Botanische Staatssammlung, Menzinger Strasse 67, 80638 München, Germany*
Author for correspondence: *Michael Hesse, michael.hesse@univie.ac.at*

Abstract Almost all systematic treatments agree that *Calla* is a puzzling case, being a highly autapomorphic taxon with obscure relationships. In molecular-based classifications the variable placements of *Calla* within Aroideae conflict strongly with those in morphologically and anatomically based systematic classifications, which treat the genus as a subfamily (Calloideae) of its own. We studied the pollen morphology and ultrastructure of *Calla* by light and electron microscopy, and mapped the relevant pollen characters as well as some flower characters to the proposed placements of *Calla* within the Araceae as indicated in the various molecular phylogenies. *Calla* pollen is extraordinary within the entire Araceae. Pollen grains are small, and basically disulcate or with a ring-like aperture. The ornamentation is psilate to perforate, and the pollen wall consists of a sporopolleninous tectate-columellate exine. These pollen characters are shared with those of several earlier-diverging aroid taxa, especially with those of subfamily Zamioculcadoideae, whereas pollen characters in members of subfamily Aroideae deviate significantly. These findings are in accordance with other floral characters. Therefore, we propose that *Calla* is best placed in a transition zone between either subfamily Zamioculcadoideae (*Stylochaeton* clade) and subfamily Aroideae (Aroideae clade) or between subfamily Zamioculcadoideae (*Stylochaeton* clade) and subfamily Lasioideae.

Keywords Araceae; flowering behaviour; pollen; sulcus; systematics; ultrastructure

Received: 15 Jan. 2013; revision received: 10 Apr. 2013; accepted: 10 July 2013. DOI: http://dx.doi.org/10.12705/624.34

Hesse M, Ulrich S. 2009. Pollen morphology and systematic position of Calla (Araceae). Seminar in „Neues aus der Ultrastrukturforschung - Neues aus Licht- und Elektronenmikroskopie“ (WS 2013/14). University Center, Vienna. 25.11.2009

APPENDIX

Araceae species investigated

Species investigated	Size	Shape hydrated	Aperture	Cell number	Raphids	Calcium oxalate Crystals	Pollen coatings (LM)	Ornamentation LM	Ornamentation SEM	Pollen reserves	Wall-Type (sensu Weber et al. 1999)	Peculiarities
Orontioideae												
Lysichiton americanus Hultén & H.St.John	medium	oblate*	sulcate	2	not detected	absent	pollenkitt (-)	reticulate	reticulate		1a (Weber et al. 1999)	pollen wall shedding
Pothoideae, Anthurieae												
Anthurium protensum Schott	small	spheroidal	inaperturate ?	2	raphids	small, cubic	absent	scabrate	perforate-reticulate	n.i.	1a (Weber et al. 1999, e.g. *A. lindmanianum*)	small cubic crystals attached on pollen surface
Pothoideae, Potheae												
Pothoidium lobbianum Schott	small	spheroidal	sulcate	2	not detected	absent	absent	reticulate	reticulate	no starch	n.i.	
Pothos junghuhnii de Vriese	small	oblate* to spheroidal	sulcate	2	raphids	absent	pollenkitt (-)	scabrate to reticulate	reticulate	no starch	1a (Weber et al. 1999, e.g. *P. scandens*), embedded*	
Monsteroideae, Monstereae												
Rhaphidophora angustata Schott	small	oblate*	sulcate	2	not detected	absent	pollenkitt (-)	scabrate	n.i.	starch	? embedded*	
Rhaphidophora decursiva (Roxb.) Schott	medium	oblate	sulcate	n.i.	raphids	absent	n.i.	n.i.	microreticulate-perforate (Halbritter, unpublished data)	n.i.	? embedded*	
Alloschemone occidentalis (Poepp.) Engl. & K.Krause	medium	spheroidal	sulcate?	2	not detected	absent	pollenkitt (+)	psilate	psilate	starch		pollen wall shedding
Stenospermation popayanense Schott	medium	spheroidal	sulcate	3	raphids	large	pollenkitt (+++)	psilate	psilate	starch	1a (Weber et al. 1999, e.g. *S. robustum*), embedded*	
Monsteroideae, Spathiphylleae												
Spathiphyllum humboldtii Schott	small	spheroidal to oblate*	inaperturate	n.i.	n.i.	n.i.	n.i.	plicate	n.i.	n.i.	1a (Weber et al. 1999, e.g. *S. blandum*), embedded*	
Spathiphyllum minor G.S.Bunting	small to medium	spheroidal to oblate*	inaperturate	2	raphids	absent	pollenkitt (-)	plicate	plicate	starch	1a (Weber et al. 1999, e.g. *S. blandum*)	
Spathiphyllum sp. nov. [Bogner J., 3002 (M)]	small	spheroidal to oblate*	inaperturate	2	raphids	absent	pollenkitt (-)	plicate	plicate	no starch	1a (Weber et al. 1999, e.g. *S. blandum*)	psilate apices
Spathiphyllum tenerum Engl.	small to medium	spheroidal to oblate*	inaperturate	2	raphids	absent	pollenkitt (+)	plicate	plicate	starch	1a (Weber et al. 1999, e.g. *S. blandum*)	
Lasioideae												
Pycnospatha palmata Gagnep.	small	oblate*	sulcate	2	raphids	absent	pollenkitt (-)	reticulate	micro-reticulate	no starch	? embedded*	
Dracontium asperum K.Koch	small to medium	oblate*	sulcate	2	not detected	absent	pollenkitt (-)	reticulate	micro-reticulate	starch	? embedded*	
Dracontium prancei G.H.Zhu & Croat	medium	oblate*	sulcate	2	raphids	absent	pollenkitt (-)	reticulate	reticulate	starch	? embedded*	
Aroidea, Zamioculcadeae												
Gonatopus boivinii Engl.	medium	"hamburger-shape"	ring-like aperture	2 (& 3)	not detected	absent	pollenkitt (-)	psilate	psilate-perforate	starch	1a (Weber et al. 1999)	
Calloideae												
Calla palustris L.	small	oblate	disulcate to ring-like aperture	2	raphids	absent	absent	scabrate	psilate-perforate, aperture membrane ornamented (verrucate-rugulate)	lipids*	1a (Weber et al. 1999)	
Aroidea, Stylochaetoneae												
Stylochaeton zenkeri Engl.	medium	spheroidal to oblate*	inaperturate	2 (& 3)	not detected	absent	pollenkitt (-)	psilate	psilate	n.i.	1a (Weber et al. 1999)	thick pollen wall
Aroideae, Callopsideae												
Callopsis volkensii	medium	spheroidal	inaperturate	2 & 3 ?	raphids	absent	pollenkitt	echinate	echinate		1b (Weber et al 1999)	
Aroideae, Anubiadeae												
Anubias gigantea A. Chev.	small	spheroidal to oblate*	inaperturate	2	raphids	absent	pollenkitt	scabrate to verrucate	verrucate	starch	2a (Weber et al 1999), embedded*	
Anubias giletii De Wild. & T.Durand	small	spheroidal to oblate*	inaperturate	2 & 3	raphids	absent	pollenkitt	scabrate to verrucate	verrucate	unclear	2a (Weber et al 1999, *A. barteri*), embedded*	
Aroideae, Nephthytideae												
Pseudohydrosme gabunensis Engl.	medium	spheroidal	inaperturate	3	absent?	absent	pollenkitt	psilate	psilate	starch	? embedded*	thick pollen wall
Anchomanes welwitschii Rendle	medium to large	spheroidal	inaperturate	3 & 2	n.o.	absent	pollenkitt	psilate	psilate	starch	? embedded*	pollen strands
Nephthytis afzelii	medium	spheroidal to oblate*	inaperturate	3	raphids	absent	pollenkitt	psilate	keine guten SEMs	starch	? embedded*	
Aroideae, Culcasieae												
Culcasia scandens P.Beauv. (Synonym *C. saxatilis*)	medium	oblate*	inaperturate	2 (&3)	raphids	absent	pollenkitt	verrucate?	verrucate?	starch	2a ? embedded*	
Cercestis afzelii Schott	medium	spheroidal	inaperturate	2?	raphids	absent	pollenkitt (+)	psilate	psialte (Halbritter, unpublished data)	starch	n.i.	
Aroideae, Homalomeneae												
Homalomena insignis	small	oblate*	inaperturate	3	not detected	absent	pollenkitt	psilate	psialte	absent	n.i.	
Homalomena rostrata Griff. (=*Homalomena paludosa* Hook f.)	medium	oblate*	inaperturate	3	not detected	absent	pollenkitt (++)	psilate	psialte	starch	embedded*	
Homalomena picturata (Linden & André) Regel	small to medium	oblate*	inaperturate	3	raphids	absent	pollenkitt	psilate	n.i.	?	n.i.	
Homalomena punctulata Engl.	small	ellipsoid	inaperturate	3 (&2)	raphids	absent	pollenkitt	psilate	n.i.	absent	n.i.	
Homalomena wallisii	medium	ellipsoid	inaperturate	3 (&2)	not detected	absent	pollenkitt (+++)	psilate	psilate (Halbritter, unpublished data)	starch	1b?	
Homalomena erythropus (Mart. ex Schott) Engl. (*Synonym of Adelonema erythropus* (Mart. ex Schott) Schott)	medium	oblate*	inaperturate	3	not detected	absent	pollenkitt (++)	psilate	psilate	starch	n.i.	
Homalomena sepearlae (Bogner & Moffler)	medium	oblate*	inaperturate	?	?	?	?	?	psilate	?	1b?	

Species investigated	Size	Shape hydrated	Aperture	Cell number	Raphids	Calcium oxalate Crystals	Pollen coatings (LM)	Ornamentation LM	Ornamentation SEM	Pollen reserves	Wall-Type (sensu Weber et al. 1999)	Peculiarities
Aroideae, Philodendreae												
Philodendron squamiferum Poepp.	medium	oblate*	inaperturate	3	raphids	absent	pollenkitt	psilate	psilate	starch	? embedded*	
Aroideae, Dieffenbachieae												
Dieffenbachia sp.	medium to large	oblate*	inaperturate	3	raphids	present	pollenkitt	psilate	psilate	starch	1a (Weber et al. 1999), embedded*	pollen wall shedding
Aroideae, Spathicarpeae												
Spathantheum fallax Hett., Ibisch & E.G.Gonc.	medium	oblate*	inaperturate	3 ?	raphids	absent	pollenkitt (+)	psilate	psilate	starch	? embedded*	thick pollen wall, pollen wall shedding
Asterostigma lividum (Lodd.) Engl.	medium	oblate*	inaperturate	2	raphids	absent	pollenkitt	echinate	echinate (Halbritter, unpublished data)	starch	? embedded*	
Taccarum caudatum	large	spheroidal to oblate*	inaperturate	2 & 3	n.o.	absent	pollenkitt (++)	psilate	psilate	starch	2a ?	pollen wall shedding
Taccarum weddellianum	large	spheroidal to oblate*	inaperturate	2 & 3	n.o.	absent	pollenkitt (++)	psilate	psilate (to granulate) (Halbritter, unpublished data)	starch	2a (Weber et al 1999)	pollen wall shedding
Spathicarpa sagittifolia	medium	oblate*	inaperturate	3	n.o.	absent	pollenkitt	psilate	psilate	starch	1b (Weber et al 1999)	
Synandrospadix vermitoxicus	medium	spheroidal	inaperturate	2 & 3	raphids	absent	pollenkitt	echinate	echinate	starch	2a (Weber et al 1999)	
Gearum brasiliense	medium	spheroidal	inaperturate	2 & 3	raphids	present	pollenkitt	psilate	psilate (Halbritter, unpublished data)	starch	n.i.	thick pollen wall, pollen wall shedding
Incarum pavonii (Schott) E.G.Gonç. *(= Asterostigma pavonii* Schott)	medium	oblate*	inaperturate	2	raphids	absent	pollenkitt	psilate ?	n.o.	starch	2a (Ulrich, unpubl. data)	
Aroideae, Philonotieae												
Philonotion americanum (A.M.E.Jonker & Jonker) S.Y.Wong & P.C.Boyce (Basionym: Schismatoglottis americana)	small	oblate*	inaperturate	3?	raphids	large	pollenkitt	psilate	psilate	no starch		
Aroideae, Cryptocoryneae												
Cryptocoryne annamica Serebryanyi	small to medium	spheroidal	inaperturate	3	absent	absent	pollenkitt (-)	psilate	psilate (Halbritter H.)	absent?	n.i.	pollen wall shedding
Lagenandra meeboldii (Engl.) C.E.C.Fisch.	small to medium	oblate*	inaperturate	3	absent	absent	pollenkitt (++)	psilate	psilate	starch	unclear	pollen droplets, thecae horns
Lagenandra praetermissa de Wit	small to medium	oblate*	inaperturate	3	raphids	absent	pollenkitt (+)	psilate	psilate	starch	n.i.	pollen wall shedding
Aroideae, Schismatoglottideae												
Schismatoglottis calyptrata (Roxb.) Zoll. & Moritzi	small	spheroidal to oblate*	inaperturate	2	raphids	small	pollenkitt (+)	scabrate	psilate, with crystals	lipids*	2a (Ulrich et al. 2012)	thin surface layer (Cuticle-like), acetolysis resistant, pollen strands
Schismatoglottis celebica Engl.	small	spheroidal to oblate*	inaperturate	2	raphids	small & large	pollenkitt (+)	scabrate	psilate, with crystals	lipids*	2a (Ulrich et al. 2012)	thin outer layer, acetolysis resistant, pollen strands
Schismatoglottis conoidea Engl.	small	spheroidal to oblate*	inaperturate	2	raphids	small	pollenkitt (+)	scabrate	psilate, with crystals	no starch	2a ?	pollen strands
Schismatoglottis ifugaoensis Bogner, P.C.Boyce & S.Y.Wong	small	spheroidal to oblate*	inaperturate	2	raphids	small	pollenkitt (+)	scabrate	psilate	no starch	2a ?	pollen strands
Schismatoglottis matangensis S.Y.Wong	small	spheroidal to oblate*	inaperturate	2 (& 3)	raphids	small	pollenkitt (+)	scabrate	psilate, with crystals	no starch	2a ?	pollen strands
Schismatoglottis modesta Schott	small	spheroidal to oblate*	inaperturate	2 (&3)	raphids	small	pollenkitt (+)	scabrate	psilate, with crystals	no starch	2a ?	pollen strands
Schismatoglottis motleyana (Schott) Engl.	small	spheroidal to oblate*	inaperturate	2	raphids	small & large	pollenkitt (+)	scabrate	psilate, with crystals	no starch	2a ?	pollen strands
Schismatoglottis multiflora Ridl.	small	spheroidal to oblate*	inaperturate	2 (& 3)	n.i.	large	pollenkitt (+)	scabrate	psilate with crystals	no starch	2a (Ulrich et al. 2012)	thin surface layer (Cuticle-like), acetolysis resistant, pollen strands
Schismatoglottis roseospatha Bogner	small	spheroidal to oblate*	inaperturate	n.i.	n.i.	absent	pollenkitt (+)	scabrate	psilate	n.i.	2a ?	pollen strands
Schismatoglottis tecturata (Schott) Engl.	small	spheroidal to oblate*	inaperturate	2 (& 3)	n.i.	small	pollenkitt (+)	scabrate	psilate (Gravum)	no starch	2a ?	pollen strands
Schismatoglottis viridissima A.Hay	small	spheroidal to oblate*	inaperturate	2 (& 3)	raphids	small	pollenkitt (+)	scabrate	psilate with crystals	no starch	n.i.	pollen strands
Schottariella mirifica P.C. Boyce & S.Y. Wong	small	spheroidal to oblate*	inaperturate	2 (& 3)	n.i.	absent..	pollenkitt (+)	scabrate	psilate	n.i.	1b ? (Ulrich et al. 2012)	thin bi-layered endexine? or thin ektexine?
Phymatarum borneense M.Hotta	small	spheroidal to oblate*	inaperturate	2	raphids	absent.	pollenkitt (+)	scabrate	psilate	little amounts of starch	? Unclear 1b or 2b??	thick intine
Piptospatha viridistigma P.C.Boyce, S.Y.Wong & Bogner	small	oblate*	inaperturate	3 (& 2)	raphids	absent..	pollenkitt (+)	scabrate	psilate	no starch	n.i.	
Piptospatha ridleyi N.E.Br ex Hook.f.	small	oblate*	inaperturate	n.i.	n.i.	absent.	?	scabrate	psilate (Halbritter, unpublished data)	n.i.	n.i.	
Bucephalandra motleyana Schott	small	oblate*	inaperturate	2 (& 3)	raphids	absent..	pollenkitt (+)	psilate	verrucate	no starch	? Unclear 1b or 2b??	droplets, thecae horns
Apoballis acuminatissima (Schott) S.Y.Wong & P.C.Boyce	small	spheroidal to oblate*	inaperturate	2 (& 3)	raphids	absent	pollenkitt (-)	echinate	echinate		2a (Ulrich et al. 2012)	thin outer layer, acetolysis resistant, polysaccharidic echini
Apoballis mutata (Hook.f.) S.Y.Wong & P.C.Boyce	small	spheroidal to oblate*	inaperturate	2	raphids	absent	pollenkitt (-)	echinate	echinate		2a (Ulrich et al. 2012)	thin outer layer, acetolysis resistant, polysaccharidic echini
Apoballis longicaulis (Ridl.) S.Y.Wong & P.C.Boyce	small	n.i.	inaperturate	n.i.	n.i.	n.i.	n.i.	echinate (Thanikaimoni, 1969)	n.i.	n.i.	n.i.	
Hestia longifolia S.Y.Wong & P.C.Boyce	small	oblate*	inaperturate	2	raphids	small	pollenkitt (+)	psilate	psilate, with crystals	no starch	n.i.	
Ooia grabowskii (Engl.) S.Y.Wong & P.C.Boyce	small	oblate*	inaperturate	3 (& 2)	raphids	absent.	pollenkitt (++)	scabrate	psilate	no starch	2a (Ulrich et al. 2012)	

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Aroideae, Thomsonieae												
Amorphophallus asterostiomatus Bogner & Hett.	large	oblate	inaperturate	3	not detected	absent	pollenkitt (-)	striate to reticulate	striate to reticulate	starch	2a (Ulrich et al. 2015)	pollen strands
Amorphophallus atrorubens Hett. & Sizemore	large	spheroidal to oblate	inaperturate	3	not detected	absent	pollenkitt (-)	striate (untypical)	striate (untypical)	starch	n.i.	pollen strands
Amorphophallus bulbifer Blume	large	oblate	inaperturate	3	not detected	absent	pollenkitt (+)	psilate	psilate	starch	2a (Ulrich et al. 2015)	pollen strands
Amorphophallus henryi N.E.Br.	large	spheroidal	inaperturate	3	not detected	absent	pollenkitt	psilate	psilate	starch	2a (Ulrich et al. 2015)	pollen strands
Amorphophallus interruptus Endl. & Gehrm.	large	oblate	inaperturate	3	not detected	absent	pollenkitt (-)	striate	striate	starch	n.i.	pollen strands
Amorphophallus konjac K.Koch	medium	spheroidal to oblate	inaperturate	3	not detected	absent	n.o.	irregularly striate	irregularly striate	starch	2a (Ulrich et al. 2015)	pollen strands
Amorphophallus krausei Endl.	medium to large	spheroidal to oblate	inaperturate	3	not detected	absent	pollenkitt (+)	striate	striate	starch	n.i.	pollen strands, pollen wall shedding
Amorphophallus longituberosus Endl. & Gehrm.	medium	oblate	inaperturate	3	not detected	absent	pollenkitt (-)	striate	striate	starch	2a (Ulrich et al. 2015)	pollen strands
Amorphophallus mossambicensis Klotzsch ex Garcke	large	spheroidal	inaperturate	3	raphids	absent	pollenkitt (-)	irregularly striate	irregularly striate (sometimes psilate)	starch	2a (Ulrich et al. 2015)	pollen strands
Amorphophallus onoscakulii Hett. & A.Galloway	medium	oblate	inaperturate	3	not detected	absent	pollenkitt (-)	striate to plicate	striate to plicate	starch	2a (Ulrich et al. 2015)	pollen strands
Amorphophallus operculatus Hett. & Sizemore	medium	oblate	inaperturate	3	not detected	absent	pollenkitt (+)	striate to plicate, with appendages	striate to plicate, with appendages	starch	2a (Ulrich et al. 2015)	pollen strands
Amorphophallus palawanensis Bogner & Hett.	medium	spheroidal to oblate	inaperturate	3	raphids	absent	pollenkitt (+)	irregularly striate	irregularly striate	starch	2a (Ulrich et al. 2015)	pollen strands
Amorphophallus polyanthus Hett. & Sizemore	medium	spheroidal	inaperturate	3	raphids	absent	absent	striate to plicate	striate to plicate	starch	2a (Ulrich et al. 2015)	pollen strands
Amorphophallus prainii Hook.f.	medium to large	spheroidal	inaperturate	3	not detected	absent	pollenkitt (+)	psilate	psilate	starch	2a (Ulrich et al. 2015)	pollen strands
Amorphophallus serrulatus Hett. & A.Galloway	medium to large	oblate	inaperturate	3	not detected	absent	absent	striate to plicate, with appendages	striate to plicate, with appendages	starch	2a (Ulrich et al. 2015)	pollen strands
Amorphophallus stuhlmannii (Engl.) Engl. & Gehrm.	large	spheroidal	inaperturate	3	not detected	absent	pollenkitt (+++)	verrucate	verrucate	starch	2a (Ulrich et al. 2015)	pollen strands
Amorphophallus taurostigma Ittenb., Hett. & Bogner	large	oblate	inaperturate	3	not detected	absent	pollenkitt	irregularly striate to fossulate	irregularly striate	starch	2a (Ulrich et al. 2015)	pollen strands
Amorphophallus tonkinensis	medium to large	spheroidal to oblate	inaperturate	3	not detected	absent	n.o.	striate	striate	starch	n.i.	n.o.
Amorphophallus yunnanensis Engl.	medium to large	oblate to spheroidal	inaperturate	3	not detected	absent	pollenkitt (+)	striate	striate	starch	2a (Ulrich et al. 2015)	pollen strands, pollen wall shedding
Amorphophallus rhizomatosus Hett.	medium	spheroidal	inaperturate	3	raphids	absent	pollenkitt (+)	irregularly striate	irregularly striate	starch	2a	pollen strands, pollen wall shedding
Amorphophallus paeoniifolius (Dennst.) Nicolson	large	spheroidal	inaperturate	3	not detected	absent	pollenkitt (+)	psilate	psilate	starch	n.i.	pollen strands
Amorphophallus maneelsdorffii Bogner	medium	spheroidal	inaperturate	3	not detected	absent	pollenkitt (-)	echinate	micro-echinate	starch	n.i.	pollen strands, pollen wall shedding
Amorphophallus mvosuroides Hett. & A.Galloway	medium	oblate	inaperturate	3	raphids	absent	pollenkitt (-)	striate to plicate	striate to plicate	starch	n.i.	pollen strands
Amorphophallus sumawongii (Bogner) Bogner & Mayo	medium	oblate	inaperturate	3	not detected	absent	n.i.	n.i.	striate (Halbritter 2005)	starch	n.i.	pollen strands
Amorphophallus lacourii Linden & André (=*Pseudodracontium lacourii* N.E.Br.)	large	oblate to spheroidal	inaperturate	3	raphids	absent	pollenkitt (-)	striate to plicate, with psilate apices	striate to plicate, with psilate apices	starch	2a (Ulrich et al. 2015)	psilate apices, pollen strands
Amorphophallus latifolius (Serebryanyi) Hett. & Claudel (=*Pseudodracontium latifolium* Serebryanyi	large	oblate to spheroidal	inaperturate	3	raphids	absent	pollenkitt (-)	striate to plicate, with psilate apices	striate to plicate, with psilate apices	starch	2a (Ulrich et al. 2015)	psilate apices, pollen strands
Aroideae, Caladieae												
Caladium steudneriaefolium Engl.	medium	spheroidal	inaperturate	3	n.o.	present	pollenkitt	psilate (to scabrate)	smooth	starch	? embedded*	
Syngonium podophyllum Schott	medium	spheroidal to oblate*	inaperturate	3	raphids	absent	pollenkitt	echinate	echinate	starch	n.i.	
Hapaline benthamiana Schott	medium	spheroidal	inaperturate	3	n.o.	absent	absent ?	echinate	echinate	starch	n.i.	
Aroideae, Zomicarpeae												
Ulearum sagittatum Engl.	medium	spheroidal	inaperturate	2 & 3	raphids	absent	pollenkitt	echinate	echinate	starch, only little amounts	2a (Weber et al. 1999)	long, thin echini
Filarum manserichense Nicolson	medium	spheroidal	inaperturate	3 & 2		absent	pollenkitt (+)	echinate	echinate	starch, only little amounts	n.i.	

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Aroideae, Caladieae												
Xanthosoma helleborifolium Schott	medium	spheroidal	inaperturate	3	raphids	present	pollenkitt (+)	psilate to scabrate	psialte	starch	? embedded*	thick pollen wall, pollen strands, pollen tetrads with callose wall??
Xanthosoma mariae Bogner & E.G.Gonç.	medium	spheroidal	inaperturate	3	raphids	present	pollenkitt (+)	psilate to scabrate	psilate	starch	? embedded*	thick pollen wall, pollen strands, pollen shed in monads
Scaphispatha gracilis Brongn. ex Schott	medium	spheroidal	inaperturate	3	raphids	present	pollenkitt	verrucate to striate?	verrucate to striate?	starch	? embedded*	
Caladium lindenii (André) Madison	medium	spheroidal	inaperturate	3	raphids	absent	pollenkitt	psilate (to scabrate)	smooth	starch	1b (Weber et al. 1999, *C*-Hybrid), embedded*	
Aroideae, Zomicarpeae												
Zomicarpella sp. nov.	small	spheroidal	inaperturate	2	raphids	absent	pollenkitt	echinate	echinate	starch	2a (Weber et al. 1999)	thick pollen wall, pollen wall shedding
Aroideae, Arisareae												
Arisarum vulgare Targ.Tozz.	small to medium	oblate*	inaperturate	2	raphids	absent	pollenkitt (-)	plicate	plicate	starch	n.i.	
Aroideae, Ambrosineae												
Ambrosina bassii L.	medium	oblate*	inaperturate	2	raphids	absent	pollenkitt (-)	plicate (polyplicate)	plicate	starch	2a (Weber et al 1999, this study)	
Aroideae, Peltandreae												
Typhonodorum lindleyanum	medium	spheroidal	inaperturate	2	raphids	absent	pollenkitt (+)	psilate	psilate (Halbritter, unpublished data)	no starch	? embedded*	
Aroideae, Arophyteae												
Carlephyton glaucophyllum Bogner	medium	spheroidal	inaperturate	3	raphids	absent	absent	echinate	echinate	absent	? embedded*	pollen wall shedding
Colletogyne perrieri Buchet	medium	spheroidal	inaperturate	3	not detected	absent	absent	echinate	echinate	starch	? embedded*	
Arophyton crassifolium (Buchet) Bogner	small to medium	spheroidal	inaperturate	3 & 2	raphids	absent	pollenkitt	echinate	echinate	no starch	2a	pollen wall shedding
Aroideae, Pistieae												
Pistia stratiotes L.	small	oblate*	inaperturate	3	raphids	absent	pollenkitt	polyplicate	polyplicate		2a (Weber et al 1999)	polysaccharidic outer layer, not acetolysis resistant
Aroideae, Colocasieae												
Alocasia acuminata Schott	small to medium	spheroidal	inaperturate	3	n.o.	absent	pollenkitt (-)	echinate	echinate	starch	2a ?	
Alocasia cuprea K.Koch	medium	spheroidal	inaperturate	3	n.o.	absent	pollenkitt (-)	echinate	echinate	starch	2a ? embedded*	
Alocasia lauterbachiana (Engl.) A.Hay	small to medium	spheroidal	inaperturate	3	raphids	absent	pollenkitt (-)	echinate	echinate	starch	2a (Weber et al 1999)	polysaccharidic outer layer, not acetolysis resistant
Colocasia antiquorum Schott	small	spheroidal	inaperturate	3	n.o.	absent	pollenkitt	echinate	echinate	starch	2a ? embedded*	
Colocasia affinis Schott	small	oblate*	inaperturate	3 (& 2)	raphids	absent	pollenkitt	psilate to scabrate	plicate	most pollen without starch	2a ? embedded*	
Colocasia gigantea (Blume) Hook.f. (=Leucocasia gigantea)	small	spheroidal	inaperturate	3	n.o.	absent	pollenkitt	scabrate	unclear	starch	2a (Weber et al 1999)	polysaccharidic outer layer, not acetolysis resistant
Steudnera assamica Hook.f.	small	oblate*	inaperturate	3 & 2	raphids	absent	pollenkitt	plicate	plicate	no starch	2a (Weber et al 1999, *Steudnera griffithii*)	polysaccharidic outer layer, not acetolysis resistant
Steudnera henryana Engl.	small	oblate*	inaperturate	3 & 2	raphids	absent	pollenkitt	plicate	plicate	no starch	2a ? embedded*	
Steudnera kerrii Gagnep.	small	oblate*	inaperturate	3 & 2	raphids	absent	pollenkitt	plicate	plicate	starch	2a ? embedded*	
Remusatia hookeriana Schott	small	spheroidal	inaperturate	3	not detected	absent	pollenkitt	echinate	echinate	starch	1b?	
Remusatia pumila (D.Don) H.Li & A.Hay	small	spheroidal	inaperturate	3	not detected	absent	pollenkitt	echinate	echinate	starch	1b?	spines acetolysis resistant
Remusatia vivipara (Roxb.) Schott	small to medium	spheroidal	inaperturate	3	not detected	absent	pollenkitt	echinate	echinate	starch	1b (Weber et al 1999)	
Aroideae, Arisaemateae												
Arisaema fargesii Buchet	small	spheroidal	inaperturate	3 & 2	absent	absent	pollenkitt	echinate	echinate	no starch	1b (Weber et al 1999, e.g. *A. tortuosum*), embedded*	
Pinellia pedatisecta Schott	small	spheroidal	inaperturate	3	raphids	absent	pollenkitt	echinate	echinate	starch	2a (Weber et al 1999, *P. ternata*), embedded*	polysaccharidic outer layer, not acetolysis resistant
Pinellia peltata C.Pei	small	spheroidal	inaperturate	3	n.o.	absent	pollenkitt	echinate	micro-echinate	starch	2a? embedded*	polysaccharidic outer layer, not acetolysis resistant
Aroideae, Areae												
Arum cylindraceum Gasp.	medium	spheroidal	inaperturate	3	raphids	absent	pollenkitt	echinate	echinate	unclear	2a (Weber et al 1999, e.g. *A. maculatum*), embedded*	polysaccharidic outer layer, not acetolysis resistant
Dracunculus vulgaris Schott	medium	spheroidal	inaperturate	3	not detected	absent	pollenkitt (-)	psilate	psilate	starch	2a (Weber et al 1999)	polysaccharidic outer layer, not acetolysis resistant
Biarum ditschianum Bogner & P.Boyce	small	oblate*	inaperturate	3	raphids	absent	pollenkitt (-)	plicate	plicate	starch	2a (Weber et al 1999)	
Biarum tenuifolium (L.) Schott	small	spheroidal	inaperturate	3	raphids	absent	pollenkitt (+)	echinate	echinate	starch	2a (Weber et al 1999)	polysaccharidic outer layer, not acetolysis resistant
Helicodiceros muscivorus Engl.	medium	spheroidal	inaperturate	3	absent	absent	pollenkitt (++)	echinate	echinate	starch	2a (Weber et al 1999)	polysaccharidic outer layer, not acetolysis resistant
Typhonium corrugatum Hett. & Rybková	medium	spheroidal	inaperturate	3	not detected	absent	pollenkitt (-)	echinate	echinate	starch	2a (Weber et al 1999, *T. trilobatum*), embedded*	
Typhonium divaricatum (L.) Blume	medium	spheroidal	inaperturate	3	raphids	absent	pollenkitt (-)	echinate	echinate	starch	2a? embedded*	
Typhonium sp. (Bestimmung W.Hetterscheid; Prehsler-112)	medium	spheroidal	inaperturate	3	raphids	absent	pollenkitt (-)	echinate	echinate	starch	2a (Weber et al 1999)	