Estrutura floral de *Guarea macrophylla* Vahl e *Trichilia claussenii* C.DC. (Meliaceae): aspectos funcionais e implicações na sistemática e evolução de Sapindales.

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Dissertação apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Mestre em Ciências, na Área de Botânica.

Orientador: Prof. Dr. José Rubens Pirani
Coorientador: Prof. Dr. Diego Demarco

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Prof. Dr. José Rubens Pirani
Orientador
Dedico a minha família,
que são indispensáveis em minha vida.
“O que vale na vida não é o ponto de partida e sim a caminhada. Caminhando e semeando, no fim terás o que colher.”

(Cora Coralina)
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ABSTRACT

The reproductive systems of angiosperms are quite diverse. In the flowers of Meliaceae (Sapindales) we found a wide diversity of these systems, including reports of monoecious, dioecious and polygamous plants. However, the sexual dimorphism of flowers in the family is very subtle, and structurally each flower always has a gynoecium and a developed androecium, even though functionally one or the other may be sterile (pistillodes or staminodes). Consequently, the determination of floral sexuality in the family is often difficult or even inconclusive in many taxa, and the available data often come from the analysis of herbarium material. This lack of detailed information on floral structure, development and function still prevent an accurate identification of floral sexuality in most members of Meliaceae, and also limits a comparison with the data known to other representatives of Sapindales, especially in relation to the structure and development of the carpels and stamens in the families most closely related, which are Rutaceae and Simaroubaceae. Studies on flower development associated with pollination studies and phylogenetic through comparative analysis, provide a better understanding on how these systems behave, and allow a more accurate classification of the sexual system present in particular species, leading to evolutionary data and assisting in clarifying kinship issues. The present dissertation addresses a detailed study of the structure of flowers of two Brazilian taxa (*Trichilia claussenii* and *Guarea macrophylla* subsp. *tuberculata*) belonging to the subfamily Melioideae, with emphasis on the organization and development of their sexual systems. Our study includes analysis of the external morphology, anatomy, vascularization, ontogeny, and histochemical tests of both species. We also analyzed the functional aspects of flowers of *T. claussenii*, related to floral sexuality, which was evaluated by means of pollination tests, associated to tests of pollen viability, stigmatic receptivity and morphoanatomic and ontogenetic analyzes. Among the main results, floral dimorphism was detected only in *T. claussenii*, which is likely gynodioecious, and provided with a peculiar sterility of the androecium in the female flower morphotype: pollen grains remain concealed by a secretion from the tapetum. In addition, we promote the discussion of pathways of the evolution of sexual systems within the Meliaceae, as well as of its main flower and fruit morphological characters, through hypotheses evaluated upon the current molecular phylogenetic framework of the family.
RESUMO
Os sistemas reprodutivos das angiospermas são bastante diversos. Nas flores de Meliaceae (Sapindales) encontramos uma grande diversidade desses sistemas, sendo relatada a presença de plantas monoicas, dioicas e polígâmicas. No entanto, o dimorfismo sexual das flores nessa família é muito sutil, e estruturalmente cada flor sempre possui um gineceu e um androceu desenvolvidos, mesmo que funcionalmente um ou outro seja estéril (pistilódio ou estaminódio). Consequentemente, a determinação da sexualidade floral na família é muitas vezes difícil ou até mesmo inconclusiva em muitos táxons, sendo a maioria dos dados disponíveis provenientes da análise de material herborizado. A carência de informações mais detalhadas sobre estrutura, desenvolvimento e função, ainda limita muito uma acurada identificação da sexualidade floral em Meliaceae, assim como impede uma comparação com os dados conhecidos sobre outros representantes de Sapindales, especialmente em relação à estrutura e desenvolvimento do androceu e gineceu, nas famílias mais proximamente aparentadas a ela, que são Rutaceae e Simaroubaceae. Estudos sobre o desenvolvimento floral, associados a estudos de polinização e filogenia, por meio de análises comparativas, propiciam melhor compreensão de como esses sistemas se comportam, permitindo uma classificação mais acurada do sistema sexual presente numa determinada espécie, além de fornecer dados evolutivos importantes e auxiliar no esclarecimento de questões de parentesco. Neste contexto, a presente dissertação aborda um estudo detalhado da estrutura das flores de dois táxons brasileiros (Trichilia clausenii e Guarea macrophylla subsp. tuberculata) pertencentes à subfamília Melioideae, com ênfase na organização e desenvolvimento do sistema sexual. Tal estudo é composto por análise da morfologia externa, anatomia, vascularização, ontogenia e testes histoquímicos em ambos táxons. Também foram feitas análises dos aspectos funcionais das flores de Trichilia clausenii, relativos à sexualidade floral, por meio de testes de polinização, testes de viabilidade polínica, receptividade estigmática e análises morfoanatómicas e ontogenéticas. Entre os principais resultados, destacamos o dimorfismo floral detectado apenas em T. clausenii, espécie ginodioica na qual a esterilidade do androceu no morfotipo floral feminino é alcançada pela impossibilidade de liberação dos grãos de pólen, retidos por uma secreção do tapete. Além disso, promovemos a discussão de transições evolutivas do sistema sexual na história da família, bem como de outros caracteres morfológicos florais e de fruto, por meio de avaliação de hipóteses estruturadas sobre a filogenia molecular atual da família.
GENERAL INTRODUCTION

The flowers are quite diverse reproductive structures, mostly bisexual and only about 10% unisexual (Barrett, 2002). Their sexual systems present a great complexity, which makes possible a series of classifications, from the spatial and temporal distributions of the reproductive organs in the plant, to self-incompatibility and variation in the length of these reproductive structures (Bawa & Beach, 1981).

Sexual systems based on spatial distribution of male and female reproductive organs can be classified as: hemaphroditism, where plants have only bisexual flowers; monoecism in which the plants have male and female flowers on the same individual; andromonoecism, where the same individual has male and bisexual flowers; gynomonoecism, in which the plant has female and bisexual flowers; dioecism, where the female flowers are in an individual different from the male flowers; androdioecism, in which individuals have male or bisexual flowers; gynodioecism, in which there are individuals who produce female flowers and individuals who produce bisexual flowers in the same population; polygamous in which the plant population produces female, male and bisexual flowers on the same plant or on different plants; subdioecism presents populations of plants with male flowers, plants with female flowers and some hermaphrodite plants (Bawa & Beach, 1981; Sakai & Weller, 1999). The diversity of these sexual systems derives from the strategy of differential allocation of female and male resources controlled by sexual selection, since male success depends on the dispersion of pollen and the female depends on the nutritional resources available for the development of embryos, fruits and seeds. Pollination has an important impact in this process, since it promotes the movement of pollen among flowers of the same species (Bawa & Beach, 1981).

The understanding of this dynamic and its complex diversity relies on the necessity of detailed studies of floral development associated with studies of pollination and reproductive biology, along with phylogenetic and evolutionary approaches. The combination of these different approaches allows us to understand more properly how sexual diversification may have taken place in certain groups and across other related lineages.
When analyzing the flowers of dimorphic taxa, if there are macroscopic differences as the presence of rudimentary organs of the opposite sex, such as staminodes (anthers smaller than functional ones and with non-viable pollen grains) or pistillodes (with aborted ovules), there is a greater facility to classify the functionality as well as the sexual system (Pennington et al., 1981; Vieira 2013). However, in cases where the dimorphism is subtle the difficulty in sexual determination increases and developmental and pollination studies are needed to clarify and classify sexual systems accurately. The presence of the opposite sex linked to functionality can be approached from a macroevolutionary perspective in order to evaluate the evolutionary rates of this vestigialisation in non-functional characters of the opposite sex (e.g. Eckhart, 1999).

In the order Sapindales (belonging in the Eudicots-Rosids-Malvids clade according to APG IV, 2016), it is common the occurrence of morphologically bisexual flowers that are functionally unisexual (e.g., Engler, 1931; Smith et al., 2004). Among the nine families of the order, several recent studies have improved the knowledge about this phenomenon (e.g., Bachelier and Endress, 2008, 2009; Bachelier et al., 2011; El Ottra et al. 2013; El Ottra, 2014; Alves et al., 2017; Gouvea et al., 2008a, 2008b), but there are still many gaps to be filled. Some of these gaps are found in relation to the flowers of the clade formed by Rutaceae, Simaroubaceae and Meliaceae, which according to Muellner-Riehl et al. (2016) is strongly supported. However, the relationship between Meliaceae and Simaroubaceae as sister to each other is moderately supported. Detailed studies of the floral structure with emphasis on the reproductive organs are available for several genera of the first two families. In contrasts, the lack of such information for most Meliaceae precludes an accurate comparison between the representatives of that clade.

Meliaceae is a mostly pantropical family, with a few taxa represented in the subtropical and temperate regions. In Brazil the two monophyletic subfamilies, Melioideae and Cedreloideae, are represented by eight genera and ca. 84 species, 30 of them endemic (Pennington & Styles, 1975;
The sexual systems of this family present a wide diversity, with dioecious, monoecious and polygamous plants being reported. Usually the flowers in the family are morphologically bisexual, but functionally they are predominantly unisexual. The sexual dimorphism of flowers is often very subtle, making it difficult to determine floral sexuality in several genera of the family (e.g. Pennington & Styles, 1975; Styles, 1972). In this way, more detailed studies of floral structure are necessary, since many of the reproductive data on floral sexuality in the family come only from morphological analysis, often from exsiccate, which makes it impossible to detect the functionality of reproductive structures and may even generate conflicting data. This lack of studies stimulated the elaboration of the present dissertation.

Meliaceae are of great economic importance, since the wood of several species is widely marketed worldwide. They have great potential for timber, such as the species of mahogany (*Swietenia* sp.) and cedar (*Cedrela* sp.) in the Neotropical region (Styles, 1972; Pennington *et al.*, 1981). Another commercial use of the family is the use of its active compounds (e.g., limonoids and meliacins) as insecticides or repellents (e.g., neem oil), which may further have fungicidal, antibacterial, antiviral (Singh *et al.*, 1988; Engelmeier *et al.*, 2000; Aboutabl *et al.*, 2000; Simmonds *et al.*, 2001; Greger *et al.*, 2001).

*Trichilia* P.Browne and *Guarea* Allamand ex L., are two of the five major genera that compose the family. There are few works related to sexuality in these genres (e.g., Moscheta, 1995; Souza *et al.* 2001, 2002; Morelato, 2004; Schmitt, 2013) and no detailed study regarding the structure and development of internal morphological surfaces of carpels and anthers.

In this dissertation we present an analysis of the sexual-functional aspects of the flower of *Trichilia claussenii*, whose sexual system is doubtful. This analysis was performed through optical microscopy and scanning electron microscopy, combined with pollen tests, pollen viability and stigma receptivity. Data from these analyzes along with their discussion and the external morphology boards, anatomical sections, anther electromicrographies and tests performed are found in Chapter 1.
We performed also an analysis on the structure and development of the gynoecium and androecium of *Trichilia claussenii* and *Guarea macrophylla* subsp. *tuberculata*, in a comparative way, trying to understand the sexual systems of these species. For this, analyzes of the external morphology, anatomy, vascularization, ontogeny, and histochemistry of both species were performed with the aid of stereomicroscopy, optical microscopy, scanning electron microscopy and histochemical tests. The data obtained from these analyzes, their discussion and the boards of the internal morphology, the anatomical sections, the electromicrographs, the vascularization diagrams and the histochemical tests are presented in Chapter 2.

Finally, we performed an evolutionary analysis on the main morphological characters of flowers in Meliaceae, based on the current phylogenetic framework. We aimed to find probable synapomorphies for some clades of the family, and to evaluated hypotheses on evolution of the reproductive structures (flowers and fruits) and of the sexual systems within the group. We coded 20 reproductive morphological characters and used 31 terminals of Meliaceae and six terminals of Simaroubaceae as outgroup, and plotted evolutionary transitions upon the molecular phylogeneny published by Muellner-Riehl *et al.* (2016). The discussion of the evolution of the sexual systems in the family and other reproductive morphological characters is found in Chapter 3.
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FINAL CONSIDERATIONS

In this work we performed the analysis on the structure, development and sexual-functional aspects of the flowers of *Trichilia claussenii* C.DC. and *Guarea macrophylla* subsp. *tuberculata* (Vell.) T.D.Penn (Meliaceae, Melioideae), in order to contribute to a better understanding of the floral and evolutionary diversity of the group. We also analyzed some morphological reproductive characters upon the current phylogenetic relationships of Meliaceae, aiming to find probable synapomorphies for some clades of the family, and to evaluate hypotheses about floral evolution and sexual systems in the group.

The comparison between the structures of the two species studied herein, and in relation to other representatives of the family, showed interesting variations in structure and development. We found some novelties, such as the existence in *Trichilia claussenii* of at least two functional floral morphotypes. Pollen viability tests, stigma receptivity, fruit formation, associated to morphoanatomical data, reveal the viability and integrity of the androecium and gynoecium in both floral morphotypes. However, in the female morphotype the anther is sterile due to the presence of the secretion of the tapetum, which forms a mass that adheres to the pollen and prevents its release and eventually causes its sterility. In addition, our pollination tests allow to characterize this species as gynodioecious. The two floral morphotypes found in *T. claussenii* do not differ in relation to their chemical structure, basic structural development and vascularization; they differ only by the external morphology and the concealed pollen grains in the female morphotype. Although sharing several morphological, anatomical and histochemical features, the studied species of *Guarea* and *Trichilia* differ in some characteristics of both the gynoecium and the androecium. Even though there are descriptions in the literature of sexual dimorphism in *Guarea*, we found no dimorphism in the population analyzed herein, since all flowers had fully developed ovules and fully developed pollen grains. Additionally, all individuals presented abundant fruit set during the study, but further detailed pollen viability and reproductive biology studies are still needed for this species.

The evolutionary hypotheses raised on the analysis upon the molecular phylogeny must be taken with caution, but they suggest some macroevolutionary
patterns that should be tested and encourage detailed structural research focused on some particular floral aspects. They also indicate possible evolutionary pathways that the reproductive structures may have undergone related to the specialization in floral biology and strategies of dispersion. In this way, we were able to increase the knowledge about the flowers of Meliaceae, mainly in relation to the structure and development of the gynoecium and androecium of two selected species of *Guarea* and *Trichilia*. Our data contribute to the improvement of the systematics of the family and to the discussions about evolution and diversification of the floral structure and sexuality within the family and in the Sapindales order.
