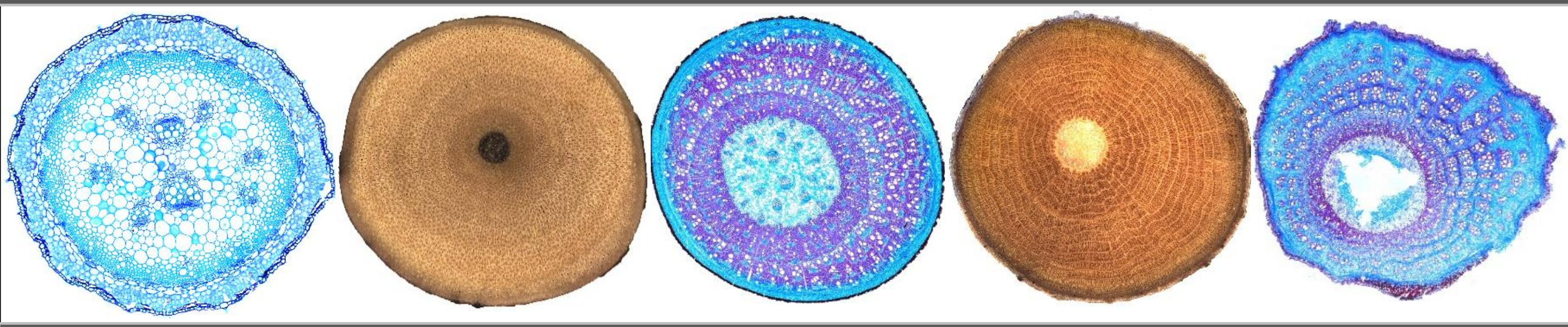

Israel Lopes da Cunha Neto

Diversidade e evolução do sistema vascular em Nyctaginaceae (Caryophyllales)

Diversity and evolution of the vascular system in Nyctaginaceae (Caryophyllales)

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Tese apresentada ao Instituto de Biociências da Universidade de São Paulo (USP), para a obtenção do título de Doutor em Ciências, na Área de Botânica.

Orientadora: Prof. Dr. Veronica Angyalossy
(Universidade de São Paulo, Brasil)

Coorientador: Prof. Dr. Marcelo R. Pace
(Universidade Nacional Autónoma de México)

São Paulo
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Comissão Julgadora:

Prof. Dr.

Prof. Dr.

Prof. Dr.

Prof. Dr. Veronica Angyalossy

Dedico este trabalho

Aos mestres que me iniciaram no mundo da ciência e que primeiro sonharam comigo o sonho de me tornar um botânico: Ana Cristina Fermino Soares e Fabiano Machado Martins

À minha família, porque sempre me encorajam a ir além e porque sentirão um orgulho enorme por esta conquista.

"The dominant position which the Dicotyledons unquestionably hold among existing forms of vegetation is probably due in a greater degree to their method of secondary growth in stem and root, than to any other single character. The ability to increase indefinitely the amount of mechanical, conducting and storing tissues in the axial organs, in proportion to the increasing development of the foliage, has more or less generally existed in all the most successful classes of plants; but it is in the Dicotyledons that the highest differentiation of the secondary tissues is attained… The study of the modifications in the secondary formation of tissue in this class is therefore an important branch of biological inquiry.

Dukinfield H. Scott & George Brenner
On the Anatomy and Histogeny of Strychnos, 1889

"The central question in every morphological investigation became twofold: it was no longer simply *what is*? it was also *how came it to be*? And this second question, be it observed, is not properly a speculative matter at all, but an historical one; it related not to an ideal or hypothetical mode of origin, but to a real process that has actually taken place in the past and is to be determined like any other historical event."

Ronald A. Jenner
Natural History Museum, London, United Kingdom
The origin of evolutionary storytelling / Perspectives on Evolutionary and Developmental Biology, 2019

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Contents

Prefácio

Este trabalho foi projetado como uma investigação sobre a diversidade dos **padrões anatômicos** encontrados nos **caules** adultos de espécies de plantas da família **nictaginácea** (ou Nyctaginaceae, nome em latim). A espécie mais conhecida desse grupo é a **primavera** ou **buganvília** (*Bougainvillea,* em latim), e tenho certeza que você já se encantou com a beleza de suas flores em algum momento da vida. Além do grande poder ornamental das buganvílias e da "maravilha" (*Mirabilis*), as nictagináceas são importantes quer seja por serem usadas como plantas modelos em estudos ecológicos ("sand verbena" - *Abronia,* uma erva dos desertos da América do Norte), como madeira para serragem ou construção de pequenas embarcações lá na Amazônia (maria-mole - *Neea*) ou como fonte de compostos químicos para fins diversos, inclusive farmacológicos (*Boerhavia, Boldoa, Colignonia, Mirabilis*). Conhecer os padrões anatômicos dos caules das nictagináceas (e outras plantas) é importante tanto para o conhecimento da nossa biodiversidade, como para usos em estudos da biologia e outras ciências aplicadas (comercialização de madeira, botânica forense, biotecnologia). Assim, o estudo da anatomia das plantas pode desempenhar um importante papel para o progresso da ciência e da nossa sociedade.

Além de identificar os padrões observados nos caules das nictáginaceas, um dos principais objetivos do meu estudo foi descobrir quais as alterações que ocorrem na anatomia desses órgãos durante as fases de crescimento da planta. Assim, estudos voltados para conhecer o **desenvolvimento** (ou ontogenia) são realizados para investigar a forma dos organismos (plantas ou animais) desde sua origem até o estágio adulto. No nosso caso, estudamos os caules lá na ponta dos ramos (origem) até atingirem as formas mais complexas nos caules adultos, isto é, nos troncos das árvores ou na base das ervas e arbustos, ou ao longo dos caules dos cipós. Ou seja, este trabalho consistiu em nada mais nada menos do que contar a **história** da formação da estrutura interna dos caules nessas plantas com diferentes hábitos de vida e que estão distribuídas nos mais variados ambientes, incluindo florestas, montanhas, desertos ou mesmo na beira da praia. A diversidade estrutural que observamos nesses caules está diretamente relacionada com a organização das células que compõem os **tecidos vasculares** (ou condutores), isto é, os tecidos que contém as células que transportam a água e os açúcares. À propósito, vale dizer que esses tecidos são fundamentais para a existência das plantas terrestres que vemos ao nosso redor – e por isso conhecê-los se torna tão importante, sendo alvo dos nossos estudos em uma escala anatômica.

Embora o objetivo principal desta pesquisa estivesse relacionado com os padrões anatômicos do sistema vascular, o olhar de anatomista e a curiosidade de pesquisador não me permitiu deixar passar despercebido uma **estrutura secretora** (=glândula que produz substâncias químicas) que ocorre em algumas plantas da família. A presença desta estrutura é tão marcante que em 1909, Paul C. Standley, um pesquisador dos Estados Unidos usou esta característica para escolher o nome científico dado a essas plantas (=*Anulocaulis*). Uma dessas espécies foi utilizada aqui para investigar esta estrutura porque ninguém havia descrito como ela de fato é em sua anatomia, e nem o tipo de substância que produz. Na natureza, a substância que é secretada é muito pegajosa, e alguns insetos ficam grudados nela e acabam morrendo. Curiosamente, algo semelhante acontece com as estruturas secretoras localizadas nos frutos de algumas espécies arbóreas (*Ceodes, Pisonia*) de ilhas do pacífico; nesse caso, as estruturas e substâncias são tão poderosas que podem grudar na asa de pássaros que não conseguem mais voar e acabam morrendo, motivo pelo qual estas plantas são conhecidas como "pegadoras de passarinhos").

O presente trabalho foi desenvolvido em parte durante a pandemia de coronavírus (COVID-19), pela qual vários planos, viagens de campo e atividades foram comprometidos enquanto uma doença infecciosa disseminada desolava o Brasil e o mundo. Parte do agravamento dessa pandemia se deve à natureza do vírus, parte ao descuido dos tomadores de decisão e, claro, ao ceticismo infundado em relação ao trabalho incansável de cientistas ao redor do globo.

Enfim, a seguir eu introduzo os vários temas relacionados à minha tese e então apresento a incrível diversidade anatômica dos caules das nictagináceas na forma de artigos científicos. Caso não seja possível ler todo esse conteúdo, espero que possa ao menos folhear as próximas páginas para se encantar com as belas imagens que a anatomia das nictagináceas nos forneceu! É tão incrível que uma dessas imagens foi destaque na revista Pesquisa FAPESP, em abril de 2020, e outra estampará a capa de um dos números do International Journal of Plant Sciences, em setembro de 2021.

Foreword

This work was originally planned as an investigation on the diversity of **anatomical patterns** found in adult **stems** of species of the **four o'clock family** (or Nyctaginaceae, name in latin). The most known plants of this group are the **paperflowers** (*Bougainvillea*) which I am certain you have been enchanted by their beauty of these flowers at some point in your life. In addition to the ornamental power of the paperflowers and four o'clocks (*Mirabilis*), the nyctaginacea are important because they are used as model plants in ecological studies ("sand verbena" – *Abronia*, an herb native to the deserts of North America), as sawdust or timber for construction of canoes in the Amazon region ("maria-mole" – *Neea*) or as source of chemical compounds for various purposes, including pharmacological (*Boerhavia, Boldoa, Colignonia, Mirabilis*). Knowing the anatomical patterns of stems of the four o'clock species (and other plants) is important for our knowledge of biodiversity, as for uses in a variety of biological studies, as well as other applied sciences (timber exploitation and marketing, forensic botany, biotechnology). Therefore, the study of plant anatomy can play an important role in the progress of science and our society.

In addition to identifying the structural patterns observed in the stems of the four o'clock plants, one of the main goals of my study was to unravel the changes occurring in the anatomy of the stems during the different stages of the growth of the plant. Thus, studies seeking to understand the **development** (or ontogeny) are carried out to investigate the form of organisms (plants or animals) from their origin until they reach the adult stage. In my case, I studied the stems from their origin (at the tip of the branches) until they reached the most complex forms in the adult stems, that is, in the trunks of trees or at the base of herbaceous plants, shrubs, or along the stems of climber plants. In other words, this work consisted of nothing more than telling the **story** of the formation of the internal structure of the stems in these plants with different habits and distributed different environments, including forests, mountains, deserts or even on the shore. The structural diversity that we observe in these stems is related to the organization of the cells that make up the **vascular tissues** (or conductive tissues), that is, the tissues that contain the cells that transport water and sugars. Additionally, it is worth noting that these tissues are fundamental for the existence of the land plants we see around us – and that is why learning of them has become an important target of my studies on an anatomical scale.

Although the main goal of this study was to understand the anatomical patterns of the vascular system, the trained eye of an anatomist and the curiosity of a researcher did not prevent me from overlooking a **secretory structure** (=gland that produces chemical substances) that occurs in some plants of the family. The presence of this structure is so incredibly remarkable that in 1909, Paul C. Standley, a researcher from the United States, used this feature to decide on the scientific name given to these plants (=*Anulocaulis*). One of these species was used in this study to investigate this structure, as no one had described what it actually looks like in its anatomy, nor the type of substance it produces. In nature, the substance that is secreted is very sticky, and some species of insects tightly bind to it causing them to dye. Interestingly, something similar happens with the secretory structures present in the fruits of some tree species (*Ceodes, Pisonia*) of the Pacific Islands; in this case, the structures and substances are so powerful that they can stick to the wing of birds impairing their flying abilities and eventually causing their death; these plants are known as "bird catchers".

This work was developed in part during the coronavirus pandemic (COVID-19) by which various plans, field trips and activities were compromised as widespread infections desolated Brazil and the world. Part of the aggravation of this pandemic is due to the nature of the virus, part due to the carelessness of decision-makers, and, of course, due to unfounded skepticism towards the tireless work of scientists around the globe.

Anyway, below I present a summary of the results of my thesis (page 15). Afterwards, I start to present the incredible anatomical diversity of stems from species of the four o'clock plants in the form of scientific articles. If you are not interested in reading all this content, I hope you can at least flip through the next few pages to be enchanted by the beautiful images that the anatomy of the four o'clock has provided us! It's so incredible that one of these images was featured in Pesquisa FAPESP magazine, in April 2020, and another image will be on the cover page of an issue of the International Journal of Plant Sciences in September, 2021.

General Introduction

General Introduction

Plant Morphology: a way to understand nature

Plant morphology can be described as the discipline that try to explain what each part of the plant is (Scott, 1906). This branch of botany encompasses the study of plant anatomy, which is concerned with the survey of the internal morphology of plants. It is a traditional field in plant science seeking to comprehend the structure and any part of living organisms. Plant morphology is, therefore, a complex concept since it encompasses the knowledge from macromolecules to whole organisms. The ways to study these structures is also variable and that is how morphology become a so ample and interesting subject. The morphological research can be divided in many fields (e.g., concept, process, developmental morphology, morphometrics) (Sattler, 1996; Weber, 2003; Sattler, 2019; Rutishauser, 2020). Currently, plant morphology plays an important role not only in describing the variety of forms in nature, but also to understand the evolutionary history of phenotypic characters and ontogenetic pathways within the framework of molecular phylogenies (Endress, 2003; Jaramillo et al., 2004; Rutishauser, 2020). This evolutionary developmental approach is the main method used in this study.

The evolutionary developmental biology is a field of study that aims to understand the processes involved in the generation of morphological diversity and evolutionary patterns of the organisms (Hall, 2003, 2012; Arthur, 2004). In this sense, the interpretation of ontogenetic data within a phylogenetic framework allows us to better understand the morphological diversity of organisms because the development is the process that generates the adult forms. Therefore, comparative studies integrating ontogeny and phylogeny in plants have become increasingly common, from seed morphology to pollination biology, allowing us to test whether developmental mechanisms such as heterochrony, heterotopy, novelty, or homeosis had been involved in the generation of morphological diversity (Jaramillo et al., 2004; Rudall and Bateman, 2006; Pryer and Hearn, 2009; Pace et al., 2009; Armbruster et al., 2013; Vasconcelos et al., 2018).

Exploring the structure of plants is fundamental in botanical science and offers essential information to look at the hypotheses of plant systematics and evolution. Specifically, the study of wood anatomy (secondary xylem) and bark anatomy (secondary phloem + periderm) has played a crucial role in our understanding of plant biology (Baas, 1982; Olson, 2007; Carlquist, 2009; Rosell et al., 2017; Frankiewicz et al., 2020), including in the interface with biomechanics (Rowe *et al.* 2004; Isnard *et al.* 2005; Gerolamo *et al.* 2020), hydraulics (Gerolamo and Angyalossy 2017; Lamarque *et al.* 2018; Dória *et al.* 2019), as well as ecophysiology (Feild and Isnard 2013; Jupa *et al.* 2016) and biotechnology/biomimetics (Fiorello et al., 2020; Gallentine et al., 2020; Soffiatti and Rowe, 2020).

Anatomy of vascular tissues: a major aspect of plant biology

Because of the vital importance of conducting tissues in vascular plants, the stele is one of the firstborn and key concepts in plant biology. The stele is the organization of the primary vascular system in stems and roots (Van tieghem 1884). The vascular tissue produces during this developmental stage is a result of the activity of the primary meristem, the procambium, and the different types of steles have long been showed to have pivotal systematic importance. As these organs continue to develop, additional vascular tissue is formed by the vascular cambium, which is a secondary meristem that differentiates in a continuum with the procambium (Esau 1943). Structurally, the vascular cambium is a meristem formed by two types of cells but organized generally as a single or few layers of cells along the stem surface, as seen in transverse view. Yet, the vascular cambium is the group of cells that produce most of the biomass of the planet, since its meristematic activity yields the wood (secondary xylem) and bark (secondary phloem) forming the tall, long, and robust stems/trunks of plants over the surface of the Earth.

The risen of vascular cambium was a key innovation accounting for the possibility of new growth forms, i.e., tall self-supporting plants like trees and shrubs, and enabling them to conquer different ecological niches. Evolutionarily, the vascular cambium appeared in the ancestors of a large group of plants, the lignophytes, which includes most of the tallest and largest plants ever known (Simpson, 2010; Decombeix et al., 2019). This group encompass both extinct and extant plants, the latter including the well-known gymnosperms and angiosperms, together known as the seed plants (Simpson, 2010). Nevertheless, one must bear in mind that this meristem is in no way uniform in origin, location, structure, or nature of action. Overall, in relation to their activity, the cambium of the seed plants is usually a bifacial meristem generating xylem centripetally and phloem centrifugally (Spicer and Groover, 2010; Chiang and Greb, 2019). However, there are plants interpreted as having a unifacial cambium (e.g., Lycopsids*,* Decombeix et al., 2019), and plants with cambium producing secondary tissues with inverted polarity due to natural and/or experimental causes (Siebers, 1971; Terrazas et al., 2011; Cunha Neto et al., 2018; Tomescu and Groover, 2019).

In addition, the stems (and some roots) can show alternative types of vascular growth, called cambial variants, which result from the differential activity of the regular/single cambium (e.g., phloem wedges, xylem and phloem in plates) or the formation of several cambia (e.g., successive cambia, compound, divided and corded stems) (Angyalossy *et al.* 2012, 2015). The various types of cambial variants are widespread across the phylogeny of angiosperms, appearing also in the gymnosperms (e.g., Gnetales) (Angyalossy *et al.* 2012, 2015).

Unlike the evolution of the vascular cambium in the lignophytes, however, the multiple evolution of cambial variants does not seem to have impacted the life of plants on earth in the same proportion. Although much has still to be investigated, there are reasons to believe that cambial variants confer important ecological advantages to vascular plants especially in lianas (scandent plants), in which they are referred to increase their mechanical strength, conductivity and flexibility (Putz and Mooney 1991; Schnitzer *et al.* 2015). The functional role of cambial variants in self-supporting plants the functional role is still waiting for clarification.

In relation to the diverse biological aspects of the cambium, the case study presented here is certainly a good example to show how liable this meristem can be even within a small lineage as it is the family Nyctaginaceae.

Nyctaginaceae (the four o'clock family): a neotropical lineage with intriguing morphological diversity

Nyctaginaceae is a monophyletic family considered a core group within the order Caryophyllales based on both morphological and molecular evidence (Bittrich and Kühn, 1993; Douglas and Manos, 2007; Hernández-Ledesma et al., 2015). Morphologically, the monophyletism of the family is indicated by the absence of corolla and the type of fruit, an achene, commonly known as 'anthocarp' (Levin, 2000; Douglas and Manos, 2007). Although the majority of the genera within Nyctaginaceae may be recognized based on the variation of the anthocarp (Douglas and Manos, 2007; Douglas and Spellenberg, 2010), the relationships inside the family are less evident, probably as a result of the absence of characters provided by the flowers (Levin, 2000; Douglas and Spellenberg, 2010).

Currently, the classification of Nyctaginaceae includes around ~300-400 species, distributed in ~34 genera, divided in seven tribes (e.g., Boldoeae, Bougainvilleeae, Caribeaeae, Colignonieae, Leucastereae, Nyctagineae and Pisonieae) (Douglas and Manos, 2007; Douglas and Spellenberg, 2010; Rossetto et al., 2019; Rossetto and Caraballo-Ortiz, 2020). While the tribal classification seems to be quite stable due to its recent re-assessment based on molecular data (Douglas and Spellenberg, 2010), the generic classification is undergoing new circumscriptions, as for the resurrection of earlier described genera (e.g., *Ceodes* and *Rockia*), that were segregated from the large genus *Pisonia* (Rossetto and Caraballo-Ortiz, 2020).

The species of Nyctaginaceae are distributed mostly in the tropics and subtropics of the New World (Bittrich and Kühn, 1993; Douglas and Manos, 2007; Hernández-Ledesma et al., 2015). In the Neotropics, one of the centres of distribution of the family, approximately 23 genera and approximately 180 species have been recorded (Damascena and Coelho 2009). In Brazil, there are 11 genera and more than 50 species distributed throughout the country (Sá *et al.* 2020); the genera *Andradea, Belemia, Bougainvillea, Guapira, Leucaster, Neea, Pisonia, Ramisia* and *Reichenbachia* have been recorded as native plants; *Andradea, Belemia, Leucaster* and *Ramisia* are endemic to the country, while *Boerhavia* and *Mirabilis* are considered naturalized genera (Sá *et al.* 2020). The second centre of distribution of the family is in the deserts of North America, where several native genera of herbs and subshrubs underwent a remarkable species radiation (Douglas and Manos, 2007).

Overall, the species of Nyctaginaceae comprise a wide diversity of growth forms, including herbs, subshrubs, shrubs, scandent-shrubs, trees and scandent plants, herbaceous or woody vines (lianas) (Bittrich and Kühn 1993; Douglas and

Spellenberg, 2010; Hernández-Ledesma et al., 2015). It is worth to note that several taxa within the family can be found sometimes as self-supporting (e.g., free-standing shrubs or trees) and sometimes as scandent individuals (i.e., scandent-shrubs or lianas), which is the case of some species of *Bougainvillea* and *Leucaster* (pers. obser.) and some *Commicarpus* (Friis et al., 2016; Thulin, 2021). Other taxa are remarkable as suffrutescent plants (=sufrutescens or sufruticosus) meaning that they are woody at the base and herbaceous throughout the branches, which are similar to subshrubs (Bittrich and Kühn 1993; Douglas and Spellenberg, 2010; Hernández-Ledesma et al., 2011, 2015; Friis et al., 2016; Blecher and Blecher, 2017). This is the case of genera such as *Colignonia, Commicarpus* and *Mirabilis* (Friis et al., 2016; pers. obser.), and likely other less known taxa, as the species *Cryptocarpus pyriformis* from the Galapagos and continental Ecuador. As we will see, this diversity of habits is accompanied by a variation in the degree of wood formation ('woodiness'), as well as in the developmental processes resulting in their adult forms characterized by cambial variants. This remarkable diversity is one reason why we use the family Nyctaginaceae as a model to investigate aspects of the diversity, distribution, and evolution of stem anatomical characters.

Stem anatomy in Nyctaginaceae: intricate diversity from stele to cambium and cambial variants

In Nyctaginaceae, the origin, development and activity of the vascular meristems have been debated since a long time. Different interpretations have been proposed in the literature and there has been no consensus on several aspects of stem development. Given the notorious diversity of vascular patterns in the family, as easily spotted from the presence of medullary bundles in young stems and mature stems with cambial variants, a variety of studies focuses on the presence and development of these various patterns of secondary growth (Chalk and Chattaway 1937; Rajput and Rao 1998; Carlquist 2004; Hernández-Ledesma *et al.* 2011). However, these works generally analyses few taxa, and it is possible to observe that different cambial variants are reported for the same genus or even the same species without a clear developmental understanding and classification. For example, Chalk and Chattaway (1937) reported that the cambial variant of the genera *Guapira, Neea* and

Pisonia, is interxylary phloem, whereas Carlquist (2004) states that they all have successive cambia. On the other hand, detailed studies in relation to both wood (secondary xylem) and the bark (all tissues outside the cambium) are still needed, since most information on stem anatomy for the family are restricted to classical works (Solereder 1908; Record and Hess 1943; Metcalfe and Chalk 1957; Roth 1981), or papers concentrating on a few taxa (Puglia and Norverto 1991; Rajput and Rao 1998; Hernández-Ledesma *et al.* 2011; Sonsin *et al.* 2014).

Studies integrating stem anatomy and ontogenetic data with phylogeny have never been performed for Nyctaginaceae. Because the family have a wide diversity of habits and types of cambial variants within these habits, multiple scenarios are possible to evaluate whether there have been shifts in rates of diversification and if the transitions of habit (as suggested by Gianoli, 2004, 2015) and habitat, as well as if the acquisition of new anatomies have impacted the family in terms of diversification.

Furthermore, the analysis of anatomical characters of the secondary xylem and phloem will allow us to contribute to the understanding of the evolution of these tissues in plants with transitions of habits and acquisition of cambial variants. In other words, with this study we will be able to answer several questions, such as: How many cambial variants are there in Nyctaginaceae and when did they arise? Are these characters ancestral or derived for the family? Was the cambial variant already present in Nyctaginaceae and its sister group, thus constituting a symplesiomorphy or alternatively the cambial variants appear/evolve within the group? If so, how many times? In the self-supporting plants, which are submitted to different selection pressures than lianas, what would be the possible functions of the cambial variants?

This thesis aims to bring together the aspects concerning the development and evolution of stem anatomical characters in Nyctaginaceae, with emphasis on the distribution and anatomical diversity of vascular patterns. We accomplish this by studying the origin, anatomy, and development of vascular tissues within a phylogenetic framework. By addressing the anatomical and developmental information upon a robust phylogenetic hypothesis, we are now able to identify the main aspects behind the evolutionary history of stem characters in the family.

Thesis outline

Our hypotheses and specific goals are investigated in each of the five chapters of the thesis. Chapters one to four have already been published, and chapter five will be submitted for publication after the thesis defense and due corrections of the manuscript. The organization of the articles follow the guidelines of the respective journals.

In **chapter 1**, we demonstrate the structure and histochemistry of a poorly known secretory structure found in the stems of *Anulocaulis.* With regard the vascular structure, Nyctaginaceae has presented an intriguing and complex morphological diversity concerning the formation of both primary and secondary anatomies. **Chapter 2** presents a case study to approach the complexity of alternative patterns of primary and secondary vascular anatomies in *Allionia.* It also encompasses a general study of the anatomy of vegetative organs aiming at to corroborate to the systematics of this genus. The facets of the primary vascular tissue organization are explored in a broader scale in **chapter 3**, where the presence of medullary bundles is investigated in the context of stele concept. In this paper the development and evolution of the different eustele types (i.e., regular and polycyclic) is presented, with emphasis to the diversity of medullary bundles. **Chapter 4** presents a comparative anatomical study of stems under secondary growth of species from different lineages of Nyctaginaceae. This study presents a new interpretation for the secondary growth in the family as unexpected developments confirm the presence of interxylary phloem for the family. These represent some of the most important results of this thesis due to the demystification of successive cambia as the universal type of cambial variant in the family, and perhaps other families of the Caryophyllales. In **Chapter 5**, we explore the evolution of development for the two newly delimited types of cambial variants in Nyctaginaceae (i.e., interxylary phloem and successive cambia). We demonstrate that there are different combinations on how the secondary growth is established in relation to the different subtypes of eusteles. The mode how these two types of cambial variants are constructed is not always the same but constitute different developments that may contain intermediate stages, as in a case of the so called "fuzzy morphology" (continuum morphology).

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Final Remarks

In this study, we used an **integrative approach** including mostly **development** and **phylogenetic comparative methods** to understand the **diversity** and **evolution** of the **vascular system** in Nyctaginaceae, a predominantly **neotropical family**. The development of this study increased our knowledge on different aspects of stem anatomy of the main lineages of Nyctaginaceae, except for Caribeaeae, which is a monotypic group, from Cuba, and likely extinct. Explanation of the most important conclusions of each chapter are summarized below.

In the first chapter, we revealed the **anatomy** and **histochemistry** of a poorly known **secretory structure** in the genus *Anulocaulis,* which seem to be present also in other genera (e.g., *Boerhavia, Cyphomeris*). We showed that the secretory structure is a group of elongated epidermal cells (or unicellular glandular trichomes) which secrete a complex exudate. From the second chapter onward, we focused on the diversity of the vascular system, which proved to be more **diverse** and **complex** than previously thought. Chapter two was dedicated to a case study using the genus *Allionia,* with only two species with unresolved **taxonomy**. In this study, we discovered that the two species have conserved vegetative anatomy and that even though being small **herbs**, the vascular system possesses interesting characteristics such as **polycyclic eustele** and **cambial variants**, characterized as **successive cambia**. In addition, we demonstrated through a detailed **ontogenetic study** that the **origin** of this cambial variant which originate in the **pericycle** contradicts the vast literature stating that a meristem derived from the **cortex** generate the successive cambia system.

From chapter three onwards, our approach changed to a broader taxonomic scale using **comparative morphological analyses** under a phylogenetic context. In chapter three we investigated the diversity and evolution of the vascular system in Nyctaginaceae and related families and showed that two subtypes of **eustele** occur in this lineage, the **regular eustele** and the **polycyclic eustele**, which consists of **medullary bundles** and a **cylindrical continuous procambium** (CCP). The polycyclic eustele develops as **vascular traces** of lateral organs and the medullary bundles may appear in various number (from eight to ~30 bundles) and arrangements (ordered or non-ordered). This type was reconstructed as the **ancestral character** for the family, and as a **symplesiomorphy** for the clade phytolaccoid of the Caryophyllales, that includes families such as Phytolaccaceae and Petiveriaceae.

In chapter four, we developed the largest comparative study of **stem anatomy** in Nyctaginaceae to begin to understand the complexity and diversity of the secondary vascular system, which is marked by the presence of **cambial variants**. Nonetheless, the types and developments of these patterns is much more intricate and complex than previously reported. For this reason, this paper was dedicated to unravelling the development of the most common type of cambial variant in the family, which is not successive cambia as stated since late nineteenth century. Because the gross morphology of the stems is achieved through the activity of a **single cambium**, the secondary growth occurring in representatives of most tribes of the family is better described as **interxylary phloem** – characterized mostly by the presence of phloem strands immersed within the secondary xylem. Despite sharing this character, the anatomy in these plants is diverse, ranging from stems with small phloem islands, to others forming patches and long bands of phloem recovered by the sheathing axial parenchyma interrupting the secondary xylem.

In chapter five, we expand our analyses of the development of cambial variants to all lineages of the family. We discovered that two patterns are present in the family, interxylary phloem and successive cambia, following four different **developmental pathways.** Unlike our expectations, interxylary phloem is the most common pattern and was reconstructed as **ancestral** in the family. Interxylary phloem occur in most lineages of Nyctaginaceae, being absent likely only in **Leucastereae** – an almost exclusive Brazilian tribe - distinguished also by being the only lineage with regular eustele instead of polycyclic eustele (chapter two). From this pattern, successive cambia evolved three times in the family, each following a different ontogeny. This **complexity** is because the ontogenies depart from different eustele subtypes and due to the appearance of unusual features such as the **extra-fascicular cambium**, as well as the well-known development of successive cambia with a ***de novo*** formation of a new meristem in the **pericycle**. For example, successive cambia may be derived from both regular eustele or polycyclic eustele, whereas interxylary phloem is always derived from polycyclic eustele. Other important findings from this study include the observation that regular secondary growth is found only during the initial growth of

Reichenbachia, which later develop successive cambia. All other Nyctaginaceae present variations in their development through the formation of polycyclic eustele + interxylary phloem (known for 19 genera in all tribes, except Leucastereae and Caribeaeae), regular eustele + successive cambia (4 genera from Leucastereae) or polycyclic eustele + successive cambia (2 genera from Nyctagineae). This observation is important because makes Nyctaginaceae one of the few families with cambial variants in all representatives.

In the evolution of the developments of cambial variants in Nyctaginaceae, **heterochrony**, **heterotopy** and **modularity** are the main **developmental processes** accounting for the increased morphological diversity in the group. In addition, we showed that the only way to understand the evolution of successive cambia from interxylary phloem is using the **fuzzy worldview**, since that there are intermediate forms between each category and between these two morphological types. This is the first time that cambial variants are discussed under the **continuum morphology** concept and that one variant can originate another. Also exciting were the results obtained with the **diversification analyses**, which showed that being a liana did not increase species diversification compared to their non-climbing sister groups. This result opens new avenues to investigate the role of cambial variants in lianescent groups, since their presence instead of the climbing habit might be the key innovation promoting **species diversification**.

Considering the anatomical diversity found in the vascular system of Nyctaginaceae, we must, therefore, recognize that the generally accepted definitions of **procambium** and **cambium** cannot be employed strictly. As we could observe from Nyctaginaceae, the primary vascular **meristem**, the **procambium**, appears in different configurations, and the development of the primary and secondary growth merges, gradually and continuously, following diverse **ontogenetic pathways** that result in **disparate morphologies** (e.g., eustele subtypes, cambial variants). These observations are remarkable because they were unnoticed for quite some time and because they show unique anatomies compared to the patterns found in most **eudicots**. Here, again, we realize that in biology there is almost not a rule not including exceptions.

In summary, anatomical and ontogenetic studies under a phylogenetic approach are essential to our understanding of the developmental processes that generate disparate morphologies.

Future research directions – To continue to expand our understanding of the anatomical diversity of the vascular system in Nyctaginaceae, an investigation of the diversity and evolution of wood and bark characters would be a great benefit. Efforts to summarize this information has already begun using our large taxonomic sampling and slide collection used in this thesis. Other research ideas deriving from this project and under progress include: i) a study of the diversity and evolution of medullary bundles within Caryophyllales; in this study we aim at integrating the anatomical data with biomechanical and/or hydraulic experiments to explore the adaptive function of medullary bundles; ii) an anatomical comparative study of vegetative organs of the emblematic genus *Belemia,* including an updated taxonomic treatment for the group.

In addition, given that now we understand better the cambial variants in Nyctaginaceae in terms of anatomy, using a model plant from this group to investigate the molecular basis underlying these patterns would put an enhanced perspective into the evolution of its diversity. Investigations on molecular genetics of plant development have become increasingly common in recent years, yet much of these plant evo-devo studies have concentrated on the flower and the leaf. Nevertheless, to understand morphological evolution, these concepts and research need to be developed across all biological systems of land plants. Therefore, investigating the developmental genetics in groups with such intriguing morphologies as it is the Nyctaginaceae and perhaps other families of the Caryophyllales would bring more light to our knowledge on the aspects of wood, bark and cambial variants development and evolution.

Resumo

Nyctaginaceae, a família da primavera, tem ampla ocorrência nos Neotrópicos, e com espécies com uma notável variedade de hábitos e padrões anatômicos vasculares em seus caules. Por exemplo, os caules dessas plantas têm sido descritos com feixe medulares e variações cambiais desde o século 19, sendo na maioria das vezes interpretadas como tendo câmbios sucessivos e em algumas poucas vezes como floema interxilemático. Todavia, pouco se sabe sobre a real diversidade, origem, desenvolvimento e evolução dessas anatomias vasculares e ainda menos conhecida é sua distribuição filogenética e se elas impactaram a diversificação dos clados dentro da família. Portanto, o presente estudo teve como objetivo compreender a diversidade anatômica, desenvolvimento, distribuição e evolução dos diferentes padrões anatômicos vasculares em Nyctaginaceae. Amostras de caules de mais de 90 espécies distribuídas em 26 dos 34 gêneros conhecidos, e pertencentes a seis das setes tribos da família foram investigadas. Nossos resultados apontam que a diversidade e complexidade do sistema vascular caulinar em Nyctaginaceae é maior do que previamente imaginado. Há dois subtipos de eustelo na família, regular e policíclico (que inclui feixes medulares e um procâmbio contínuo), e dois tipos de variações cambiais, câmbios sucessivos e floema interxilemático. Nós vimos que caules com feixes medulares e floema interxilemático representam a condição mais comum e ancestral para a família. Entretanto, a evolução de variações cambiais não é contingente em relação ao sistema vascular primário, e nem aos hábitos. Vimos também que Nyctaginaceae se destaca como uma das poucas famílias onde representantes de todas as linhagens apresentam variações cambiais. Finalmente, a diversidade dessas anatomias complexas é discutida sob o conceito de morfologia contínua, dado que a evolução desses padrões em Nyctaginaceae ocorreu com transições intermediárias entre as diferentes categorias. O impacto dos hábitos, habitats e variações cambiais na diversificação da família também são discutidos. Este trabalho demonstra que estudos ontogenéticos realizados em um contexto filogenético continuam sendo um excelente método para desvendar a enorme diversidade morfológica encontrada nos organismos, sua evolução e seus impactos na diversificação das espécies.

Abstract

Nyctaginaceae, the four o'clock family, has a broad occurrence in the Neotropics, containing species with an outstanding diversity of habits and stem vascular anatomical patterns. For instance, the stems in Nyctaginaceae have been described with medullary bundles and cambial variants since the 19th century, being the majority of times interpreted as having successive cambia, and a few times with interxylary phloem. However, little is known about the real diversity, origin, development and evolution of these vascular anatomies, but even less understood is its phylogenetic distribution and if they impacted the diversification of clades within the family. For that, stem samples from more than 90 species distributed in 26 from the 34 genera currently recognized and belonging to six out of the seven tribes of the family were investigated. Our results indicate that the diversity and complexity of the stem vascular system in Nyctaginaceae is larger than previously anticipated. There are two subtypes of eustele in the family, regular and polycyclic (which includes medullary bundles and a continuous procambium), and two types of cambial variants, successive cambiums and interxylary phloem. We have seen that, stems with medullary bundles and interxylary phloem represent the most common and ancestral condition for the family. However, the evolution of cambial variants is not contingent on the primary vascular system, nor on habits. We have also seen that Nyctaginaceae stands out as one of the few families where representatives of all lineages have cambial variants. Finally, the diversity of such complex anatomies is discussed under the concept of continuum morphology, given that the evolution of these patterns in Nyctaginaceae occurred with transitions of intermediate forms between the different categories. The impacts of habits, habitats and cambial variants in the diversification of the family are also discussed. Thus, this work demonstrates that ontogenetic studies carried out in a phylogenetic context continue to be an excellent method to unravel the immense morphological diversity observed in organisms, their evolution and their impact on species diversification.

Extra files

Extra file I – First page of the published articles with results from this project

Chapter 1

WHAT ARE THE "STICKY RINGS" ON STEMS OF *ANULOCAULIS* AND RELATED TAXA (NYCTAGINACEAE) FROM ARID REGIONS?

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ABSTRACT

Anulocaulis, commonly known as "ringstem," is a small, unusual genus restricted to the Chihuahuan, Sonoran, and Mojave deserts of North America. Here we combined light microscopy and histochemical tests to characterize for the first time the "sticky structures" (here called secretory rings) found on the stem internodes of *Anulocaulis.* The secretory rings were shown to be groups of epidermal cells, or unicellular glandular trichomes, which largely differ from their neighboring cells both in structure and histochemistry. The cells start to differentiate in early stages of stem development. They begin as regular epidermal cells, but later their anticlinal and external tangential walls start to enlarge. At maturity the cells become remarkably elongated, even balloon-like, with dense cytoplasmic content. Although the secretory rings have been reported as "mucilaginous structures" based on morphological observations, preliminary histochemical analyses showed that its exudate is complex, including a mixture of mucilage, proteins, and phenolic compounds. Future investigations are needed to compare the anatomy of the secretory rings within related genera of Nyctaginaceae and characterize the chemical components of their exudate more specifically to search for potential homologies and adaptive functions of these structures.

RESUMEN

Anulocaulis, comúnmente conocido como "ringstem," es un género pequeño que se encuentra restringido a los desiertos de Chihuahua, Sonora y Mojave de América del Norte. En este estudio, usamos microscopía óptica y pruebas histoquímicas para caracterizar por primera vez las "sticky structures" (aquí denominadas "secretory rings") que se encuentran en los entrenudos del tallo de *Anulocaulis.* Se demostró que los anillos secretores son grupos de células epidérmicas, o tricomas glandulares unicelulares, que se diferencian en gran medida de sus células vecinas tanto en estructura como en histoquímica. Las células comienzan a diferenciarse en las primeras etapas del desarrollo del tallo. Comienzan como células epidérmicas normales, pero luego sus paredes anticlinal y tangencial externa comienzan a agrandarse. En la madurez, las células se vuelven notablemente alargadas, incluso como globos, con un contenido citoplasmático denso. Aunque los anillos secretores han sido descritos como "estructuras mucilaginosas" basadas en observaciones morfológicas, los análisis histoquímicos preliminares mostraron que su exudado es complejo, incluyendo una mezcla de mucílago, proteínas y compuestos fenólicos. Son necesarias investigaciones futuras que permitan estudiar comparativamente la anatomía de los anillos secretores en los géneros de Nyctaginaceae y así mismo caracterizar los componentes químicos de su exudado más específicamente para buscar posibles homologías y funciones adaptativas de estas estructuras.

Key Words: Anatomy, Caryophyllales, Nyctagineae, secretory structures, Chihuahuan Desert, glandular trichomes

INTRODUCTION

Anulocaulis is a small genus of perennial herbs with just five species. It is included in tribe Nyctagineae, which is the largest and most diverse tribe in the family Nyctaginaceae (Douglas & Spellenberg 2010). The genus is endemic to arid regions of North America (e.g., Chihuahuan, Sonoran, and Mojave Deserts) and is distributed from northern Mexico to southeastern California in the United States of America (Spellenberg 1993; Douglas & Spellenberg 2010). *Anulocaulis* may be divided into two groups based on fruit morphology (Spellenberg 1993). The first encompasses *A. annulatus, A. hintoniorum,* and *A. eriosolenus,* which have smooth anthocarps. *Anulocaulis annulatus* is restricted to low, hot elevations near Death Valley in the Mojave Desert, while *A. hintoniorum and A. eriosolenus* are restricted to the Chihuahuan Desert in Mexico and Texas. The second group comprises *A. leiosolenus* (considered to have four varieties, Spellenberg 1993) and *A. reflexus.* These are characterized by their variously winged and wrinkled anthocarps and are primarily gypsum endemic plants

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Chapter 2

ANATOMY OF VEGETATIVE ORGANS IN *ALLIONIA* (NYCTAGINACEAE), WITH EMPHASIS ON THE VASCULAR SYSTEM

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ABSTRACT

Allionia is a small genus within the tribe Nyctagineae (Nyctaginaceae) which has a controversial, infrageneric delimitation. Here, we investigated the two known species of *Allionia* in order to characterize the anatomy of leaves, stems and roots, with further notes on vascular system development. Additionally, the present study aimed to broaden our knowledge of stem vascular diversity and to survey for anatomical features with diagnostic value in distinguishing *A. choisyi* from *A. incarnata*. Leaf anatomy of other Nyctagineae taxa was also analysed. Anatomical and ontogenetic observations from the vegetative organs in *Allionia* revealed no diagnostic features to distinguish the two species. We illustrated the occurrence of Kranz anatomy, which in Nyctaginaceae is only known in *Allionia, Boerhavia,* and *Okenia*. The stem primary vascular system was unusual in showing a polycyclic eustele (medullary bundles + continuous concentric procambium). Likewise, mature stems and roots show vascular cambial variants (successive cambia) that arise from the pericycle. The anatomy and histochemistry of multicellular glandular trichomes observed in aerial organs were presented. Raphids were seen in all organs. Although no strong xerophytic features were observed in *Allionia*, several characteristics can be associated with their arid habitats. Our findings on the vascular system of *Allionia* showed the two species to be much the same and reinforced earlier findings that the stem anatomy of Nyctaginaceae is complex and intriguing.

RESUMEN

Allionia es un género pequeño dentro de la tribu Nyctagineae (Nyctaginaceae) con delimitación infragenérica controversial. Analizamos las características anatómicas de hojas, tallos y raíces de las dos especies conocidas de *Allionia* e incluimos comentarios sobre el desarrollo del sistema vascular. El presente estudio pretende, examinar características diagnósticas entre *A. choisyi* y *A. incarnata* y de esta forma ampliar el conocimiento sobre la diversidad vascular del tallo. Adicionalmente, analizamos la anatomía foliar de otros taxa de Nyctagineae. Las observaciones anatómicas y ontogenéticas de los órganos vegetativos en *Allionia* no mostraron características diagnósticas que permitieran diferenciaran entre las dos especies. La anatomía Kranz para Nyctaginaceae, restringida únicamente a *Allionia, Boerhavia* y *Okenia* fue ilustrada. Presentamos la anatomía e histoquímica de tricomas glandulares multicelulares observados en órganos aéreos. El sistema vascular primario del tallo era incomum al mostrar un eustele policíclico (haces medulares + procambio concéntrico continuo). Así mismo, tallos y raíces maduras mostraron variantes cambiales vasculares (cambios sucesivos) que surgen en el periciclo. Todos los órganos presentaron rafidios. No fueron observadas características xerofíticas en *Allionia*, sin embargo, varias características pueden estar relacionadas con ambientes áridos. Estos hallazgos esclarecen y corroboran la complejidad anatómica de las especies de Nyctaginaceae, y muestran la intrigante diversidad de patrones anatómicos caulinares.

Key Words: *Allionia choisyi, Allionia incarnata,* Caryophyllales, cambial variants, Nyctagineae, ontogeny

INTRODUCTION

Nyctaginaceae have about 30 genera and 400 species which include trees, shrubs, subshrubs, lianas and herbs (Douglas & Manos 2007; Douglas & Spellenberg 2010; Hernández-Ledesma et al. 2015). The species are distributed mostly in the tropics and subtropics of the New World, except for some genera that occur in the Old World (e.g., *Boerhavia, Commicarpus, Pisonia, Phaeoptilum,* and *Mirabilis*) (Hernández-Ledesma et al. 2015). In the most recent classification, the family has been divided into 7 tribes: Nyctagineae, Boldoeae, Leucastereae, Bougainvilleeae, Pisonieae, Colignonieae, and Caribeeae (Douglas & Spellenberg 2010).

Allionia L. belongs to tribe Nyctagineae and comprises species of annual or perennial herbs with procumbent, decumbent or prostrate stems (Fig. 1). Two species are recognized *A. choisyi* Standl. and *A. incarnata* L. which are very similar morphologically, differing only in some fruit characteristics (e.g., number of lateral expansions, length of glands) (Spellenberg 2003), which makes the delimitation of infrageneric categories

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Chapter 3

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RESEARCH ARTICLE
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Diversity, distribution, development, and evolution of medullary bundles in Nyctaginaceae

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PREMISE: Medullary bundles, i.e., vascular units in the pith, have evolved multiple times in vascular plants. However, no study has ever explored their anatomical diversity and evolution within a phylogenetic framework. Here, we investigated the development of the primary vascular system within Nyctaginaceae showing how medullary bundles diversified within the family.

METHODS: Development of 62 species from 25 of the 31 genera of Nyctaginaceae in stem samples was thoroughly studied with light microscopy and micro-computed tomography. Ancestral states were reconstructed using a maximum likelihood approach.

RESULTS: Two subtypes of eusteles were found, the regular eustele, lacking medullary bundles, observed exclusively in representatives of Leucastereae, and the polycyclic eustele, containing medullary bundles, found in all the remaining taxa. Medullary bundles had the same origin and development, but the organization was variable and independent of phyllotaxy. Within the polycyclic eustele, medullary bundles developed first, followed by the formation of a continuous concentric procambium, which forms a ring of vascular bundles enclosing the initially formed medullary bundles. The regular eustele emerged as a synapomorphy of Leucastereae, while the medullary bundles were shown to be a symplesiomorphy for Nyctaginaceae.

CONCLUSIONS: Medullary bundles in Nyctaginaceae developed by a single shared pathway, that involved the departure of vascular traces from lateral organs toward the pith. These medullary bundles were encircled by a continuous concentric procambium that also constituted the polycyclic eustele, which was likely a symplesiomorphy for Nyctaginaceae with one single reversion to the regular eustele.

KEY WORDS Caryophyllales; evo-devo; Nyctaginaceae; ontogeny; primary growth; stem anatomy; trait evolution; vascular bundles.

Medullary bundles are complete vascular bundles located in the pith and may be arranged in two or more concentric rings or as bundles scattered within the pith in addition to the bundles of the stele ring (de Bary, 1884; Esau, 1967; Ogura, 1972; Beck et al., 1982; Schmid, 1982; Mauseth, 1988; Beck, 2010; Isnard et al., 2012). Such organization of the vascular tissue constitutes the "polycyclic eustele" subtype in the stele classification by Beck et al. (1982) and Schmid (1982). Medullary bundles have also been addressed in the context of "anomalous structures" (de Bary, 1884; Eames and MacDaniels, 1925; Metcalfe and Chalk, 1950; Beck, 2010; Yang and Chen, 2017), a concept not followed here, since we consider the so-called anomalous vascular structures to be a variant type of secondary growth (Carlquist, 2001; Angyalossy et al., 2012, 2015). Regardless, medullary bundles are a remarkable feature of vascular plants, which also contributes to their complexity and morphological diversity (Eames and MacDaniels, 1925; Beck et al., 1982).

Medullary bundles have evolved multiple times in the history of vascular plants, being present in ferns (e.g., Cyatheaceae and Dennstaedtiaceae [*Pteridium*], Ogura, 1927, 1972; Eames and MacDaniels, 1925; Lucansky, 1974), and more frequently in the flowering plants, where they have been recorded in approximately 60 families, including magnoliids (Isnard et al., 2012; Trueba et al., 2015) and eudicots (Wilson, 1924; Lambeth, 1940; Boke, 1941; Holwill, 1950; Metcalfe and Chalk, 1950; Davis, 1961; Esau, 1967; Pant and Bhatnagar, 1975; Raj and Nagar, 1980, 1989; Kirchoff and Fahn, 1984; Mauseth, 1993, 2006; Costea and DeMason, 2001; Schwallier et al., 2017; Kapadane et al., 2019). In some families, this character is found in just a few representatives (e.g., *Nepenthes*,

Chapter 4

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A NEW INTERPRETATION OF THE SUCCESSIVE CAMBIA OF SOME NYCTAGINACEAE AS INTERXYLARY PHLOEM

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q1 *Premise of research*. The alternative patterns of secondary growth (vascular cambial variants) in stems of Nyctaginaceae are outstanding and have been widely investigated since the late nineteenth century. However, there are controversial interpretations in the literature regarding the existence of either one or two types of cambial variants in this family (successive cambia vs. interxylary phloem). We aim to explore the anatomical diversity of stems in Nyctaginaceae to document the real nature of the cambial variant present in most species of the family.

Methodology. We analyzed 60 species, focusing on 18 species from 12 genera, for developmental studies. Anatomical and ontogenetic features were characterized from images produced by standard plant techniques for macro- and microscopic analyses.

Pivotal results. Our analyses reveal that most species of Nyctaginaceae present stems with polycyclic eusteles, which later develop a single cambium that produces secondary xylem and secondary phloem at unequal rates around the stem circumference. This unusual activity results in the absence of a regular cylinder of secondary vascular tissues and in the formation of secondary phloem strands (surrounded by variable amounts of sheathing axial parenchyma) embedded within the secondary xylem. In cross section, adult stems can exhibit different tissue arrangements (i.e., phloem islands/strands, patches, or concentric bands) that result from differences in rates of production of phloem and associated sheathing axial parenchyma forming the strands. The cambial variant in these stems is described as interxylary phloem, as similarly observed in other eudicot lineages.

Conclusions. Our examination of the stem development of Nyctaginaceae confirms the presence of interxylary phloem, which has been overlooked in the family as most previous studies have reiterated descriptions of successive cambia as the common cambial variant within the family. These findings emphasize the importance of developmental studies encompassing a representative number of genera to further our understanding of stem macromorphologies and to highlight the complexity and diversity of stem architectures in Nyctaginaceae.

Keywords: cambial variant, Caryophyllales, ontogeny, polycyclic eustele, secondary growth, stem anatomy.

Online enhancement: supplemental table.

Introduction

The prevalence of families with cambial variants in the order Caryophyllales, compared with other angiosperm orders, has been reported since the first treatises on the subject (de Bary 1884; Schenck 1893; Pfeiffer 1926). Cambial variants are reported in at least 19 of the 39 families currently recognized in the Caryophyllales (Gibson 1994; Carlquist 2010; Hernández-Ledesma et al. 2015). Until the late twentieth century, the majority of papers on the formation of alternative vascular anatomies in this order followed the traditional view (Schenck 1893; Pfeiffer 1926) that regarded the main cambial variant in the family as successive cambia (i.e., additional increments of vascular tissue through the formation of new cambia outside the first cambium). More recently, Carlquist's outstanding contributions to the understanding of vascular anatomies in Caryophyllales (Carlquist 1991, 1999, 2000, 2001, 2003, 2004, 2007, 2010) have concurred with earlier views that successive cambia is the cambial variant occurring in the order. This view has been subsequently shared by other authors (Rajput and Rao 1998; Rajput et al. 2009, 2012; Hernández-Ledesma et al. 2011, 2015; Rajput and Marcati 2013; Rajput 2015; Myśkow et al. 2019; Zumaya-Mendoza et al. 2019).

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Extra file II - List of works presented in meetings with results from this project

1. CUNHA NETO, I.L., PACE, M.R., ANGYALOSSY, V. When what seems successive cambia in Nyctaginaceae (Caryophyllales) is actually a new type of interxylary phloem. Botanical Society of America Conference - Botany 2021, Online, USA. 2021. (Oral-video).
2. CUNHA NETO, I.L., Anatomia do caule em plantas vasculares: o crescimento secundário nas espermatófitas. II Webnário Botânico da Faculdade de Educação, Ciências e Letras de Igatu (FECLI), da Universidade Estadual do Ceará, May 2021. Brazil (Oral-online).
3. CUNHA NETO, I.L., PACE, M.R., DOUGLAS, N.A., NEE, M.H., SÁ, C.F.C., MOORE, M.J., ANGYALOSSY, V. Stele diversity and evolution in Nyctaginaceae: medullary bundles across the family. Botanical Society of America Conference - Botany 2020, Online, USA. 2020. (Oral-video).
4. CUNHA NETO, I.L., DOUGLAS, N.A., ANGYALOSSY, V. "First report on the structure and histochemistry of the "sticky rings" in stems of Anulocaulis (Nyctaginaceae)". Botanical Society of America Conference - Botany 2019, Tucson, AZ, USA. 2019. (Poster).
5. CUNHA NETO, I.L. "A developmental and evolutionary perspective on the vascular system of Nyctaginaceae". Caryophyllales 2018 Conference, Universidade Nacional Autónoma de México, México City, México. 2018. (Oral).
6. CUNHA NETO, I. L. "Stem anatomy and development as subsidy to vascular plants systematics". IX Winter Botany Course, August 2019, University of São Paulo, SP, Brazil.
7. CUNHA NETO, I. L. "Diversity and evolution of the vascular system in Nyctaginaceae". 69° Congresso Nacional de Botânica (69° CNBot), July 2018, Cuiabá, MS, Brazil.

Extra file III - Awards obtained during the Ph.D.

- 2018 – Cuatrecasas Travel Award, National Museum of Natural History, Smithsonian Institution, Washington D.C., USA.

- 2020 - I.W. Bailey Award 2020, IAWA Journal/ Brill Publishers – awarded by joint candidature with Dr. Joyce G. Chery (Cornell University).

Sobre o autor

Israel L. da Cunha Neto nasceu em Santo Antônio de Jesus, Bahia, Brasil, em 04 de fevereiro de 1991. O interesse pelo mundo natural surgiu principalmente pela vida no interior. Em 2008, ingressou na Universidade Federal do Recôncavo da Bahia (UFRB) para cursar Agronomia. Já no terceiro semestre, se encantou pela botânica de um modo tão profundo que recusou outras oportunidades, até conseguir uma chance no Laboratório de Anatomia Vegetal e Histoquímica, sob orientação do Dr. Fabiano Machado Martins, em 2009. Durante a graduação desenvolveu vários estudos em anatomia e histoquímica de plantas cultivadas e não cultivadas, até conhecer o mundo da anatomia de madeira, lianas e variações cambiais. Entre 2013 e 2014, realizou intercâmbio na Corvinus University of Budapest, Hungria, onde também desenvolveu pesquisa em anatomia vegetal. Em 2014, concluiu o bacharelado com prêmio de aluno destaque daquele ano.

Depois de se graduar, mudou-se para o Rio de Janeiro, onde iniciou o mestrado em botânica no glorioso Museu Nacional da Universidade Federal do Rio de Janeiro (UFRJ), em março de 2015. Neste período realizou pesquisa com foco na anatomia do sistema vascular de lianas da família Sapindaceae, desenvolvendo atividades no Jardim Botânico do Rio de Janeiro (JBRJ) sob supervisão da Dr. Neusa Tamaio. Completou o mestrado em fevereiro de 2017 e, posteriormente, Israel iniciou o doutorado na Universidade de São Paulo (USP) em junho do mesmo ano. Desta vez, sua pesquisa é dedicada ao sistema vascular em plantas da família Nyctaginaceae, sob supervisão da Dr. Veronica Angyalossy. Para este estudo, Israel realizou diversas expedições de coleta no Brasil e no exterior (Bolívia, Estados Unidos, México), além de desenvolver parte dos trabalhos no Museu de História Natural do Smithsonian Institution (Washington, D.C, EUA). Os resultados desta pesquisa já foram apresentados em conferências no Brasil (2018), México (2018) e EUA (2020, 2021) (remotamente).

A lista de publicações do autor pode ser encontrada em seu currículo nas plataformas online Lattes / Orcid / Google Acadêmico / ResearchGate.

About the author

Israel L. da Cunha Neto was born on the 4th of February 1991 in Santo Antônio de Jesus, Bahia, Brazil. Interest in the natural world came from life in the countryside. He joined the Federal University of Recôncavo da Bahia in 2008 to pursue a bachelor's degree in Agronomy. In the third semester, he was fascinated by botany in such a profound way that he refused several internship opportunities, until he was accepted to work at the Laboratory of Plant Anatomy and Histochemistry, under the direction of Dr. Fabiano Machado Martins, from October 2009. During graduation he developed several studies in anatomy and histochemistry of cultivated and non-cultivated plants, until he developed an interest in wood anatomy, lianas and cambial variants. In the meantime, he did an exchange program at Corvinus University of Budapest, Hungary, between 2013 and 2014, where he also developed research in plant anatomy. In 2014, he completed his bachelor's degree obtaining the Outstanding Student Award.

After graduating, he moved to Rio de Janeiro, where he began his master's degree in botany at the celebrated National Museum of the Federal University of Rio de Janeiro (UFRJ), in March 2015. During this period, he conducted research focusing on the anatomy of the vascular system of lianas from the Sapindaceae family, developing activities at the Rio de Janeiro Botanical Garden under the supervision of Dr. Neusa Tamaio. He completed his master's degree in February 2017. Subsequently, Israel began his doctorate at the University of São Paulo in June of the same year. This time, his research is dedicated to the vascular system in plants of the Nyctaginaceae (Four O'clock Family), under the supervision of Dr. Veronica Angyalossy. For this study, Israel carried out several fieldtrips in Brazil and abroad (Bolivia, United States, Mexico), in addition to developing part of the studies at the National Museum of Natural History of the Smithsonian Institution (Washington, DC, USA). The results of this research have already been presented at conferences in Brazil (2018), Mexico (2018) and the USA (2020, 2021) (remotely).

The author's list of publications can be found in his curriculum in online platforms such as Lattes / Orcid / Google Scholar / ResearchGate.

Cover

Micro- (colored) and macro- (brownish) photographs of stems of Nyctaginaceae in different developmental stages.

Cover: *Acleisanthes chenopodioides; Pisonia aculeata; Pisoniella glabrata; Reichenbachia hirsuta; Colignonia glomerata.*

Back cover: *Reichenbachia hirsuta; Bougainvillea berberidifolia; Leucaster caniflorus; Commicarpus scandens; Pisoniella glabrata.*