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# When males and hermaphrodites coexist in a population: Reproductive Biology of *Picconia excelsa* (Ait.) DC

## INTRODUCTION

## Flowering Phenology. Are Sexual Phenotypes real? Or are they Phenological stage?

## MATERIALS & METHODS

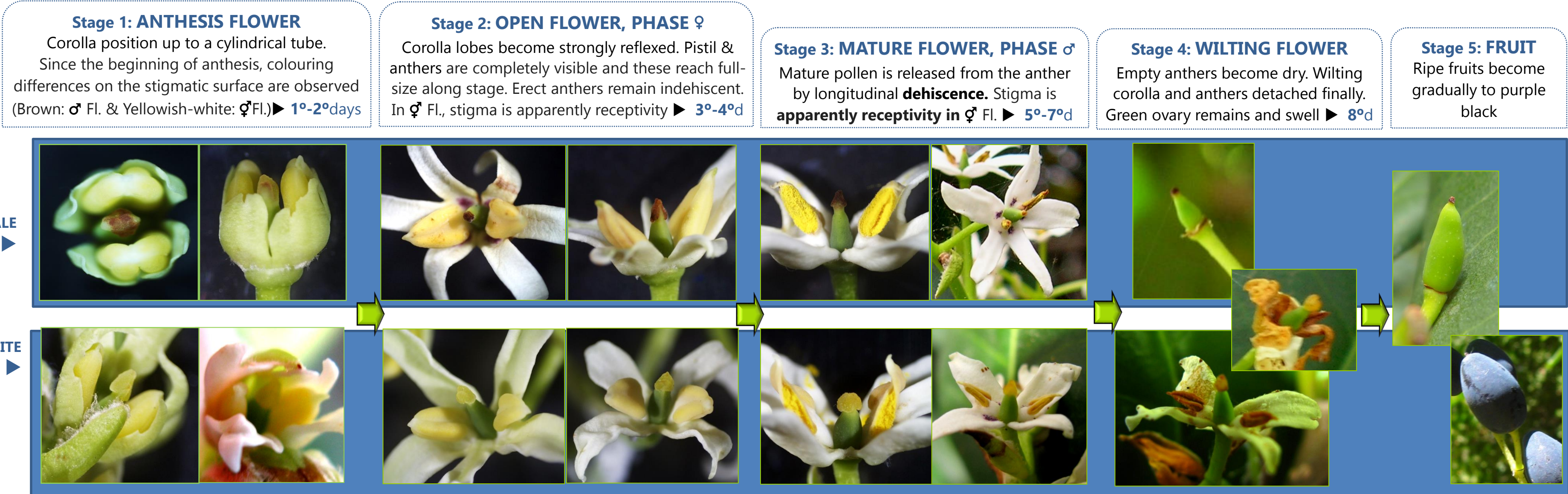
**Seasonal Phenology:** In **TILOS de MOYA** (Natural Population), 34 different individual plants were randomly marked with labels in order to record monthly phenological events.

**Floral Phenology:** In **JBCVC** (Cultivation Area), single flower buds in tagged inflorescences short before opening were marked with coloured threads (2 inflo./tree; 4 ind.). Observations were carried on daily during 20 days. Flowering period, flower lifespan and timing of anther dehiscence were recorded, in order to aid understanding of phenological patterns of *Picconia* flowering (dichogamy) and to evaluate particularly the consistency of the presence of two Sexual Phenotypes.

## RESULTS

### Floral Phenology

In this study, flower phenology was divided into **five** stage for both Sexual Phenotypes.



## MATERIALS & METHODS

Male tree/hermaphrodite tree proportion (sex ratio) of *P. excelsa* were calculated in **TILOS de MOYA** and in **JBCVC** from data obtained during the phenological survey.

## RESULTS

## Sex Ratio & Fruit set. Is this condition maintained in the natural environment?

## RESULTS

Our census of sex ratios have confirmed the presence of the two sexual phenotypes (Pérez de Paz *et al.*, 2017) in natural environment (see table below).

Slightly biased sex ratios were found in our preliminary prospect, being higher the proportion of male individual in JBCVC while hermaphrodite individuals dominated slightly in TILOS de MOYA.

Population	N	♂ Ind. (Brownish Stigma)	♀ Ind. (Whitish Stigma)	Sex Ratio (♂/♀)
TILOS de MOYA (Natural Population)	34	47,06%	55,88%	0,84
JBCVC (Cultivation area)	59	59,32%	40,68%	1,46

In the most of ♂ individuals, a high level ovary abortion was detected. However, some ♂ individuals occasionally borne greenish fruits. Occasionally, they reached a full size before the abscission. It is unknown if these fruits have a viable embryo.

♀ individuals borne fully formed fruits with mature seeds

## MATERIALS & METHODS

To determinate Pollen/ovule ratio (P/O) in ♂ and ♀ flowers, the number of pollen grains per theca was estimated using "NisElements" imaging software, and ovule numbers per flower were counted directly (in all cases, it was 4 ovules). Anthers with closed thecae from flower buds shortly before opening were used for that purpose.

The P/O ratio per flower was calculated dividing the mean number of pollen grains of at least ten flowers by their mean number of ovules

## RESULTS

The amount of pollen per anther varied considerably between ♂ and ♀ flowers and within each Sexual Phenotype (see left table).

P/O ranges between 21951 and 69512 for ♂ flowers, while it varies from 27442 to 82415. According to Cruden (1977), these high P/O ratios detected in both Sexual Phenotypes, is characteristic of **Xenogamous** species.

Sexual Phenotype	Population	Ind	Pollen/Theca	Pollen/Anther (2 Thecas/Ant)	Pollen/Flower (2 Ant./Fl)	P/O
♂ Individuals (Brownish Stigma)	TILOS de MOYA	PET2	21951	43902	87804	21951
		BGL4	39218	78436	156872	39218
		EPN1	44992	89984	179968	44992
	JBCVC	J14	---	69512	278048	69512
		JR13	56458	112916	225832	56458
♀ Individuals (Whitish Stigma)	TILOS de MOYA	JR9	51807	103614	207228	51807
		PET4	49511	99022	198044	49511
		JL1	34884	54884	109768	27442
	JBCVC	JR26	56735	113470	226940	56735
		JR19	82415	164830	329660	82415

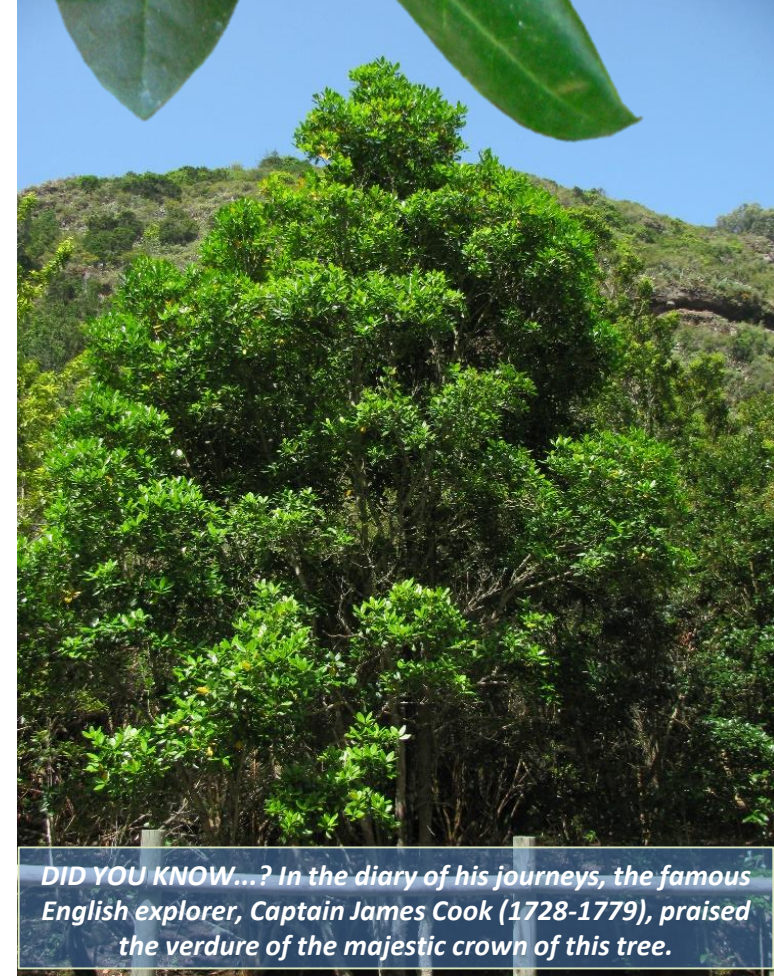
## DISCUSSION & CONCLUSIONS:

Through the parameters analyzed in the present study, it has been possible to confirm the presence of two Sexual Phenotypes for *Picconia excelsa* (♂ and ♀ individuals), aspect already detected in previous works (Pérez de Paz *et al.*, 2013, 2017), as well as to achieve a better knowledge about phenological & morphological characteristics and frequency of both Sexual Phenotypes. *Picconia excelsa* is one of the few cases of androdioecy in Canary Islands

- Seasonal phenology (Flowering & Fructification):**
  - At the level of seasonal phenology, differences between both sexual phenotypes were not detected.
  - Flowering, with a duration of ~2 months, achieved a peak when the climatic conditions are more benevolent (in spring).
  - Flowering intensity is variable from year to year, being eventually very abundant which supposes a punctual increase in the quantity of the floral resources available for pollinators
  - Fruiting are overlapping to flowering period and took place during the dry period (summer), providing large amount of food to natural dispersers (Valido, 1999; Arteaga *et al.*, 2006; Traveset, 2001).
- Floral phenology (Inflorescence & flowers):**
  - In general, inflorescence had an acropetal maturation
  - From the beginning of bud phase, ♂ and ♀ flowers can be morphologically distinguished by the appearance of their stigmas.
  - Five stages have been established for the flowers of both Sexual Phenotypes. In both cases, flower lifespan was around 7 days.
  - All hermaphrodite flowers showed a female phase (stigma attains apparently receptivity and maturation; it will be confirmed), with a duration of ≈ 2 days, before the dehiscent anthers begin to shed their pollen grains (protogynous dichogamy)
- Sex Ratio & Fruit set.**
  - A conservation level, this aspect (sex ratio) is very important and essential for mass propagation, multiplication and conservation of threatened plants.
  - Our sampling efforts have revealed a slight bias of the proportion of the Sex phenotype, being higher the proportion of male individual in JBCVC while hermaphrodite individuals dominated slightly in Los TILOS de MOYA. These coincides with others Oleaceae genus with functional androdioecia, as *Phillyrea*, *Osmanthus*, *Chionanthus* and *Fraxinus*, (Domínguez *et al.*, 1999; Hao *et al.*, 2011; Billiar *et al.*, 2015; Song *et al.*, 2016; Vernet *et al.*, 2016). It is also possible the presence of a homomorphic system of self-incompatibility (diallelic) among hermaphrodite individuals as in *Phillyrea angustifolia* (Samitou-Laprade *et al.*, 2010) and the confirmation of this aspect should be a future goal for this genus.
- morphological features:**
  - In general, all morphological features analyzed in hermaphrodite flowers (androecium and gynoecium sizes) are larger than those detected in male flowers.
  - In *Picconia*, the putative male flowers are morphologically hermaphrodites, showing two smaller anthers compared to those of hermaphrodites flowers, and gynoecium of smallest size and with brownish stigmatic surface (papillae), presumably non-functional (without fruit set), which is considered homologous to the "pistillodes" of their relatives (Xu *et al.*, 2014; Pérez de Paz *et al.*, 2017).
- P/O Ratio.**
  - According to results of P/O ratios (Cruden, 1977), *P. excelsa* is considered Xenogamous species.
  - The great number of pollen grains detected in *Picconia* is supported floral design (architecture and dicogamy).

As part of this project, we have undertaken several studies on micro-morphological attributes of flowers, flowering phenology and sex ratio of *Picconia excelsa* (Ait.) DC. This species was previously assessed as Vulnerable (Bañares *et al.* 1998, Oldfield *et al.* 1998), although not included in recent versions of the Spanish Red List.

## DISTRIBUTION



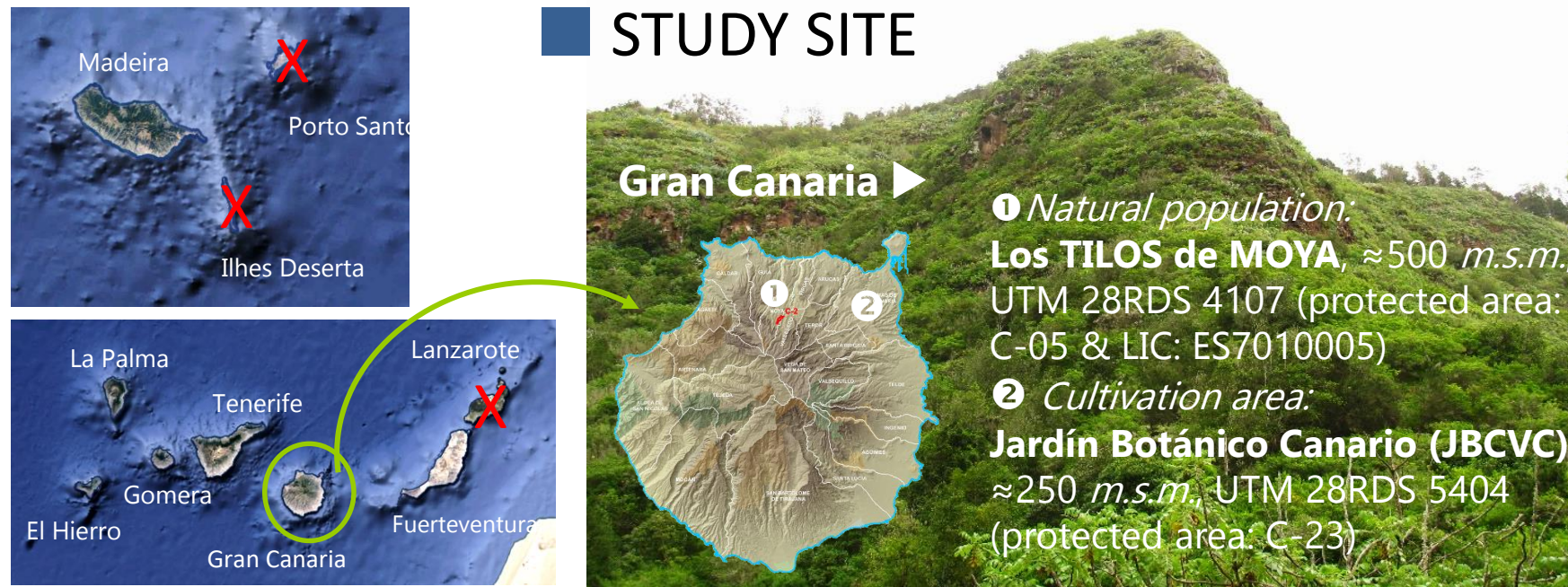
*Picconia* (Oleaceae) is an endemic genus to the Macaronesian Region with only two species, morphologically very similar:  
***Picconia excelsa*** (Ait.) DC., occurring in Madeira and almost all Canarian islands (as exception of Lanzarote)  
***Picconia azorica*** (Tutin) Knobl. in the Azores.

## STUDY SPECIES

***Picconia excelsa***, an important element of the laurisilva forests, is a very elegant evergreen tree, with 10-15 (20) m tall, and characterised by its white-grey, very rough bark. The leaves are leathery, opposite arrangement. For more basic species information, use app: ArbolappCanarias or [www.arbolappcanarias.es](http://www.arbolappcanarias.es)

***P. excelsa***,  $2n=46$  (Briggs, 1970), has flowers with 4 (5-6) white corolla lobes (fused in the base: inconspicuous corolla tubes), 2 (3-4) anthers and superior ovary that are joined into small inflorescence. Their flowers have mainly entomophily syndrome (and possibly anemophily; Pérez de Paz *et al.* 2017). The fleshy fruits are drupes, up to 2 cm in length which are dispersed by barochory, birds, endemic lizards and rats (Valido, 1999; Arteaga *et al.*, 2006; Traveset, 2001). Each fruit contains a single nutlet with a seed inside, and its greenish coloration changes to purple black when ripe.

## STUDY SITE



## Inflorescence Phenology

- On both ♂ and ♀ trees, flowers in an inflorescence opened simultaneously.
- On the inflorescences of both Sexual Phenotype, the last open flowers were principally located at the apical position. Therefore, flowers in different stage were observed at the same time on the inflorescences (relative asynchronous maturation of flowers on the inflorescences -from bottom to top).

- Our observation confirmed that these Sexual Phenotypes were conserved along different phenological stages
- This phenological study indicated that both ♂ and ♀ trees have identical flowering phenologies.
- The anthers of ♀ flowers dehiscence 5<sup>th</sup> day after flower opening (anthesis), indicating that these flowers are **protogynous** and with an exclusively female phase, which can extend around 2 days.
- In ♂ individuals, stigma remained with brown color along all phenological stages from buds stage. It was apparently non functional. Although the stigmatic receptivity has not been tested, gynoecium could be considered as pistillodes in ♂ flowers, as it has been suggested (Pérez de Paz *et al.*, 2017).

## MATERIALS & METHODS

Key morphological traits of flowers were measured to characterize the two Sexual Phenotypes

Each flower was observed individually under stereomicroscope and subsequently, all floral whorls were separated and arranged over a millimetric paper in order to make subjected to morphometric evaluation. Images of them were taken with digital camera (Nikon digital D5-U1) connected to stereomicroscope (Olympus SZ-CTV). All floral measurements were carried out using "NisElements" imaging software.

These analyses included 8 male (♂) and 7 hermaphrodite (♀) individuals, and at least two or three flowers per individual.

Non-parametric Mann-Whitney U-test was used in order to test possible statistical differences between Sexual Phenotypes ( $\alpha=0.05$ ). Statistical analysis was performed using XLSTAT.

## RESULTS

Nineteen biometric characters were recorded for the flowers of *P. excelsa* (6 for size of corollas, 7 for anthers and 6 for gynoecium). Principal morphological characteristics of the flowers are summarized for each sexual phenotype in different tables

### Corolla

There were no significant differences between ♂ and ♀ flowers in the most morphological characters analyzed for *Picconia* COROLLA. Only corolla tube width differed significantly between both sexual phenotype (see below table).

	N	Major Lobes		Minor Lobes		Inconspicuous Tube	
		Length (A)	Width (B)	Length (C)	Width (D)	Length (E)	Width (F)
♂ Individuals (Brownish Stigma)	16	6,78±0,42 (3,24-12,14)	2,67±0,12 (1,59-3,78)	6,41±0,42 (2,84-11,46)	2,6±0,14 (1,13-4,17)	1,77±0,07 (1,23-2,38)	4,83±0,14 (3,92-6,62)
♀ Individuals (Whitish Stigma)	18	7,19±0,31 (4,4-11,38)	2,7±0,09 (1,32-3,82)	6,91±0,3 (4,19-10,44)	2,68±0,11 (1,4-3,2)	1,86±0,07 (1,11-2,78)	5,16±0,1 (3,9-6,4)
Mann-Whitney U-test (p)		511 n.s. (0,428)	526 n.s. (0,543)	482,5 n.s. (0,253)	530 n.s. (0,716)	504,5 n.s. (0,383)	U=405,5* (0,037)

▲ **Descriptive statistics** for the two sexual phenotypes of the studied population and results of **Mann-Whitney U tests** ( $\alpha:0,05$ ). Numbers indicate sample size (N: total number of flower analyzed), mean ± SE and range (mm) of COROLLA size.

### Androecium

With the exception of widths of filaments and thecas, rest of morphological characters analyzed in ANDROECIUM differed very significantly between both sexual phenotype. Generally, anthers, filaments and thecae were longer in ♀ flowers than ♂ flowers (see below table). Likewise, ♀ flowers showed anther appendage biggest sizes (length & width).

	N	Anther		Filament		Theca		Appendage	
		Length (A)	Width (B)	Length (C)	Width (D)	Length (E)	Width (F)	Length (G)	Width (H)
♂ Individuals (Brownish Stigma)	16	4,31±0,1 (3,51-5,58)	2,23±0,06 (1,81-3,07)	1,03±0,02 (0,81-1,3)	3,11±0,07 (2,49-3,8)	0,8±0,02 (0,56-0,95)	0,55±0,03 (0,28-0,84)	0,57±0,02 (0,32-0,73)	0,32±0,03
♀ Individuals (Whitish Stigma)	18	4,95±0,05 (3,93-5,54)	2,52±0,06 (1,4-3,11)	1,08±0,02 (0,87-1,38)	3,4±0,03 (2,88-3,67)	0,79±0,01 (0,67-0,94)	0,71±0,02 (0,52-0,95)	0,67±0,02 (0,43-0,85)	0,29±0,01
Mann-Whitney U-test (p)		171,5*** (<0,0001)	255,5*** (0,001)	383,5 n.s. (0,126)	267,5** (0,002)	568,5 n.s. (0,315)	168,5*** (<0,0001)	220,5*** (<0,0001)	0,0***

▲ **Descriptive statistics** for the two sexual phenotypes of the studied population and results of **Mann-Whitney U tests** ( $\alpha:0,05$ ). Numbers indicate sample size (N: total number of flower analyzed), mean ± SE and range (mm) of ANDROECIUM size.

### Gynoecium

All morphological characters analyzed for GYNOCYCIUM differed very significantly between both Sexual Phenotype, being generally longer the gynoecium (length & width) and the style in ♀ flowers than ♂ flowers (see below table). Likewise, the stigmatic surfaces of ♀ flowers are bigger than ♂ flowers ones. Moreover, ♀ flowers showed wider stigma and stigmatic papillae than ♂ flowers.

	N	Gynoecium		Style Length (C)	Stigmatic surface Length (D)	Stigma Width (E)	Stigmatic Papillae Width (F)
		Length (A)	Width (B)				
♂ Individuals (Brownish Stigma)	16	3±0,06 (2,56-3,39)	1,07±0,03 (0,87-1,24)	0,7±0,02 (0,52-0,83)	1,14±0,04 (0,88-1,46)	0,49±0,02 (0,37-0,65)	0,14±0,01 (0,07-0,15)
♀ Individuals (Whitish Stigma)	18	3,45±0,04 (3,06-3,78)	1,15±0,02 (1,07-1,32)	0,88±0,02 (0,73-1,02)	2,27±0,06 (1,75-2,74)	0,85±0,02 (0,73-1)	0,29±0,01 (0,22-0,37)
Mann-Whitney U-test (p)		17,0*** (<0,0001)	72,0*** (0,012)	18,0*** (<0,0001)	0,0*** (<0,0001)	0,0*** (<0,0001)	0,0***

▲ **Descriptive statistics** for the two sexual phenotypes of the studied population and results of **Mann-Whitney U-tests** ( $\alpha:0,05$ ). Numbers indicate sample size (N), mean ± SE and range (mm) of GYNOCYCIUM (pistils) size.

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