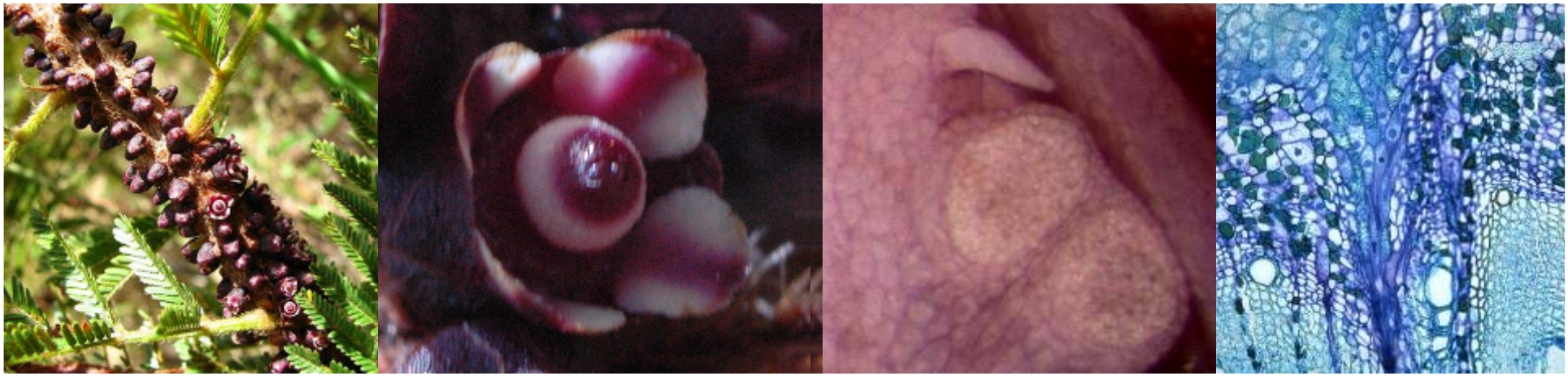


Skewed "Sex Ratios" in the peculiar holoparasite *Pilostyles* (Apodanthaceae - Cucurbitales)

Bianca de Azevedo BRASIL[1]

Teresa TERRAZAS[2]

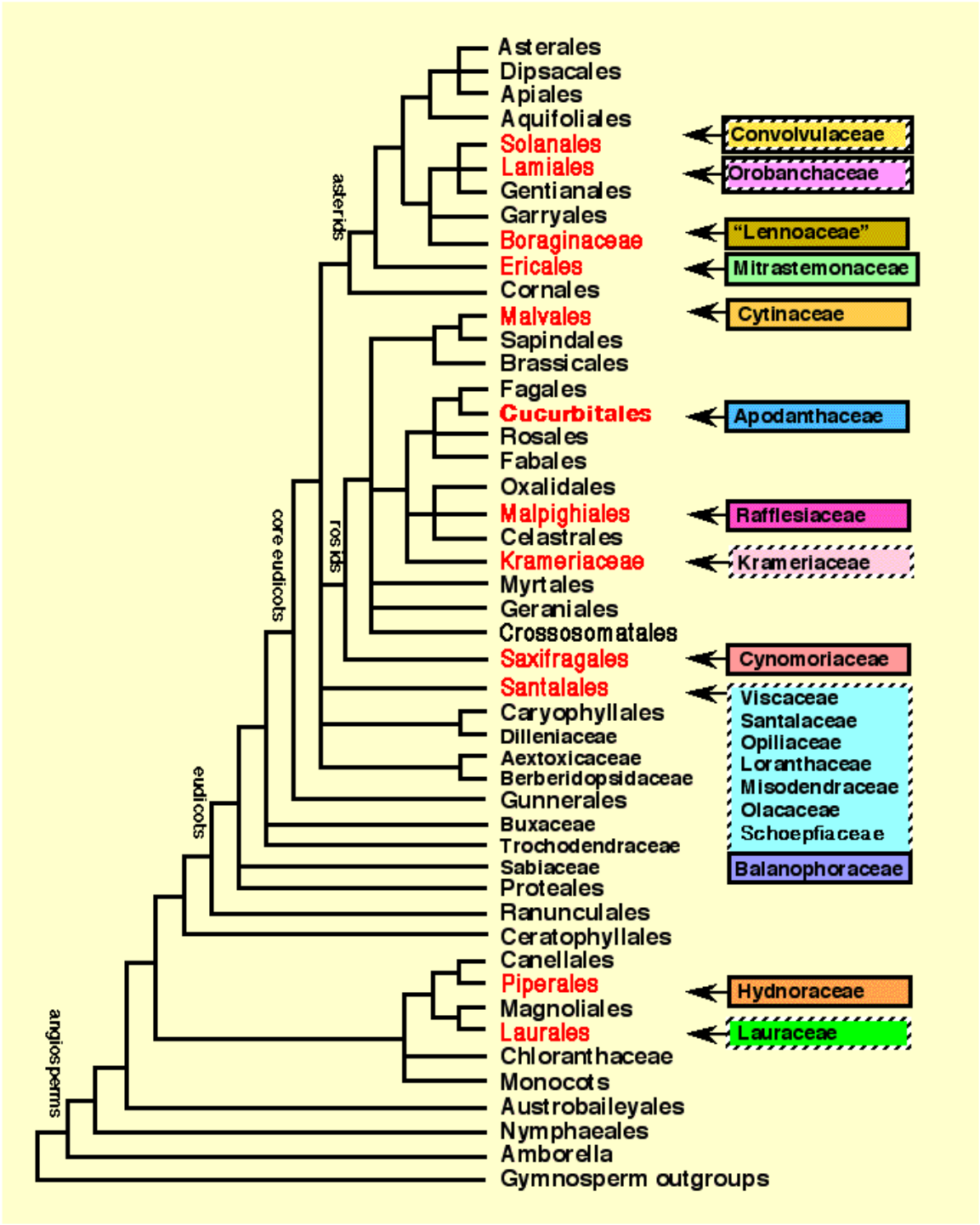
Jorge Ernesto MARIATH[3]

Gregório CECCANTINI[1]

[1]Departamento de Botânica – Instituto de Biociências - USP

[2]Dp Botánica – Instituto de Biologia – UNAM

[3 1]Dep. de Botânica – Instituto de Biologia - UFRGS



Hemiparasites

Holoparasites

Holoparasites

Convergent Evolution in 12 lineages

http://www.parasiticplants.siu.edu/Relation-Flowering.html
(Nickrent 2010)

Apodanthaceae

- Filipowicz & Renner (2010)

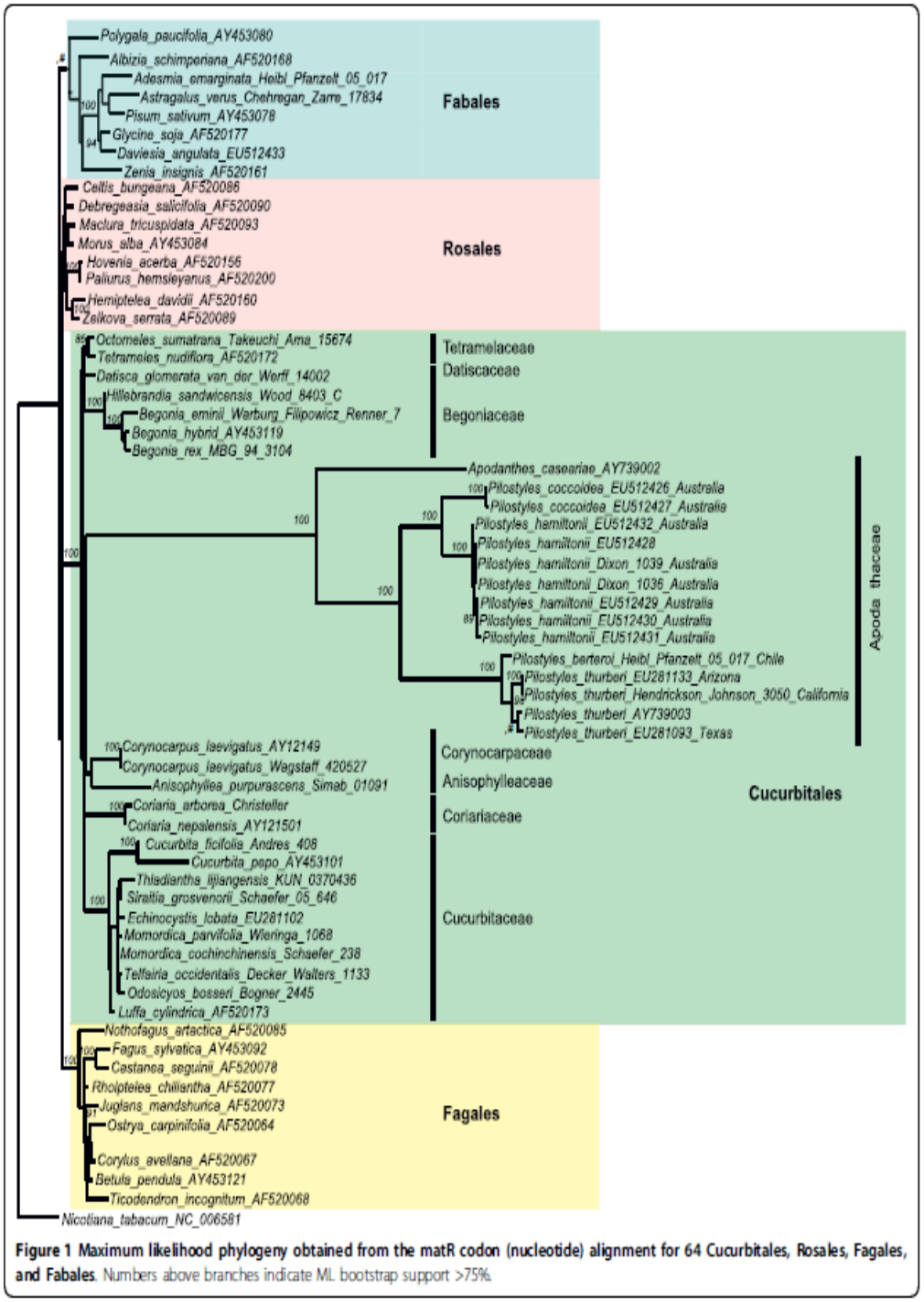


Figure 1 Maximum likelihood phylogeny obtained from the matR codon (nucleotide) alignment for 64 Cucurbitales, Rosales, Fagales, and Fabales. Numbers above branches indicate ML bootstrap support >75%.

Family Apodanthaceae

- Etymology

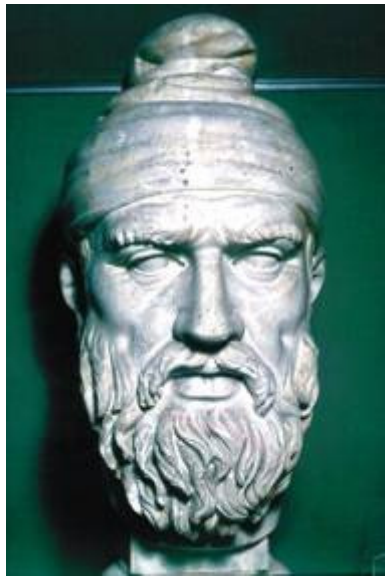
Apodanthes

Apodes = feetless
+
Anthes = flower

- Aclorophyllated plants, holoparasites of stems and rooth
- Vegetative extremly reduced to endophyte
- Genera: *Apodanthes*, *Berlinianche* e ***Pilostyles***

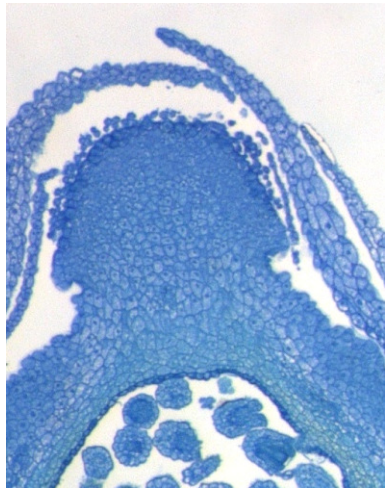
Pilostyles

Pileus (lat.) = kind of hat just to the head
+
Stylos (lat.) = Column



- Diversity
  ≈ 20 spp. In Americas, Middle East and Southwestern Australia

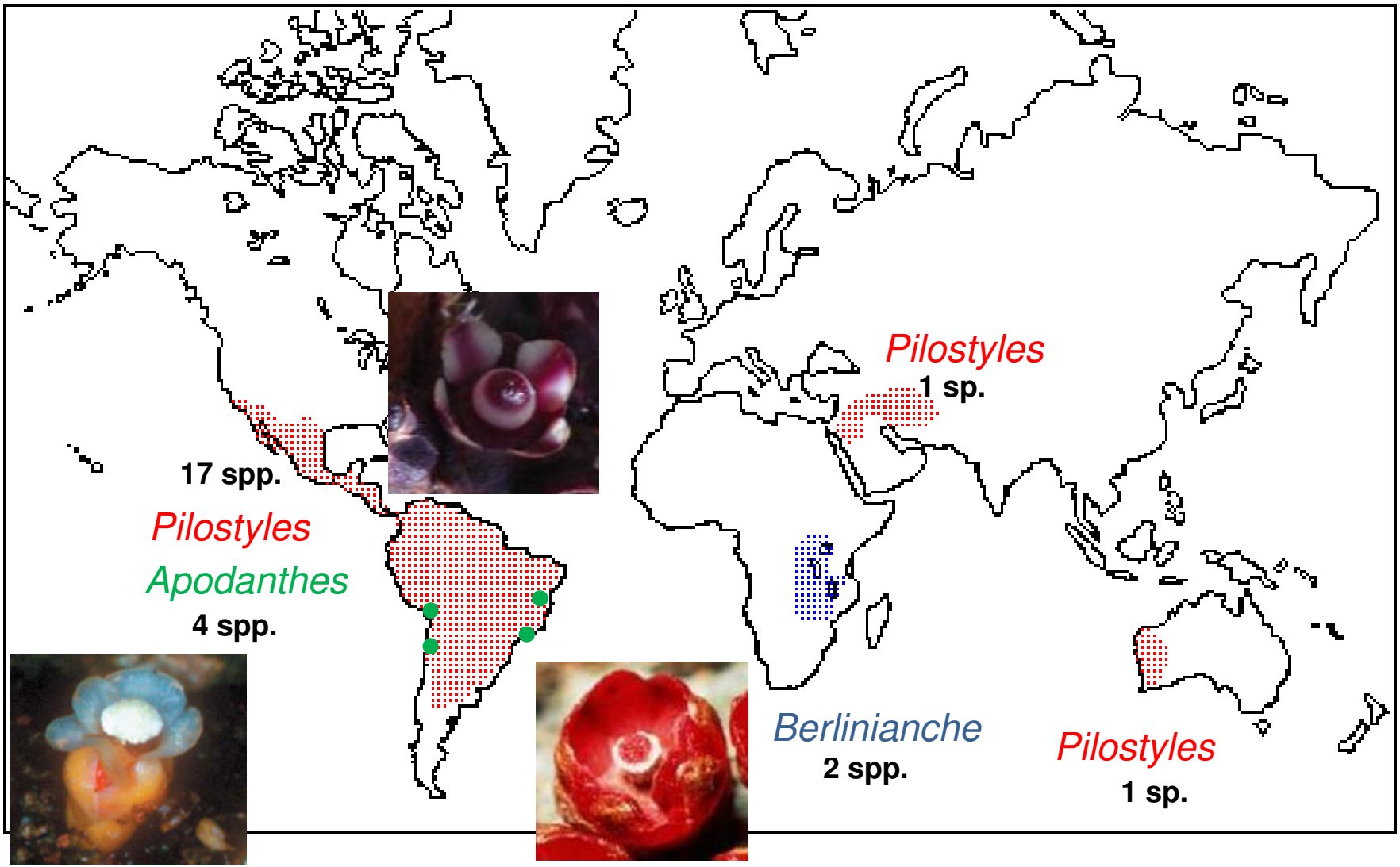
- Hosts
  Bauhinia (Caesalpinioideae)
  Adesmia, *Galactia*, Galea *Patagonium*, *Parosela* (Papilionoidae)
  Calliandra e *Mimosa* (Mimosoideae)



Vattimo (1971) e Vattimo (1972)

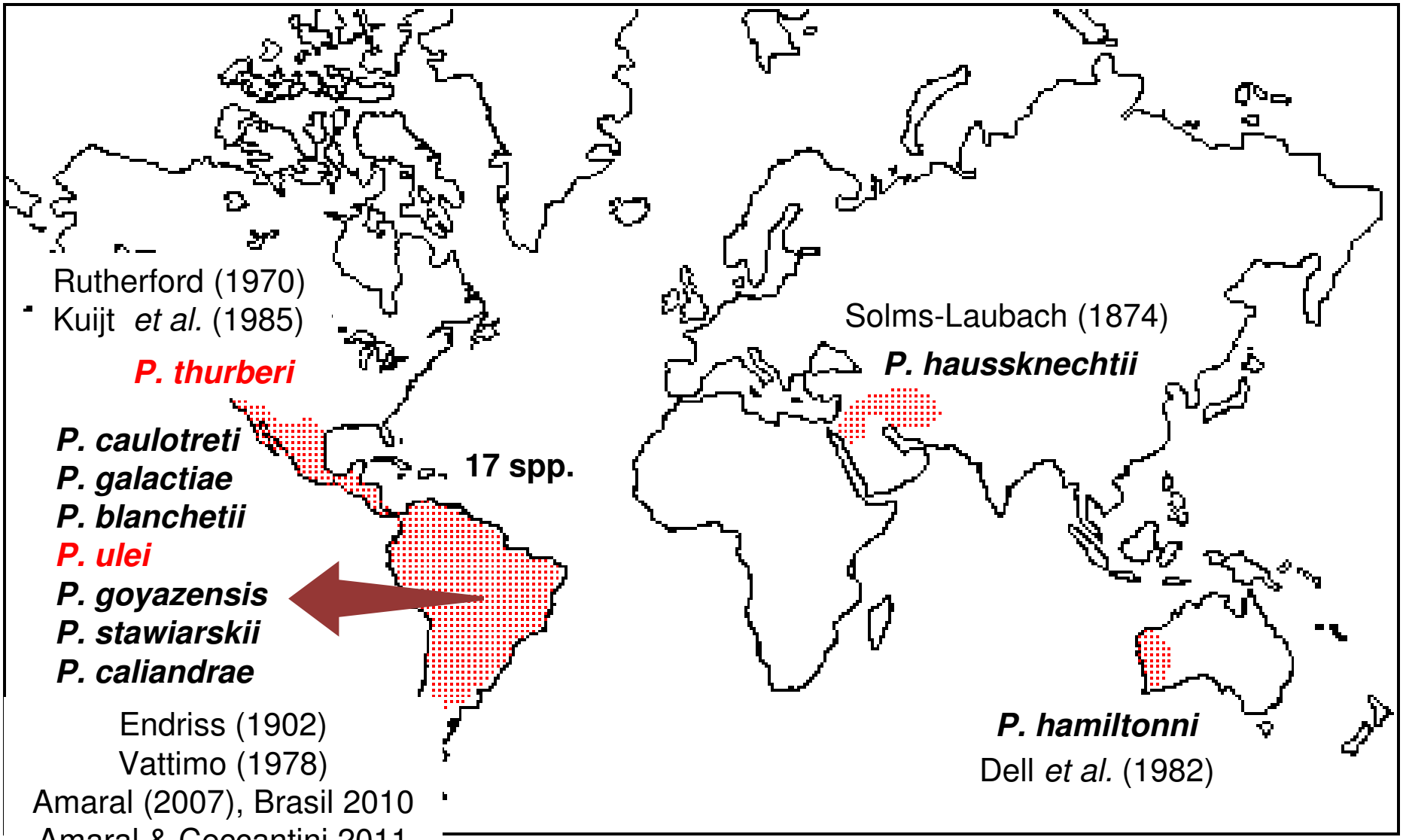
http://www.roembus.org/english/images/istoria_nationala/geto-dac.jpg

Apodanthaceae - distribution



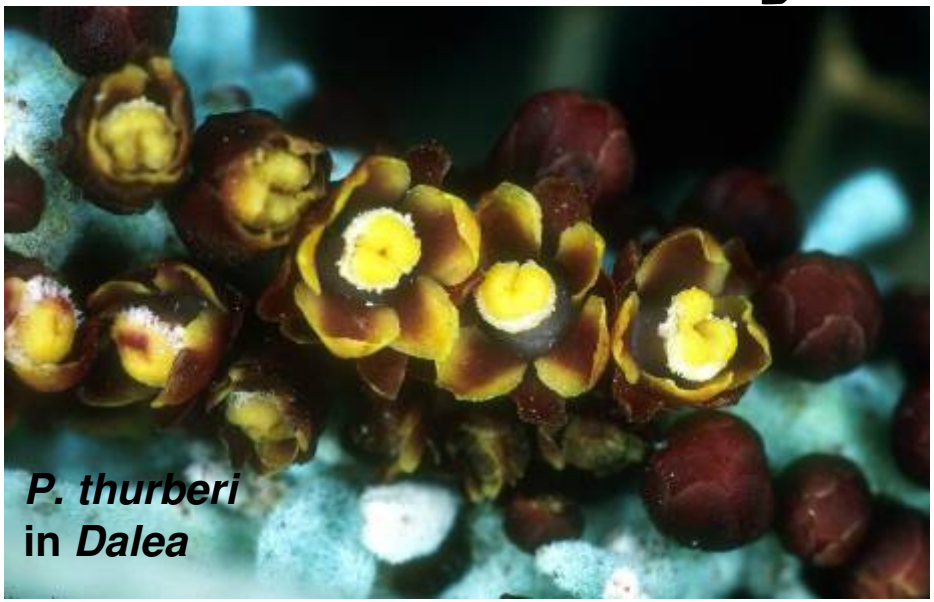
http://www.parasiticplants.siu.edu/Relation-Flowering.html

Pilostyles - distribution



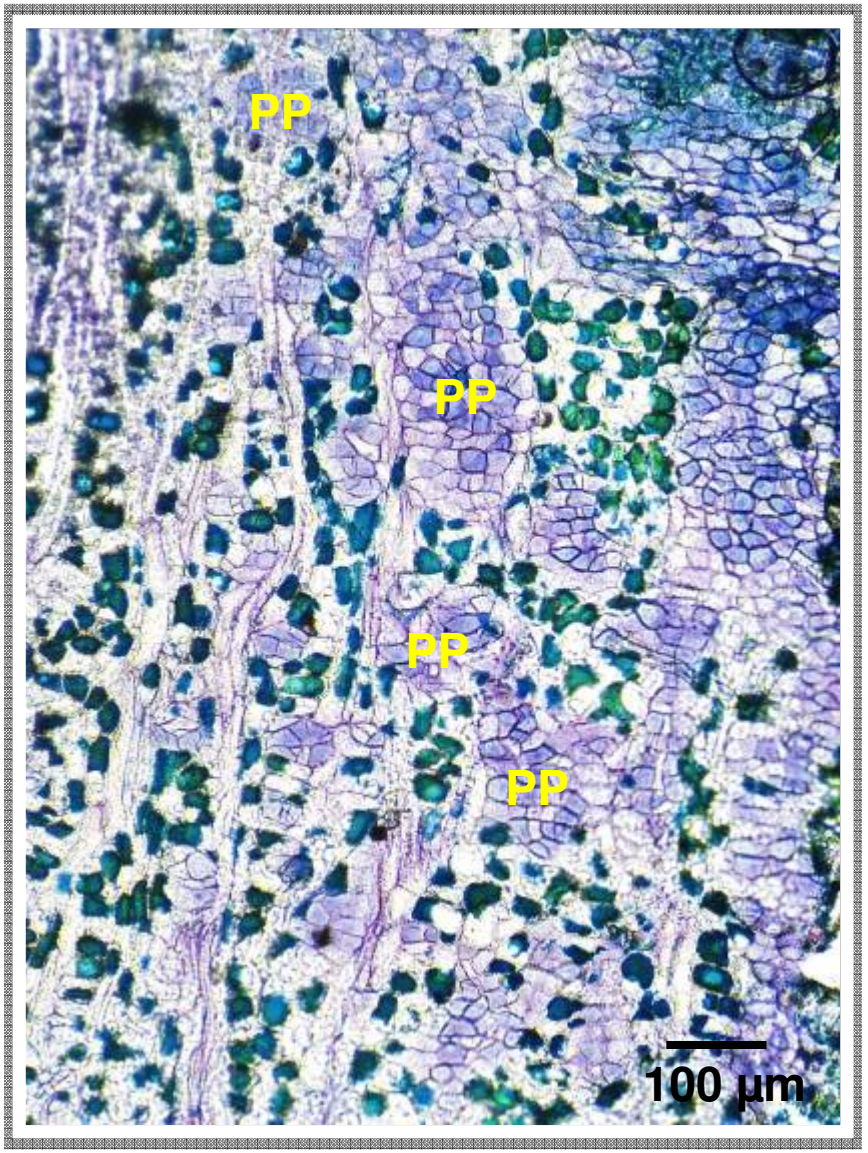
http://www.parasiticplants.siu.edu/Relation-Flowering.html

Genera *Pilostyles* - diversity



Endophytic System

- anastomozed bundles or masses of parenchimatic cells in a complex network (Amaral, 2007)
- Nuclei relatively larger and with two nucleola (Rutherford, 1970)
- Pmembrane with broad surface (Dell *et al*, 1982)
- penetrating sinkers



longitudinal tangencial slice of *Pilostyles ulei* in *Mimosa maguirei*

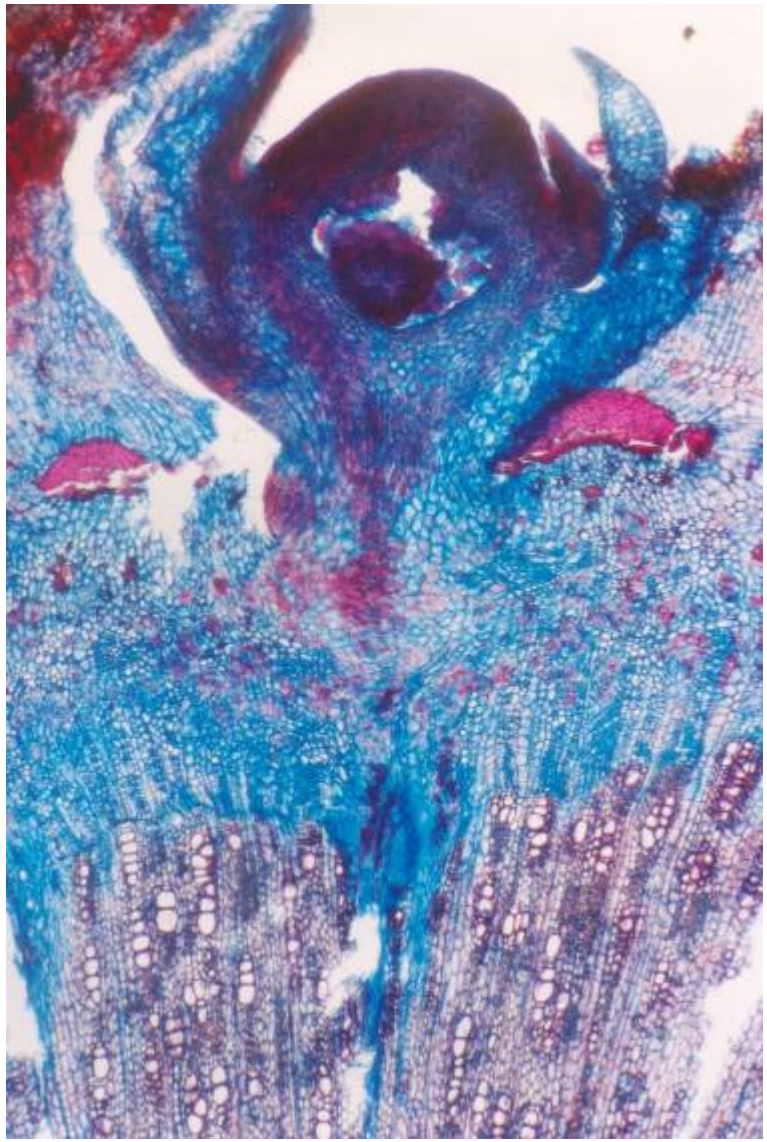
Endophytic System

- anastomozed bundles or masses of parenchimatic cells in a complex network (Amaral, 2007)

- Nuclei relatively larger and with two nucleola (Rutherford, 1970)

- Plasmatic membrane with broad surface (Dell *et al*, 1982)

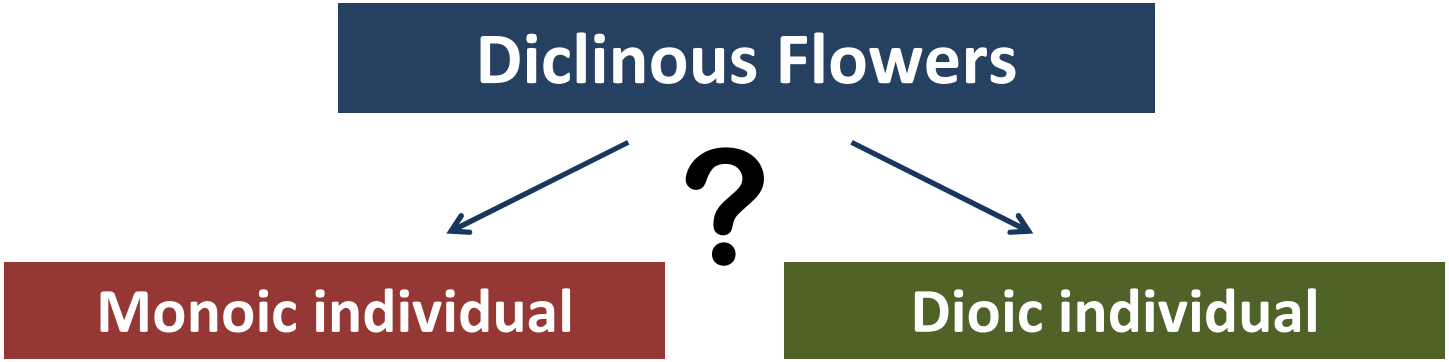
- penetrating sinkers



Family Apodanthaceae

SPORITY – SEX RATIO – SPORITY RATIO

• It is difficult to distinguish more than one parasite in the same host



• Kuijt (1969), Rutherford (1970), Vattimo (1971), Meijer (1993) e Groppo *et al*. (2007) – Dioic condition

• Kuijt (1969) – dioecy evolved recently (rudiments)

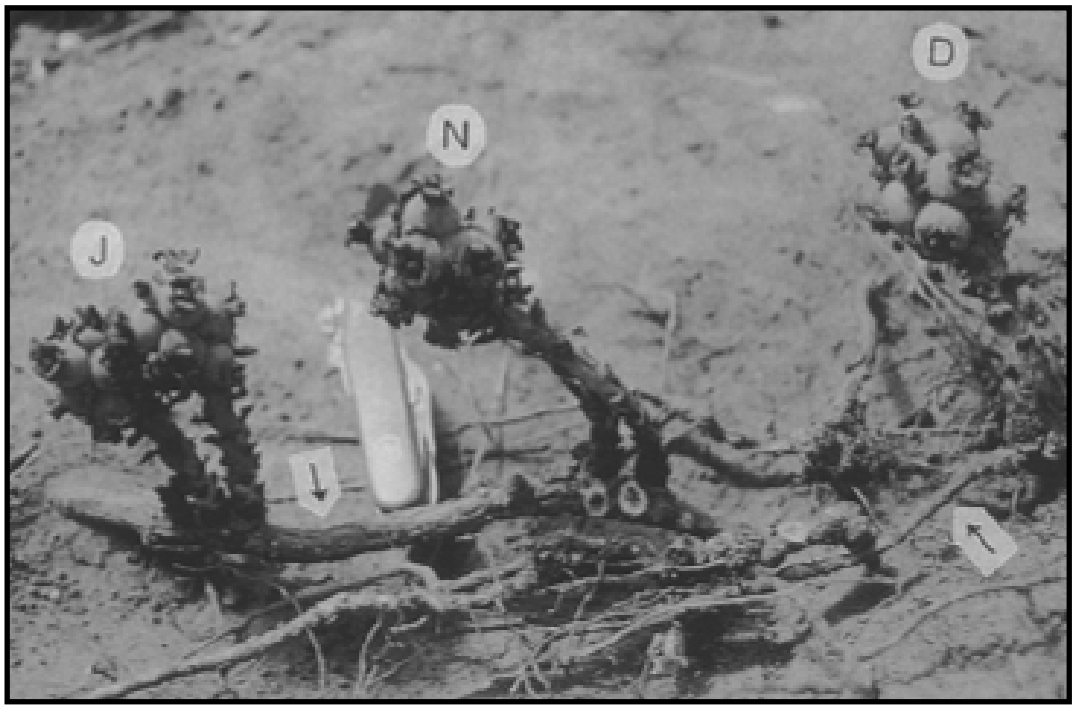
Pl. Syst. Evol. 210: 271–288 (1998)

Genetic variation, genetic structure and effective population size in the tropical holoparasitic endophyte *Bdallophyton bambusarum* (*Rafflesiaceae*)

José G. García-Franco, Valeria Souza, Luis E. Eguiarte, and Victor Rico-Gray

Received October 23, 1996; in revised version May 27, 1997

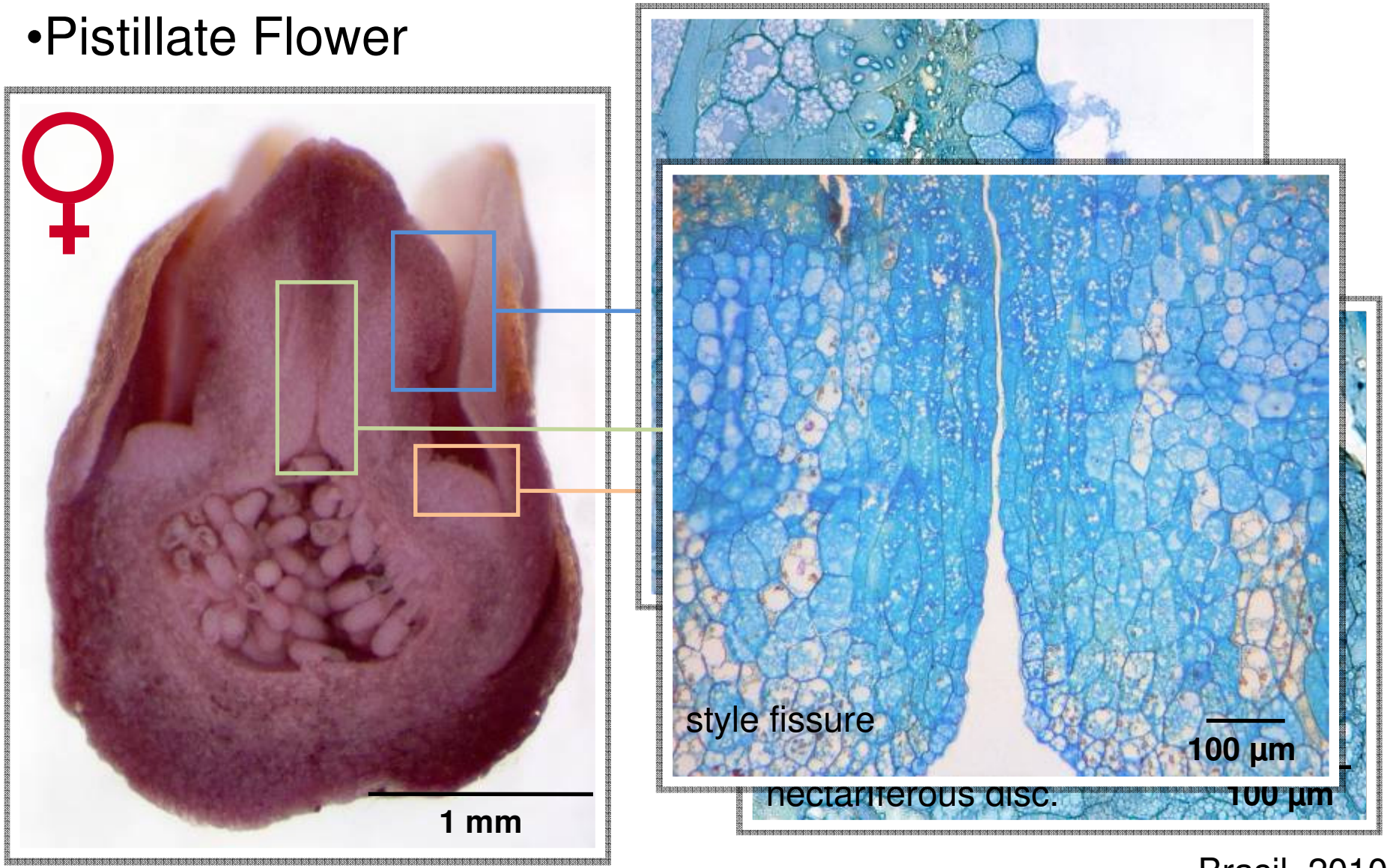
- Root Holoparasite
- 7 Aloenzyme Analyses
- Result: each inflorescence belongs to one single individual
- Dioiceous species



Bdallophyton bambusarum (Cytinaceae): inflorescences together, near and distant.

Flower anatomy

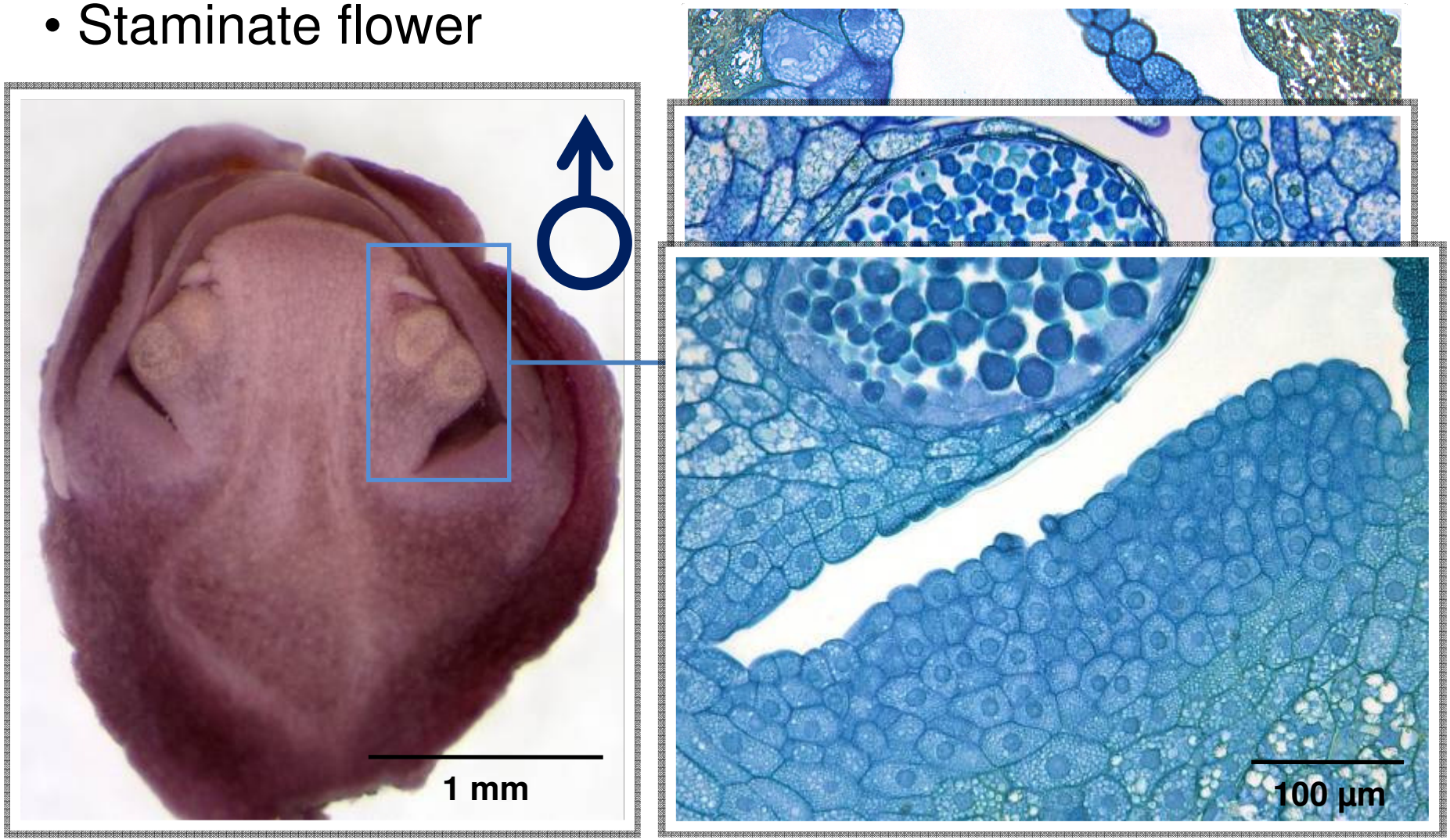
•Pistillate Flower



Brasil, 2010

Flower anatomy

- Staminate flower

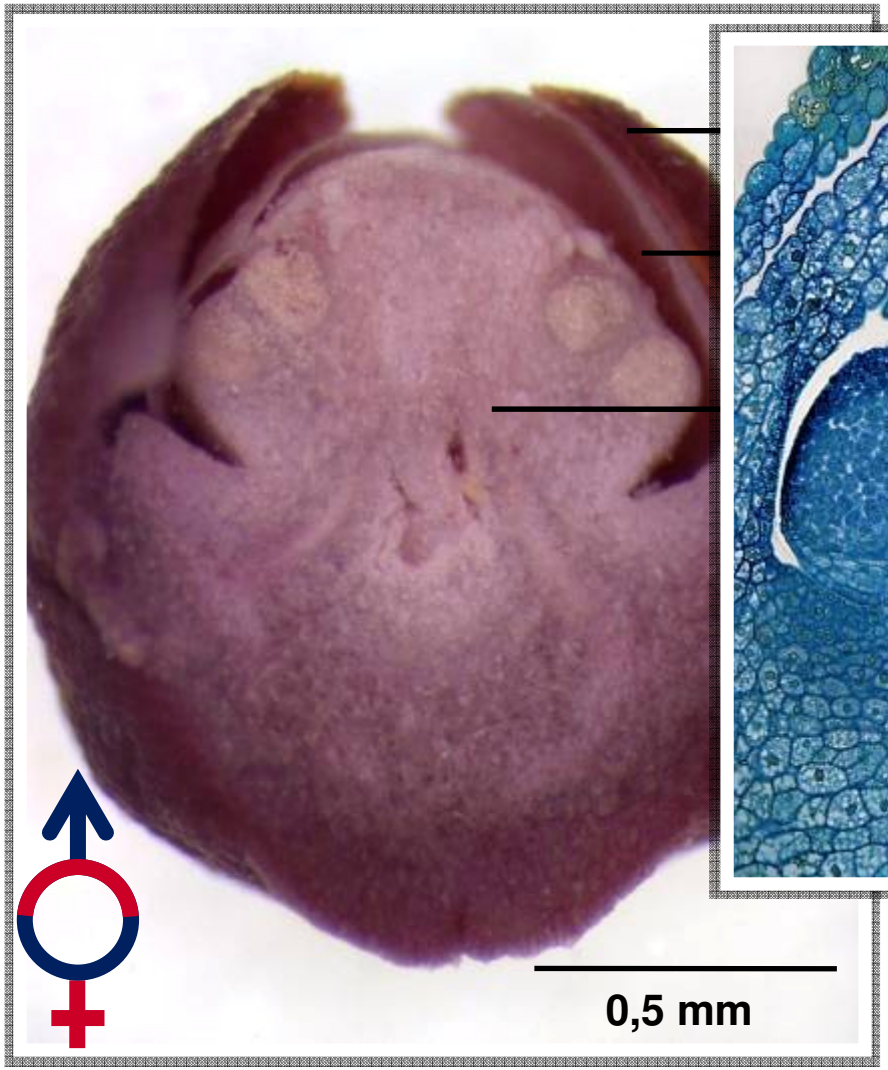


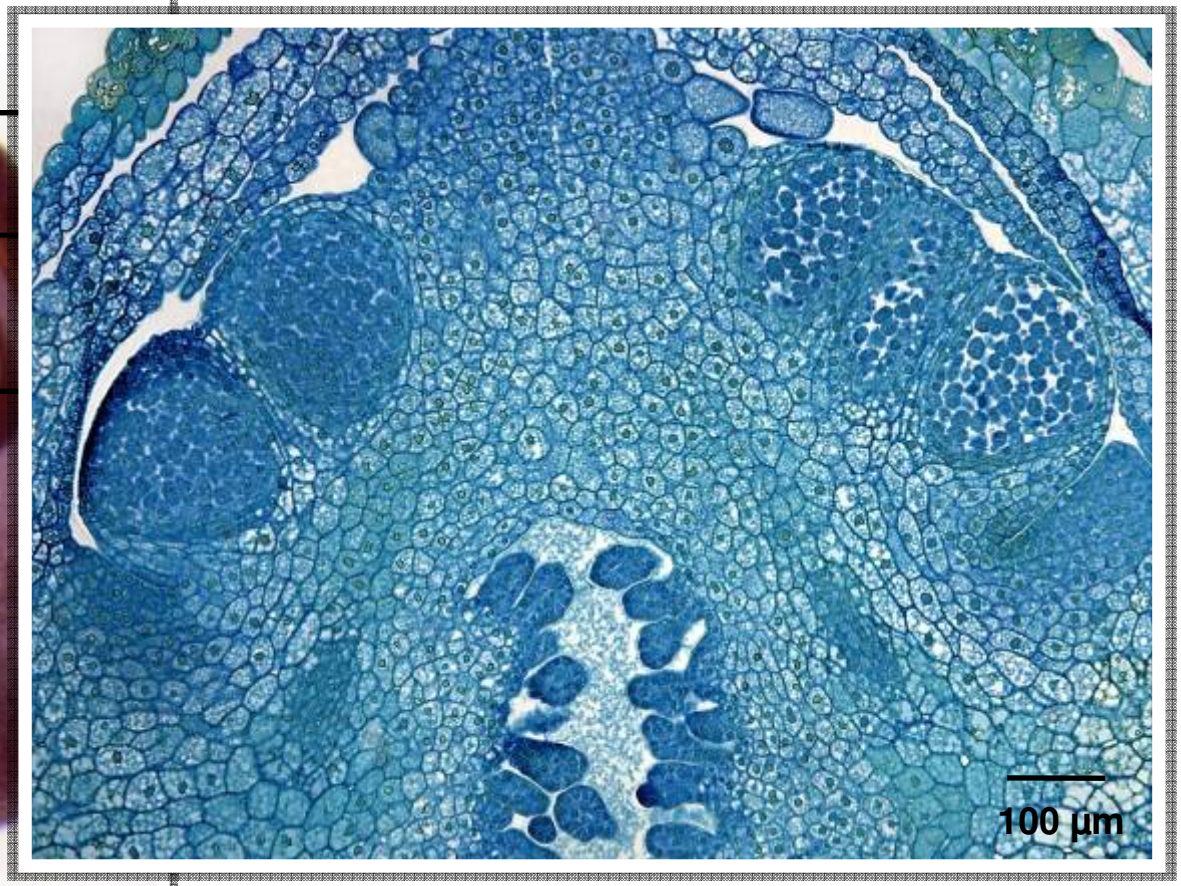
Nectariferous disk

Brasil, 2010

Flower anatomy

- Monoclinous flower



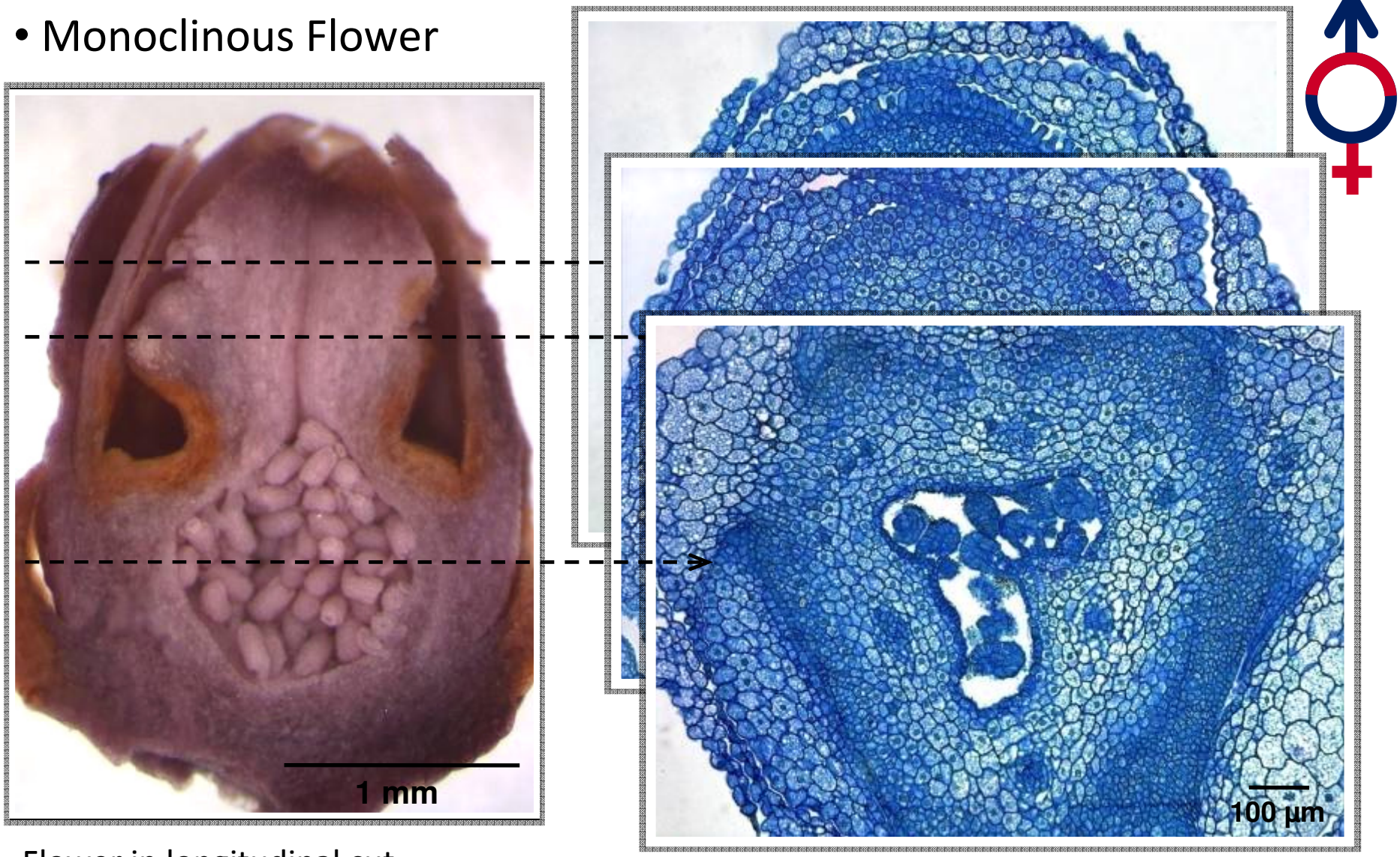


Flower in longitudinal cut

Brasil, 2010

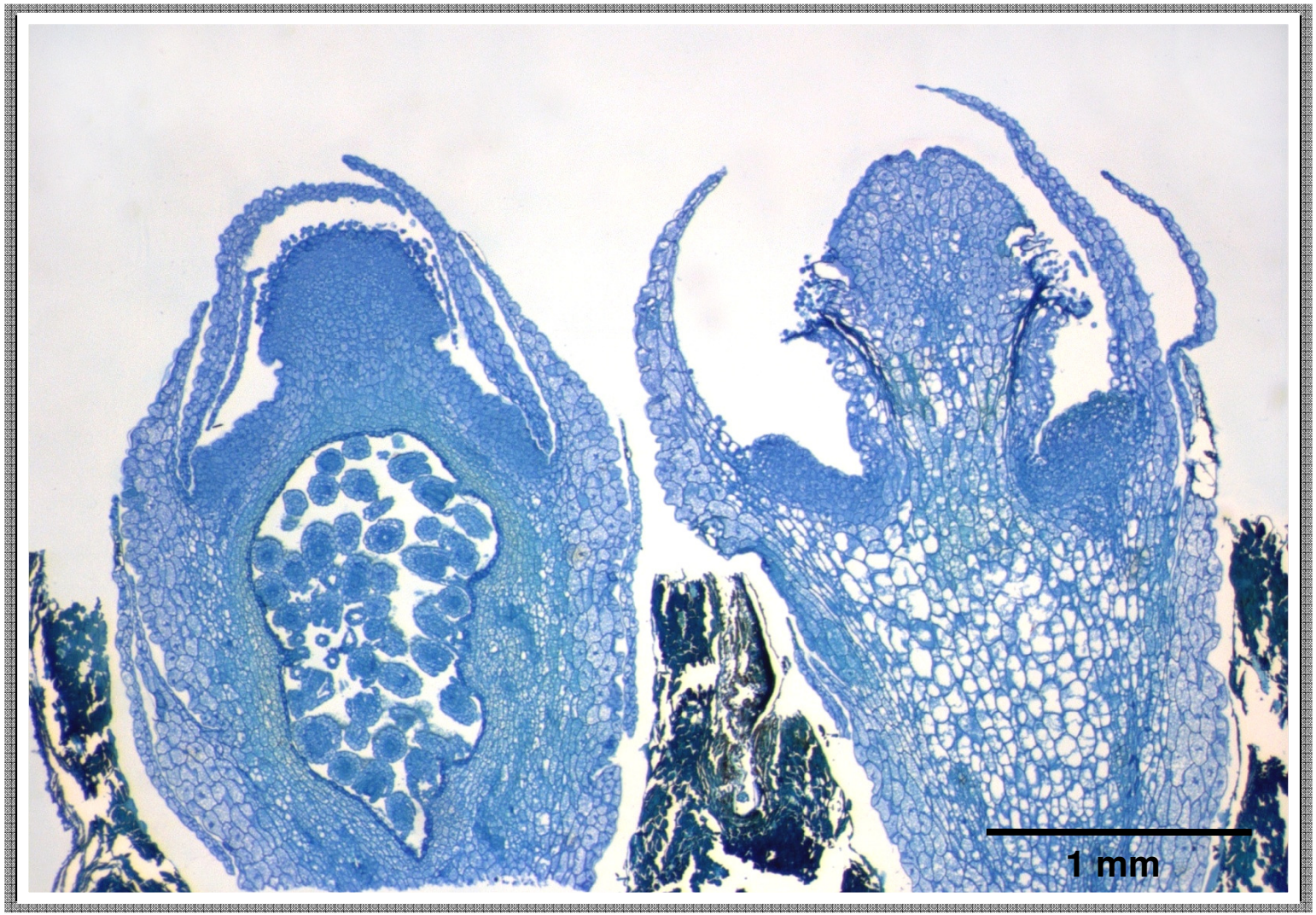
Flower anatomy

- Monoclinous Flower



Flower in longitudinal cut

Flower distribution in branches



Pistillate Flower and staminate staminate side by side in the same host branch.

Objectives

- To understand flower distribution and species sex ratio
- To evaluate the effect of site and host in flower morphogenesis
- To understand reproductive system and life cycle

Material

Pilostyles ulei

- Host – *Mimosa multipinna*

Groppo, Amaral & Ceccantini (2007)

Amaral (2007), Brasil 2010

Amaral & Ceccantini 2011

Material

Pilostyles thurberi

- Host – *Dalea bicolor* and *D. melantha*

Kuijt. et al., 1985

Rutherford, 1970


Dalea bicolor


D. bicolor


D. melantha


D. bicolor

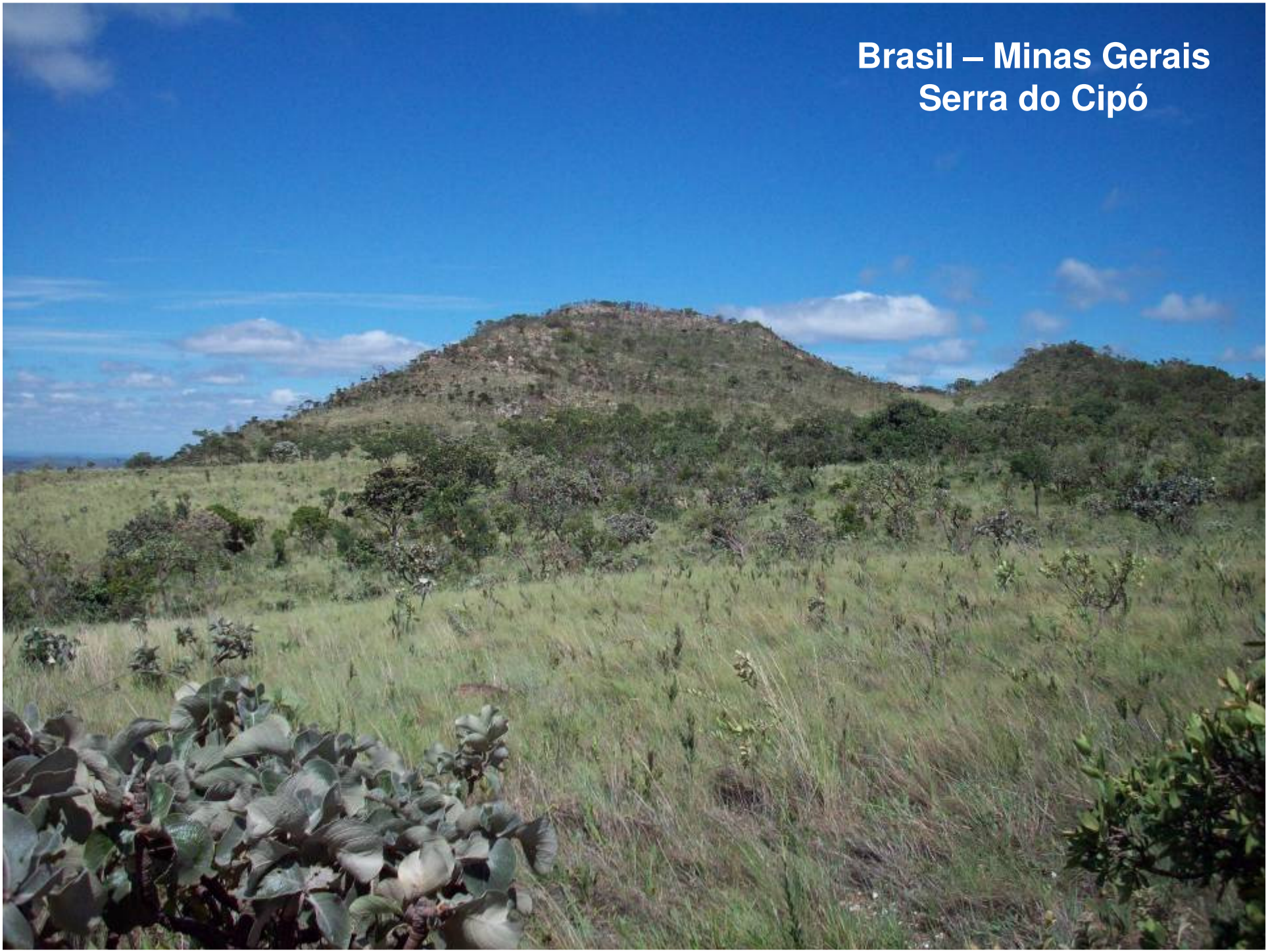
Populations

Pilostyles thurberi

- Hacienda El Ciervo – HC
- Los Trigos – LT
- Santa Isabel – SI

Pilostyles ulei

- Serra do Cipó - SC


Brasil – Minas Gerais
Serra do Cipó


Queretaro - Los Trigos - LT


Puebla - Santa Isabel - SI


Queretaro – El Ciervo - EC


Puebla - Santa Isabel - SI

Spority study

Difficulties

- Finding infected hosts - GPS

- Difficult to verify “flower sex” (spority) only sectioning under stereomicroscope

Material

Species	Locality	Vegetation type	Geographic Coordinates (deg, min, sec)	Flower per host	Ind.	Total	Host species
Pilostyles ulei *Solms. Laub.* GC 2022	Serra do Cipó, Minas Gerais, Brazil (SC)	Campo Rupestre	S 19°18' 27,8" W 43° 36'11,6"	50	100	**5000**	***Mimosa foliolosa*** var *multipinna* (Benth.) Barneby
Pilostyles thurberi Gray TT918	Hacienda El Ciervo, Querétaro, Mexico (HC)	Matorral Scrub	N 20° 27' 05,9" W 99° 52' 09,3"	50	10	**500**	***Dalea bicolor*** Willd.
Pilostyles thurberi Gray TT916	Los Trigos, Querétaro, Mexico (LT)	Matorral sub-montano	N 20° 54' 63,8" W 100°12'01,0"	50	50	**2500**	***Dalea bicolor*** Willd.
Pilostyles thurberi Gray TT927	Santa Isabel, Puebla, Mexico (SI)	Matorral sub-montano	N 18° 46' 26,2" W 97° 28' 27,0"	50	50	**2500**	***Dalea melantha*** var *melantha* S.Schauer

Methods

FLORAL ANATOMY

Usual histological techniques

Pilostyles ulei – colletion and fixation

Histologycal Preparation:

- Vacuum fixation
- Dehydratation
- Historesin inclusion
- Sectioning
- Staining
- Mounting

Flower distribution in branches

- Ascending distribution

following towards the apex,

following host branch growing

- Endophytic system present

only in branches

“isophasic pattern”

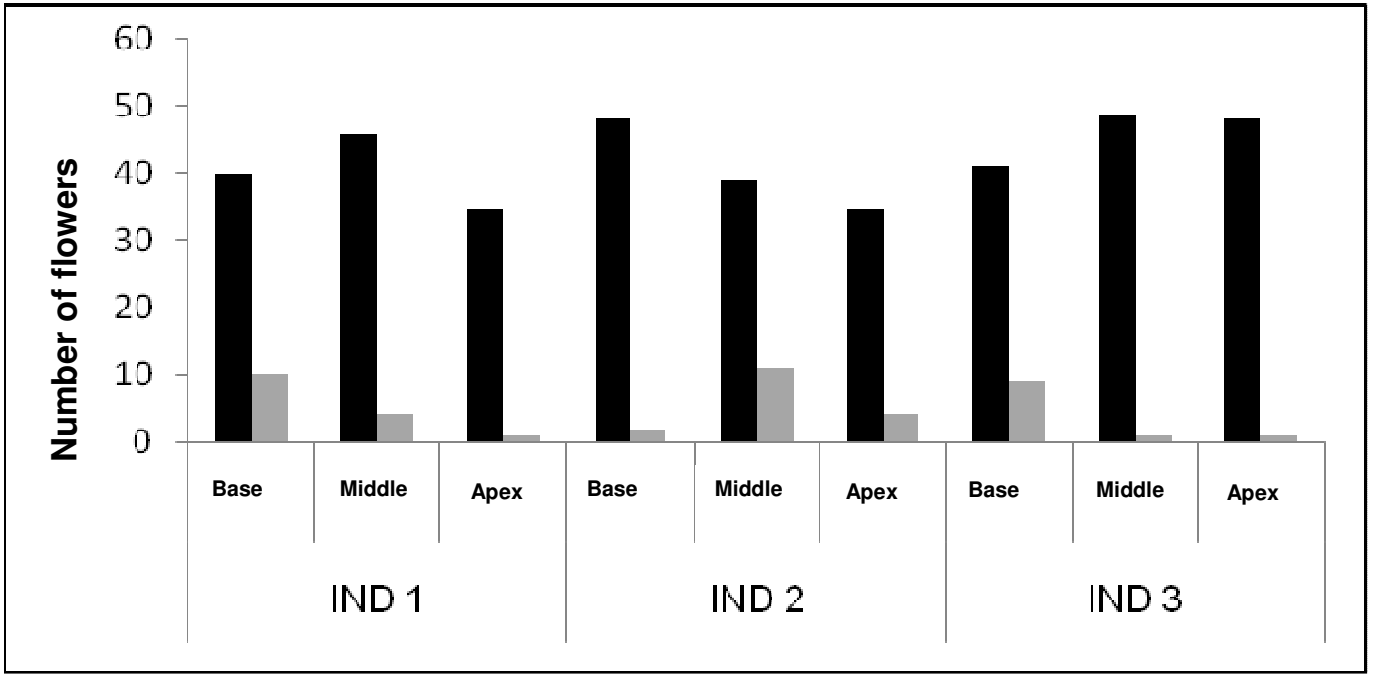
Flower distribution in branches

- Vattimo (1972) – *Pilostyles stawiarskii*

  The presence of the flowers of the both sexes is rare

  “contiguous, but distinct areas”

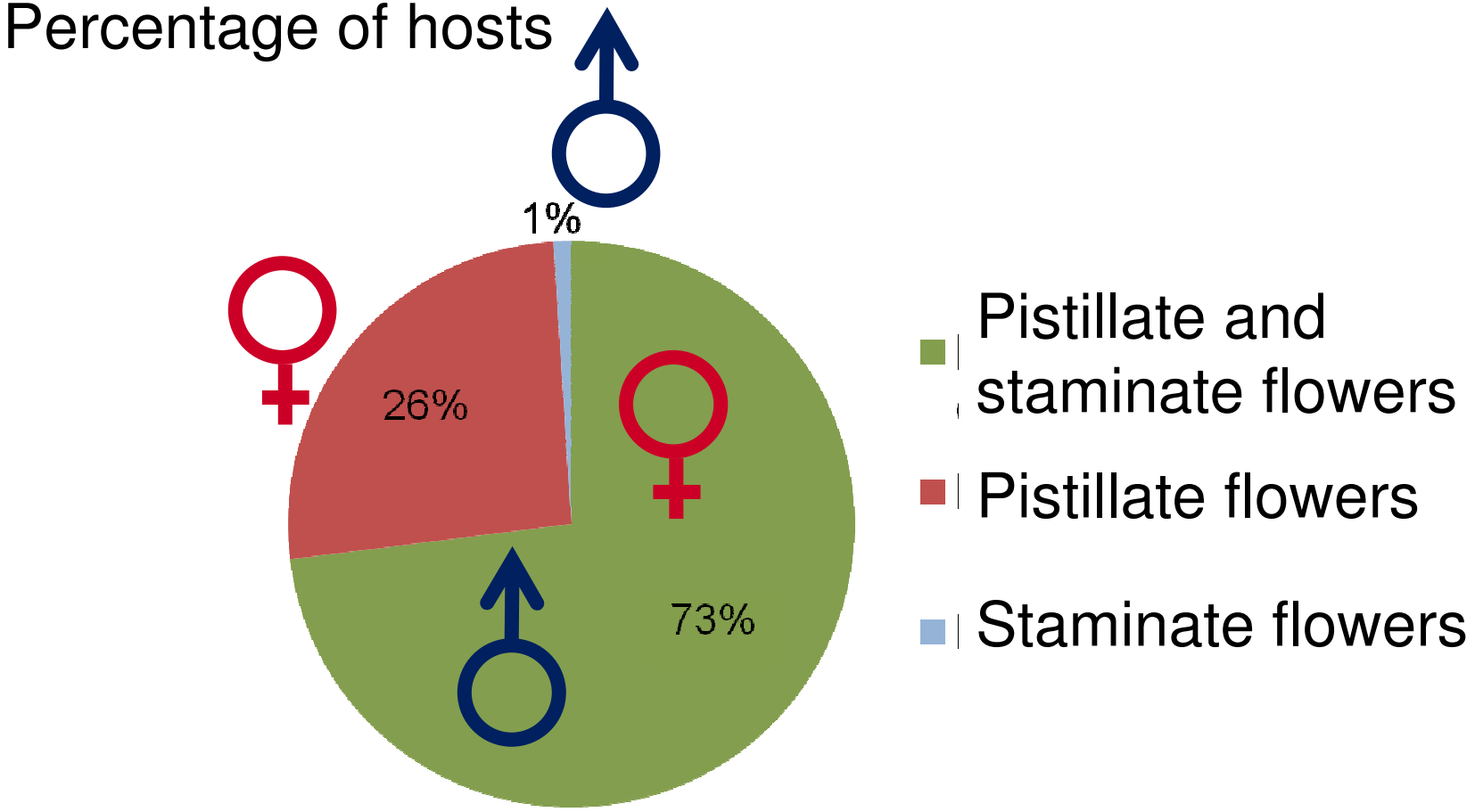
- Survey in three individuals of *M. foliolosa*:



(■) Pistillate Flowers; (■) Staminate flowers.

Flower distribution in branches

Pilostyles ulei **-** 5000 = 50 flowers per individual in 100 individuals of *Mimosa foliolosa*.



Flower distribution in branches

- Dell & Burbidge (1981) – *Pilostyles hamiltonii*

Presence of one parasite per host

Daviesia

Pistillate and staminate flowers in different hosts

↓

parasite dioic

Jacksonia, Oxylobium e *Castrolobium*

Pistillate flowers and staminate flowers in the same branch

↓

monoic parasite

Sex (Spority) Ratio ♀ : ♂

pistillate/staminate

Species	Locality	Host	Distribution	Sex ratio (♀ : ♂)
Pilostyles ulei	SC	*Mimosa foliolosa*	Most hosts with both flower type	7.9 : 1
Pilostyles thurberi	SI	*Dalea melantha*	Hosts with the two flowers	2.3 : 1
	HC	*Dalea bicolor*	Host with only one flower type	0.6 : 1
	LT	*Dalea bicolor*	Most hosts with one flower type	0.8 : 1

Monoclinous flowers

Species	Locality	Host	Monoclinous flower (%)	Hosts (%)	Min - Max (♀ : ♂)
Pilostyles ulei	SC	*Mimosa foliolosa*	1.29	48.8	1 – 3
Pilostyles thurberi	SI	*Dalea melantha*	1.76	50.0	1 – 4
	HC	*Dalea bicolor*	0.65	20.0	1 – 2
	LT	*Dalea bicolor*	1.75	18.6	1 - 14

Sexual Ratio (pistillate/staminate)

Result by host ⟶ **high : low** pis:sta

- Do these flowers represent one individual?
- Is the parasite monoic diclinous?
- Variable?

Is pollen restriction occurring?

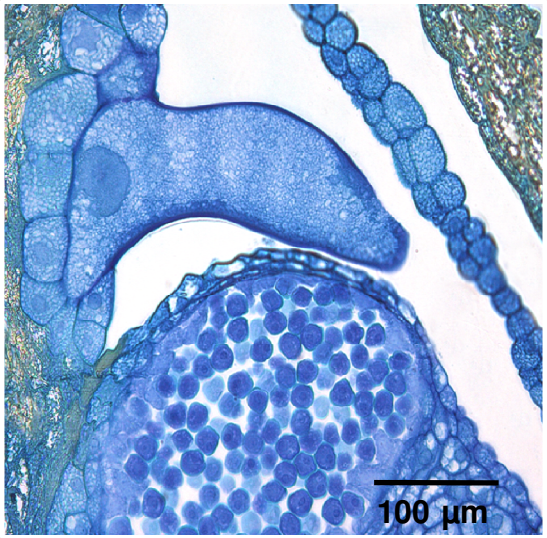
Vesicular tricomes

• Entomophlous pollination – psilate pollen grain; guaranteed transport by the presence of adhesive substance

• Similar tricomas in other Cucurbitales

Cyclanthera (Cucurbitaceae)

Ibervillea (Cucurbitaceae)

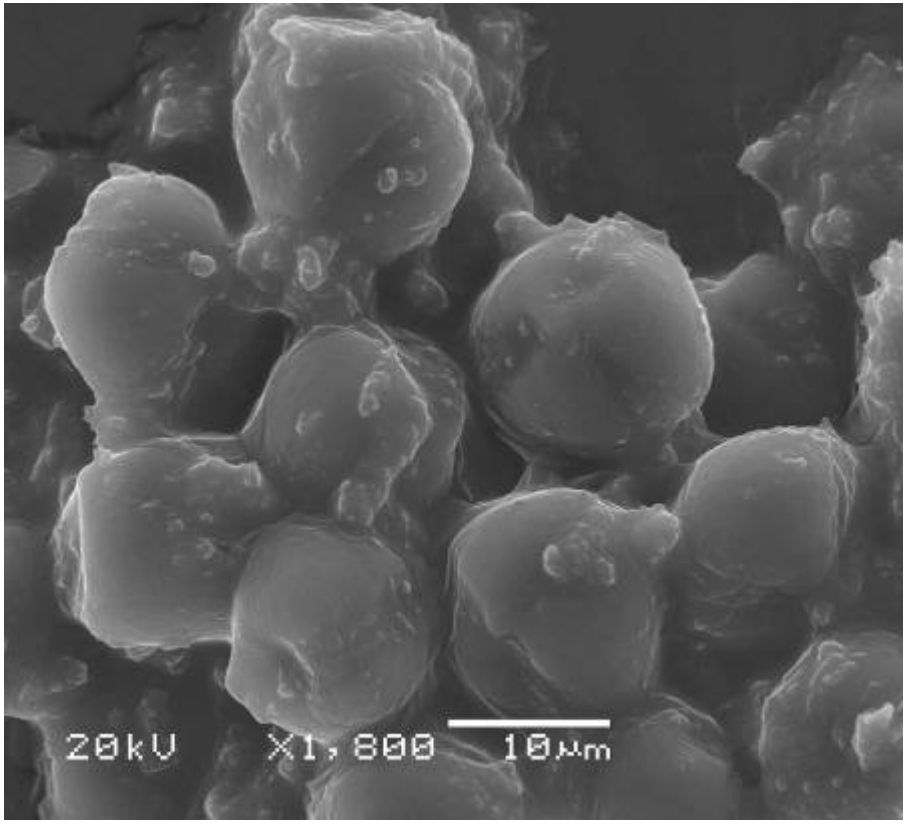


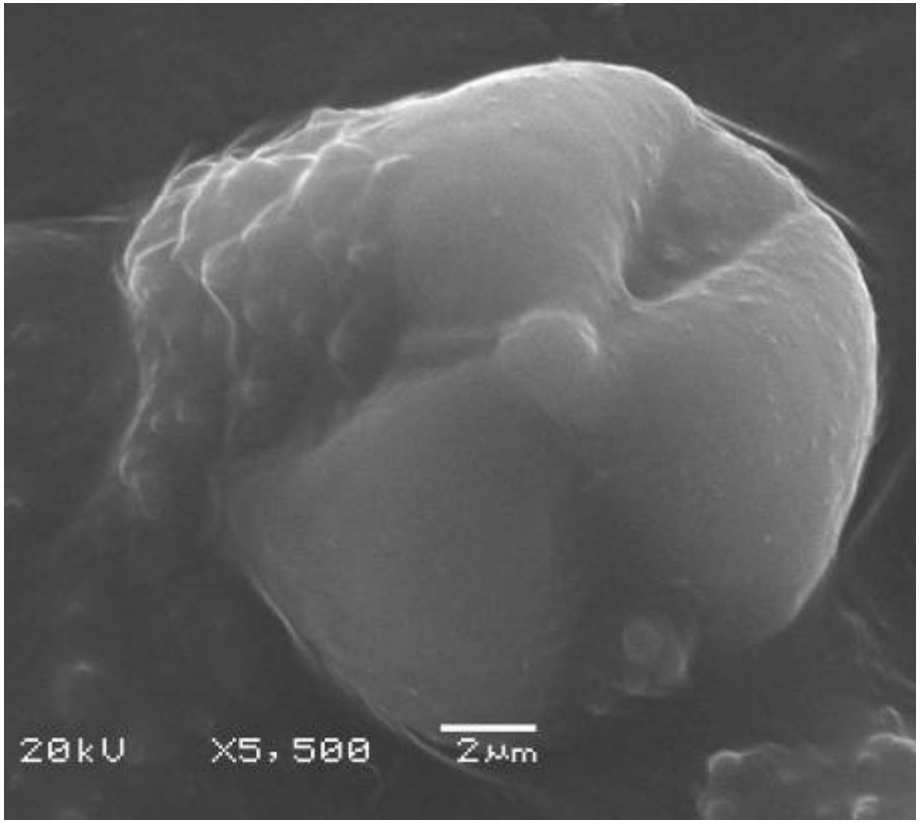
Vesicular tricomes

Pilostyles ulei (Apodanthaceae)

Pollen grain

Scanning Electron Microscopy





- peach shape
- tricolpate, psilate e suboblate

Unproportional Sexual Ratio

Possible explanation:

1. Differential host mortality

   Hosts infected by male parasites die or become weak (Vattimo 1971)

2. Staminate flowers short-lived

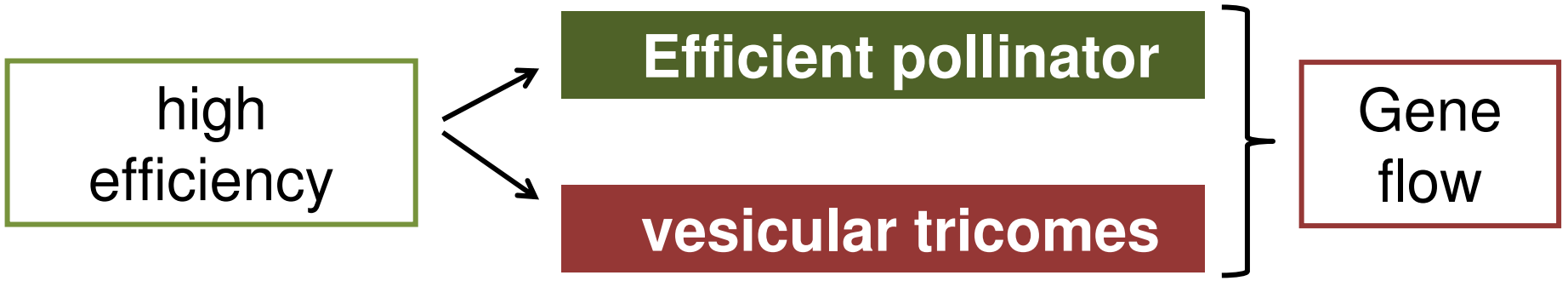
   Difficult to collect (Dell & Burbidge 1981)

3. Excess of pollen

   Each staminate flower produces enough pollen to fertilize up to 8 female flowers

Sexual Ratio

• If it is true that each staminate flower produces pollen enough to fertilize up to 8 pistillate flowers. Probably it might exist an extremelly efficient pollination system.



Spority

Monoclinous flower → **Percentage of 1,15%**

- The maximum number per host – individual is 3?
- Hypotesis:

The species are monoecious

The species are trimononoecious?

The species are subdioecious?

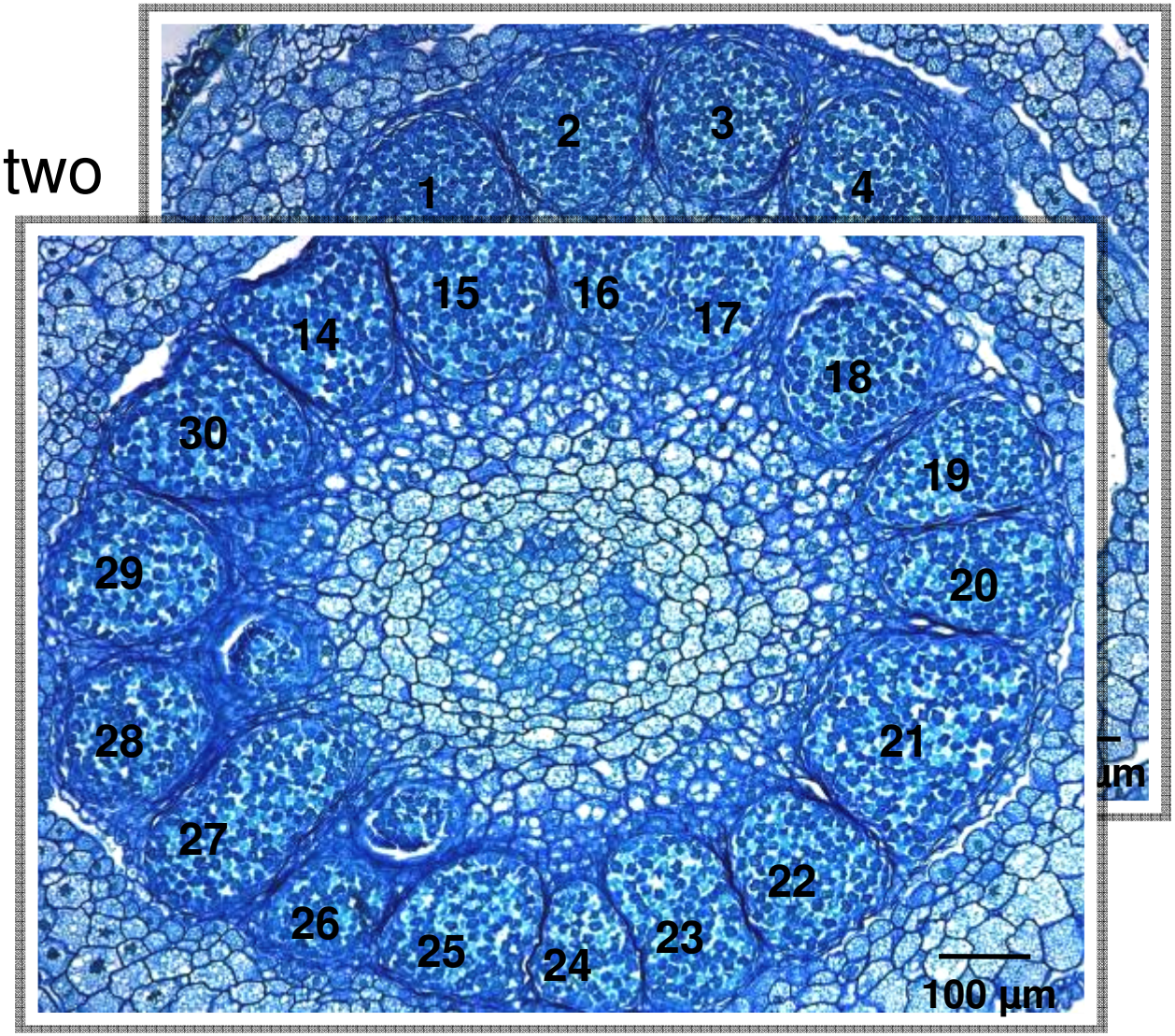
The species are dioecious

The species are triecious?

?

Floral Anatomy – staminate fl.

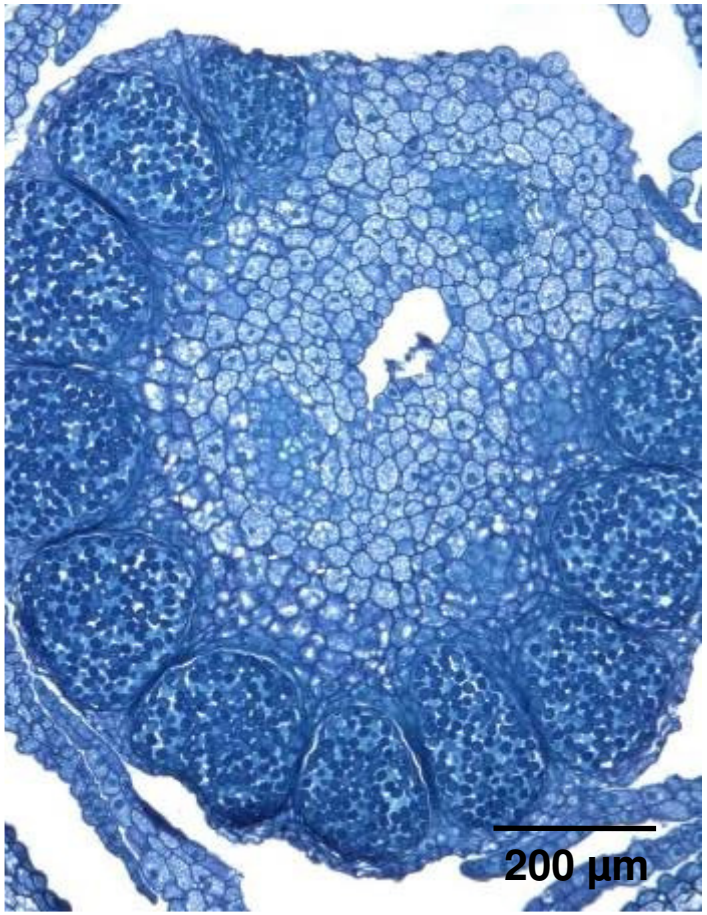
- pollen sacs in two rings (levels)
- Superior = 13
- Inferior = 17
- No alternancy



inferior ring

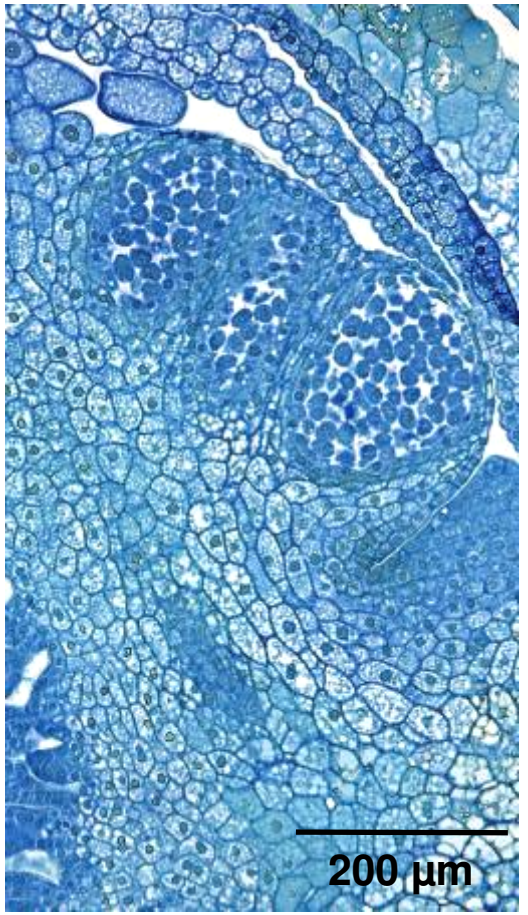
Floral anatomy - clues

- Staminate flower - VARIABLE



INCOMPLETE RINGS

ABORTED SACS



TREE RINGS

Pilostyles spp

Monoclinous flower → **μ =1.15%**

- Complementary hypothesis

Monoclinous flowers are “inconstant males”.

If the species are dioic, dioicy evolved recently

Dioicy evolved from gynogioicy

New Phytologist

'Inconstant males' and the maintenance of labile sex expression in subdioecious plants

- "Web of Science" e "Google Scholar"
- selection: monoclinous (5%)
- 32 Subdioic spp (21 families)
- 78% of spp the inconstant sex is male, producing monoclinous flowers

Evolution by gynodioicy

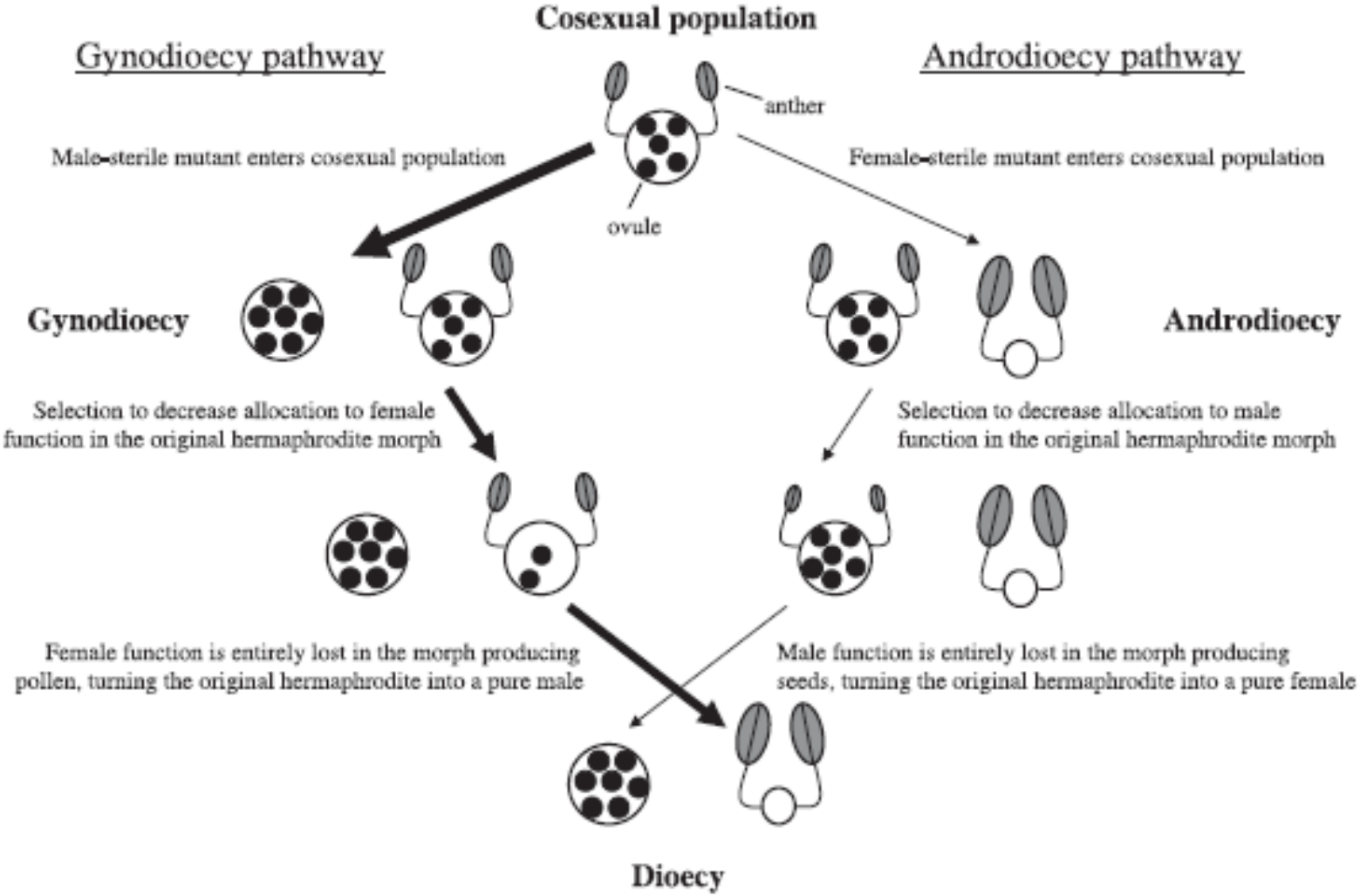
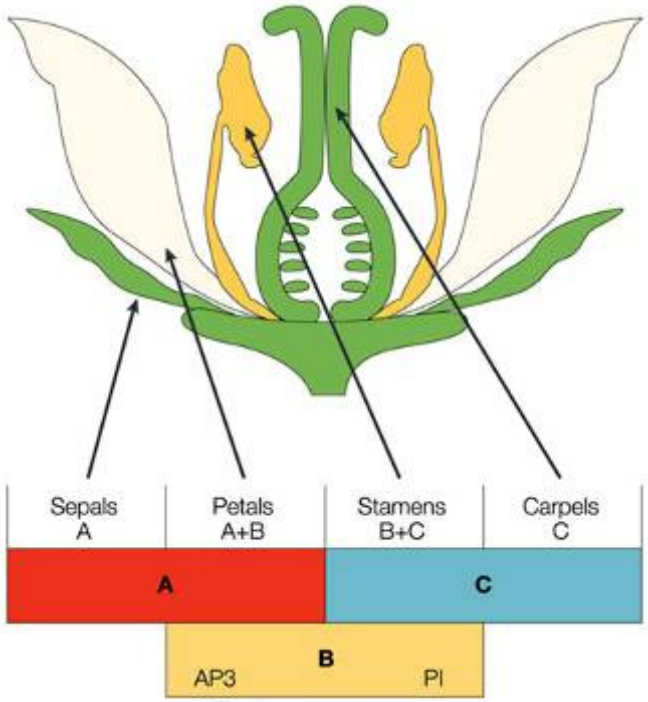


Fig. 1 The evolutionary pathways from cosexuality (shown here as hermaphroditism) to dioecy via gynodioecy and androdioecy are depicted as they are traditionally modeled. Arrow thickness indicates frequency of occurrence (thicker = more common). The evolution of dioecy is a two-step process in each case: invasion of a unisexual mutant, followed by a decrease and eventual elimination in the original hermaphrodites of the sex function provided by the unisexuals. Different species reduce investment in female or male function in different ways. For example, a

New Phytologist

Intersex inflorescences of *Rumex acetosa* demonstrate that sex determination is unique to each flower

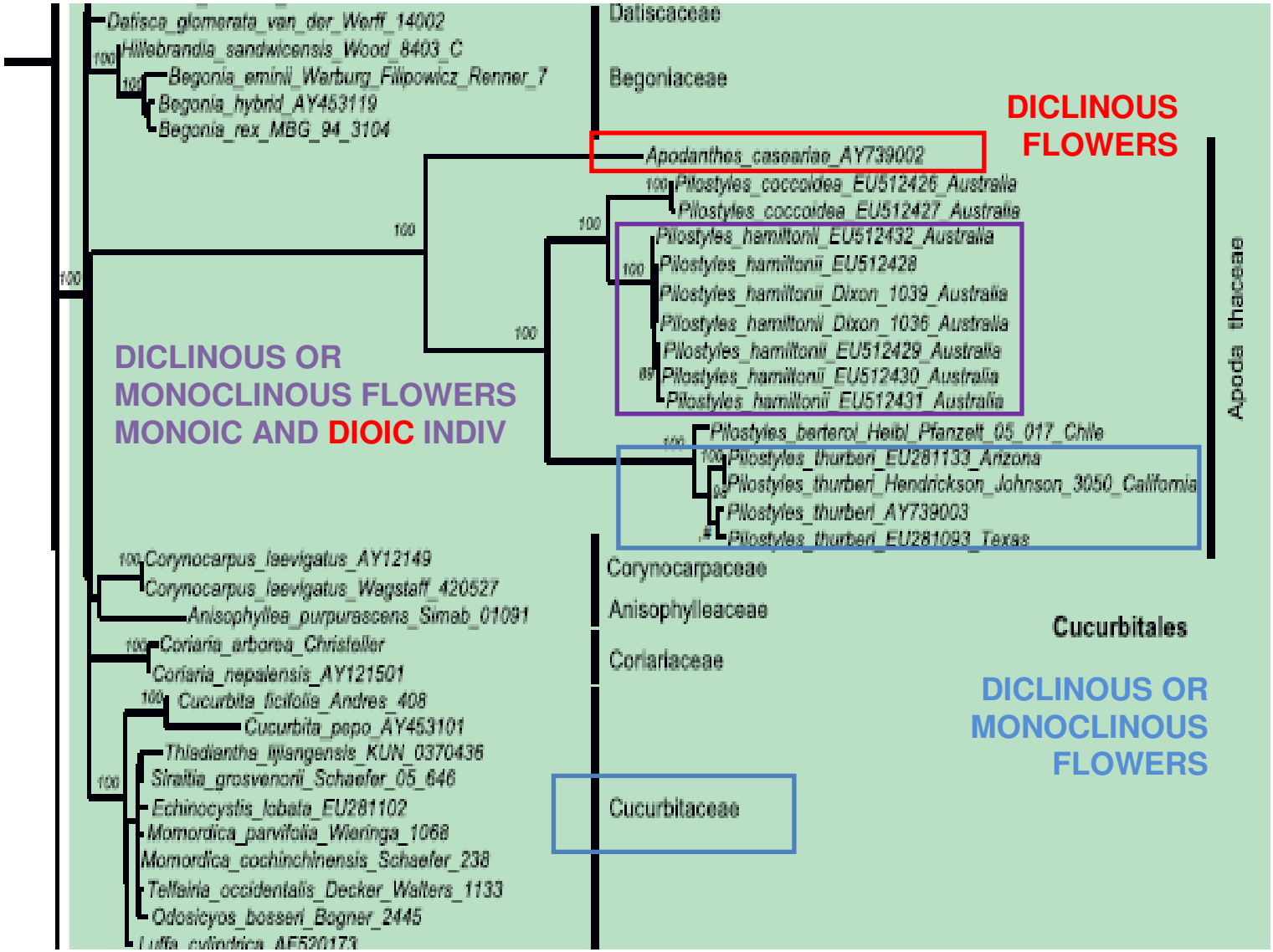
- *Rumex* (Polygonaceae) – dioecius – sex ratio : autossomic
- Triploid species – staminate, pistylate, monoclinous flowers
- variable gynoecium in monoclinous flowers
- gynoecious development is variable
- Independent on location



- Cgene expression control
- homeotic genes - ABC
- C genes expression

Apodanthaceae

- Filipowicz & Renner (2010)



Bianca and Teresa Vandalizing Mexican populations

Biana and Greg hunting pollinators


Grazie!
Thahks !