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Floral Structure and Pollination Biology of *Axinaea* (Melastomataceae)

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Abstract

Pollen as the only reward for pollinating bees is characteristic for most Neotropical Melastomataceae. For eight genera belonging to four different tribes, however, nectar secretion by stomatal openings, pseudo-tubular flowers and both vertebrate and invertebrate pollinators other than bees have been reported. The pseudo-tubular flowers of the mainly Andean genus *Axinaea* (tribe Merianieae) are characterized by distinctive bulbous connective appendages. It has been hypothesized that these appendages may bear nectar secreting structures, may play a key role in the process of pollination, or may serve as food-bodies to attract pollinators other than bees. To test these hypotheses, I have investigated floral the structure of five *Axinaea* species in detail using MicroCT, Scanning Electron Microscopy and Light Microscopy. Field studies on the breeding system and pollinator monitoring of *Axinaea confusa* have been conducted in a montane rainforest in southern Ecuador. Like nectar producing Merianieae, *Axinaea* bears stomatal openings on the inner wall of the hypanthium, but the flowers are not nectariferous. The main finding of my investigations is that instead of a nectar reward, the pollination mechanism of *Axinaea* involves floral food-bodies in combination with bird pollination. Different species of tanagers and flower piercers (Thraupidae) are attracted by the brightly coloured bulbous connective appendages in the flowers. However, these appendages do not only function as attractant and food reward for the pollinating birds, but are also an integral part of a complex pollination mechanism which is best described as a “bellows-mechanism”. The bulbous appendage, composed of loose parenchymatic tissue, has to be imagined as a balloon. Compression by the bill when the bird grips the appendage, forces the air contained in the large intercellular spaces into the hollow thecae, thus causing clouds of powdery pollen to be ejected from the poricidal anthers. The foraging bird is dusted in pollen on the head and bill and by accidental contact with the stigma successfully transfers pollen. The fruit-set on plants was high and as tanagers were the only observed visitors capable of activating this mechanism, they can be recognized as the legitimate pollinators. Stamens are usually removed one by one and about 60% of the flowers are visited two to three times until all stamens have been removed. While this strategy could augment cross-fertilization, *Axinaea confusa* has been shown to be self-compatible. Selfing might be regarded as a means to assure reproductive success in harsh environments like the rain-affected Andean forests, but also as a strategy to reconcile risks related to this highly specialised pollination mechanism. The evolution of the bellows-mechanism and bird pollination in *Axinaea* serves as another example for shifts in pollination syndromes correlated with growth at higher altitudes where bees are less efficient pollinators than birds.

Keywords: Melastomataceae, pollination, Thraupidae, floral food bodies, anther morphology, nectar, Andes, Ecuador

Abstract – German

Ein Charakteristikum neotropischer Melastomataceae sind Pollenblumen, in denen Pollen als einzige Belohnung für die bestäubenden Bienen geboten wird. Acht Gattungen weisen jedoch zusätzliche Nektarsekretion mittels Spaltöffnungen und damit verbunden halb-glockige Blüten sowie ein breiteres Bestäuberspektrum (Vertebraten sowie Invertebraten) auf. Die vorwiegend andine Gattung *Axinaea* (Tribus Merianieae), deren Blüten ebenfalls halb-glockig sind, ist durch knollig vergrößerte, dorsale Anhängsel des Konnektivs gekennzeichnet. Die Vermutung liegt nahe, dass *Axinaea* zu den wenigen nektarproduzierenden Melastomataceae gehört, somit auch einen Bestäuberwechsel durchgemacht hat und dass die Konnektivanhängsel eine fundamentale Rolle in der Bestäuberanlockung sowie im Bestäubungsmechanismus spielen. Mittels MicroCT, Rasterelektronenmikroskopie und Lichtmikroskopie wurden die Blütenstruktur von fünf Arten im Detail analysiert sowie Feldstudien an einer Art (*Axinaea confusa*) in einem Bergregendwald in Südecuador durchgeführt. So wie nektarproduzierende Merianieae, besitzt auch *Axinaea* Spaltöffnungen an der Innenwand des Hypanthiums, jedoch konnte keine Nektarsekretion festgestellt werden. An Stelle von Nektar fungieren die kräftig gefärbten Konnektivanhängsel als Futterkörperchen, die von verschiedenen Tangararten (Thraupidae) gefressen werden. Die Konnektivanhängsel dienen jedoch nicht nur der Anlockung und Belohnung von Vögeln, sondern übernehmen tatsächlich eine wichtige funktionelle Rolle im Bestäubungsvorgang. Das Innere der vergrößerten Anhängsel besteht aus lockerem, interzellularreichem Gewebe, das mit Luft gefüllt ist. Wie bei einem Blasebalg führt das Zusammendrücken der Anhängsel durch den Vogelschnabel dazu, dass die interzelluläre Luft durch die einzige Öffnung entweicht: die röhrenförmige Anthere. Dieser Luftstrom genügt, um eine Wolke pulverigen Pollens aus der Pore an der Antherenspitze zu blasen. Die Pollenwolken landen auf Schnabel und Kopf der Vögel, die beim Herauslösen des nächsten Staubblatts zufällig die Narbe berühren und so die Bestäubung durchführen. Da der Fruchtsatz hoch und Tangare die einzigen beobachteten Blütenbesucher waren, die den Blasebalgmechanismus auslösen konnten, müssen sie als legitime Bestäuber angesehen werden. Generell werden Staubblätter einzeln herausgelöst und etwa 60% der Blüten werden zwei- oder mehrmals besucht, bis alle Staubblätter gefressen sind. Obwohl diese Strategie eine höhere Wahrscheinlichkeit für Fremdbestäubung bieten könnte, ist *Axinaea confusa* selbstkompatibel. Selbstkompatibilität wird jedoch vielfach als reproduktive Absicherung unter instabilen Wetterbedingungen, wie denen der regenreichen

Bergregenwälder an-gesehen und reduziert außerdem das mit Bestäuberspezialisierung verbundene Reproduktionsrisiko in Zeiten von niedriger Bestäuberabundanz. Die Evolution des Blasebalgmechanismus stellt ein weiteres Beispiel eines spezialisierten Bestäuberwechsels in Verbindung mit dem Vorkommen in höheren Lagen dar, wo Vögel als zuverlässigere Bestäuber gelten als Bienen.

Introduction

Flowers with poricidal anthers and dry pollen are tightly associated with the buzz-pollination syndrome where floral visitors cause pollen release through high-frequency vibrations of their flight muscles (Vogel, 1978; Buchmann, 1983). Such buzz-pollination, which is effected by bees, occurs in ca. 98% of the Melastomataceae, a pantropically distributed family of around 5005 species in 118 genera (APG III (22.04.2013)). The constancy in pollination strategies within this large family has been explained by an adaptive peak the family has reached in terms of reproductive success (Macior, 1971). The family is characterized by its flowers with poricidal anthers, pollen as the only reward to the pollinating bees, and various strategies such as heteranthy to avoid excess pollen loss (Vogel, 1978; Luo et al., 2008). Nevertheless, nectar as an additional reward has evolved in eight genera belonging to four tribes of the Melastomataceae, three of which are strictly neotropical (Blakeeae, Merianieae, Miconieae) and one that is pantropical (Melastomeae), although nectar has only been detected in the neotropical genera (Renner et al., 2001; Michelangeli et al., 2013). According to Renner (1989), nectar production necessitates morphological changes in the flowers as the nectar-collecting floral visitors do not perform buzzing, thus pollen release requires a different mechanism. Most nectariferous species have retained poricidal anthers though the pores are enlarged, allowing pollen to be ejected more easily. Not surprisingly, the pollinator guild of the nectar producing species has shifted from bees to both vertebrate (e.g. Penneys & Judd, 2005; Almeda, 2000; Muchhala & Jarrin-V, 2002, Vogel, 1957, Lumer, 1980) and other invertebrate pollinators (e.g. Stiles et al., 1992). It has been hypothesized that this switch in pollinator guild is correlated with growth at higher altitudes where bees are less efficient pollinators than in the lowlands because of the raised energy costs poikilothermic animals face at lower temperatures (Cruden, 1972). Another morphological trait that has evolved in parallel with nectar producing melastomes are pseudo-tubular corollas while pollen-only species have bowl-shaped flowers or often even reflexed petals (Varassin et al., 2008). This allows some predictability of pollination syndromes in Neotropical Melastomataceae, although especially in the Miconieae, enlarged pores and shortened anthers have led to more generalized pollination systems as some species in that tribe are frequented both by buzzing bees and non-vibrating visitors

(Goldenberg et al., 2008). The combined presence of nectar production, pseudo-tubular corollas, and enlarged anthers, however, seems to be a strong indicator for specialized bird, bat, or rodent pollination (Varassin et al., 2008).

In the tribe Merianieae, nectar production in connection with bat and bird pollination has been reported for four species of *Meriania* and *Centronia*. However, in several studies no nectar secreting structures could be localized in these taxa (Vogel, 1988; Vogel 1997; Muchhala & Jarrin-V, 2002). Therefore, it has been hypothesized that nectar release occurs from non-structural nectaries like filament slits (Stein & Tobe, 1989; Renner, 1989). Stein & Tobe (1989) had related the exceptionally large vascular bundles of Melastomataceae stamens with nectar secretion. Vogel (1997), who also observed nectar release from filament slits in *Meriania phlomoides* Triana, argues that the slits described by Stein & Tobe (1989) might be the mere consequence of rapid filament extension, which causes ruptures in the tissue and incisions in the thickened phloem bundles. Finally, Varassin et al. (2008) detected localized stomatal nectaries on the stamens and the inner wall of the hypanthium in some nectariferous species of *Meriania*.

Both dorsal and ventral connective appendages as well as apical connective protrusions are common in Melastomataceae. In buzz-pollinated species, it has been observed that visiting bees grasp these appendages with their legs and use them as landing platforms when buzzing the flowers (e.g. Renner, 1989). In nectariferous melastomes, connective appendages tend to be reduced or absent. In part, this morphological trend has a phylogenetic explanation as pronounced anther connective appendages are generally lacking in the Blakeeae and Miconieae. The tribe Merianieae, on the other hand, is characterized by dorsal-basal anther connective appendages of varying size and shape (Clausing & Renner, 2001). Acute, blunt, cylindrical, and bifurcated appendages are found throughout the tribe, while *Axinaea*, a group of ca. 40 species of high-elevation, Andean trees and shrubs, has globose connective appendages (Mendoza-Cifuentes & Fernández-Alonso, 2010). As nectar producing melastomes generally have vertebrate pollinators or are pollinated by insects probably incapable of buzzing, the question arises what the function of the conspicuous appendages of *Axinaea* might be. It has been observed on herbarium samples and in the field, that the connective appendages show marks of having been gnawed on or even are chewed up completely (personal observation, pers. com. Darin S. Penneys, Carmen Ulloa, Balslev-Cotton, 2003, also see Mendoza-Cifuentes & Fernández-Alonso (2010) for *Meriania*). This gives support to the thought that the appendages contain nutritive tissues which are consumed by animals. Another important question is how pollen is released from these poricidal anthers if no buzzing is applied. While Renner (1989) argued that alternative modes of pollination tend to be related to enlarged apical pores in Melastomataceae anthers, the pores of *Axinaea* do not appear significantly enlarged. A study on nectar robbing

flowerpiercers (Diglossa, Thraupidae) detected decent amounts of *Axinaea* pollen to be carried by these birds (Rojas-Nossa, 2007).

Axinaea provides an interesting case for investigating the probable functional link between the conspicuous bulbous anther connective appendages, possible nectar production, and pollination syndromes. I hypothesized that (1) the bulbous appendages are derived from connective tissue and (2) that they bear nectar producing structures supplied with large vascular bundles. As Varassin et al. (2008) point out that nectary location differs between the genera but is consistent within, I expected to (3) find stomatal openings in an arrangement unique for *Axinaea*. Connective appendages in melastomes have been shown to function as handles for buzzing bees (Renner, 1989). I therefore assumed that also in this case, the bulbous connective appendages plays a key role in the process of pollination and it was the aim of the field study to (4) discover the pollination mechanism and detect the main pollinator of *Axinaea*. Based on the observations mentioned above, I hypothesized that (5) the bulbous connective appendages serve as food-bodies for attracting pollinators other than bees.

Material and Methods

Structural Studies

Study species

Flowers and buds of *Axinaea costaricensis* Cogn. were collected at the Finca Truchas Selva Madre, Costa Rica, at an altitude of ca. 3000 m in February 2012 by J. Schönenberger (JS 937). Samples of *A. macrophylla* Triana (Darin S. Penneys, # 1598) were taken in the Province Morona-Santiago in eastern Ecuador at an elevation of 2400 m in September 2003. *Axinaea confusa* (Balslev-Cotton, 2003), the main study species for the field experiments, and *A. sclerophylla* Triana were found in the Podocarpus National park, Province Zamora-Chinchipe, southern Ecuador at an altitudinal range of 1800 – 2200 m and 2600 – 2800 m respectively. For *A. sclerophylla*, no anthetic flowers with stamens could be collected; only opened flowers with filament-remnants have been sampled. *A. affinis* Cogn. has been collected in the Province Azuay, Ecuador, in the Bosque Cristal at 3500 m. For all morphological studies, the samples were fixed in FAA (Formaldehyde Acetic Acid) and transferred to 70% ethanol afterwards. Specimens of *A. confusa*, *A. macrophylla* and *A. sclerophylla* have been deposited in the herbaria of the Universidad Técnica Particular de Loja (UTPL), and the Universität Wien (WU). A specimen of *A. affinis* has been deposited at the Universidad del Azuay (HA). Species identification was based on Balslev-Cotton's

(2003) revision of the genus *Axinaea* (unpublished master thesis). Although the species circumscriptions of this revision have not been officially recognized, they were considered to best describe the taxa encountered in the field.

Floral structure

The floral structure of all five species was analysed and compared. Information on *A. macrophylla* is based on literature (Balslev-Cotton, 2003) as no fresh flowers were available. Character traits considered especially important for pollinator attraction (e.g. Proctor et al., 1996) are listed in Table 1. Furthermore, petal length and diameter of the corolla, and stamen and style length were measured on ten fresh flowers of *A. confusa*, shown in Table 2.

Scanning Electron Microscopy

Scanning Electron Microscopy was done for mature stamens, the gynoecium, inner hypanthium wall, as well as for individual petals of all five species. Furthermore, different developmental stages of the androecium of *A. costaricensis* were scanned, bud sizes ranged from mature buds (approx. 1.2 cm in length) to very young buds (approx. 1 mm). The samples were dehydrated over an ethanol series (70%, 85%, 96%), critical point dried (CP Autosamdri-815), and coated with gold using a Sputter Coater (SCD 050). They were mounted onto stubs and scanned for nectar producing structures in a JEOL JSM-6390. The number of stomatal openings was recorded and their size (diameter parallel to the guard cells) measured (see Varassin et al., 2008).

Light microscopy

For light microscopy, single stamens (with appendage either intact or compressed), stamens before anthesis, and gynoecia (with hypanthium) were extracted from mature buds, infiltrated (Technovit 7100, hardener I) in the fridge overnight and embedded the next day (Technovit 7100, hardener II). Cross and longitudinal serial sections of 6-7 μm thickness of both antesealous and antepetalous stamens as well as cross sections of hypanthium and gynoecium were prepared at a rotary microtome. 0.2% – Ruthenium red – 0.5% – Toluidine blue was used as a multiple general stain (RT). In order to detect the presence of carbohydrates, a periodic acid-Schiff reagent (PAS) was used (see Varassin et al., 2008). A positive reaction causes a strong red-pink coloration of the respective cells. A Ponceau-stain (modified from Ruzin (1999)) was used for the detection of proteins. In the Ponceau-stain, protein bodies stain brilliant red. Slides were mounted with Entellane and observed on an Olympus BX 50 microscope. Photographs were taken with a Nikon DS-F11. The ratio of the radial thickness of the vascular bundle to the radial thickness of the filament was measured

in sections of the basal part of the anther (see Varassin et al., 2008, Stein & Tobe, 1989) and are given in Table 3.

Micro-Computer Tomography

Freshly opened flowers and mature buds of *A. costaricensis* and *A. confusa* were prepared in 1% FDA – 70% Ethanol, the solution was changed daily for a week. Samples were then mounted for the MicroCT. Additionally, single stamens and a single flower of *A. confusa* were dehydrated (1% FDA – 96% Ethanol), transferred to acetone and CP-dried prior to M-CT [source settings: 35 kV, 68 μ A; optical magnification: 4.0033; binning: 1; exposure time: 10.0000 s; pixel size: 2.4088 μ m].

Field Methods

Study site and species

The experiments were conducted in the Podocarpus National park in the Loja and Zamora-Chinchipe Provinces, Ecuador, from mid September 2012 through the end of November 2012. Four different species of *Axinaea* were found, *Axinaea confusa*, *A. macrophylla*, *A. sclerophylla* and *Axinaea* sp. No flowering individuals of *A. macrophylla* could be found, although heavy flowering had been observed in October to December in the previous year (pers. com. Jürgen Homeier, Florian Bodner). Two individuals of *Axinaea* sp. were located. However, their buds were consumed by larvae before opening. Therefore, field experiments and observations were limited to two species, *Axinaea confusa* and *Axinaea sclerophylla*.

Axinaea confusa is a tree of 5 – 15 metres producing abundant flowers. It grows in the montane rainforests of southern Ecuador at elevations of 1500 – 3100 m (Balslev-Cotton, 2003). Experiments and observations were carried out at two different sites. Site 1 is located on the steep NW slope along the Rio San Francisco at 1800 – 1900 m, 03°58'20''S, 79°04'31''W. 35 fully grown individuals were found along an 800 metres transect on the Camino Canal trail. The construction of a water line in 1952/53 and a new electricity line in 1997 caused high disturbance in the area, resulting in partly opened, secondary forest with a high proportion of bamboo and other graminoids (Lohr, 1998). Further upstream along the Rio San Francisco, Site 2 stretches 600 metres across a SE slope at 2050 – 2200 m, 03°58'33''S, 79°06'01''W. The entire area is used for cattle farming and the old, abandoned road Loja-Zamora runs through the study site. More than 40 individuals were found, many shrubby and smaller in stature. At both sites, almost all individuals were flowering although flowering ended in late October at Site 1 while it was still going on at the end of November at Site 2. Due to the extremely steep slopes and thin trunks (mostly < 10 cm in diameter),

the accessibility of the plants was limited. At Site 1, seven individuals were used for experiments on the mating system, pollinator observations, and observations of patterns in the stamen removal (described below). At Site 2, ten plants were available for functional experiments, pollinator observations, and stamen removal experiments.

Axinaea sclerophylla is a few-flowered species occurring from 1700 m to 3400 m above sea level (Wurdack (1988), Balslev-Cotton, 2003). A population of *A. sclerophylla* was found on the windswept pass, El Tiro, following the old road from Loja to Zamora between 2600 m and 2770 m, 03°58'57''S, 79°08'33''W. Most of the individuals either bore fruits or young buds and only four individuals flowered in late October/November. Thus, work was restricted to pollinator observations.

Unless otherwise stated, the field methods refer to *A. confusa* only.

Flowering phenology

In order to study the course and duration of anthesis, single flowers of *A. confusa* were selected and monitored daily until petals dropped, as well as checked for pollen availability.

Floral rewards

The presence of nectar, oil, and lipids was tested in fresh flowers. In each trial, the bulbous connective appendages of five stamens were squeezed to extract the sap and checked for sugar content using a refractometer. A self-made sugar solution of 38% served as a reference. As hypanthial nectaries have been reported for other Merianieae (Varassin et al., 2008), stomatal nectar secretion was tested by inserting a glass capillary into the hypanthium of freshly opened buds. Tests for the presence of oils and lipids were conducted by placing granules of Sudan IV onto the fluid of five connective appendages or rubbed against their undamaged surface (Steiner and Whitehead, 2002). If oils or lipids are being secreted, the Sudan IV should turn red while it remains unchanged if the liquid is watery.

Pollinator monitoring

Besides direct visual observations, two cameras (Sony HDR-CX 190) were used to monitor floral visitors of *A. confusa* and *A. sclerophylla* during the day (6:00 – 18:00). The cameras were stationed at inflorescences with at least one freshly opened flower, and frequently several additional inflorescences were visible in the background so that possible visitors at more than one inflorescence would be detected. Flowers were observed on four nights (20:00 – 22:00) for possible nocturnal visitors.

Stamen Removal

In order to detect patterns in the removal of stamens from the flowers by floral visitors, individual flowers were tagged on the first day of opening. The flowers were monitored once a day until all ten stamens had been removed and the maximum number of days for complete stamen removal was noted. A second experiment was conducted to test if there is a higher fruit set in flowers where more stamens have been removed. Open, visited flowers with between one and nine stamens present were selected, the number of stamens present recorded, and the remaining stamens removed with tweezers. As in the other experiments, the resulting fruit set was recorded after two to four weeks. Obviously, this set-up is problematic as it is built upon the assumption that the number of removed stamens is positively correlated with the number of visits; the more stamens removed, more visits to the flower will have happened and thus chances of successful fertilization are higher. To account for this imprecision, data was grouped prior to analyses into “single visit” (1 – 4 stamens removed), “two visits” (5 – 7 stamens removed) and “more visits” (8 – 10 stamens removed).

Mating System

As the inflorescences of *A. confusa* are very dense (> 25 flowers), entire inflorescences were bagged with bridal veil to exclude floral visitors. Five different treatments were conducted to investigate the mating system: (1) autogamy – inflorescences were bagged at pre-anthesis, then left untouched; (2) apomixis – stamens or style of bagged flowers were clipped; (3) hand self-pollination – pollen was manually transferred to the stigma of the same bagged flower; (4) hand cross-pollination – pollen from different individuals of *A. confusa* was transferred to the stigma of a bagged flower; (5) open pollination – single flowers were tagged and left exposed to natural conditions for pollination. As mentioned above, for experiment (2), either stigmatic surfaces were clipped or stamens removed. Goldenberg and Sheperd (1998) argue that the clipping of stigmatic surfaces can be applied for apomixis experiments if the risk of pollen shedding is high. In *Axinaea*, the risk of pollen clouds of other flowers reaching the stigma of an experiment (2) flower was expected to be high, thus both methods were applied. In experiments (3) and (4), flowers were emasculated to prevent autonomous selfing. In order to avoid nearest-neighbour mating, pollen from the respective individual at furthest distance from the current experiment (4) flowers was used. Small jeweller’s paper-tags were used to mark individual flowers. To avoid accidental cross pollination, the tweezers were cleaned with 96% ethanol between the trials. The experimental inflorescences were checked every one to three days, the abortion of flowers and fruits was noted, and after four to six weeks, the fruit set for all treatments was recorded. To account for disturbances caused by these experiments, the fruit set of

untagged flowers was recorded as well. A self-compatibility index (SCI) was calculated and a ratio < 0.2 was considered self-incompatible (Arroyo & Uslar, 1993; Etcheverry et al., 2008). Comparison of fruit-sets from experiment (4) and experiment (5) provide an indication of pollen limitation (deWaal et al., 2012).

To assess the risk of self pollination, autonomous pollen deposition following pollen ejection from the anthers was analysed. In virgin flowers, all ten connectives were squeezed with tweezers to cause pollen expulsion. The resulting fruit set was recorded after two to four weeks.

Statistical Analyses

Chi-square tests were used to assess differences in the fruit-set between the five experiments of the mating system study and between the individual plants. Chi-square tests were also used to unravel differences in the fruit-sets from the stamen removal experiment and the pollen deposition experiment. Descriptive methods (box-plots) were applied to depict the pattern of stamen removal. All statistics were conducted using R. Unfortunately, too little data was available to conduct statistical analyses on the floral visitors and therefore these results are restricted to descriptions only.

Results

Floral Structure

Axinaea costaricensis and *A. confusa* possess dense, multiflorous inflorescences (Fig. 1E, M) while the inflorescences of *A. sclerophylla* and *A. affinis* are small and few-flowered (Fig. 1G). The former two species have delicate flowers less than 2 cm in length, while the flowers of *A. sclerophylla* are 1.5 – 3 cm long and those of *A. affinis* measure 1.5 – 2.5 cm in length and are more robust. For details on sizes of different floral organs of *A. confusa*, see Table 2. All the species observed have actinomorphic, 5-merous flowers, though 6-merous flowers are occasionally found on *A. affinis*. As is typical for the genus, a hypanthium surrounds the free ovary and the flowers are thus perigynous. The calyx is short and almost completely synsepalous (Fig. 2K). While they are hardly protruding in *A. costaricensis*, distinct, hardened teeth are visible on the back of the calyx in *A. confusa*, less so in *A. sclerophylla*. The petals, as is typical of Melastomataceae, are free from each other. Corolla colours vary with the petals in *A. costaricensis* coral red, while all other species have purple, pink to almost white petals (Fig. 1). Both *A. confusa* and *A. sclerophylla* have oblong to elliptic petals with distinctly emarginate apices. In addition,

Table 1. Floral traits considered important in pollinator attraction of the five study species, shape of connective appendage and mode of stamen removal.

	petal colour	corolla shape	stamen arrangement	connective colour	shape of connective appendage	filament colour	anther colour	stamen removal	style colour
<i>A. affinis</i>	glossy, whitish adaxially; bright pink abaxially	opened, petals reflexed	irregular, scattered	dark yellow - orange	spherical, basally apiculate	white	blackish violet	tip or half of filament	pinkish-white
<i>A. confusa</i>	light pink	almost closed	regular, circular	glossy yellow, longer ones darker	spherical, apiculate longer: ellipsoidal, shorter: more globular	pink	dark pink	tip of filament	pinkish-white
<i>A. costaricensis</i>	coral red	almost closed	regular, circular	white - yellow	spherical, basally strongly apiculate	coral red	wine red	base of filament	coral red
<i>A. macrophylla</i>	adaxially white - pink; abaxially darker ¹	almost closed	regular, circular	bright yellow orange, shiny ²	dorsally flattened, opposite pyramidal	white ³	cream, tinged with dark purple ²	n.a.	white ³
<i>A. sclerophylla</i>	light pink/whitish	almost closed, more open when older	regular, circular	bright yellow	dimorphism, longer: spherical; shorter: ellipsoidal	dark violet	pink	tip of filament	white

¹ Information retrieved from Balslev-Cotton (2003).

² Information retrieved from herbarium specimen Cotton 1707 (QCA).

³ Information retrieved from herbarium specimen Lewis & Bruneau 3540 (QCA).

No fresh flowers or pictures of *A. macrophylla* were available to decide the mode of stamen removal.



Figure 1. Buds and virgin flowers of *Axinaea* and flowers from which stamens have been removed. (A), (B) *Axinaea affinis*. (C), (D) *A. confusa*. (E), (F) *A. costaricensis*. (G) *A. sclerophylla*. (H) *A. confusa*, stamens partly removed, petals removed artificially. (I) *A. confusa* with Curculionidae. (J) *A. confusa*, 1st day flower (bottom) and older flower. (K) – (M) Yellow-throated Tanager (*Iridosornis analis*) removing stamens from flowers of *A. confusa*.

the petals in *A. sclerophylla* are fleshy. The other three species have rather oblong to obovate petals and the degree of emargination in the apices is variable. In *A. confusa*, *A. costaricensis* and *A. macrophylla* the petals do not open much and largely cover the anthers also at anthesis (Fig. 1C, E, Table 1). Flowers of *A. sclerophylla* still are campanulate at the beginning of anthesis and only open more when they age (Fig. 1G), Table 1). In *A. affinis*, however, the petals open wider and the stamens are clearly exposed in young flowers as well as in older ones (Fig. 1A, Table 1).

Table 2. Mean sizes of floral organs of *Axinaea confusa*, measured on fresh material.

	diameter corolla	length petal	length style	stamen short	stamen long
mean	1.30	1.02	1.23	0.58	0.83
std	0.05	0.12	0.29	0.06	0.08

Along with four other genera of Merianieae (*Adelobotrys*, *Graffenrieda*, *Meriania* and *Centronia*), *Axinaea* shares an unusual feature regarding the androecium: non-geniculate stamens (Mendoza-Cifuentes & Fernández-Alonso, 2010, see Fig. 5, p. 155). In *Axinaea*, the sturdy bulbous connective appendage prevents the stamens from unfolding during anthesis. Thus, stamens with acute angles between the filament and the anther are produced. In contrast to geniculate stamens where stamens unfold so that the anther tip points out of the flowers, anther tips usually point to the floral centre in non-geniculate stamens. Please note that the term “dorsal” will be used in the sense of geniculate anthers here, this is to say, anthers which unfold normally. Therefore, the side of the anther facing the style, ventral on first glimpse, will be referred to as dorsal. For more detailed information, see Mendoza-Cifuentes & Fernández-Alonso (2010) and Penneys & Judd (2011).

Stamens are attached on the torus and are basifixed. All species possess anisometric stamens, this is to say that there are size differences between the two stamen whorls with the stamens of the inner whorl being bigger (antepetalous) than those of the outer whorl (antesepalous). There also are differences in the shape of the connective appendages between the two whorls, but also within each whorl (see also Balslev-Cotton, 2003). The androecial arrangement in the flowers varies between the species from stamens aggregating regularly to form a semi-circle in *A. confusa*, *A. costaricensis*, *A. macrophylla* and *A. sclerophylla* to stamens arranged more or less irregularly around the style in *A. affinis* (Fig. 1A, C, E, G; Table 1). The style curves out to the opposite direction of the stamens and is exerted. The point of strongest curvature is 2 - 3 mm proximal to the stigma. The different forms of aggregation of the stamens and the curvature of the styles make the flowers zygomorphic. In all species, the brightly coloured bulbous connective appendages strongly contrast with other floral organs, while filaments and anthers are similar to the colour of the

corolla. Appendage colour can change with the age of the flower, they usually get darker or brownish (Fig. 1I). In *A. confusa* the two series of stamens have slightly different colouration in older flowers with the antepetalous stamens turning orange. While the appendage in the antepetalous stamens usually is more elongate (Fig. 4K), it is more globular in the shorter, antesepalous stamens (Fig. 4J) and both types have a pointed tip. Spherical, basally slightly pointed connective appendages are also found in *A. affinis* while they are dorsally slightly flattened and narrow down gradually towards the anther in *A. macrophylla* (Fig. 2C). The apex of the appendage is more pointed in *A. costaricensis* and *A. sclerophylla* shows a clear appendage dimorphism with the longer stamens being spherical and the shorter ones ellipsoidal (Table 1). Viewed on a more detailed scale, also the shapes of the appendages vary between individual stamens and will be presented in more detail below. All species have dorsiventrally flattened filaments which entirely remain in the flowers of *A. confusa* and *A. sclerophylla* after the anther-appendage complex has been removed (Fig. 1D, G, Table 1). In *A. affinis*, the filaments come off at the top or in the middle so that most of the filament remains left in the flower. In *A. costaricensis* on the other hand they come off at the base (Fig. 1B, F, Table 1). All anthers have a single apical pore, which opens at anthesis. In all five species, the syncarpous ovary is pentamerous with five locules, and axile placentation. The curved style is fleshy and has a punctiform stigma, and is coloured similarly to the corolla. All species of *Axinaea* produce loculicidal capsules (Balslev-Cotton, 2003).

Floral Morphology and Anatomy (SEM, LM, MicroCT)

The stamens of the five *Axinaea* species analysed are morphologically very similar. From the bulbous appendage, connective tissue extends ca. 2/3 of the length between the thecae. In younger anthers, two pollen sacs per theca can be discerned (Fig. 4A). The septa collapse some time before anthesis, so that only one pollen-chamber per theca is present when the pore opens (Fig. 4B, C). There is a single pore for both thecae, which internally unite toward the apex of the anther (Fig. 4D).

When screened for nectar producing structures, none could be found on the connectives of any species. As shown in Fig. 2A, B, the connective appendages can be folded in or have small flaps, their surface, however, is generally smooth. Connective appendages of *A. costaricensis* possess two small auriculate structures around the filament insertion (Fig. 2D). In *A. confusa*, these structures are larger and more rounded, resulting in an even more globular appendage. The serial sections showed that the bulbous connective appendage is composed of tissue with extremely loosely arranged cells in all species with a high proportion of intercellular spaces. These parenchymatic cells appear irregular in shape, mis-

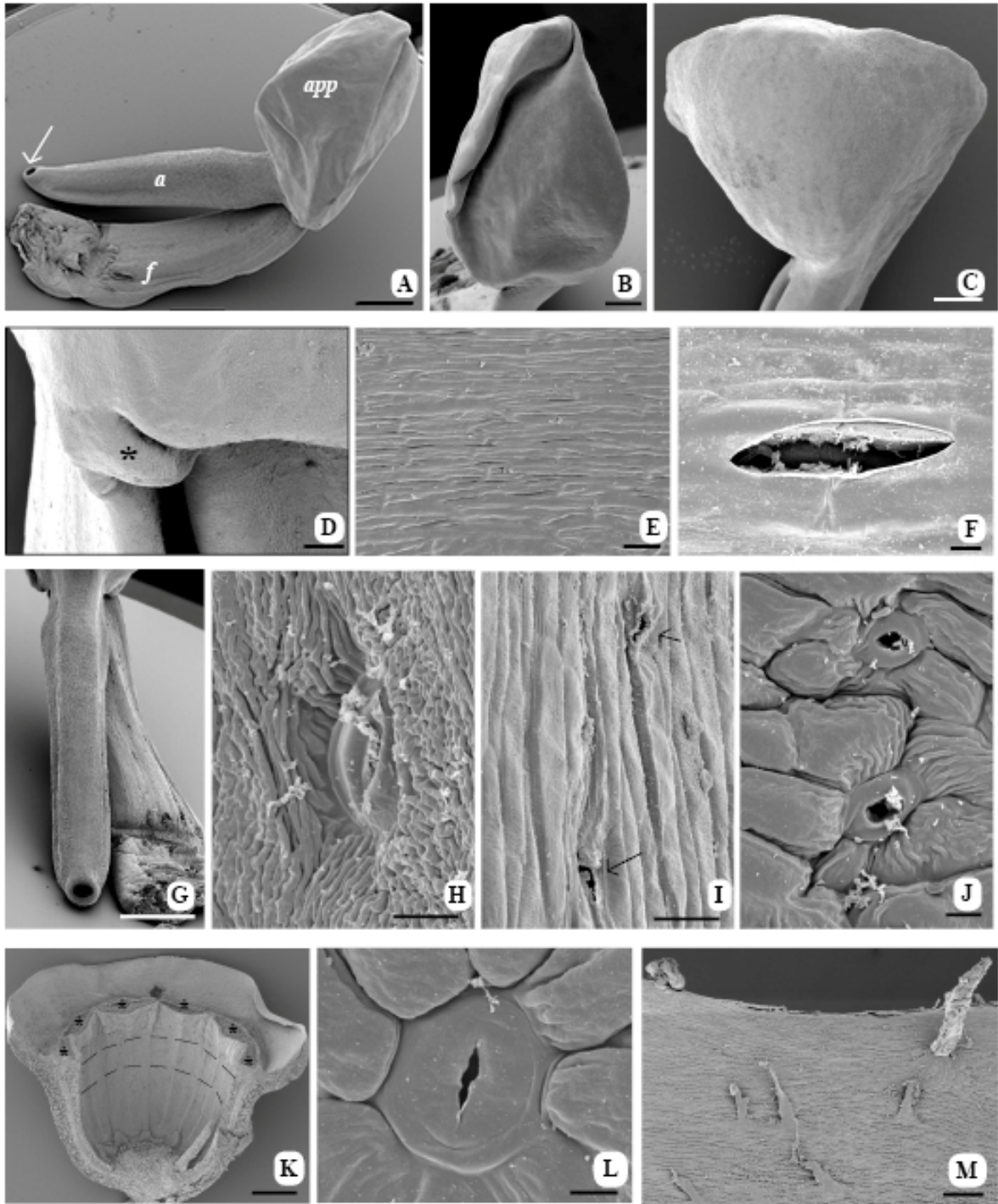


Figure 2. Scanning Electron Microscopy. (A) *Axinaea costaricensis*, entire stamen, arrow indicates apical pore, *app* = appendage, *a* = anther, *f* = filament, 1 mm. (B) *A. costaricensis*, appendage with plication, 500 μ m. (C) *A. macrophylla*, appendage, 500 μ m. (D) *A. costaricensis*, * indicates auriculate structures, 200 μ m. (E) *A. confusa*, depressions between cells on anther, 20 μ m. (F) *A. affinis*, stomata on filament, 20 μ m. (G) *A. costaricensis*, filament and anther with pore from the front, 1 mm. (H) *A. macrophylla*, stoma at base of anther, 10 μ m. (I) *A. sclerophylla*, arrows indicate holes on filament, 20 μ m. (J) *A. confusa*, stomata on the inner wall of the hypanthium, 10 μ m. (K) *A. costaricensis*, hypanthium, black lines indicate zone of stomata, * indicate filament attachment on the torus, 1 mm. (L) *A. costaricensis*, stoma on the hypanthium, 5 μ m. (M) *A. sclerophylla*, trichomes on inner surface of calyx, 100 μ m.

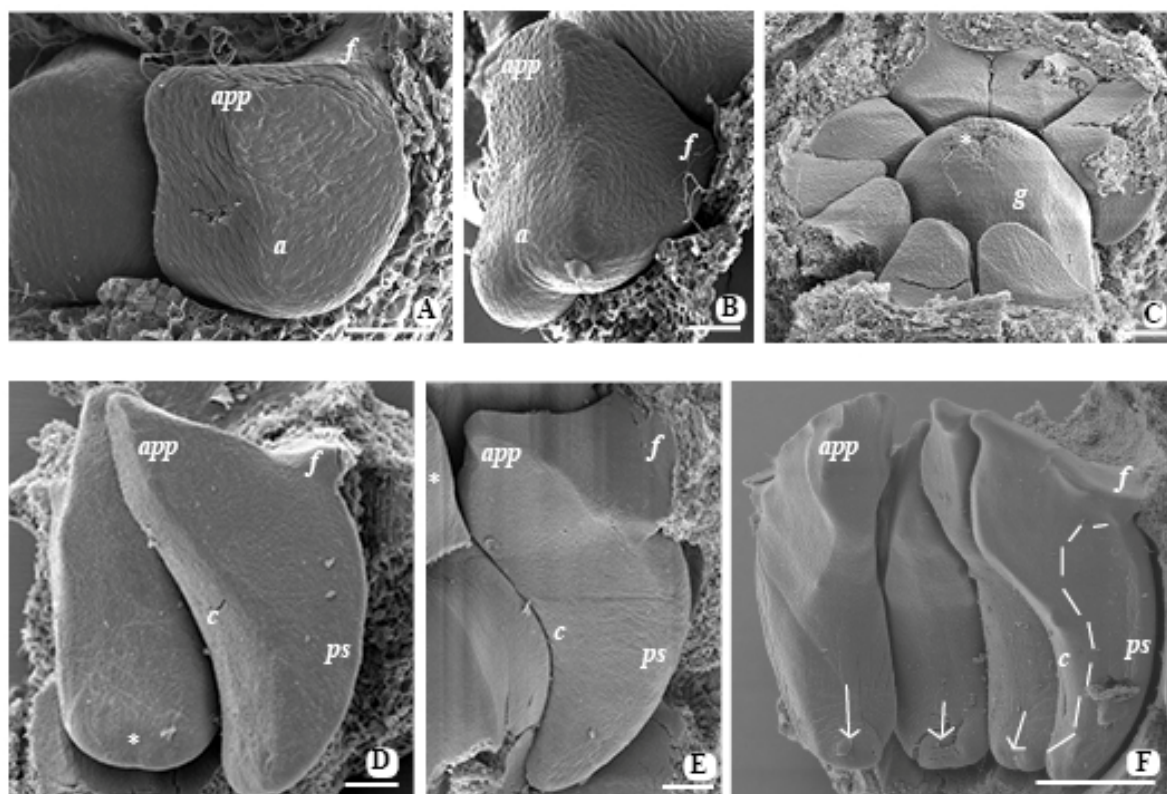


Figure 3. Androecial development of *A. costaricensis*. (A) Cubical young stamen, bud size 1 mm, scale bar 50 μm . (B) Two thecae visible, bud size 1.2 mm, scale bar 40 μm . (C) Undifferentiated gynoecium in centre, stamens tightly arranged around ovary, bud size 1.4 mm, scale bar 100 μm . (D) Appendage elongated, filament short, thecae well developed, * indicates future pore, bud size 1.8 mm, scale bar 100 μm . (E) * indicates initiation of stylar outgrowth, bud size 2 mm, scale bar 100 μm . (F) Appendage well developed, dashed lines approximate zone of pollen sacs, arrows indicate future pores, bud size 3 mm, scale bar 500 μm . *a* = anther, *app* = appendage, *c* = connective, *f* = filament, *g* = gynoecium, *ps* = pollen sac.

Table 3. The average number of stomata, their location in the hypanthium, their average size and the ratio of the radial thickness of the vascular bundle to the radial thickness of the filament.

	average number of stomata ¹	location of stomata ²	stomata (μm)	ratio of vascular bundle : filament size
<i>A. affinis</i>	6	upper 1/3, 1 in 3/3	10	0.44
<i>A. confusa</i>	32	all zones	12	0.35
<i>A. costaricensis</i>	18	from 1/3 to upper 2/3	9	0.27
<i>A. macrophylla</i>	6	1/3, sporadically 3/3	13	0.36
<i>A. sclerophylla</i>	1	1/3	10	0.31

¹ the hypanthium was divided into 10 zones in extension of the stamens, numbers refer to these zones.

² the hypanthium is divided into thirds, starting from the upper rim (1/3) to the attachment zone of the ovary (lower 3/3).

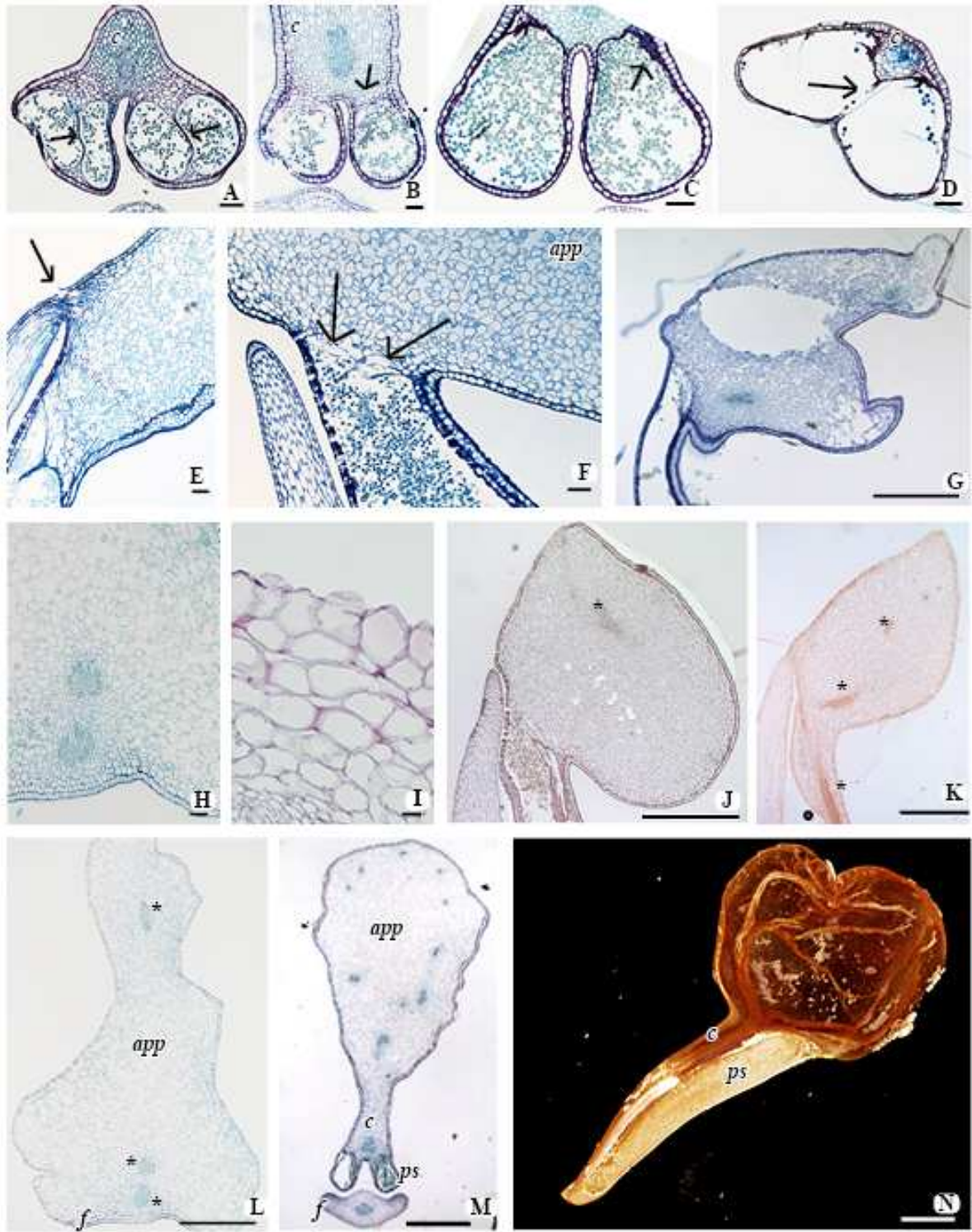


Figure 4. Light Microscopy and MicroCT. (A) – (D) cross sections of anthers, RT-stain. (A) *Axinaea confusa*, young stamen, arrows indicate septa between pollen sacs. (B) *A. costaricensis*, two thecae, septa collapsed, arrow indicates lack of thickened cell layer separating appendage from thecae. (C) *A. costaricensis*, section at the middle of the anther, arrow indicates remnants of septa. (D) *A. costaricensis*, anther tip, thecae are unite in this region. (E) – (G) longitudinal sections, RT-stain. (E) *A. confusa*, arrow indicates abscission zone at filament tip. (F) *A. confusa*, appendage parenchyma, arrows represent direction of air flow when bellows mechanism is activated. (G) *A. confusa*, compressed stamen, parenchyma squashed with empty spaces in between.

(H) *A. costaricensis*, parenchymatic cells and two vascular strands. (I) *A. costaricensis*, PAS-stain, inner hypanthium wall in zone of stomata. (J), (K) longitudinal sections of appendage (J) *A. confusa*, PAS-stain, antesealous stamen, * indicates part of stained vascular bundle. (K) *A. confusa*, Ponceau-stain, of antepetalous stamen, * indicates part of stained vascular bundle. (H), (L), (M) cross sections of appendage, RT-stain (L) *A. costaricensis*, * indicate three vascular bundles in appendage. (M) *A. affinis*, note high number of vascular bundles, filament and connective bundle at the bottom. (N) *A. confusa*, M-CT, ramified vascular bundle in bulbous appendage, vascular strand going down in connective strand (*c*). *app* = appendage, *c* = connective strand, *ps* = pollen sacs, *f* = filament. (A) – (F) 100 μ m, (I) 10 μ m, (G), (H), (J) – (M) 1000 μ m, (N) 1 mm.

matching with the adjacent cells and have very thin cell walls (Fig. 4E, F). They seem to be quite empty and their cytoplasm does not show high activity (Fig. 4H). The bulbous appendage has a one layered epidermis with relatively thick cell walls (darker stained), only on the lower dorsal side from where the connective strand leads to the anther up to five cell layers are sometimes present. It is particularly noteworthy that in mature anthers there are no cell layers separating the internal tissue of the appendage from the two pollen-chambers of the anther thecae (Fig. 4F) as cells in this area have apparently collapsed before anthesis. In other parts of the anther, a continuous epidermis and underlying cell layers surround each pollen chamber, thus separating the thecae from the connective strand. After compression of the connective appendage by tweezers, the parenchymatic tissue appears partly ruptured (Fig. 4G). At the junction of the connective appendage with the anther, a small portion of the cells lining the pollen sac walls have been damaged, but no connective tissue has been pressed into the thecae. There is a notable difference in the cells making up the wall of the thecae and those of the connective tissue. While the thecae-cells are rounded, those of the connective are elongate and similar to the cells of the filament. In the transition zone of these cells on the basal part of the anther, one to two stomatal openings have been detected on stamens in *Axinaea macrophylla* (Fig. 2H). *A. confusa* shows fine slits on the anther which might merely be depressions between the cells (Fig. 2E). In *A. affinis* and *A. sclerophylla*, on the other hand, small longitudinal ruptures or pores were found on the filaments (Fig. 2F, I). At its top, where the bulbous appendage is situated, the filament seems to be thinner in *A. confusa*. Small ruptures can be seen at the sides of the filament-tip (Fig. 4E), the zone where complete rupture occurs when one pulls at the bulbous appendage to take out the anther-appendage complex from the flower (comp. Fig. 1B, D, F, G). Possibly, this area forms an abscission zone.

In the PAS reaction for carbohydrates, the epidermis and the vascular bundles in the filament and the connective were weakly stained (Fig. 4J). This might indicate low concentrations of carbohydrates, but no distinct nectar secretion. Assuming that the compression of the appendage might force out carbohydrate-rich phloem sap contained in the vascular bundles into the parenchyma of the connective appendage, compressed stamens were analyzed. No difference in colouration of the appendage content was observed.

The Ponceau stain for proteins resulted in slightly more strongly stained epidermis cells and vascular bundles (Fig. 4K).

The developmental analyses of the flower of *A. costaricensis* give support to the hypothesis that the bulbous appendage is derived from connective tissue. Young developmental stages show that stamens are strongly incurved in bud with the morphological apex directed towards the base of the hypanthium (Fig. 3A). The pollen sacs are directed towards the hypanthium (Fig. 3D – F) and the appendage develops from the connective at the morphological base of the anther. However, as the stamens are incurved, the appendages are directed upwards in the flower. As shown in Fig. 3A, B, very young buds carry rather cubical stamens where a slight protrusion marks the future bulbous connective appendage. The filaments remain short during most of floral development and the minute anthers are attached more or less directly to the torus. In slightly older buds (Fig. 3B), the connective appendage has already enlarged and the two separate thecae are clearly visible in the anther. In the next stage (Fig. 3C), the anthers are significantly elongated and the appendages larger and more pointed. However, the developing connective is still not clearly separated from the thecae. A difference in the size of the appendages of antepetalous and antesealous stamens is perceivable at this stage. In the bigger stages this difference is displayed more distinctly (Fig. 3D). On the ventral side of the anthers, the two thecae are now visible along the whole length and a slight depression on the dorsal side of the anther tip indicates the future pore. The appendage is clearly more elongate but not particularly bulbous yet. At this stage, the filament is well developed but still very short; it elongates very late in floral development. The gynoecium is distinctly lobed but the style has not emerged yet. At 2 mm bud size, the style begins to elongate, the stamens are very similar to the previous stage (Fig. 3E). At a bud size of 3 mm, the anisometric condition between antepetalous and antesealous stamens is evident (Fig. 3F). The antepetalous stamens are longer, broader, and slightly more variable in the shape of their connective appendage apex, which tends to be more pointed and irregular. The antesealous stamens are shorter and more constant in connective appendage apex shape. Also, at this stage, the appendage starts expanding into a bulbous appendage. It acquires its final bulbous shape shortly before anthesis. Serial sections show that the parenchyma of the appendage is much more densely packed in younger stamens. The large intercellulars are formed during the rapid expansion of the appendage. At this time, the anther pore opens and the elongation of the filament is only completed at anthesis.

As seen both with the MicroCT and light microscopy, in *Axinaea costaricensis* and *A. affinis*, the vasculature of the stamen is composed of one primary bundle extending through the filament up to the base of the connective appendage. At the dorsal side of the bulbous appendage, it splits into two. One vascular strand reaches up to the tip of the

appendage, and a second one follows the connective tissue distally between the thecae (Fig. 4L, see also Wilson, 1950; Mendoza-Cifuentes & Fernández-Alonso, 2010). In *Axinaea confusa* and *A. macrophylla* (and presumably *A. sclerophylla* (see Wilson, 1950)), the vascular bundle of the connective appendage is ramified into three (later five and seven) and eight single strands, respectively (Fig. 4M, N). The ratio of the radial thickness of the vascular bundle to the radial thickness of the filament is given in Table 3.

Stomata were located on the inner wall of the hypanthium in all species (Fig. 2J - L). In the PAS reaction, the stomatal openings in the hypanthium did not stain noticeably stronger than the surrounding tissue (Fig. 4I). The number of stomata varied greatly among the species. Table 3 gives detailed information about their size, number and location. While the sizes of the stomata are smaller than in other Merianieae (Varassin et al., 2008), the ratios of vascular bundle to filament radia are similar. With 0.27, *Axinaea costaricensis* has the smallest ratio while *A. affinis* shows a thick vascular bundle (Table 3). Stomata were scarce in *A. sclerophylla* and small trichomes have been found on the calyx lobes (Fig. 2M).

Field work

Flowering Phenology

In *Axinaea confusa*, styles are exerted from mature buds while the corolla is otherwise still closed. This has led observers to think of *A. confusa* as being protogynous (pers. com. Darin S. Penneys). The flowers open before sunrise on the first day of anthesis, though they remain globular and half closed (Fig. 1J). Pollen is already ejectable from the anthers and about 70% of flowers are visited on the first day (see below). On days two through four, the petals open a little more to their maximum (Fig. 1C, I) so that the bulbous connectives are more clearly visible. On days five through seven, the petals and filaments start dropping if the flowers had been visited previously. On day eight, the style drops. It has also been observed that flowers which were not visited shed all floral organs more or less simultaneously on the ninth day. Floral scent was not detected at any stage of flowering.

Floral rewards

The test on sugary content of the bulbous connectives of *A. confusa* was negative. Only one sample from one plant yielded concentrations of 12%. This sample consisted of older stamens which had been collected on the previous day. No other sample of older stamens tested contained any sugar. Also, no nectar was found in the hypanthium. According to the Sudan IV-test, the appendage sap neither contains lipids nor oils.

Pollination Mechanism

Different species of Thraupidae remove stamens in *Axinaea confusa* and *A. sclerophylla* and thereby effect pollination. With their bill, the birds grip and remove the bulbous connective appendage together with the anther while the filament usually rips at the top. As the appendage is compressed by the bill, a jet of pollen shoots out of the apical pore of the anther. The birds consume the entire bulbous appendage and the now emptied anther, only the filaments remain in the flower (Fig. 1D, H). The birds approach the flowers from above, below, or to one side and usually remove the stamens one by one, rarely do they remove two at a time (Fig. 1K - M). The ejected pollen lands on their bill, front head or neck. Expelled pollen likely also lands on the stigma or on stigmas of neighbouring flowers and buds with exerted styles. Also, the semi-closed shape of the corollas might funnel pollen jets coming both from the stamens arranged right beneath the style and from the lateral ones. This mechanism can be imitated using tweezers to compress the appendages. It does not work properly, however, if the epidermis of the bulbous appendage is damaged beforehand. Small portions of pollen can sometimes be released when the base of the anther is squeezed, especially in older stamens. Also, the option of buzz-pollination can be ruled out as no pollen was shed when the flowers were approached with tuning forks, a method also used by Renner (1989). It was once observed, after a stormy night with heavy rain, that small quantities of pollen had been shed in bagged virgin flowers; heavy raindrops are presumed to have caused pollen shedding (comp. Almeda, 1977).

Jürg Schönenberger (pers. com) reported observations by locals of frequent bird visits on *Axinaea costaricensis*. Although no observations have been made on the other *Axinaea* species discussed in this work, the presence of flowers from which the stamens had been removed (compare Fig. 1B, D, F, G) indicates that this mechanism is functioning throughout the genus. The findings of Rojas-Nossa (2007) of flowerpiercers carrying pollen from *Axinaea macrophylla*, support the tanagers' status of legitimate pollinators. Also, the bulbous connective appendages are the most vividly coloured and thus the most visible part in the flowers of all species of *Axinaea* (Balslev-Cotton, 2003), many displaying a strong colour contrast, which is the most important attractory cue in bird pollination (comp. e.g. Stiles, 1981).

Pollinator Monitoring and Stamen Removal

Four different species of tanagers were observed feeding on the flowers of *Axinaea confusa*. The Yellow-throated Tanager, *Iridosornis analis* Tschudi, was the most frequent visitor. The Lacrimose Mountain-Tanager, *Anisognathus lacrymosus* Du Bus & Gisignies, has only been recorded once. Two other species of tanagers were observed feeding on *A. confusa*, the Blue-winged Mountain Tanager, *Anisognathus somptuosus* Lesson, and the much smaller

Orange-throated Tanager, *Euphonia xanthogaster* Sundevall, but these were never filmed. Other bird species are expected to contribute to the pollination as well and a more detailed study focusing on the pollinator community should be conducted in the future.

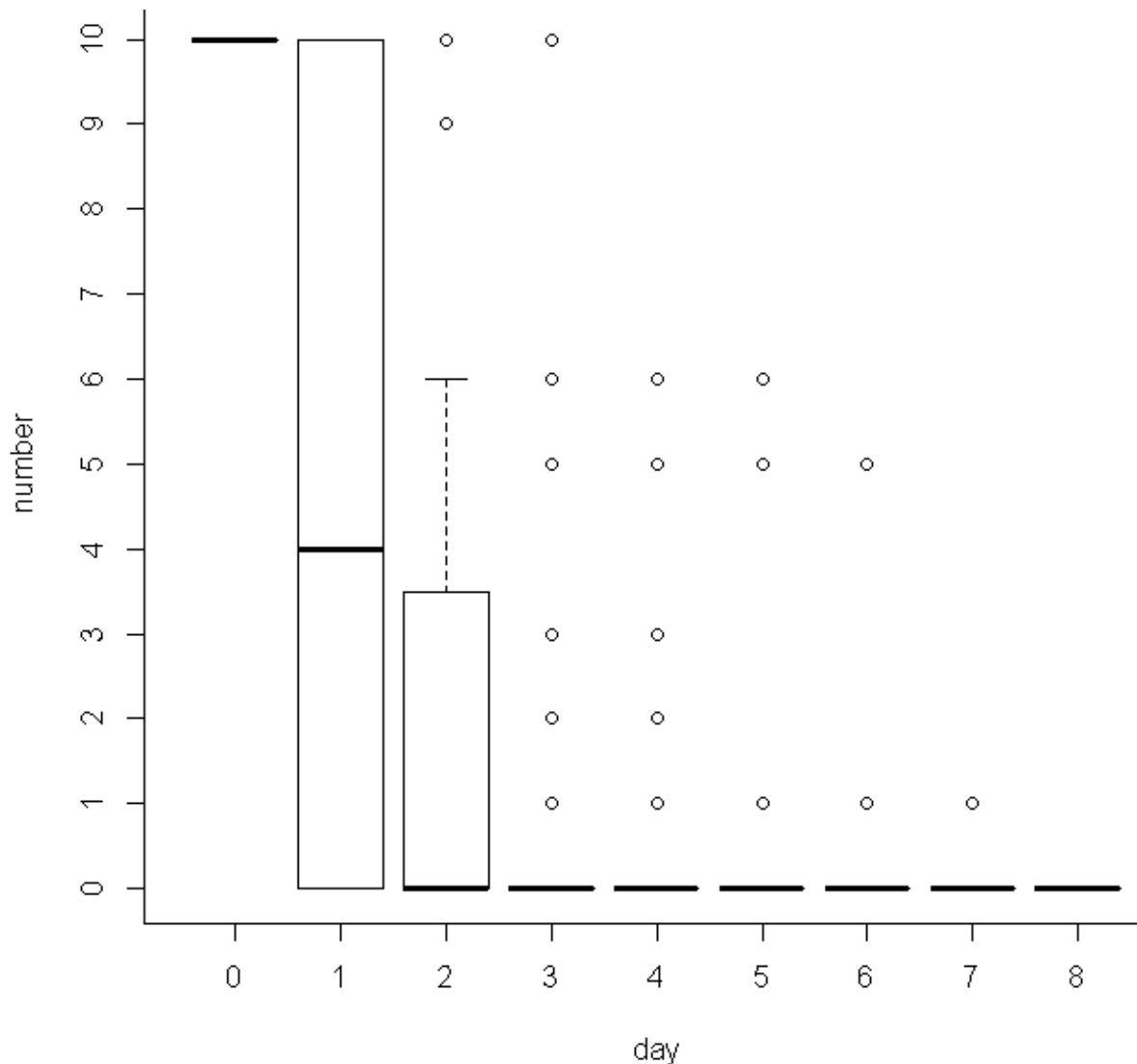


Figure 5. Pattern of stamen removal over the flowering period. Day 0 is the morning of the first day when all flowers have ten stamens, day 1 is morning of the second day (= status after day 1). Note the large variation after day 1 and day 2 (bars = median, boxes = 25%-75%, whiskers = zone without outliers, ° = extreme values).

Video monitoring showed that in contrast to many other flower-visiting birds (e.g. Arizmedi et al., 1996) there is no time of peak activity, and birds approach *Axinaea* flowers at any time of day. Mixed-species flocks of eight to ten tanagers have been observed foraging in the foliage together. The maximum number of tanagers observed feeding on a plant of *A. confusa* simultaneously was four. Nevertheless, only visits by single birds have been recorded by camera. During one visit, a bird approaches different inflorescences on one plant, sometimes returning to inflorescences and flowers previously visited. The bird

removes the stamens one by one but it usually does not take out all ten stamens during a single visit. Generally, a bird leaves one to eight stamens inside which will be consumed on subsequent visits. In almost all flowers, all stamens were removed before the end of anthesis. Figure 5 depicts this pattern of stamen removal and shows that the majority of flowers is emptied after the second day. The experiment on stamen removal showed that almost as many flowers are emptied completely (approx. 35%) as remain unvisited (approx. 32%) on the first day of anthesis (Fig. 1H). In the other 33% of flowers, one to nine stamens are removed and a second or third visit will occur in the following days until all stamens have been consumed. On the second day, more than 60% of the flowers are emptied completely. Eight days was the maximum observed until complete stamen removal in *A. confusa*. It was experimentally tested if more visits lead to higher fruit set. This does not seem to be the case ($n = 100$, $\chi^2 = 2.47$, $df = 2$, $p = 0.29$).

The overall visitation rate of flowers was low. In 176 hours of video observation at Site I, only six bird visits were recorded. At Site II, no visits were filmed (38 h). Despite this low visitation rate, the fruit set on the plants was high. The loss of big buds and freshly opened flowers overnight has been noticed on two individuals of *Axinaea confusa*. As both plants had branches close to the forest floor, this could be seen as an indication of nocturnal floral visitors that remove the entire flowers and thus are not contributing to pollination.

Apart from the tanagers, small black Curculionidae (Fig. 1I), one Elateridae, and a few Heteroptera were observed on the flowers of *Axinaea confusa*. As these insects are incapable of activating the pollen-expulsion mechanism, they are not regarded as pollinators.

On *Axinaea sclerophylla*, only one Thraupidae has been observed, the Masked Flowerpiercer, *Diglossopsis cyanea* Lafresnaye. It removed the stamens in the same manner as described above for the tanagers on *A. confusa*.

Mating System

The results of the mating system experiment are shown in Table 4. There is a highly significant difference in the fruit set between the five trials ($\chi^2 = 143.27$; $df = 4$; $p = 2.2 \cdot 10^{-16}$). As no fruits developed in the apomixis-trials (exp. (2)), the possibility of asexual reproduction can be ruled out in this system. Also, the fruit set in the exclusion trials (exp. (1)) was very low. The reproductive success between open pollination (controls, exp. (5)) and hand-cross pollination (exp. (4)) is almost the same. Thus, the system is not pollen limited (e.g. Knight et al., 2005; DeWaal et al., 2012). Self-pollination by hand (exp. (3)), however, yielded the highest fruit set. Still, there was no significant difference between the fruit sets of the hand-cross pollination and the hand-self pollination ($\chi^2 = 1.2$; $df = 1$;

$p = 0.27$). A Self-compatibility Index (SCI) of 1.14 indicates that *Axinaea confusa* is self-compatible. There was no significant difference in fruit-sets between the plants for each experiment. Only the naturally openly pollinated experiment (exp. (5)) did give a significant result, which is due to high flower-loss on one individual where nocturnal flower robbers have been notice ($\chi^2 = 14.7$; $df = 5$; $p = 0.01$).

Table 4. Fruit sets in the different pollination experiments are given, n is the number of experimental flowers used.

treatment	fruits	n
(1) Autogamy	15	90
(2) Apomixis	0	88
(3) Hand-Self	67	95
(4) Hand-Cross	55	89
(5) Natural	57	99

In the experiment of pollen deposition on the style by merely pressing the bulbous appendage, 32 (n = 90) fruits developed. This is significantly less than when own pollen is transferred by hand (67, n = 96) or flowers are naturally pollinated (57, n = 99; $\chi^2 = 23.26$; $df = 2$; $p = 8.9 \cdot e^{-06}$).

Discussion

The pollination system of *Axinaea* by Thraupidae is an extraordinary case of a pollinator shift within the mostly buzz-pollinated Melastomataceae. No similar mechanism is known from any other group of angiosperms. The essential mechanics of this system are here summarized via a functional approach. When removing the stamens, tanagers did not pull at the anthers but rather they gripped and compressed the bulbous connective appendages, thus causing pollen shedding. This action could be replicated artificially using tweezers. Sometimes, a small amount of pollen is shed when the base of an aged anther is squeezed. Strong wind or rain that caused violent shaking of the flowers is another action capable of causing some pollen shedding, pollen generally lands in the floral centre in these cases. The serial sections of *Axinaea*-connectives prove that the appendages are composed of extremely loose, almost sponge-like tissue and that there is no specific cell-layer separating the pollen chambers from the internal tissue of the appendage. This lack of an endothecium in mature anthers is a morphological synapomorphy of the Melastomataceae above *Pternandra* (Clausing & Renner, 2001) and can also be reported for *Axinaea*. This trend has generally been observed in flowers with poricidal anthers and has often been correlated with buzz-pollination (Endress, 1996).

The bellows mechanism

How then can the expulsion of pollen clouds be effected? A “bellows hypothesis” is proposed here to describe the pollination mechanism observed. This bellows-hypothesis postulates that the bulbous anther connective appendage functions like a bellows such that when that structure is compressed, the air contained in the spongy intracellular spaces is forced into the pollen chambers of the anther thecae, and the jet of air flushes the pollen out of the common pore located at the anther apex. Doubt arises, however, when one recalls that the epidermis can be ripped by sharp objects like a bird’s bill or tweezers. The amount of air pressed through the thecae would consequently be reduced as the rupture forms a secondary outlet. This might be the explanation, however, for the fact that the amount of pollen expelled can vary greatly among the stamens. Compression with little damage to the epidermis causes larger pollen clouds than when a bulbous appendage is gripped from the side, where it usually is squashed completely. Damage to the epidermis prior to activating the mechanism will hinder it from functioning optimally. The serial sections of squeezed stamens give support to the bellows-hypothesis. In the sections, most of the pollen grains have been expelled, the parenchymatic tissue has been squashed and cells are packed somewhat more densely creating larger intercellular spaces, presumably filled with air. In the video-observations of the tanagers, not every stamen removal causes a visible pollen cloud. It is also possible that pollen shedding often happens inside the pseudo-tubular flower, spraying pollen onto the bird’s beak only and is thus not perceivable with the camera.

Reports on “bellows-like” pollination mechanisms are longstanding (e.g. Delpino, 1873; Lagerheim, 1899). Ziegler (1925) describes a bellows-mechanism for the melastome genera *Brachyotum*, *Centradenia* and *Rhexia*, although he emphasizes his uncertainty about how the mechanism functions. Ziegler (1925) assumed that pressure applied to the thecae releases pollen clouds from the pore, which thereby functions as a pulverizer (also compare Lagerheim, 1899). The bat pollination of *Meriania phlomoides* is reminiscent of the syndrome observed in *Axinaea*. In the latter species, copious nectar is produced and accumulates at the base of the filaments. By reaching for this nectar with their tongues, the pollinating bats push against the anthers and thus cause the expulsion of pollen clouds (Vogel, 1997). In the rodent pollination syndrome of nectar producing *Blakea*, a similar mechanism has been observed. The nocturnal rodents effect the shedding of pollen clouds by applying pressure to the outside of the petals or to the base of the stamens (Lumer, 1980). In these systems, the application of pressure onto the surface of some part of the stamen, mostly the base of the anthers, is the source of pollen release; in none of those examples is there any specific structure that is being compressed, nor is there any bellows-organ developed. In the cases described above, the bellows-mechanism can be repeated several

times. In *Axinaea* on the other hand, the mechanism is not repeatable as the bellows are being destroyed when compressed. There also are differences in the precision of the mechanism. Pollen expulsion in *Meriania phlomoides*, for example, is a by-product of nectar-foraging and is not directed at a single stamen, rather the entire androecium is contacted by the bat's tongue. In *Axinaea*, the foraging is focused upon a single stamen at a time, rendering this mechanism much more specialized and effective. Redundancy of the bellows mechanism, which is guaranteed by repeatability in the other cases described, is reached by the independent provision of pollen by each single stamen in *Axinaea*. In their work on a bellows-mechanism in *Cyphomandra* (Solanaceae), Sazima et al. (1993) briefly outline different functional possibilities of tubular anthers. Amongst other things, they point out that “no air stream operates” in buzz-pollination while “a principle of air flow (...) underlies the bellows-like mechanism” (Sazima et al., 1993, p. 80). The descriptions of pollination named “bellows-like” in Melastomataceae generally are too unclear to securely judge if they really are based on air streams (Ziegler, 1925). It might also be that pollen is merely shaken out of rigid anthers due to vibrations when floral visitors accidentally touch the stamens, very similar to the principle of buzz-pollination (as found in some Ericaceae, compare Sazima et al., 1993). A bellows-like pollination mechanism described (Legett, 1881) for *Rhexia virginica* L. is reminiscent to that in *Axinaea*. Legett (1881) stated that pollen puffs are expelled from the anther pores when a bee tramples the inflated sacs at the base of the anther, and that piercing these sacs with sharp pins will spoil the bellows, as in *Axinaea*.

Nectar production and morphology

There are numerous similarities in the comparative anatomy of *Axinaea* with nectar-producing Neotropical Melastomataceae (Varassin et al., 2008). Besides a pseudo-tubular corolla, *Axinaea* possesses stomatal openings of an anomocytic type where the two kidney shaped guard cells are surrounded by undifferentiated epidermal cells (Gaffal et al., 1998; Renner, 1993). The size of these stomata are smaller than in the two *Meriania* species analysed, but are similar to sizes found in other sub-families (compare Table 1 in Varassin et al., 2008). Stomata located on the inner wall of the hypanthium could be a synapomorphy for *Axinaea* and *Meriania*. It should be noted that the Myrtales, to which the Melastomataceae belong, generally bear hypanthial and gynoeical nectaries (Bernadello, 2007) and that anomocytic leaf-stomata are basic in the order (Dahlgren & Throne, 1984). However, nectar does not seem to be secreted in *Axinaea*. The stomata in the inner hypanthium did not stain more strongly than the surrounding tissue in the PAS-reaction, and the underlying cells are undifferentiated from the surrounding tissue. The staining of the

epidermis and the vascular bundles in the anthers can be due to certain concentrations of carbohydrates, but only in *A. macrophylla* are there actual stomata at the base of the anther. Given the pollination mechanism of *Axinaea* described above, nectar secretion on the stamens would be in vain as birds remove the entire bulbous connective appendage and anther anyways and nectar displayed at the outer surface of the stamen would only attract nectar robbers. Thus, the hypothesis of (staminal) non-structural nectaries advocated by Stein & Tobe (1989) can also not be applied to *Axinaea*. It is likely that nectar is not produced by the connective itself, and if only present in very low concentrations, it is not secreted. The radial ratio of vascular bundle to filament size is comparable to ratios found in nectar-secreting melastomes (Varassin et al., 2008, Table 1). Due to the fact that vascular bundles stretch to the tip of the connective or are highly ramified in some species such as *Axinaea affinis* and *A. confusa* (Wilson, 1950), sucrose-rich phloem sap could be present in the bulbous connective appendage. The actual composition of the appendage content will have to be analysed chemically. Neither sugars nor oils or lipids were detected using a refractometer and the Sudan IV test, respectively. The rapid enlargement and the loosely arranged cells of the bulbous appendage at the end of floral development could be an indication of low nutrient concentrations.

Reproductive systems in nectar producing Melastomataceae

Although recorded bird visits on *Axinaea* were few, the analyses of the mating system experiment show that the system is not pollen limited (deWaal, 2012), and that the flowers are self-compatible. Despite the herkogamous construction of the flowers, the pollen deposition experiment proved that pollen clouds, once expelled, can reach the stigma of the same flower autonomously. This is the case both when the mechanism is triggered (by a bird or forceps) and probably by wind or heavy raindrops (comp. experiment (1); Almeda, 1977). Self-compatibility is widespread in the Melastomataceae (e.g. Santos et al., 2012, Andrade et al., 2007, Goldenberg & Varassin, 2001, Renner, 1989), especially in the tribes Melastomeae and Microliceae (Goldenberg & Sheperd, 1998). 80% of the 20 species growing in the Campo Rupestre in Brazil, a montane savanna with rocky outcrops and a high amount of endemics, are self-compatible. In a compiled study of 124 Melastomataceae species for which information on the breeding-system was available, almost 43% were self-compatible (Santos et al., 2012). Within the Merianieae, two self-compatible species have been reported, *Adelobotrys rachidotricha* Brade and *Graffenrieda latifolia* (Naudin) Triana (Renner, 1989; Sobrevila & Arroyo, 1982); no other studies of mating systems in the Merianieae have been published. Despite this lack of investigation in the breeding systems of Merianieae, it is not surprising that *Axinaea confusa*, and perhaps the entire genus, is self-

compatible. While numerous cases of apomixis have been reported for the Melastomataceae (e.g. Santos et al., 2012, Goldenberg & Varassin, 2001), *A. confusa* did not set fruits after apomixis treatments. The methodologies employed in the current investigation do not enable differentiation between truly self-compatible sexual species and pseudogamic apomictic ones where asexual embryos only form after fertilization and the formation of at least one sexual embryo (Mendes-Rodrigues et al., 2005) or fertilization of the polar nuclei (Mogie, 1992). Confirmed bird visits were few, but during those visits high amounts of flowers were visited repeatedly until stamen removal was completed, thus augmenting the chance that pollen is shed onto the stigma or the bird. Pollen-loads of a single stamen are sufficient to fertilize more than one sexual embryo per flower, thus pseudogamic apomixis is unlikely in *Axinaea*. A molecular assessment of the proportion of cross-pollinated seeds in *Axinaea* is desirable (deWaal et al., 2012). Visiting birds will spend a few minutes on one plant, probing several flowers and thus will mostly convey pollen within this specific individual, augmenting geitonogamy. Lloyd & Schoen (1992) describe prepotency, where cross-pollen succeeds in fertilizing more ovules than would be expected in an entirely stochastic event. Given this model, germination of pollen brought by a bird from another *Axinaea* individual is potentially more likely than fertilization by pollen from the same individual currently being visited. As about 60% of flowers are being visited at least twice, chances are high that a decent amount of pollen from a different individual reaches the stigma.

Bird pollination and floral food bodies

The behaviour of visiting tanagers in this investigation corresponds to observations on fruit-choice behaviour of several species in Costa Rica. Tanagers have been reported to be highly selective in the fruits they choose, making berry-to-berry choices based on fruit characteristics like ripeness and accessibility of fruits (Moermond & Denslow, 1983). Tanagers observed visiting fruiting trees frequently move around the plant and re-examine infructescences (or inflorescences in *Axinaea*) before picking a berry (or stamen in *Axinaea*). Although daily bird-feeding activity usually peaks during the morning and the late afternoon (Campbell & Lack, 1985), tanager visits to *Axinaea confusa* did not follow this pattern. Tanagers have been observed foraging in mixed-species flocks in the canopy and on *A. confusa*. While Arbeláez-Cortés et al. (2011) hypothesize that tanagers might have a cohesive function in mixed-bird flocks, tanager-association was shown to be loose in a secondary forest in Ecuador (Poulsen, 1996). A rather loose association could explain why all video-records of tanagers visiting *A. confusa* only always show one bird at a time.

Patterns documented in other bird-pollinated plants may aid the understanding of the processes underlying the reproductive system of *Axinaea*. Bird pollination is thought to have evolved independently in approximately 65 angiosperm families (deWaal et al., 2012), with three bird families regarded as flower specialists: Trochilidae (hummingbirds), Nectariniidae (sunbirds) and Meliphagidae (honey-eaters) (Proctor et al., 1996). Meliphagidae are largely restricted to Australia (and range up to Hawaii and New Zealand), Nectariniidae are important pollinators in Africa and Asia, and Trochilidae are the main representatives of the bird-pollinator guild in South and North America (Cronk & Ojeda, 2008). Flower-visiting of less-specialized birds has been reported for at least 50 bird families, and especially in the Americas, Icteridae (American Orioles) and Thraupidae (Tanagers) are important groups (e.g. Graves, 1982; Morton, 1979; Steiner, 1979). For these groups, as in hummingbirds, the presence of high quantities of energy rich, dilute nectar is the main reason to visit flowers (Cronk & Ojeda, 2008). Besides legitimate pollination, many species can be classified as nectar-robbers (e.g. deWaal et al., 2012, Morton, 1979). Among these are the predominantly Andean flowerpiercers (Diglossa and Diglossopsis), belonging to the Thraupidae (Nickolson, 2007), which have also been observed pollinating the otherwise hummingbird-pollinated genus *Brachyotum* (Stiles et al., 1992) and carrying pollen of *Axinaea macrophylla* (Rojas-Nossa, 2007).

There have been very few previous reports of angiosperms providing floral food body rewards to bird pollinators, and none of them involve modifications to the androecium. Sársic & Cocucci (1995) report pollination by the Least Seedsnipe, *Thinocorus rumicivorus* Eschscholtz (Thinocoridae, Charadriiformes) of the Patagonian *Calceolaria uniflora* Lam. (Calceolariaceae). The birds feed on the fleshy, glucose-rich corolla appendages, which, similar to the connective appendages in *Axinaea*, are composed of parenchyma with large intercellular spaces and do not visibly secrete any nectar. Further examples of floral food-bodies consumed by birds are edible bracts in *Freycinetia funicularis* Merr. (Porsch, 1923) fed on by bulbuls (Pycnonotidae), and deceit fruits situated between flowers in *Boerlagiodendron* (*Osmoxylon*, Araliaceae), which attract pigeons (van der Pijl, 1961 (orig. Beccari, 1877)). Within Myrtaceae, there are two cases known where frugivorous passerines feed on sweet, juicy petals of nectarless flowers (Roitman et al., 1997; Sazima & Sazima, 2007). Both in *Myrrhinium atropurpureum* Schott and in *Acca sellowiana* Burret, tanagers are important pollinators where these birds grab the petals and remove them entirely or in pieces. As the filaments are long in *Myrrhinium* and *Acca*, and the anthers exerted at anthesis, the birds are dusted with pollen on their breast and head when removing the petals (Roitman et al., 1997; Sazima & Sazima, 2007). In all of these systems, birds are deterred from consuming the reproductive parts of the flower but direct their foraging to brightly coloured petals or fruit-mimics, which are the floral rewards. In

Axinaea, where no nectar secretion has been found, tanagers consume the very part of the flower that provides the male reproductive function: the stamens. This is an exceptional case in the framework of pollination, where the tendency is to prevent flower visitors from consuming reproductive organs. It was stated above that nectar secretion in the hypanthium is not present in *Axinaea* as tanagers would not trigger pollen release by only inserting their tongues into the hypanthium. Furthermore, this would direct the birds' foraging towards the gynoeceum potentially damaging that reproductive organ as well. It is clear that the evolution of the bellows-pollination mechanism was crucial to enable this pollination system to function. I hypothesize that *Axinaea* arose from a nectar-producing ancestor similar to hummingbird-pollinated representatives of *Meriania*. The hypanthial stomata might have been functioning in nectar secretion and also the stomata found on stamens of *A. macrophylla* could be vestigial nectaries. They could also just have been mechanical devices transmitting the movements of the birds to the stamens in order to shake out pollen from the anthers. In a hypothetical transitional ancestor, directing foraging activity on the nectar secreting appendages could have increased the pollen expulsion. As the appendages were sugary, they could also have attracted other birds like the normally insect- and fruit-eating tanagers. It has been observed that insectivorous birds also take nectar when encountering such while looking for insects sitting in inflorescences (Cronk & Ojeda, 2008). Thus, probably mere nectar-robbers at the start, tanagers gradually became legitimate pollinators. Nectar-production in the hypanthium then was unnecessary and was lost. Stein & Tobe (1989), who speak of an ancestral loss of the structural myrtalean nectary type (Eyde, 1967) in the melitophilous Melastomataceae species, have interpreted the nectaries of vertebrate-pollinated present-day species as derived re-invention. What we observe in *Axinaea* then would come up to a loss of function of the localized nectaries, although the stomata still are present.

Reward or deception?

With regard to floral-food bodies, bird pollination, and mimicry, a very interesting case of flowerpecker-pollination has been described in several mistletoe species in India (Davidar, 1983). Fruiting- and flowering periods partly overlap in these species and the flowerpeckers, usually feeding on fruits, will take both fruits and flowers. These are very much alike, both brownish-green. A special pollination mechanism has evolved where flowerpeckers will actively open buds that would otherwise remain closed and by opening these, pollen is released "in a burst" onto the bird (Davidar, 1983; org. Kannan, 1966). Davidar (1983) argues that this is a case of facultative mimicry (sensu Dafni & Ivri, 1981), where the flowers provide a nectar reward but their chance of visitation is enhanced by the

simultaneous presence of fruits. All cases of Batesian mimicry, where the mimic obtains a one-sided advantage over the model by imitating it, is built upon this simultaneous presence of the model and the mimic (Dafni, 1984). This does not seem to be the case in *Axinaea*. While it is thought to be crucial for Batesian mimicry to work that the mimic occurs infrequently and that the compensatory reward of the model is abundant, no possible “model” was found for *A. confusa*. This would have had to be a species with purple to pink fruits with fleshy yellow seeds, fruiting when *A. confusa* is flowering. It is therefore hypothesized that low quantities of sugars are present in *Axinaea*'s bulbous appendages and tanagers are in fact rewarded when visiting the flowers. It might be that the energetic cost of pumping small amounts of phloem sap into the bulbous appendage is lower than specific nectar production. Visual cues have been considered the most important attractant in food-deceptive systems (Schiestl, 2005). The vividly contrasting colours of *Axinaea* flowers thus might trick the tanagers to a certain degree, probably reminding them of similar fruit sources not present at the moment and promising a better reward than actually provided. However, the system does not correspond to mechanisms observed in totally deceptive ones. It thus can be assumed that the food-bodies of *Axinaea* are a welcome alternative food-source to tanagers experiencing a period of fruit scarcity and would otherwise be restricted to feeding solely on insects. Changes towards mainly insect feeding during the breeding season have been reported for tanagers in Costa Rica, for example (Naoki, 2003). This hypothesis will need further investigation both on the side of chemical components in the appendages as well as on fruit-rewards taken by tanagers in the respective areas.

The ecogeographic hypothesis

In their study, Varassin et al. (2008) conclude that there is strong evidence for a correlation between nectar production in Melastomataceae and growth in montane environments. These tend to have more extreme weather conditions with strong winds and heavy rain, in which poikilothermic bees are more affected than birds (Cruden, 1972). The ecogeographic scenario proposed by Thomson & Wilson (2008) acts on the assumption that visitor frequencies change due to an extrinsic ecogeographical change and thus trigger shifts in the pollinator community. An example from the Andes is given by Arroyo et al. (1982), who observes a gradual shift from a primarily melittophilous syndrome to alternative pollinators along an altitudinal gradient. It has to be noted, however, that in this study, bees are still the most abundant pollinators at elevations where *A. confusa* grows in Ecuador, but other species of *Axinaea* can be found at higher altitudes. From an evolutionary perspective, once nectar-production occurred in the Melastomataceae, hummingbirds could have been attracted and proven more efficient in pollen transfer, thus destabilizing the bee-pollination

system. Numerous ecological scenarios come to mind (e.g. unusually wet season, absence of regular food-sources of tanagers, etc.), which then can have induced this pollinator shift. In addition to the reduced efficiency of bees in high montane habitats, abundances of pollinators are lower there in general, resulting in decreased chances of successful zoophilous cross-pollination (Arroyo et al., 1985). It has been widely accepted that pollinator specialization has been shaped by selective pressures favouring cross-pollinated offspring due to their higher fitness (Zhang et al, 2005; Proctor et al., 1996). The conclusion lies at hand that more specialized systems will more easily become subject to reproductive failure if the pollinator they depend on is absent (Waser et al., 1996). Therefore, strategies like autonomous selfing or apomixis might serve to reconcile this failure or uncertainty to guarantee reproductive success (e.g. Fenster & Martén-Rodríguez, 2007). Over time, a self-compatible system with limited gene-flow could give rise to population specialization and endemism (Lowry & Lester, 2006). For the Campo Rupestre melastomes mentioned above, a relation between self-compatibility and narrow distribution has been found (Santos et al., 2012). Most of the *Axinaea* species recognized by Balslev-Cotton (2003) are similarly narrowly distributed with a high rate of endemism. Contradictory results state, however, that there is no correlation between autonomous breeding systems and the degree of specialization (Fenster & Martén-Rodríguez, 2007). Arroyo et al. (1985) warn from generalizations such as that self-compatibility and growth at high altitudes are correlated. In fact, most Melastomataceae will face the problem that even if they are self-compatible, they usually require a floral visitor to expel pollen from the poricidal anthers, only occasionally wind or heavy rain will take over the role of pollen shedding. With the relatively low rates of autonomous selfing, this also is the case in *Axinaea confusa*. We thus speak of facilitated selfing (Lloyd & Schoen, 1992), where the transfer of self-pollen to the stigma is an unintended by-product of potential cross-pollination. Facilitated selfing can be seen as a beneficial mode of reproductive assurance when mates are scarce, not when pollinators are limited (Goodwillie et al., 2005). There might also be alternative strategies to secure successful reproduction such as simultaneous flowering of higher-altitude populations (Brito & Sazima, 2012). Especially in species-rich areas, phenological shifts in flowering species competing for the same pollinator can be useful in order to minimize effects of pollen limitation (Vamosi et al, 2006). In *Axinaea confusa*, such co-flowering was the case and has also been observed in the population of *A. affinis* and reported for a population of *A. macrophylla* (pers. com. Jürgen Homeier, Florian Bodner).

Conclusion

With its bird pollinated flowers, *Axinaea* conforms to other vertebrate-pollinated melastomes with pseudo-tubular corollas, although, contrasting to the trend, there is no nectar-secretion in the genus. The trend that ornithophily replaces melittophily at higher altitudes in order to optimize pollinator efficiency paired with the fact that the mating system of *A. confusa* is self-compatible maximizes reproductive potential. As reflected by the low rates of fruit-set after autonomous-selfing, *Axinaea* usually needs a trigger to expel pollen which creates a reproductive uncertainty (Wilson & Thomson, 1991). With ten stamens available as independent sources of male gametes and the pollen-load of a single stamen potentially sufficient for successful pollination, pollinator specialization can be reconciled in the sense of Fenster & Martén-Rodríguez (2007). In *A. confusa*, there might be competition between cross- and self-pollination and in the presence of both, outcrossing might be favoured to avoid inbreeding depression and assure genetic diversity in the population (Proctor et al., 1996). This mechanism of facultative selfing may be beneficial especially as many species of *Axinaea* tend to be patchily distributed due to their growth in disturbed landscapes such as forest gaps and pastures (Balslev-Cotton, 2003; pers. obs.). The high number of repetitive bird visits to the same flower over several days augments chances of outcrossing. The combination of androecial food bodies, the elaborate bellows-mechanism with a self-compatible system, and equal distribution of the male reproductive function to ten independent possibilities of pollen transfer within one flower has resulted in a very efficient and successful reproductive system.

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Zusammenfassung

Der beobachtete Trend, dass neotropische Melastomataceae mit halbglockenförmigen Blüten einen Bestäuberwechsel von Bienen zu Vögeln, Säugern oder anderen Insekten durchgemacht haben, bestätigt sich auch bei *Axinaea*. Vier Tangararten konnten an den Blüten von *Axinaea confusa* im Feld beobachtet werden, eine *Diglossa*-Art an *A. sclerophylla*. Die Blüten der Gattung *Axinaea* sind durch Stamina mit knollig vergrößerten Konnektivanhängseln gekennzeichnet, die von den Vögeln gefressen werden. Die Tangare packen jeweils ein Staubblatt, das an der Filamentspitze abreißt, sodass der Vogel den Konnektiv-Antheren-Komplex aus der Blüte herauslöst und frisst. In dem Moment, wo der Tangar das beerenartige Konnektivanhängsel mit dem Schnabel packt und zusammendrückt, schießt eine Pollenwolke aus der kleinen apikalen Pore der Anthere und landet auf Kopf und Schnabel des Vogels oder direkt auf dem Stigma der Blüte. Beim Herauslösen weiterer Staubblätter berührt der Vogel unabsichtlich das Stigma und führt so die Bestäubung durch. Die morphologischen Untersuchungen haben ergeben, dass die Konnektivanhängsel aus sehr lockerem Gewebe bestehen. Außerdem ist keine verdickte Zellschicht an der Basis der Anthere vorhanden, die das Konnektivanhängsel von den pollengefüllten Theken abtrennt. Der beobachtete Ausstoß von Pollenwolken beim Zusammendrücken der Konnektivanhängsel wurde daher durch einen Blasebalg-Mechanismus erklärt, bei dem die im lockeren parenchymatischen Konnektivanhängselgewebe enthaltene Luft in die röhrenförmigen Theken gepresst und dadurch der Pollen hinausgeblasen wird. Nektarsekretion, die bei anderen Melastomataceae mit halbglockigen Blüten und dem erwähnten Bestäuberwechsel in Verbindung gebracht wird, kann für die fünf morphologisch untersuchten *Axinaea*-Arten nicht bestätigt werden. Vielmehr ersetzen aber die knollig vergrößerten Konnektivanhängsel Nektar als Bestäuberbelohnung. Da in vorläufigen Untersuchungen weder Zucker noch Lipide, Öle oder Proteine im Gewebe gefunden wurden, erhebt sich die Frage, worin die Bestäuberbelohnung besteht. Möglicherweise handelt es sich bei dem beobachteten System um einen teilweisen Bestäuberbetrug, wo die durch die beerenartigen Konnektivanhängsel angelockten Tangare nur eine geringe nutritive Belohnung erhalten. Neben der Bestäuberbelohnung übernehmen die kräftig gefärbten Anhängsel, die einen starken Farbkontrast innerhalb der Blüten erzeugen, die Funktionen der Bestäuberanlockung sowie der Pollenverbreitung mittels Blasebalgmechanismus. Untersuchungen zum Reproduktionssystem von *Axinaea confusa* haben gezeigt, dass die Art selbstkompatibel, aber nicht autogam ist. Die Verbindung des

spezialisierten Bestäubungsmechanismus durch Vögel, die in höheren Lagen als effizientere Bestäuber als Bienen gelten, mit der potentiellen Selbstkompatibilität von *Axinaea*, können als reproduktive Optimierung verstanden werden. Der geringe Fruchtsatz bei autonomer Selbstbestäubung zeigt, dass bei *Axinaea* generell zumindest ein Auslöser benötigt wird, um Pollen aus den kleinen Poren zu schleudern. Die dadurch entstehende Abhängigkeit von der Anwesenheit von genügend Bestäubern kann jedoch durch zwei Faktoren minimiert werden. Zum einen stellen die zehn Staubblätter jeder Blüte, die durch die Vögel zumeist einzeln herausgelöst werden, zehn voneinander unabhängige Möglichkeiten des erfolgreichen Pollentransfers dar. Zum anderen ist die Pollenmenge eines einzelnen Staubblattes groß und die ausgelösten Pollenwolken können problemlos die Narbe der eigenen Blüte erreichen. Möglicherweise entsteht dadurch bei Vorhandensein von sowohl fremdem als auch eigenem Pollen Konkurrenz zwischen denselben, wobei Fremdbefruchtung, die vor inbreeding depression schützt und genetische Diversität sichert, begünstigt sein könnte. Da viele *Axinaea*-Arten eine fragmentierte Verbreitung aufweisen und vielfach in landwirtschaftlich genutzten Flächen oder Störungsflächen vorkommen, könnte diese Strategie der fakultativen Selbstbestäubung von großem Vorteil sein. Außerdem könnte die hohe Anzahl an wiederholten Tangarbesuchen der gleichen Blüte über den Zeitraum mehrerer Tage bis zum vollständigen Verzehr aller zehn Staubblätter die Fremdbestäubung zusätzlich erhöhen. Die Kombination von staminalen Futterkörperchen und dem raffinierten Blasebalgmechanismus mit einem an sich selbstkompatiblen System und der gleichmäßigen Aufteilung der männlichen reproduktiven Funktion auf zehn voneinander unabhängige Möglichkeiten zum Pollentransfer innerhalb einer einzigen Blüte, schafft ein sehr effizientes und erfolgreiches Reproduktionssystem bei *Axinaea*.

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Ausbildung

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- seit 2010 /09 Masterstudium Syn- und Landschaftsökologie, Universität Wien, 2010/2011 Erasmus-Auslandsaufenthalt (Universität Lund, Schweden), 2012/10: Feldkurs Tropical Ecology (TBA, Borneo)
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Arbeitserfahrung und Wissenschaftliche Tätigkeiten

- 2005 /06 Work experience auf einer Papageifarm, England (Shrewsbury).
- 2007 /06 Bioinformatisches Praktikum in einem Labor für Neurobiologie, Medizinische Universität, Wien.
- 2008 /07 Volontariat beim Internationalen Bartgeierprojekt, Nationalpark Hohe Tauern; Beobachtung der Jungvögel sowie Besucher-information.
- 2010 /07 Datenaufnahme für ein Projekt zu *Senecio carniolicus* (Department für Biogeographie, Universität Wien).

2010 /07-08	Praktikum im Bereich Umweltbildung im Nationalpark Gesäuse; Führungen, Besucherinformation, Veranstaltungen.
2011 /06-07	Feldassistenz bei einem Projekt zur Ökologie brütender Watvögel in Süd-Lappland, Schweden; Nestkontrollen, Beringen, eigenständige Vegetations- und Habitatsklassifizierung (Universität Lund).
2011 /08-09	Praktikum beim Internationalen Bartgeierprojekt, NP Hohe Tauern; Besucherbetreuung und Brutvogelmonitoring.
2012 /03-06	Tutorium im Kurs „Diversität und Systematik der Höheren Pflanzen“ (Universität Wien)
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Sprachkenntnisse

Deutsch: Muttersprache	Spanisch: fortgeschritten
Englisch: verhandlungsfähig	Schwedisch: fließend
Französisch: fließend	Latein

Sonstiges

Microsoft Office Programme, Europäischer Computerführerschein
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