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Evolution of trap pollination in the Araceae

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CONTENTS

ABSTRACT

Floral traps are among the most sophisticated devices that evolved in angiosperms in the context of pollination. However, the evolution of trap pollination has not yet been studied in a phylogenetic context. The aim of this thesis is to determine the evolutionary history of trap pollination in the family Araceae. Inflorescence morphology was investigated to identify trapping devices and to classify traps into functional types. Phylogenetic relationships in the family were inferred with maximum likelihood and Bayesian methods. In addition, the character evolution of trapping devices, trap types, and pollinator types was reconstructed with maximum parsimony and Bayesian methods and a possible association of trap pollination with specific pollinator types was investigated. Moreover, the interactions between pollinators and aroids were studied in the field using the genus *Colocasia* as a model system. The present thesis demonstrates that trap pollination in the Araceae is more common than previously thought. Inflorescence traps have evolved independently in 10 different clades, and, at least in some clades, imperfect traps served as a precursor for the evolution of more elaborate traps. Several organs of the inflorescence are involved in trapping and the morphology and composition of these trapping devices differ significantly among the various groups of aroids. These differences are partly due to adaptations to different types of pollinators. As shown for the genus *Arum*, the different pollinator types select for differences in the size and the density of the trapping devices. In general, trap pollination in Araceae is correlated with pollination by flies. Several inflorescence traps have probably evolved from rewarding ancestors that offered a brood site for the pollinating flies. This syndrome was also found in extant *Colocasia*, in which drosophilid pollinators are rewarded with a site for mating, breeding, and hatching of the larvae. Moreover, the flies are arrested within the inflorescence during the male phase of anthesis, apparently in order to secure pollen export. In conclusion, preadaptations of the spathe and the spadix facilitated the multiple evolution of trap pollination. Different insect pollinators are likely to have selected for differences in the size and number of trapping devices. Therefore, changes to these devices are an important and previously little recognised variable in the design of pollination traps. The present thesis shows that studying the morphology of structures related to pollination is indispensable for understanding the evolutionary processes that drive the interactions between flowers and their pollinators.

ZUSAMMENFASSUNG

Kesselfallenblumen gehören zu den komplexesten Gebilden, die innerhalb der Angiospermen im Zusammenhang mit der Bestäubung entstanden sind. Bis jetzt wurde die Entstehung dieser Fallenblumen aber noch nicht mithilfe phylogenetischer Methoden untersucht. Das Ziel der vorliegenden Dissertation ist es, die Evolution der Kesselfallenblumen innerhalb der Familie der Araceae nachzuzeichnen. Um Kesselfallenblumen zu identifizieren und zu typifizieren, wurde die Morphologie der Infloreszenzen in allen Einzelheiten studiert. Gleichzeitig wurde mit maximum likelihood und Bayesian-Methoden eine molekulare Phylogenie der Araceae erstellt, auf deren Basis die Evolution der einzelnen Fallenstrukturen und –typen ebenso wie die Evolution der verschiedenen Bestäubertypen rekonstruiert werden konnte. Zudem wurde eine mögliche Korrelation von Bestäubertypen und Kesselfallenblumen untersucht. Um die Interaktion zwischen Aronstabgewächsen und Bestäubern genauer zu beleuchten, wurden Freilanduntersuchungen an mehreren Arten der Gattung *Colocasia* durchgeführt. Die vorliegende Doktorarbeit zeigt, dass Kesselfallen bei den Araceen häufiger vorkommen als man bisher angenommen hat. Sie sind innerhalb der Familie in mindestens 10 verschiedenen Gruppen unabhängig voneinander entstanden. Zumindest in einigen dieser Gruppen sind komplex aufgebaute Fallen aus weniger elaborierten Halbfallen entstanden. Strukturen, die dem Insektenfang dienen, kommen in insgesamt 27 Gattungen vor und haben sich aus unterschiedlichen floralen und superfloralen Organen entwickelt. Ihre Morphologie und Zusammensetzung unterscheidet sich beträchtlich zwischen den verschiedenen Taxa. Diese Abweichungen beruhen vermutlich zum Teil auf Anpassung an unterschiedliche Bestäubertypen. So zeigte sich in der Gattung *Arum*, dass verschiedene Bestäubertypen auf Unterschiede in Größe und Anzahl von Fallenstrukturen selektionieren. Im Allgemeinen ist die Evolution von Kesselfallenblumen innerhalb der Araceen mit der Bestäubung durch Fliegen korreliert. Die Rekonstruktion der ursprünglichen Bestäubertypen deutet darauf hin, dass die letzten gemeinsamen Vorfahren einiger Kesselfallenblumen eine symbiotische Beziehung mit Fliegen eingegangen sind, wobei der Blütenstand den Fliegen als Brutplatz diente. Brutplatz-Bestäubung tritt im Besonderen bei rezenten Arten der Gattung *Colocasia* auf. Deren Infloreszenzen werden von Fruchtfliegen als Paarungs- und Brutplatz genutzt, die Fruchtfliegen werden aber trotzdem während der männlichen Anthesephase im Blütenstand gefangen gehalten. Dies offenbar deshalb, um den Export des produzierten Pollens sicherzustellen. Zusammenfassend lässt sich sagen, dass verschiedene

Voranpassungen – so z.B. papillöse Zellen, die ursprünglich vermutlich der Produktion von Düften dienten – eine wichtige Rolle für die mehrfache Entstehung von Kesselfallenblumen gespielt haben. Die unterschiedlichen Fallenstrukturen haben sich im Laufe der Evolution zum Teil an verschiedene Besäubertypen angepasst. Änderungen in diesen Strukturen spielen daher eine wichtige und bisher unterschätzte Rolle für die Bestäubung von Kesselfallenblumen. Wie diese Arbeit zeigt, sind morphologische Untersuchungen von Blütenorganen für das Verständnis der evolutionären Prozesse, welche die Interaktionen zwischen Blüten und Bestäubern steuern, unverzichtbar.

CHAPTER 1

GENERAL INTRODUCTION

1.1. AIMS

The overall aim of the present thesis is to shed light on the origin and elaboration of one of the most sophisticated reproductive structures in angiosperms, namely trap flowers. In order to investigate the drivers for the evolution of trap flowers I use the family of Araceae as a model-system. The structural diversity of the aroid inflorescence in different clades including open as well as chamber blossoms and the occurrence of different pollination syndromes make this family an ideal object for studying the evolution of trap pollination. The thesis combines morphological and ecological data gathered in field studies, in the laboratory and from literature, which are evaluated on the basis of the phylogenetic tree of the family.

In the first part of my thesis (Chapter 2), I examine the macroevolutionary patterns that have led to the evolution of traps in Araceae. To assess the frequency of trap pollination in the family, I examined the morphology of trapping devices in various taxa spanning the majority of clades. In addition, I reconstructed the phylogeny of Araceae based on the molecular data of Cusimano *et al.* (2011), complemented by sequences of one additional taxon. The character states for the different trapping devices, trap type, and pollinator type were then mapped onto the phylogeny and the ancestral states were reconstructed with maximum parsimony and Bayesian inference. In particular, the following questions were addressed: (1) In which taxa of Araceae do trapping devices occur and how did they evolve? (2) Did the different types of traps evolve from a common trap-ancestor and did perfect traps evolve from imperfect traps? (3) Is trap pollination in the Araceae associated with specific insect groups?

The second part of my thesis (Chapter 3) is a case study dealing with the adaptations for trap pollination in four species of the genus *Colocasia*. These species combine features typical for rewarding and for trapping aroids. *Colocasia* is known to be visited by drosphilid flies of the genus *Colocasiomyia* (Toda & Lakim 2011). In closely related aroids drosophilid flies act as pollinators that get rewarded with a brood-site, indicating the presence of a rewarding system in *Colocasia* (Takenaka *et al.* 2006). At

the same time, some species of *Colocasia* exhibit spathe movements that resemble the trap mechanism of *Sauromatum* and *Typhonium* (Dakwale & Bathnagar 1985). My aim was to investigate the adaptations for the retention of pollinators and possible drivers for their evolution. I studied the pollination biology of four species of *Colocasia* in the field (in tropical South-West China). In addition, I investigated the morphology and anatomy of the spathe in three of these species. I attempt to answer the following questions: (1) Which insects are the pollinators of *Colocasia*? (2) Is the relationship between *Colocasia* and its pollinators mutualistic or has it shifted to deceptive trap pollination? (3) What is the role of the spathe movements and the papillate cells forming the adaxial spathe epidermis?

The third part of the thesis (Chapter 4) deals with the design of the inflorescence traps and the size of the slippery surfaces in the genus *Arum*. During the work on the present thesis it became clear that trapping devices vary between different genera but appear to be rather uniform within a given genus. Whether pollinators might affect the design of the traps and the size of the trapping devices has not been studied yet. Therefore, I investigated the bauplan of pollination traps in 15 species of *Arum* and measured the size of the trapping devices under scanning electron microscopy. The research questions are: (1) Is trap pollination a stable condition within the genus *Arum*? (2) Do different pollinators select for differences in the design of the trap or the size of the trapping devices?

The fourth part of the thesis (Chapter 5) reports on spathe movements in *Apoballis acuminatissima*. Besides slippery surfaces, spathe movements are an important adaptation for trapping pollinators. In absence of information on pollination biology, I investigated the spathe movements in *Apoballis* and compared them with taxa of the closely related genus *Schismatoglottis* and other aroids in order to explain their possible function.

Finally, the results of the four chapters and their significance for the evolution of trap pollination are reviewed in the general discussion (Chapter 6).

1.2. TRAP POLLINATION

Deceptive pollination is found in about 32 angiosperm families, totalling about 4% of all species (Renner 2006). In several groups, pollinators are not only deceived but also trapped within the flower (or inflorescence) for a certain time in order to exploit them as pollen vectors. Such trap blossoms (‘Kesselfallenblumen’ sensu Vogel 1965)

Fig. 1. Trap blossoms of different angiosperm families. **A,** *Arum elongatum* (Araceae). Note that the frontal part of the spathe tube has been removed in order to show the flowers inside. **B,** *Aristolochia arborea* (Aristolochiaceae). **C,** C*eropegia* sp. (Apocynaceae).

predominantly occur in the early diverging angiosperm lineages up to the Monocots (Dafni 1984, Faegri & Van der Pijl 1971, Proctor *et al.* 1996) (Fig. 1). The most important precondition for the evolution of a floral trap is a chamber that encloses the floral organs and facilitates the capture of insect pollinators. Trap blossoms share many similarities with rewarding chamber blossoms (sensu Faegri & Van der Pijl 1971), and in some families such as Araceae and Aristolochiaceae both types co-occur (Sakai 2002a, Gibernau *et al.* 2010). Shared features are protogyny, thermogenesis, coloured tepals, floral odours and pollen being used as a food reward, while the presence of nectar is rare (Thien *et al.* 2009). Pollination in rewarding taxa is mainly achieved by flies and beetles which often use the inflorescence as site for mating and/or breeding (Bernhardt 2000, Toda & Lakim 2011). In deceit-pollinated chamber blossoms, the plants attract saprophilous flies and beetles by mimicking their oviposition substrates such as dung (Diaz & Kite 2002), carrion (Stensmyr *et al.* 2002) and fungi (Vogel 1978). In contrast to sexually-deceptive and food-deceptive blossoms where pollinators stay only for a short moment, in many brood-site-mimicking chamber blossoms pollinators get trapped and are forced to stay for a certain period of time (Dafni 1984).

Trap blossoms use specific sensory cues that activate the insects' instinctive behaviour in order to attract pollinators. Odours play a central role for attraction. Decaying organic matter is imitated by compounds such as sulfides, phenol and indol derivates and terpenes (Kite *et al.* 1998, Jürgens *et al.* 2006, van der Niet *et al.* 2011). Visual cues include dull colouration, light-windows and flickering hairs. Dull colours are thought to imitate the faeces- or carrion-models (Faegri & Van der Pijl 1971). Flickering hairs mimick aggregating flies thereby stimulating passing flies to join in (Vogel 1961).

Light-windows in the chamber walls come into play when insects enter the blossom. As flies behave positively phototropic, they will try to escape from a dark chamber towards the light. The light-windows guide them away from the entrance towards the floral organs (Vogel 1965, Dafni 1984). In general, these sensory cues are also found in several non-trapping deceptive blossoms, for example in orchids and several stapeliads (Jersáková *et al.* 1994, Meve & Liede 1994).

The key elements in true trap blossoms are specialised structures which ensure that insects will be trapped inside the blossom and hindered from untimely escape. Most important are slippery surfaces which cause insects to glide into the chamber. Slippery surfaces usually consist of downward-pointing papillae and/or epicuticular wax crystalloids (Vogel 1965, Vogel & Martens 2000). Such wax crystalloids can be found throughout the angiosperms (Barthlott *et al.* 1998) and have evolved repeatedly in various contexts of plant-pollinator interactions (Eigenbrode 2004, Gaume *et al.* 2004, Quek *et al.* 2004). Through their three-dimensional structure, they reduce the surface to which insect's legs can attach and thus impede adhesion. Moreover, the crystalloids also can break off and stick to the insect's adhesive pads (Gaume *et al.* 2004). In Araceae and Aristolochiaceae, some traps also block the exit by hairs or elongated sterile flowers (Knoll 1926, Vogel 1961, Oelschlägel *et al.* 2009). Pollinators are arrested for the time of anthesis and get released after pollen extrusion. In most cases, getaway is facilitated by the wilting of the trapping structures, while in a few taxa a secondary opening of the blossom provides a new exit (Vogel 1965). Some deceptive flowers are intermediate between true traps and non-trapping cheaters. In these taxa the insects slip into the chamber and are forced to take a predetermined way out of the blossom, but they are not arrested for a certain time-interval. Such imperfect trap blossoms are also called 'semi-traps' (Faegri & Van der Pijl 1971).

1.3. ARACEAE

1.3.1. *Systematics and distribution*

My study system was the Araceae, a diverse family with >3300 species in about 126 genera (Boyce & Croat 2012) (Fig. 2). Based on morphological characters, Mayo *et al.* (1997) recognised seven subfamilies (i.e. Gymnostachydoideae, Orontioideae, Pothoideae, Monsteroideae, Lasioideae, Calloideae, and Aroideae). The Zamioculcadoideae were added as an eighth subfamily by Bogner & Hesse (2005). More recent molecular phylogenies confirm large parts of the classification of Mayo *et al.*

Fig. 2. Members of the Araceae. **A,** *Lysichiton americanus* (Orontioideae). **B,** *Anthurium dactylifer* (Pothoideae). **C,** *Stenospermation popayanense* (Monsteroideae). **D,** *Dracontium prancei* (Lasioideae). **E,** *Stylochaeton bogneri* (Zamioculcadoideae). **F-P,** Aroideae: **F,** *Philodendron martianum*. **G,** *Gorgonidium* cf. *intermedium*. **H,** *Lagenandra praetermissa*. **I,** *Schismatoglottis calyptrata*. **J,** *Amorphophallus bulbifer*. **K,** *Caladium steudneriifolium*. **L,** *Arisarum vulgare*. **M,** *Alocasia odora*. **N,** *Colletogyne perrieri*. **O,** *Arisaema ghaticum*. **P,** *Dracunculus vulgaris*.

(1997), but add the duckweeds (Lemnoideae) as an additional subfamily (Cabrera *et al.* 2008, Cusimano *et al.* 2011). The systematic position of the monotypic Calloideae is still doubtful (Chartier 2011, Cusimano *et al.* 2011). The youngest subfamily Aroideae is the most diverse clade regarding genera and species numbers (>1700). The most outstanding genus by means of species diversity is is *Anthurium* (Pothoideae) with 905 species recorded so far, followed by *Philodendron* (482 spp; Aroideae).

The Araceae are an ancient family that was already present in the Early Cretaceous (Friis *et al.* 2004). The early aroids probably first evolved in wet habitats, presumably with Laurasia as an important centre of distribution (Friis *et al.* 2010, Nauheimer *et al.* 2012a). While early lineages still occur in Laurasia, the extension of the distribution range of several lineages into Africa, South America, South-East Asia and Australia has happened much later. Some lineages, such as the Lasioideae, got extinct in the Northern Hemisphere after climate cooling during the Oligocene and only survived in tropical regions (Nauheimer *et al.* 2012a). Today, the Araceae have a cosmopolitan distribution. Diversity is highest in the tropics, with hotspots in America and Asia, while Africa is comparatively species-poor. Besides, aroids also occur in temperate as well as boreal regions, the Mediterranean, Australia and Madagascar (Mayo *et al.* 1997).

The Araceae display a broad variety of live forms including epiphytes (e.g. *Anthurium* and *Philodendron*), primary and secondary hemiepiphytes (especially Pothoideae and Monsteroideae), free floating aquatics (Lemnoideae, *Pistia*), rheophytes (e.g. Schismatoglottideae), helophytes (e.g. *Montrichardia*, *Typhonodorum*) and terrestrial herbs (especially Aroideae) (Croat 1988, Boyce & Wong 2012). Several aroids are geophytic (e.g. *Arum*, *Amorphophallus*, *Dracontium*) and survive the dry season as underground tubers.

1.3.2. *Inflorescence morphology*

A typical feature of the aroid inflorescence is that flowers are arranged along an axis, called spadix, which bears a modified bract, called spathe (Mayo et al. 1997). The shape of the spathe is subject to much variation. It is expanded in *Anthurium* and *Spathiphyllum*, boat-shaped in several Monseroideae, sometimes with overlapping margins (e.g. Lasioideae). In several taxa of the Aroideae, the spathe forms a basal tube which is separated from the expanded spathe blade by a constriction. In some taxa such as *Gymnostachys* and *Orontium* the spathe is inconspicuous or absent. Moreover, the

colours of the spathe vary considerably, ranging from green to red, white, yellow as well as brown or purple (Grayum 1990).

The spadix is quite uniform in the early-diverging lineages as it bears bisexual flowers only. These flowers are usually two- or three-merous and surrounded by one or two whorls of tepals. In *Calla palustris*, the flowers are bisexual, but a perigone is absent. In contrast, in the subfamily Zamioculcadoideae the flowers are unisexual, with pistillate flowers at the base of the spadix and staminate flowers above, but they bear a perigone (Bogner & Hesse 2005). In the Aroideae, the flowers are consistently aperigoniate and unisexual. The pistillate flowers are situated at the lower part of the spadix, and the staminate flowers above. In several taxa, sterile flowers are present between the pistillate and the staminate flowers. In many later-diverging Aroideae (e.g. Schismatoglotideae, Arisaemateae, Areae), the upper part of the spadix (called 'appendix') is sterile (i.e. without flowers) (Grayum 1990).

In conclusion, the spathe, the spadix, and the flowers are subject to various modifications and show increasing synorganisation, in particular within the Aroideae subfamily, that led to the formation of a pseudanthium.

1.3.3. *Pollination biology*

One of the most important features of aroid inflorescences with regard to pollination biology is that the whole family is protogynous (Gibernau 2011). While in taxa with bisexual flowers receptive stigmas and anthers extruding pollen are found on the spadix at the same time (but in different flowers), in taxa with unisexual flowers the anthesis of the whole inflorescence is separated into a distinct pistillate and staminate phase. In order to ensure pollination, these aroids either have to attract new pollinators during the staminate phase of anthesis or to retain the pollinators that arrived during the pistillate phase until pollen release. In some taxa, pollinators get deceived by the inflorescence that mimicks a brood site and they are often trapped until anther dehiscence. In rewarding aroids the inflorescences offer food bodies, odour, a mating site or a brood site for the pollinators (Gibernau *et al.* 2010, Chartier 2011). The main groups of pollinators are Hymenoptera, Coleoptera and Diptera (Gibernau 2011). Some *Anthurium* species are even thought to be pollinated by hummingbirds (Kraemer & Schmitt 1999).

Bee pollination is essentially restricted to the subfamilies Monsteroideae and Pothoideae. In *Anthurium* and *Spathiphyllum*, scent-collecting euglossine bees are the

main pollinators (Vogel 1966a, Hentrich *et al.* 2010). Male bees visit the inflorescences and get rewarded with odour compounds that are used for the attraction of females (Vogel 1966b). In *Monstera deliciosa*, *Trigona* bees have been observed collecting gums from the flowers (Ramirez & Gomez 1978) but it is not clear whether they are the legitimate pollinators. In *Monstera obliqua*, not bees but nitidulid beetles have been observed pollinating the flowers (Chouteau *et al.* 2007).

Beetle pollination is much more common in the Araceae than bee pollination. In the neotropics, scarab beetles of the subfamily Dynastinae – especially the genus *Cyclocephala* - play a prominent role as pollinators. They are found for example in *Caladium* (Maia & Schlindwein 2006), *Dieffenbachia* (Young 1988), *Montrichardia* (Gibernau *et al*. 2003), *Philodendron* (Gottsberger & Silberbauer-Gottsberger 1991) and *Taccarum* (Maia 2011). The beetles usually feed on plant tissue (e.g. sterile flowers) and mate within the spathe chamber. As they have a nocturnal life cycle, they usually arrive at the inflorescence during the dusk and stay for one or two days, before they leave again. They are attracted by a strong scent, which may imitate sexual pheromones in some taxa (Maia 2011). Odour production is often associated with thermogenesis, which enhances odour emission but also serves as heat reward for the beetles (Seymour *et al.* 2003). Despite the close interaction, scarab beetles and aroids probably have not coevolved, but several aroids have adapted to the beetles' behaviour (Schiestl & Dötterl 2012). Several other - often saprophilous - beetles are associated with aroid inflorescences, including Nitidulidae, Staphylinidae and Hybosoridae.

Besides beetles, flies are the most common pollinators in the Araceae. Several aroids (e.g. *Alocasia*, *Furtadoa*, *Steudnera*) live in close association with drosophilid flies of the genus *Colocasiomyia* (Mori & Okada 2001, Toda & Lakim 2011, Takenaka Takano *et al.* 2012). These small flies use the inflorescences as mating- and brood-site. Similarly, in the North American *Peltandra virginica* chloropid flies act as pollinators and get rewarded with a brood-site and pollen (Patt *et al.* 1995). A number of aroids also lure and trap saprophilous flies and midges.

Several taxa of Araceae (e.g. *Lysichiton*, *Symplocarpus*) show no specialisation to a certain pollinator group. They attract various species of flies and beetles which probably feed on pollen (Pellmyr & Patt 1986, Uemura *et al.* 1993).

1.3.4. *Trap pollination in the Araceae*

Trap-pollination is well known in several clades of subfamily Aroideae (e.g. Ørgaard & Jacobsen 1998, Vogel & Martens 2000, Gibernau *et al.* 2004). Attraction is by means of deception. The inflorescences mimic carcass, dung or fungi in order to lure saprophilous insects (Vogel 1965). The most common pollinators are flies (e.g. Calliphoridae, Sarcophagidae and Sphaeroceridae, midges (e.g. Ceratopogonidae, Mycetophilidae, Psychodidae, Sciaridae), and beetles (e.g. Histeridae, Nitidulidae, Scarabaeidae, Staphylinidae) (Gibernau 2003, 2011).

Early observations on trap pollination in *Arum*, *Dracunculus* and *Helicodiceros* have been made by the two Italian botanists Giovanni Arcangeli (1883) and Federico Delpino (1890). They found that the inflorescences of these species imitated dung or carcass in order to attract saprophilous insects. Arcangeli could show that the pollinators of *Dracunculus vulgaris* are saprophilous beetles (e.g. Histeridae) which were trapped inside the inflorescence as they could not walk on the 'smooth' spathe epidermis. The first thorough studies on the trap mechanism were performed by Fritz Knoll in the early 20th century, who investigated the epidermal surfaces of several Araceae. Unfortunately, many observations were never published except for the detailed and extensive study on trap pollination in *Arum nigrum* (1926). Previous investigations of different angiosperms proved that flower surfaces can be slippery for insects (Knoll 1914, 1922). Knoll could show that this is also the case in *Arum nigrum*, which is pollinated by saprophilous flies (e.g. Sphaeroceridae) and beetles (Scarabaeidae, Staphylinidae). He found that the slippery epidermis of the spathe and the spadix is formed by downward-pointing papillate cells that exude small droplets of oil. Knoll observed that also the sterile flowers were slippery and helped to prevent the insects' escape. Moreover, Knoll postulated that these hairs act like a sieve preventing large insects from entering the spathe chamber.

Besides Knoll's study there were only few observations on trap pollination at that time (e.g. Troll 1928, Schmucker 1930, Van der Pijl 1937). As Knoll published in German, his findings remained unknown or were misinterpreted by non-german-speaking botanists (Dormer 1960). It was up to Stefan Vogel, who still knew Knoll personally, to revive research on trap pollination. Vogel's studies were devoted to different groups including *Ceropegia* (Apocynaceae), *Aristolochia* (Aristolochiaceae) and various Araceae (Vogel 1965). In the latter, he focused on fungus-gnat pollination in *Arisarum* (Vogel 1978) and *Arisaema* (Vogel & Martens 2000). These fungus-mimicking plants attract midges that belong mainly to the families Mycetophilidae and Sciaridae. Vogel

found that *Arisarum* has a slippery surface (consisting of downward-pointing papillate cells), but insects can escape by their own attempt without being imprisoned for a certain period of time. This was the first'semi-trap' that has been described for the Araceae (see also Koach & Galil 1986). In the dioecious *Arisaema*, Vogel investigated the unusual trap-and-lure mechanism. Fungus-gnats do get released by the staminate inflorescences through a basal opening of the spathe in order to disperse pollen. In contrast, the pistillate inflorescences do not provide such an exit for the flies. They are prone to die unless they manage to escape via the spathe entrance. Vogel also found that in most of the species of *Arisaema* studied the slippery surfaces consisted of epidermal wax crystalloids while only few species had papillate cells like those of *Arum*. Vogel also was the first to make observations on *Zomicarpa riedelianum* in its natural habitat in Brasil, discovering remarkable analogies to the inflorescence traps of *Arisaema* (Vogel & Martens 2000). Moreover, Vogel probably was the first to raise the question whether and how the different types of traps in Araceae have evolved from a common ancestor (Vogel, pers. comm.). However, he could not draw firm conclusions as a robust phylogeny was not available at that time and therefore his considerations remained unpublished.

There are a few studies that deal with the role of spathe movements for trapping pollinators. Such movements were for example found in *Sauromatum* and *Typhonium* (Armstrong 1979, Dakwale & Bhatnagar 1982, 1985). These movements serve to occlude the floral chamber during anthesis and open it only after insects have become dusted with pollen. Similar movements were also found in *Colocasia* (Cleghorn 1913), but their function was not yet studied in detail.

Detailed studies of the morphology of the slippery surfaces exist for *Arisaema* (Vogel & Martens 2000), *Arum* (Knoll 1926, Bermadinger-Stabentheiner & Stabentheiner 1995), *Cryptocoryne* and *Lagenandra* (Ørgaard & Jacobsen). The latter two taxa also bear downward-pointing, papillae. However, unlike in *Arum* or *Arisaema* they are hair-like and produce substances of unknown chemical composition (Knoll, unpublished data). Information on further aroids is provided by a review of Poppinga *et al.* (2010) on slippery surfaces in angiosperms. However, some of their taxa (i.e. *Alocasia*, *Xanthosoma*) do not possess the typical slippery surfaces and it remains doubtful whether they actually function as traps.

There are several further studies by various authors dealing with pollination in trapping Araceae, many of which focus on the Areae clade. Several studies exist for the genera *Arum* (Gibernau *et al.* 2004, Albre & Gibernau 2008, Quilichini *et al.* 2010, Stökl

et al. 2010), *Theriophonum* (Dakwale & Bhatnagar 1997) and *Typhonium* (Banerji 1947, Van der Pijl 1953). Some authors also have examined the pollination of sapromyiophilous species of *Amorphophallus* (Thomsonieae) (Beath 1996, Jung 2006, Grimm 2009, Punekar & Kumaran 2010). Nevertheless, the focus of most studies is on the general pollination biology rather than on the specific trap mechanism. One of the few studies that emphasised the significance of the trap mechanism for the reproductive success was carried out by Lack & Diaz (1991) for *Arum maculatum*.

Despite a long tradition of research on trap pollination in aroids, several questions on the evolution, the structure, and the function of the trap mechanisms remain unanswered. For example, did trapping devices evolve *de novo* or have there been preadaptations? Are traps restricted to the members of the Aroideae subfamily or do they occur in the earlier diverging subfamilies as well? Did different types of traps evolve from a common ancestor? If not, what were the drivers for a parallel and multiple evolution of traps in the Araceae? These are some of the questions that I will try to answer in the following chapters.

CHAPTER 2

RECONSTRUCTING THE ORIGIN AND ELABORATION OF INSECT-TRAPPING INFLORESCENCES IN THE ARACEAE

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Angabe zum Eigenanteil des Dissertanten: David Bröderbauer hat die Daten für den vorliegenden Artikel gesammelt, ausgewertet, und den Text für das Manuskript (in Zusammenarbeit mit A. Diaz und A. Weber) verfasst.

ABSTRACT

• Premise of the study: Floral traps are among the most sophisticated devices that have evolved in angiosperms in the context of pollination, but the evolution of trap pollination has not yet been studied in a phylogenetic context. We aim to determine the evolutionary history of morphological traits that facilitate trap pollination and to elucidate the impact of pollinators on the evolution of inflorescence traps in the family Araceae.

• Methods: Inflorescence morphology was investigated to determine the presence of trapping devices and to classify functional types of traps. We inferred phylogenetic relationships in the family using maximum likelihood and Bayesian methods. Character evolution of trapping devices, trap types, and pollinator types was then assessed with maximum parsimony and Bayesian methods. We also tested for an association of trap pollination with specific pollinator types.

• Key results: Inflorescence traps have evolved independently at least 10 times within the Araceae. Trapping devices were found in 27 genera. On the basis of different combinations of trapping devices, six functional types of traps were identified. Trap pollination in Araceae is correlated with pollination by flies.

• Conclusions: Trap pollination in the Araceae is more common than was previously thought. Preadaptations such as papillate cells or elongated sterile flowers facilitated the evolution of inflorescence traps. In some clades, imperfect traps served as a precursor for the evolution of more elaborate traps. Traps that evolved in association with fly pollination were most probably derived from mutualistic ancestors, offering a brood-site to their pollinators.

INTRODUCTION

Changes in flower morphology have been of key importance for the diversification of angiosperms (Friis *et al.* 2006, Endress 2011). The modification of floral organs, their increasing synorganization, and the evolution of new floral structures have enabled adaptation to a wide array of pollinators (Claßen-Bockhoff *et al.* 2004, Whittall & Hodges 2007, Alcantara & Lohmann 2010). In some groups, the interplay of these processes led to the evolution of very complex flowers and inflorescences (Harris 1999, Rudall & Bateman 2002). The reconstruction of the evolutionary history of such morphological changes in a phylogenetic context allows a better understanding of the general patterns of plant–pollinator interactions (Fenster *et al.* 2004, DeWitt Smith 2010).

Floral traps ("Kesselfallenblumen" sensu Vogel 1965, 1999) are among the most sophisticated devices that have evolved in angiosperms in the context of pollination. Their key innovation is the formation of a chamber, which encloses the sexual organs. The inner epidermis of the chamber entrance is slippery, causing insects—commonly attracted by means of deception—to slip and to fall into the chamber. The slippery surface consists of downward-pointing papillae and/or is covered with epicuticular wax crystalloids (Vogel & Martens 2000, Poppinga *et al.* 2010). In some floral traps, the exit can be blocked either by hairs or elongated sterile flowers (Knoll 1926, Sakai 2002b, Coombs *et al.* 2011) or by active closure of the floral chamber (Armstrong 1979, Dakwale & Bhatnagar 1985). Floral traps are almost always protogynous (Vogel 1961, Dafni 1984, Thien *et al.* 2009). Pollinators are arrested for a defined period of time during the pistillate phase and can escape only during or after pollen release. In most cases, the escape is facilitated by the wilting of the trapping structures, while in few taxa a secondary opening of the floral trap creates a new exit (Vogel 1965). In addition to perfect traps just described, imperfect traps (so-called semitraps after Faegri & Van der Pijl 1971) also exist. In imperfect traps, insects are forced to exit the flower via a predetermined route so that pollen is deposited on their body. However, they are not arrested for a defined period of time. Perfect floral traps have evolved in at least eight unrelated families, predominantly in the basal angiosperms and the monocots (Dafni 1984, Thien *et al.* 2009, Urru *et al.* 2011). Well-known examples are *Aristolochia* (e.g., Sakai 2002b) and *Arum* (e.g., Knoll 1926, Gibernau *et al.* 2004). Although a number of studies have shed light on the interactions of floral traps and their pollinators (Vogel

1961, Diaz & Kite 2002, Bolin *et al.* 2009), how these complex traps have evolved from nontraps is still unknown.

The present study is the first to analyze the evolution of floral traps in a phylogenetic context. Our overall aim is to determine the evolutionary history of morphological traits that facilitate trap pollination and to elucidate the impact of pollinators on the evolution of traps. Our study system is the Araceae, a diverse family comprising over 3300 species in 126 genera (Boyce & Croat 2012). The most characteristic feature in this family is the inflorescence, which consists of a thickened flower-bearing spike, called the spadix, and a single, usually conspicuous bract, called the spathe (Fig. 1A). The spathe, the spadix, and the flowers are subject to various modifications and increasing synorganization. While the spathe is often inconspicuous or simply expanded in basal clades such as Gymnostachydoideae and Orontioideae, it frequently surrounds the spadix and forms a chamber around the flowers in higher clades such as Lasioideae and Aroideae (note that terms such as basal or higher used in the text for taxa refer to the topology of the phylogenetic tree and do not indicate primitiveness/advancement of any given character; Crisp & Cook 2005). Moreover, in the majority of clades the spadix is completely covered by bisexual flowers, while in the Aroideae the flowers are unisexual and arranged in distinct zones: the pistillate flowers are situated on the lower portion of the spadix and the staminate flowers on the upper portion, often separated by a zone of sterile flowers. In higher taxa of Aroideae such as *Arum* and allies the upper part of the spadix becomes sterile and serves as an osmophore (Fig. 1B). The whole family is protogynous. In clades with bisexual flowers, anthesis lasts for several days, while in monoecious taxa of Aroideae anthesis usually ceases after 1–2 d. Pollinators of Araceae are Diptera, Coleoptera, and Hymenoptera (Gibernau 2011). Interactions include food reward, mating mutualism, nursery mutualism, and deception (Gibernau *et al.* 2010, Chartier 2011). Trap pollination is known from several clades of the subfamily Aroideae and includes both perfect as well as imperfect traps (Ørgaard & Jacobsen 1998, Vogel & Martens 2000, Gibernau *et al.* 2004), but most of the genera of Araceae apparently have no traps. The diversity of inflorescence forms and the occurrence of different pollination syndromes make the Araceae an ideal object for studying the evolution of trap pollination.

In this paper, we specifically address the following questions: (1) In which taxa of Araceae do trapping devices occur and how did they evolve? (2) Did the different types of inflorescence traps evolve from a common trap-ancestor and did perfect traps evolve

Fig. 1. Inflorescence morphology of selected Araceae. **(A)** *Anthurium digitatum* (Jacq.) Schott, spadix with bisexual flowers and an expanded spathe. **(B)** *Arum nigrum* Schott, spadix with unisexual and sterile flowers and a spathe separated into a tube and a blade. The front part of the spathe tube is removed for better visibility of the spadix; a = pistillate flowers; b = elongated sterile flowers; c = staminate flowers; d = sterile appendix. Scale bars = 1 cm.

from imperfect traps? (3) Are traps associated with specific insect groups? To assess the frequency of trap pollination in the family, we examined the presence and structure of trapping devices in taxa from all major clades. In addition, we reconstructed the phylogeny of Araceae based on the molecular data of Cusimano *et al.* (2011), complemented by sequences of one additional taxon. Trapping structures, trap types, and pollinator types were then mapped onto the phylogeny and the ancestral states were reconstructed with maximum parsimony and Bayesian inference.

MATERIALS AND METHODS

Plant material — We studied taxa from all genera available covering all tribes of the family (sensu Mayo *et al.* 1997) except for the monotypic subfamily Gymnostachydoideae, for which no samples were available (and which with certainty does not form traps). Inflorescences were collected during anthesis in the field and in botanical gardens. The samples were stored in 70% alcohol as well as dried at room temperature. We also used material already stored in ethanol from various wet collections. Voucher specimen information is listed in Appendix 1.

Occurrence, structure, and function of traps — *Trapping devices* — Based on the trap characters defined by Vogel (1965, 1999), we examined the four morphological characters that—alone or in combination—are essential to trap and detain pollinators and thus allow us to infer the presence of a trap from the inflorescence morphology: (1) Spathe shape: We used the typification of spathe type by Grayum (1990). Additional information on missing or misclassified genera in Grayum's study was taken from the more recent descriptions of spathe shape in Mayo *et al.* (1997). Grayum's spathe types are: type 1 = unmodified, bractlike; type 2 = expanded and/or colored (including boat-shaped Monsteroideae), type 3 = enclosing spadix, i.e., spathe margins convolute at least in the lower part of spadix; type 4 = constricted, i.e., the spathe more or less completely surrounds the spadix, forming a basal chamber and an apical blade. Only taxa with spathe type 3 or 4 can form traps as the presence of an at least rudimentary chamber is essential for retaining insects. (2) Slippery surface: The presence of epicuticular wax crystalloids and/or downward pointing papillate cells on the epidermis of the spathe was studied with scanning electron microscopy (SEM) (JEOL JSM6390, Akishima, Japan). We investigated samples of 142 species in 76 genera. Samples were taken from all different regions of the organ after preliminary investigation under light microscope (Olympus BX50, Tokyo, Japan). Samples used for the assessment of cell shape were dehydrated in an increasing series of ethanol from 70% to 85% to 96% for 20 min in each solution and then transferred to acetone. Consecutively, samples were critical-point-dried and sputter-coated with gold and investigated with SEM. Samples used for the examination of epicuticular wax crystalloids only were air-dried before sputter-coating, because ethanol and heat would alter the crystal structure of the wax (Barthlott & Wollenweber 1981). (3) Elongated sterile flowers: Information on the presence of elongated sterile flowers was taken from Mayo *et al.* (1997). (4) Temporary closure of the spathe during anthesis: Spathe movements in specimens cultivated in the Botanical Garden of Vienna were recorded during daily observations. We also used a Nikon Coolpix P 5000 camera (Tokyo, Japan) to take images automatically every 10 min. In addition, information on spathe movements was also taken from Mayo *et al.* (1997).

Functional types of traps — In our study, we define a genus as having a trap when the spathe shape is "enclosing the spadix" (type 3) or "constricted" (type 4) and when one or more of the aforementioned trapping devices were also present. For our results, we identify and classify the range of functional types of traps resulting from the different combinations of these trap characters. The functional types also relate to the

mode of operation of traps based on the following stages (sensu Vogel 1965): (1) mode of capture, (2) mode of retention, and (3) mode of release of pollinators.

Reconstruction of the evolutionary history — *Molecular phylogeny* — We reconstructed the phylogeny of the Araceae using the molecular matrix of Cusimano *et al.* (2011) , which includes 113 genera of Araceae and three outgroup taxa (Acorus, Hedyosmum, Tofieldia). The alignments of multiple chloroplast markers (rbcL, matK, partial trnK intron, partial tRNA - Leu gene, trnL – trnF spacer, and partial tRNA - Phe gene) were obtained from TreeBase (study 11083, tree Tr26254). We added sequences for one taxon, namely *Colocasia gigantea* (Blume) Hook.f., which had been shown to be more closely related to *Alocasia* than to the other taxa of *Colocasia* (Renner & Zhang 2004, Nauheimer *et al.* 2012b). Sequences of *Colocasia gigantea* were downloaded from GenBank (EU193194.1, EU886581.1, EU193409.1, EU193321.1) (Cusimano *et al.* 2008, 2010, Mansion *et al.* 2008) and aligned manually. The new matrix of the combined regions consisted of 117 taxa and 4498 aligned characters.

Sequence data were analyzed with maximum likelihood and Bayesian methods following Cusimano *et al.* (2011). The best fitting model of evolution was determined as GTR + Γ by the Akaike information criterion (Akaike 1974) as implemented in the program jModelTest v0.1.1 (Posada 2008). For maximum likelihood analysis, we used the software RAxML 7.2.8 (Stamatakis *et al.* 2008) available through CIPRES Science Gateway (Miller *et al.* 2010). RAxML uses the GTRCAT approximation of the GTR + Γ model, with the gamma shape parameter having 25 rate categories. Bootstrap values were obtained by running 1000 replicates. The Bayesian analysis was run with the program MrBayes (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003), available at the Bioportal cluster (http://www.bioportal.uio.no). We performed four runs of eight million generations, with trees sampled every 500th generation. The convergence diagnostic in MrBayes was used to assess the convergence of all runs. For each run, the first 25% of the resulting 40 000 trees were discarded as burnin. For consecutive analyses, we sampled 10 000 Bayesian trees from two different runs. A 50% majority-rule consensus tree was reconstructed for which polytomies were randomly resolved, and a length of 10^{-7} was assigned to branches with zero or negative length using the software Mesquite 2.0 (Maddison & Maddison 2007).

Character evolution — Character states of all trapping devices and the functional types of traps were mapped onto the Bayesian 50% majority-rule consensus tree, and

ancestral states were reconstructed applying maximum parsimony (MP) in Mesquite and Bayesian analysis in the software SIMMAP 1.5 (Bollback 2006). The outgroup taxa (i.e., *Acorus*, *Hedyosmum*, *Tofieldia*) were excluded from the analyses. Molecular rates of evolution are correlated with generation time (Smith & Donoghue 2008). Since we have a wide range of generation times in Araceae, we chose to use the phylogram instead of an ultrametric tree for both analyses. In SIMMAP, we used all 10 000 Bayesian trees to calculate posterior probabilities (PP) for ancestral states, thus taking into account phylogenetic uncertainties. To assess how prior choice may influence the posterior results (Schultz & Churchill 1999), we used two different sets of priors for all characters studied. One set of priors was calculated with the MCMC approach offered in the software, the second set consisted of the program's default priors. The advantage of the MCMC approach is that overall rate values are sampled and the best fitting gamma distribution can be found, instead of guessing and trying a large number of different priors. However, one has to keep in mind that the results of ancestral state reconstructions always depend on the underlying assumptions and that inferences may fail if the model applied is unrealistic (see Crisp & Cook 2005). Results are shown for calculations with the MCMC prior if not stated otherwise. As SIMMAP does not allow polymorphic character states, we coded them as ambiguous. For the mapping of the character trap type, we used an additional approach in Mesquite: it can be argued that transitions between character states are more likely to be imbalanced in complex characters due to asymmetric gain-loss probabilities (for a detailed discussion, see Kohn *et al.* 1997, Omland 1999). Thus, transitions between different types of traps may occur with higher probability than transitions between nontraps and traps. Therefore, we made a step matrix for the MP reconstruction in Mesquite, where each transition between two trap types costs one step, while a transition between a trap and a nontrap costs two steps. The average number of changes between the different types of traps (including nontraps) was estimated with the "summarize changes" option in Mesquite for 10 000 Bayesian trees reconstructed with the MP method.

Association between inflorescence traps and pollinators — *Pollinators —* Information on pollinator types in Araceae was taken from reviews by Gibernau and his coworkers (Gibernau 2003, 2011, Gibernau *et al.* 2010). In addition, information on pollination of *Dracunculus vulgaris* Schott in H. W. Schott & S. L. Endlicher was taken

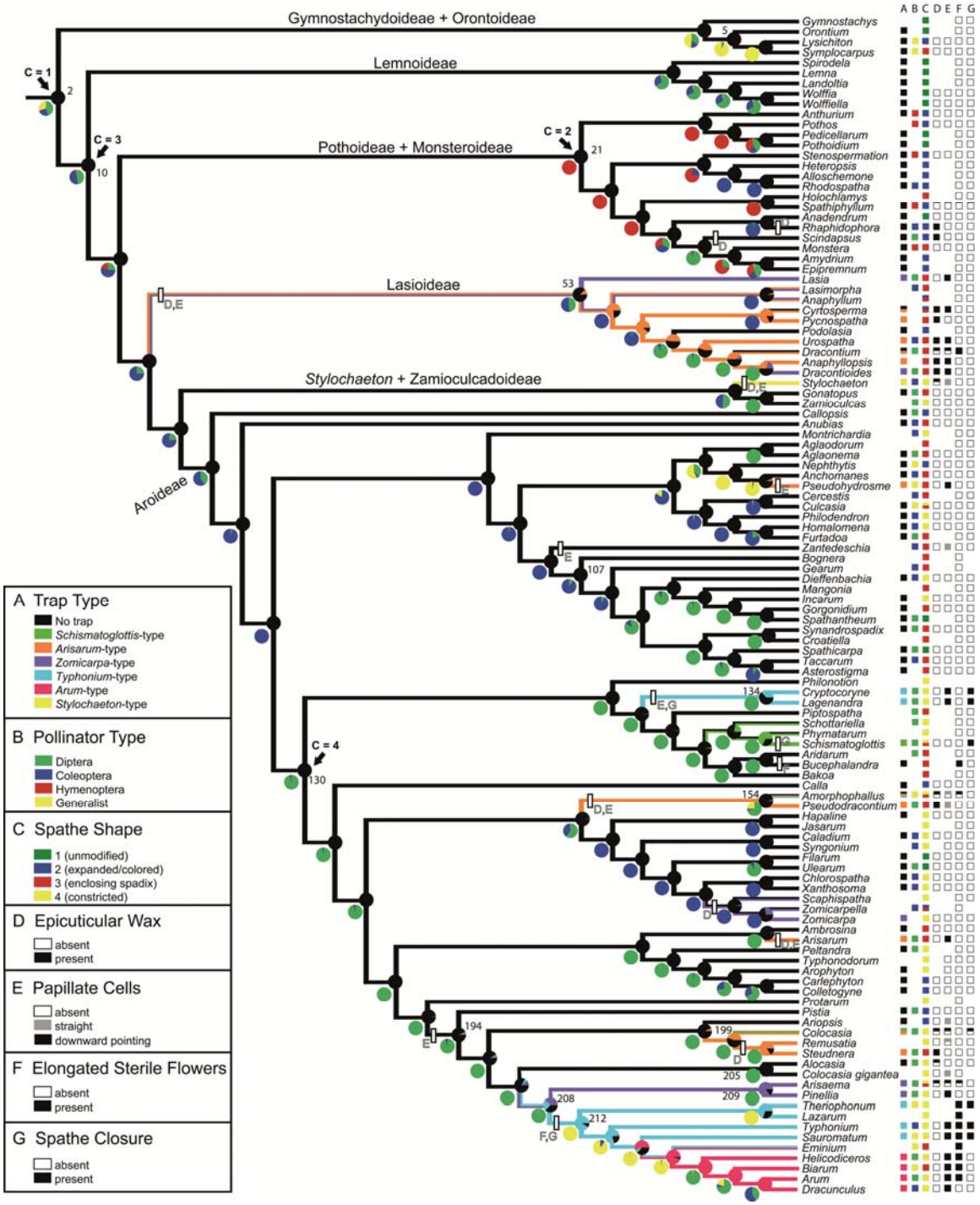

Fig. 2. Ancestral state reconstructions of trap pollination in Araceae. Colors on lines indicate reconstruction of trap types (A) with maximum parsimony in Mesquite 2.0. In cases where lines have more than one color, ancestral states could not be resolved unambiguously. Pie charts on the nodes display the posterior probabilites of trap types calculated with Bayesian inference in SIMMAP 1.5. Pie charts below the nodes display the posterior probabilites of pollinator types (B). Arrows point at the earliest appearance of the four types of spathe shape (C). Bars indicate the appearance of characters D-G along the phylogeny. Coding for all characters is shown at the right. Node numbers are referred to in the text.

from Schmucker (1930) and Meeuse & Hatch (1960). *Lasia spinosa* (L.) Thwaites has been observed to be visited by flies in Xishuangbanna Tropical Botanical Garden, Yunnan, China (Yin J. T., personal communication). Pollinators were classified into four

categories: Diptera, Coleoptera, Hymenoptera, and generalist (i.e., more than one type of pollinators). Ancestral states of pollinator type were reconstructed using the methods described above.

Character association — We tested for correlated evolution of trap pollination with pollinator type using the programs SIMMAP 1.5 and MacClade 4 (Maddison & Maddison 2000). The different trap types were summed up under the character trap presence with the states trap and nontrap. For all correlations, we used the Bayesian 50% majority-rule consensus tree. All taxa with unknown character states were removed, resulting in a matrix with 54 taxa. SIMMAP allows multiple comparisons of characters with an unlimited number of character states. The software calculates the time that characters spend in particular states along the tree as a measure for association. The observed distribution of the character states is then compared to a predicted distribution to assign a *P* value. We ran 1000 simulations and drew 500 predictive samples to calculate the *P* value. To check whether prior choice influences the results, we made all calculations using the priors calculated with the MCMC approach offered by the software, as well as with the default priors. The Character Correlation Test (CCT) in Mac-Clade 4 only allows two binary characters to be correlated. Therefore, we coded pollinators as Diptera/other or Coleoptera/other, and correlated each of these two characters schemes with trap presence. The CCT tests whether changes in the dependent character (i.e., trap presence) are more concentrated than expected on those branches that have a particular state in the independent character (i.e., fly/beetle pollination). We used ACCTRAN and DELTRAN options to resolve equivocal reconstructions of ancestral character states and applied MINSTATE and MAXSTATE reconstructions for the calculation of correlated evolution. For each run, 100 000 simulations were performed.

RESULTS

Occurrence of traps — *Trapping devices* — The coding of all characters studied is shown in Fig. 2. Character sampling covers the majority of clades but is not complete. The reader therefore must be aware that inferences may vary if missing taxa are added or scored. The most common spathe shapes found across the 114 genera were type 3 (spathe enclosing spadix) (found in 37genera) and type 4 (spathe constricted) (36 genera). Both these types were especially abundant in the Aroideae subfamily. Unmodified spathes (type 1) were most common in Gymnostachydoideae, Orontioideae, and Lemnoideae (14 genera). Expanded/colored spathes (type 2) were found in 20 genera, 11 of which belong

to the subfamilies Monsteroideae and Pothoideae. In addition, seven genera contain species with different spathe shapes.

Of 76 genera studied under SEM, 31 possessed either epicuticular wax crystalloids, papillate cells, or both on their adaxial spathe surface (Fig. 3). Papillate cells in *Amorphophallus*, *Colocasia*, *Pseudodracontium*, *Stylochaeton*, *Ariopsis*, *Colocasia gigantea*, *Remusatia*, and *Zantedeschia* were not downward pointing but orientated horizontally. Because the latter four taxa did not have any additional trap characters, they were coded as equivocal for trap type. Epicuticular wax crystalloids formed platelets (Fig. 3A, F), tubules (Fig. 3B), threads (Fig. 3B), or branched rodlets (Fig. 3.C). Downward pointing papillae were found in 16 genera (Fig. 3C–E). In Lasioideae, downward pointing papillae usually formed imbricate rows like roof tiles (Fig. 3E). Moreover, several lasioids had an additional epicuticular wax layer (Fig. 3C). In *Pycnospatha*, the cells were flattened, and papillae were no longer recognizable. In this case, the function of a slippery surface was completely transferred to the epicuticular wax crystalloids. In the subfamily Aroideae, downward pointing cells were not fused (Fig. 3D). In contrast with slippery surfaces in the Lasioideae, the co-occurrence of an epicuticular wax layer was found to be rare in the Aroideae, except for a few taxa such as *Amorphophallus* (Fig. 3A), where straight papillate cells and epicuticular wax crystalloids are present. Moreover, wax crystalloids were also detected on the sterile appendix of the spadix in several species of *Amorphophallus*. In several taxa, cuticular folds were present on papillate cells (Fig. 3E). These folds also occurred in nontraps with tabular or convex epidermal cells, for example, in several genera of tribe Spathicarpeae (Fig. 2, node 107). A detailed description of cell shape, structure of wax crystalloids, and presence of cuticular folds in all taxa studied is presented in Appendix S1.

Elongated sterile flowers were found in 12 genera, eight of which belong to the tribe Areae. Temporary closure of the spathe during anthesis occurred in seven genera. In *Colocasia* (Fig. 4A–C) and *Schismatoglottis*, the entire spathe blade closes. In *Sauromatum*, *Theriophonum*, and *Typhonium* (Fig. 4D–F), only the constriction closes, thereby secluding the basal chamber.

Functional types of traps — On the basis of the presence and combination of trapping devices, we could identify six types of traps (Fig. 5). (1) In the *Schismatoglottis* type (Fig. 5A), insects are trapped by temporary closure of the spathe blade, thereby enclosing the whole spadix. A slippery surface is not present. The spathe is constricted,

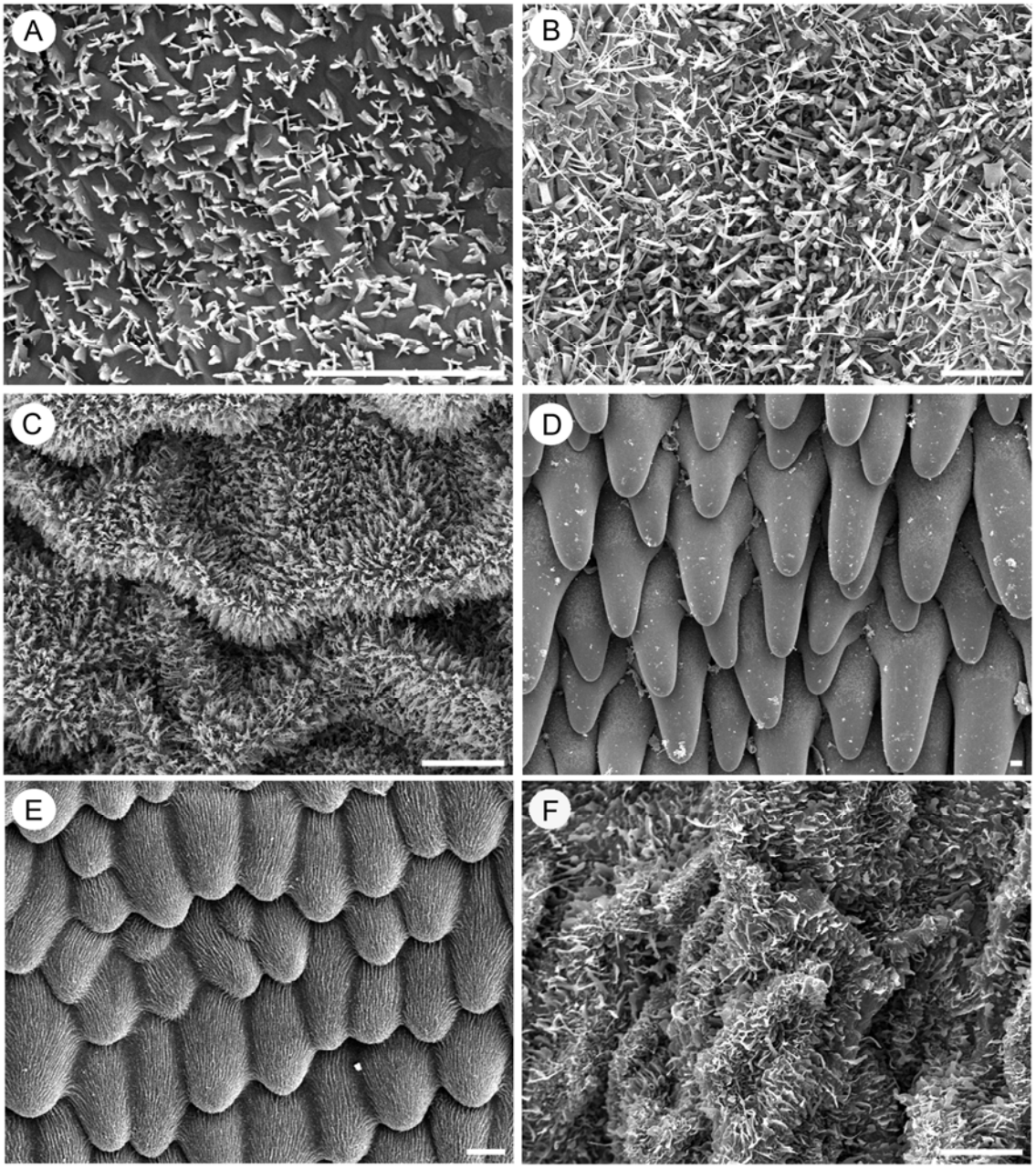

Fig. 3. Slippery surfaces on the adaxial spathe epidermis in Araceae. **(A)** Wax platelets (*Steudnera kerrii* Gagnep.). **(B)** Wax tubules and threads (*Amorphophallus taurostigma* Ittenbach, Hett. & Bogner). **(C)** Imbricate downward pointing papillate cells and branched wax rodlets [*Urospatha sagittifolia* (Rudge) Schott]. **(D)** Downward pointing papillate cells (*Helicodiceros muscivorus* L.f.). **(E)** Imbricate downward-pointing papillate cells with cuticular folds (*Dracontium asperum* K. Koch). **(F)** Wax platelets on perpendicular papillate cells (*Stylochaeton* cf. *hypogaeus* Lepr.). *Note:* Cells in samples A–C and F have shrunk due to drying at room temperature. Scale bars = 10 μm.

forming a chamber around the pistillate flowers at the lower part of the spadix. The spathe blade usually opens a small slit only during pistillate phase. The constriction closes after insects have left the chamber and moved to the upper part of the inflorescence. In the subsequent staminate phase, the spathe blade expands and often bends back abruptly, thus exposing the upper part of the spadix. The spadix itself is composed of a sterile zone located between pistillate and staminate flowers. In several

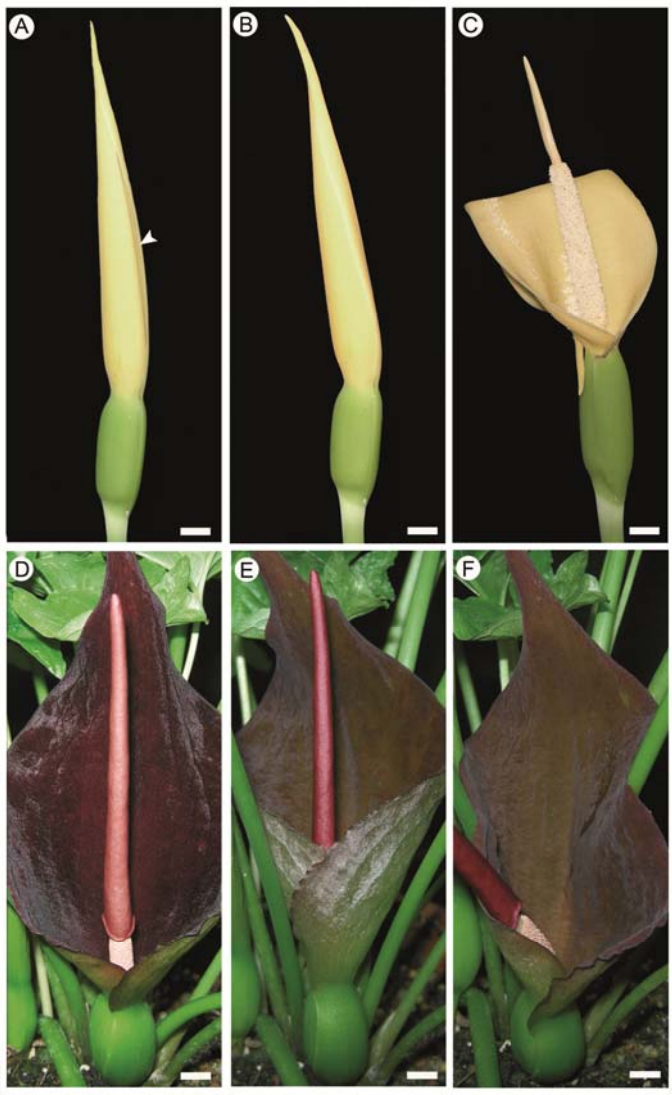

Fig. 4. Spathe closure during anthesis. **(A–C)** *Colocasia fontanesii* Schott. **(A)** The spathe blade opens a narrow slit (arrowhead) during the pistillate phase. **(B)** The spathe blade closes at the end of the pistillate phase. **(C)** The spathe blade reopens and reflexes during the staminate phase. The constriction above the spathe chamber is now closed. **(D–F)** *Typhonium trilobatum* (L.) Schott. **(D)** The spathe constriction above the floral chamber opens at the beginning of the pistillate phase and spadix tilts forward. **(E)** The constriction closes at the end of the pistillate phase; the spadix is erect. The color of the spathe blade gradually turns from red to brown. **(F)** The constriction reopens during the staminate phase, and the spadix tilts forward again. Scale bars = 1 cm.

taxa, a sterile appendix above the staminate flowers is also present. Anthesis lasts for about 24 h. The *Schismatoglottis* type was found in only two genera of Aroideae, *Schismatoglottis* and *Colocasia*. (2) In the *Arisarum* type (Fig. 5B), no spathe closure occurs, but slippery surfaces (wax, downward pointing papillae) are present on the spathe. Spathe shape usually is an “enclosing-spadix” type. This trap type occurs in the subfamilies Lasioideae and Aroideae. In the *Arisarum* type traps of the Lasioideae, spathe margins are convolute only in the lower part of the spathe, while in the Aroideae the floral chamber makes up more than half of the spathe and encloses at least a part of the staminate section of the spadix. Anthesis lasts for several days to weeks. The spadix does not contain any elongated sterile flowers, which might serve as a barrier and the spathe is usually not constricted. Therefore, insects will glide down into the lower part of the spathe but are not arrested and can escape by climbing the spadix and/or flying out of the chamber. Thus, traps of the *Arisarum* type represent imperfect traps. (3) Traps of the

Zomicarpa type (Fig. 5C) are similar in shape to the *Arisarum* type, but the spathe margins are always convolute to the upper third of the spathe, and the fertile part of the spadix is completely hidden inside. The entry to the floral chamber often is masked by a hooded spathe blade. This type of trap occurs in subfamily Lasioideae as well as in Aroideae tribe Arisaemateae (Fig. 2, node 209) and the genus *Zomicarpa*. Slippery surfaces often consist of wax crystalloids, which can also be present on the sterile appendix in *Arisaema*. In several taxa, the epidermis of the spathe can also consist of downward pointing papillate cells. Insects cannot escape until the spathe margin bulges out at the lower spathe or opens completely and builds a secondary exit. *Arisaema* is unique in being dioecious (Vogel & Martens 2000). Only the male inflorescences provide an exit for insects. The female inflorescences remain closed, and the captured insects cannot escape. Anthesis usually lasts for several days to weeks. (4) Traps of the *Typhonium* type (Fig. 5D) are found in the tribes Cryptocoryneae (Fig. 2, node 134) and Areae (Fig. 2, node 212). Here, slippery surfaces are made up by papillate cells. The spathe closes temporarily. There are two means by which the floral chamber is secluded. In Cryptocoryneae an extension of the spathe margin occludes the floral chamber, while in Areae a twist of the spathe causes the closure of the constriction between the floral chamber and the blade. All taxa of the *Typhonium* type have monoecious inflorescences. In most taxa, the floral chamber also encloses the staminate flowers, while in *Typhonium* they are situated above the constriction. Therefore, pollen does not fall into the floral chamber but is deposited on the constriction. After the spathe has opened again and the slippery surface ceased to be slippery, insects can escape. Anthesis usually lasts for 24 h. (5) In the *Arum* type (Fig. 5E), which is restricted to four taxa of the tribe Areae, traps do not close their constriction. During anthesis, escape is prevented by the presence of downward pointing papillae on the spathe and slippery elongated sterile flowers on the spadix. After anthesis, these parts wither, and the insects can leave the trap through climbing. Elongated sterile flowers can occur in one or two whorls, and their shape is subulate to filiform. Pistillate and staminate flowers are enclosed by the floral chamber. Anthesis usually lasts for 24 h. (6) *Stylochaeton* appears to have a unique trapping mode (Fig. 5F). The gliding surface consists of straight papillate cells and an epicuticular wax layer. At the beginning of anthesis, the spadix is hidden inside the spathe. When pollen is released, the spadix starts to grow above the spathe chamber, forming a ladder that presumably facilitates the escape of insects. Anthesis lasts for one to a few days.

Reconstruction of the evolutionary history of traps — *Phylogeny* — The topologies of the ML and Bayesian analyses proved consistent with those of Cusimano *et al.* (2011). The additional taxon *Colocasia gigantea* is grouped with *Alocasia*. The branch is strongly supported with a bootstrap support of 100 and a Bayesian posterior probability of 1 (Fig. 2, node 205).

Character evolution — Ancestral state reconstructions of trap type and selected transitions in other trap characters are shown in Fig. 2 , the 50% majority-rule consensus tree of 10 000 Bayesian trees sampled from two runs. Detailed ancestral state reconstructions of all trap characters not displayed in the main figures are shown in Appendix S2–S6. Results of MP and Bayesian approach were consistent overall. Different reconstructions were found in the character trap type in the common ancestors of four clades (Fig. 2, nodes 53, 134, 154, 199). In the MP analysis, these nodes were reconstructed as having traps because the step matrix favored transitions between different trap types. In contrast, in the Bayesian analysis, these nodes were reconstructed as nontraps from which different types of traps were derived. Thus, the Bayesian approach shows a higher number of changes between traps and nontraps. Two ways of handling the choice of prior in Bayesian analysis did not affect the reconstruction of character history except for the character spathe shape. The parameters for the default Γ -prior for multistate characters were $\alpha = 1.25$, $\beta = 0.25$, and $\alpha = 1.00$ for the default B-prior for two-state characters. The priors calculated with the MCMC- approach for the various characters are: trap type (Γ: $\alpha = 8.58$, $\beta = 0.24$), spathe shape (Γ: $\alpha = 16.62$, $\beta = 0.37$), epicuticular wax (B: $\alpha = 11.55$), papillate cells (B: $\alpha = 11.28$), elongated sterile flowers (B: $\alpha = 2.65$), and spathe closure (B: $\alpha = 5.05$). For spathe shape, the calculations with the default prior yielded results more similar to the MP reconstruction than the calculations with the MCMC prior. The common ancestor of Araceae (Fig. 2, node 2) was most likely type 1 (unmodified) (PP = 48%) with the default prior (followed by enclosing spadix, PP = 39%), while it was most likely type 3 (enclosing spadix) with the MCMC prior (PP = 46%) (followed by constricted, PP = 39%). The majority of nodes did not change in their reconstructed ancestral state with a different choice of prior. In the MP approach, node 2 is reconstructed as spathe type 1 in accordance with the Bayesian reconstruction using the default prior (Appendix S2). Apart from Lasioideae and Aroideae, where spathe shape 3 is more common, it is only present in one extant taxon of subfamily Orontioideae (Fig. 2, node 5) and two taxa of subfamily Monsteroideae, while spathe shape 1 is more common in the latter clades. We present the results for spathe

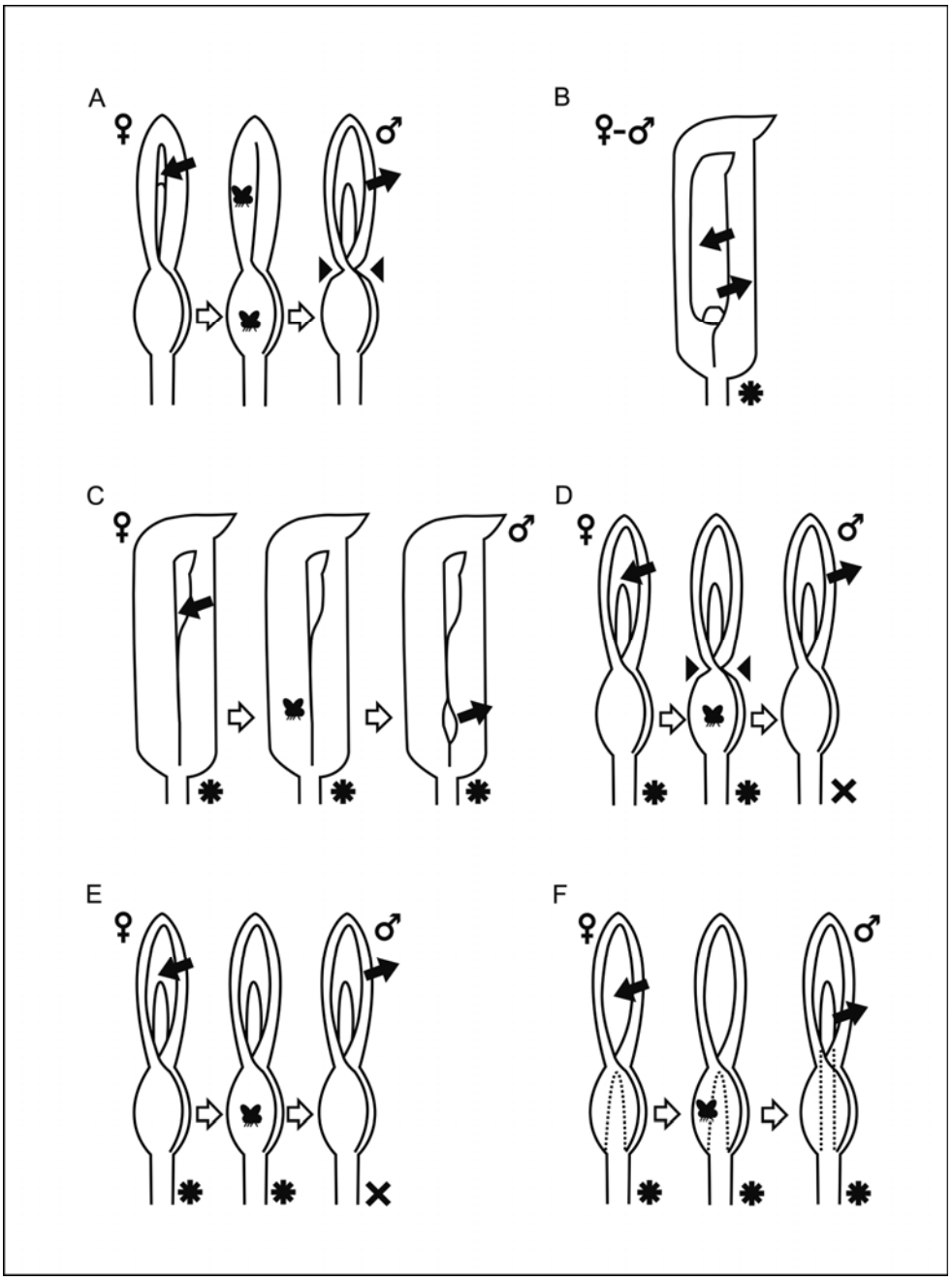

Fig. 5. Functional types of traps in Araceae. **(A)** *Schismatoglottis* type. The insects are retained by spathe movements; slippery surfaces are absent. **(B)** *Arisarum* type. An imperfect trap with a slippery spathe surface. Insects slip and fall into the spathe chamber but can escape unhampered by climbing the spadix or flying off. **(C)** *Zomicarpa* type. Insects are trapped inside the inflorescence by slippery surfaces and are released through a secondary exit formed by a movement of the spathe. **(D)** *Typhonium* type. Insects glide down slippery surfaces and are retained in the floral chamber by closure of the spathe constriction. During the staminate phase, the constriction reopens, and slippery surfaces cease to be slippery. **(E)** *Arum* type. The insects are trapped by slippery spathe surfaces and sterile flowers on the spadix that partially occlude the spathe chamber. Insect release is facilitated by withering of the slippery organs. **(F)** *Stylochaeton* type. Insects are trapped by slippery spathe surfaces. In the pistillate phase, the spadix is enclosed in the spathe chamber. During the staminate phase, the spadix grows out of the chamber, and insects can escape via climbing. ***Gender symbols*** indicate pistillate and staminate phase of anthesis. ***Black arrows*** indicate arrival and departure of pollinators. ***Arrowheads*** indicate closure of the spathe constriction. The ***insect symbol*** indicates the pollinator's residence during arrest. ***Asterisks*** indicate the presence of an intact slippery surface; ***crosses*** indicate that the slippery surface has withered and ceased to be functional.

shape calculated with the default prior in the Bayesian analysis in Fig. 2. In all reconstructions, inflorescences that have at least a rudimentary floral chamber had already evolved very early in the history of Araceae, possibly in the branch preceding node 10 (PP = 43%). Spathe shape 4 (constricted) only occurs in subfamily Aroideae. We found that it evolved several times within basal clades of the subfamily and formed the ancestral state of a large clade including all remaining taxa of Aroideae (Fig. 2, node 130). Within this clade, spathe shape 4 was lost several times. Epicuticular wax crystalloids, papillate cells, elongated sterile flowers, and spathe closure during anthesis have evolved repeatedly in various clades (Appendix S3–S6). In Lasioideae, *Stylochaeton* and *Arisarum* the former two traits occur simultaneously. In most cases, they are associated with trap pollination. *Rhaphidophora* and *Scindapsus* had the only two nontraps with a pronounced epicuticular wax layer in our study. However, the wax layer does not contain three-dimensional crystalloids, but forms an irregular crust. Furthermore, their spathe shape is rather boat-shaped without convolute spathe margins. Elongated sterile flowers occur in *Amorphophallus*, *Arisaema*, *Dracontium*, *Bucephalandra*, and in the tribe Areae.

Inflorescence traps have evolved at least 10 times within the family (Fig. 2). The common ancestor of Araceae does not have a trap (PP = 100%). Moreover, traps do not occur in any genus of the basal clades. Among the basal subfamilies with inflorescence having a lower degree of synorganization, Lasioideae is the only clade where traps have been found. Most traps occur in the Aroideae where at least seven independent events have led to the formation of traps in at least 19 genera. With the exception of the *Arum* type and the *Stylochaeton* type, all functional types of traps have evolved in more than one clade. The *Stylochaeton* type is restricted to a single genus and has evolved from a nontrap. The *Arisarum* and the *Zomicarpa* type are the most widespread types, being also present in Lasioideae. In this subfamily, the latter type is derived from the former in *Dracontioides*. The trap type of the common ancestor of all Lasioideae (Fig. 2, node 53) could not be resolved due to the different states in *Lasia* and the core lasioids. In the Bayesian analysis, it is inferred to be a nontrap (PP = 88%), implying that traps have evolved twice in Lasioideae, i.e., once in *Lasia* and once separately in the remaining clade. For MP, the node is reconstructed as a trap (equivocal for the *Arisarum* type and the *Zomicarpa* type). In the Areae clade, the *Arum* type is derived from the *Typhonium* type. The common ancestor of the clades Areae and Arisaemateae cannot be resolved unambiguously. In the Bayesian analysis, the *Zomicarpa* type has the highest probability

(PP = 39%) followed by the *Typhonium* type (PP = 23%), while in MP it is equivocal (*Zomicarpa* or *Typhonium* type).

The average number of changes between traps and nontraps (mean ± SD) over 10000 trees was 9.7 ± 0.73 changes from nontraps to traps and 2.8 (± 0.99) reversals. Changes occurred most often from nontraps to imperfect traps of the *Arisarum* type (4.20 ± 0.41) and from the latter to the *Zomicarpa* type (1.34 ± 0.35). In all other transitions, ≤ 1.0 changes occurred.

Association between trap pollination and pollinators — *Pollinators* — Prior choice in the Bayesian approach had an impact on the ancestral state reconstruction of pollinator types. In the reconstruction using the MCMC prior (Γ: $\alpha = 2.86$, $\beta = 0.01$), pollination by Diptera prevailed in the majority of clades, even if the extant taxa were not pollinated by flies (Appendix S7). The reconstruction using the default prior was considerably different, as fly pollination was less common except for subfamily Aroideae. Here we focus on discussing the ancestral state reconstruction of the Bayesian analysis calculated with the default prior in Fig. 2 since it is closer to the results of MP analysis in Mesquite. It was not possible to reconstruct the pollinator type of the common ancestor of Araceae unambiguously. In the Bayesian analysis, the most probable common ancestor was Diptera with a posterior probability of 46%, followed by generalist pollinators (30%) and Coleoptera (24%). The reconstruction with MP was equivocal, too (Appendix S7). Bee pollination is restricted to the subfamilies Pothoideae and Monsteroideae (Fig. 2, node 21). The common ancestor of subfamily Aroideae was most probably pollinated by beetles (PP = 60%). Subsequently, a change from beetle to fly pollination occurred in the branch leading to node 130 (Fig. 2). With the exception of tribes Caladieae (Coleoptera) and Areae (generalist pollination by flies and beetles), all higher clades of Aroideae have Diptera as the ancestral state. Of 10 clades containing traps, six clades most probably had a fly-pollinated ancestor, whereas beetle pollination was ancestral only once (i.e., *Zomicarpa*). In two clades, the ancestral state was ambiguous, and in one clade, pollination was probably achieved by more than one type of pollinator.

Character association — Results of the correlation analyses are shown in Table 1. Regardless of the method of reconstruction applied, pollination by Diptera was significantly correlated with trap pollination in CCT. In contrast, there was no significant correlation between Coleoptera and trap pollination. Likewise, regardless of prior choice considered here, SIMMAP found a correlation between trap pollination and Diptera,

TABLE 1. Correlation of trap pollination and pollinator type in Araceae calculated with CCT in MacClade 4 and the character association test in SIMMAP 1.5. *P*-values for results in SIMMAP before Bonferroni-correction are given in brackets.

Test	Diptera	Coleoptera	Hymenoptera	Generalist
CCT ACCTRAN	$P < 0.01$	$P = 0.22$	-	-
CCT DELTRAN	$P < 0.01$	$P = 0.17$	-	-
SIMMAP	n.s. ($P = 0.04$)	n.s. ($P = -0.09$)	n.s. ($P = -0.16$)	n.s. ($P = 0.46$)

although the correlation became nonsignificant after application of Bonferroni-correction for multiple comparisons. Pollination by Coleoptera, Hymenoptera, or generalists was never correlated with trap pollination.

DISCUSSION

The evolution of floral traps depends on the presence of several morphological traits that facilitate the capture and retention of pollinators (Vogel 1965). To understand the drivers for the evolution of trap pollination in the Araceae, we studied (1) the occurrence and function of trapping devices, (2) the emergence of different types of traps, and (3) the association between traps and the pollinating fauna.

Occurrence of traps and the origin of trapping devices — This study demonstrates that trap pollination is more widespread in Araceae than was previously thought. Inflorescence traps are present in at least 27 genera. We found that they are not restricted to several clades of the subfamily Aroideae, but also occur in *Stylochaeton* (*Stylochaeton* clade sensu Cusimano *et al.* 2011) as well as in several genera of the subfamily Lasioideae.

The precondition for the evolution of traps was the presence of a floral chamber formed by the spathe. Although we cannot completely exclude other possibilities, our ancestral state reconstructions indicate that the common ancestor of Araceae most likely had a bract-like spathe. Subsequently, a floral chamber had already evolved in the early history of the family. Nevertheless, this key innovation was not immediately followed by the evolution of traps. Therefore, it is probable that the spathe chamber is a preadaptation that originally served another function. As a bract, the spathe surrounds and thus protects the developing inflorescence. This was most likely its ancestral—and only—function (Grayum 1990). In extant aroids, there are many further functions. In several taxa, the spathe is expanded and colored, an aid in attracting pollinators (Grayum 1990, Kraemer & Schmitt 1999). In other taxa, the spathe base remains furled round the flowers to form a floral chamber throughout flowering and seed set (e.g., *Alocasia*, *Caladium*,

Dieffenbachia, *Philodendron*). Here, it often serves as a mating chamber or brood site (Gibernau *et al.* 2000, Miyake & Yafuso 2005, Maia & Schlindwein 2006). Provision of such rewards is essential for pollination success, as these guarantee that the insects will stay in the inflorescence until the staminate phase. Young (1986) showed that beetles that fed on sterile flowers in the spathe chamber of *Dieffenbachia longispatha* left the inflorescence before pollen-shedding when these food bodies were removed. The evolution of the ancestral spathe chamber thus probably included functions found in extant species such as shelter, food rewards, and/or a mating site for its pollinators (Chartier 2011). Plant–pollinator interactions in such rewarding taxa differ fundamentally from true traps, and one should be cautious to deduce the presence of trap pollination from the shape of the inflorescence alone, because trapping depends on further trapping devices.

The most common device for trapping insects is a slippery plant surface. Such surfaces are composed of an epicuticular layer of wax crystalloids and downward pointing papillate cells (Poppinga *et al.* 2010). In Araceae, both traits have evolved multiple times, in some cases concurrently (Fig. 2). We found slippery surfaces with wax crystalloids of various shapes ranging from scale-like platelets to long threads. Through their three-dimensional structure, the crystalloids reduce the surface to which insect's legs can attach and thus impede adhesion. Moreover, the crystalloids also can break off and stick to the insect's adhesive pads (Gaume *et al.* 2004). Such wax crystalloids can be found throughout the angiosperms (Barthlott *et al.* 1998). They have evolved repeatedly in various contexts of plant–pollinator interactions (Eigenbrode 2004, Gaume *et al.* 2004, Quek *et al.* 2004) and are also found on the foliage leaves of some Araceae (Koch *et al.* 2008). Because wax crystalloids are easily formed and are absent in many taxa it is most likely that they evolved de novo in the context of trap pollination.

Downward pointing papillae not only function as slippery surfaces because of their shape, but also secrete oil, which increases slipperiness (Knoll 1926, Yadav 1998). After pollen release, they often collapse, thus facilitating the escape of pollinators (Dakwale & Bhatnagar 1982, Lack & Diaz 1991, Bermadinger-Stabentheiner & Stabentheiner 1995). We found several aroid taxa with papillate cells on the adaxial surface of the spathe, which did not point downward but projected perpendicularly to the spathe surface. Whether this kind of "straight" papillae can also form a slippery surface is not clear. Ivancic *et al.* (2004) mention that the (papillate) spathe surface of *Colocasia esculenta* was slippery for flies. However, according to our own field observations in the

same species as well as in *Colocasia fontanesii*, drosophilid pollinators can walk along the spathe (Bröderbauer et al., unpublished manuscript). "Straight" papillate cells also occur in *Zantedeschia*. In *Zantedeschia* var. *elliotiana*, we observed (in the Botanical Garden of Vienna) trapped wild bees that were unable to climb the lower papillate portion of the inner spathe (Bröderbauer, unpublished manuscript). However, experimental proof that such cells can form a slippery surface is still missing. If they produce oil they might easily be slippery without pointing downward.

Papillate cells also might serve another function. While the spadix is the most common organ for scent-production, the spathe has also been shown to be an osmophore in some aroid taxa such as *Arisaema*, *Cryptocoryne*, and *Dracontium* (Vogel 1963, Mayo *et al.* 1997, Zhu & Croat 2004). During our study, we found that most of the papillate slippery surfaces also emitted faecal odours, often similar to those of the spadix and changing during the course of anthesis. In fact, osmophoric plant surfaces reported by other researchers (Vogel 1963, Stpiczynska 2001, García *et al.* 2007, Płachno *et al.* 2010) often resemble the "straight" papillate cells in Araceae. Whether ("straight") osmophoric papillae are ancestral and subsequently changed their function toward slippery surfaces has yet to be demonstrated. However, in the '*Pistia* clade' (sensu Renner & Zhang 2004, Fig. 2, node 194), which contains two clades in which traps have evolved independently (Fig. 2, nodes 199 and 208), the common ancestor of the *Pistia* clade apparently did not have a trap but already possessed papillate cells. This would imply that papillate cells were present before the emergence of slippery surfaces.

A trend similar to that in papillate cells can also be observed in elongated sterile flowers of the tribe Areae. In *Sauromatum*, sterile flowers situated below the staminate flowers act as osmophores (Hadacek & Weber 2002). Moreover, we also found that sterile flowers of *Typhonium* produce scent and stain intensively after treatment with neutral red (Bröderbauer, unpublished data), which is used to detect osmophores (Vogel 1963). In both taxa, sterile flowers are located within the floral chamber below the constriction of the spathe. By contrast, in *Arum*, the sterile flowers, which are present below and above of the staminate zone, are part of the trap (Knoll 1926). They produce oil and are slippery, thereby preventing trapped insects from escape. Moreover, they act like a sieve that gives access to the spathe chamber only to insects of a certain size. Thus, sterile flowers apparently have shifted in function from osmophores to trapping devices in the Areae clade. The function of elongated sterile flowers in general varies in different clades. In *Arisaema*, sterile flowers present on the appendix help in the attraction of

pollinators (Vogel & Martens 2000), while in *Bucephalandra* they probably serve as protecting structures for the developing fruits (P. Boyce, Universiti Sains Malaysia, personal communication). In *Dracontium* and *Amorphophallus* the function of sterile flowers is unclear, but judging from their shape and position, a role in trapping insects seems unlikely in most species.

Movements of the spathe during or after anthesis are ubiquitous in Araceae (Mayo *et al.* 1997). In genera such as *Dieffenbachia* (Young 1986) and *Alocasia* (Miyake & Yafuso 2003), the constriction closes after the pollen release. These movements are thought to force the pollinators to leave the inflorescence and also to protect developing fruits (Mayo *et al.* 1997). The closure of the inflorescence during anthesis to imprison pollinators might result simply from a change in the timing of the spathe closure. In *Cryptocoryne* and *Lagenandra*, the spathe margins are connate and are not able to constrict. Instead, the seclusion of the chamber is achieved by the movement of a specialized extension of the spathe margin, the so-called flap (Ørgaard & Jacobsen 1998). Besides their function in trapping, spathe movements can also be important for the release of pollinators. In *Arisaema* and *Pinellia*, insects are set free from the trap by spathe movements that result in the formation of a secondary opening (Vogel & Martens 2000). This is necessary because in these traps slippery surfaces (i.e., epicuticular wax crystalloids) do not wither, thus preventing the insect's escape through the still slippery entrance of the chamber.

Evolutionary history of functional types of traps — We found that inflorescence traps have evolved at least 10 times independently in the Araceae. Traps are not restricted to taxa with highly synorganized inflorescences but also occur in the subfamily Lasioideae. In this clade and in several other lineages, the spadix is not differentiated and bears bisexual flowers only. Moreover, the spathe only forms a rudimentary chamber without a constriction in most taxa of Lasioideae. Unisexual flowers appear in the *Stylochaeton* clade and are prevalent in the Aroideae. Here, an increasing synorganization of spadix and spathe can be observed, with the pistillate flowers being enclosed in the spathe chamber, the sterile flowers leveling with the spathe constriction and the staminate flowers facing the spathe blade. Despite these morphological differences, convergent evolution has led to the formation of traps that function in a similar way in distinct clades. Perhaps the most astonishing examples for convergent evolution are the traps of the *Zomicarpa* type in *Dracontioides* (bisexual flowers),

Zomicarpa, and *Arisaema* (unisexual flowers) (Vogel & Martens 2000). A second trap type, which is present in bisexual (i.e., Lasioideae) and unisexual (i.e., Aroideae) clades, is the *Arisarum* type. This type represents an imperfect trap because insects glide down slippery surfaces and fall into the spathe chamber. They are, however, not imprisoned inside because they can escape by climbing the spadix (Vogel 1978). The *Arisarum* type prevails in subfamily Lasioideae. We suppose that the evolution of perfect traps is less probable in this clade due to the lower degree of synorganization of spathe and spadix. Nevertheless, a transition from imperfect to perfect traps occurred within Lasioideae in *Dracontioides desciscens*. In contrast to the imperfect traps of the same clade, the spadix is completely hidden inside the spathe, and a secondary exit is formed by the opening of the convolute spathe margins. This example shows that imperfect traps may serve as a precursor for perfect traps. This tendency is supported by the number of transitions, which occurred most frequently from nontraps to traps of the imperfect *Arisarum* type and next most frequently from the *Arisarum* type to the *Zomicarpa* type.

The purpose of imperfect traps is to ensure that insects lured to an inflorescence will have contact with flowers before departing, leading to pollen transfer (Faegri & Van der Pijl 1971). However, pollination success will be greatly improved in traps in which the insects are forced to stay inside the floral chamber, thus depositing cross pollen on the stigmas and removing pollen from the anthers more effectively (Lack & Diaz 1991). Therefore, traits that ensure the retention of pollinators may be favored by selection in imperfect traps, facilitating the evolution of true traps. However, the presence of such a precursory imperfect stage could not be found in *Stylochaeton* and subfamily Aroideae. It remains uncertain whether it simply did not exist or it transitioned rapidly into a perfect trap. Nevertheless, we can still observe different degrees of synorganization. For example, *Arum* type traps are derived from the *Typhonium* type, in which the sterile flowers serve as osmophores not involved in trapping. The closure of the floral chamber is reached by a narrowing of the spathe constriction. In contrast, sterile flowers have become part of the trap in the *Arum* type, replacing the function of the spathe movements. Moreover, in the *Arum* type, the fertile part of the spadix is completely hidden within the spathe chamber, while at least in some taxa of the *Typhonium* type, staminate flowers are situated above the constriction of the spathe chamber.

Shifts from traps to nontraps are rare within Araceae. The only known example is found in the genus *Arum*, which mainly consists of deceptive traps pollinated by flies and beetles (Gibernau *et al.* 2004). *Arum creticum*, however, has shifted to bee pollination

and rewards its visitors with pollen during the staminate phase of anthesis (Diaz & Kite 2006). However, bees are still trapped during the pistillate phase to secure the transfer of outcross pollen onto the stigma. The absence of true transitions from traps to rewarding inflorescences indicates that trap pollination is an evolutionary stable condition within the Araceae.

Association between traps and pollinators — The ancestral pollinator type of Araceae could not be resolved unambiguously. Most clades originated from beetle- or fly-pollinated ancestors, with flies prevailing in the clades occurring after node 130 (Fig. 2), and beetles prevailing in the remaining clades. Bees serve as pollinators only in the subfamilies Monsteroideae and Pothoideae. The inflorescence traps in Araceae are known to be pollinated by beetles or flies and in some taxa by both occurring together. In most cases, these are saprophilous species (Gibernau 2003). An obvious reason for the evolution of trap pollination is a change toward deceptive pollination, as insects will soon leave a flower when putative rewards are revealed to be a fake (Faegri & van der Pijl 1971, Dafni 1984). Chartier (2011) showed that, in Araceae, deceit pollination was derived from mating mutualism involving beetle pollination as well as from nursery mutualism involving flies, as was postulated by Stebbins (1970). But does pollination by a certain type of insect make a change to a deceptive trap more likely? We found that trap pollination in Araceae is correlated with pollination by flies rather than beetles. According to our ancestral state reconstructions, the common ancestors of clades with traps were pollinated by flies in the majority of cases. Interestingly, most changes from nontraps to traps were not associated with a simultaneous change in pollinator type but happened within flypollinated clades. Gibernau *et al.* (2010) showed that in several taxa with traps floral traits match those of mutualistic taxa pollinated by flies, indicating that trap pollination is embedded in the pollination syndrome of myophily. For example, traps of the *Schismatoglottis* type in *Schismatoglottis* and *Colocasia* are embedded in clades where nursery pollination involving flies prevails (Chartier 2011). In contrast to other traps in Araceae, their pollinators (flies of the drosophilid genus *Colocasiomyia*) are not deceived. Their reward is a brood site (Toda & Okada 1983, Takenaka *et al.* 2006, Toda & Lakim 2011). The flies lay their eggs between the flowers, and larvae develop inside the decaying inflorescence. Contrary to the situation in other trap types, adult flies can move freely within the inflorescence during the pistillate phase. However, after some time, the spathe closes completely and thus imprisons the drosophilids. After pollen

release, the spathe opens abruptly and the flies depart (Cleghorn 1913, Boyce & Wong 2007, Bröderbauer, unpublished manuscript). We conclude that in this case trapping is more important for ensuring efficient pollen export than for the pollen deposition on the stigma, which in any case is achieved by egg-laying flies.

A scenario with nursery mutualism as a precursor to trap pollination is also probable in other clades. Based on Chartier's (2011) reconstruction of plant–pollinator interactions in Araceae we can infer that nursery mutualism was also present in the common ancestor of traps in the *Arum* clade. An example for trap pollination through deception of drosophilids in an extant member of Areae is found in *Arum palaestinum* (Stökl *et al.* 2010). However, deception of fruit flies in this species is probably derived from trap pollination by saprophilous flies (Linz *et al.* 2010). Nursery pollination by drosophilid flies is also found in *Aristolochia* (Sakai 2002b). While most species of *Aristolochia* form deceptive traps, *Drosophila* spp. pollinating *A. maxima* do not get retained but deposit their eggs in the flowers. These findings suggest that transitions between nursery mutualism and brood-site mimicry could be a common phenomenon. A shift to saprophilous pollinators can be achieved by simple changes in floral scent (Shuttleworth & Johnson 2010). As floral odors are very diverse in the Araceae (Kite *et al.* 1998, Stökl *et al.* 2010, Schiestl & Dötterl 2012) such changes in floral scent have probably occurred independently several times.

Further hypotheses that could explain our finding of a correlation between flies and trap pollination relate to the differential behavior of flies and beetles. Knoll (1926) and Bown (2000) argue that flies are much more agile and therefore have to be arrested to transfer pollen. In contrast, beetles tend to stay in flowers for longer intervals voluntarily (Dafni 1984, Willmer 2011). In addition, many chamber flowers/inflorescences offer solid food rewards for beetles (Proctor *et al.* 1996, Gibernau *et al.* 1999, Bernhardt 2000), which cannot be consumed by flies.

Conclusions — The repeated emergence of morphological traits that facilitate trap pollination has led to the evolution of inflorescence traps at least 10 times, such that it is found in at least 27 genera of Araceae. On several occasions, the formation of trapping devices resulted from a shift of function in already existing inflorescence characters. Various functional types of traps evolved independently in different clades. In at least some of these clades, imperfect traps predated the evolution of perfect traps, and elaborate traps were derived from less complex ancestors. The evolution of traps is

correlated with fly pollination. Nursery mutualism between aroid inflorescences and drosophilid flies is likely to be a precursor for the evolution of traps. Further studies on plant–pollinator interactions in such nursery mutualisms are needed to detect drivers for the evolution of floral traps in Araceae and elsewhere.

ACKNOWLEDGEMENTS

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APPENDIX 1. List of plant material investigated under light and scanning electron microscope. Specimens stored in spirit collections are indicated by an asterisk.

Taxon;Voucher (Herbarium).

Aglaonema modestum Schott ex Engl.; 0064899 (WU)*. ***Aglaonema nebulosum*** N.E. Br.; 0064900 (WU)*. ***Alocasia acuminata*** Schott; 0064901 (WU)*. ***Alocasia lauterbachiana*** (Engl.) A. Hay; 0064902 (WU)*. ***Alocasia odora*** (Lindl.) K. Koch; 0064903 (WU)*. ***Alocasia portei*** Schott; Bogner 1768 (M). ***Ambrosina bassii*** L.; 0064905 (WU) *.

Amorphophallus atrorubens Hett. & Sizemore; 0064906 (WU)*. ***Amorphophallus henryi*** N.E. Br.; 0064908 (WU)*. ***Amorphophallus konjac*** K. Koch; 0064910 (WU) *. ***Amorphophallus longituberosus*** (Engl.) Engl. & Gehrm.; 0064912 (WU)*. ***Amorphophallus mossambicensis*** (Schott ex Garcke) N.E. Br.; 0064913 (WU)*. ***Amorphophallus myosuroides*** Hett. & A. Galloway; 0064914 (WU)*. ***Amorphophallus palawanensis*** Bogner & Hett.; 0064917 (WU)*. ***Amorphophallus polyanthus*** Hett. & Sizemore; 0064918 (WU)*. ***Amorphophallus stuhlmannii*** (Engl.) Engl. & Gehrm.; 0064919 (WU)*. ***Amorphophallus taurostigma*** Ittenbach, Hett. & Bogner; 0064920 (WU)*. ***Amorphophallus variabilis*** Blume; 0064921 (WU)*. ***Amorphophallus yunnanensis*** Engl.; 0064922 (WU)*. ***Anadendrum affine*** Schott; 012384 (NCY)*. ***Anaphyllopsis americana*** (Engl.) A. Hay; Barabé et al. 258 (MT). ***Anchomanes dalzielii*** N.E. Br.; 0064924 (WU)*. ***Anchomanes difformis*** (Blume) Engl.; 012388 (NCY)*. ***Anchomanes giganteus*** Engl.; 012389 (NCY)*. ***Anthurium magnificum*** Engl.; 0064925 (WU)*. ***Anthurium nymphaeifolium*** K. Koch & C.D. Bouché; Bogner 762 (M). ***Anthurium pedatum*** (Kunth) Engl. ex Kunth; Bogner 2956 (M). ***Anubias gigantea*** A. Chev. ex Hutch.; 0064928 (WU)*. ***Anubias gilletii*** De Wild. & T. Durand; Bogner 108 (M). ***Arisaema fargesii*** Buchet; 0064931 (WU)*. ***Arisaema ghaticum*** (Sardesai, S.P. Gaikwad & S.R. Yadav) Punekar & Kumaran; 0064932 (WU)*. ***Arisarum proboscideum*** (L.) Savi; 0064933 (WU)*. ***Arisarum vulgare*** O. Targ. Tozz.; 0064934 (WU)*. ***Arophyton crassifolium*** (Buchet) Bogner; 0064935 (WU)*. ***Arophyton humbertii*** Bogner; 0064936 (WU)*. ***Arum cylindraceum*** Gasp. in G. Gussone; 0064941 (WU)*. ***Arum italicum*** Mill.; 0064947 (WU)*. ***Arum nigrum*** Schott; 0064949 (WU)*. ***Asterostigma lividum*** (Lodd.) Engl.; 0064951 (WU)*. ***Biarum carratracense*** (Willk.) Font Quer; 0064951 (WU)*. ***Biarum tenuifolium*** (L.) Schott in H.W. Schott & S.L. Endlicher; 0064954 (WU)*. ***Caladium bicolor*** (Aiton) Vent.; RMP 3137 (FRP). ***Caladium lindenii*** (André) Madison; Bogner 2338 (M). ***Caladium steudneriifolium*** Engl.; 0064958 (WU)*. ***Calla palustris*** L.; 0064959 (WU)*. ***Callopsis volkensii*** Engl.; 0064960 (WU)*. ***Carlephyton glaucophyllum*** Bogner; RMM 124 (FRP). ***Chlorospatha croatiana*** Grayum; 0064963 (WU)*. ***Colletogyne perrieri*** Buchet; 0064964 (WU)*. ***Colocasia affinis*** Schott; 0064966 (WU)*. ***Colocasia esculenta*** (L.) Schott in H.W. Schott & S.L. Endlicher; 0064967 (WU)*. ***Colocasia fallax*** Schott; 0064968 (WU)*. ***Colocasia fontanesii*** Schott; 0064969 (WU)*. ***Colocasia gigantea*** (Blume) Hook.f.; Bogner 427 (M). ***Culcasia saxatilis*** A. Chev.; Bogner 2727 (M). ***Cryptocoryne longicauda*** Becc. ex Engl.; 0064971 (WU)*. ***Cryptocoryne pontederiifolia*** Schott; Bogner 1739 (M)*. ***Cyrtosperma ferox*** N.E. Br. & Linden; Bogner 2131 (M). ***Cyrtosperma johnstonii*** (N.E. Br.) N.E. Br.; 1978.3.532 (NCY). ***Dieffenbachia bowmannii*** Carrière; 012504 (NCY)*. ***Dieffenbachia seguine*** (Jacq.) Schott in H.W. Schott & S.L. Endlicher; 012506 (NCY)*. ***Dieffenbachia oerstedii*** Schott; 0064978 (WU)*. ***Dracontioides desciscens*** (Schott) Engl.; 1994.3.770 (NCY). ***Dracontium amazonense*** G.H. Zhu & Croat; H.AR.83 (FRP). ***Dracontium asperum*** K. Koch; Bogner 2793 (M). ***Dracontium bogneri*** G.H. Zhu & Croat; 0064982 (WU)*. ***Dracontium nivosum*** (Lem.) G.H. Zhu in R.H.A. Govaerts & D.G. Frodin; 012516 (NCY)*. ***Dracontium polyphyllum*** L.; 0064984 (WU)*. ***Dracontium prancei*** G.H. Zhu & Croat; Bogner 1132 (M). ***Dracontium soconuscum*** Matuda; RMP 2233 (FRP). ***Dracontium spruceanum*** (Schott) G.H. Zhu; RMP 2162 (FRP). ***Dracunculus canariensis*** Kunth; 0064041 (WU)*. ***Dracunculus vulgaris*** Schott in H.W. Schott & S.L. Endlicher; 0064988 (WU)*. ***Filarum manserichense*** Nicolson; 0064990 (WU)*. ***Gonatopus boivinii*** (Decne.) Engl. in A.L.P. de Candolle & A.C.P. de Candolle; 0064991 (WU)*. ***Gorgonidium cf. intermedium*** (Bogner) E.G. Gonç.; 0064992 (WU)*. ***Hapaline cf. benthamiana*** Schott; 0064993 (WU)*. ***Helicodiceros muscivorus*** (L.f.) Engl. in A.L.P. de Candolle & A.C.P. de Candolle; 0064994 (WU)*. ***Homalomena picturata*** (Linden & André) Regel; 0064996 (WU)*. ***Homalomena wallisii*** Regel; 0064998 (WU)*. ***Incarum pavonii*** (Schott) E.G. Gonç.; 0064999 (WU)*. ***Lagenandra praetermissa*** de Wit; 0065000 (WU)*. ***Lasia spinosa*** (L.) Thwaites; 0065001 (WU)*. ***Lysichiton americanus*** Hultén & St. John; 0065002 (WU)*. ***Monstera adansonii*** Schott; 1981.3.587 (NCY). ***Monstera obliqua*** Miq.; 2003.3.214 (NCY). ***Nephthytis afzelii*** Schott; Bogner 2998 (M). ***Nephthytis hallaei*** (Bogner) Bogner; 012564 (NCY)*. ***Nephthytis sp.*** 0065004 (WU)*. ***Philodendron martianum*** Engl.; 0065006 (WU)*. ***Philodendron pedatum*** (Hook.) Kunth; 0065007 (WU)*. ***Philodendron sodiroi*** N.E. Br.; 0065008 (WU)*. ***Philodendron squamiferum*** Poepp. in E.F. Poeppig & S.L. Endlicher; Bogner 1958 (M). ***Pinellia cordata*** N.E. Br.; 0065011 (WU)*. ***Pinellia peltata*** C. Pei.; 0065012 (WU)*. ***Pinellia ternata*** (Thunb.) Makino; 0065013 (WU)*. ***Piptospatha ridleyi*** N.E. Br. ex Hook.f.; 012664 (NCY)*. ***Pistia stratiotes*** L.; 0065014 (WU)*. ***Pothos junghuhnii*** de Vriese in F.A.W. Miquel; Bogner 1550 (M). ***Pseudodracontium latifolium*** Serebryanyi; 0065014 (M). ***Pseudodracontium sp.*** 0065016 (WU)*. ***Pseudohydrosme gabunensis*** Engl.; 0065019 (WU)*. ***Pycnospatha palmata*** Gagnep.; 0065020 (WU)*. ***Rhaphidophora angustata*** Schott; Bogner 2989 (M). ***Rhaphidophora decursiva*** (Rox.) Schott; 0065022 (WU)*. ***Remusatia hookeriana*** Schott; 0065023 (WU)*. ***Remusatia pumila*** (D. Don) H. Li & A. Hay; 0065024 (WU)*. ***Remusatia vivipara*** (Roxb.) Schott in H.W. Schott & S.L. Endlicher; 0065025 (WU)*. ***Sauromatum venosum*** (Dryand. ex Aiton) Kunth; 0065026 (WU)*. ***Schismatoglottis calyptrata*** (Roxb.) Zoll. & Moritzi in H. Zollinger; 0065028 (WU)*. ***Schismatoglottis multiflora*** Ridl.; Bogner 1453 (M). ***Schismatoglottis subundulata*** (Zoll. ex Schott) Nicolson; 0065030 (WU)*. ***Scindapsus lucens*** Bogner & P.C. Boyce; 012699 (NCY)*. ***Spathicarpa hastifolia*** Hook.; Bogner 2546 (M). ***Spathiphyllum cannifolium*** (Dryand. ex Sims) Schott; 0065032 (WU)*. ***Spathiphyllum wallisii*** Regel; 0065033 (WU)*. ***Stenospermation popayanense*** Schott; Bogner 463 (M). ***Steudnera henryana*** Engl.; 0065035 (WU)*. ***Steudnera kerrii*** Gagnep.; 2000.3.441 (NCY). ***Stylochaeton bogneri*** Mayo; Bogner 216 (M). ***Stylochaeton cf. hypogaeus*** Lepr.; 0065038 (WU)*. ***Stylochaeton zenkeri*** Engl.; 0065039 (WU)*. ***Symplocarpus foetidus*** (L.) Salisb. ex W.P.C. Barton; 0065040 (WU)*. ***Synandrospadix vermitoxicus*** (Griseb.) Engl.; 0065040 (WU)*. ***Syngonium macrophyllum*** Engl.; 012708 (NCY)*. ***Syngonium podophyllum*** Schott; 012709 (NCY)*. ***Taccarum caudatum*** Rusby; 0065042 (WU)*. ***Typhonium blumei*** Nicolson & Sivad.; 0065043 (WU)*. ***Typhonium sp. nov.*** 0065047 (WU)*. ***Typhonium trilobatum*** (L.) Schott; 0065046 (WU)*. ***Typhonodorum lindleyanum*** Schott; 0065048 (WU)*. ***Ulearum sagittatum*** Engl.; 0065049 (WU)*. ***Urospatha grandis*** Schott; RMP 1306 (FRP). ***Urospatha sagittifolia*** (Rudge) Schott; Bogner 2770 (M). ***Urospatha tonduzii*** Engl.; Bogner 1115 (M)*. ***Xanthosoma cubense*** (Schott) Schott; 0065053 (WU)*. ***Xanthosoma mariae*** Bogner & E.G. Gonç.; 0065055 (WU)*. ***Zamioculcas zamiifolia*** (Lodd.) Engl.; 0065056 (WU)*. ***Zantedeschia aethiopica*** (L.) Spreng.; 0065057 (WU)*. ***Zantedeschia albomaculata*** (Hook.) Baill.; 012714 (NCY)*. ***Zomicarpa riedelianum*** Schott;Vogel54(WU).

APPENDIX S1. Shape and cover of epidermal cells on the adaxial side of the spathe.

Species	Cell shape	Wax crystalloids	Cuticular folds
Aglaonema modestum	Tabular	Tubules & threads	
A. nebulosum	Tabular		
Alocasia acuminata	Convex		+
A. lauterbachiana	Tabular		
A. odora	Dome-shaped	Tubules & threads	+
A. portei	Tabular		+
Ambrosina bassii	Convex		
Amorphophallus atrorubens	Convex	Platelets	+
A. henryi	Papillate, straight		+
A. konjac	Papillate, straight	Platelets	+
A. longituberosus	Dome-shaped	Granules & tubules	+
A. mossambicensis	Dome-shaped		+
A. myosuroides	Papillate, straight	Rods	+
A. palawanensis	Papillate, straight	Platelets	
A. polyanthus	Tabular		+
A. stuhlmannii	Papillate, straight	Platelets	+
A. taurostigma	Papillate, straight	Tubules & threads	+
A. variabilis	Convex	Transitional	+
A. yunnanensis	Papillate, straight		+
Anadendrum affine	Convex		+
Anaphyllopsis americana	Papillate, downward-pointing	Granules	+
Anchomanes dalzielii	Convex		
A. difformis	Convex		+
A. giganteus	Convex		+
Anthurium magnificum	Convex		
A. nymphaeifolium	Convex		
A. pedatum	Convex		
Anubias gigantea	Convex		
A. gilletii	Tabular		
Ariopsis peltata[a]	Papillate, straight		
Arisaema fargesii	Convex		
A. ghaticum	Convex	Rods & threads	
Arisarum proboscideum	Papillate, downward-pointing		+
A. vulgare	Papillate, downward-pointing		+
Arophyton crassifolium	Tabular		+
A. humbertii	Convex		+
Arum cylindraceum	Papillate, downward-pointing		
A. italicum	Papillate, downward-pointing		
A. nigrum	Papillate, downward-pointing		
Asterostigma lividum	Convex		+
Biarum carratracense	Papillate, downward-pointing		
B. tenuifolium	Papillate, downward-pointing		
Caladium bicolor	Convex		
C. lindenii	Tabular		
C. steudneriifolium	Convex		
Calla palustris	Convex		+
Callopsis volkensii	Convex		+
Carlephyton glaucophyllum	Convex		+
Chlorospatha croatiana	Convex		
Colletogyne perrieri	Convex		
Colocasia affinis	Convex	Platelets	

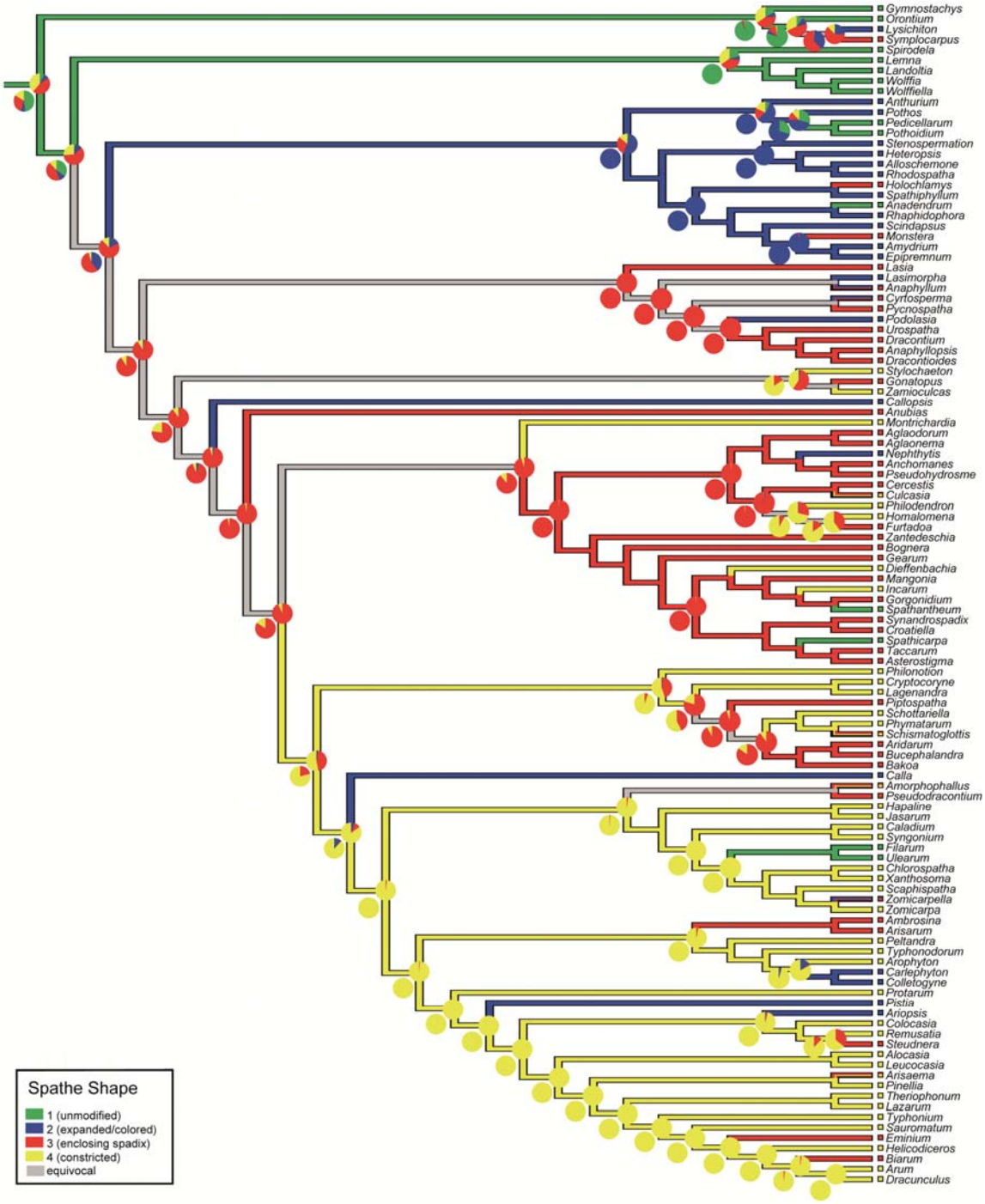
APPENDIX S1. *continued.*

Species	Cell shape	Wax crystalloids	Cuticular folds
C. esculenta	Papillate, straight		
C. fallax	Tabular		
C. fontanesii	Papillate, straight		
C. gigantea	Papillate, straight		
Culcasia saxatilis	Tabular		
Cryptocoryne longicauda	Trichomes, downward-pointing		
C. pontederiifolia	Trichomes, downward-pointing		
Cyrtosperma ferox	Papillate, downward-pointing	Granules & rods	
C. johnstonii	Papillate, downward-pointing	Granules & rods	
Dieffenbachia bowmannii	Tabular		
D. oerstedii	Tabular		
D. seguine	Tabular		
Dracontioides desciscens	Papillate, downward-pointing	Tubules	+
Dracontium amazonense	Papillate, downward-pointing		+
D. asperum	Papillate, downward-pointing		+
D. bogneri	Papillate, downward-pointing	Granules	+
D. nivosum	Papillate, downward-pointing		+
D. polyphyllum	Papillate, downward-pointing	Granules	+
D. prancei	Papillate, downward-pointing		+
D. soconuscum	Tabular		+
D. spruceanum	Papillate, downward-pointing		+
D. canariensis	Papillate, straight		
D. vulgaris	Papillate, downward-pointing		
Filarum manserichense	Convex		
Gonatopus boivinii	Tabular		+
Gorgonidium cf. intermedium	Tabular		+
Hapaline cf. benthamiana	Tabular		
Helicodiceros muscivorus	Papillate, downward-pointing		
Homalomena picturata	Tabular		
H. wallisii	Tabular		
Incarum pavonii	Tabular		+
Lagenandra praetermissa	Trichomes, downward-pointing		
Lasia spinosa	Papillate, downward-pointing		+
Lysichiton americanus	Convex		+
Monstera adansonii	Tabular		
M. oblliqua	Tabular		
Nephthytis afzelii	Tabular		
N. hallaei	Convex		
N. sp.	Tabular		
Philodendron martianum	Convex		
P. pedatum	Tabular		
P. sodiroi	Tabular		
P. squamiferum	Papillate, downward-pointing		

APPENDIX S1. *continued.*

Species	Cell shape	Wax crystalloids	Cuticular folds
Pinellia cordata	Papillate, downward-pointing		+
P. peltata	Papillate, downward-pointing		+
P. ternata	Papillate, downward-pointing		+
Piptospatha ridleyi	Tabular		+
Pistia stratiotes	Convex		
Pothos junghuhnii	Convex		
Pseudodracontium latifolium	Papillate, straight	Platelets	+
P. sp.	Papillate, straight	Transitional	+
Pseudohydrosme gabunensis	Papillate, downward-pointing		+
Pycnospatha palmata	Tabular, imbricate	Filaments	+
Rhaphidophora angustata	Convex	Fissured	+
R. decursiva	Tabular	Fissured	+
Remusatia hookeriana	Papillate, straight		+
R. pumila	Papillate, straight		+
R. vivipara	Convex		
Sauromatum venosum	Papillate, downward-pointing		
Schismatoglottis calyptrata	Convex		
S. multiflora	Convex		
S. subundulata	Convex		
Scindapsus lucens	Convex	Fissured	
Spathicarpa hastifolia	Convex		+
Spathiphyllum cannifolium	Convex		
S. wallisii	Convex		
Stenospermation popayanense	Convex		
Steudnera henryana	Convex	Platelets	
S. kerrii	Convex	Platelets	
Stylochaeton bogneri	Convex	Platelets & rods	
S. cf. hypogaeus	Papillate, straight	Platelets	
S. zenkeri	Papillate, straight		
Symplocarpus foetidus	Convex		
Synandrospadix vermitoxicus	Tabular		+
Syngonium macrophyllum	Convex		
S. podophyllum	Convex		
Taccarum caudatum	Convex		
Typhonium blumei	Papillate, downward-pointing		
T. sp. nov.	Papillate, downward-pointing		
T. trilobatum	Papillate, downward-pointing		
Typhonodorum lindleyanum	Convex		
Ulearum sagittatum	Convex		
Urospatha grandis	Papillate, downward-pointing	Rodlets	+
U. sagittifolia	Papillate, downward-pointing	Rodlets	+
U. tonduzii	Papillate, downward-pointing	Rodlets	+
Xanthosoma cubense	Convex		
X. mariae	Convex		
Zamioculcas zamiifolia	Convex		
Zantedeschia aethiopica	Papillate, straight		+
Z. albomaculata	Tabular		+
Zomicarpa riedelianum[b]	Convex	+ (structure unknown)	

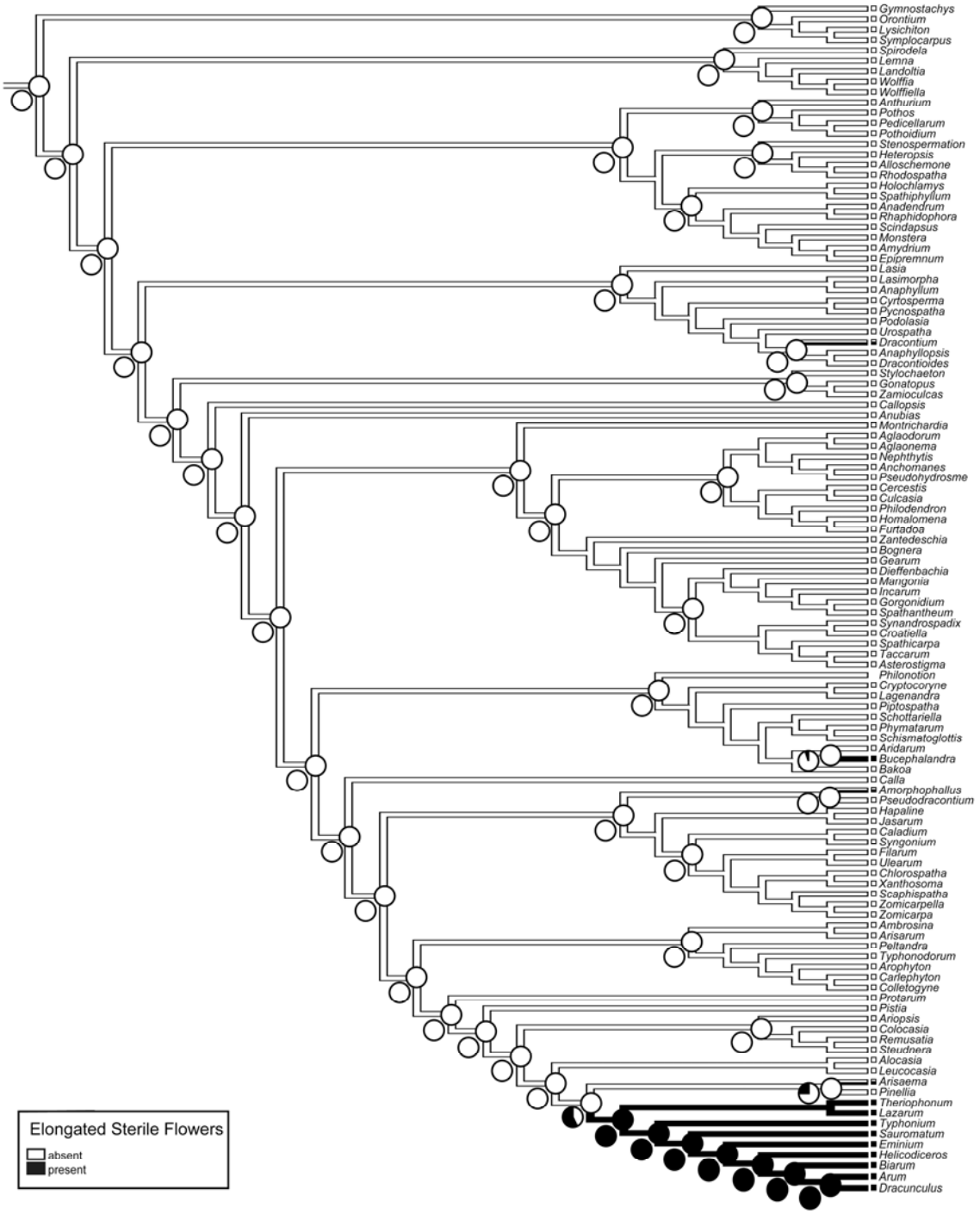
[a] Information taken from unpublished data of F. Knoll (1924); [b] Information from Vogel and Martens (2000).



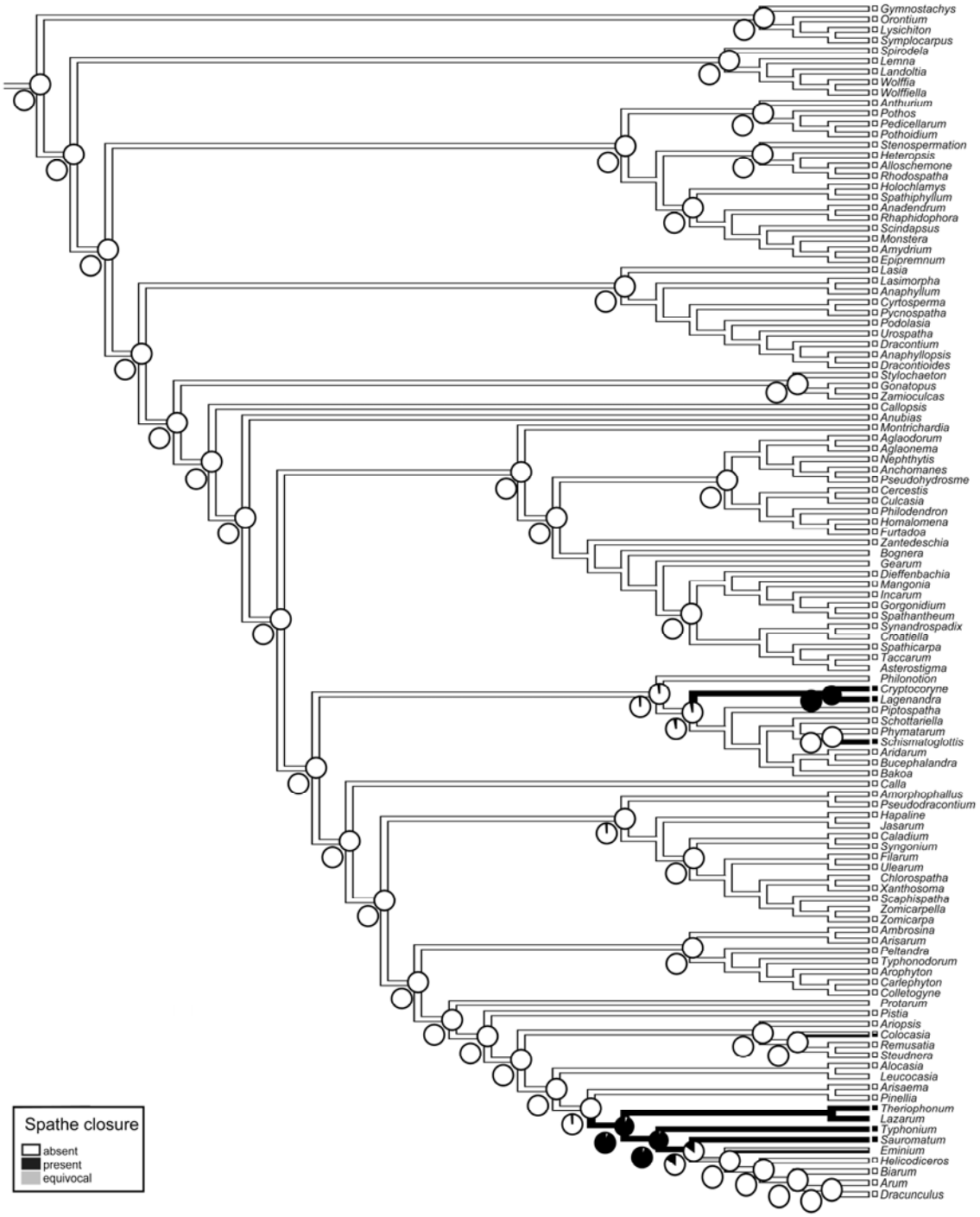
APPENDIX S2. Ancestral state reconstruction of spathe shape. Colors on lines indicate maximum parsimony reconstruction in Mesquite 2.0. Branches shaded in grey indicate equivocal reconstruction. Pie charts on the nodes display the posterior probabilites of trap types computed with Bayesian inference using the MCMC-prior (Γ: ά = 16.62, β = 0.37) in SIMMAP 1.5. Pie charts below the nodes show the results calculated with the program's default prior (Γ: ά = 1.25, β = 0.25).

APPENDIX S3. Ancestral state reconstruction of epicuticular wax. Colors on lines indicate maximum parsimony reconstruction in Mesquite 2.0. Branches shaded in grey indicate equivocal reconstruction. Pie charts on the nodes display the posterior probabilites of trap types computed with Bayesian inference using the MCMC-prior (B: $\acute{\alpha}$ = 11.55) in SIMMAP 1.5. Pie charts below the nodes show the results calculated with the program's default prior (B: $\acute{\alpha}$ = 1.00).

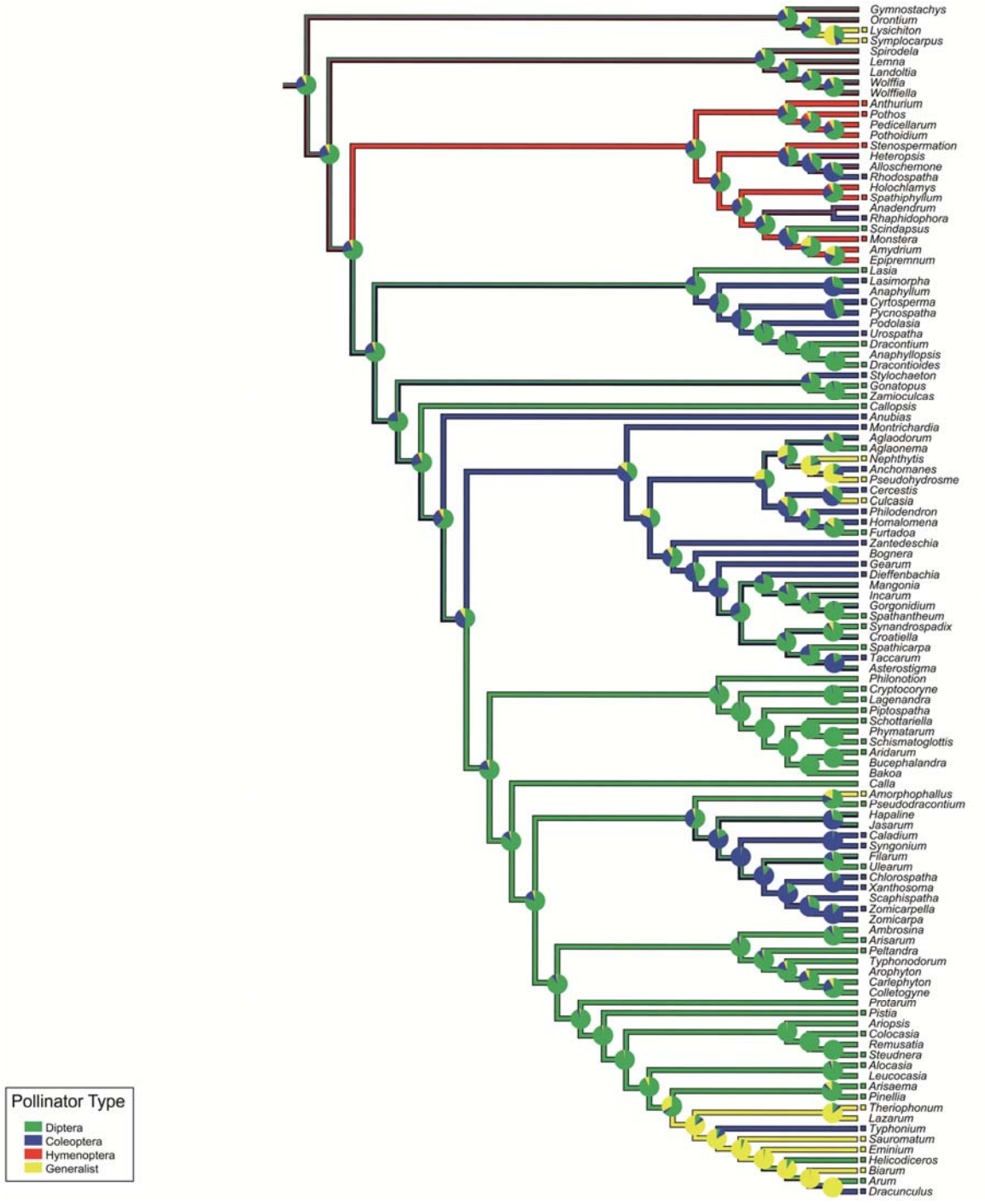
APPENDIX S4. Ancestral state reconstruction of papillate cells. Colors on lines indicate maximum parsimony reconstruction in Mesquite 2.0. Branches shaded in grey indicate equivocal reconstruction. Pie charts on the nodes display the posterior probabilites of trap types computed with Bayesian inference using the MCMC-prior (B: ά = 11.28) in SIMMAP 1.5. Pie charts below the nodes show the results calculated with the program's default prior (B: ά = 1.00).



APPENDIX S5. Ancestral state reconstruction of elongated sterile flowers. Colors on lines indicate maximum parsimony reconstruction in Mesquite 2.0. Branches shaded in grey indicate equivocal reconstruction. Pie charts on the nodes display the posterior probabilites of trap types computed with Bayesian inference using the MCMC-prior (B: ά = 2.65) in SIMMAP 1.5. Pie charts below the nodes show the results calculated with the program's default prior (B: ά = 1.00).



APPENDIX S6. Ancestral state reconstruction of spathe closure during anthesis. Colors on lines indicate maximum parsimony reconstruction in Mesquite 2.0. Branches shaded in grey indicate equivocal reconstruction. Pie charts on the nodes display the posterior probabilites of trap types computed with Bayesian inference using the MCMC-prior (B: ά = 5.05) in SIMMAP 1.5. Pie charts below the nodes show the results calculated with the program's default prior (B: ά = 1.00).



APPENDIX S7. Ancestral state reconstruction of pollinator types. Colors on lines indicate maximum parsimony reconstruction in Mesquite 2.0. Branches with multiple colors indicate equivocal reconstruction. Pie charts on the nodes display the posterior probabilites of trap types computed with Bayesian inference using the MCMC-prior (Γ: $\acute{\alpha} = 2.86$, $\beta = 0.01$) in SIMMAP 1.5.

CHAPTER 3

THE INFLORESCENCE OF *COLOCASIA* (ARACEAE): A PRISON OR THE LAND OF MILK AND HONEY?

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ABSTRACT

The Araceae include both taxa with rewarding and lure-and-trapping 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 species proved 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 with lure-and-trapping pollinated 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 de novo in *Colocasia* and is supposed to facilitate a more efficient pollen export. *C. affinis* differs from the other three species examined in spathe morphology and timing of anthesis. This corroborates polyphyly of the genus *Colocasia* and the placement of *C. affinis* in the genus *Steudnera* as recently suggested by molecular phylogenies.

INTRODUCTION

Brood-site pollination is a highly specialised type of plant pollinator interaction occurring in various angiosperm families (Sakai 2002a, Armbruster 2012). In some lineages the presence of a brood-site is only faked. Here, flowers mimic decaying organic matter such as dung or carcass in order to lure saprophilous flies and beetles not adapted to flower visits (Urru *et al*. 2012). In such deceptive pollination systems the insects often get trapped to ensure transfer of pollen to the stigma (Dafni 1984). In few families such as Annonaceae, Araceae and Aristolochiaceae, brood-site pollination and brood-site deception co-occur (Endress 1994, Sakai 2002b, Gibernau *et al*. 2010), often sharing similar features such as thermogenesis and odour-production by specialised osmophores (Vogel 1963, Thien *et al*. 2009). Whether brood-site pollination and brood-site mimicry are closely related syndromes and direct shifts between these two occur is still unclear.

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. The aroid family is diverse with more than 3300 species in 126 genera (Boyce & Croat 2012). The later diverging clades possess a highly elaborate 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 a 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).

Brood-site deception occurs in several aroid clades and usually involves the trapping of pollinators (Bröderbauer *et al.* 2012) which is achieved by slippery surfaces that consist of downward-pointing papillate cells and/or an epicuticular layer of wax crystalloids (Knoll 1926, Vogel & Martens 2000). The exploitation of aroid inflorescences as brood-sites by drosophilid flies of the genus *Colocasiomyia* has been observed in at least seven genera (e.g. *Colocasia*, *Schismatoglottis*, *Homalomena*) (Carson & Okada 1980, Toda & Lakim 2011). That these flies effectively act as pollinators has so far been shown only for *Alocasia* and *Steudnera* (Miyake & Yafuso 2005, Takenaka *et al*. 2006, Takenaka Takano *et al*. 2012). The genus *Colocasia* is estimated to comprise 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). In some species of *Colocasia* spathe movements (Cleghorn 1913) and papillate epidermal cells on the spathe (Poppinga *et al*. 2010) -

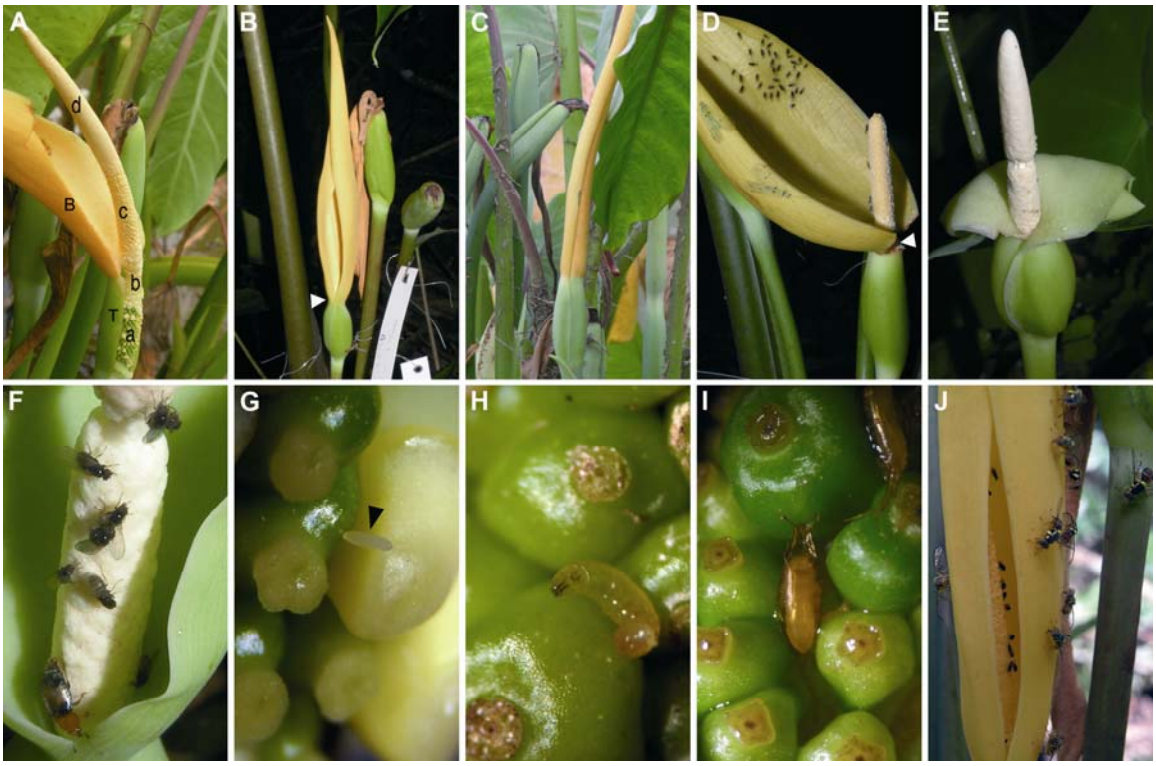


Fig. 1. Inflorescences and insect visitors of *Colocasia* spp. **A,** *C. esculenta*, spadix; a = pistillate and sterile flowers, b = intermediate sterile flowers, c = staminate flowers, d = sterile appendix. Note that parts of the spathe blade (B) and the spathe tube (T) were removed for better visibility 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*.

resembling trapping devices in brood-site mimicking taxa of Araceae - have been observed. However, the function of these structures as well as the role of the drosophilid visitors in pollination has not been studied in detail so far.

In this paper we address the following questions: 1) Which insects pollinate *Colocasia*? 2) Is the relationship between *Colocasia* and its pollinators mutualistic or has it shifted to deceptive trap pollination? 3) What is the role of spathe movements and papillate cells forming the adaxial spathe epidermis? 4) Do our data support polyphyly of *Colocasia* as was suggested by recent molecular analyses?

MATERIALS AND METHODS

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 the area the climate is seasonal, with most rainfalls 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 rainforest and tropical montane rainforest, a major part of which has been converted to rubber plantations during the last decades (Zhang & Cao 1995).

***Study species* —** Four species of *Colocasia* have been investigated: (1) *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. (2) *C. lihengiae* C.L.Long & K.M.Liu. This species has been considered conspecific with *C. fontanesii* by Li *et al.* (2010), but it 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 origin of the material is the same as in *C. fontanesii*. (3) *C. esculenta* (L.) Schott. This species is widely cultivated in the tropics as a starchy food crop. It probably originates from Southeast Asia (White & O'Connell 1982), but the natural distribution range is unknown. Study site: ponds at XTBG. (4) *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). Three 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 affinis* WU0064966, *Colocasia esculenta* WU0064967, *Colocasia fontanesii* WU0064969.

***Field work* —** Fieldwork was carried out from 30 June to 19 August 2010 in and around XTBG.

The *course of anthesis* in *C. esculenta* (n = 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 two days in August for one hour 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, respectively. 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 five minutes.

Spathe movements — In addition to the observations on anthetic inflorescences in the field, spathe movements in specimens of *C. affinis*, *C. esculenta* and *C. fontanesii*, cultivated in the glasshouses of the Botanical Garden of Vienna, were recorded with a Nikon Coolpix P 5000 camera, automatically taking pictures every 10 minutes.

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 Acknowledgments).

Bagging experiments — Thirteen inflorescences of C. *esculenta* were covered with organdy 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 10 fruits per inflorescence.

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

Odour emission — Inflorescences were submerged in neutral red (1:10000 neutral red:tap water) and checked for staining in one hour intervals (Vogel 1963, Dobson *et al.* 2005). Moreover, 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 in intervals of 30 minutes (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. Consecutively, 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 wax (Barthlott & Wollenweber 1981).

Light microscopy (LM) — For investigation under LM spathes were fixed in FAA for at least seven 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) and cut at 6 mm with a Thermo Scientific rotary microtome (Microm HM355S). The sections were stained with Ruthenium red and Toluidin blue. In addition, the presence of starch and lipids in fresh spathes of *C. fontanesii* was checked by 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), postfixed with 1% osmiumtetroxide (OsO_4) and 0.8% potassium hexacyanoferrate ($K_4Fe(CN)_6 \bullet 3H_2O$). Fixed material was dehydrated in 2,2-dimethoxypropane and then embedded in Agar's low viscosity resin (LV-Resin) (Agar Scientific, 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 (Thiéry 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

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 proved protogynous, with the anthesis lasting for about 24 hours. In *C. esculenta*, *C. fontanesii* and *C. lihengiae* the inflorescence opened before dawn between 02:00 and 05:00, concomitantly to the emission of an intense fruity odour with a musty component (Fig. 1B). At 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 (Fig. 1C). Between 17:00 and 20:00 the spathe constriction closed, thereby occluding the passage between the lower and the 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 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 minutes (Fig. 1D).

In contrast to the above mentioned species, the spathe blade of *C. affinis* was observed to open only by narrow slit. The opening presumably takes place in the evening before anthesis. The pistillate phase lasted for the whole next day, with the inflorescence emitting a strong odour similar to that of unripe banana and freshly cut grass. The narrow slit of the spathe blade remained open for the entire duration of anthesis. Around 15:00 the constriction above the floral chamber started to close while the spathe blade expanded further. Between 16:00 to 18:00, pollen was released and the spathe blade reflexed and curled (Fig. 1E).

Thermogenesis — Thermogenesis in *C. esculenta* and *C. fontanesii* occurred during the pistillate and the staminate phase of anthesis (Fig. 2). In both species peaks of heat production occurred in the appendix and the staminate flowers during the first and the second morning reaching 6 to 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).

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 10 to 30 individuals (60 in one inflorescence of *C. fontanesii*) could be 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 latter three taxa, but was regularly present in inflorescences of *Colocasia affinis* (Fig. 1F). The flies arrived at the onset of anthesis. They landed on the outside of the spathe blade and quickly walked

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)
C. affinis (3)	29 (10)	0	0	0
C. esculenta (14)	3 (1)	20 (10)	255 (190)	17 (13)
C. fontanesii (4)	1 (0)	6 (1)	67 (48)	36 (23)
C. lihengiae (3)	0	2 (2)	38 (27)	3 (3)

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

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 the 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. 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 hours and developed between the pistillate and sterile flowers inside the lower floral chamber without damaging the developing fruits (Fig. 1H, I).

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 never could be observed mating or laying eggs. After pollen extrusion, they fed on pollen and then left the inflorescence. As to our short observations beetles seem to be present more regularly in *C. affinis*, but - as in the other *Colocasia* species - only in low numbers per inflorescence.

Regular visitors of the anthetic inflorescences of all *Colocasia* species were an unidentified species of the Chrysopidae family (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 get in contact with the flowers.

Bagging experiments — The inflorescences of *C. esculenta* contained on average 184±27 pistillate flowers (n = 17), of which 26% produced fruits after open pollination.

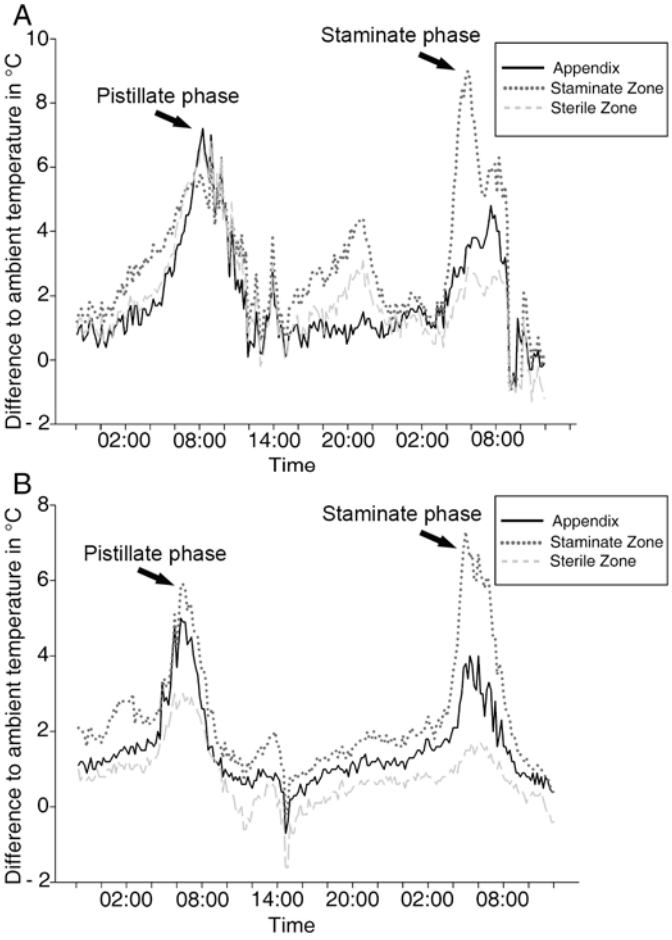


Fig. 2. Heat production during anthesis in different zones of the spadix relative to the ambient temperature A: *Colocasia esculenta*. B: *Colocasia fontanesii*.

Pollinated ovaries contained 2.2±2.5 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 ovules per ovary. *C. lihengiae* (n = 8) contained 162±25 pistillate flowers, of which 81% produced fruits after open pollination. Pollinated ovaries contained 11.2±11.8 ovules on average.

Morphology and anatomy of the spathe — In all three species tested for osmophoric activity, the spathe showed a positive reaction to staining with neutral red. While in *C. esculenta* and *C. fontanesii* the whole inside of the spathe (i.e. the adaxial epidermis) stained more or less uniformly red, in *C. affinis* only the inner epidermis of the spathe tube and the lower part of the spathe blade stained intensively red. In all taxa 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 all three species by smelling after storage of samples in separate glass vials. In *C. esculenta* and *C. fontanesii* the spathe blade in particular produced a strong sweet-musty smell.

►►►

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. affinis*, adaxial spathe blade. Note that the epidermal cells are covered with wax platelets. Cells have shrunk due to drying. SEM. **D,** *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. **E,** *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. **F,** *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. **G,** *C. fontanesii*, papilla of the adaxial spathe epidermis. Note the dense cytoplasm and high intracellular activity (asterisk). TEM, U+Pb. **H,** *C. fontanesii*, detail of a papilla of the adaxial spathe blade during the pistillate phase of atnhesis. Abbrevations: lipid droplets (L), mitochondria (M), dictyosomes (D), polyribosomes (arrowhead), vesicles (arrow), and smooth endoplasmatic reticulum (sER). TEM, U+Pb. **I,** Detail of H: vesicles (arrow) are transported from the cell to the cuticle (C). TEM, U+Pb. **J,** *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. **K,** *C. esculenta*, amyloplasts appear dark (electron dense) in the parenchymatic cells of the spathe blade adjoining the adaxial epidermis. TEM, Thìery-Test. **L,** *C. fontanesii*, papilla on the adaxial spathe blade after anthesis. Note the big vacuole (V) and the low intracellular activity (asterisk). TEM, U+Pb. Scale bars = 100 µm (E-F), 10 µm (A-D, K), 1 µm (G-J, L).

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-sweet odour, while the staminate flowers first smelled musty and in a later stage of anthesis often produced a foul smell.

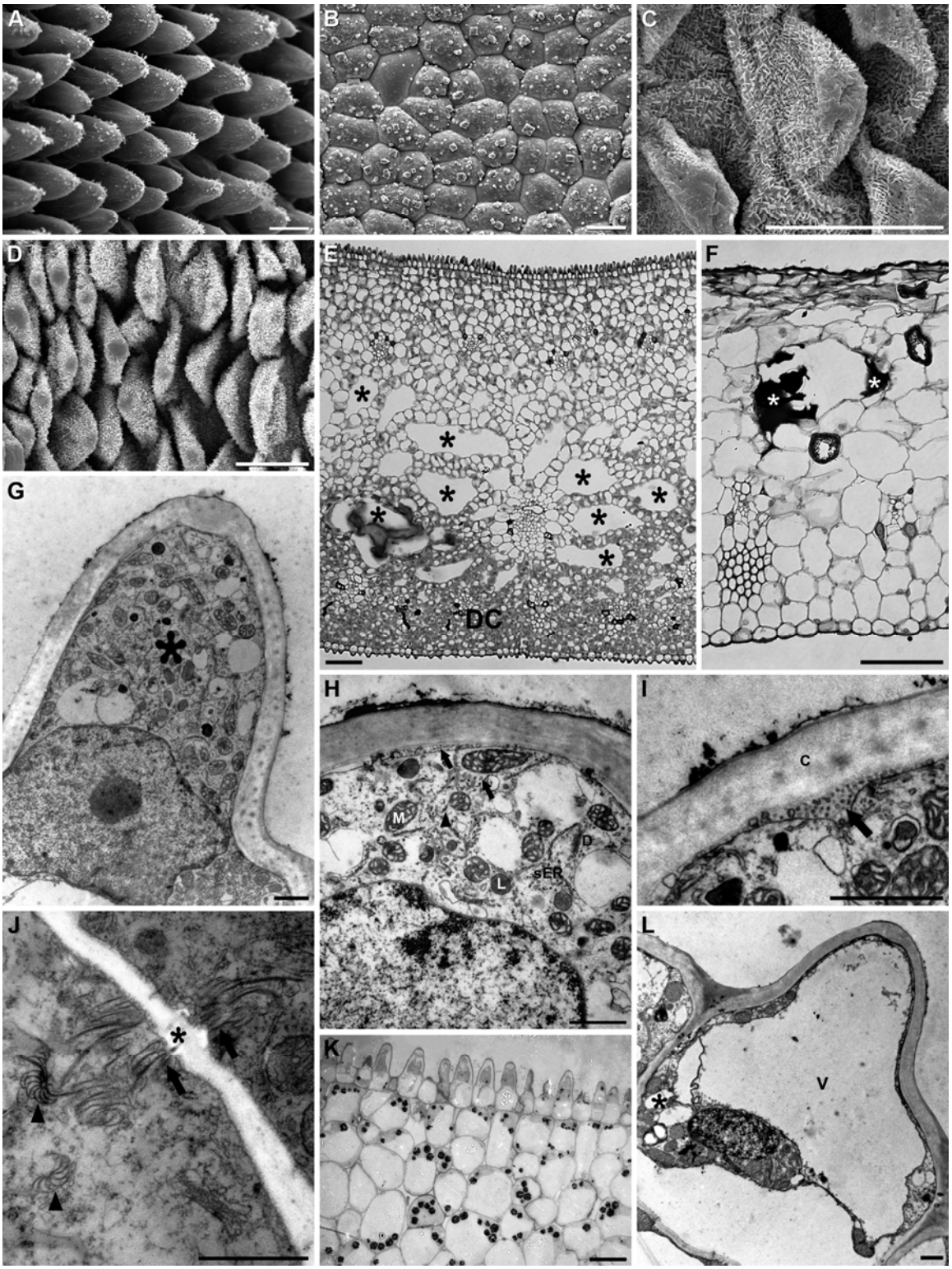
C. affinis differed from the other two species in that the tube appeared to be the main source for odour production in the spathe (sweetish, like peas). Moreover, the odour produced by the appendix was acetonic rather than musty-sweet.

SEM — In *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).

In *C. affinis*, the epidermal cells of the adaxial spathe blade were not papillate but convex and covered with wax platelets (Fig. 3C). Sparse dome-shaped cells were restricted to the apical part of the blade and were entirely covered by a prominent epicuticular wax-layer.

All three species had in common that the abaxial epidermal layer of the spathe blade consisted of papillate cells bearing cuticular folds, covered with wax platelets (Fig. 3D). These papillae where less densely packed than the papillae of the adaxial spathe epidermis in *C. esculenta* and *C. fontanesii*. Moreover, they did not collapse after anthesis.

LM — Lipids could be detected in the epidermis of *C. fontanesii* but not in the parenchymatic cells. As indicated by the 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 parenchymatic cells adjoining the abaxial epidermis of the spathe blade during the pistillate phase of anthesis (Fig 3E). In *C. affinis*, the cytoplasm of the adaxial epidermis and the parenchyma was less dense. In all

three taxa, the mesophyll of the spathe blade contained lacunar tissue (Fig. 3E). In the spathe tube, lacunar tissue was less widespread and the lacunae were often filled with mucilage (Fig. 3F).

TEM — In *C. esculenta* and *C. fontanesii*, epidermal papillae of the adaxial spathe tube showed a dense cytoplasm and high intracellular activity (Fig. 3G). Besides the presence of amyloplasts and lipids, the cells contained numerous mitochondria, dictyosomes, polyribosomes and smooth endoplasmatic reticulum (sER) (Fig. 3H). Moreover, vesicles were transported from the cell to the cuticle (Fig. 3I). Such activity was also observed to a lesser extent in epidermal cells of the abaxial spathe blade and the adaxial spathe tube. In papillate cells of *C. fontanesii*, unusual gorgon-head-shaped endoplasmatic reticulum (ER), not documented so far to the best of our knowledge, was common (Fig. 3J). Amyloplasts were particularly abundant in the parenchymatic cells of the spathe blade (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 and also occurs in *Colocasia*. Moreover, as in most taxa of the large subfamily Aroideae, anthesis lasts for two days only (Mayo *et al*. 1997). In three of the four *Colocasia* species studied (i.e. *C. esculenta*, *C. fontanesii*, *C. lihengiae*) the attraction of pollinators, the receptivity of the stigmas and the pollen extrusion occur during the early morning hours, as is also the case in other fly-pollinated Araceae (Mori & Okada 2001, Takenaka *et al*. 2006). In contrast, in aroids pollinated by scarab beetles the major events occur during dusk (Gibernau *et al*. 2000, Maia & Schlindwein 2006). Although *C. affinis* is pollinated by drosophilid flies too, the timing of anthesis differs. Here, pollen release and curling of the spathe blade take place during the afternoon rather than in the morning (but not at dusk as for species pollinated by beetles).

Thermogenesis — Thermogenesis is a common phenomenon in Araceae (Barthlott *et al*. 2008, Seymour *et al*. 2009). It does not only enhance odour emission but also serves as a heat reward for departing insects, facilitating the warm-up before takeoff (Seymour *et al*. 2003). In *C. esculenta* and *C. fontanesii* the peaks of heat production occur in the first and the 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 reach 6 - 8°C above ambient temperature. During the second morning, heat

production is mostly due to 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 to the staminate flowers extruding pollen. Simultaneously, the heat possibly stimulates the flies to leave the inflorescence after their body temperature has reached its optimum. In *C. esculenta* an additional - but weaker - thermogenetic 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, this 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 region (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*. 2007).

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 arrestment 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 pollinated pistillate flowers. In the latter case the spathe movements occur during the pistillate phase in order to arrest the insects inside the spathe tube. In *Colocasia*, closure of the spathe constriction after anthesis was observed in all species studied. However, the temporary occlusion of the lower floral chamber during anthesis has only been observed in *C. esculenta, C. fontanesii* and *C. lihengiae*, but not in *C. affinis*. Here, the spathe remains open for the entire duration of anthesis. In trapping inflorescences of *Typhonium* and *Theriophonum* the constriction occludes the spathe tube comprising the pistillate flowers (Armstrong 1979, Dakwale & Bhatnagar 1997). In contrast, it is the spathe blade that closes again in the three taxa of *Colocasia*, thereby occluding the entire spadix, while the constriction between the spathe tube and the spathe blade initially remains open. 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). Similar to some Schismatoglottids, the spathe blade of *Colocasia* species reflexes very fast after pollen release. 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 *Colocasia* spp. except for *C. affinis*. *Colocasiom. sp.* 3 *aff. colocasiae* is so far known to visit *C. esculenta* in Vietnam (M.J. Toda, pers. comm.). *Colocasiom. alocasiae* and *Colocasiom. xenalocasiae* are known as pollinators of *Alocasia odora* and *A. cucullata* (Yafuso 1994).

As far as we can conclude from our short observations, *C. affinis* was only visited by *Colocasiom. steudnerae*, which otherwise was 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 (M. J. Toda, pers. comm.). In contrast to *Steudnera colocasiifolia* and other *Colocasia* species with anthesis starting during the morning hours, anthesis in *C. affinis* starts in the afternoon. We suggest that the different timing could serve 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 and laying eggs mainly between the pistillate flowers (Miyake & Yafuso 2005). The importance of the brood-site as a major reward for drosophilids was also reflected by a bias towards female specimens in the most abundant pollinating species (Table 1). After the drosphilid flies have oviposited, larvae quickly hatch (usually within the next day). They stay mainly between the pistillate flowers. Sterile flowers situated between and/or below the pistillate flowers decay and form a mucilaginous substrate for the larvae. In inflorescences of *C. esculenta* in which flies were excluded by organdy bags these sterile flowers remained intact. Thus, the sterile pistillate flowers probably are an adaptation of the plant that facilitates the development of the fly larvae. The fertile pistillate flowers remain undamaged unlike in well know examples of brood-site pollination such as in *Ficus* or *Yucca* (Armbruster 2012). The larvae do not leave the inflorescence before pupation. As in *Alocasia*

macrorrhizos, they develop within the ripening infructescences (Takenaka Takano *et al.* 2012).

Bagging experiments — Due to their abundance and behaviour, flies of the genus *Colocasiomiya* appear to be the most important pollinators of *Colocasia*. Our bagging experiment in *C. esculenta* proves that inflorescences do not bear fruits 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 due to selection for vegetative traits affecting reproductive traits during human cultivation.

Due to protogyny, self-fertilisation is avoided in *Colocasia*. Moreover, the spathe constriction above the female flowers closes before pollen extrusion, thereby preventing pollen from falling onto the 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 highly intimate pollination mutualism in which the inflorescences of *Colocasia* serve as mating and breeding sites for the flies. Despite of the fact that the flies obtain rewards for their pollination services, they also get 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 lure-and-trap pollinated Araceae is remarkable. According to ancestral state reconstructions the trap mechanism has evolved de novo in *Colocasia* (Bröderbauer *et al.* 2012). The convergent evolution of trap pollination in different clades of Araceae is probably due to protogyny. Rewardless inflorescences have to retain the insects that have arrived during the pistillate phase of anthesis in order to secure pollen export during the staminate phase. In *Colocasia*, the reward (i.e. the brood site) is only available during the pistillate phase until the spathe constriction narrows and occludes the lower floral chamber. Then, flies have to progress into the upper floral chamber containing the staminate flowers, which at that time are still undehisced. Therefore, the trapping of the pollinators may be necessary in order to secure their presence until pollen is released. A comparable case is known in *Arum creticum* where bees are rewarded with pollen during the male phase but have to be arrested in the rewardless pistillate phase (Diaz & Kite 2006). The closure of the flower during anthesis also occurs in other rewarding protogynous lineages, mainly within the ‘basal angiosperms’ such as *Calycanthus* (Grant 1950) or *Magnolia* (Gottsberger *et al.* 2012). Therefore, we suggest

that protogyny is an important basis for the convergent evolution of retention mechanisms in several early angiosperm lineages.

In *C. affinis*, the spathe does not close during anthesis. Nevertheless, the drosophilids remain inside the inflorescence. Trapping is thus probably not an indispensable precondition for successful pollen export in *Colocasia*. We suppose that trapping in combination with the rapid reflexing of the spathe blade increases the probability that flies will depart at the right time (i.e. during pollen release), whereby a more efficient pollen transfer is enabled. Consequently, adaptations for the retention and release of pollinators in *Colocasia* probably have evolved in order to increase male rather than female reproductive success.

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, Procheş & Johnson 2009). As in *Colcoasia*, many of these taxa have flowers/inflorescences forming a pollination chamber and/or producing a fruity odour and heat. A close relative of *A. humeralis*, *A. concolor* Macleay, 1872, has been observed to visit *Gossypium tomentosum* on Hawai'i (Burraston *et al.* 2005). In Araceae, nitidulids have been found on inflorescences of *Amorphophallus*, *Cyrtosperma*, *Typhonium* and *Urospatha* (Gibernau 2003, Punekar & Kumaran 2009). However, no detailed studies on their behaviour in the inflorescences exist.

Aethina humeralis behaved similar to the drosophilids in the inflorescences of *Colocasia*. It entered the inflorescence at the first morning of anthesis and only departed on the second morning after pollen release. 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 investigation under light microscope. Thus, the beetle might transfer pollen between inflorescences successfully. 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 (A.G. Kirejtshuk, pers. comm.). Nevertheless, an important role as pollinator of *Colocasia* spp. seems unlikely due to 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 three 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 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. Unlike other osmophores (Vogel 1963), in *Colocasia* there are no specialised cell layers for the storage of starch, which is distributed in parenchymatic and epidermal cells. The intense osmophoric 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* are not pointing downwards. The drosophilid flies as well as 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 as osmophores only. 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).

In *C. affinis* the spathe blade only contained few papillate cells contrary to *C. esculenta* and *C. fontanesii*. As far as can be judged from smelling, odour was mainly produced by the spathe tube. Furthermore, immersion in neutral red caused intensive staining in the adaxial spathe tube but not in the blade. Epidermal cells of the adaxial tube were convex. Nevertheless, smelling and neutral red staining indicated that odour emission was of similar intensity as that of the osmophoric cells of the spathe blade in *C. esculenta* and *C. fontanesii*. Despite the absence of odour (at least to the human nose), the epidermis of the spathe tube in *C. esculenta* and *C. fontanesii* also showed a positive

staining reaction with neutral red. Thus, the cells also might produce odour or other substances which we could not perceive.

In all taxa studied, the epidermis of the abaxial spathe blade seemed to act as an osmophore too, showing a similar albeit weaker intracellular activity compared to the adaxial epidermal cells. Concordantly, osmophoric activity was only indicated by a weak staining with neutral red in all species studied. We conclude that the spathe emits odours in different parts, probably in 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 to influence 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 a more efficient pollen transfer. Possibly, such an odour gradient, in combination with the second thermogenetic 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 the pollen extrusion. Such an effect of the 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 concentration of odour compounds emitted by the spathe (Patt *et al.* 1995).

A prominent feature in spathe blades of the three taxa examined under LM was the presence of aerenchym-like lacunar tissue. Such tissue is known from the specialised osmophoric appendices in several members of Araceae (Vogel 1963). The intercellulars 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 the spathe of *Colocasia* indicates that in Araceae not only the spadix but also the spathe can be a highly elaborate osmophore.

Systematic position of C. affinis — According to the molecular phylogeny of Nauheimer *et al.* (2012b), *C. affinis* is more closely related to *Steudnera* than to the species of *Colocasia*. The sharing of the same pollinator in *S. colocasiifolia* and *C. affinis* might reflect this close relationship. We also found that the epicuticular wax layer on the adaxial spathe blade in *C. affinis* resembles that of *Steudnera* spp. (Bröderbauer *et al.* 2012). Moreover, *C. affinis* differs from other species of *Colocasia* through the absence

of papillate epidermal cells and the temporary closure of the spathe during anthesis. These observations support a placement of *C. affinis* as sister to *Steudnera*. However, the pollen of *C. affinis* is echinate like that of *C. esculenta*, and *C. fontanesii*, while pollen of *Steudnera* spp. is plicate (Hesse 2001, Bröderbauer & Ulrich, unpublished data). Thus, the different characters provide conflicting evidence. Further studies are needed to clarify the relationships within *Colocasia* and related genera.

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CHAPTER 4

INSECT POLLINATORS SELECT FOR THE DESIGN OF TRAPPING DEVICES IN POLLINATION TRAPS OF THE GENUS *ARUM* (ARACEAE)

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Angabe zum Eigenanteil des Dissertanten: David Bröderbauer hat die Daten für den vorliegenden Artikel gesammelt, ausgewertet (die statistischen Analysen mit SPSS und Primer wurden von A. Diaz durchgeführt), und den Text für das Manuskript (in Zusammenarbeit mit A. Diaz und A. Weber) verfasst.

ABSTRACT

Pollinators have long been known to select for floral traits but the nature of this relationship has been little investigated in trap pollination systems. We investigated the trapping devices of 15 trapping species of *Arum*, a genus characterised by pollination traps, and compared these features with the types of insects trapped. The species examined had a similar general design with trap chamber walls covered in downward-pointing papillate cells, and lacunate cells improving oxygen supply within the chamber and with elongated sterile flowers partially blocking the constriction of the trap. However, there was significant variation in many features particularly in the size of papillate cells but also in the area of lacunae and the number of sterile flowers. Furthermore, these differences related to the type of pollinator trapped. Species pollinated by midges had small papillae, a larger lacunate area and more sterile flowers than species pollinated by flies and beetles while sterile flowers were almost or completely absent in bee visited *Arum creticum* and *A. idaeum*. We conclude that trap pollinated systems evolve in response to the type of insect trapped and that changes to the slippery surfaces of the chamber wall are an important and previously little recognised variable in the design of pollination traps.

INTRODUCTION

It is well known that pollinators select for floral traits in flowers and thus affect the size and shape of floral organs (e.g. Schemske & Bradshaw 1999, Sletvold *et al.* 2012). In rewarding flowers, stabilising selection on advertisement traits is thought to ensure recognition by pollinators that learn to remember floral characters (Ackermann *et al.* 2011). In contrast, in deceptive flowers variability in floral traits is presumed to be higher so that pollinators are not able to learn to discriminate between the deceptive flower and the imitated rewarding model (Ayasse *et al.* 2000).

Some deceptive flowers not only mimic rewards but also trap their pollinators in order to ensure pollen transfer (Vogel & Martens 2000). Such pollination traps have evolved in various angiosperm lineages. They are characterised by a chamber formed by tepals or modified bracts that enclose the flowers (Vogel 1965). Different morphological adaptations enable the trapping of the insect pollinators inside the chamber. For example, slippery surfaces covering the chamber walls occur in several clades (Poppinga *et al.* 2010). These surfaces usually consist of downward-pointing papillate cells or an epicuticular wax layer that disable the insect's attachment organs and cause it to slip into the floral chamber (Gaume *et al.* 2004). Some pollination traps bear hairs on the chamber walls that block the exit of the floral chamber (Oelschlägel *et al.* 2009). In some taxa, the entire floral chamber becomes temporarily occluded by a constriction of the chamber wall (Ulrich *et al.* 2012). Insects can escape from the floral chamber only after pollen release, when the exit reopens and/or after the trapping devices have wilted (Bröderbauer *et al.* 2012). However, the extent to which these trapping devices are under selection based on the type of pollinator caught is currently an entirely unexplored question.

The genus *Arum* offers an excellent opportunity to explore the relationship between pollinators and floral structure as it comprises of 29 species (Boyce 1993, Linz *et al*. 2010) which attract various types of pollinators (reviewed in Gibernau *et al.* 2004). All species of *Arum* have highly synorganised inflorescences consisting of a flower-bearing spadix (with pistillate, staminate as well as sterile flowers) that is surrounded by a modified bract, the so-called spathe (Fig. 1). The adaxial spathe epidermis consists of downward-pointing papillate cells that are slippery and cause the insects to glide into the floral chamber (Knoll 1926) which is formed by the inflated spathe tube. Intercellular spaces in the wall of the spathe tube, called lacunae, support the oxygen supply in the floral chamber which is believed to prevent the suffocation of pollinators during their arrest (Bermadinger-Stabentheiner & Stabentheiner 1995). Elongated sterile flowers

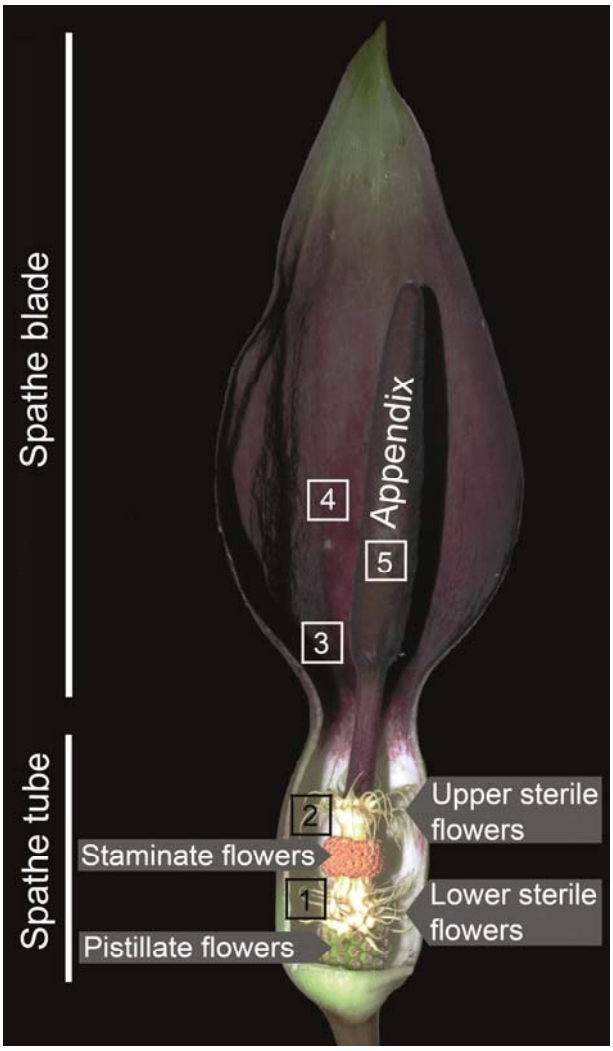


Fig. 1. Inflorescence of *Arum elongatum* consisting of the flower bearing spadix and the surrounding spathe. The numbers in boxes indicate the parts of the spathe from which samples were taken (i.e. 1, lower spathe tube; 2, upper spathe tube; 3, spathe blade one cm above the spathe constriction; 4, central spathe blade; 5, central part of the appendix). The frontal part of spathe tube has been removed to show the flowers inside.

situated on the spadix below the appendix and the staminate flowers (Fig. 1) are also slippery and hinder the escape of the trapped insects (Knoll 1926). In addition, papillate cells are also found on the sterile appendix that sits atop of the staminate flowers (Fig. 1) and produces heat and odour (Mayo *et al.* 1997). In all species studied so far, the pollinators are trapped within the floral chamber for about 24h (e.g. Diaz & Kite 2002, Quilichini *et al.* 2010, Stökl *et al*. 2010). The various types of pollinators include saprophilous flies and beetles, midges, and bees (e.g. Gibernau *et al.* 2004).

The overall aim of the present study is to compare the relationship between trapping devices and types of pollinators in species of the genus *Arum* (Araceae). The specific questions are: (1) How variable is the overall design of the trapping inflorescences within the genus *Arum*? (2) Are differences in the design of the respective trapping devices related to differences in the type of insects trapped?

MATERIALS AND METHODS

Comparison of trap design — To compare the trap design of the *Arum* species examined, we investigated all inflorescence structures that contribute to the trapping of the pollinators. These are (1) the papillate epidermal cells that cover the spathe and the spadix, (2) the lacunae in the spathe tissue of the spathe tube, and (3) the elongated sterile flowers situated below and above the staminate flowers. Despite best efforts no more than 15 of the 29 species of *Arum* were available for our study. These species cover the majority of the clades in the genus (Espindola *et al.* 2010) and represent all pollination syndromes found in *Arum* (Linz *et al.* 2010). Voucher specimens are preserved in the spirit collections of the Herbarium of the University of Vienna (WU) (see Appendix).

Relationship between pollinators and trapping devices — Inflorescences were collected during anthesis and were preserved in 70% alcohol. For the investigation under scanning electron microscopy (SEM) samples were taken from five different regions of the spathe: (1) lower spathe tube - at the level of the lower sterile flowers; (2) upper spathe tube - at the level of the upper sterile flowers; (3) 1cm above the spathe constriction; (4) from the central part of the spathe blade; and (5) from the central part of the spadix-appendix (Fig. 1). Samples were dehydrated in a graduated series of ethanol and then infiltrated with acetone. Afterwards samples were critical-point-dried, sputter-coated with gold and investigated with a JEOL JSM6390 SEM.

Interspecific variation in trapping devices — To compare the nature of the slippery surfaces of each species we measured the basal area of papillae (n = 10) and the length of papillae (n = 10) for the upper spathe tube, the lower and the central spathe blade, and the spadix-appendix. In addition, we estimated the average area of the upper spathe tube covered with lacunae (n = 10) by multiplying the average lacuna size (n = 10) by the number of lacunae given for an area under 500 μm magnification. As the lower spathe tube did not contain trapping devices, it was excluded from the further statistical analyses.

Univariate analyses were carried out using SPSS version 15. We tested for differences in the design of the slippery surfaces between all 15 species and between the pollinator types using the Kruskal-Wallis-test. For the latter analysis, the four species with unknown pollinators were removed as they could not be assigned to any group. Multivariate analyses were carried out using Primer version 6 (Clarke & Gorley 2006).

Firstly we gained a visual representation of the combined differences in plant pollination traits between species by applying a non-metric multi-dimensional scaling (NMDS) analysis based on the Bray-Curtis similarity index with no data transformation and no normalisation. Then, in order to test whether there were significant differences between plant species and insect groups, we conducted a one-way analysis of similarity (ANOSIM) with 999 permutations. The global R statistic that results from ANOSIM represents similarity and generally ranges from 0 (total similarity) to 1 (total dissimilarity). As the measures for the area and the length of papillae could not be taken at one time for the same papilla during investigation under SEM, we had to combine data on length and area originating from different papillae. Therefore, the data for a single point in the NMDS stem from different papillae, whereby every point becomes a pseudo-individual. Nevertheless, our results indicate that the data are representative, as the pseudo-individuals of the respective species always grouped together.

In addition, we used a second approach, where we produced a reduced data set using the mean of all 10 measurements, thus only having a single data point for every given species (instead of 10). The latter analyses using the means enabled us to include data on elongated sterile flowers measured by us (i.e. number of elongated sterile flowers) and taken from the literature (Boyce 1993). As Boyce does not provide averages but a data range for his measurements (i.e. the number of whorls, the length of the sterile flowers and the length of the sterile zone) we used the mid-range of the data range recorded by him. In order to test for a possible difference in the importance of the slippery surfaces versus the elongated sterile flowers, we made three analyses: (1) all data included, (2) only slippery surface data included, (3) only elongated sterile flowers data included. Two species with unknown pollinator type, *Arum besserianum* and *A. megobrebi*, were excluded from these analyses as no quantitative information on the number of sterile flowers was available for them.

Identification of pollinators — Information on pollinators was taken from the literature (Table 1). We grouped the species of *Arum* according to the composition of their pollinating fauna as follows: (1) Bees (Hymenoptera); (2) Flies & beetles (Diptera-Brachycera & Coleoptera); (3) Midges (Diptera-Nematocera).

Table 1. Species of *Arum* investigated and their pollinators. Note: Taxa in brackets represent visitors that are most likely not involved in pollination according to the more recent literature

Species	Pollinator type	Taxa	Source
A. balansanum R.R.Mill	unknown	unknown	
A. besserianum Schott	unknown	unknown	
A. concinnatum Schott	flies & beetles	Chironomidae, Drosophilidae, Psychodidae, Sciaridae, Sphaeroceridae, Staphylinidae	Drummond & Hammond, 1993; Gibernau *et al*., 2004; Urru *et al*., 2010
A. creticum Boiss. & Heldr.	bees	Halictidae (Miridae, Chrysomelidae, Melyridae, Scarabeaidae)	Diaz & Kite, 2006 (Drummond & Hammond, 1993; Gibernau *et al*., 2004)
A. cylindraceum Gasp.	midges	Culicidae, Psychodidae	Gibernau *et al*., 2004
A. dioscoridis Sm.	flies & beetles	Scarabaeidae, Sphaeroceridae, Staphylinidae	Kullenberg, 1953; Papp & Rohacek, 1987; Drummond & Hammond, 1991; Gibernau *et al.*, 2004
A. elongatum Steven	midges	Ceratopogonidae	Braverman & Koach, 1982; Koach, 1985
A. euxinum R.R.Mill	midges	Psychodidae, Sphaeroceridae	Gibernau *et al*., 2004; Linz *et al.*, 2010
A. hygrophilum Boiss.	midges	Psychodidae	Koach, 1985; Gibernau *et al.*, 2004
A. idaeum Coustur. & Gand.	bees	Halictidae,(Miridae, Chrysomelidae, Melyridae, Mordellidae)	Diaz & Kite, 2006 (Gibernau *et al*., 2004)
A. italicum Mill.	midges	Chironomidae, Psychodidae, Ceratopogonidae, Drosophilidae	Diaz & Kite, 2002; Albre, Quilichini & Gibernau, 2003; Gibernau *et al.*, 2004
A. maculatum L.	midges	Psychodidae	Rohacek, Beck-Haug & Dobat, 1990; Lack & Diaz, 1991; Diaz & Kite, 2002; Gibernau *et al*., 2004
A. megobrebi Lobin, M.Neumann, Bogner & P.C.Boyce	unknown	unknown	
A. nigrum Schott	flies & beetles	Sphaeroceridae, Staphylinidae	Knoll, 1926; Gibernau *et al*., 2004
A. purpureospathum P.C.Boyce	unknown	unknown	

RESULTS

Comparison of trap design — Apart from *Arum creticum* and *A. idaeum*, spathes of the species of *Arum* studied showed a clear zonation and a consistent set of features. The lower spathe tube consisted of unspecialised tabular to convex epidermal cells, often with small intercellulars in the cell corners (Fig. 2A). In the upper part of the spathe tube cells were papillate and downward-pointing and lacunae (i.e. large intercellulars) occurred in the corners of the papillate cells (Fig. 2B). In *Arum creticum* and *A. idaeum* the spathe tube lacked papillate cells and lacunae (Fig. 2C). Moreover, in *A. idaeum* papillae where absent in the entire spathe (Fig. 2D). In the other species, the epidermis of spathe blade was made up of downward-pointing papillae and lacunar tissue was absent (Fig. 2E). While in some species papillae covered the whole cell surface (Fig. 2E) (i.e. *Arum concinnatum*, *A. creticum*, *A. dioscoridis*, and *A. nigrum*), in the other species the papillae emerged from tabular cell surfaces (Fig. 2F). The appendix was covered with papillae in all species except *Arum idaeum*, but in contrast to the papillae covering the

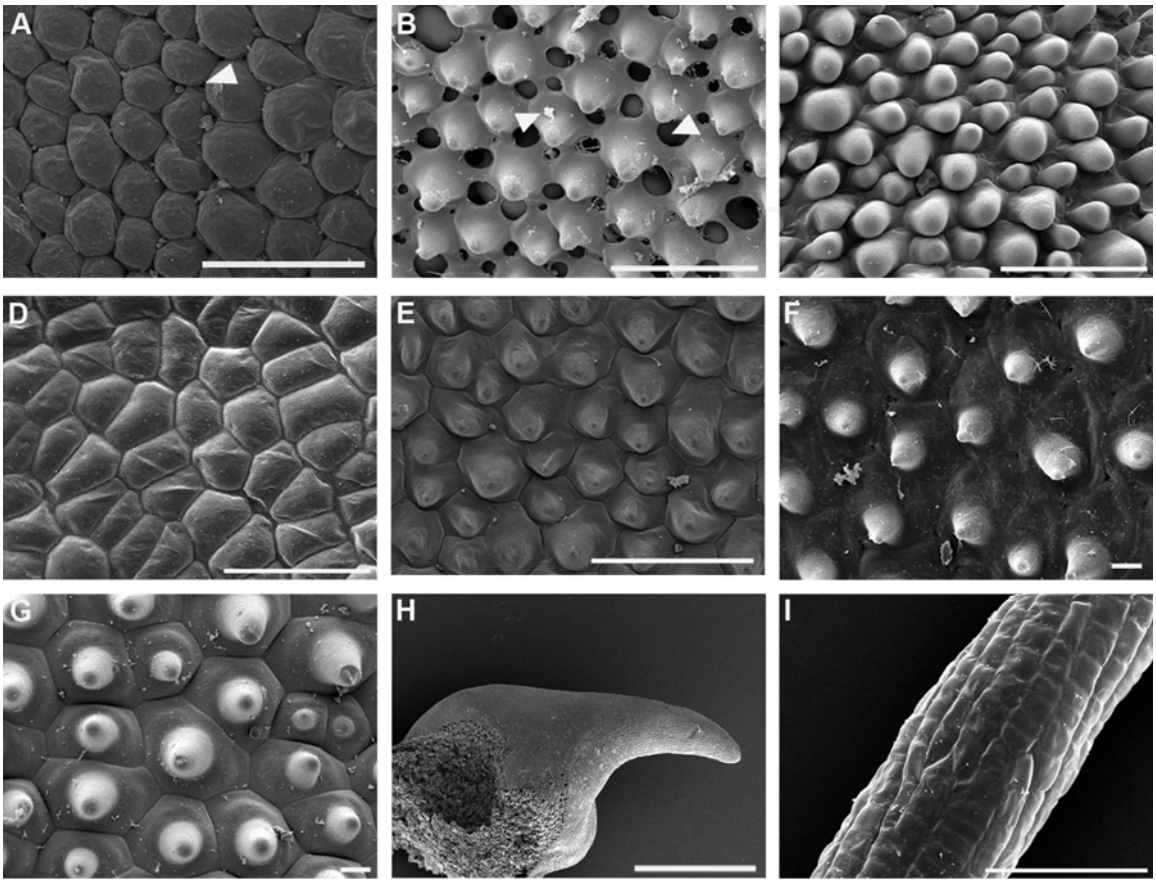


Fig. 2. Trapping devices in *Arum*. **A,** *A. megorbrebi*, lower spathe tube, convex epidermal cells. Note the small lacunae in the cell corners (arrowhead); **B,** *A. besserianum*, upper spathe tube, downward-pointing papillate epidermal cells with lacunae in the cell corners (arrowheads); **C,** *A. creticum*, upper spathe tube, downward-pointing papillate epidermal cells. Note that lacunae are absent; **D,** *A. idaeum*, spathe blade, tabular epidermis cells; **E,** *A. concinnatum*, spathe blade, downward-pointing papillate epidermal cells; **F,** *A. italicum*, spathe blade, downward-pointing papillae emerging from tabular epidermal cells; **G,** *A. euxinum*, appendix, straight papillae; **H,** *A. nigrum*, elongated sterile flower; **I,** *A. cylindraceum*, tabular epidermis of the elongated sterile flower. Scale bars = 1000 μm (K), 100 μm (A-E, H, I), 10 μm (F, G).

spathe they were perpendicular to the appendix surface or only slightly downward-pointing (Fig. 2G). The epidermis of the elongated sterile flowers was tabular (Fig. 2H, I). In all species, stomata were rare on the adaxial (i.e. inner) spathe epidermis, but common on the abaxial epidermis, especially along the spathe tube.

Relationship between pollinators and trapping devices — The means±standard deviation (SD) of measurements of slippery surfaces are shown in Table 2. Species pollinated by flies & beetles had larger papillae than those pollinated by midges, while the lacunate area was larger in the latter group. The two species pollinated by bees had no lacunae at all and also lacked elongated sterile flowers. While papillae were absent in *Arum idaeum*, papillae of *A. creticum* appeared to be intermediate in size between the group of species pollinated by flies & beetles and the group pollinated midges. In general

Table 2. Size of papillate cells and lacunae in *Arum* spp.

Species (n = 10)	Length of papillae (µm) Section 2	Length of papillae (µm) Section 3	Length of papillae (µm) Section 4	Length of papillae (µm) Section 5	Basal area of papillae (µm²) Section 2	Basal area of papillae (µm²) Section 3	Basal area of papillae (µm²) Section 4	Basal area of papillae (µm²) Section 5	Area of lacunae (µm²) Section 2
A. balansanum R.R.Mill	31.80 ±3.88	20.60 ±5.34	34.00 ±7.01	31.80 ±4.83	490.40 ±91.49	176.30 ±42.31	1047.30 ±294.26	391.00 ±101.91	2873.70 ±391.05
A. besserianum Schott	22.00 ±3.71	13.10 ±6.66	11.90 ±2.56	32.00 ±5.16	264.80 ±78.30	279.10 ±74.81	347.30 ±72.31	416.40 ±191.96	8449.74 ±595.37
A. concinnatum Schott	31.00 ±2.11	24.70 ±4.32	22.80 ±4.08	25.00 ±5.27	669.60 ±122.38	598.40 ±221.61	625.40 ±108.99	553.90 ±165.28	7782.21 ±743.35
A. creticum Boiss. & Heldr.	41.90 ±5.36	28.00 ±3.68	29.90 ±3.98	19.40 ±4.14	527.00 ±63.56	681.20 ±225.74	679.30 ±161.50	708.20 ±207.85	0
A. cylindraceum Gasp.	21.60 ±3.50	18.20 ±1.75	18.30 ±2.79	25.00 ±5.81	441.30 ±96.39	206.00 ±39.64	332.60 ±67.04	357.50 ±64.01	5883.54 ±994.40
A. dioscoridis Sm.	45.10 ±4.77	42.90 ±6.47	41.20 ±7.35	37.60 ±3.8	1297.50 ±360.88	1411.10 ±315.05	1510.90 ±366.7	764.30 ±251.23	5077.80 ±755.35
A. elongatum Steven	45.30 ±10.90	26.10 ±2.56	21.60 ±3.72	16.00 ±1.25	650.10 ±165.71	396.50 ±59.58	394.70 ±74.85	507.00 ±196.10	4656.24 ±731.63
A. euxinum R.R.Mill	17.00 ±3.43	13.50 ±1.65	13.50 ±1.72	19.20 ±4.57	248.60 ±40.93	172.60 ±34.56	151.70 ±39.23	234.00 ±57.23	8001.99 ±653.73
A. hygrophilum Boiss.	18.30 ±3.02	10.60 ±1.90	10.60 ±2.17	8.00 ±1.70	292.30 ±123.08	50.50 ±19.75	55.60 ±15.09	69.50 ±17.05	3367.20 ±316.68
A. idaeum Coustur. & Gand.	0	0	0	0	1046.10 ±278.67	964.50 ±162.65	1452.40 ±289.79	898.50 ±198.28	0
A. italicum Mill.	23.20 ±3.19	15.20 ±1.69	16.50 ±1.58	31.90 ±4.79	266.40 ±43.79	166.00 ±50.22	149.20 ±42.01	425.40 ±116.71	5619.38 ±327.40
A. maculatum L.	29.40 ±2.59	28.30 ±2.71	23.60 ±3.17	33.80 ±3.88	441.40 ±71.70	396.70 ±62.50	370.70 ±81.14	292.20 ±87.42	9162.28 ±804.51
A. megobrebi Lobin, M.Neumann, Bogner & P.C.Boyce	29.90 ±5.49	17.80 ±2.86	0	18.70 ±2.26	442.20 ±82.91	502.30 ±82.42	587.20 ±108.28	347.60 ±142.74	5364.05 ±433.27
A. nigrum Schott	69.80 ±8.04	43.30 ±10.60	32.40 ±7.18	29.80 ±6.96	1779.30 ±600.00	856.50 ±320.68	747.50 ±285.11	914.50 ±206.98	990.28 ±114.36
A. purpureospathum P.C.Boyce	31.40 ±8.04	27.60 ±3.10	25.30 ±3.95	32.20 ±3.61	449.80 ±114.72	365.90 ±120.61	502.70 ±129.50	624.20 ±153.14	5745.39 ±641.94

Note: Numbers are means ± standard deviation.

Table 3. Measures of elongated sterile flowers in *Arum* spp.

Species	Number of the upper sterile flowers (N)	Number of the lower sterile flowers (N)	Length of the upper sterile flowers	Length of the lower sterile flowers	Width of the upper sterile zone	Width of the lower sterile zone	Number of whorls (upper sterile flowers)	Number of whorls (lower sterile flowers)
A. balansanum R.R.Mill	61.5 (2) ±13.4	9.5 (2) ±2.1	5	4	4.75	4.25	3	4.5
A. besserianum Schott	-	-	-	-	-	-	-	-
A. concinnatum Schott	86.6 (5) ±9.5	21.5 (5) ±7.2	6.5	3.5	12	4	6	2
A. creticum Boiss. & Heldr.	1.3 (10) ±2.4	0.8 (10) ±2.2	0	0	0	0	0	0
A. cylindraceum Gasp.	39.7 (6) ±7.6	16.17 (6) 3.9	5.5	3.5	3	4	2.5	4
A. dioscoridis Sm.	28 (1)	8 (1)	5.75	4.5	4.75	6.25	2.5	4
A. elongatum Steven	53 (1)	16 (1)	3.5	3	3.75	3.25	3.5	3.75
A. euxinum R.R.Mill	76 (1)	7 (1)	4.25	3	5	2.2	4	1
A. hygrophilum Boiss.	24 (1)	11 (1)	4.5	4.5	2.75	2.5	2.5	2.5
A. idaeum Coustur. & Gand.	0 (4)	0 (4)	0	0	0	0	0	0
A. italicum Mill.	66.5 (4) ±10.0	17 (4) ±1.2	4.75	4.25	7	2.75	3.5	3.5
A. maculatum L.	68 (1)	15 (1)	5.5	2.75	6	4.75	5.5	2
A. megobrebi Lobin, M.Neumann, Bogner & P.C.Boyce	-	-	-	-	-	-	-	-
A. nigrum Schott	15 (2) ±2.8	12 (2) ±2.8	6	6	3	1.75	2	1
A. purpureospathum P.C.Boyce	40 (1)	34 (1)	8.5	7.5	5.5	4	3	3

Note: Numbers of upper and lower sterile flowers are means ± standard deviation, while the remaining numbers are mid-ranges of data from Boyce (1993).

the number of elongated sterile flowers and whorls of flowers was greater in species pollinated by midges than in species pollinated by flies & beetles with the exception of *Arum concinnatum*, which had that highest number of sterile flowers out of the l3 species measured (Table 3). The size of papillae and the area covered by lacunae differed significantly in the species compared (Table 4). Moreover, these differences were also significant when compared across the three pollinator groups (Table 5).

The interspecific variation in the trapping devices found in the Kruskal-Wallis-tests was also evident in the NMDS-analyses. The individual measurements of the size of slippery surfaces grouped according to species identity and were in most cases distinguishable from other species (Fig. 3). Only in *Arum cylindraceum* and *A. italicum* we found a large overlap. The two species pollinated by bees (i.e. *Arum creticum* and *A. idaeum*) grouped closely together and were clearly distinct from the rest. The three species pollinated by flies & beetles did not form a uniform cluster. While *Arum concinnatum* overlapped with species pollinated by midges, *A. dioscoridis* and *A. nigrum* occurred more distant from the midges-group. The midge-pollinated species themselves formed a larger cluster. Only *A. hygrophilum* appeared isolated from the other taxa.

Table 4. KruskalWallis-ANOVA for size of slippery surfaces and lacunae in 15 species of *Arum*.

	Length of papillae (µm) Section 2	Length of papillae (µm) Section 3	Length of papillae (µm) Section 4	Length of papillae (µm) Section 5	Basal area of papillae (µm²) Section 2	Basal area of papillae (µm²) Section 3	Basal area of papillae (µm²) Section 4	Basal area of papillae (µm²) Section 5	Area of lacunae (µm²) Section 2
H (df = 3)	148.26 ***	129.89 ***	137.92 ***	121.24 ***	126.94 ***	133.19 ***	135.48 ***	108.52 ***	139.63 ***

Note: Significant differences in the Kruskal-Wallis analyses are indicated as *** (P < 0.001).

Table 5. KruskalWallis-ANOVA for size of slippery surfaces and lacunae of *Arum* spp. visited by different types of pollinators.

Spathe surface	Flies & beetles (n = 30)	Midges (n = 60)	Bees (n = 20)	H (df = 3)
Length of papillae (µm) Section 2	48.63 ±17.16	25.80 ±10.92	20.95 ±21.81	35.26 ***
Length of papillae (µm) Section 3	36.97 ±11.47	18.65 ±6.83	14.00 ±14.59	43.26 ***
Length of papillae (µm) Section 4	32.13 ±9.81	17.35 ±5.15	14.95 ±15.58	38.85 ***
Length of papillae (µm) Section 5	30.80 ±7.49	22.32 ±9.85	9.70 ±10.35	35.80 ***
Basal area of papillae (µm²) Section 2	1248.80 ±608.55	390.02 ±170.90	786.55 ±331.07	65.66 ***
Basal area of papillae (µm²) Section 3	955.33 ±443.72	231.38 ±134.99	822.85 ±240.40	74.27 ***
Basal area of papillae (µm²) Section 4	961.27 ±479.04	242.42 ±141.30	1065.85 ±457.62	79.83 ***
Basal area of papillae (µm²) Section 5	744.23 ±252.96	314.27 ±174.11	803.35 ±220.49	62.86 ***
Area of lacunae (µm²) Section 2	4616.76 ±2901.05	6115.10 ±2072.82	0	51.75 ***

Note: Numbers are means ± standard deviation; significant differences in the Kruskal-Wallis analyses are indicated as *** (P < 0.001).

Three species with unknown pollinator type (i.e. *Arum besserianum*, *A. megobrebi*, *A. purpureospathum*) occurred among the midge-pollinated species, while *A. balansanum* formed a distinct cluster. According to the ANOSIM-analysis pollinator types differed significantly among each other. Only between species pollinated by midges and species with unknown pollinator type there was no significant difference, indicating that the latter species might also be pollinated by midges.

The results of the NMDS-analysis performed with the reduced data set (i.e. the means of the measurements) were in agreement with the extended analysis (Fig. 4A). This was also the case when the data on the elongated sterile flowers were included (Fig. 4B). When only the sterile flower data were used, the results differed in some parts (Fig. 4C). Here, *Arum hygrophilum* grouped with *A. dioscoridis* and *A. nigrum*, while *A.*

euxinum and *A. purpureospathum* appeared more distant from the midge-pollinated taxa than in the previous analyses.

DISCUSSION

Comparison of trap design — Trap pollination by different types of insects has been recorded in different species of *Arum* (Gibernau 2003, Quilichini *et al.* 2010). However, few studies have dealt with the trapping devices that secure successful pollination (Knoll 1926, Lack & Diaz 1991). We found that the overall design of the pollination traps and the zonation of the trapping devices were very uniform among the particular species. A uniform bauplan with the presence of a basal inflated chamber, a narrow tube and an apical expanded section, also occurs in species of other pollination traps, e.g. in *Aristolochia* (Aristolochiaceae) and *Ceropegia* (Apocynaceae) (Vogel 1965, Oelschlägel *et al.* 2009). This uniformity is probably due to the common requirements for attraction, trapping and retaining of the pollinators (Vogel 1965).

Nevertheless, in *Ceropegia* and *Aristolochia* there is considerably higher variation in the size and shape of the trap (Ollerton *et al.* 2009) as well as the zonation and composition of trapping devices (Vogel 1961, 1965). Different species of the latter two genera contain either both trapping hairs (analogous to the sterile flowers in *Arum*) and slippery surfaces or only one of the two features. Moreover, trapping hairs often occur on different parts of the floral tube. In contrast, we found that in *Arum* sterile flowers almost always co-occur with slippery surfaces and that the latter always occur in the same zones of the trap. A major difference between *Arum* and the above-mentioned genera is that the trap in *Arum* is not a flower, but an inflorescence. While in *Ceropegia* and *Aristolochia* slippery surfaces as well as trapping hairs are formed by the epidermis of the perianth, the trapping devices of *Arum* are formed by different organs (i.e. the spathe and the sterile flowers of the spadix). These organs need to be synorganised in order to ensure successful trapping of the pollinators. As shown for several angiosperms, variation in floral traits is often lower in flowers with a higher degree of synorganisation (Armbruster *et al*. 2009a), and this might also be the reason for the conserved bauplan and zonation of trapping devices in *Arum*.

The species of *Arum* that deviated most from the core design were the two closely related species *Arum creticum* and *A. idaeum*. *A. creticum* rewards pollinating bees with pollen during the staminate phase of anthesis but has to trap the bees in the rewardless

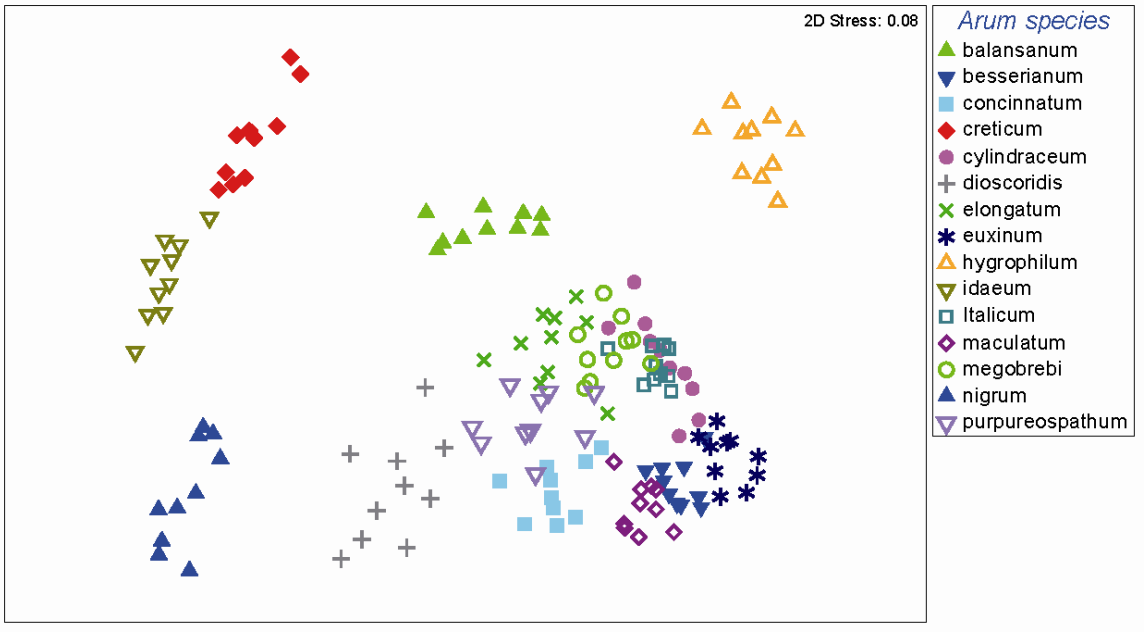


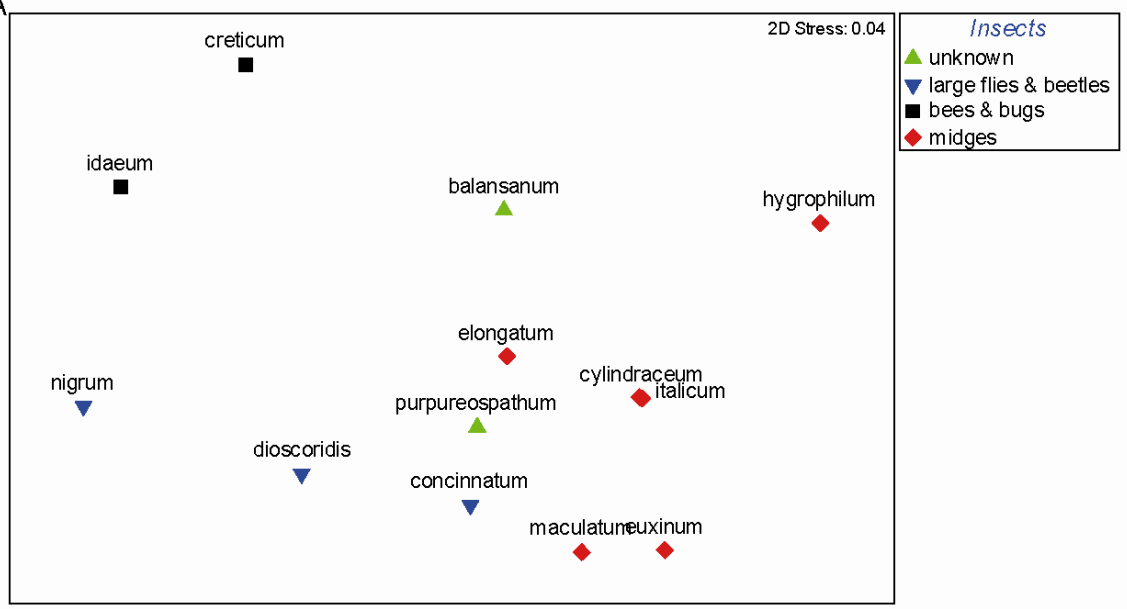
Fig. 3. NMDS of trapping devices (slippery surfaces and lacunae) representing 15 species of *Arum*.

pistillate phase in order to ensure the transfer of pollen to the stigmas (Diaz & Kite 2006). Results from the current study indicate that the switch to a semi-rewarding pollination system coincides with trait changes that may represent a causal relationship, i.e. a reduction of lacunae and sterile flowers but maintenance of the slippery papillae which are still necessary to make the bee glide into the lower spathe tube containing the pistillate flowers (Diaz & Kite 2006). The sister species *A. idaeum*, an endemic species confined to mountain tops in Crete, also attracts bees at the lower altitude margins of its distribution range (Diaz & Kite 2006) but is also capable of autogamy by a loss of dichogamy (Diaz, unpublished data). Results from the current study suggest this reduction of selective pressure for trapping may result in a concomitant reduction of all trapping devices.

Slippery surfaces made up by downward-pointing papillate cells occur not only in *Arum* but also in several other genera of Araceae (Bröderbauer *et al.* 2012), in pollination traps of other angiosperms and even in the pitcher traps of carnivorous plants (Poppinga *et al.* 2010). In *Arum*, the papillae cover the upper spathe tube and the spathe blade. In contrast, in *Ceropegia* the slippery surfaces are usually restricted to the trap entrance (Vogel 1961). Here, the slippery surfaces ensure that the insects slip, but the subsequent arrest of insects is secured by different trapping devices (Vogel 1961). The presence of a

Fig. 4. NMDS of mean values of trapping devices (slippery surfaces, elongated sterile flowers, and lacunae) displayed for pollinator type. **A,** All trapping structures; **B,** Slippery surfaces and lacunae; **C,** Elongated sterile flowers. Note: *Arum creticum* and *A. ideaum* are not displayed in 4C as they lack sterile flowers. ►►►

A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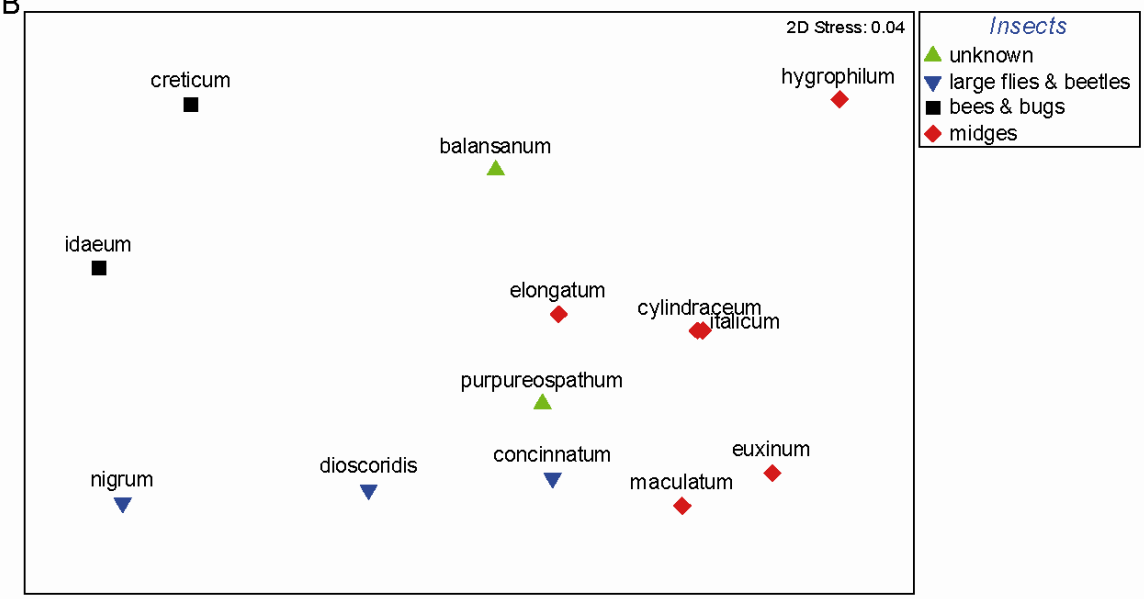
idaeum
balansanum
hygrophilum
elongatum
nigrum
cylindraceum
italicum
purpureospathum
dioscoridis
concinnatum
maculatum
euxinum
Insects
unknown
large flies & beetles
bees & bugs
midges

B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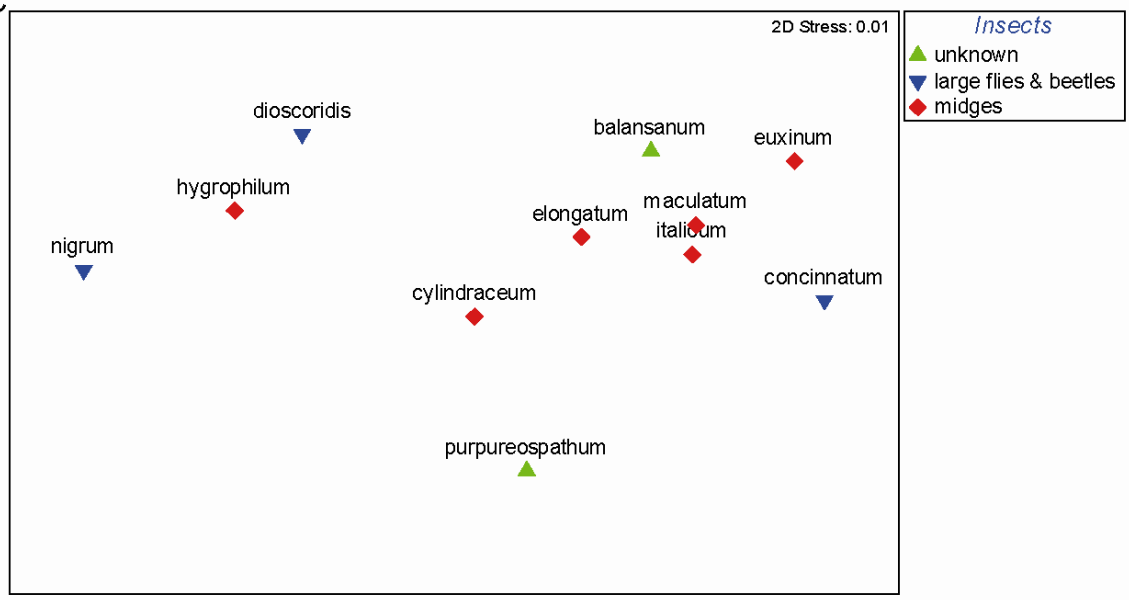
hygrophilum
balansanum
idaeum
elongatum
cylindraceum
italicum
purpureospathum
nigrum
dioscoridis
concinnatum
euxinum
maculatum
Insects
unknown
large flies & beetles
bees & bugs
midges

C


2D Stress: 0.01
dioscoridis
balansanum
euxinum
hygrophilum
maculatum
elongatum
italicum
nigrum
concinnatum
cylindraceum
purpureospathum
Insects
unknown
large flies & beetles
midges

slippery surface inside the spathe tube of *Arum* indicates that it has a dual function. First, insects slip on the spathe blade, then they are hindered from climbing the spathe walls and escaping by the slippery surfaces inside the spathe tube. We found that in contrast to the papillae of the spathe the papillae on the spadix-appendix are hardly downward-pointing. The primary function of the appendix is apparently the production of heat and odours (Vogel 1963, Seymour *et al.* 2003). In addition, already Knoll (1926) observed that insects in *Arum nigrum* tend to slip usually on the spathe blade, indicating that the the appendix is less important for trapping. We conclude that papillate cells in *Arum* have various functions, including to cause the slipping of insects but also to ensure their retention.

We found that the elongated sterile flowers always face the slippery surface inside the spathe tube. We suppose that both parts have to act together in order to avoid the escape of insects. In *Aristolochia* and *Ceropegia*, the trapping hairs are unicellular or multicellular trichomes that replace the slippery surfaces in parts of the floral tube (Vogel 1961). By blocking the entrance, they alone hinder the insects from escaping (Oelschlägel *et al.* 2009). In *Arum*, the organs function in a different way. They do not block the entrance completely but they produce slippery oil so insects cannot climb them (Knoll 1926). Consequently, the interplay with the slippery spathe epidermis appears to be indispensable in *Arum*, as insects otherwise could simply pass by the sterile flowers through climbing the spathe wall.

Like other adaptations for insect trapping, lacunate cells have evolved convergently in pollination traps of different angiosperm families (Vogel 1961). In *Arum*, these lacunae were so far only known for *Arum maculatum* and *A. nigrum*. We found that lacunae are present in most species studied. They are commonly interpreted as supporting the O_2-uptake of the trap chamber in order to avoid the suffocation of trapped insects (Knoll 1923, Bermadinger-Stabentheiner & Stabentheiner 1995). However, there is no definitive proof for this hypothesis and other explanations have been proposed too, favouring a mere structural role not related to pollination (Vogel 1961). Nevertheless, our finding that lacunae only have been reduced in those species of *Arum* that have shifted to a very unusual pollination mode (i.e. bee pollination *Arum creticum* and *A. idaeum*) supports Knoll's original hypothesis that lacunae represent an adaptation for trapping pollinators.

The presence of trapping devices in most species studied (except for *Arum idaeum* that appears to have lost its pollinator) and their uniform zonation indicates that

trap pollination is a stable condition within the genus *Arum*, similar to other aroid taxa with pollination traps (Bröderbauer *et al.* 2012). In *Aristolochia* and *Ceropegia*, switches to reward pollination probably have occurred more often (Sakai 2002b, Ollerton *et al.* 2009) and therefore trap pollination might be a less stable condition in these genera. A direct comparison between *Arum* and the latter two genera is however difficult, as both taxa are much more species-rich (*Aristolochia* >120 spp., *Ceropegia* >180 spp.) and have a much wider distribution range than *Arum*. Moreover, the flowers of *Ceropegia* are not protogynous unlike most pollination traps (Dafni 1984, Thien *et al.* 2009) and they are highly adapted for an efficient pollen export through the presence of pollinia (Wyatt 1978). Therefore, a switch to a non-trapping pollination syndrome appears to be easier in that family.

Relationship between pollinators and the design of trapping devices — Although different types of insects have been found to pollinate plants with floral traps (Proctor *et al.* 1996), it is not known whether pollination traps show specific adaptations to the respective pollinator groups. We found that traps in the genus *Arum* pollinated by different types of insects differ significantly in the size of the slippery surfaces and lacunae. These differences are not likely to be a result of common ancestry, as our sample species belong to different clades of the genus and pollination syndromes in these clades have been shown to have evolved in convergence independently of the phylogenetic relationship (Linz *et al.* 2010). Therefore, we conclude that the differences in trapping devices in *Arum* are due to adaptation to the respective pollinator types. Many studies have shown already that selection through pollinators affects floral colours, odours and shapes (Chittka *et al.* 2001, Fenster *et al.* 2004, Parachnowitsch, Raguso & Kessler 2012). Adaptations of the floral epidermis to the insects' attachments organs have so far mostly been studied with respect to functional aspects (Bohn & Federle 2004, Gaume *et al.* 2004). Our results indicate that, as for other floral organs, the design of epidermal cells is under selection by different types of pollinators, and that their role in flowers may have been underestimated. This may be particularly true for insect pollinators as they display a high diversity of attachment organs adapted to locomotion on various surfaces (Gorb 2001).

Our NMDS-analyses show that the bee-pollinated species (i.e. *Arum creticum* and *A. idaeum*) form a distinct cluster. This appears to be a general trend in Araceae also observed for several other floral traits (Gibernau *et al.* 2010). The morphological

differences are probably due to the fundamentally different behavioural and cognitive abilities that separate bees from saprophilous flies and beetles which are primarily not adapted to flower visitation (Faegri & Van der Pijl 1971). Differences between species of *Arum* pollinated by midges and flies & beetles were not obvious in the case of *A. concinnatum*, which clustered with the midge-pollinated species. The species is visited by staphylinid beetles and various midges from different families (Urru *et al.* 2010) and was therefore coded as pollinated by flies & beetles. Nevertheless, our analyses indicate that *A. concinnatum* is more similar to midge-pollinated species. We postulate that the beetles may exert a low selective pressure on the inflorescences as they may visit the inflorescence only at the end of anthesis foraging for fallen pollen and thus may not be efficient pollinators. By contrast, inflorescences or flowers of the same species visited by different types of pollinators will be under divergent selection for different floral traits (Gomez *et al.* 2008, Martén-Rodriguez *et al.* 2011). This might also be the case in the two species of *Arum* pollinated by both beetles and flies (i.e. *A. disocoridis* and *A. nigrum*). These two species are markedly different from the midge-pollinated species but do not cluster closely together too, indicating different selective pressures which are probably exerted by the different flies and beetles. Nevertheless, they show similarities in the design of their trapping devices, especially the large size of papillate cells and the low number of elongated sterile flowers, that distinguish them clearly from the midge-pollinated *Arum* species.

Pollination by midges is the most common system in *Arum* and our results indicate a strong grouping of morphological traits for all midge pollinated species except for *Arum hygrophilum*. This species has the smallest papillate cells of all taxa studied. Like other species of *Arum* it is pollinated by midges of the Psychodidae family (Koach 1985). However, anthesis in *A. hygrophilum* lasts up to 10 days and the midges remain trapped in the inflorescence during the whole time (Koach 1985). In contrast, a two-day-anthesis is the standard in *Arum* (Gibernau *et al.* 2004). Whether this difference could have an impact on the size of the slippery surfaces remains unclear. Another difference between *A. hygrophilum* and other midge pollinated species is that in our analysis of mean data per species it groups with *A. dioscoridis* and *A. nigrum* (flies & beetles) in terms of number of sterile flowers because all three species have a low number of sterile flowers. In general, we found that midge-pollinated species appear to have denser whorls of sterile flowers than the beetle-pollinated species. Knoll (1926) suggested that the sterile flowers hinder insects of a certain size to pass them. Therefore, midge-pollinated

Arum species could prevent large flies and beetles from entering the floral tube by their dense sterile flowers. An alternate explanation would be that the whorls have to be more densely packed in order to prevent the smaller midges (compared to larger flies and beetles) from escaping. Either way, the lower number of sterile flowers in *A. hygrophilum* is anomalous and the precise reason unknown.

Our study shows that the trapping devices in pollination traps of *Arum* have adapted to different types of pollinators. There may be several reasons why different insects select for a different size of papillate cells. First, different insect pollinators have attachment organs that differ in the degree of elaboration and adaptation for climbing surfaces (Knoll 1926, Gorb 2001). The ability to attach to steep surfaces also depends on the animal's body mass. The heavier the animal, the higher is the number of attachment hairs required for climbing steep surfaces (Federle *et al.* 1997, Arzt *et al.* 2003). Therefore, adaptations of slippery surfaces for trapping small midges probably have to be different from those for larger and heavier flies or beetles. Moreover, the various insects differ in their behaviour on flowers as flies are generally more agile than beetles (Willmer 2011). This may also influence the way the insects are trapped best. Thus, although the overall design of pollination traps is very uniform in most species of *Arum*, variation in the size and number of trapping devices does occur as a consequence of pollination by different types of pollinators. We conclude that the number, size and shape of the so far little recognised trapping devices are important variables in the reproductive ecology of floral traps.

ACKNOWLEDGEMENTS

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APPENDIX. Voucher specimens of *Arum* spp. deposited in the herbarium of the University of Vienna (WU) and the private spirit collection of the first author (BRO).

A. balansanum R.R.Mill WU 0064937. *A. besserianum* Schott WU 0064939. *A. concinnatum* Schott BRO 11092012. *A. creticum* Boiss. & Heldr. WU 0064940. *A. cylindraceum* Gasp. WU 0064941. *A. dioscoridis* Sm. WU 0064942. *A. elongatum* Steven WU 0064943. *A. euxinum* R.R.Mill WU 0064946. *A. hygrophilum* Boiss. BRO 11092016. *A. idaeum* Coustur. & Gand. WU 0064945. *A. italicum* Mill. WU 0064947. *A. maculatum* L. BRO 11092014. *A. megobrebi* Lobin, M.Neumann, Bogner & P.C.Boyce WU 0064948. *A. nigrum* Schott WU 0064949. *A. purpureospathum* P.C.Boyce WU 0064950.

CHAPTER 5

SCHISMATOGLOTTIS AND *APOBALLIS* (ARACEAE: SCHISMATOGLOTTIDEAE): A NEW EXAMPLE FOR THE SIGNIFICANCE OF POLLEN MORPHOLOGY IN ARACEAE SYSTEMATICS

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Angabe zum Eigenanteil des Dissertanten: David Bröderbauer hat die Daten zu den „spathe movements“ erhoben und die betreffenden Teile in der Introduction, Materials and Methods („the course of anthesis…“), Results („Spathe movements of *Apoballis acuminatissima*“) und Discussion („Spathe movements“) verfasst.

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*.

INTRODUCTION

The genera of Araceae display a high morphological diversity, which extends to pollen wall morphology and exine sculpturing (Grayum 1992, Mayo *et al.* 1997, Hesse 2006). Tribe Schismatoglottideae is a well circumscribed basal clade within subfamily Aroideae (French *et al.* 1995, Hay 1996, Mayo *et al.* 1997, Hay & Yuzammi 2000, Keating 2002, 2004, Cabrera *et al.* 2008, Cusimano *et 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 *et 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 *et al.* 2008, Cusimano *et 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 *et al.* 1998, 1999), or polysaccharidic echini (Pacini & Juniper 1983, Weber *et 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 *et al.* 1998). It was also reported for *Pistia stratiotes* L., in which there are polysaccharidic plicae (ribs), and an additional thin polysaccharidic layer (Weber *et 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) and Sannier *et 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 *et 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.

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

Apoballis acuminatissima (Schott) S.Y. Wong & P.C. Boyce, comb. nov.	Basionym: *Schismatoglottis acuminatissima* (Alderw.)
Apoballis belophylla (Alderw.) S.Y. Wong & P.C. Boyce, comb. nov.	Basionym: *Schismatoglottis belophyll* (Alderw.)
Apoballis brevipes (Hook. f.), S.Y. Wong & P.C. Boyce, comb. nov.	Basionym: *Schismatoglottis brevipes* (Hook. f.)
Apoballis grandiflora (Alderw.) S.Y. Wong & P.C. Boyce, comb. nov.	Basionym: *Schismatoglottisgrandiflora* (Alderw.)
Apoballis hastifolia (Hallier *f.* ex Engl.) S.Y. Wong & P.C. Boyce, comb. nov.	Basionym: *Schismatoglottis hastifolia* (Hallier *f.* ex Engl.)
Apoballis javanica (Engl.) S.Y. Wong & P.C. Boyce, comb. nov.	Basionym: *Schismatoglottis javanica* (Engl, in Endl. & Krause)
Apoballis longicaulis (Ridl.) S.Y. Wong & P.C. Boyce, comb. nov.	Basionym: *Schismatoglottis longicaulis* (Ridl.)
Apoballis mutata (Hook. f.) S.Y. Wong & P.C. Boyce, comb. nov.	Basionym: *Schismatoglottis mutata* (Hook. f.)
Apoballis okadae (M. Hotta) S.Y. Wong & P.C. Boyce, comb. nov.	Basionym: *Schismatoglottis okadae* (M. Hotta)
Apoballis ovata (Schott) S.Y. Wong & P.C. Boyce, comb. nov.	Basionym: *Schismatoglottis ovata* (Schott)
Apoballis rupestris (Zoll. & Moritzi ex Zoll.) S.Y. Wong & P.C. Boyce, comb. nov.	Basionym: *Schismatoglottis rupestris* (Zoll. & Moritzi ex Zoll.)
Apoballis sagittifolia (Alderw.) S.Y. Wong & P.C. Boyce, comb. nov.	Basionym: *Schismatoglottis sagittifolia* (Alderw.)

For transmission electron microscopy (TEM), anthers were rehydrated and fixed in 3% glutaraldehyde (GA), postfixed with 1% osmiumtetroxide (OsO4) and 0.8% potassium hexacyanoferrate (K4Fe(CN)6 • 3H2O). 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 (KMnO4) (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.

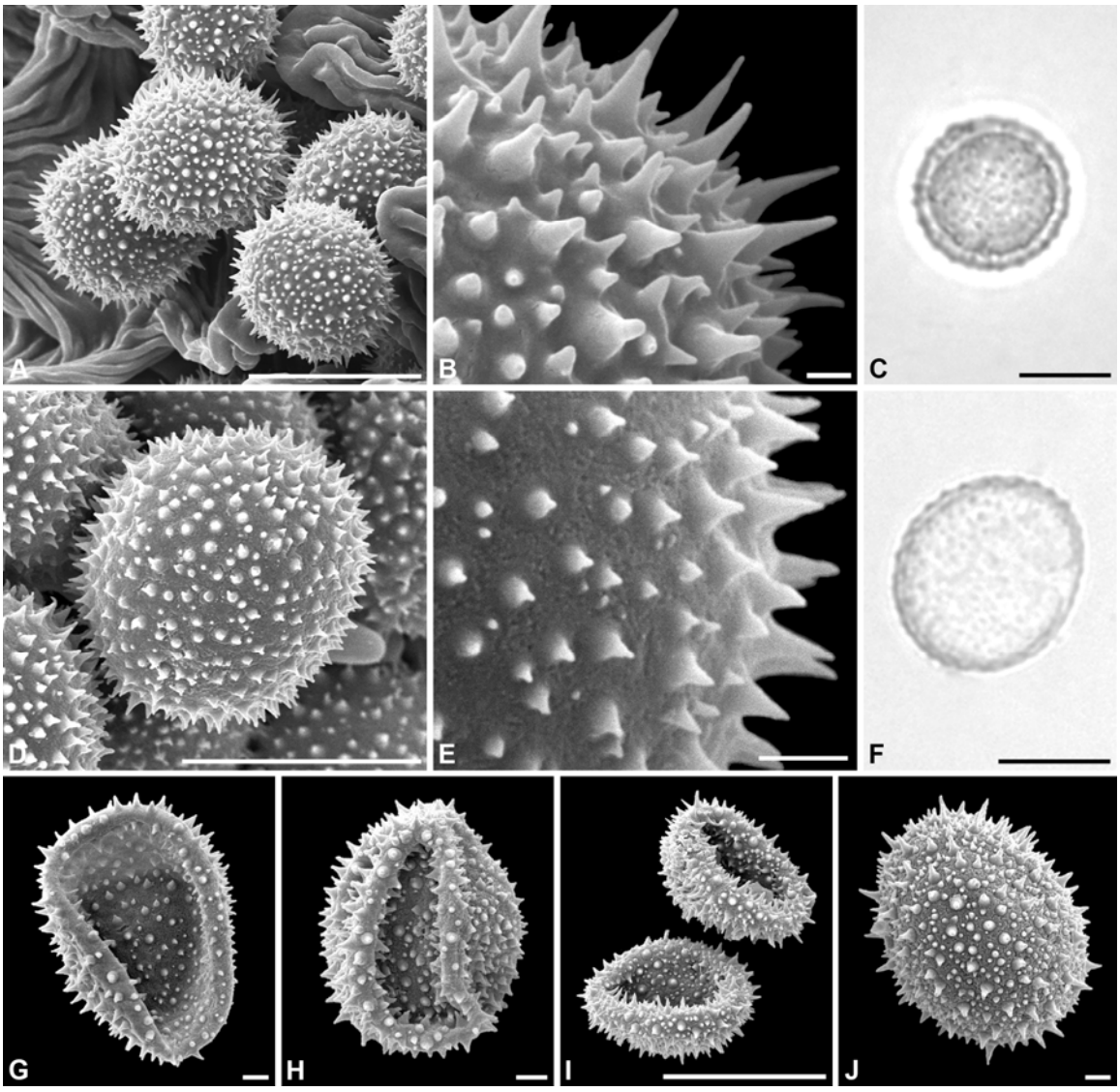

Fig. 1. Echinate pollen typical for *Apoballis*. **A–C,** *Apoballis acuminatissima*: **A,** pollen grains under SEM, air-dried; **B,** detail of pollen surface, air-dried; **C,** hydrated pollen grain in LM. **D–F,** *Apoballis mutata*: **D,** hydrated pollen grains under SEM, critical point–dried; **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).

RESULTS

Pollen analyses — Pollen of *Apoballis* (Fig. 1, Table 2) and *Schismatoglottis* (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

Table 2. Summary of the relevant pollen characters of all investigated species of Schismatoglottidae

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)
	A. longicaulis (Ridl.) S.Y.Wong & P.C.Boyce	small	n.i.	inaperturate	echinate (Thanikaimoni)
Bucephalandra Schott /3	***B. motleyana*** Schott	small	elliptic	inaperturate	psilate
Hestia S.Y.Wong & P.C.Boyce /1	***H. longifolia*** 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 Bogner, P.C.Boyce & S.Y.Wong	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	***Schottariella mirifica*** P.C. Boyce & S.Y. Wong	small	elliptic	inaperturate	Scabrate

(n.i. = not investigated)

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	Fig.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	Fig. 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	
n.i.	n.i.	absent.	psilate (Halbritter unpubl. data)	n.i.	n.i.	n.i.	
2 (& 3)	n.i.	small	psilate (Grayum)	n.i.	n.i.	n.i.	
2 (& 3)	raphids	small	psilate with crystals			n.i.	
2 (& 3)	n.i.	absent	psilate	bi-layered	continuous, spongy	thin outer layer (ektexine); holes between ektexine and endexine	

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 thelight 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 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 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 flowersare 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 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

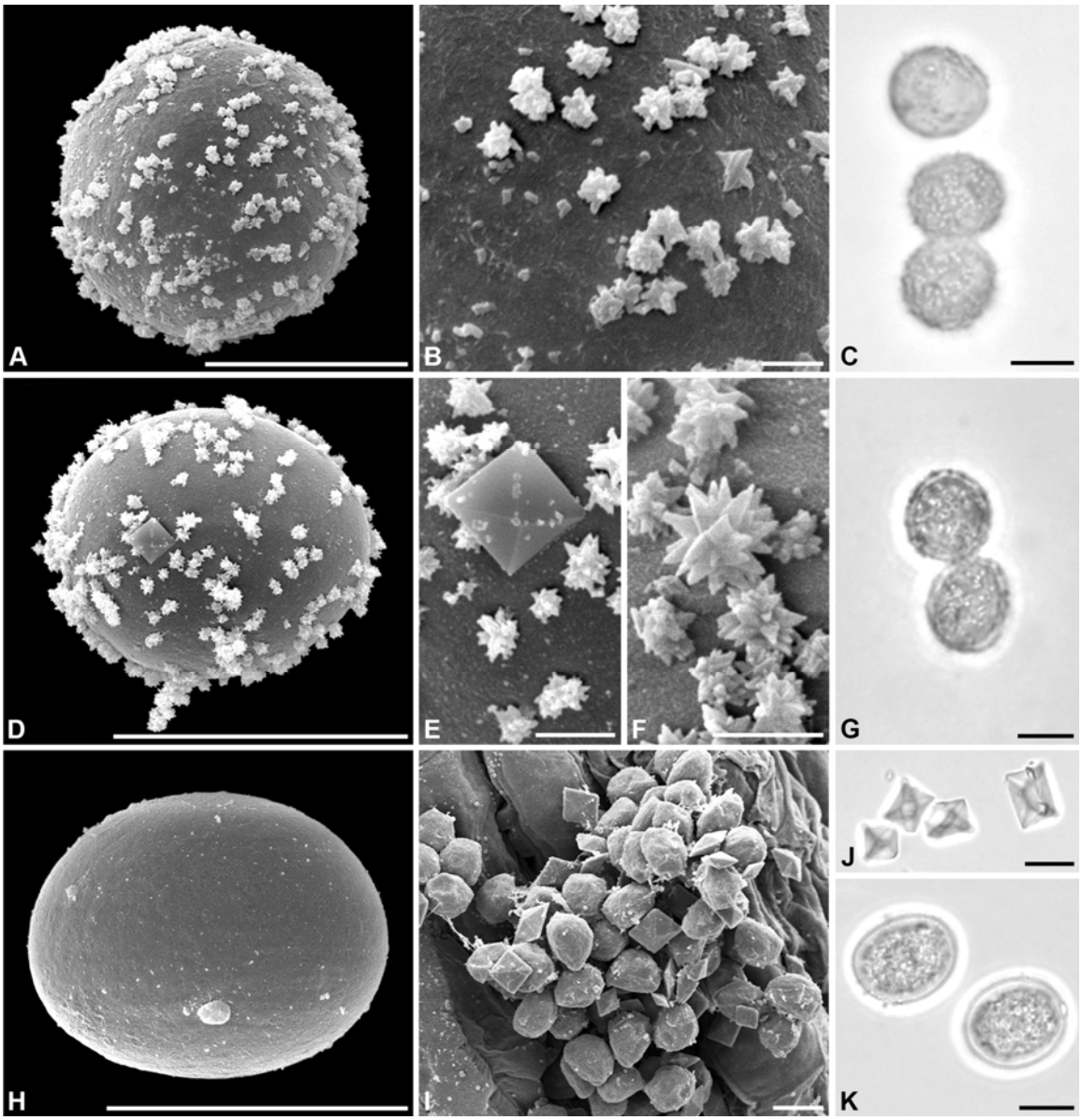

Fig. 2. Psilate pollen typical for *Schismatoglottis*. **A–C,** *Schismatoglottis celebica*: **A,** hydrated pollen grains under SEM, critical point–dried; **B,** detail of pollen surface; note the crystals covering the pollen surface; **C,** hydrated pollen grains under LM. **D–G,** *Schismatoglottis 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).

(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

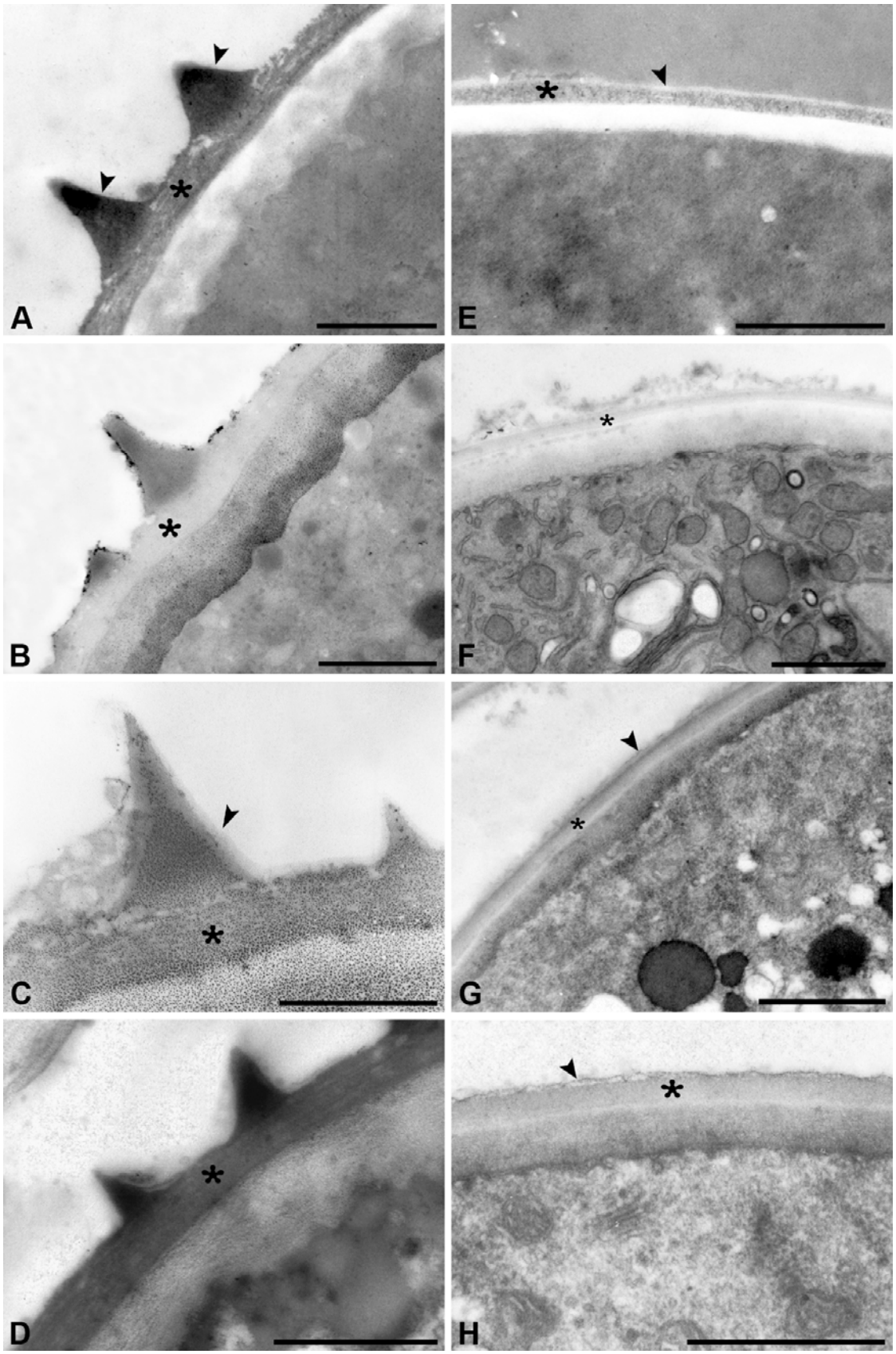

Fig. 3. Cross-sections of pollen walls of *Apoballis* and *Schismatoglottis* using different staining methods. **A–D,** *Apoballis acuminatissima*; **E–H,** *Schismatoglottis celebica*. **A, E,** pollen wall after potassium permanganate staining; **B, F,** pollen wall after modified Thiéry-test; **C, 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.

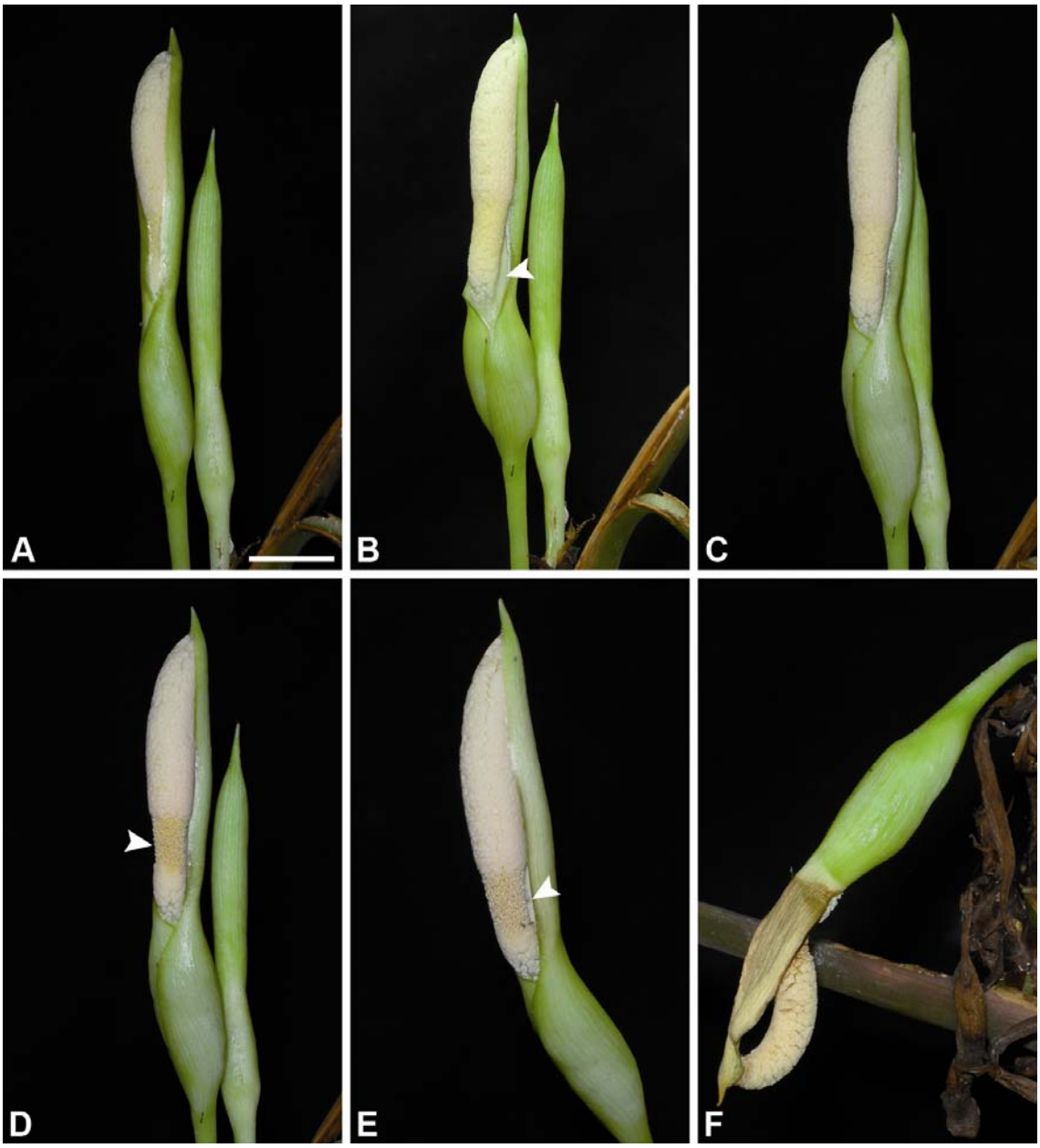

Fig. 4. Spathe movements of *Apoballis acuminatissima*. **A,** preanthesis (1 day before anthesis); **B,** pistillate phase (day 1 of anthesis, 12:10 h), arrowhead indicates spathe opening; **C,** pre-staminate phase (day 2, 10:38 h); **D,** pollen shedding (day 2, 16:58 h), arrowheads indicate anthers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

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 *et al.* 1997, Hay et Yuzammi 2000, Keating 2002, 2004, Cabrera *et al.* 2008, Cusimano *et 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 *et 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 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 *et al.* 1998). Pollen of all *Schismatoglottis* species and species within the recently resurrected New World genus *Philonotion* Schott (Wong *et al.* 2010), so far studied by us (Appendix), is psilate, in accordance with literature reports (Grayum 1992, Wong *et 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 *et 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 *et al.* 1996, Barabé *et 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 t ransferred 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 *et 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 *et 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 *et 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 *et 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 *et 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.

ACKNOWLEDGEMENTS

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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).

CHAPTER 6

GENERAL DISCUSSION

6.1. MULTIPLE EVOLUTION OF TRAP POLLINATION

Deceptive pollination occurs in only c. 4% of all angiosperms (Renner 2006). Taxa with pollination traps, most of which are embedded in clades exhibiting deceptive pollination syndromes, are even rarer (Vogel 1965, Dafni 1984). Nevertheless, as demonstrated in the present thesis, trap pollination is common in the Araceae and even more widespread than was previously thought. I found that inflorescence traps are present in at least 27 of the ca. 126 genera (Chapter 2). They are not derived from a common ancestor, but have evolved at least 10 times independently in different clades of the Araceae – a surprisinlgy high number in view of the low number of pollination traps in angiosperms. So far, traps were only known to occur in subfamily Aroideae (Vogel & Martens 2000). This study shows that also in other lineages, i.e. the subfamilies Zamiculcadoideae and Lasioideae, traps evolved. Inflorescenes in the latter clade bear several ancestral characters such as an undifferentiated spadix with bisexual flowers. Moreover, in most taxa of Lasioideae the spathe only forms a rudimentary chamber without a constriction. Thus, traps are not restricted to taxa with highly synorganised inflorescences, but also occur in early diverging lineages which still have 'primitive' inflorescence characters (e.g. bisexual flowers).

The trapping inflorescences within Araceae were found to have various adaptations for the trapping of pollinators. Consequently, at least six different functional types of traps can be recognised within the Araceae (Chapter 2). In some cases, convergent evolution in distinct clades has led to the formation of traps that function in a similar way. Perhaps the most astonishing examples for convergent evolution are the traps of the *Zomicarpa* type found in *Dracontioides* (bisexual flowers), *Zomicarpa* and *Arisaema* (unisexual flowers) (Vogel & Martens 2000). Therefore, I conclude that – similar to other pollination syndromes (Fenster *et al.* 2004) – selection through specific functional groups of pollinators (i.e. saprophilous insects) shaped a distinct 'trap-pollination syndrome' (also see Vogel 1965) that has evolved in convergence in unrelated groups independent of genetic similarity.

The stepwise evolution of complex traits, initially starting with 'trials and errors', is a trend observed in the floral evolution of angiosperms as a whole (Endress 2001). A similar process probably caused the evolution of complex inflorescence traps in the Araceae, as we can observe transitions between traps that show different degrees of synorganisation. For example, in traps of the *Arum* type single floral organs as well as the entire spadix and the spathe are much more synorganised and this trap type is clearly derived from the *Typhonium* type, which still has less elaborate adaptations.

A stepwise evolution of trap pollination is also plausible because imperfect traps of the *Arisarum* type were found to be a precursor for the evolution of perfect traps in some clades. The purpose of imperfect traps is to ensure that insects lured to a flower are forced to get in contact with the floral organs before departure, thereby depositing cross pollen on the stigmas and removing pollen from the staminate flowers (Faegri & Van der Pijl 1971). However, pollination success will be greatly improved in dichogamous traps, if the insects are forced to stay inside the floral chamber, thus depositing cross pollen on the stigmas and removing pollen from the anthers more effectively (Lack & Diaz 1991). Therefore, traits that ensure the retention of pollinators may be favoured by selection in imperfect traps, facilitating the evolution of perfect traps. In the Araceae, imperfect traps prevail in subfamily Lasioidae, a clade with a low degree of synorganisation of spathe and spadix. This less elaborate bauplan of the inflorescence possibly complicated the evolution of perfect traps. Nevertheless, a transition from imperfect to perfect traps occurred within Lasioideae in *Dracontioides desciscens*. This can be seen as a proof that imperfect traps are a transitional step in the evolution of perfect traps. In addition, this trend is supported by the finding that most of the transitions between the different trap types occurred from non-traps to imperfect traps of the *Arisarum* type, followed by transitions from the *Arisarum* type to the *Zomicarpa* type.

Shifts from traps to non-traps are rare within the Araceae (Chapter 2). The only known example is found in the genus *Arum* which mainly consists of deceptive traps (Chapter 4). Pollinators are flies and beetles (Gibernau *et al*. 2004). *A. idaeum* has probably lost its legitimate pollinator due to the colonisation of harsh mountain habitats and has switched to autogamy (A. Diaz, unpublished data). The closely related *Arum creticum* has shifted to bee pollination and rewards the insects with pollen during the staminate phase of anthesis (Diaz & Kite 2006). Nevertheless, the bees still have to be trapped during the rewardless pistillate phase to secure the transfer of outcross pollen onto the stigmas. The absence of transitions from traps to rewarding inflorescences

indicates that trap pollination is an evolutionary stable condition within the Araceae. This is also corroborated by my observations regarding the other taxa of *Arum* (Chapter 4). Most of them share a very uniform trap design. I suppose that variation in the zonation of slippery surfaces would be detrimental for the plants as variation in floral organs can cause inaccuracy in the process of pollination (Armbruster *et al*. 2009a), which could decrease the success in trapping insect pollinators. Moreover, variation in floral traits appears to be generally lower in flowers with higher integration of floral organs (Armbruster *et al*. 2009a). Thus, stabilising selection may favour the maintenance of traps at least in traps of the later diverging clades which are characterised by highly synorganised inflorescences. However, this is not necessarily a general rule for all pollination traps. In *Ceropegia* (Apocynaceae) and *Aristolochia* (Aristolochiaceae) reversals to rewarding pollination have probably occurred more often (Sakai 2002b, Ollerton *et al.* 2009)

6.2. ADAPTATIONS FOR TRAP POLLINATION

The multiple evolution of inflorescence traps has probably been facilitated by various preadaptations. I found that several adaptations that are indispensable for trapping (e.g. presence of a spathe chamber, elongated sterile flowers, spathe movements, papillae) already were present in non-trapping aroid ancestors before traps evolved (Chapter 2). Therefore, several of these adaptations are probably exaptations that ancestrally had a different function. Exaptations also play an important role in derived plant-pollinator interactions of other angiosperms (Armbruster *et al.* 2009b). In fact, many key innovations that facilitated the evolution of higher land plants appear not to have occurred in a short time but in a stepwise process, and some of these key innovations represent exaptations (Donoghue 2005).

A ***floral chamber*** formed by the spathe was the precondition for the formation of traps in Araceae. It had already evolved in the early history of the family. Nevertheless, this key innovation was not associated with trap pollination in the ancestral taxa, but likely served another function. In several extant taxa the spathe base remains furled around the flowers to form a floral chamber throughout anthesis and seed set (e.g. *Alocasia*, *Caladium*, *Dieffenbachia*, *Philodendron*). Here, it often serves as a mating chamber or brood site (Gibernau *et al.* 2000, Miyake & Yafuso 2005, Maia & Schlindwein 2006). This was probably also its original function in the common ancestor that developed a spathe chamber.

Slippery surfaces in Araceae consist of downward-pointing papillae and/or epicuticular wax crystalloids. I found that both traits have evolved multiple times, in some cases concurrently (Chapter 2). As wax crystalloids are easily formed and are absent in many taxa, it is most likely that they evolved *de novo* in the context of trap pollination. Downward-pointing papillae are already known from several aroids (Knoll 1926, Dakwale & Bhatnagar 1982, Yadav 1998). I discovered that in several taxa the papillate cells on the adaxial surface of the spathe do not point downwards, but project perpendicularly to the spathe surface. Such 'straight' papillate cells occur, for example, in *Zantedeschia*. In the Botanical Garden of Vienna I observed trapping of wild bees in *Zantedeschia var. elliotiana*. They were unable to climb the lower papillate portion of the inner spathe (Bröderbauer, pers. obs.). However, experimental proof that such 'straight' papillate cells can form a slippery surface is still missing. If they produce oil as do other (downward-pointing) papillae (e.g. in *Arum*, Knoll 1926) they might easily be slippery without pointing downwards. Straight papillate cells also occur in *Colocasia* (Poppinga *et al.* 2010). Ivancic *et al.* (2004) mention that the spathe surface of *Colocasia esculenta* is slippery for flies. However, according to my own field observations in the same species, and in *Colocasia fontanesii*, the drosophilid pollinators are able to walk along the spathe without slipping (Chapter 3). Instead, the papillate cells of the spathe epidermis serve for the production of odour. The papillae in the *Colocasia* species studied show the typical osmophoric activity (e.g., numerous mitochondria, amyloplasts and smooth endoplasmatic reticulum) (Hadacek & Weber 2002, Wiemer *et al.* 2009, Pansarin & Pansarin 2011). Also their shape is similar to the odour emitting surfaces of various other angiosperms (Vogel 1963, Garcia *et al.* 2007, Płachno *et al.* 2010). Although the spadix is the most common organ for scent-production in Araceae, there are several taxa (e.g. *Cryptocoryne*, *Dracontium* and *Peltandra*) for which the spathe has already been shown to be an osmophore (Mayo *et al.* 1997, Zhu & Croat 2004, Patt *et al.* 1995). During the work on this thesis I found that papillate slippery surfaces of most trapping species also emit foetid odours, often similar to those of the spadix and changing during the course of anthesis. Whether ('straight') osmophoric papillae are ancestral and subsequently changed their function towards slippery surfaces remains to be seen. The results of the ancestral state reconstructions of papillae indicate that such a change might have occurred in the '*Pistia* clade' (sensu Renner & Zhang 2004). The clade contains two lineages in which traps have evolved independently. The common ancestor of the *Pistia* clade apparently did not have a trap but already possessed papillate

cells. This would imply that papillate cells were present before the development of slippery surfaces and therefore must have served another function.

Sterile flowers probably also have shifted in function from osmophores to trapping devices in the Areae clade (Chapter 2). In *Sauromatum*, sterile flowers situated below the staminate flowers produce odours (Hadacek & Weber 2002). Moreover, also in *Typhonium* sterile flowers produce scent and stain intensively after treatment with neutral red (Bröderbauer, unpubl. data). In both taxa, sterile flowers are located within the floral chamber below the constriction of the spathe. In contrast, in *Arum* the sterile flowers which are present below and above of the staminate flowers, are part of the trap as they prevent insects from escape (Knoll 1926). The function of elongated sterile flowers generally varies in the different clades. In *Arisaema*, sterile flowers present on the appendix facilitate the attraction of pollinators (Vogel & Martens 2000), while in *Bucephalandra* they probably serve as protecting structures for the developing fruits (P. Boyce, pers. comm.). In *Dracontium* and *Amorphophallus*, the function of the sterile flowers is unclear, but judging from their shape and position, a role in trapping insects seems unlikely in most species.

Movements of the spathe during or after anthesis are ubiquitous in the Araceae (Mayo *et al.* 1997) (Chapter 2). In genera such as *Dieffenbachia* (Young 1986) and *Alocasia* (Miyake & Yafuso 2003), the constriction closes after pollen release. Such movements are thought to force the pollinators to leave the inflorescence and also to protect developing fruits (Mayo *et al.* 1997). The closure of the inflorescence during anthesis in order to imprison pollinators might result simply from a change in the timing of the spathe closure. In *Cryptocoryne* and *Lagenandra*, the spathe margins are connate and are not able to constrict actively. Instead, the seclusion of the chamber is achieved by the movement of a specialised extension of the spathe margin, the so-called ‘flap’ (Ørgaard & Jacobsen 1998). Besides their function in trapping, spathe movements can also be important for the release of pollinators. In *Arisaema* and *Pinellia* insects are released from the trap by spathe movements that result in the formation of a secondary opening (Vogel & Martens 2000). This is necessary because in these traps slippery surfaces (i.e. epicuticular wax crystalloids) do not wither, thus preventing the insects’ escape through the still slippery entrance of the chamber. The spathe movements observed in *Colocasia*, *Schismatoglottis* and *Apoballis* differ from other trapping Araceae (Chapters 2, 3 & 5). While in *Typhonium* and *Sauromatum* the constriction occludes only the floral chamber, it is the spathe blade that locks the entire inflorescence

during anthesis in the above mentioned species. *Apoballis* is unique in that the bending of the spadix is part of the trap- and release-mechanism. A feature typical for some species of *Colocasia* is the rapid reflexing and curling of the spathe after pollen release. These special adaptations of the spathe are probably related to the trapping of *Colocasiomyia* flies (Drosophilidae) in both taxa.

6.3. THE IMPACT OF POLLINATORS ON THE EVOLUTION OF TRAP POLLINATION

Deceptive pollination by means of brood-site mimicry has been supposed to be mainly correlated with pollination by saprophilous flies rather than beetles (Faegri & Van der Pijl 1971, Proctor *et al.* 1996). However, this hypothesis has never been tested. In the inflorescence traps of Araceae both beetles and flies – most of which are saprophilous – act as pollinators (Gibernau 2003). I found that trap pollination in Araceae is correlated with pollination by flies rather than beetles (Chapter 2), thus confirming the above hypothesis. According to my ancestral state reconstructions, the common ancestors of clades with traps were pollinated by flies in most cases. This finding is also corroborated by Gibernau *et al.* (2010), who showed that in several trap-pollinated aroids floral traits match those of mutualistic taxa pollinated by flies, indicating that trap pollination is embedded in the pollination syndrome of myiophily.

Moreover, most changes from non-traps to traps were not associated with a simultaneous change in pollinator type (e.g. from beetles to flies) but happened within fly-pollinated clades. For example, traps of the *Schismatoglottis* type in *Schismatoglottis* and *Colocasia* are embedded in clades in which nursery pollination involving flies prevails (Chartier 2011) (Chapters 2, 3 & 5). In contrast to other traps in Araceae, their pollinators (flies of the drosophilid genus *Colocasiomyia*) are not deceived but rewarded with a brood site (Toda & Okada 1983, Takenaka 2006, Toda & Lakim 2011). However, observations that insects also get trapped (Cleghorn 1913), already indicated that these rewarding inflorescences have some adaptations for trap pollination.

My field studies showed that brood-site pollination is prevalent in *Colocasia*. I observed different species of *Colocasiomyia* laying their eggs between the flowers of *Colocasia* spp. (Chapter 3). The importance of the brood-site as a major reward for drosophilids was (inter alia) reflected by a bias towards female specimens in the most abundant pollinating species. Unlike in well know examples of brood-site pollination, such as in *Ficus* or *Yucca* (Armbruster 2012), the *Colocasiomyia* larvae do not harm the

developing fruits. Despite of the fact that the drosophilid flies get rewarded for their pollination services, they also get arrested in *Colocasia esculenta*, *C. fontanesii* and *C. lihengiae*. The reasons for trapping insects in *Colocasia* are not fully understood, but the resemblance to trap mechanisms in lure-and-trap pollinated Araceae is remarkable (Chapter 2). In rewardless inflorescences, the insects that have arrived during the pistillate phase of anthesis have to be retained in order to secure pollen export during the staminate phase. In *Colocasia*, the reward in the form of a breeding site is only available during the pistillate phase, i.e. until the spathe constriction narrows and thereby occludes the lower floral chamber. Then, the flies have to proceed into the upper floral chamber containing the staminate flowers, which at that time are still undehisced. Consequently, the trapping of the pollinators may be necessary in order to secure that they stay until pollen is released.

A scenario with nursery mutualism as a precursor to trap pollination is also probable in other clades. Based on Chartier's (2011) reconstruction of plant-pollinator interactions in Araceae we can infer that nursery-mutualism was also present in the common ancestor of traps in the *Arum* clade. Thus, at least in some clades trap mechanisms may have evolved in rewarding taxa in order to ensure male reproductive success. A similar trend can be observed in the unrelated Aristolochiaceae. While most species of *Aristolochia* form deceptive traps, nursery-pollination by drosophilid flies is found in *Aristolochia maxima* (Sakai 2002b). The pollinators do not get trapped but deposit their eggs in the flowers. These findings suggest that transitions between nursery mutualism and brood-site mimicry could be a common phenomenon in angiosperms. A shift to saprophilous pollinators can be achieved by simple changes in floral scent (Shuttleworth & Johnson 2010). As floral odours are very diverse in the Araceae (Kite *et al.* 1998, Stökl *et al.* 2010, Schiestl & Dötterl 2012), such a shift has probably occurred several times in the family.

Further hypotheses that could explain our finding of a correlation between flies and trap pollination relate to the differential behaviour of flies and beetles (Chapters 2 & 4). Knoll (1926) and Bown (2000) argue that flies are much more agile and therefore have to be arrested in order to transfer pollen. In contrast, beetles are lethargic and tend to stay in flowers voluntarily for longer intervals (Dafni 1984, Willmer 2011). In addition, many chamber blossoms offer solid food rewards for beetles (Proctor *et al.* 1996, Gibernau *et al.* 1999, Bernhardt, 2000), which cannot be consumed by flies.

Insect pollinators do not only have a different impact on the evolution of trap pollination in general. I found evidence that different pollinators select for differences in the size, the shape and the number of trapping structures (Chapter 4). Traps in species of *Arum* pollinated by midges differ from those pollinated by beetles and large flies. In general, the latter have larger papillate cells and less dense whorls of sterile flowers. The bee-pollinated *Arum creticum* and its sister species *A. idaeum* show even more diverging traits. Both species lack lacunae in the spathe that serve for oxygen supply in the floral chamber (Knoll 1923). As *Arum creticum* only traps bees during the pistillat phase of anthesis (Diaz & Kite 2006), an additional oxygen supply may not be necessary. *Arum idaeum* has probably switched to autogamy and consequently reduced the papillate slippery surface.

There might be several reasons, why different slippery surfaces must have a different size and shape in order to trap different types of insects (Chapters 2 & 4). First, different insect pollinators have attachment organs that differ in the degree of elaboration and adaptation for climbing surfaces (Knoll 1926, Gorb 2001). The ability to attach to steep surfaces also depends on the animal's body mass. Heavier animals need a higher number of attachment hairs in order to compensate for the increased body weight (Federle *et al.* 1997, Arzt *et al.* 2003). Thus, slippery surfaces of taxa pollinated by small midges probably need to have different adaptations than in taxa pollinated by larger and heavier flies or beetles. The higher agility of flies compared to beetles (Dafni 1984, Willmer 2011) probably also influences the way the insects are trapped best. Thus, although the bauplan of inflorescence traps is uniform in *Arum*, there is considerable variation of the size of the slippery papillae due to the different types of pollinators.

The present study was the first to analyse the evolution of floral traps in a phylogenetic context. So far, no detailed information on the emergence of floral traps was available. The combination of a morphological approach with data on the pollination ecology enabled the detailed reconstruction of the evolutionary history of trap pollination for the entire family of Araceae. Thus, it was possible to account for differences in the various clades regarding inflorescence design and pollinating fauna. Such an approach would not be possible in specific case studies that only focus on a few taxa. The reconstruction of the evolutionary history of trapping structures shows that the trap pollination syndrome did not simply evolve in response to pollination by saprophilous insects, but was shaped by the interplay of morphological constraints and ecological drivers, i.e. by exaptation of floral structures and adaptation to saprophilous insects. As

shown in the present thesis, preadaptations may play an important role in the evolution of complex floral syndromes and facilitate the convergent evolution in unrelated lineages. Consequently, trap mechanisms have evolved surprisingly often in various clades of the Araceae, resulting in the evolution of different trap types. Reversals to non-traps seem to be extremely rare. Therefore, trap pollination is considered a stable condition in the Araceae.

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APPENDIX

APPENDIX. Epidermal surfaces of the adaxial spathe of all Araceae species studied under scanning electron microscopy. * indicates that the sample has been air-dried instead of critical point drying.

1. ***Aglaonema modestum*** Schott ex Engl. Spathe, tabular cells; micron bar = 100 μm.

2. ***Aglaonema nebulosum*** N.E. Br. Spathe*, tabular cells; micron bar = 100 μm.

3. ***Alocasia acuminata*** Schott. Spathe blade, tabular cells; micron bar = 10 μm.

4. ***Alocasia clypeolata*** A. Hay. Spathe blade, convex cells; micron bar = 10 μm.

5. ***Alocasia lauterbachiana*** A. Hay. Spathe blade, convex cells with crystals; micron bar = 10 μm.

6. ***Alocasia odora*** K. Koch. Spathe blade, epicuticular wax crystalloids (rodlets); micron bar = 10 μm.

7. ***Alocasia portei*** Schott. Spathe blade, slightly concave cells; micron bar = 10 μm.

8. ***Ambrosina bassii*** L. Spathe chamber*, shrunken hair; micron bar = 100 μm.

9. ***Amorphophallus atrorubens*** Hett. & Sizemore. Spathe*, epicuticular wax crystalloids (platelets); micron bar = 10 μm.

10. ***Amorphophallus henryi*** N.E. Br. Basal spathe tube*, perpendicular papillae with cuticular folds; micron bar = 10 μm.

11. ***Amorphophallus konjac*** K. Koch. Spadix-appendix*, tabular cells with epicuticular wax crystalloids (platelets); micron bar = 1 μm.

12. ***Amorphophallus longituberosus*** (Engl.) Engl. & Gehrm. Spathe tube*, tabular cells with epicuticular wax crystalloids (granules and tubules); micron bar = 10 μm.

13. ***Amorphophallus mossambicensis*** (Schott ex Garcke) N.E. Br. Spathe blade, perpendicular papillae with cuticular folds; micron bar = 10 μm.

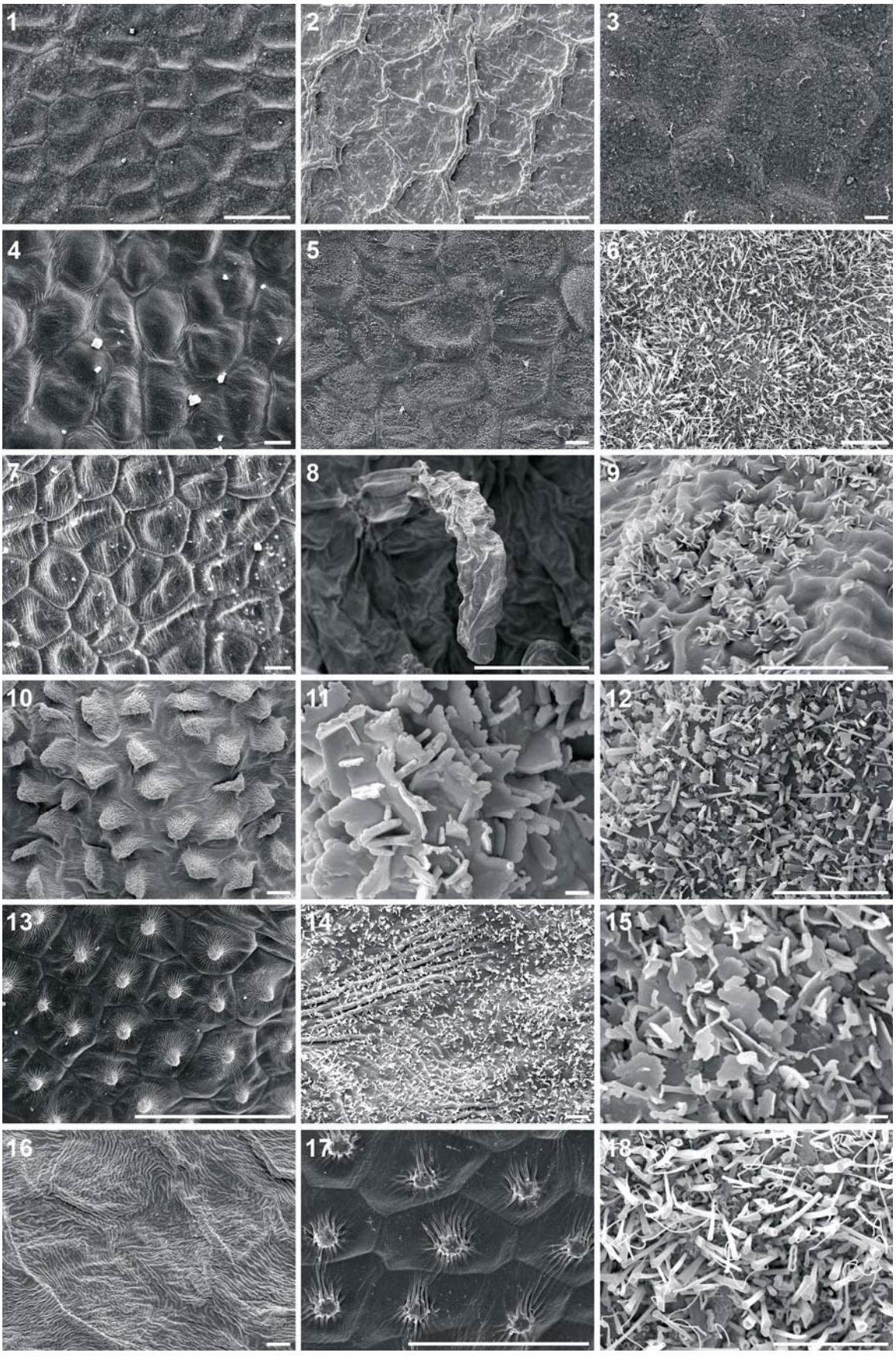
14. ***Amorphophallus myosuroides*** Hett. & A. Galloway. Spathe blade*, tabular cells with cuticular folds and epicuticular wax crystalloids (rodlets); micron bar = 10 μm.

15. ***Amorphophallus palawanensis*** Bogner & Hett. Spathe blade*, tabular cells with cuticular folds and epicuticular wax crystalloids (platelets); micron bar = 1 μm.

16. ***Amorphophallus polyanthus*** Hett. & Sizemore. Spathe blade*, tabular cells with cuticular folds; micron bar = 10 μm.

17. ***Amorphophallus stuhlmannii*** (Engl.) Engl. & Gehrm. Spathe tube, papillae with cuticular folds; micron bar = 10 μm.

18. ***Amorphophallus taurostigma*** Ittenbach, Hett. & Bogner. Spathe tube*, tabular cells with cuticular folds and epicuticular wax crystalloids (tubules and threads); micron bar = 10 μm.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19. ***Amorphophallus variabilis*** Blume. Spathe tube*, tabular cells with cuticular folds and epicuticular crystalloids (platelets, rodlets, and rods); micron bar = 10 μm.

20. ***Amorphophallus yunnanensis*** Engl. Basal spathe blade*, tabular cells with cuticular folds; micron bar = 10 μm.

21. ***Anadendrum affine*** Schott. Spathe, tabular cells with cuticular folds; micron bar = 10 μm.

22. ***Anaphyllopsis americana*** A. Hay. Spathe*, papillae with cuticular folds and epicuticular wax crystalloids (granules); micron bar = 10 μm.

23. ***Anchomanes difformis*** (Blume) Engl. Spathe, convex cells; micron bar = 100 μm.

24. ***Anchomanes giganteus*** Engl. Spathe, convex cells; micron bar = 100 μm.

25. ***Anthurium magnificum*** Engl. Spathe, convex cells; micron bar = 100 μm.

26. ***Anthurium pedatum*** (Kunth) Engl. ex Kunth. Spathe*, shrunken cells; micron bar = 100 μm.

27. ***Anubias gigantea*** Chev. ex Hutch. Spathe, convex cells; micron bar = 100 μm.

28. ***Anubias giletii*** De Wild. & T. Durand. Spathe, tabular cells; micron bar = 100 μm.

29. ***Arisaema fargesii*** Buchet. Spathe tube*, tabular cells with epicuticular wax crystalloids (granules); micron bar = 10 μm.

30. ***Arisaema ghaticum*** (Sardesai, S.P. Gaikwad & S.R. Yadav) Punekar & Kumaran. Spathe tube*, tabular cells with epicuticular wax crystalloids (threads); micron bar = 10 μm.

31. ***Arisaema*** **sp.** Spathe tube*, tabular cells with epicuticular wax crystalloids (threads); micron bar = 10 μm.

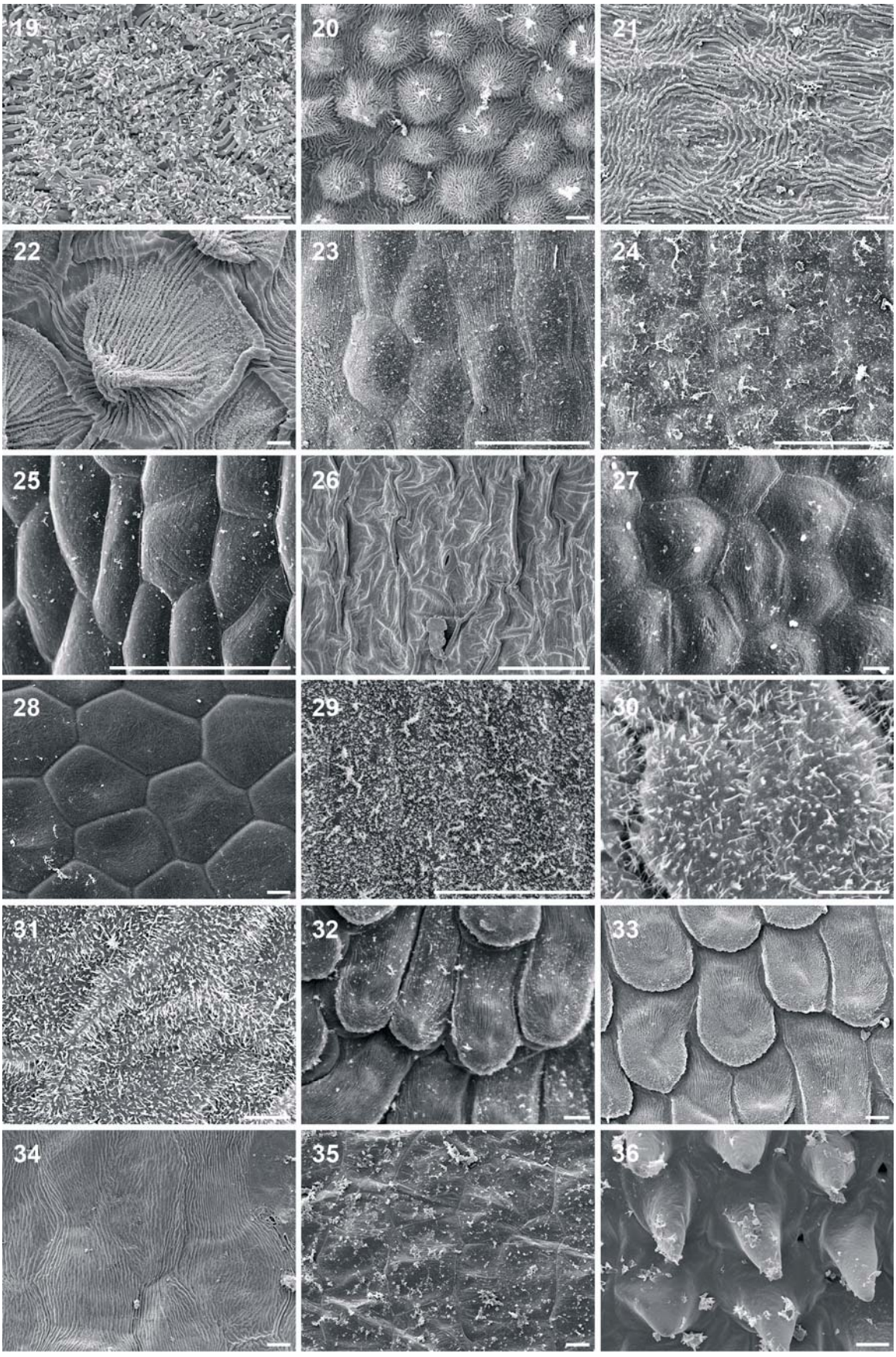
32. ***Arisarum proboscideum*** Savi. Spathe tube*, flat downward-pointing papillae with cuticular folds; micron bar = 10 μm.

33. ***Arisarum vulgare*** O. Targ. Tozz. Spathe tube, flat downward-pointing papillae with cuticular folds; micron bar = 10 μm.

34. ***Arophyton crassifolium*** (Buchet) Bogner. Spathe tube, tabular cells with cuticular folds; micron bar = 10 μm.

35. ***Arophyton humbertii*** Bogner. Spathe blade, tabular cells; micron bar = 10 μm.

36. ***Arum balansanum*** R.R.Mill. Spathe blade, spathe, downward-pointing papillae; micron bar = 10 μm.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37. ***Arum besserianum*** Schott. Basal spathe tube, dome-shaped cells with lacunae in the cell corners; micron bar = 10 μm.

38. ***Arum concinnatum*** Schott. Upper spathe tube, spathe, downward-pointing papillae; micron bar = 10 μm.

39. ***Arum creticum*** Boiss. & Heldr. Upper spathe tube, spathe, downward-pointing papillae; micron bar = 10 μm.

40. ***Arum cylindraceum*** Gasp. Spathe blade, spathe, downward-pointing papillae; micron bar = 10 μm.

41. ***Arum dioscoridis*** Sibth. & Sm. Spathe blade, downward-pointing papilla; micron bar = 10 μm.

42. ***Arum elongatum*** Steven. Upper spathe tube, downward-pointing papillae with lacunae in the cell corners; micron bar = 10 μm.

43. ***Arum euxinum*** R.R.Mill. Spathe blade, downward-pointing papillae; micron bar = 10 μm.

44. ***Arum hygrophilum*** Boiss. Spathe blade, downward-pointing papillae; micron bar = 10 μm.

45. ***Arum idaeum*** Coustur. & Gand. Spathe blade, tabular cells; micron bar = 10 μm.

46. ***Arum italicum*** Mill. Upper spathe tube, downward-pointing papillae with lacunae in the cell corners; micron bar = 10 μm.

47. ***Arum maculatum*** L. Upper spathe tube, downward-pointing papillae with lacunae in the cell corners; micron bar = 10 μm.

48. ***Arum megobrebi*** Lobin, M.Neumann, Bogner & P.C.Boyce. Upper spathe tube, downward-pointing papillae with lacunae in the cell corners; micron bar = 10 μm.

49. ***Arum nigrum*** Schott. Spathe blade, downward-pointing papillae; micron bar = 100 μm.

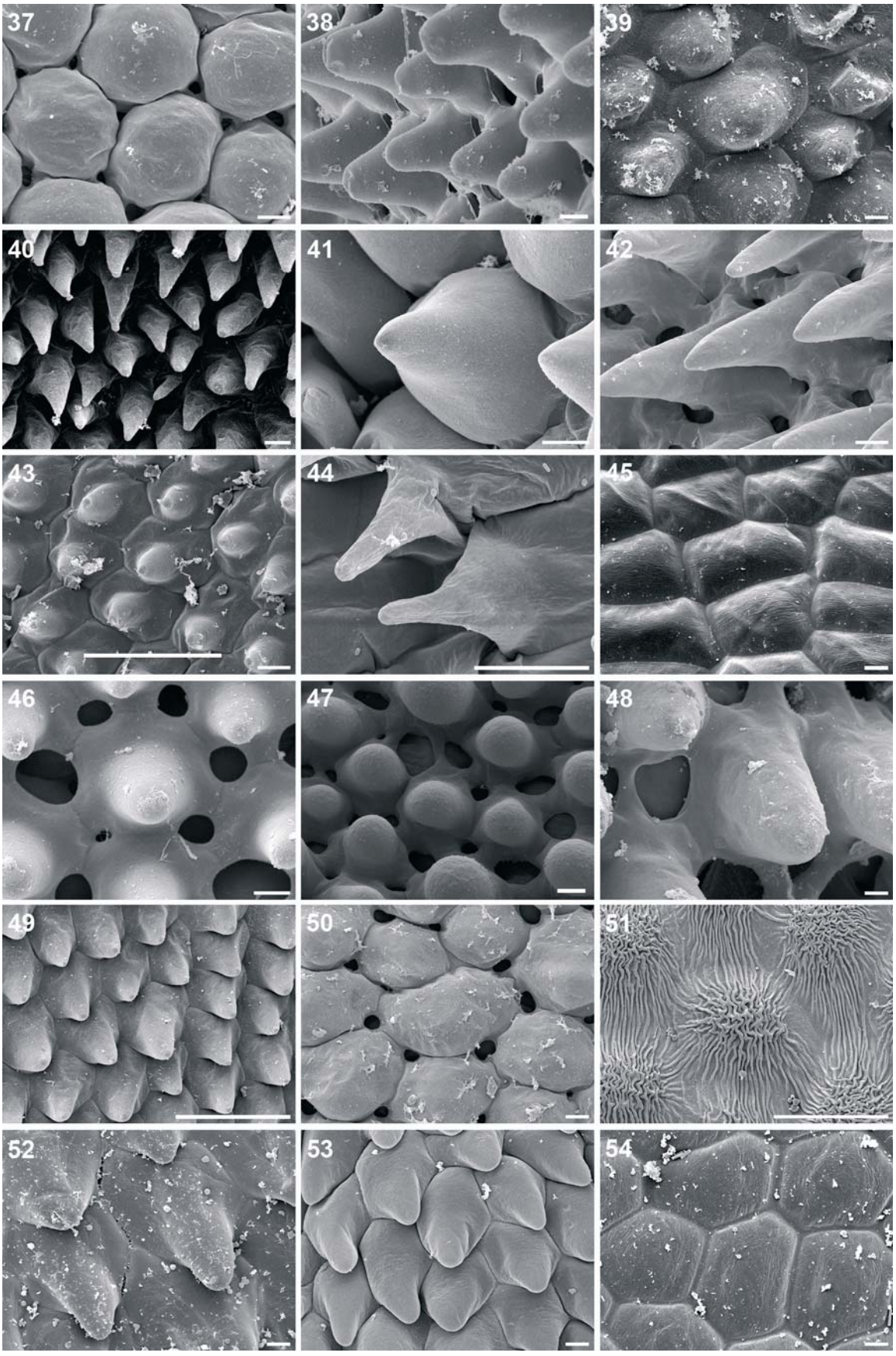
50. ***Arum purpureospathum*** P.C.Boyce. Lower spathe tube, convex cells with lacunae in the cell corners; micron bar = 10 μm.

51. ***Asterostigma lividum*** (Lodd.) Engl. Spathe, convex cells with cuticular folds; micron bar = 10 μm.

52. ***Biarum carratracense*** (Willk.) Font Quer. Spathe blade, downward-pointing papillae; micron bar = 10 μm.

53. ***Biarum tenuifolium*** (L.) Schott. Spathe blade, downward-pointing papillae; micron bar = 10 μm.

54. ***Bucephalandra motleyana*** Schott. Spathe tube, tabular cells; micron bar = 10 μm.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55. ***Caladium bicolor*** (Aiton) Vent. Spathe tube, convex cell; micron bar = 10 μm.

56. ***Caladium lindenii*** (André) Madison. Spathe blade, tabular cells; micron bar = 10 μm.

57. ***Caladium steudneriifolium*** Engl. Spathe blade, convex cells; micron bar = 10 μm.

58. ***Calla palustris*** L. Spathe*, shrunken cells with cuticular folds; micron bar = 10 μm.

59. ***Callopsis volkensii*** Engl. Spathe, convex cells with cuticular folds; micron bar = 10 μm.

60. ***Carlephyton glaucophyllum*** Bogner. Spathe, convex cells; micron bar = 10 μm.

61. ***Chlorospatha croatiana*** Grayum. Spathe blade, convex cells; micron bar = 10 μm.

62. ***Colletogyne perrieri*** Buchet. Spathe, tabular cells; micron bar = 10 μm.

63. ***Colocasia affinis*** Schott. Spathe blade, perpendicular papillae with cuticular folds, hidden under a smooth wax layer; micron bar = 100 μm.

64. ***Colocasia esculenta*** Schott. Spathe blade, perpendicular papillae; micron bar = 10 μm.

65. ***Colocasia fallax*** Schott. Spathe, tabular cells; micron bar = 10 μm.

66. ***Colocasia fontanesii*** Schott. Spathe blade, perpendicular papillae; micron bar = 10 μm.

67. ***Cryptocoryne longicauda*** Becc. ex Engl. Spathe tube, downward-pointing papillae; micron bar = 10 μm.

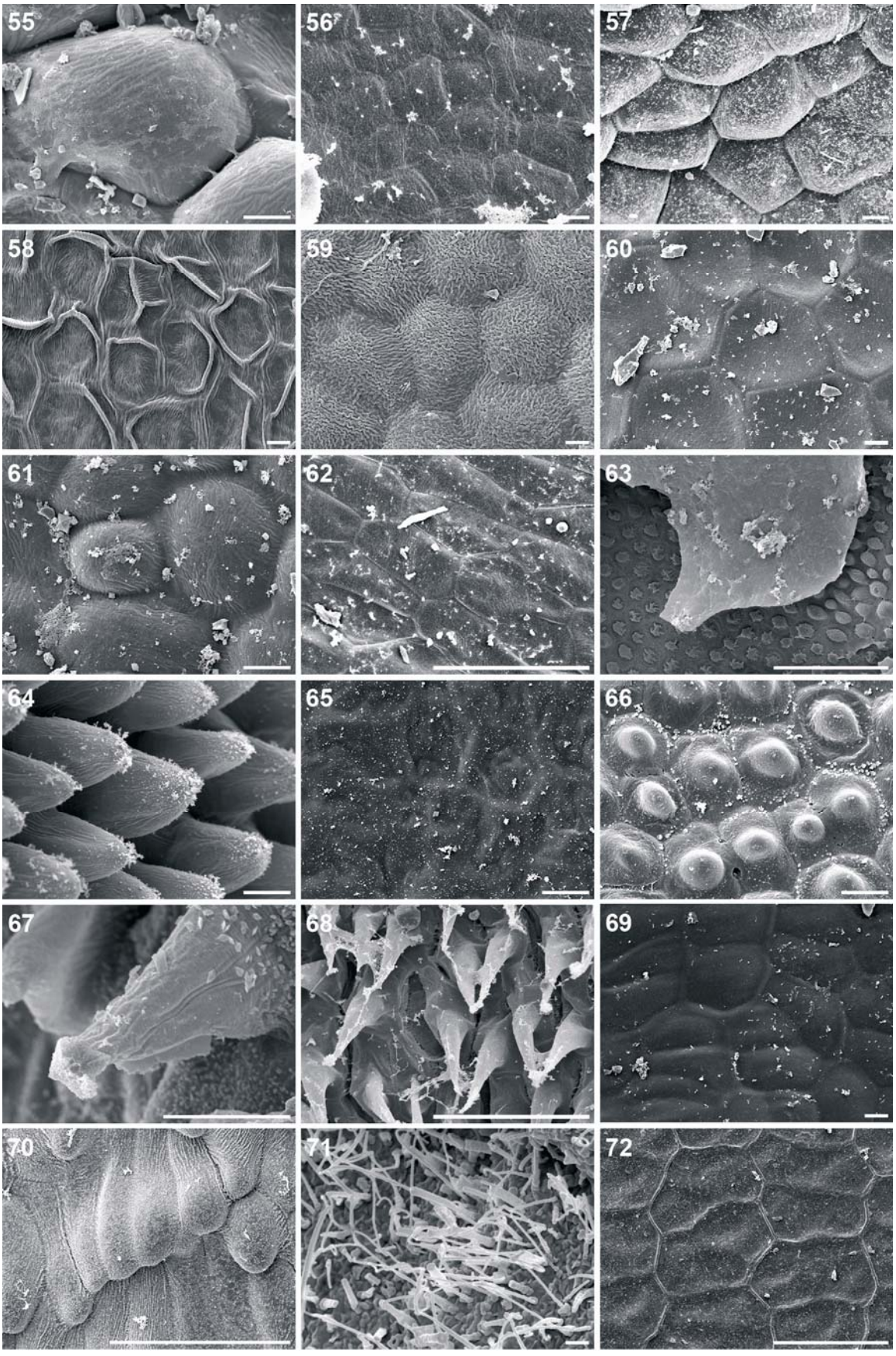
68. ***Cryptocoryne pontederiifolia*** Schott. Spathe tube, downward-pointing papillae; micron bar = 10 μm.

69. ***Culcasia saxatilis*** A. Chev. Spathe, tabular cells; micron bar = 10 μm.

70. ***Cyrtosperma ferox*** N.E. Br. & Linden. Spathe, downward-pointing papillae; micron bar = 10 μm.

71. ***Cyrtosperma johnstonii*** (N.E. Br.) N.E. Br. Spathe, tabular cells with epicuticular wax crystalloids (granules, tubules and threads); micron bar = 1 μm.

72. ***Dieffenbachia bowmannii*** Carrière. Spathe tube, tabular cells; micron bar = 100 μm.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73. ***Dieffenbachia oerstedii*** Schott. Spathe blade, tabular cells; micron bar = 10 μm.

74. ***Dieffenbachia seguine*** Schott. Spathe blade, tabular cells; micron bar = 100 μm.

75. ***Dracontioides desciscens*** (Schott) Engl. Spathe tube, downward-pointing papillae; micron bar = 10 μm.

76. ***Dracontium amazonense*** G.H. Zhu & Croat. Upper spathe, fused perpendicular papillae with cuticular folds; micron bar = 10 μm.

77. ***Dracontium asperum*** K. Koch. Central spathe, fused downward-pointing papillae with cuticular folds; micron bar = 10 μm.

78. ***Dracontium bogneri*** G.H. Zhu & Croat. Central spathe, fused downward-pointing papillae with cuticular folds; micron bar = 10 μm.

79. ***Dracontium nivosum*** (Lem.) G.H. Zhu. Central spathe, fused downward-pointing papillae with cuticular folds; micron bar = 100 μm.

80. ***Dracontium polyphyllum*** L. Central spathe*, epicuticular wax crystalloids (granules); micron bar = 1 μm.

81. ***Dracontium prancei*** G.H. Zhu & Croat. Central spathe*, shrunken, fused downward-pointing papillae with cuticular folds; micron bar = 10 μm.

82. ***Dracontium soconuscum*** Matuda. Spathe, fused perpendicular papillae with cuticular folds; micron bar = 100 μm.

83. ***Dracontium spruceanum*** (Schott) G.H. Zhu. Spathe, fused downward-pointing papillae with cuticular folds; micron bar = 100 μm.

84. ***Dracunculus canariensis*** Kunth. Spathe blade, shrunken papillae; micron bar = 100 μm.

85. ***Dracunculus vulgaris*** Schott. Spathe blade, downward-pointing papillae with cuticular folds; micron bar = 10 μm.

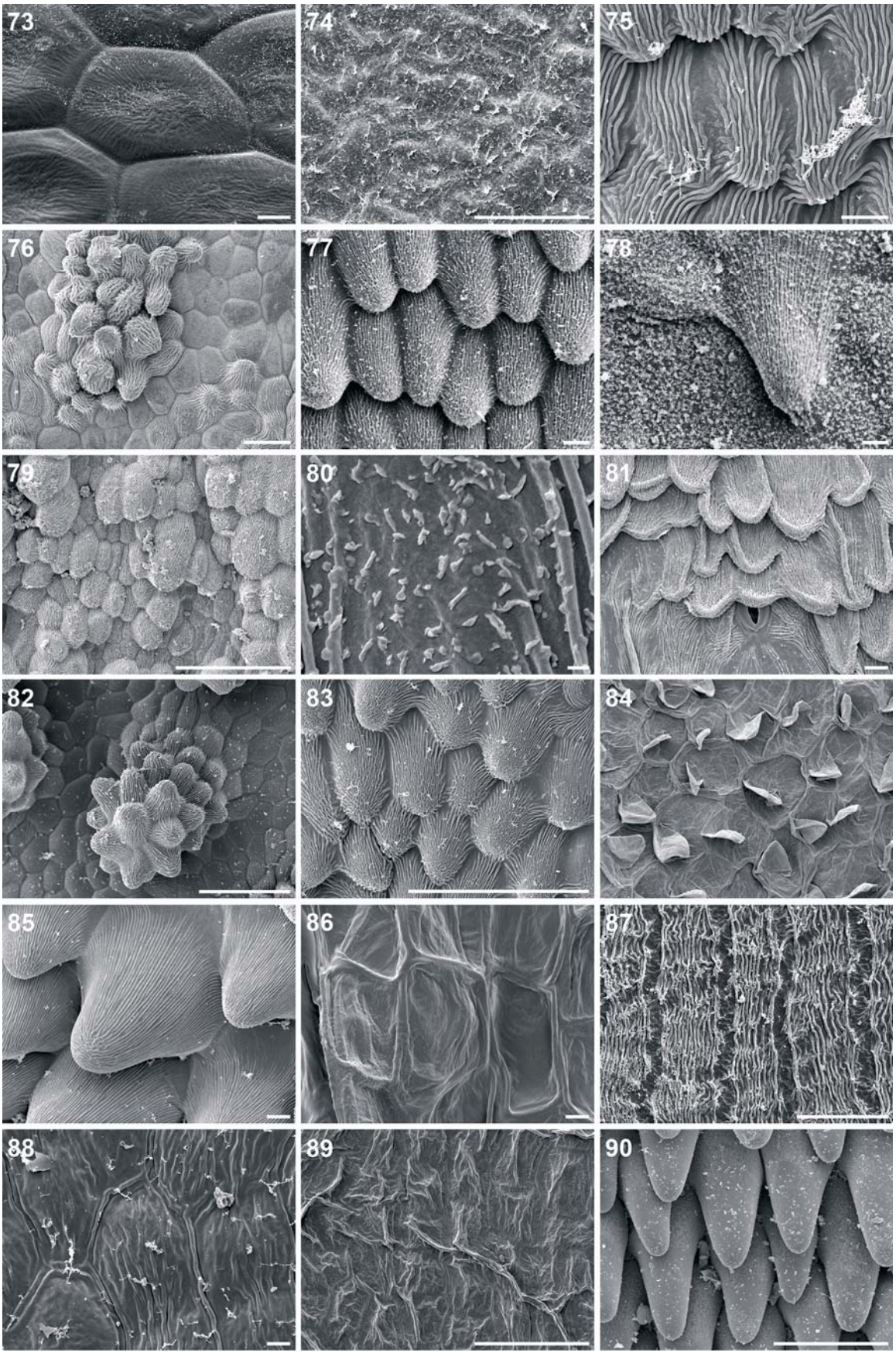
86. ***Filarum manserichense*** Nicolson. Spathe*, shrunken cells; micron bar = 10 μm.

87. ***Gonatopus boivinii*** Engl. Spathe tube, tabular cells with cuticular folds; micron bar = 10 μm.

88. ***Gorgonidium*** **cf.** ***intermedium*** (Bogner) E.G. Gonç. Spathe, tabular cells with cuticular folds; micron bar = 10 μm.

89. ***Hapaline*** **cf.** ***benthamiana*** Schott. Spathe, shrunken cells; micron bar = 100 μm.

90. ***Helicodiceros muscivorus*** (L.f.) Engl. Spathe tube, downward-pointing papillae; micron bar = 100 μm.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91. ***Homalomena picturata*** (Linden & André) Regel. Spathe tube, tabular epidermis; micron bar = 10 μm.

92. ***Homalomena wallisii*** Regel. Spathe blade, tabular epidermis; micron bar = 10 μm.

93. ***Incarum pavonii*** (Schott) E.G. Gonç. Spathe, tabular cells with cuticular folds; micron bar = 10 μm.

94. ***Lagenandra praetermissa*** de Wit. Spathe tube, downward-pointing papillae; micron bar = 10 μm.

95. ***Lasia spinosa*** (L.) Thwaites. Spathe tube, downward-pointing papillae with cuticular folds; micron bar = 10 μm.

96. ***Leucocasia gigantea*** *Schott*. Spathe blade, perpendicular papillae with cuticular folds; micron bar = 10 μm.

97. ***Lysichiton americanus*** Hultén & St. John. Spathe, convex cells with cuticular folds; micron bar = 10 μm.

98. ***Monstera adansonii*** Schott. Spathe, tabular cells with smooth wax layer; micron bar = 10 μm.

99. ***Monstera obliqua*** Miq. Spathe*, shrunken tabular cells; micron bar = 100 μm.

100. ***Nephthytis afzelii*** Schott. Spathe*, tabular cells with smooth wax layer; micron bar = 10 μm.

101. ***Nephthytis hallaei*** (Bogner) Bogner. Spathe*, tabular cells with smooth wax layer and crystals; micron bar = 10 μm.

102. ***Nephthytis*** **sp.** Spathe*, shrunken tabular cells; micron bar = 10 μm.

103. ***Philodendron martianum*** Engl. Spathe tube, convex cells; micron bar = 10 μm.

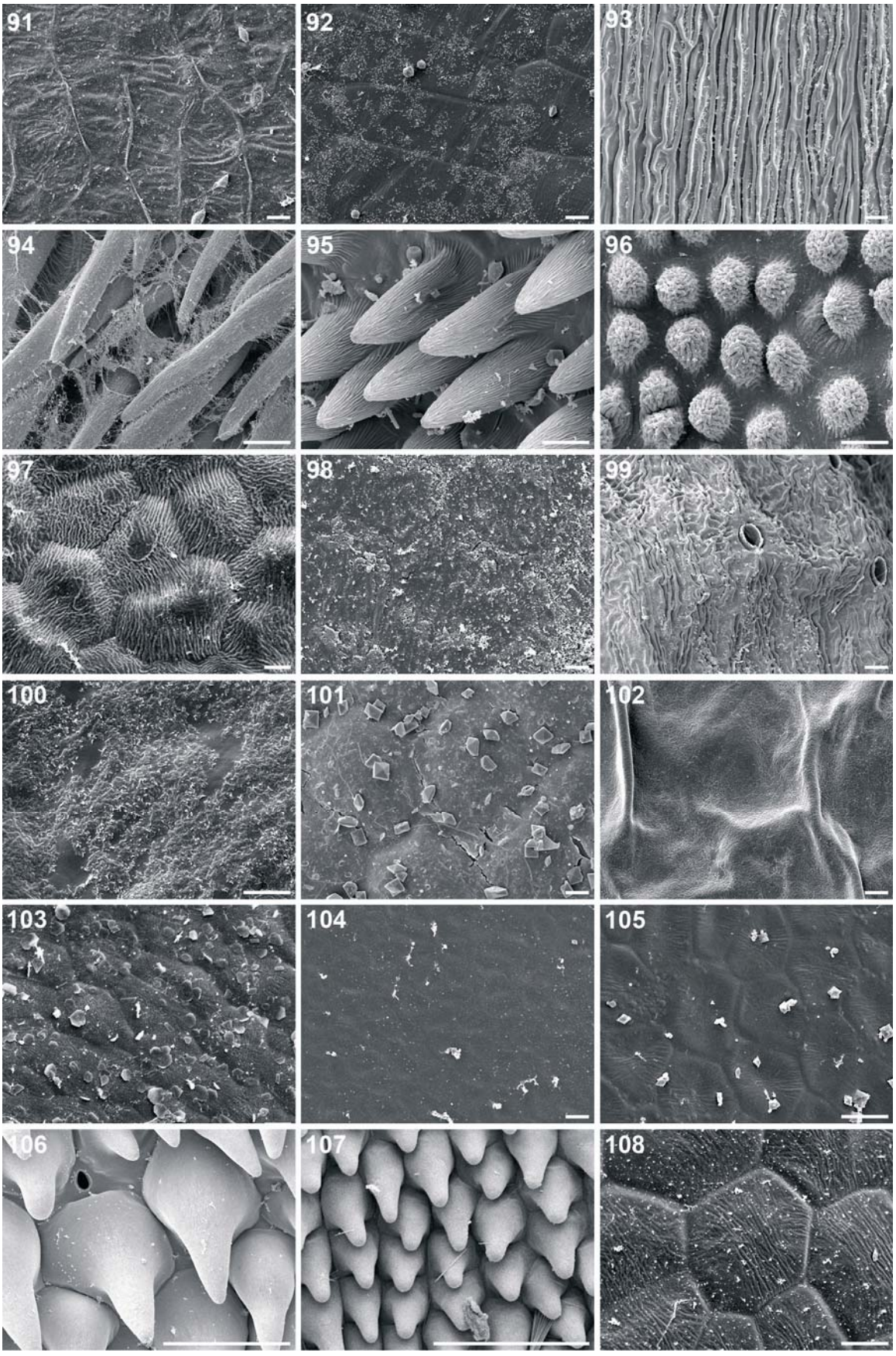
104. ***Philodendron pedatum*** (Hook.) Kunth. Spathe blade, tabular cells; micron bar = 10 μm.

105. ***Philodendron sodiroi*** N.E. Br. Spathe tube, tabular cells; micron bar = 10 μm.

106. ***Pinellia peltata*** C. Pei. Spathe blade, downward-pointing papillae with cuticular folds; micron bar = 100 μm.

107. ***Pinellia ternata*** (Thunb.) Makino. Spathe blade, downward-pointing papillae with cuticular folds; micron bar = 10 μm.

108. ***Piptospatha ridleyi*** N.E. Br. ex Hook.f. Spathe, tabular cells; micron bar = 10 μm.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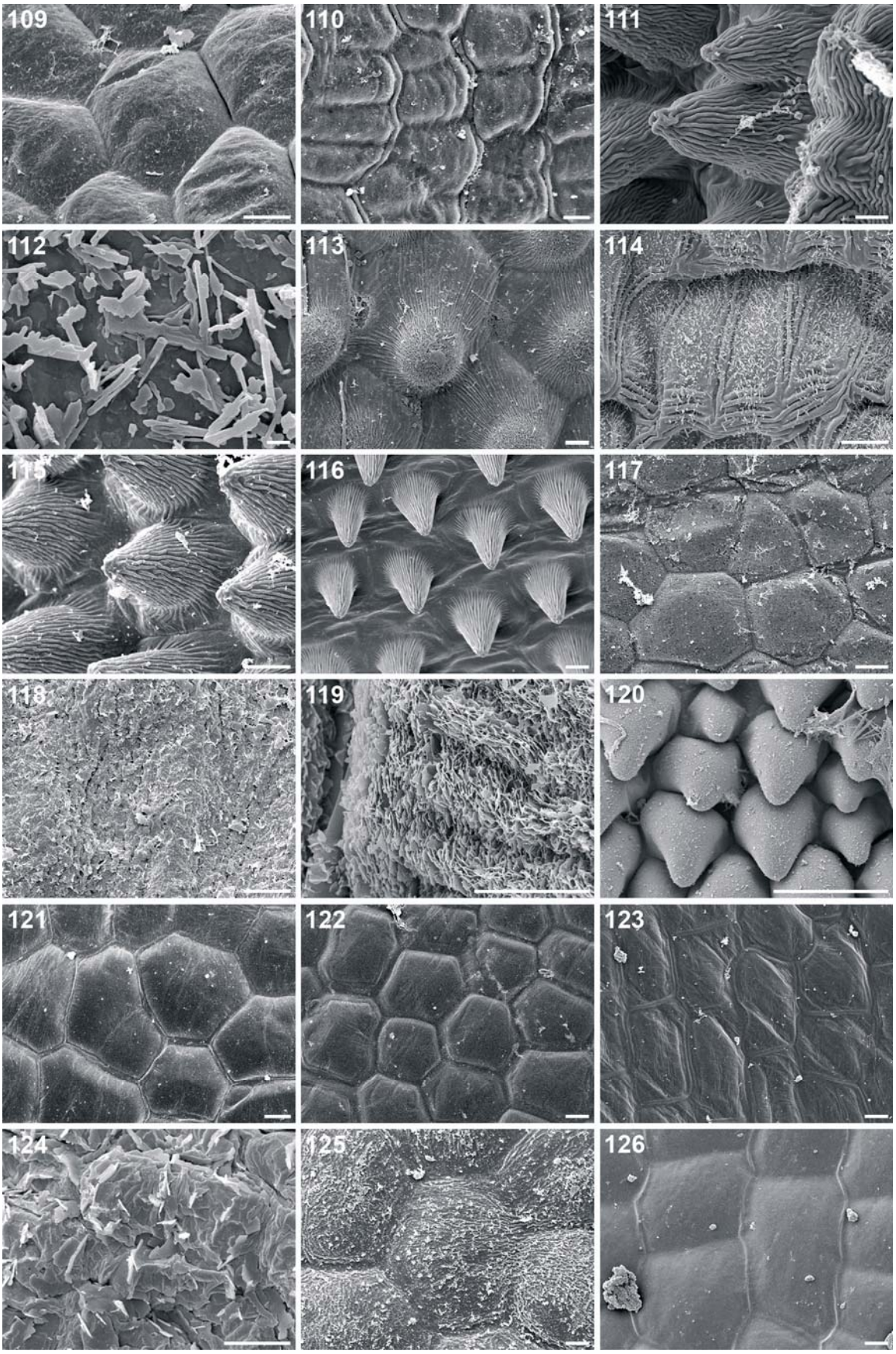
109. ***Pistia stratiotes*** L. Spathe, convex cells; micron bar = 10 μm.
110. ***Pothos junghuhnii*** de Vriese in F.A.W. Miquel. Spathe, convex epidermis; micron bar = 10 μm.
111. ***Pseudodracontium latifolium*** Serebryanyi. Basal spathe, perpendicular papillae with cuticular folds; micron bar = 10 μm.
112. ***Pseudodracontium*** **sp.** Upper spathe, tabular cells with cuticular folds and epicuticular wax crystalloids (platelets); micron bar = 1 μm.
113. ***Pseudohydrosme gabunensis*** Engl. Spathe, downward-pointing papillate cells with cuticular folds; micron bar = 10 μm.
114. ***Pycnospatha palmata*** Gagnep. Spathe, fused cells with cuticular folds and epicuticular wax crystalloids (rodlets); micron bar = 10 μm.
115. ***Remusatia hookeriana*** Schott. Spathe blade, perpendicular papillae with cuticular folds; micron bar = 10 μm.
116. ***Remusatia pumila*** (D. Don) H. Li & A. Hay. Spathe blade, perpendicular papillae with cuticular folds; micron bar = 10 μm.
117. ***Remusatia vivipara*** (Roxb.) Schott. Spathe blade, tabular cells with smooth wax layer; micron bar = 10 μm.
118. ***Rhaphidophora angustata*** Schott. Spathe*, thick amorphous wax crust; micron bar = 10 μm.
119. ***Rhaphidophora decursiva*** (Rox.) Schott. Spathe*, epicuticular wax crystalloids (platelets); micron bar = 10 μm.
120. ***Sauromatum venosum*** (Dryand. ex Aiton) Kunth. Spathe tube, downward-pointing papillae; micron bar = 100 μm.
121. ***Schismatoglottis calyptrata*** (Roxb.) Zoll. Spathe blade, tabular cells; micron bar = 10 μm.
122. ***Schismatoglottis multiflora*** Ridl. Spathe blade, tabular cells; micron bar = 10 μm.
123. ***Schismatoglottis subundulata*** (Zoll. ex Schott) Nicolson. Spathe blade, tabular cells; micron bar = 10 μm.
124. ***Scindapsus lucens*** Bogner & P.C. Boyce. Spathe, tabular cells with amorphous epicuticular wax layer; micron bar = 10 μm.
125. ***Spathicarpa hastifolia*** Hook. Spathe, convex cells; micron bar = 10 μm.
126. ***Spathiphyllum cannifolium*** (Dryand. ex Sims) Schott. Spathe, tabular cells; micron bar = 10 μm.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127. *Spathiphyllum wallisii* Regel. Spathe, tabular epidermis; micron bar = 10 μm.

128. *Stenospermation popayanense* Schott. Spathe, concave cells; micron bar = 10 μm.

129. *Steudnera henryana* Engl. Spathe*, epicuticular wax crystalloids (platelets); micron bar = 1 μm.

130. *Steudnera kerrii* Gagnep. Spathe*, epicuticular wax crystalloids (platelets); micron bar = 1 μm.

131. *Stylochaeton bogneri* Mayo. Spathe tube*, epicuticular wax crystalloids (platelets and threads); micron bar = 10 μm.

132. *Stylochaeton* cf. *hypogaeus* Lepr. Spathe tube, perpendicular papillae covered with an epicuticular wax layer; micron bar = 10 μm.

133. *Stylochaeton zenkeri* Engl. Spathe tube, perpendicular papillae; micron bar = 10 μm.

134. *Symplocarpus foetidus* (L.) Salisb. ex W.P.C. Barton. Spathe, convex cells; micron bar = 10 μm.

135. *Synandrospadix vermitoxicus* (Griseb.) Engl. Spathe, tabular cells with cuticular folds; micron bar = 10 μm.

136. *Syngonium macrophyllum* Engl. Spathe tube, tabular cells with remnants of a smooth wax layer; micron bar = 10 μm.

137. *Syngonium podophyllum* Schott. Spathe blade, convex cells; micron bar = 10 μm.

138. *Taccarum caudatum* Rusby. Spathe, dome-shaped cells; micron bar = 10 μm.

139. *Typhonium blumei* Nicolson & Sivad. Spathe tube, short downward-pointing papillae; micron bar = 10 μm.

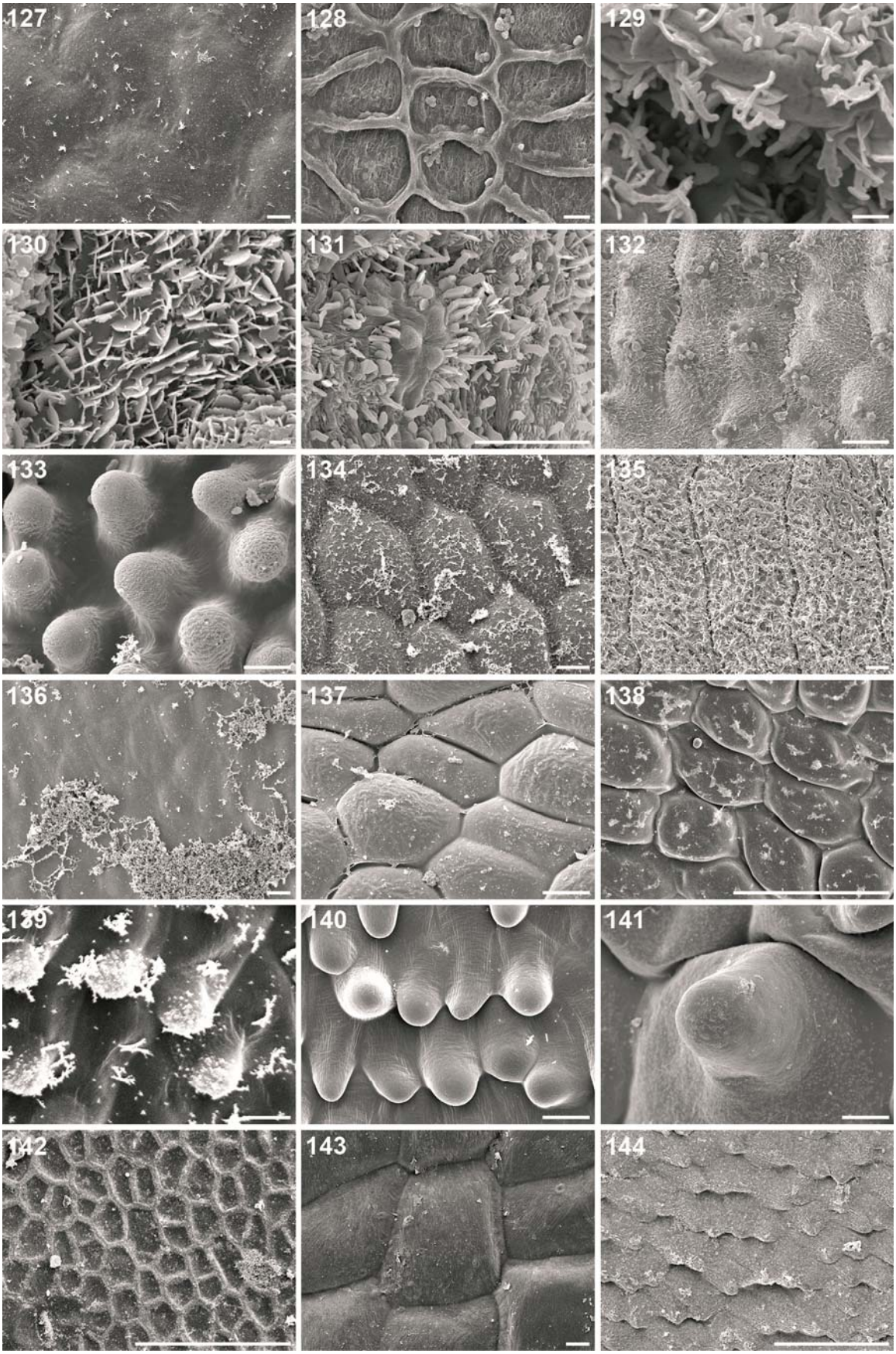
140. *Typhonium* sp. Spathe blade, downward-pointing papillae; micron bar = 10 μm.

141. *Typhonium trilobatum* (L.) Schott. Spathe blade, downward-pointing papillae; micron bar = 10 μm.

142. *Typhonodorum lindleyanum* Schott. Spathe blade, concave cells; micron bar = 10 μm.

143. *Ulearum sagittatum* Engl. Spathe, convex cells; micron bar = 10 μm.

144. *Urospatha grandis* Schott. Spathe, fused downward-pointing papillae with cuticular folds; micron bar = 10 μm.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145. ***Urospatha sagittifolia*** (Rudge) Schott. Spathe, epicuticular wax crystalloids (branched rodlets); micron bar = 1 μm.

146. ***Urospatha tonduzii*** Engl. Spathe, diagonally arranged fused papillae with cuticular folds; micron bar = 10 μm.

147. ***Xanthosoma cubense*** (Schott) Schott. Spathe tube, tabular cells; micron bar = 10 μm.

148. ***Xanthosoma mariae*** Bogner & E.G. Gonç. Spathe blade, tabular cells; micron bar = 10 μm.

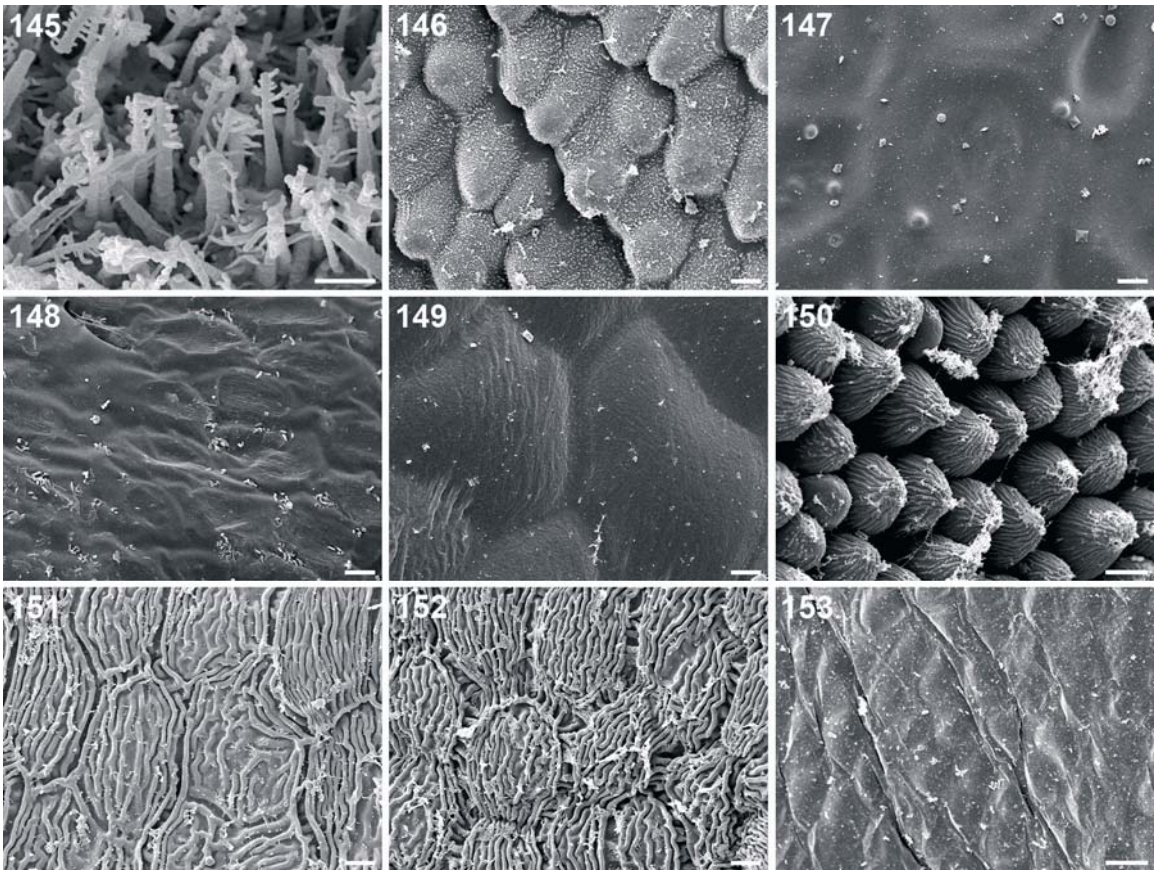
149. ***Zamioculcas zamiifolia*** (Lodd.) Engl. Spathe blade, convex cells; micron bar = 10 μm.

150. ***Zantedeschia aethiopica*** (L.) Spreng. Spathe, perpendicular papillae with cuticular folds; micron bar = 10 μm.

151. ***Zantedeschia albomaculata*** (Hook.) Baill. Spathe, tabular cells with cuticular folds; micron bar = 10 μm.

152. ***Zantedeschia rehmannii*** Engl. Spathe, tabular cells with cuticular folds; micron bar = 10 μm.

153. ***Zomicarpa riedelianum*** Schott. Spathe, convex cells; micron bar = 10 μm.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09/2008 Diplomarbeit zum Thema „Spatial distribution of primary hemiepiphytes in different Costa Rican rainforests”.

10/2002 – 09/2008 Studium der Biologie und Ökologie (Schwerpunkt Naturschutz und Vegetationsökologie) an der Universität Wien, Magister rer. nat. mit ausgezeichnetem Erfolg.

Auslandserfahrung

07/2010 – 09/2010 Feld- und Laborarbeit im Xishuangbanna Tropical Botanical Garden, Yunnan, China. Untersuchung von Bestäuber-Pflanzen-Interaktionen in einem Biosphärenreservat.

01/2007 – 05/2007 Feldforschung im Rahmen der Diplomarbeit in Costa Rica. Erhebung von Verbreitung und Vielfalt von Aufsitzerpflanzen in natürlichen und gestörten Regenwäldern in verschiedenen Naturschutzgebieten.

Förderungen/Preise

2012 2. Platz beim Schreibwettbewerb ‘uni international’ der Universität Wien.

2007 Universität Wien – Förderung für Forschungsaufenthalt in Costa Rica (Diplomarbeit).

Publikationen

Bröderbauer D, Diaz A, Weber A. 2012. Reconstructing the origin and elaboration of insect-trapping inflorescences in the Araceae. *American Journal of Botany* 99: 1666-1679.

Ulrich S, Hesse M, Bröderbauer D, Wong SY, Boyce PC. 2012. *Schismatoglottis* and *Apoballis* (Araceae: Schismatoglottideae): A new example for the significance of pollen morphology in Araceae systematics. *Taxon* 61: 281-292.

Bröderbauer D. 2008. Spatial distribution of primary hemipiphytes in different Costa Rican rainforests. Universität Wien, Diplomarbeit.

Wien, Dezember 2012