Revisiting the gynoecium of *Cabralea canjerana* and the concept of syncarpy in some species of the clade Melioideae

Revisitando o gineceu de *Cabralea canjerana* e o conceito de sincarpia em algumas espécies do clado Melioideae

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**ABSTRACT**
A reanalysis of the gynoecium of *Cabralea canjerana* was performed, and compared with floral studies of *Guarea kunthiana*, *Guarea macrophylla*, *Trichilia catigua*, *Trichilia elegans* and *Trichilia pallida*, all species belonging to the clade Melioideae. Our study structurally emphasizes the compitum and eusyncarpy. Floral buds and flowers were investigated according to usual techniques in light microscopy and scanning electron microscopy. *Cabralea canjerana* has a discoid stigma, pollen tube transmitting tract only in the lower part of the style, and a semi-inferior ovary with five carpels and locules and two ovules per locule with axile placentation. *Guarea* and *Trichilia* species have discoid and capitate stigmas, respectively, superior ovary, four carpels and locules in the former and three in the latter, two ovules per locule and axile placentation. Both genera have a transmitting tract along the length of the style. *Cabralea canjerana* and the other species are eusyncarpous, with compitum on the style and upper portion of the ovary.

**Keywords:** compitum, eusyncarpy, pollen tube transmitting tract, stigma, style.

**RESUMO**
A reanálise do gineceu de *Cabralea canjerana* foi feita e comparada com estudos florais de *Guarea kunthiana*, *Guarea macrophylla*, *Trichilia catigua*, *Trichilia elegans* e *Trichilia pallida*, todas espécies pertencentes ao clado Melioideae. Nosso estudo enfatiza o compitum e a eussincarpia. Botões florais e flores foram investigadas de acordo com técnicas usuais em microscopia de luz e microscopia eletrônica de varredura. *Cabralea canjerana* tem estigma discoide, tecido transmissor de tubo polínico somente na parte inferior do estilete e ovário semi-infero com cinco carpelos e lóculos, dois óvulos por lóculo e placentação axial. As espécies de *Guarea* e *Trichilia* tem estigma discoide e capitado, respectivamente, ovário superior, quatro carpelos em *Guarea* e três em...
Trichilia, dois óvulos por lóculo e placentação axial. Cabralea canjerana e as outras espécies são eussincárpicas, com compitum no estilete e na porção superior do ovário.

**Palavras-chave:** compitum, estigma, estilete, eussincarpia, tecido transmissor.

1 INTRODUCTION

Meliaceae is a family widely distributed in tropical and subtropical regions, where many of its species are a source of hardwood, and has 51 genera and 550 species (Judd et al., 2009). Species of Meliaceae can be used in the restoration of forests that suffer anthropic action. Cedrela odorata L., for example, was considered one of the five best performing species for growth (diameter and height) in an Amazon Forest restoration study (Araújo, 2020).

*Cabralea canjerana* (Vell.) Mart. often occurs in forest remnants in northern Paraná, Brazil, and its arboreal size can reach 30 meters in height. The species belongs to the Meliaceae, which is mostly pantropical family, and currently comprise 778 species distributed in 51 genera (*sensu* Gama et al., 2020). In the study of the evolution of reproductive characters of Meliaceae, Gama et al. (2020) include *Cabralea* A. Juss. into Melioideae clade, along with other genera, such as *Guarea* F. Allam. and *Trichilia* P. Browne, which have representatives in these forest remnants.

A precise knowledge of the structure of the flower of *Cabralea canjerana* has been made by Moscheta et al. (2002). In this work, the authors describe in detail the structure of the perianth, nectary, androecium and gynoecium. Other species of the clade Melioideae, belonging to the genera *Trichilia* and *Guarea*, had their flowers investigated by Souza et al. (2001) and Souza et al. (2002), respectively. More recently, the structure of *Guarea* and *Trichilia* flowers has been analyzed, as *Guarea macrophylla* subsp. *tuberculata* (Vell.) T. D. Penn. and *Trichilia claussemii* C.D.C. (Gama, 2018), and the gynoecium structure of *Trichilia pallens* C.D.C. (El Ottra et al., 2022).

The gynoecium consists mostly of carpels, which are more or less united (syncarpous gynoecium; probably more than 80% of angiosperm species) (Endress, 1994). Earlier, Carr and Carr (1961) have emphasized that the gynoecia of flowering plants may be classified as apocarpous, pseudo-syncarpous or eusyncarpous, in which the pseudo-syncarpous one is functionally apocarpous. The eusyncarpous type has a compitum, which is considered by the authors as a connection between the carpels which allows pollen tubes from grains germinating on any stigma to fertilize ovule belonging to more than one carpel.

After germination of the pollen grains on the stigma, the pollen tubes grow through the pollen tube transmitting tract, a specially differentiated tract between stigma and ovule (*sensu* Endress, 1994). In most syncarpous gynoecia this author distinguishes two zones, one called symplicate region, in which there is a common transmitting tract, and another already in the ovary where the...
compitum ends, which is the synascidiate region. Based on some studies of Meliaceae flowers carried out by other authors, El Ottra et al. (2022) report the possibility of secretion in the pollen tube transmitting tract, and that there seems to be a high degree of syncarpy in Meliaceae.

In the present work, the structure of the gynoecium of *Cabralea canjerana* is rediscussed with emphasis on the degree of syncarp, and compared with some species of *Guarea* and *Trichilia* belonging to the Meliioideae clade.

2 MATERIAL AND METHODS

**Plant samples** - Flower buds and flowers of *Cabralea canjerana* were collected at “Horto Florestal Dr. Luiz Teixeira Mendes”, which is owned by “Companhia Melhoramentos Norte do Paraná” (23°43′45.9″ S, 51°96′78.5″ W), with an area of 37 hectares, and on the campus of the State University of Maringá (UEM) (23°24′28.0″ S, 51°56′14.2″ W), Brazil. The plant material collected was prepared for mounting exsiccates and studying the gynoecium under a light microscope and scanning electron microscope (SEM). The exsiccates were deposited in the Herbarium of the State University of Maringá (HUEM), under registration HUEM 36360 and HUEM 39327. The structure of the gynoecium of *Cabralea canjerana* was compared with the gynoecium of previously studied species of *Guarea kunthiana* A. Juss. and *Guarea macrophylla* Vahl. (Souza et al., 2002) and *Trichilia catigua* A. Juss., *Trichilia elegans* A. Juss. and *Trichilia pallida* Sw. (Souza et al., 2001).

**Floral analysis in light microscope** – Flower buds and flowers were fixed in FAA 50 (formaldehyde, acetic acid and ethyl alcohol), and later dehydrated using the series of ethyl alcohol with concentrations of 80%, 90% and 100%. The plant samples were embedded in Leica historesin, according to the manufacturer's specification. The samples were sectioned on a rotation microtome, with sections obtained 6-8 µm in thickness being stained in Toluidine Blue in phosphate buffer (pH = 6.8) (O'Brien et al., 1965). The sample illustrations were made using a Leica ICC50 light microscope with an attached digital camera, using the Leica Application Suite software, version 1.8. Photomicrographs were prepared according to the scale corresponding to the observed increase.

**Floral analysis in scanning electron microscope** – Flowers were fixed at 2.5% glutaraldehyde, 0.1 M phosphate buffer, pH 7.2 for approximately 48 hours. After, the flowers were dehydrated in an ethylic series (70, 80, 90 and 100%), and partially dissected under a stereoscopic microscope (Leica EZ4). The material was critical point dried using CO₂ (Leica CPD300). It was then mounted on metal stubs for sputtering (Sputter Coater, FDU 010, Bal-Tec, Balzers, Liechtenstein), with a layer of about 10nm of colloidal gold. The samples were examined in a Scanning Electron Microscope Quanta 250 (Fei–Oxford Instruments, Oxfordshire, United Kingdom) operating between 15-25kV, and the images were captured digitally.
3 RESULTS AND DISCUSSION

Flower types and gynoecium structure

Cabralea canjerana has pistillate flowers with anterodes and staminate flowers with pistillodes, with white corolla and staminal tube (Figure 1A). Moscheta et al. (2002) have described similar morphology for flowers of Cabralea canjerana. Guarea kunthiana and Guarea macrophylla are dioecious plants, with pistillate and staminate flowers (Souza et al., 2002). In the case of Trichilia species, Souza et al. (2001) report that Trichilia catigua, Trichilia elegans and Trichilia pallida are unisexual, but strictly dioecious specimens were not found in the studied area, all of them being monoecious to a greater or lesser degree. Gama et al. (2020) mentioned the sexual systems of Cabralea, Guarea and Trichilia in a study of reproductive traits in Meliaceae. These authors indicate dioecious plants for Cabralea, dioecious and hermaphroditic plants for Guarea and dioecious, hermaphroditic and polygamous plants for Trichilia. These variations concerning the reproduction systems of Cabralea, Guarea and Trichilia were probably not found because collections were restricted to floral anatomy purposes, and the limited size of forest remnants in the northwest region of Paraná, Brazil.

The gynoecium of Cabralea canjerana has a discoid stigma with a star-shaped canal, evidencing the five carpels (Figures 1B,C; 2B). The morphology of the stigma reveals two regions, one in which the epidermis is papillose, probably non-secretory, and another of a hairy nature, possibly secretory (Figures 1C; 2B,C). In this last stigmatic region, it is possible to find adhered pollen grains (Figure 1D). Similarly, both species of Guarea (Guarea kunthiana and Guarea macrophylla) also exhibit discoid stigma with the possibly secretory region located in its lateral region (Souza et al., 2002). The three species of Trichilia (Trichilia catigua, Trichilia elegans and Trichilia pallida) have capitate stigma, but like the species of the other two genera, they show the secretory region laterally. In the analysis of reproductive traits in Meliaceae, Gama et al. (2020) considered discoid stigma for the related genera Cabralea and Guarea and the capitate type for Trichilia.

The style of Cabralea canjerana consists of uniseriate epidermis (Figure 2D), glabrous near the stigma (Figure 1C) and hairy towards the base (Figures 1E; 2A). It also contains parenchyma, numerous vascular bundles and five separate stylar canals with pollen tube transmitting tract (Figure 2D). Close to the ovary, the stylar canals and pollen tube transmitting tract demonstrate continuity between the carpels (Figure 2E). The common transmitting tract of the carpels is also found in the upper region of the ovary (Figure 2F). Further down, in the placental region, the pollen tube transmitting tract separate again for each carpel. Both species of Guarea, unlike Cabralea canjerana, have a star-shaped stylar canal and a common transmitting tract along the whole style.
The styles of the three species of *Trichilia* exhibit a single stylar canal and common transmitting tract (Souza et al., 2001).

It must be pointed out here that Endress (1994), based on Hanf (1936), formulated a pollen tube transmitting tract classification proposal. According to the proposal regarding the form of the inner morphological surface of the carpels, *Cabralea canjerana*, the two species of *Guarea*, and the three species of *Trichilia* have stylar canal with small lumen but large inner surface.

Figure 1 – Flower morphology of *Cabralea canjerana* under light microscope (A) and scanning electron microscope (B-F). A – Whole pistillate flower (sg=stigma; tu=staminal tube). B – Top view of the stigma. C - Upper portion of flower bud showing corolla (co), anthers (an) and stigma and style; white arrow indicates subapical hairy secretory region. D - Stigma in lateral view with pollen grains. E - Basal region of style densely hairy. F - Basal region of the flower showing a semi-inferior ovary (ne=nectary; st=style). Scale bars: 2mm (A), 500µm (B-F), 300µm (D).
The ovary of *Cabralea canjerana* is semi-inferior (Figure 1F), pentacarpellate with five loculi (Figure 2F). Each locule contains two ovules with axile placentation (Figure 2F). *Guarea kunthiana* and *Guarea macrophylla* have a superior ovary with four carpels and loculi, containing two ovules, sometimes one in the latter species, and axile placentation (Souza et al., 2002). The three species of *Trichilia*, in turn, exhibit three carpels and loculi in the superior ovary, and two ovules per locule with axile placentation (Souza et al., 2001). Axile placentation typically occurs in eusyncarpous ovaries (Endress, 1994). According to Gama et al. (2020) Meliaceae ancestor had ovary with four or five loculi, and the phylogenetic reconstruction supports that the number of ovules per locule is an important character to recognize to the genera of Melioidae, which generally have one or two ovules.
Compitum and syncarpy

In the upper portion of the style of *Cabralea canjerana* there is no compitum, a region comprising a common pollen tube transmitting tract. In this zone the transmitting tract of each carpel is separated from one another (Figure 2C,D). The symplicate region with common transmitting tract occurs at the base of the style and upper portion of the ovary, where the compitum is present (Figure 2E,F). On the other hand, the transmitting tract is common to carpels along the whole style and upper portion of the ovary in species of *Guarea* and *Trichilia*. In these species of *Guarea* and *Trichilia* the compitum ends at the ovary when the transmitting tract separates at each carpel (synascidiate part of ovary for Endress, 1994). Similar results were found in the gynoecium of *Trichilia pallens* by El Ottra et al. (2022), in which the carpels are symplicate from the stigma up to the upper third of the ovary.

The gynoecia of *Cabralea canjerana* and the other species of the Melioideae clade mentioned here are syncarpous with a single style, as already reported by Endress (1994) for Meliaceae family. All species are eusyncarpous, but the degree of eusyncarpy can be considered different between the referred species. *Cabralea canjerana* has the upper part of the gynoecium (stigma and style) with a separate transmitting tract for each carpel, whereas *Guarea* and *Trichilia* species have the common transmitting tract between the carpels throughout the length of the style and upper part of the ovary.

4 CONCLUSIONS

*Cabralea canjerana* and *Guarea* and *Trichilia* species are eusyncarpous and exhibit compitum in the style and upper region of the ovary. *Cabralea canjerana* differs from these species by having a semi-inferior ovary, a greater number of carpels and a pollen tube transmitting tract in the lower half of the style.
REFERENCES


