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**Forma y función de los estambres de *Meriania macrophylla* (Benth.) Triana
(Melastomataceae) e implicaciones en su polinización**

Tesis para optar por el grado de Licenciatura en Biología con énfasis en Zoología

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“This is the part when I break free...”

Ariana Grande

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ABSTRACT

Heteranthery is the condition in which a flower has two different types of stamens that can differ in form and function. This phenomenon is explained by the division of labor hypothesis in which one set of stamens has a reproductive role and the other serves as a reward for pollinators. In the family Melastomataceae, heteranthery is a frequent phenomenon, particularly in the tribe Merianieae. Within this tribe, the species *Meriania macrophylla* has dimorphic bulbous-acuminate stamens (poorly represented in the tribe) that differ in the size of their connective (thick and thin stamens). The existing descriptions of *M. macrophylla* morphology are very basic and there is no information about reproductive biology (morphology suggest bird-pollination, but the flowers also have traits associated with bee-pollination). In this project I aim to describe the reproductive biology of *M. macrophylla*, focusing on the form and function of the two types of stamens. I also report the flower visitors, describe the mechanism of flower-pollinator interactions and present a caloric content analysis of the stamens. I conducted the study in Vara Blanca, Heredia, Costa Rica. In the field, flowers were collected and preserved, and direct observations of floral visitors were made during 10 days. In addition, video cameras were placed in the field to record the flower visitors. In the laboratory, the stamens and pollen were prepared for SEM, and the caloric content of the stamens was determined. *M. macrophylla* had two sets of stamens that differ in shape, size of the connective and slightly in pollen grain size (both are larger in the thick stamens), but not in color, tissue arrangement or pollen morphology. The stamens presented a bellows-like mechanism of pollen dispersion and frugivorous birds are reported as the effective pollinators. The set of thick stamens function as a food-body reward for birds (with a high caloric content), but the thin stamens, although producing pollen and having caloric value, are not consumed by birds and remain in the flowers. Insects and hummingbirds are reported as visitors but not as pollinators. In conclusion, there is no evidence supporting the division of labor hypothesis, so another evolutionary context is needed to explain the role of heteranthery in *M. macrophylla*.

KEY WORDS: Division of labor hypothesis, Heteranthery, Merianieae, Pollen, Thraupidae

RESUMEN

La heteroanteria es la condición en la que una flor tiene dos verticilos diferentes de estambres que pueden diferir tanto en forma como en función, este fenómeno se puede explicar según la hipótesis de división de labores que postula que un juego de estambres tiene una función reproductiva (polen fértil) y el otro sirve como recompensa (polen alimenticio). En la familia Melastomataceae, la heteroanteria es un fenómeno frecuente y particularmente en la tribu Merianieae. Dentro de esta tribu, la especie *Meriania macrophylla* presenta estambres heteroantéricos bulboso-acuminados (una morfología poco representada en la tribu) los cuales difieren en el tamaño del conectivo (estambres gruesos y delgados). Las descripciones de *M. macrophylla* son muy básicas y no hay información sobre la biología reproductiva (su morfología sugiere polinización por aves, pero hay rasgos asociados a polinización por abejas). En este proyecto describí la biología reproductiva de *M. macrophylla*, enfatizando en la forma y función de los estambres. Además, reporté los visitantes florales, describí las interacciones flor-polinizador y caractericé el contenido calórico de los estambres. La investigación la realicé en Vara Blanca, Heredia, Costa Rica. En el campo realicé la colecta de flores y observaciones directas de los visitantes durante 10 días. También, coloqué cámaras de video para grabar las interacciones entre los visitantes y las flores. En el laboratorio realicé la preparación de los estambres y el polen de las flores colectadas, para microscopía electrónica de barrido, e hice los análisis de contenido calórico de los estambres. *M. macrophylla* presentó dos tipos de estambres que difieren en tamaño, forma, tamaño del conectivo y ligeramente en el tamaño del polen, estas características mayores para los estambres gruesos; por otro lado, no hubo diferencias en color, disposición de tejidos y morfología del polen. Los estambres presentaron un mecanismo como fuelles en el que el polen es expulsado cuando un pájaro aplica presión con el pico. Se reportaron aves frugívoras como los polinizadores efectivos. Los estambres gruesos funcionaron como recompensa para las aves (con un alto contenido calórico), mientras que los estambres delgados, aunque producen polen y tienen valor calórico, no son utilizados por las aves ni otros animales. Insectos y colibríes también visitaron las flores, pero no se consideraron polinizadores. Se concluyó que no hay evidencia para apoyar la hipótesis de división de labores, por lo que otro contexto evolutivo es necesario para explicar el papel de la heteroanteria en las flores de *M. macrophylla*.

INTRODUCTION

The disposition of flower stamens often varies across families and across species within families. For instance, heteranthery is the condition in which a flower has two different types of stamens that can differ in form and function (Müller, 1883; Barrett, 2002; Barrett, 2010). Darwin (1862) proposed the division of labor hypothesis to explain the differences in form and function of the two types of stamens. This hypothesis postulates that one set of stamens has a reproductive function (fertile pollen) and the other set provides a reward to the pollinators (food pollen) (Luo, Zhang & Renner, 2008; Lu, Wu, Wang, Li & Wang, 2009; Vallejo-Marín, Manson, Thomson & Barrett, 2009; Vallejo-Marín, Da Silva, Sargent & Barrett, 2010). Heteranthery is also strongly associated with poricidal anther dehiscence and buzz-pollination (Vallejo-Marín et al. 2009). In buzz-pollinated flowers, pollen can only be released from these poricidal anthers through the application of high-frequency vibrations by pollinating bees (Renner, 1989). Heteranthery in buzz-pollinated flowers seems to have evolved as a strategy to reduce pollen lost by the action of other flower visitors (Vallejo-Marín et al. 2009).

In the family Melastomataceae, heteranthery and buzz-pollination with pollen rewards is a frequent phenomenon, particularly the tribe Merianieae which shows a wide diversity of stamen arrangement and morphology (Renner, 1989; Almeda, 1993; De Luca & Vallejo-Marín, 2013). Dellinger *et al.* (2019) report six types of stamens in this tribe, and associate these morphologies with three pollination syndromes: passerine birds (staminal food bodies as rewards), buzzing bees (pollen as reward) and a mixed vertebrate system (nectar as reward).

Among the species of the tribe Merianiae, *Meriania macrophylla* (Benth.) Triana has dimorphic bulbous-acuminate stamens that differ in the size of their connective: antepetalous stamens have a thick connective and antesepalous stamens have a thin connective (Almeda, 1993). Bulbous-acuminate stamens are poorly represented within this tribe (Dellinger *et al.*, 2019). There are two other species of *Meriania* that are known to have a similar morphology: *Meriania franciscana* C. Ulloa & Homeier and *Meriania peltata* L. Uribe (Ulloa & Homeier, 2008). Literature about *M. macrophylla* includes only a basic description of the flowers (Almeda, 1993; Almeda, 2007), and characteristics of the calyx and stamens in the tribe (Mendoza-Cifuentes & Fernández-Alonso 2010). However,

there is no detailed description of the internal morphology and histology of the stamens, the morphology of the pollen or the differences between the two types of stamens.

Moreover, there is no information on the reproductive biology of *M. macrophylla*, and both the pollinators and the mechanism of pollen dispersal are unknown. Based on the morphology of the bulbous-acuminate stamen, urceolate corollas and the absence of nectar, Dellinger *et al.* (2019) predicted that *M. macrophylla* is a bird-pollinated species, specifically by passerine birds which are rewarded with the bulbous nutritive stamen appendages, as occurs in the genus *Axinaea* (also in the tribe Merianieae) which is characterized by bulbous, non-heterantheric stamens (Dellinger *et al.*, 2014). This prediction has not been verified by empirical observations, and it should be considered that the presence of strong heteranthery is very uncommon in non-bee-pollinated flowers.

In this project I aim to describe the reproductive biology of *M. macrophylla*, focusing on the form and function of the stamens using detailed descriptions of their internal morphology and histology, the morphology of the pollen and comparison between the two types of stamens in a context of functional division. I also report the possible flower visitors (some could be pollinators) and describe the mechanism of flower-pollinator interactions. In addition, I present a caloric content analysis of the stamens in a comparative context to characterize the energetic contribution of the stamens as a reward to the pollinators.

MATERIALS & METHODS

Study site

I conducted this investigation in a forest remnant between pastures used for livestock activities in Vara Blanca, Heredia province, Costa Rica (10°09' N, 84°09' W, 1860 m a.s.l.). The site is classified as a montane forest (Holdridge, 1987). The average annual rainfall varies between 3000 and 5000 mm, and the annual temperature varies between 15 and 22 °C (Pérez-Umaña, 2017).

Focal species

Meriania macrophylla is a tree 6-21 m tall with peltate elliptic leaves (9.8-34 x 10-28 cm) and terminal panicles with hermaphroditic flowers (1.5 -1.8 x 0.9 - 1.2 cm). The flowers have an urceolate green corolla, five magenta petals and two sets of five purple stamens: antepetalous stamens with a thickened white connective and antesealous stamens with a flat white connective (thick and thin stamens hereafter). The style is magenta, positioned in the center of the flower and can be seen above the stamens (Almeda, 1993) (Fig. 1). The species' distribution ranges from Mexico to Guatemala and from Costa Rica to Venezuela in tropical forests between 1400 and 2600 m a.s.l. (Almeda, 1993; Calderón-Sáenz & Mendoza-Cifuentes, 2000). *M. macrophylla* is a rare species throughout its distribution, with few known populations, with low densities, and probably one of the most endangered species in the family in the countries where it is present (Almeda, 1993; Almeda, 2007). Almeda (1993) also reports that the flowers bloom from September to January, and fructification takes place from January to April.

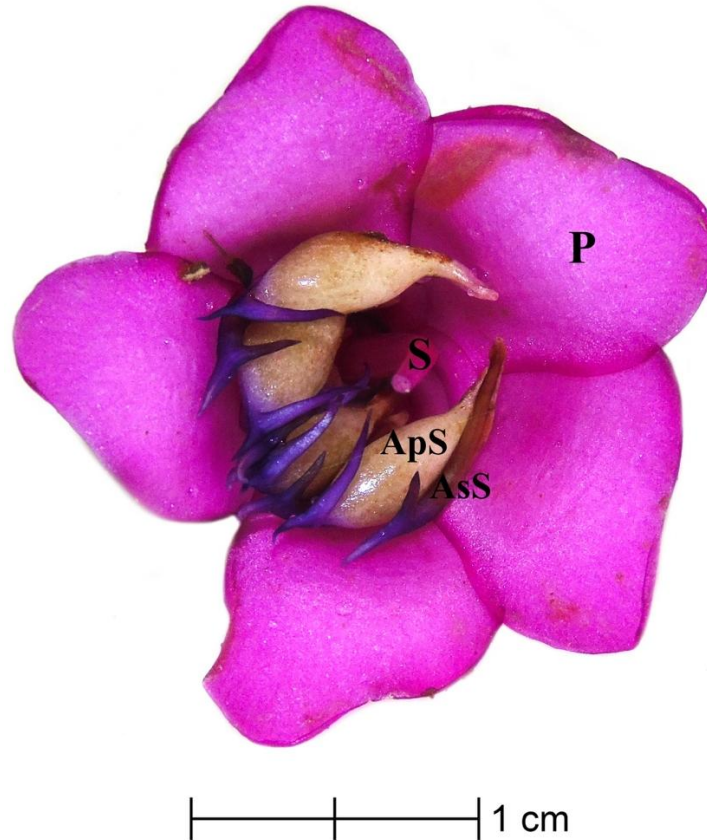


Fig.1. Flower of *M. macrophylla*, the thick stamens are clearly visible by the inflated whitish connective. ApS= Antepetalous stamen (thick stamen), AsS= Antesepalous stamen (thin stamen), P= Petal, S= Style.

Fieldwork

Fieldwork was conducted from October 11 to October 16, and from November 2 to November 4, 2018. Two populations of the species were located in Vara Blanca, but only the one that had reproductive individuals during the study period was used.

I observed the inflorescences from 5:00 to 15:00 h. Observations were made with binoculars, and four video cameras that were placed to record a site with at least one inflorescence with anthetic flowers. The video cameras recorded for two 4 hour periods, hence the video observations do not capture the full 10 hours of direct observation time. I made observations for 10 days and on each day the cameras were installed to record different areas, while the direct observations were made on all the flowers. Each bird that interacted with the flowers was determined to species with the app Merlin Bird ID (Cornell University, 2018), and the number of flowers visited was recorded.

Flowers were collected for morphological analysis and fixed in situ with FAA (formaldehyde, alcohol and acetic acid). The flowers were preserved in FAA for a week and then transferred them to 75% ethanol. Only inflorescences from the lower branches of the tree were sampled due to the difficulties in reaching the top of the tree. From each inflorescence all open flowers were collected.

Sample processing for electron microscopy

Three stamens of each type were prepared for SEM (Scanning Electron Microscopy). The stamens were washed with 0.2 M phosphate buffer for 15 min each, and post-fixation was done with 2% Osmium Tetroxide (OsO_4) for 2 h, followed by five washes with distilled water, 10 min each.

To prepare the stamens I used the cryofracture technique, following the protocol by Tánaka (1989). Stamens were treated with Dimethyl Sulfoxide (DMSO) at concentrations of 25% and 50%, 30 min each, then frozen in a metal plate with liquid nitrogen, and broken with a frozen hammer and scalpel. The broken and frozen tissue was treated with DMSO at 50% and 25% to defrost and then washed five times with distilled water, 10 min each. For the thick stamens, dissections were done using only a scalpel. I also included eight additional stamen samples, four of each type: four stamens were kept whole (two for each type), and the other four were cut using a scalpel; no post-fixation was done with these samples.

Dehydration of all samples, with or without post-fixation, was done with an ethanol series, using increasing concentrations from 30% to 100% (30%, 50%, 70%, 80%, 90%, 95% and two baths of 100%). Then, two baths were done, one with Isoamyl Acetate ($\text{C}_7\text{H}_{14}\text{O}_2$) and ethanol (1:1) for 15 min, and then with 100% Isoamyl Acetate. To dry the samples, a Leica EM CPD300 critical point dryer (Leica Mikrosysteme GmbH, Austria) was used. Samples were mounted on 50 mm plates and covered with gold using a Quorum EMS 150RS coater (Quorum Technologies Ltd, United Kingdom). Samples were photographed using a Hitachi 3700N scanning electron microscope (SEM) in the laboratory in the CIEMIC (Centro de Investigación en Estructuras Microscópicas, Universidad de Costa Rica).

Stamen morphology and functionality

I photographed the stamens under an Olympus SZX16 stereoscope and the photos were used to measure the length and width of the stamens using ImageJ (Schneider, Rasband & Eliceiri, 2012). The length was measured as the diagonal between the apex of the anther and the beginning of the appendage, and the width was measured in the widest part of the connective (Fig. 2).

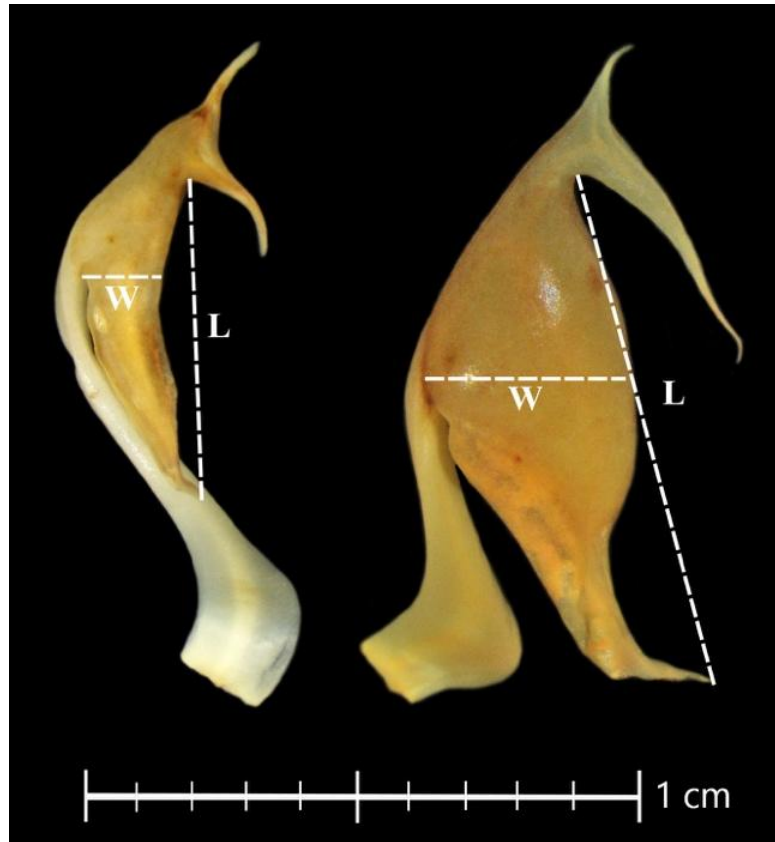


Fig.2. Measurements made on the stamens of *M. macrophylla*. L: length, W: width

To compare the two types of stamens 45 stamens of each type were measured and a Wilcoxon signed-rank test was used. Also, images under both light microscope and SEM were used to describe the internal and external morphology of the stamens. To show the expulsion of pollen from the anthers I used forceps to compress the stamens, emulating a bird's beak.

Pollen morphology

Images obtained from the SEM were used to describe and measure the pollen. The description was based on the grain polarity, presentation, scope, and type and number of openings. The shape was also described according to Erdtman (1969) using the polar axis and equatorial diameter ratio of 10 pollen grains. I used the measurements of the equatorial diameter to define the grain size, and to standardize the measurement only grains visible in equatorial view were measured. Pollen sizes were compared between the two types of stamens using a Student's t-test.

Video analysis

In total, I analyzed 105.60 video recording hours. In each video the visiting bird species was identified and the time of visitation was recorded. In addition, interactions with flowers (potential pollination events) were distinguished from interactions with buds. When a bird visited an open flower, the number of stamens it removed from the flower was recorded. In non-bird interactions I only recorded the identity of the visiting animal.

Measurements of caloric content of stamens

Single stamens of each stamen type were dried for 2 min using a microwave oven. Four different samples were then prepared: two samples containing either entire thick or thin stamens and two samples containing only the appendages of thick or the appendages of thin stamens. I removed the appendages from the rest of the stamens using a scalpel. Each of the four samples was pulverized and then compressed into a small pellet. The calorimetric content of each pellet was measured separately using an IKA calorimeter C 2000 basic Version 1 (IKA®-Werke GmbH & Co. KG, Germany). This analysis was done in the Department of Structural and Functional Botany of the University of Vienna. All dimensions are presented as mean \pm standard deviation. For the statistical tests and data representation, the software R (R Core Team, 2016) was used.

RESULTS

Stamen morphology

The flowers of *M. macropylla* presented two types of stamens arranged in two whorls: five stamens with a large, inflated white connective appendage (thick stamens) and five stamens with a small connective (thin stamens). Both types of stamens had a magenta filament with a purple anther and a prominent bifurcated purple appendage (Fig. 3).

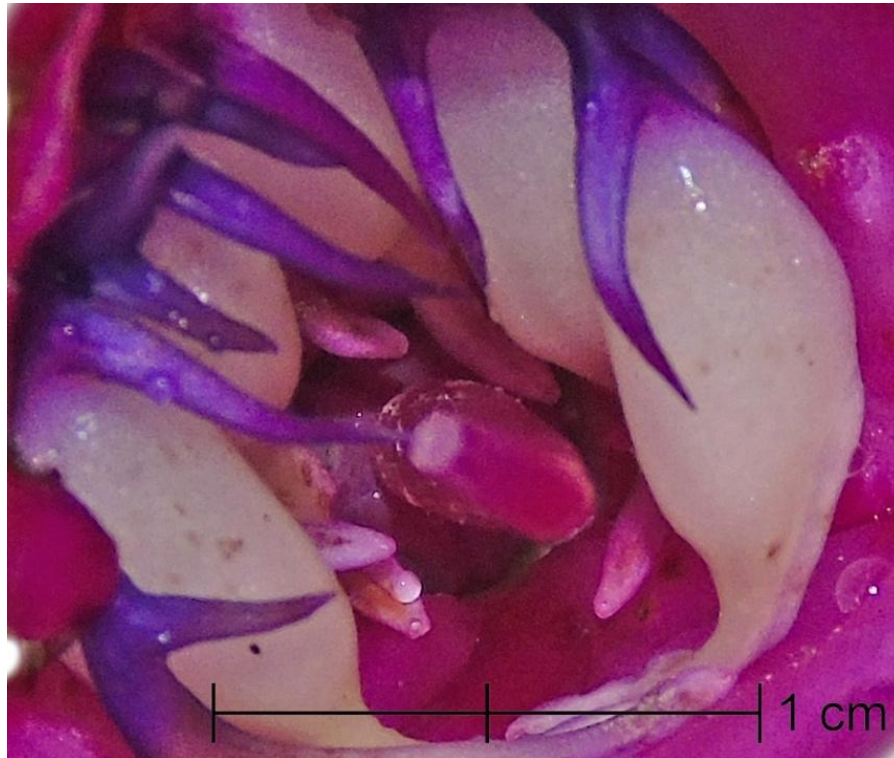


Fig.3. Details of the stamen arrangement in a flower of *M. macropylla*.

Each stamen had two thecae, which contain the pollen in fused chambers. The thecae merge at the apex where the pore is located (Figs. 4, 5, 6). The anthers (excluding the appendages) of the thin stamens were $8.1 (\pm 1.3)$ mm long and $1.6 (\pm 0.3)$ mm wide and the anthers of the thick stamens were $9.1 (\pm 1.3)$ mm in length and $3.6 (\pm 0.5)$ mm in width. The length ($W=533.5$, $p\text{-value} < 0.001$) and width ($W=3$, $p\text{-value} < 0.001$) of the thick stamens are greater than the thin ones (Fig. 7).

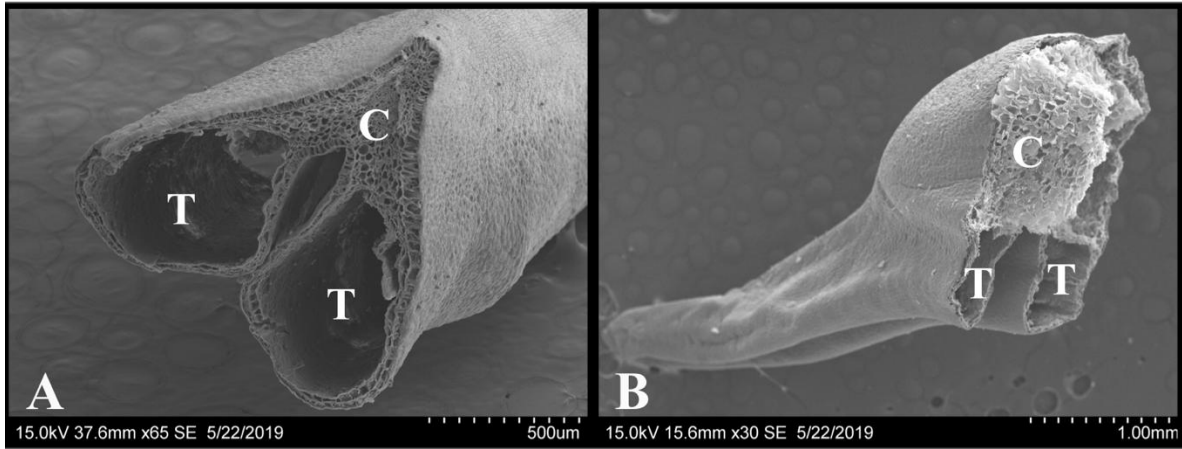


Fig.4. Thecae and connective of the anther in a thin (A) and thick (B) stamen of *M. macrophylla*. SEM images of a cross-section of the stamens treated with OsO₄. C= connective, T= thecae

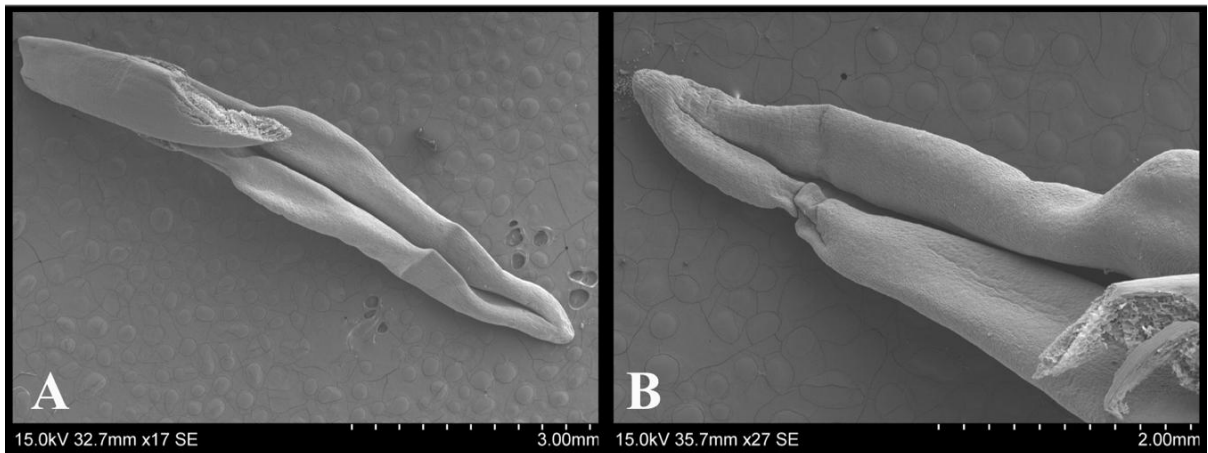


Fig.5. Ventral view of the thecae, converging at the apex of the anther in the thin (A) and thick (B) stamen of *M. macrophylla*. SEM images of the whole stamens not treated with OsO₄. Deformation in the thecae of the thick stamen caused by the manipulation of the sample.

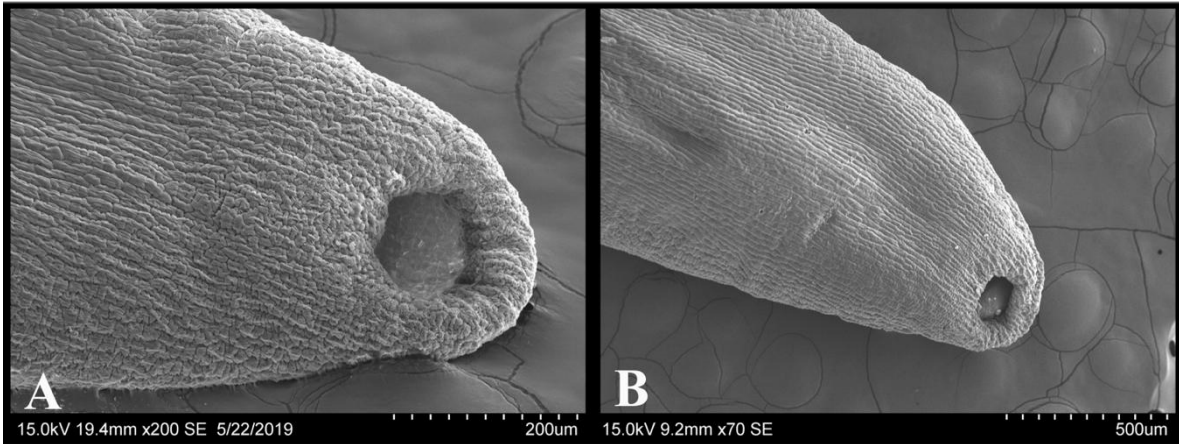


Fig.6. Pore at the apex of the anther in a thin (A) and a thick (B) stamen of *M. macrophylla*. SEM images of the whole stamens treated with OsO₄.

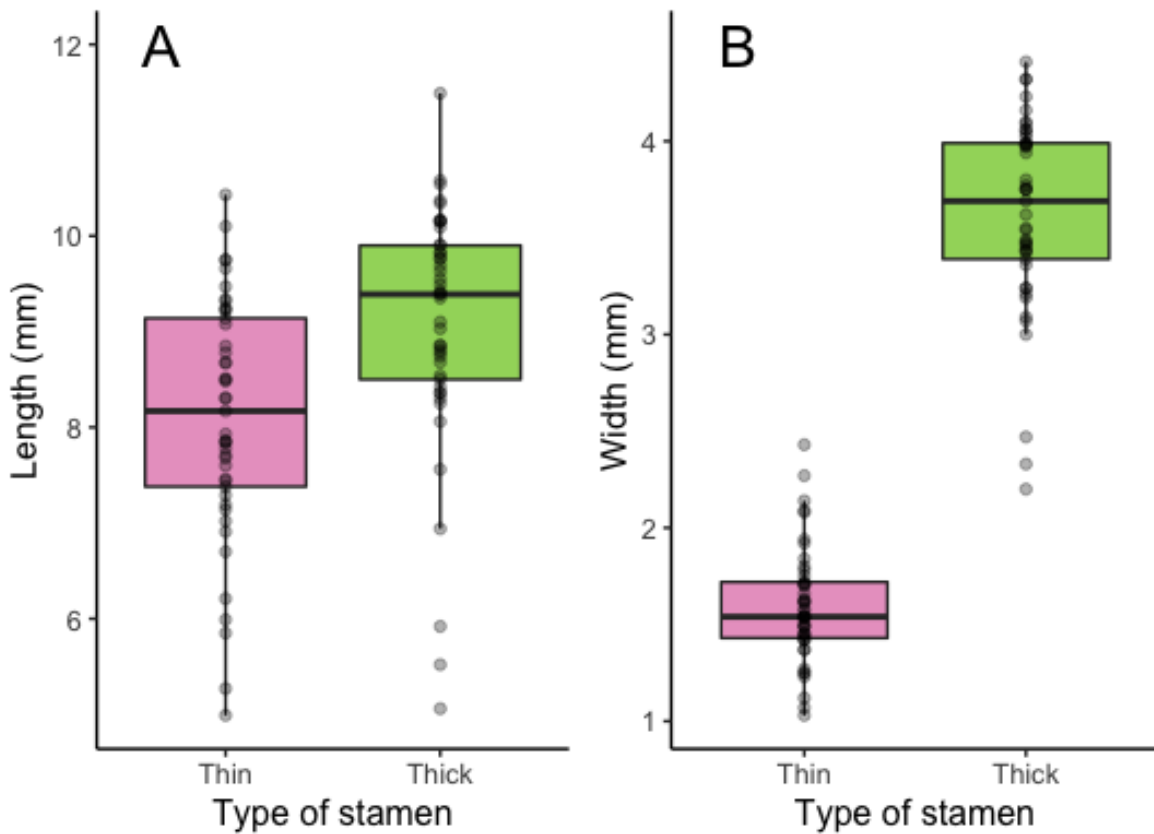


Fig.7. Length (A) and width (B) of the two types of stamens. The box represent the interquartile range, the horizontal line in the box represents the median and the whiskers represent the distribution of the 98% of the data. Every point represents a measurement.

Histologically, the stamen is composed of parenchyma and is covered with an epidermis, which also covers the inner walls of the thecae. The vascular bundle can be identified in the ventral region of the connective and is surrounded by a thin layer of collenchyma (Fig. 8). Structurally, the only difference between the two types of stamens is the size of the connective, defined by the volume of parenchyma (Fig.4).

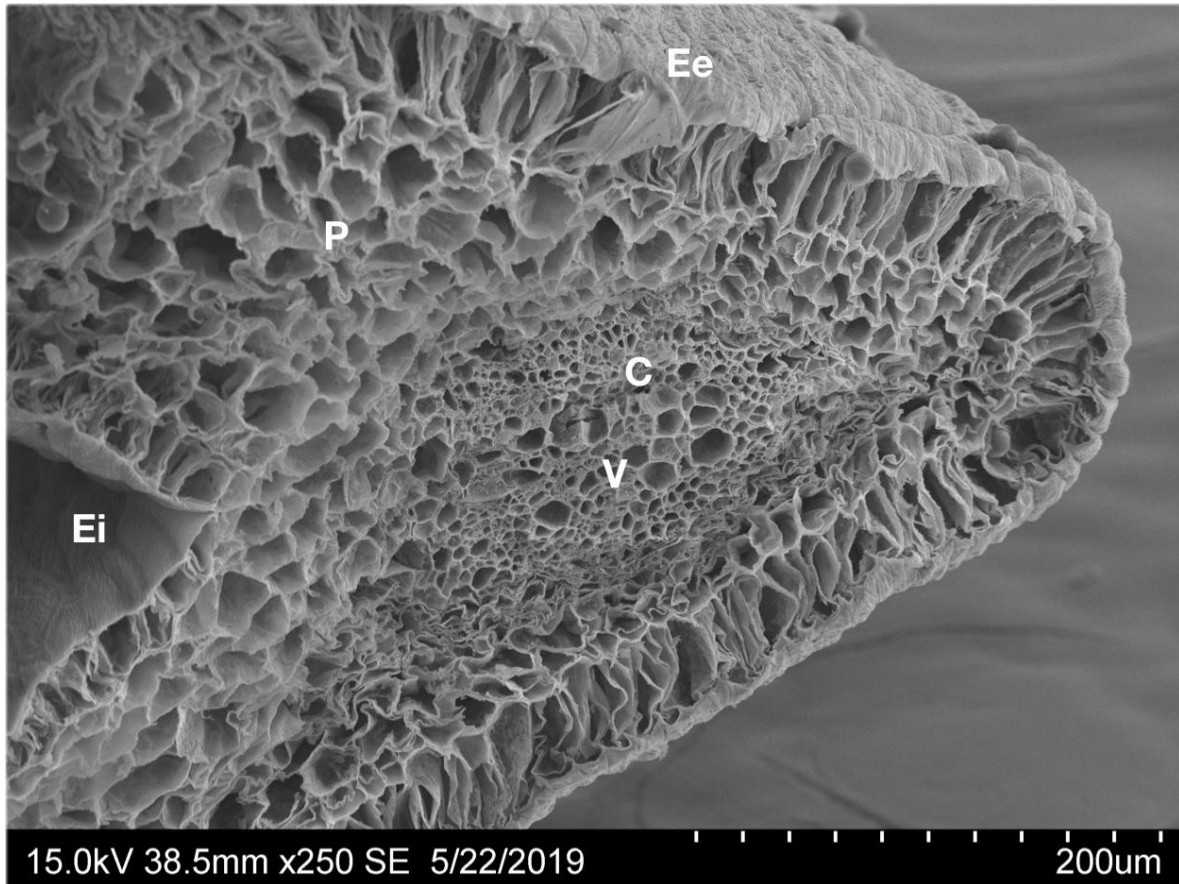


Fig.8. Tissue arrangement in a stamen of *M. macrophylla*. SEM image of a cross-section of a thin stamen treated with OsO₄. C= Collenchyma, Ee= External epidermis, Ei= internal epidermis, P= Parenchyma, V=Vascular bundle

Stamen function

Thick stamens had a bellows-like mechanism in which the pollen is expelled from the pore of the anther, along with an unidentified liquid, when pressure is applied to the connective. In the thin stamens, the pollen was not expelled when pressure was applied with forceps.

Pollen morphology

Both thin and thick stamens produced pollen. For both types of stamens, the pollen is an isopolar monad with a spherical contour and six colpi (Fig.9). The grain has an oblate-spheroidal shape, based on a mean ratio of $0.98 (\pm 0.06) \mu\text{m}$. Pollen grains did not differ in shape between the two stamen types. Average pollen grain size was $12.08 (\pm 0.61) \mu\text{m}$ for the thin stamens and $12.35 (\pm 0.52) \mu\text{m}$ for the thick stamens (Fig.10; $t=-2.14, d.f.=82.19, p\text{-value}<0.05$).

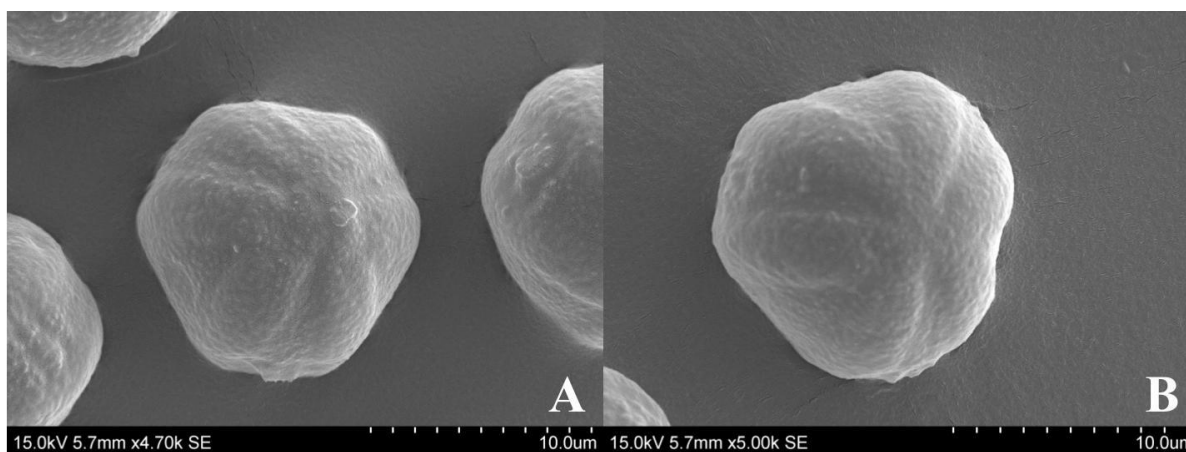


Fig.9. SEM images of a whole grain of pollen from the thin (A) and thick (B) stamens.

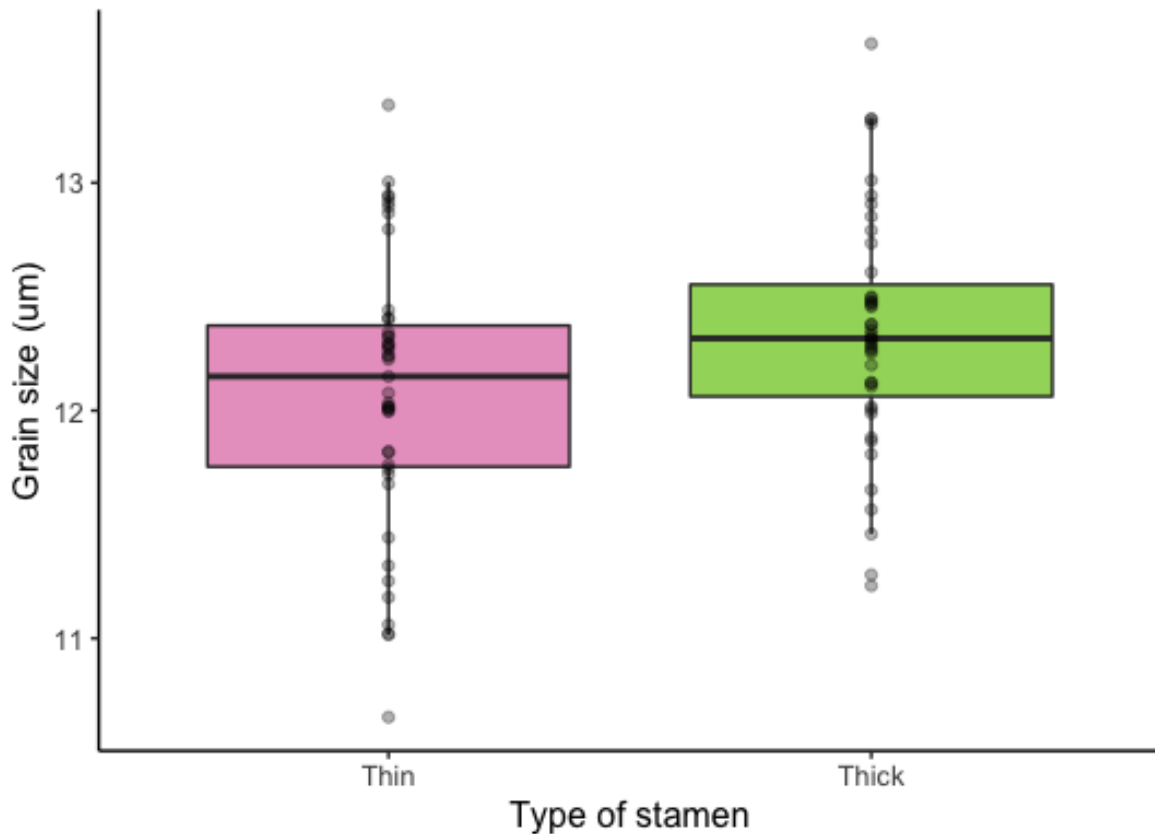


Fig.10. Size of pollen grain in the two types of stamens. The box represents the interquartile range, the horizontal line in the box represents the median and the whiskers represent the distribution of the 98% of the data. Each point represents a measurement.

Flower visitors

Flies, bumblebees, wasps, lepidopterans and birds visited the flowers, but only birds were observed removing stamens from the flowers of *M. macrophylla*. The insects approached the flowers, but were never observed extracting pollen. Detailed examination of flowers revealed larvae of flies and lepidopterans in the flowers, and scars made by oviposition in the petals and calyx. Six bird species were identified, including two hummingbirds and five frugivorous birds (Table 2).

Table 1. Birds species that visit the flowers of *M. macrohylla*.

Order	Family	Species	Stamen removal
Apodiformes	Trochilidae	<i>Elvira cupreiceps</i> (Lawrence, 1867)	No
		<i>Lampornis calolaemus</i> (Salvin, 1865)	No
Passeriformes	Fringillidae	<i>Chlorophonia callophrys</i> (Cabanis, 1861)	Yes
	Thraupidae	<i>Chlorospingus ophthalmicus</i> (De Bus de Gisignies, 1847)	Yes
		<i>Tangara dowii</i> (Salvin, 1863)	Yes
		<i>Tangara icterocephala</i> (Bonaparte, 1851)	Yes

The common bush tanager, *Chlorospingus ophthalmicus* had 51 effective visits during the 10 days of sampling (a visit was considered effective if the bird removed at least one stamen). The silver-throated tanager, *Tangara icterocephala*, was a recurrent visitor while golden-browed chlorophonia, *Chlorophonia callophrys*, and the spangle-cheeked tanager, *T. dowii* visited only a few flowers on a single day (Fig.11). Two hummingbird species (Trochilidae) were observed approaching the flowers, although there was no evidence of pollen removal.

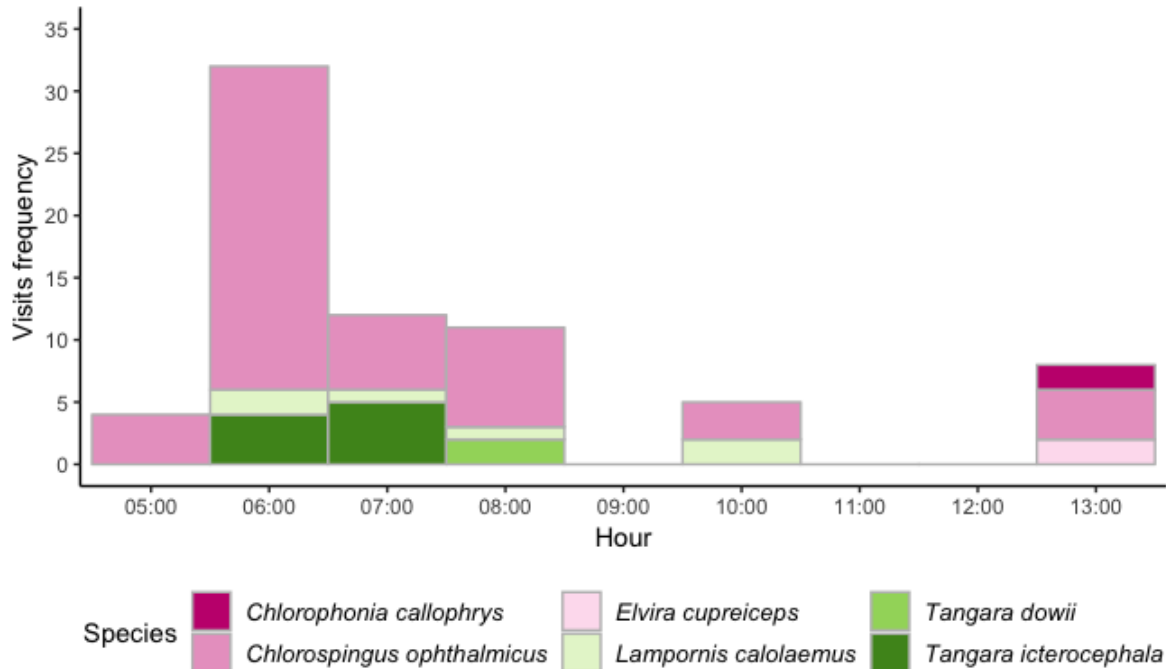


Fig.11. Frequency of the bird visits by hour to *M. macrophylla* flowers , during 10 days of sampling. Hours after 13:00 were excluded due to the lack of visits.

Stamen removal

Birds remove the stamens following a relatively stereotypic sequence. The perching bird introduces part of its head into the flower to pick up a thick stamen with its beak and in the process touches the stigma. Based on all observations, *C. ophthalmicus* removed a mean of $1.31 (\pm 1.52)$ stamens from each visited flower and a mean of $4.14 (\pm 2.57)$ flowers per visit. Data of *C. ophthalmicus* were not compared with those of other bird species due to the low visitation of the other birds. The stamens were removed one by one and every stamen removed was chewed and then spit out. The pressure produced by the compression on the bulbous connective by the beak resulted in the expulsion of a cloud of pollen, and liquid, from the stamen. This pollen landed on the bird's face. The thin stamens were never removed and remained in the flowers where they withered (Fig.12). *C. ophthalmicus* was the species used to describe the interaction due to the greater number of visits among all the birds, but all the frugivorous species behaved in a similar way.



Fig.12. Flower of *M. macrophylla* after thick stamens removal, thin stamens remain in the flowers.

Caloric content of stamens

The calorimetric analysis resulted in an energetic contribution of 460.8 J for each flower, considering only the ten stamens (Table 1). The analysis proved that the energetic contribution of the thick stamens is greater than that of the thin stamens (39.24 J more in thick stamens). The appendage content of the thin stamens could not be analyzed separately due to the very low weight of the sample. For a bird eating only the thick stamens of a flower, there is a maximum energetic input of 328 J/flower (0.08 kcal/flower) (five stamens) and a mean input of 86.07 J (0.02 kcal/visit) (based on the mean of stamens removed).

Table 2. Energy contribution from both types of stamens of *M. macrophylla*

Stamen	Sample	Weight of the sample (g)	Weight of one stamen (g)	Energy (J/g)	Energy (J/stamen)
Thick	Whole	1.21690	0.0043	15279	65.7
	Appendage	0.12850	0.0020	16136	35.49
Thin	Whole	0.19910	0.0015	17642	26.46
	Appendage	-	-	-	-

DISCUSSION

Stamen form and function

The two types of stamens in *M. macrophylla* differed in several respects, as expected for a heterantherous flower (Müller, 1883; Barrett, 2002; Barrett, 2010). They differ in size and shape of the connective, and although connectives are white in both stamen types, thick stamens show more contrast than thin stamens. Hence, thick stamens are likely more attractive to pollinators than thin stamens (Spaethe, Tautz & Chittka, 2001). A pore was observed at the apex of both types of stamens, a morphology that is congruent with pollen release via a buzz mechanism, but the evidence is insufficient.

Contrary to *Axinaea* (Dellinger *et al.*, 2014), the bulbous connective of *M. macrophylla* does not have aerenchyma and therefore the expulsion of pollen depends exclusively on the pressure applied to the parenchymatic tissue. Bellows-like anthers, depending merely on pressure applied to a parenchymatic tissue, are reported for the bee-pollinated genus *Cyphomandra* (Solanaceae) (Sazima, Vogel, Cocucci & Hausner, 1993) and the rodent-pollinated genus *Blakea* (Melastomataceae) (Lumer, 1980). For *Cyphomandra* even a slight pressure will activate the mechanism (like a bee perching over the stamen) (Sazima *et al.*, 1993). For the stamens of *M. macrophylla* it seems unlikely that a weak pressure could activate the mechanism because a strong compression with the forceps was necessary to expel pollen. Nevertheless, measurements of the force required to compress the stamen's connective are necessary to discard this possibility.

The nature of the liquid expelled with the pollen is unknown and more detailed chemical analyses is required to determine its composition. It could be expected that it has a high hexose composition as is known to occur in bird-pollinated flowers (Baker, Baker & Hodges, 1998). Nectar production, though rare among melastomes, is described for at least 80 species including species from the tribe Merianieae; *Meriania phlomoides* (Triana) Almeda, *M. tomentosa* (Cogn.) Wurdack (Varassin, Penneys & Michelangeli, 2008), *M. sanguinea* Wurdack and *M. furvanthera* Wurdack (Dellinger *et al.* 2019b). SEM images of the stamens did not reveal any sign of nectaries, but a detailed microscopic and histologic analysis is necessary to safely discard their presence in the anthers. Therefore, the mechanism described here is possibly the first bellows-like pollination mechanisms with a liquid component.

Pollen morphology

Pollen morphology was the same for the two anthers, but there was a very small difference in size. Mesquita-Neto, Costa & Schlindwain (2017) reported larger, but not statistically significant differences between pollen size in dimorphic stamens of *Senna reniformis* (G. Don) H.S. Irwin & Barneby (Fabaceae), but no differences in pollen performance. On the contrary, Vallejo-Marin *et al.* (2009) reported smaller and statistically significant differences between pollen grains in the heterantheric species, *Solanum rostratum* Dunal (Solanaceae), though they did not report information about viability. In *Clarkia unguiculata* Lindl. (Onagraceae) Peach & Mazer (2019) found that anther type explains only a small portion of the variation in pollen performance, with environmental and biogeographical variables being more important for explaining differences in pollen performance. It is necessary to check pollen viability to determine if both stamens have fertile pollen or only pollen from thick stamens can germinate (Li *et al.*, 2015; Mesquita-Neto *et al.*, 2017).

Visitation and pollination

Several insect and bird species visited the flower of *M. macrophylla*. Butterflies, bumblebees, wasps and flies visited the flowers. Drosophilid flies (Diptera: Drosophilidae) and lepidopterans used the stamens as an oviposition site and the larvae consume the connective tissue and ovary as they grow. Oviposition and subsequent feeding on flowers have been reported for thrips (Childers & Achor, 1991; Teulon & Penman, 1991; Kiers, De Kogel, Balkema-Boomstra & Mollema, 2000) and moths (Aker & Udovic, 1981). The activity of wasps when visiting the flowers was unclear, but they are probably parasitoids or predators of other insects (Patt, Hamilton & Lashomb, 1997; Généau, Wäckers, Luke & Balmer, 2013).

Pollination by bumblebees is common in Melastomataceae, due to the presence of poricidal anthers in many melastomes (De Luca & Vallejo-Marín, 2013). Bumblebees that pollinate melastomes usually collect pollen to feed their brood, and they collect pollen from one type of stamen while the other type of stamen release pollen, that gets attached to the bee, through vibrations (Buchman, 1983; De Luca & Vallejo-Marín, 2013). However, there

was no evidence of a bumblebee obtaining a pollen reward from *M. macrophylla* flowers, because there was no damage in the stamen indicating pollen consumption. Thus it is possible that bumblebees came only to the flowers attracted by the color (Dyer, Spaethe & Prack, 2008).

Tanagers (Thraupidae) and finches (Fringillidae) were the only birds observed activating the bellows-like mechanism of the flower, so they were considered to be the legitimate pollinators of *M. macrophylla* in this study. As predicted, the pollination system of *M. macrophylla* matches the passerine syndrome described by Dellinger *et al.* (2014) in *Axinaea costaricensis* Cogn. and both species share the same families of birds as pollinators.

Although hummingbirds were recorded visiting some flowers, there was no evidence of pollen extraction, so hummingbirds were not considered as effective pollinators. Varassin *et al.* (2008) reported hummingbird visitations in *M. phlomoides* and *M. tomentosa*, and Dellinger *et al.* (2019b) confirmed hummingbird pollination, as well as bat and rodent pollination for *M. sanguinea* and *M. furvanthera*. Hummingbirds visit flowers for nectar, which accumulated on the abaxial surface of the filaments and on the petals of the pendant, pseudo-campanulate flowers (Dellinger *et al.* 2019b). It remains unclear why hummingbirds approached flowers of *M. macrophylla*, where no nectar was secreted. Hummingbirds likely approach the flowers only because they were attracted by the color of the petals (Stiles, 1976), or seeking insects to eat (Young, 1971).

Stamens as rewards

The thick stamens function as the main, and probably only reward provided by *M. macrophylla* to pollinators. There are other examples of floral parts serving as a reward for a vertebrate pollinator (Simpson & Neff, 1981). The inflorescences of *Freycinetia funicularis* (Savigny) Merr. (Pandanaaceae) has juicy bracts removed by bats (van der Pijil, 1956), while in *Feijoa sellowiana* (O. Berg) O. Berg and in *Myrrhinium atropurpureum* Schott (Myrtaceae) fleshy sweet petals are eaten by birds (Roitman, Montaldo & Medan, 1997; Sazima & Sazima, 2007). In the flowers of the slipperwort, *Calceolaria uniflora* Lam. (Calceolariaceae), a corolla appendage is eaten by birds. Within Melastomataceae, the

genus *Axinaea* is known for its bulbous stamens, which serve as a food reward eaten by frugivorous birds (Dellinger *et al.*, 2014).

The most interesting feature of the stamens is their high caloric content. With 15.279 J/g (3.65 kcal/g), a gram of thick stamens exceeds the caloric value of many fruits (Schaefer, Schmidt & Wesenberg, 2002; Vinson, Zubik, Bose, Samman & Proch, 2005), and slightly exceeds what was reported for *Axinaea* (Dellinger *et al.*, 2014). In contrast to the bird-pollinating behavior reported for *Axinaea*, in *M. macrophylla* birds do not swallow the stamens, so is possible that the caloric content is provided mainly by the secreted liquid. Tanagers feed on fruits and insects (Snow & Snow, 1971; Valburg, 1992), so stamens of *M. macrophylla* may function as a supplements to the birds' diet (Dellinger *et al.* 2014).

Evolutionary framework

The mechanism described for *M. macrophylla* does not support the division of labor hypothesis suggested to explain strongly dimorphic heterantherous flowers (Peach & Mazer, 2019). In *M. macrophylla*, thick stamens fulfill feeding and reproductive functions (possibly including attractiveness to pollinators), while the thin stamens, even though they have a caloric contribution and might have fertile pollen, are not used by pollinators and wither in the flower.

So why is there a set of stamens that is not used in reproduction, but is likely costly for the plant in terms of pollen production and caloric input? One possibility is the eventual partial or total loss of function of the unused stamens. Evidence of stamen loss and vestigial staminal forms can be found in many families of flowering plants (Wilson, 1982).

Dellinger *et al.* (2019) reported buzzing bees as the main pollinators for the tribe Merianieae, including heterantherous species and species with isomorphic stamens. In the mixed-vertebrate syndrome, most species have isomorphic stamens. Species of the genus *Axinaeae*, *M. macrophylla* and *Meriania inflata* Michelang. & R. Goldenb are grouped into the passerine pollination syndrome. *M. inflata* has pyramidal stamens as do many other species in the genus and actual pollinator observations are still lacking. Potentially, the thin stamens are on their way to becoming vestigial structures after the shift from buzz-pollination by bees to passerine pollination.

In order to clarify the function of the thin stamens, additional morphological and experimental studies are needed, which might also provide a better understanding of the ecological and biogeographical context of their evolution (Peach & Mazer, 2019). For now, there is no evidence on the possible function associated with heteranthery in *M. macrophylla*.

Conclusions

M. macrophylla is a bird-pollinated species with two sets of dimorphic stamens, but the evidence suggests that only one set of stamens is functional. Frugivorous birds seem to be the effective pollinators of this plant species. The stamens differ in shape, size of the connective (defined by the parenchyma volume) and slightly in pollen grain size (both are larger in the thick stamens), but not in color, tissue arrangement or pollen morphology. The set of thick stamens function as a food-body reward for birds (with a high caloric content) and also have a bellows-like mechanism activated when birds seize the stamens. This bellows-mechanism is responsible for pollen expulsion. The thin stamens produce pollen and also have some caloric value but are not consumed by birds and remain in the flowers as they wither. Finally, there is no evidence supporting the division of labor hypothesis, so another evolutionary context is needed to explain the role of heteranthery in *M. macrophylla*.

REFERENCES

- Aker, C. L., & Udovic, D. (1981). Oviposition and pollination behavior of the yucca moth, *Tegeticula maculata* (Lepidoptera: Prodoxidae), and its relation to the reproductive biology of *Yucca whipplei* (Agavaceae). *Oecologia*, 49(1), 96-101.
- Almeda, F. (1993). An evaluation of the Mesoamerican species of *Meriania* (Melastomataceae: Merianieae). *Proceedings of the California Academy of Sciences*, 48(7), 141–152.
- Almeda, F. (2003). Melastomataceae, pp. 394-575. In: Hammel, B.E., Grayum, M.H., Herrera, C. & Luer, C. A., eds., Manual de Plantas de Costa Rica, Volumen vi. Missouri Botanical Garden Press, St. Louis, Missouri.
- Baker, H. G., Baker, I., & Hodges, S. A. (1998). Sugar Composition of Nectars and Fruits Consumed by Birds and Bats in the Tropics and Subtropics 1. *Biotropica*, 30(4), 559-586.
- Barrett, S. C. (2002). Evolution of sex: the evolution of plant sexual diversity. *Nature Reviews Genetics*, 3(4), 274-284.
- Barrett, S. C. (2010). Darwin's legacy: the forms, function and sexual diversity of flowers. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 365(1539), 351-368.
- Buchmann, S. L. (1983). Buzz pollination in angiosperms. In C. E. Jones, & R. J. Little (Eds.), *Handbook of Experimental Pollination Biology* (pp. 63-113). United States, New York: Van Nostrand Reinhold Company
- Calderón-Sáenz, E., & Mendoza-Cifuentes, H. (2000). Melastomatáceas de los géneros *Axinaea*, *Blakea*, *Castratella*, *Centronia*, *Killipia*, *Meriania*, *Monochaetum*, *Ossaea* y *Tibouchina* en Colombia. *Biota Colombiana*, 1(3), 336–356.
- Childers, C. C., & Achor, D. S. (1991). Feeding and oviposition injury to flowers and developing floral buds of 'Navel' orange by *Frankliniella bispinosa* (Thysanoptera: Thripidae) in Florida. *Annals of the Entomological Society of America*, 84(3), 272-282.

- Cornell University. (2018). Merlin Bird ID by Cornell Lab (1.5) [Mobile application software]. Retrieved from <https://apps.apple.com/us/app/merlin-bird-id-by-cornell-lab/id773457673>
- Darwin, C. (1862). Letter to Asa Gray, 22 January. Available at URL: <https://www.darwinproject.ac.uk/letter/?docId=letters/DCP-LETT3404.xml;query=;brand=default> , accessed 23 October 2019.
- De Luca, P. A., & Vallejo-Marín, M. (2013). What's the 'buzz'about? The ecology and evolutionary significance of buzz-pollination. *Current Opinion in Plant Biology*, 16(4), 429-435.
- Dellinger, A. S., Chartier, M., Fernández-Fernández, D., Penneys, D. S., Alvear, M., Almeda, F., ... & Schönenberger, J. (2019a). Beyond buzz-pollination—departures from an adaptive plateau lead to new pollination syndromes. *New Phytologist*, 221(2), 1136-1149.
- Dellinger, A. S., Penneys, D. S., Staedler, Y. M., Fragner, L., Weckwerth, W., & Schönenberger, J. (2014). A specialized bird pollination system with a bellows mechanism for pollen transfer and staminal food body rewards. *Current Biology*, 24(14), 1615-1619.
- Dellinger, A. S., Scheer, L. M., Artuso, S., Fernández-Fernández, D., Sornoza, F., Penneys, D. S., ... & Schönenberger, J. (2019b). Bimodal Pollination Systems in Andean Melastomataceae Involving Birds, Bats, and Rodents. *The American Naturalist*, 194(1), 000-000.
- Dyer, A. G., Spaethe, J., & Prack, S. (2008). Comparative psychophysics of bumblebee and honeybee colour discrimination and object detection. *Journal of Comparative Physiology A*, 194(7), 617.
- Erdtman, G. (1969). *Handbook of palynology. Morphology, taxonomy, ecology. An introduction to the study of pollen grains and spores*. New York: Hafner.
- Géneau, C. E., Wäckers, F. L., Luka, H., & Balmer, O. (2013). Effects of extrafloral and floral nectar of *Centaurea cyanus* on the parasitoid wasp *Microplitis mediator*: olfactory attractiveness and parasitization rates. *Biological Control*, 66(1), 16-20.

- Holdridge, L.R. 1987. Ecología basada en zonas de vida. San José, Costa Rica, IICA, 216 p.
- Kiers, E., De Kogel, W. J., Balkema-Boomstra, A., & Mollema, C. (2000). Flower visitation and oviposition behavior of *Frankliniella occidentalis* (Tysan., Thripidae) on cucumber plants. *Journal of Applied Entomology*, 124(1), 27-32.
- Li, J. K., Song, Y. P., Xu, H., Zhang, Y. W., Zhu, J. Y., & Tang, L. L. (2015). High ratio of illegitimate visitation by small bees severely weakens the potential function of heteranthery. *Journal of Plant Ecology*, 8(2), 213-223.
- Lu, G., Wu, W., Wang, R., Li, X., & Wang, Y. (2009). Division of labor of heteromorphic stamens in *Melastoma malabathricum*. *Biodiversity Science*, 17(2), 174-181.
- Lumer, C. (1980). Rodent pollination of *Blakea* (Melastomataceae) in a Costa Rican cloud forest. *Brittonia*, 32(4), 512-517.
- Luo, Z., Zhang, D., & Renner, S. S. (2008). Why two kinds of stamens in buzz-pollinated flowers? Experimental support for Darwin's division-of-labour hypothesis. *Functional Ecology*, 22(5), 794-800.
- Mendoza Cifuentes, H., & Fernández Alonso, J. L. (2010). Evaluación de caracteres del cáliz y de los estambres en la tribu Merianieae (Melastomataceae) y definición de homologías. *Revista de la Academia Colombiana de Ciencias*, 34(131), 143-171.
- Mesquita-Neto, J. N., Costa, B. K. P., & Schlindwein, C. (2017). Heteranthery as a solution to the demand for pollen as food and for pollination—Legitimate flower visitors reject flowers without feeding anthers. *Plant Biology*, 19(6), 942-950.
- Müller, F. (1883). Two kinds of stamens with different functions in the same flower. *Nature*, 27(694), 364.
- Patt, J. M., Hamilton, G. C., & Lashomb, J. H. (1997). Foraging success of parasitoid wasps on flowers: interplay of insect morphology, floral architecture and searching behavior. *Entomologia Experimentalis et Applicata*, 83(1), 21-30.

- Peach, K., & Mazer, S. J. (2019). Heteranthery in *Clarkia*: pollen performance of dimorphic anthers contradicts expectations. *American Journal of Botany*, 106(4), 598-603.
- Pérez Umaña, D. J. (2017). Evaluación del potencial turístico de geomorfositos del Parque Nacional Volcán Poás (Tesis de licenciatura). Universidad Nacional, Heredia, Costa Rica.
- R Core Team. 2016. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing
- Renner, S. S. (1989). A survey of reproductive biology in Neotropical Melastomataceae and Memecylaceae. *Annals of the Missouri Botanical Garden*, 76, 496-518.
- Roitman, G. G., Montaldo, N. H., & Medan, D. (1997). Pollination biology of *Myrrhinium atropurpureum* (Myrtaceae): sweet, fleshy petals attract frugivorous birds. *Biotropica*, 29(2), 162-168.
- Sazima, I., & Sazima, M. (2007). Petiscos florais: pétalas de *Acca sellowiana* (Myrtaceae) como fonte alimentar para aves em área urbana no Sul do Brasil. *Biota Neotropica*, 7(2), 307-311.
- Sazima, M., Vogel, S., Cocucci, A., & Hausner, G. (1993). The perfume flowers of *Cyphomandra* (Solanaceae): pollination by euglossine bees, bellows mechanism, osmophores, and volatiles. *Plant Systematics and Evolution*, 187(1-4), 51-88.
- Schaefer, H. M., Schmidt, V., & Wesenberg, J. (2002). Vertical stratification and caloric content of the standing fruit crop in a tropical lowland forest1. *Biotropica*, 34(2), 244-253.
- Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7), 671.
- Sérsic, A. N., & Cocucci, A. A. (1996). A remarkable case of ornithophily in *Calceolaria*: food bodies as rewards for a non-nectarivorous bird. *Botanica Acta*, 109(2), 172-176.
- Simpson, B. B., & Neff, J. L. (1981). Floral rewards: alternatives to pollen and nectar. *Annals of the Missouri Botanical Garden*, 301-322.

- Snow, B. K., & Snow, D. W. (1971). The feeding ecology of tanagers and honeycreepers in Trinidad. *The Auk*, 88(2), 291-322.
- Spaethe, J., Tautz, J., & Chittka, L. (2001). Visual constraints in foraging bumblebees: flower size and color affect search time and flight behavior. *Proceedings of the National Academy of Sciences*, 98(7), 3898-3903.
- Stiles, F. G. (1976). Taste preferences, color preferences, and flower choice in hummingbirds. *The Condor*, 78(1), 10-26.
- Tánaka, K. (1989). High resolution scanning electron microscopy of the cell. *Biology of the Cell*, 65(2), 89-98.
- Teulon, D. A. J., & Penman, D. R. (1991). Effects of temperature and diet on oviposition rate and development time of the New Zealand flower thrips, *Thrips obscuratus*. *Entomologia Experimentalis et Applicata*, 60(2), 143-155.
- Ulloa, C. U., & Homeier, J. (2008, December). *Meriania franciscana* (Melastomataceae), a new species of the Andes of Ecuador. *Anales del Jardín Botánico de Madrid*, 65(2), 383-387.
- Valburg, L. K. (1992). Feeding preferences of common bush-tanagers for insect-infested fruits: avoidance or attraction?. *Oikos*, 29-33.
- Vallejo-Marín, M., Da Silva, E. M., Sargent, R. D., & Barrett, S. C. (2010). Trait correlates and functional significance of heteranthery in flowering plants. *New Phytologist*, 188(2), 418-425.
- Vallejo-Marín, M., Manson, J. S., Thomson, J. D., & Barrett, S. C. (2009). Division of labour within flowers: heteranthery, a floral strategy to reconcile contrasting pollen fates. *Journal of Evolutionary Biology*, 22(4), 828-839.
- van der Pijl, L. (1956). Remarks on pollination by bats in the genera *Freycinetia*, *Duabanga* and *Haplophragma*, and on chiropterophily in general. *Acta Botanica Neerlandica*, 5(2), 135-144.
- Varassin, I. G., Penneys, D. S., & Michelangeli, F. A. (2008). Comparative anatomy and morphology of nectar-producing Melastomataceae. *Annals of Botany*, 102(6), 899-909.

- Vinson, J. A., Zubik, L., Bose, P., Samman, N., & Proch, J. (2005). Dried fruits: excellent in vitro and in vivo antioxidants. *Journal of the American College of Nutrition*, 24(1), 44-50.
- Wilson, C. L. (1982). Vestigial structures and the flower. *American Journal of Botany*, 69(8), 1356-1365.
- Young, A. M. (1971). Foraging for insects by a tropical hummingbird. *The Condor*, 73(1), 36-45.