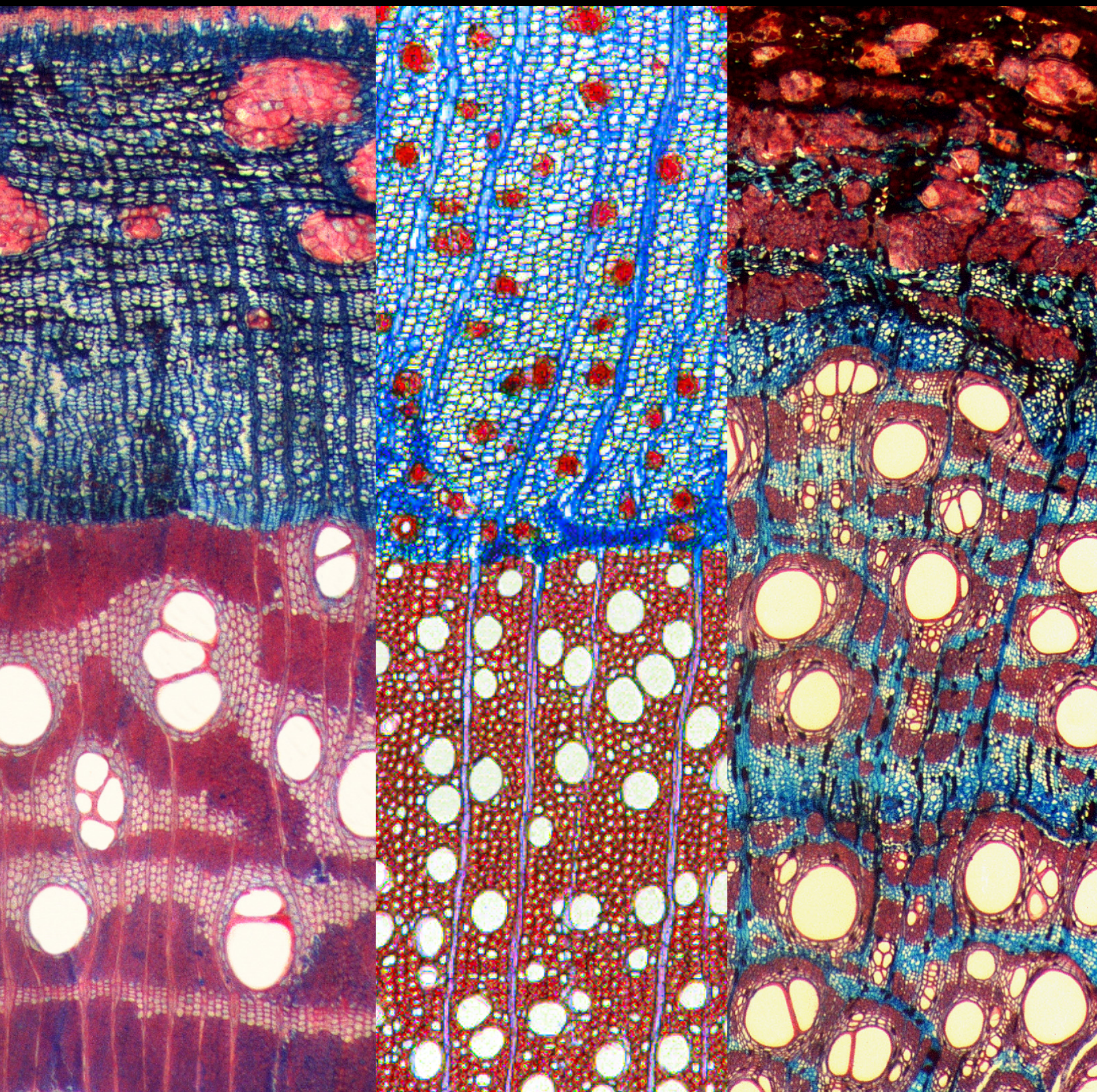


EVOLUTION OF THE VASCULAR SYSTEM IN LINEAGES THAT CONTAIN LIANAS

MARCELO RODRIGO PACE



MARCELO RODRIGO PACE

EVOLUTION OF THE VASCULAR SYSTEM IN
LIANEAGES THAT CONTAIN LIANAS

SÃO PAULO
2015

MARCELO RODRIGO PACE

EVOLUÇÃO DO SISTEMA VASCULAR EM
LINHAGENS QUE CONTÊM LIANAS

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

Orientação: Prof. Dr. Veronica Angyalossy

SÃO PAULO
2015

PACE, MARCELO RODRIGO

2015

EVOLUTION OF THE VASCULAR SYSTEM

IN LINEAGES THAT CONTAIN LIANAS

240 PÁGINAS

Tese (Doutorado) – Instituto de Biociências da Universidade de São Paulo, Departamento de Botânica.

1. Diversificação 2. Evolução 3. Madeira 4. Caule 5. Floema secundário
6. Traqueófitas 7. Bignoniaceae 8. Malpighiaceae

COMISSÃO JULGADORA

Prof. Dr.

Prof. Dr.

Prof. Dr.

Prof. Dr.

Prof. Dr. Veronica Angyalossy

*Nothing in biology makes sense except in the
light of evolution.*

Theodosius Dobzhansky
The American Biology Teacher 35

A Veronica Angyalossy

À mestre que me guiou na construção do conhecimento aqui expresso, pelos 14 anos de convivência e por sempre exigir o máximo de mim, acreditar em mim e me ajudar a superar até mesmo minhas próprias expectativas.

À amiga, que sempre teve tempo e entendeu uma mão, fosse para sorrir ou chorar juntos,

dedico este trabalho

ABRAÇARE AGRADECER

*Chegar para agradecer e louvar.
Louvar o ventre que me gerou
O orixá que me tomou,
E a mão da doçura de Oxum que consagrou.
Louvar a água de minha terra
O chão que me sustenta, o palco, o massapé,
A beira do abismo,
O punhal do susto de cada dia.
Agradecer as nuvens que logo são chuva,
Sereniza os sentidos
E ensina a vida a reviver.
Agradecer os amigos que fiz
E que mantém a coragem de gostar de mim, apesar de mim...
Agradecer a alegria das crianças,
As borboletas que brincam em meus quintais, reais ou não.
Agradecer a cada folha, a toda raiz, as pedras majestosas
E as pequeninas como eu, em Aruanda.
Agradecer o sol que raia o dia,
A lua que como o menino Deus espalha luz
E vira os meus sonhos de pernas pro ar.
Agradecer as marés altas
E também aquelas que levam para outros costados todos os males.
Agradecer a tudo que canta no ar,
Dentro do mato sobre o mar,
As vozes que soam de cordas tênues e partem cristais.
Agradecer os senhores que acolhem e aplaudem esse milagre.
Agradecer,
Ter o que agradecer.
Louvar e abraçar!*

Maria Bethânia

Agradecimentos

Quatro anos parecem voar, mas quando busco na memória tudo o que ocorreu nesses tempo, dou-me conta de que não foram poucas coisas. Este doutorado é para mim a realização de um sonho e que foi possível graças a muitos elementos e pessoas, que tentarei fazer jus agradecendo nominalmente aqui, esperando não ter-me esquecido de alguém. Agradeço portanto:

À **Universidade de São Paulo**, especialmente ao **Laboratório de Anatomia Vegetal** do Departamento de Botânica do Instituto de Biociências pela oportunidade de me formar aqui com excelente infra-estrutura, cursos e oportunidades e um corpo docente de professores apaixonados pelo que fazem e ensinam que me atraíram para a botânica, para minha própria surpresa.

Às agências de fomento **FAPESP** (2012/01099-8; 2013/10679-0) e **CNPq** pelo financiamento dessa tese e tudo o que ela envolveu, incluindo idas ao campo, ida a congressos, contratação de técnicos TT3 para realização de trabalhos práticos e compra de materiais.

À minha orientadora **Veronica Angyalossy**, que sem sombra de dúvidas foi a pessoa mais importante em todo meu processo de formação e que se envolveu plenamente com essa tese, da sua concepção à última linha da conclusão. Veronica, te agradeço por ter sempre acreditado em mim, por ter assinado embaixo nas minhas idéias mais megalomaniacas e sobretudo pelas inúmeras oportunidades que você me brindou ao longo desses quatro anos (ou 14), incluindo-me em palestras, comitês, projetos, como colaborador no seu curso de anatomia da madeira e me estimulando para ir além até de onde achei que pudesse chegar. Obrigado também pela sensibilidade com que sempre me recebeu em momentos pessoalmente mais conturbados. Tenho profunda admiração por você como profissional e como ser humano e me sinto afortunado por ter tido a oportunidade de te conhecer e de conviver tantos anos com você e sei que só cheguei aqui graças a você.

Aos meus amados pais **Adriana De Grandis Pace** e **Héctor Eduardo Pace** agradeço imensamente, tanto pelos pais maravilhosos que são, como por toda ajuda financeira que me deram, sobretudo nesses últimos 3 anos, pelo apoio incondicional para a realização dessa tese, e sobretudo pelo imenso carinho que recebo de vocês em olhares, palavras e ações quotidianas.

À minha amada irmã **Gabriella Pace**, pela amiga e companheira que é e que sempre fez questão de ser, que sempre está presente na minha vida em todas suas nuances e cuja ajuda foi crucial para a realização desse sonho. Obrigado!

À **Lisana Rezende**, pelas técnicas e apoios que hoje carrego no meu repertório básico de sobrevivência e sem os quais eu não seria a mesma pessoa. Você é especial.

Aos **Verônicos André Carvalho Lima, Caian Gerolamo, Carolina Lopes Bastos, Erica Moniz e Mariana Victorio** por partilhar da orientação, pelas risadas e pelo excelente convívio diário.

Menção especial à querida amiga, colega e roomie **Carolina (Carou) Lopes Bastos**, que se tornou tão especial, uma hiper amiga e que compartilhou comigo um lar pelos últimos 2 anos e inúmeras horas e horas de apoio e terapia mútuos que sem dúvidas influenciaram essa tese. Realmente muito obrigado!!!!

À **Gisele Costa e Tássia dos Santos**, técnicas do Laboratório de Anatomia Vegetal, por toda a ajuda e presteza com que sempre receberam todos meus pedidos e pela excelente convivência diária.

À grande ajuda técnica que tive e que permitiu que mais de uma centena de espécies e seus indivíduos fossem cortados e processados, envolvendo os mais diversos métodos laboratoriais e plantas incortáveis. Sem esse apoio a realização dessa tese seria inviável. Obrigado **Erica Moniz, Mariana Victorio, Rachel Koch, Marli Botânico, André Carvalho Lima e Carolina Lopes Bastos**.

Agradeço a todos os amigos e colegas do Laboratório de Anatomia Vegetal dos últimos quatro anos: **Aline Siqueira Nunes, André Carvalho Lima, Caian Gerolamo, Carolina Lopes Bastos, Fernanda Cordeiro, Gisele Costa, Giuliano Locosselli, José Hernandes, Keyla Rodrigues, Luiza Teixeira, Mariana Victorio, Natalie Capelli, Rafael Cruz, Raquel Koch, Renata Cassimiro de Lemos, Tássia C. dos Santos, Thalia Gama, Vitor Barão e Yasmin Hirao**.

Àqueles amigos do Departamento de Botânica que se tornaram muito especiais, como **Anselmo Nogueira, Juliana Lovo, Mariane de Souza-Baena e Suzana Alcantara**, que contribuíram sem dúvidas para o meu aprendizado e compleição dessa tese, com discussões, debates, conselhos, risadas e muito companheirismo.

Ao **Cassiano Luiz Mecchi** pelos quase 11 anos que compartilhamos e por ter sempre apoiado meus sonhos e vibrado com minhas conquistas. Espero que você seja sempre muito feliz, na parte do mundo onde esteja. Conte comigo para o que precisar sempre!

Aos amigos da Finlândia, Helsinki University, Viikki Biocenter 3, Suomen Kielen Kurssi I e que apoiaram que eu realizasse esse doutorado e que, apesar dos meus temores e inseguranças, hoje vejo que foi a melhor decisão que poderia ter tomado. Grazie **Tahira Anwar e Riccardo Siligato**, Kiitos paljon **Anne-Maarit Bågman ja Vesa Nousiainen**, Hvala vam **Milica Maksimovic, Спасибо Katja Shalkovskaya**, Obrigado **Aime Virkkilä Accorsi**. Que bom conhecer vocês.

Aos colaboradores mais recentes, **André Marcio Amorim** e **Suzana Alcantara**. Obrigado André por me receber em Ilhéus para sem dúvidas a coleta mais bem sucedida que já participei, com quase 40 espécies de Malpighiaceae coletadas em menos de uma semana e por sempre enviar materiais de excelente qualidade e estar aberto a discussões científicas. À **Suzana Alcantara** por me receber em Florianópolis para dias de trabalho intenso e eficiente em conjunto, comilanças e até um pouco de praia.

Aos membros da minha banca de qualificação pelos preciosos comentários e recomendações, **Carmen Marcati**, **José Rubens Pirani** e **Nanuza Luiza de Menezes**.

À **Carmen Marcati** também pela amizade e companheirismo, hospedagens em Botucatu, risadas.

À querida amiga **Neusa Tamaio**, que tive o prazer de conviver em campo, congressos e cursos e que sempre foi uma grande incentivadora do meu trabalho e com quem sempre pude contar, além de profunda conhecedora das lianas.

Aos professores do Laboratório de Anatomia Vegetal, **Diego Demarco**, **Gladys Flávia Melo de Pinna**, **Gregório Ceccantini** e **Nanuza Luiza de Menezes**. Em especial ao **Gregório** por ter sempre trazido amostras valiosas para mim do campo e incentivado meu trabalho constantemente e à **Nanuza** pelo imenso carinho que sinto emanar dela sempre que nos encontramos, pelo maravilhoso curso de pós-graduação que nos deu e que nos permitiu entender melhor as plantas como um todo, pela profissional primorosa e ser humano excepcional! Obrigado.

Ao **William Anderson** (*in memoriam*), com o qual pelos primeiros anos da tese troquei e-mails semanais e que me ajudou com seu profundo conhecimento e incomensurável paciência, pois eu não sabia absolutamente nada, a entender as Malpighiaceas, mudanças taxonômicas ocorridas nas últimas décadas, identificação de muitas das plantas que coletei e pelas trocas de CDs de ópera, outra paixão além das plantas que logo descobrimos compartilhar. Agradeço ainda sua esposa, **Christiane Anderson**, que gentilmente identificou algumas plantas e também sempre foi extremamente carinhosa em seus e-mails, mesmo em períodos em que ela estava enfrentando momentos difíceis.

À **Lúcia G. Lohmann** e **Richard Olmstead** por terem aceitado participar da segunda parte do Capítulo I e publicação do artigo correspondente, pelos comentários e dicas que enriqueceram o manuscrito final.

Aos membros do **IAWA Bark Committee**, composto majoritariamente de membros sênior ou experientes botânicos já bem estabelecidos, pela acolhida de um aluno dentre eles como igual e pelos agradáveis dias em Brotas (SP), **Alan Crivellaro**, **Alexei Oskolski**, **Carmen Marcati**, **Carolina Mittelstaedt**, **Ekaterina Kotina**, **Frederic Lens**, **Leo Junikka**, **Pieter Baas**, **Nadezda Nikolaeva**, **Solange Mazzoni-Viveiros** e **Teresa Terrazas**.

Ao **Alex C. Wiedenhoeft**, **Michael Wiemann** e **Regis Miller** por ter-nos recebido de maneira tão generosa no *Forest Products Laboratory* em Maddison (Wisconsin), deixando a coleção inteira à nossa disposição para que pudéssemos analisar o material, inclusive noite adentro e nos finais de semana. O **Regis** foi ainda fundamental para que pudéssemos descobrir a origem de algumas das espécies analisadas.

Ao querido mestre e amigo **Antonio Carlos Franco Barbosa**, que me ensinou a trabalhar no laboratório ainda no mestrado e cujos ensinamentos temos podido passar adiante e que nos permite obter materiais de qualidade para as análises anatômicas. Agradeço ainda pelo corte de *Stereospermum* (Bignoniaceae) que fez às pressas e com o primor de sempre.

Ao **Pieter Baas**, por me receber e me hospedar mais de uma vez em sua casa em Leiden/Leiderdorp (Holanda), pelas revisões e sugestões no primeiro capítulo, pela influência no tema do segundo capítulo e pela doação de amostras de Malpighiaceae do Velho Mundo, presente na xiloteca de Leiden.

À **Michelle Zhjra** pela identificação de algumas madeiras de Coleeae (Bignoniaceae), que ela mesma trouxe de Madagascar e depositou em Madison (WI).

À **Maria José Miranda** e **Rafael Pigozzo** por permitirem que fotografasse e analisasse as amostras de *Tabebuia*, *Handroanthus* e *Sparattosperma* (Bignoniaceae) da coleção do *Instituto de Pesquisas Tecnológicas* (IPT-SP).

Ao **Andrew Groover**, pelo convite em participar da edição especial do *International Journal of Plant Sciences* que resultou no Capítulo I e pelo incentivo em me dar o *grant* para o congresso em Ohio, que mudou minha perspectiva para sempre.

Aos colegas e amigos que revisaram cuidadosamente versões anteriores dos manuscritos/capítulos da tese e deram sugestões valiosas que foram incorporadas, **André Carvalho Lima**, **Andrew Groover**, **Caian Gerolamo**, **Dewey Litwiller**, **Giuliano Locosselli**, **Guillermo Angeles**, **Pieter Baas** e quatro revisores anônimos.

Ao **Harri Lorenzi** por permitir que por mais de uma vez fôssemos coletar no Instituto Plantarum e que sempre nos recebeu com entusiasmo.

Ao **Augusto Francener** pela ajuda na identificação das Malpighiaceas, especialmente *Byrsonimas* e outras espécies do cerrado.

Ao querido **Rafael Felipe de Almeida** pelas discussões sobre Malpighiaceae, por coletar materiais valiosos e ajudar na identificação das espécies. Obrigado também pelo incentivo constante e risadas

Agradeço também às amigas de décadas que me acompanharam todos esses anos - escola, graduação, mestrado, doutorado, viagens - e que sempre me apoiaram e apoiam **Fernanda Castanho Pereira dos Santos, Juliana Sette Sabbato e Marianne Thamm de Aguiar**. Vocês são para lá de especiais na minha vida.

Agradeço aos amigos mais recentes e mais antigos, do dia a dia de São Paulo, pelas viagens, festas, risadas, *meriendas*, dramas e compreensão pelo último ano e especialmente últimos meses em que tive que dizer mais “nãos” que “sins” pra conseguir terminar a tese e que até disso me ajudaram a rir criando o *hashtag* **#drama_tesis**. Obrigado/gracias **Abner Mendonça, Andrea Calero, Andrés Mesa, Adriana Ferro, Belén Sala Torres, Danilo Prado, Gustavo Caixeta, Lida Marica Fierro, Marcio Miranda Perez, Muriel Valencia, Pablo Sarmiento, Pilar Marcela Afanador, Rocío Lobo Machín, Viviana Peña**.

Por fim, a todos que me ajudaram a coletar as espécies utilizadas nesse trabalho, diretamente participando em expedições de coleta ou enviando materiais, **Alcides Sáenz (Isla Martín García, Argentina), Alexandre Zuntini, André Amorim, Anselmo Nogueira, Arnildo Pott, Arno Fritz, Berta Villagras, Cairo Faleiros, Carolina Lopes Bastos, Carolina Madero (México), Daniel Bazzano (Isla Martín García, Argentina), Daniel Villavoel (Chuquisaca, Bolívia), Erica Moniz, Genise Somner, Geraldo Damasceno Jr., Gonzalo Castillo, Diana Sampaio, Guillermo Angeles (México), Gregório Ceccantini, Ivone Vázquez Briones (Tarapoto, Peru), Jefferson Prado, Joan Miró Ortega, Juliana Lovo, Julio Majcher, Kamila Drequeceler, Luiz Carlos Jesus Gomes, Lukas Tadeu Halla Daneu, Marccus Alves, Márdel Lopes, Maria Ana Farinaccio, Milton Groppo Jr., Neusa Tamaio, Pablo Cabanillas (Isla Martín García, Argentina), Renata Udulutsch, Rosani Arruda, Suzana Alcantara, Rafael Felipe de Almeida, Ricardo Zárate (Iquitos, Peru)**.

Como dito anteriormente, todas essas pessoas contribuíram direta ou indiretamente para que essa tese pudesse existir e portanto têm minha imensa gratidão.

Obrigado!

ABSTRACT

The vascular system of lianas, especially the xylem, has been repeatedly shown to be different, with lianas having a set of features shared among even distantly related lineages, such as the presence of cambial variants, wide and long vessels, more abundant axial parenchyma, frequently non-lignified, taller and wider rays, which are generally heterocellular. In spite of this amount of knowledge, few works have investigated the impact for the vascular system of the evolution of this habit within lineages whose ancestors are not lianas, but self-supporting plants. Therefore, in this dissertation we explored wood, phloem and overall stem-anatomy evolution in lineages that contain lianas and self-supporting plants, using well-supported phylogenies and detailed anatomical investigations. Within Bignoniaceae (Lamiales), we thoroughly investigated the wood anatomy, delimiting character states and mapping them onto the last phylogeny for the group, encountering that eco-physiological and habit transition were the main drivers of modifications in the wood anatomy in the family. Ring-porous and semi-ring porous woods and helical thickening was found in plants either growing in higher latitudes or with marked seasonal water regimes, and septate fibres correlated with scanty axial parenchyma, which are eco-physiological drivers. Evolution of lianas, in turn, drove an increase in vessel diameter; wide vessels accompanied by very narrow ones, presence of perforated ray cells, scanty axial parenchyma and cambial variants. Despite the great wood anatomical diversity within the family, major clades have quite predictive wood anatomy and 9 possible anatomical synapomorphies were raised in this work to clades previously delimited exclusively by molecular characters. Within the tracheophytes, we investigated 26 phylogenetically controlled pairs of lianas and their self-supporting relatives within all major lineages of tracheophytes (except lycophytes), in order to seek characters evolving in correlation with the lianescent habit. We found that the sieve elements and sieve pores were always wider in the lianas, and that the rays were always taller and heterocellular. However, all the main characters of the phloem of the lianas remained conserved with that of their self-supporting relatives. This evidenced that although a more efficient photosynthetic conductive system evolved in the phloem of lianas, overall anatomy conserved a high phylogenetic signal. Within Malpighiaceae, (Malpighiales), lianas are abundant and many cambial variants are present. However, nothing was known regarding how many types of cambial variants there were in the family and how they were distributed. We were able to delimit 6 different types of cambial variants that evolved at least 8 times independently in the family, which ancestrally lacks a cambial variant. Many of these types share common stages of development and some variants that are anatomically very similar derive from different ontogenetic trajectories. Within the genera, the variants are conserved, and even between sister groups in the new and old world, evidencing that cambial variants may be a good indicator of relationships within the family. Overall, we conclude that lianas greatly impact the evolution of the vascular system in the lineages where they have evolved, and these modifications normally result in a more efficient water and photosynthates conduction system and an increased flexibility for climbing.

RESUMO

O sistema vascular das lianas, em especial o xilema, mostrou-se repetidas vezes distinto nas lianas, com aspectos compartilhados mesmo dentre linhagens distantemente relacionadas, tais como a presença de variações cambiais, vasos mais largos e longos, parênquima axial mais abundante - frequentemente não-lignificado - raios mais altos e largos - geralmente heterocelulares. Não obstante todo esse conhecimento, poucos trabalhos investigaram o impacto da evolução do hábito lianescente no sistema vascular em linhagens cujos ancestrais não são lianas e sim plantas auto-suportantes. Portanto, nesta tese exploramos o lenho, o floema e a anatomia caulinar como um todo em linhagens que contêm lianas e plantas auto-suportantes, utilizando filogenias bem sustentadas e investigações anatômicas detalhadas. Em Bignoniaceae (Lamiales), investigamos em detalhe a anatomia do lenho, delimitando caracteres e estados de caráter e mapeando-os na filogenia mais recente do grupo, encontrando que modificações eco-fisiológicas e transições de hábito tiveram grande impacto na evolução do lenho na família. Anéis porosos e semi-porosos, bem como espessamento espiralado foram encontrados em plantas crescendo em latitudes mais altas ou em regimes hídricos fortemente sazonais, ao passo que fibras septadas apareceram correlacionadas com a presença de parênquima axial escasso. A evolução de lianas, por sua vez, parece ter levado a um aumento no diâmetro dos vasos, contudo dimórficos, células perfuradas de raio, parênquima axial mais escasso e surgimento de variações cambiais. Apesar da enorme diversidade dentro de Bignoniaceae, os grandes clados possuem uma anatomia bastante preditiva e 9 possíveis sinapomorfias morfológicas são sugeridas para clados delimitados somente com base em dados moleculares. Dentro das traqueófitas, investigamos 26 pares filogeneticamente controlados de lianas espécies auto-suportantes relacionadas pertencentes a todas as principais linhagens de traqueófitas (exceto licófitas), a fim de buscar caracteres que tenham evoluído em correlação com o hábito lianescente. Encontramos que os elementos crivados e os poros das placas crivadas têm sempre maior calibre nas lianas, e que os raios são mais altos e heterocelulares. Contudo, as principais características do floema das lianas se mantêm conservadas em relação às espécies auto-suportantes relacionadas, evidenciando que as lianas teriam evoluído um sistema de condução de fotossintetatos mais eficiente, porém preservando um alto sinal filogenético. Em Malpighiaceae, lianas são abundantes, tal como as variações cambiais. Contudo, pouco se sabe sobre o número de variações presentes na família ou como elas estariam distribuídas. Aqui delimitamos 6 diferentes tipos de variação cambial, que teriam evoluído independentemente 8 vezes na família, cujo ancestral é reconstruído como tendo caule simples. Muitas dessas variações compartilham estágios de desenvolvimento, ao passo que variações anatomicamente muito similares derivam de trajetórias ontogenéticas distintas. Dentro dos gêneros as variações se mostraram conservadas e mesmo dentre grupos irmãos do novo e velho mundo, evidenciando que as variações cambiais seriam bons indicadores de relações na família. De maneira geral, podemos concluir que lianas impactam significativamente o sistema vascular nas linhagens onde ocorrem e que tais modificações em geral resultam em um sistema de condução hídrico e de fotossintetatos mais eficiente e também mais flexível para a escalada.

Contents

General Introduction

Lianas and their vascular system	2
References	8

Chapter I. Diversity and evolution of the secondary xylem in Bignoniaceae

Preface	13
Section I	
Wood anatomy and evolution: a case study in the Bignoniaceae	
Resumo & Abstract	15
Introduction	16
Material & Methods	21
Results	28
Discussion	49
References	56
Section II	
Wood anatomy of major Bignoniaceae clades	
Resumo & Abstract	63
Introduction	66
Material & Methods	68
Results	69
Discussion	90
References	97
Apendix I, for sections I and II	100
General conclusions	105

Chapter II. The phloem of lianas: a comparative study across the tracheophytes

Resumo & Abstract	108
Introduction	109
Material & Methods	114
Results	135
Discussion	155
References	166

Chapter III. Diversity of the cambial variants and their distribution in Malpighiaceae

Resumo & Abstract	176
Introduction	177
Material & Methods	178
Results	180
Discussion	203
References	210
Appendix I	214

Conclusão Geral	219
-----------------------	-----

Extra file I. First page of the manuscripts published with results from this work	221
---	-----

Extra file II. List of works presented in meetings with results from this work	226
--	-----

GENERAL INTRODUCTION

General Introduction

“Plants become climbers, in order, as it may be presumed, to reach the light, and to expose a large surface of their leaves to its action and to that of the free air. This is effected by climbers with wonderfully little expenditure of organized matter, in comparison with trees, which have to support a load of heavy branches by a massive trunk. Hence, no doubt, it arises that there are so many climbers in all quarters of the world, belonging to so many different orders.”

(Charles Darwin, 1865, pages 107-108 in
The Movements and Habits of Climbing Plants)

The first land plants were likely erect self-supporting plants (Bennici 2008), from which lianas evolved multiple times since the appearance of tracheophytes (= vascular plants) at around 433 mya (Silvestro et al. 2015). The first fossil records of lianas dates back to the Carboniferous (Mississippian, ~ 335 mya), with peaks of diversity in the Pennsylvanian (a more recent period of the Carboniferous) and the Eocene (~50 mya; Burnham 2009, 2015). Lianas are here treated as any climbing plant that germinates on the soil and that by a range of different mechanisms climb up other plants or vertical supports in search of light (Muller-Dombois & Ellenberg 1974). Lianas are present in all major tracheophyte lineages (Fig. 1), being present in lycophytes (e.g., *Lycopodiella cernua*, Lycopodiaceae, occasionally is a climber; Rowe et al. 2004), ferns (e.g., *Lygodium*, Schizaeaceae), extinct progymnosperms (e.g., *Medullosa steinii*, Medullosaceae; Dunn et al. 2003), gymnosperms (e.g., *Gnetum*, Gnetaceae), and a diverse range of angiosperms, including palms (e.g., the richest genus of the palm family being lianescent, *Calamus*; Dransfield et al. 2014), magnoliids (e.g., black pepper, Piperaceae; *Tasmannia cordata*, Winteraceae; Feild et al. 2012) and many eudicots (e.g., peas, beans and wild roses being a few examples).

Architecturally, however, the question is what modifications in the *bauplan* of a plant have to occur to make a liana a liana? First of all, climbing can only be achieved either by twining flexible stems or specialized structures for grasping. Also, lianas ought to have narrower and yet longer stems, since growing into as massive heavy organisms as trees can impose excess weight and mechanical failure on their support, while having a higher length is both a side effect of twining and a means of exploring various tree canopies in search of sunlight. Both these aspects of the liana *bauplan* seem to have been conquered multiple times in the transitions from self-supporting to lianas across evolution (at least 133 families contain a few climbers; Gentry 1991), since both morphological modifications adapted for climbing and taxa with much narrower and longer stems are the rule rather than the exception in lianescent taxa (Bhambie 1972, Putz 1983, Ewers & Fisher 1991, Rowe et al. 2006), being a textbook case of convergent evolution (Futuyma 2009).

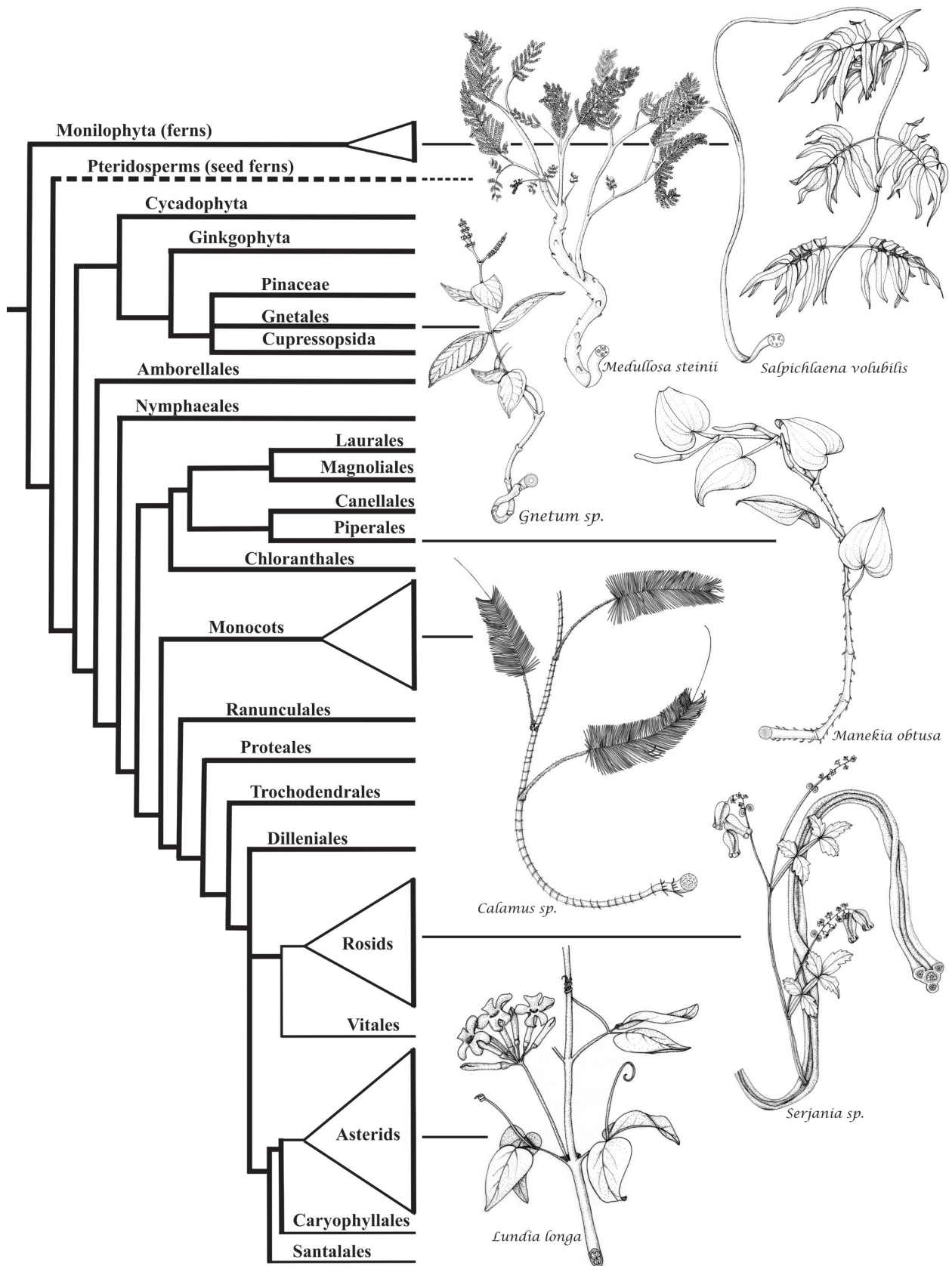


Fig. 1 Cartoon illustrating the phylogeny of tracheophytes (excluding lycophytes), with examples of lianas that evolved independently in all its major lineages. In monilophytes, *Salpichlaena volubilis* (Blechnaceae). Within the extinct pteridosperms *Medullosa steinii* (Medullosaceae). Within the gymnosperms, *Gnetum* (Gnetales). Within the magnoliids, *Manekia obtusa* (Piperaceae). Within the rosids, *Serjania* sp. (Sapindaceae) and within the asterids *Lundia longa* (Bignoniaceae). Not to scale. Reproduced from Angyalossy et al. 2015, original drawing from Luciana W. Gussella.

The most conspicuous morphological modifications are the different mechanisms for climbing. While the majority of lianas use a rather simple mechanism of ascent, which are twining stems (Fig. 2A; Darwin 1865, Schenck 1892, Putz & Holbrook 1991, Lehnebach 2012; e.g., *Ipomoea*, Convolvulaceae; *Mikania*, Asteraceae), other taxa have evolved much more elaborate structures for climbing, derived from modifications in one of the three plant organs: roots, stems or leaves. These structures are: (i) irritable petioles (e.g., *Clematis*, Ranunculaceae), (ii) tendrils, which may be derived from modified leaflets (Fig. 2B; e.g., Lianas of Bignoniaceae, Bignoniaceae; *Cobaea*, Polemoniaceae), stipules (Fig. 2C; e.g., Smilacaceae) or even branches (Fig. 2D; e.g. Vitaceae), or part of a branch (the floral axis in *Antigonon leptopus*, Polygonaceae; all lianescent Sapindaceae tribe Paullinieae); (iii) hooks (e.g., *Strychnos*, Loganiaceae; *Phanera*, Leguminosae); (iv) adventitious roots (Fig. 2E; e.g., *Hedera helix*, Araliaceae; *Hoya*,

Apocynaceae-Asclepiadoideae), (v) spines or prickets (Fig. 2E; e.g., *Calamus* palms, Arecaceae; *Smilax*, Smilacaceae); (vi) or even angled stems and stiff leaves designed to attach to the vegetation around (e.g. *Combretum bracteosum*, Combretaceae).

Modifications on anatomy, in turn, have accompanied these morphological changes. Lianas are known to undergo an increase in flexibility along development (reduction of the Young's Module – the opposite of what happen in trees and shrubs, which get stiffer along development; Rowe & Speck 1996, Gallenmüller et al. 2001, Rowe et al. 2004, 2006), and this increase in flexibility has been shown to be correlated with the transition from a stiff young phase, when the liana stem is either initially self-supporting or a rigid searcher branch (Caballé 1998, Putz & Hoolbrook 1991, Rowe & Speck 1996, Gallenmüller et al. 2001, Rowe et al. 2004, 2006) standing firmly

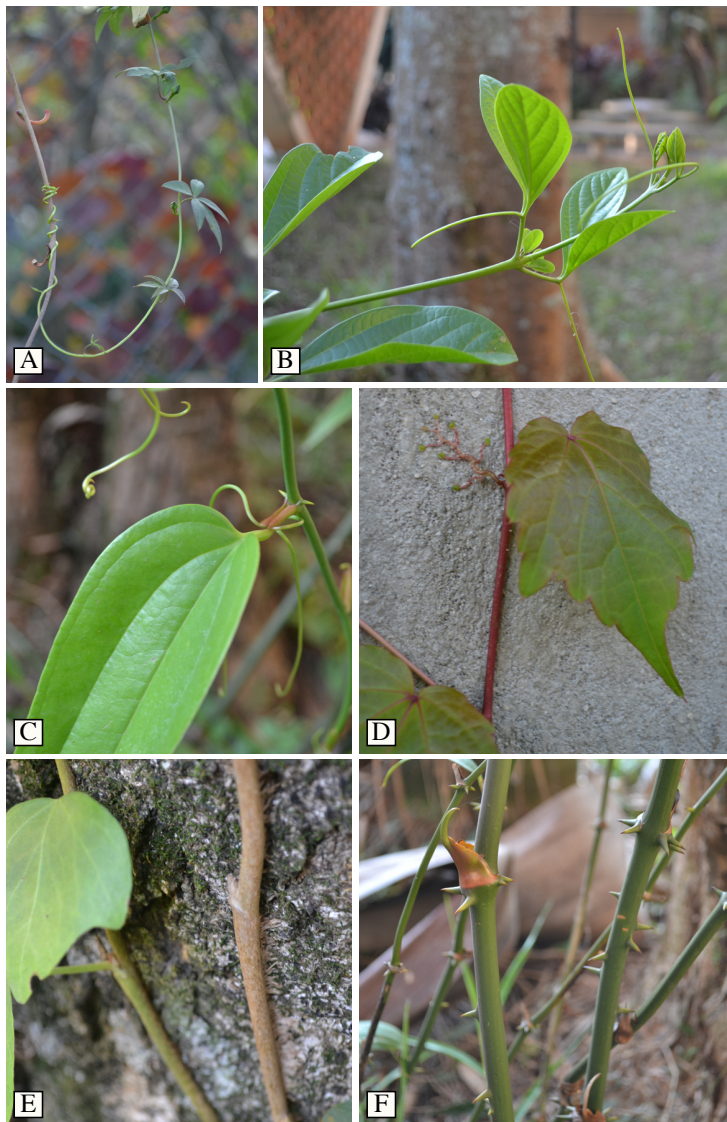


Fig. 2 Different mechanisms for climbing. A. *Ipomoea cairica* (Convolvulaceae), twining stem. B. *Bignonia magnifica* (Bignoniaceae), tendrils derived from one of the leaflets of a triphid leaf. C. *Smilax quinquenervia* (Smilacaceae), tendrils derived from stipules. D. *Parthenocissus* sp. (Vitaceae), tendril derived from a branch, with adhesive discs at its tips. H. *Hedera helix* (Araliaceae), which climb with the aid of stem-borne adventitious roots. F. *Smilax quinquenervia* (Smilacaceae), prickets around the node and internode. Photos shot with André C. Lima.

in the air, circumnutating in search of a support (Darwin 1965, Putz & Holbrook 1991, Isnard & Silk 2009). Whenever the support is found, however, an abrupt change occurs anatomically, resulting in this decrease in stiffness and increase in flexibility (Putz & Holbrook 1991, Rowe & Speck 1996, Rowe et al. 2004). Specifically, at the initial self-supporting phase the xylem anatomy is marked by the presence of narrow vessels, scanty axial parenchyma and the prevalence of fibres (Fig. 3A-B; Rowe et al. 2004, 2006; Angyalossy et al. 2012, 2015). However, as soon as the plant switches to the lianescent phase, flexibility increases by the formation of more axial parenchyma (Fig. 3A), which is frequently non-lignified (Fig. 3B), less fibres, sometimes gelatinous, and wider vessels in the xylem (Fig. 3A-B), summed to the common appearance of cambial variants at this stage of development (Carquist 1985, Rowe & Speck 1996, Rowe et al. 2004, Bowling et al. 2009, Crivellaro et al. 2012, Angyalossy et al. 2012, 2015). Cambial variants are alternative forms of secondary growth that generally results in soft tissues (phloem and/or non-lignified parenchyma) mixed with the rigid secondary xylem, such as the xylem furrowed by phloem wedges in Bignoniaceae and Polemoniaceae (Fig. 3E) or the successive cambia in Menispermaceae and Convolvulaceae (Fig. 3F; Schenck 1893, Dobbins 1971, Mennega 1982, Carlquist 2001, Tamaio et al. 2009, Angyalossy et al. 2015). Lianas typically have also higher and wider rays (Fig. 3D), generally heterocellular mixed or more heterocellular than their close-related counterparts (Pace & Angyalossy 2013, Angyalossy et al. 2015). The presence of more parenchyma and less fibres within the xylem is possible only because lianas do not sustain their own bodies, leaning on their supports (Carlquist 1985, Ewers 1985, Stevens 1987, Gartner 1991, Crivellaro et al. 2012).

Longer and narrower stems, but supporting canopies as large or larger than those of trees (Putz 1983, Ewers & Fisher 1991), in turn, create a demand for efficient water and photosynthates transport over long distances, given liana's high length-low width stem ratio (Putz 1984, Kurzel et al. 2006). This is achieved by the presence of very wide and long vessels, in fact the widest and longest known in plants (some up to 500 μm wide and 8 m long; Zimmermann & Jeje 1981, Ewers 1985, Ewers & Fisher 1989, 1991, Isnard & Feild 2015) and with perforation plates that converged to be simple, even in families where the self-supporting species have scalariform perforation plates (Ayensu & Stern 1964; Carlquist 1991, Lens et al. 2008). Such features make up an extremely efficient hydraulic system, shown to transport approximately 3 times more water than that of self-supporting plants (Isnard & Feild 2015). However, because wide vessels are known to be more vulnerable to embolism, it is also widespread the presence of narrow vessels associated with the wide vessels of lianas (Fig. 3A-C; Carlquist 1985, Santiago et al. 2015), a phenomenon named vessel dimorphism by Carlquist in his work with the carnivore liana *Nepenthes* (1981). The presence of these narrow vessels associated with the wide ones act as a guarantee that the water column will not be broken in the event of embolism in the vulnerable wide vessels, with the narrow vessels acting as a water

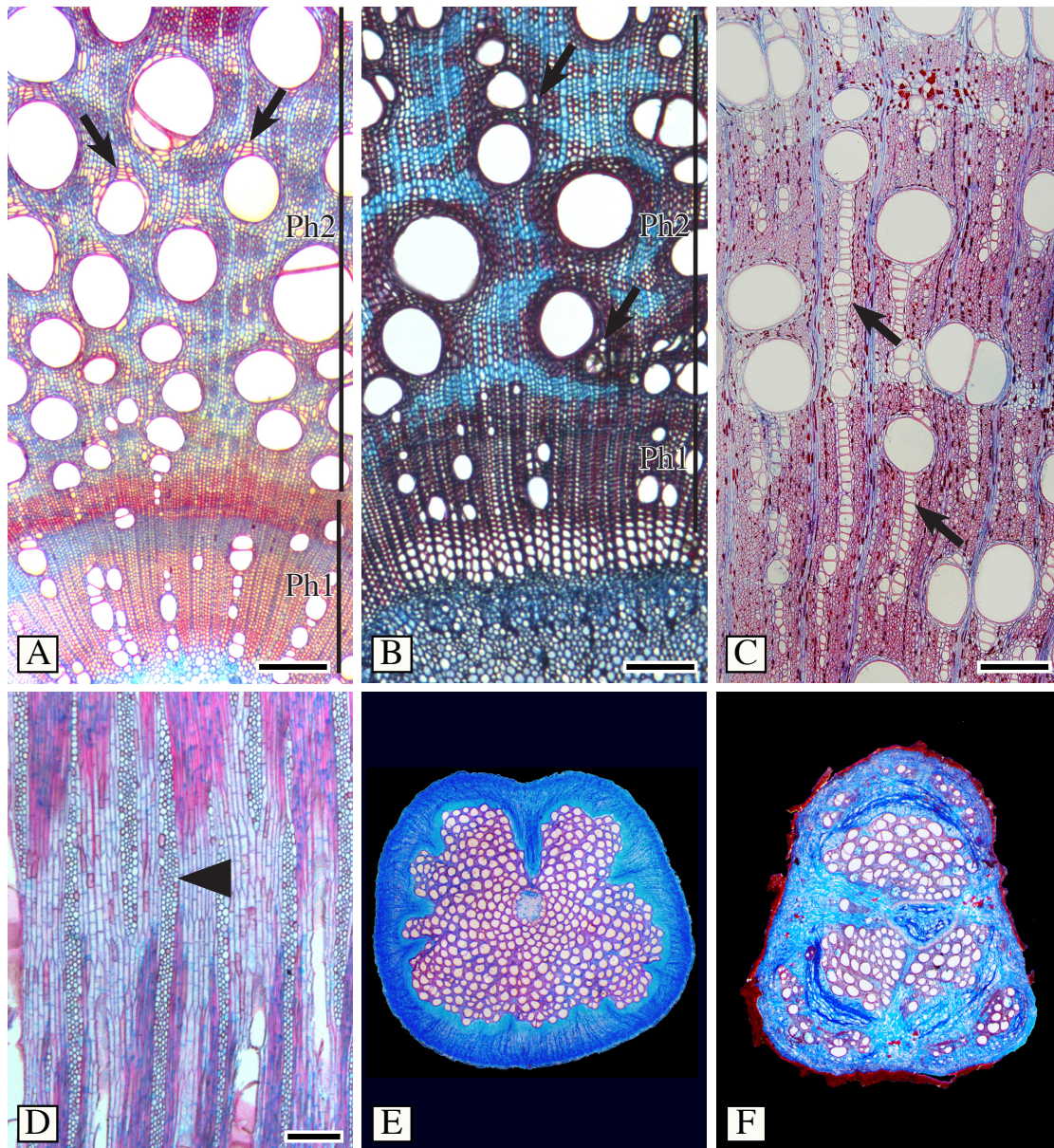


Fig. 3 Lianescent secondary xylem. A. *Gouania mollis* (Rhamnaceae) transverse section (TS), self-supporting stage of growth (Ph1) marked by the presence of narrow vessels, scanty axial parenchyma and more fibres. Lianescent stage of growth (Ph2) marked by wide vessels associated with narrow ones (arrows), more axial parenchyma and less fibres. B. *Peltastes peltatus* (Apocynaceae) TS, self-supporting stage of growth (Ph1) marked by the presence of narrow vessels, scanty axial parenchyma and more fibres. Lianescent stage of growth (Ph2) marked by wide vessels associated with narrow ones (arrows), non-lignified axial parenchyma and less fibres. C. *Heteropterys intermedia* (Malpighiaceae) TS, secondary xylem marked by the presence of wide vessels associated with narrow vessels in long radial chains (arrows). D. *Ficus pumila* (Moraceae) tangential section, high and wide rays (arrowhead). E. *Cobaea scandens* (Polemoniaceae), xylem furrowed by phloem wedges. F. *Ipomoea cairica* (Convolvulaceae), stem with successive cambia and wide rays. Scale bars: A-C = 250 μ m, D-E = 100, μ m, F = 50 μ m. Photo B from Carolina Lopes Bastos.

by-pass (Santiago et al. 2015), a phenomenon also used to justify the more common presence of perforated ray cells in lianas, connecting vessels on both sides of the rays, even in lineages where they are not found in the self-supporting members (Angyalossy et al. 2012, 2015; Pace & Angyalossy 2013). Wider sieve elements in the phloem have likewise been repeatedly stressed in the phloem of lianas, and is presumed to contribute to a more efficient photosynthate conduction (Roth 1981, Carlquist 1975, 1991, Angyalossy et al. 2015). The combination of all these anatomical characters has recently been coined “lianescent vascular syndrome” (Angyalossy et al. 2015), and it represents a strong case of convergence, which result in very similar xylem anatomies among lianas of even distantly related

lineages.

What all the previous works convey is that lianas are special both morphologically and anatomically. These special features have been noted since the XIX century (Darwin 1865, Van Tieghem 1884, Avetta 1887, Schenck 1893, Pfeiffer 1926) and stimulated large treatises on the subject (Schenck 1892, 1893, Obaton 1960, Putz and Mooney 1991, Schnitzer et al. 2015). What at the present are still meagre are studies that address the evolution of lianas within a phylogenetic framework, both on a narrow and a wide scale, in order to detect exact how the evolutionary transition from self-supporting to lianas impact the anatomical diversification of the vascular system, considering xylem, phloem and the overall stem anatomy. Therefore, the main aim of this PhD dissertation was to explore xylem, phloem and overall stem architecture evolution within well-supported phylogenies.

In the first chapter we carried out an in-depth analysis of wood evolution within Bignoniaceae, a pantropical family with a myriad of different habits, including lianas, shrubs and trees, and occupying diverse habitats, from humid tropical forests to savannas and temperate forests (Gentry 1980, Fischer et al. 2004). Our aim was to explore all the wood variable characters in the family to launch hypotheses on what would be the main drivers of evolution and diversification in this group, how the diversity in wood structure is distributed in the family and ultimately how wood characters supported clades in the family, whose relationships have been reconstructed with basis in molecular sequence data.

In the second chapter, we carried a detailed study of the phloem across all major lineages of tracheophytes (except lycophytes) in order to investigate a number of taxa of lianas and their self-supporting relatives to explore whether there is any correlation similar to what described to the xylem, summarized in the 'lianescent vascular syndrome', including confirming or refuting the hypothesis that lianas would always have wider sieve elements. Also, it included an investigation of taxa in which two phloem types co-occur: a regular and a variant phloem, resulting from two types of cambial variants (interxylary phloem and furrowed xylem) and that has been hypothesized as undergoing a subfunctionalization, with the regular phloem specializing in storage and the variant phloem specializing in conduction (Pace et al. 2011, Carlquist 2013).

In the third chapter, we explored the stem anatomical diversity in the pantropical family Malpighiaceae, aiming at delimiting how many different cambial variants are present in the family, how they are distributed in the phylogeny of the family, their ontogeny, and possible common underlying features shared between them.

With these studies we expect to shed light on the main convergent modifications undergone in the vascular system of plants when they transition from self-supporting to lianas.

LITERATURE CITED

- Angyalossy V, Angeles G, Pace MR, Lima AC, Dias-Leme CL, Lohmann LG, Madero-Vega C. 2012. An overview of the anatomy, development and evolution of the vascular system of lianas. *Plant Ecology & Diversity* 5: 167-182.
- Angyalossy V, Pace MR, Lima AC. 2015. Liana anatomy: a broad perspective on structural evolution of the vascular system. In: Schnitzer, SA, Bongers F, Burnham R, Putz FE, eds. *Ecology of Lianas*. Wiley-Blackwell Publishers, Oxford.
- Avetta C. 1887. Contribuzione allo studio delle anomalie di struttura nelle radici delle dicotiledoni. *Annuario del Reale Istituto Botanico di Roma* 3: 3-19.
- Ayensu ES, Stern WL. 1964. Systematic anatomy and ontogeny of the stem in Passifloraceae. *Contributions of the United States National Herbarium* 34: 45-72.
- Bennici A. 2008. Origin and early evolution of land plants: problems and considerations. *Communicative & Integrative Biology* 1: 212-218.
- Bhambie S. 1972. Correlation between form, structure and habit in some lianas. *Proceedings of the Indian Academy of Sciences* 75: 246-256.
- Bowling AJ, Vaughn KC. 2009. Gelatinous fibers are widespread in coiling tendrils and twining vines. *American Journal of Botany* 96: 719-727.
- Burnham RJ. 2009. An overview of the fossil record of climbers: bejucos, sogas, trepadoras, lianas, cipós, and vines. *Revista Brasileira de Paleontologia* 12: 149-160.
- Burnham RJ. 2015. Climbing plants in the fossil record: Paleozoic to present. In: Schnitzer, SA, Bongers F, Burnham R, Putz FE, eds. *Ecology of Lianas*. Wiley-Blackwell Publishers, Oxford.
- Caballé G. 1998. Le port autoportant des lianes tropicales: une synthèse des stratégies de croissance. *Canadian Journal of Botany* 76: 1703-1716.
- Carlquist S. 1975. Ecological strategies of xylem evolution. University of California Press, Berkeley.
- Carlquist S. 1981. Wood anatomy of Nepenthaceae. *Bulletin of the Torrey Botanical Club* 108: 324-330.
- Carlquist S. 1985. Observations on functional wood histology of vines and lianas: vessel dimorphism, tracheids, vascentric tracheids, narrow vessels, and parenchyma. *Aliso* 11: 139-157.
- Carlquist S. 1991. Anatomy of vine and liana stems: a review and synthesis. Pages 53-73 in FE Putz, HA Mooney, eds. *The biology of vines*. Cambridge University Press, Cambridge.
- Carlquist S. 2001. Comparative wood anatomy, 2nd edn. Springer, Berlin.
- Carlquist S. 2013. Interxylary phloem: diversity and functions. *Brittonia* 65: 477-495.
- Crivellaro A, McCulloh K, Jones FA, Lachenbruch B. 2012. Anatomy and mechanical and hydraulic needs of

- woody climbers contrasted with subshrubs on the island of Cyprus. *IAWA Journal* 33: 355-373.
- Darwin C. 1865. On the movements and habits of climbing plants. *Journal of the Linnean Society*, London, pp. 1-18.
- Dobbins DR. 1971. Studies on the anomalous cambial activity in *Doxantha unguis-cati* (Bignoniaceae). II. a case of differential production of secondary tissues. *American Journal of Botany* 58: 697-705.
- Dransfield J, Uhl NW, Asmussen CB, Baker WJ, Harley M, Lewis C. 2008. *Genera Palmarum: the evolution and classification of palms*. Kew Press, London.
- Dunn MT, Krings M, Mapes G, Rothwell GW, Mapes RH, Keqin S. 2003. *Medullosa steinii* sp nov., a seed fern vine from the Upper Mississippian. *Review of Palaeobotany and Palynology* 124: 307-324.
- Ewers FW. 1985. Xylem structure and water conduction in conifer trees, dicot trees, and lianas. *IAWA Bulletin* n.s. 6: 309-317.
- Ewers FW, Fisher JB. 1989. Variation in vessel length and diameter in stems of six tropical and subtropical lianas. *American Journal of Botany* 76: 1452-1459.
- Ewers FW, Fisher JB. 1991. Why vines have narrow stems: histological trends in *Bauhinia* (Fabaceae). *Oecologia* 88: 233-237.
- Feild TS, Chaletet DS, Balun L, Schilling EE, Evans R. 2012. The evolution of angiosperm lianesence without vessels – climbing mode and wood structure in *Tasmannia cordata* (Winteraceae). *New Phytologist* 193: 229-240.
- Fischer E, Theisen I, Lohmann LG. 2004. Bignoniaceae. In: K. Kubitzki and J.W. Kadereit (eds). *The Families and Genera of Vascular Plants VII. Dicotyledons. Lamiales (Except Acanthaceae including Avicenniaceae)*. Springer-Verlag, Heidelberg.
- Futuyma DJ. 2009. *Evolution*. 2nd. ed. Sinauer, Sunderland.
- Gallenmüller F, Müller U, Rowe N, Speck T. 2001. The growth form of *Croton pullei* (Euphorbiaceae) - Functional morphology and biomechanics of a neotropical liana. *Plant Biology* 3: 50-61.
- Gartner BL. 1991. Structural stability and architecture of vines vs. shrubs of poison oak, *Toxicodendron diversilobum*. *Ecology* 72: 2005-2015.
- Gentry AH. 1980. Bignoniaceae: Part I (Crescentia and Tourrettieae). *Flora Neotropical Monograph* 25 (1), The New York Botanical Garden, New York.
- Gentry AH. 1991. The distribution and evolution of climbing plants. In F.E. Putz, and H.A. Mooney (eds), *The Biology of Vines*. Cambridge University Press, New York.
- Isnard S, Silk WK. 2009. Moving with climbing plants from Charles Darwin's time into the 21st century. *American Journal of Botany* 96: 1205-1221.
- Isnard S, Feild TS. 2015. The evolution of angiosperm lianesence: a perspective from xylem structure-function. In: Schnitzer, SA, Bongers F, Burnham R, Putz FE, eds. *Ecology of Lianas*. Wiley-Blackwell Publishers, Oxford.
- Kurzel BP, Schnitzer SA, Carson WP. 2006. Predicting liana crown location from stem diameter in three Panamanian lowland forests. *Biotropica* 38: 262-266.

- Lehnebach R. 2012. Evolution et diversité des traits morpho-anatomiques chez les lianes tropicales. Master thesis, Université Montpellier II, Montpellier.
- Lens F, Kårehed J, Baas P, Jansen S, Rabaey D, Huysmans S, Hamann T, Smets E. 2008. The wood anatomy of polyphyletic Icacinaceae s.l., and their relationship within asterids. *Taxon* 57: 525-552.
- Mennega AW. 1982. Stem structure of the New World Menispermaceae. *Journal of the Arnold Arboretum* 63: 145-172.
- Muller-Dombois D, Ellenberg H. 1974. Aims and methods of vegetation ecology. John Wiley. New York.
- Obaton M. 1960. Les lianes ligneuses a structure anormale des forêts denses d'Afrique Occidentale. *Annales des Sciences Naturelles Botanique et Biologie Végétale* 12: 1-220.
- Pace MR, Angyalossy V. 2013. Wood anatomy and evolution: a case study in the Bignoniaceae. *International Journal of Plant Sciences* 174: 1014-1048.
- Pace MR, Lohmann LG, Angyalossy V. 2011. Evolution of disparity between the regular and variant phloem in Bignoniaceae (Bignoniaceae). *American Journal of Botany* 98: 602-618.
- Pfeiffer H. 1926. Das abnorme Dickenwachstum. In: Lisbauer K. (ed.), *Handbuch der Pflanzenanatomie*. Gebrüder Bornstraeger, Berlin.
- Putz FE. 1983. Liana biomass and leaf area of a tierra firme forest in the Rio Negro basin, Venezuela. *Biotropica* 15: 185-189.
- Putz FE. 1984. The natural history of lianas on Barro Colorado Island, Panama. *Ecology* 65: 1713-1724.
- Putz FE, Holbrook NM. 1991. Biomechanical studies in vines. In F.E. Putz, and H.A. Mooney (eds), *The Biology of Vines*. Cambridge University Press, New York.
- Putz FE, Mooney HA. 1991. The biology of vines. Cambridge University Press. Cambridge.
- Roth I. 1981. Structural patterns of tropical barks. In: Braun HJ, Carlquist S, Ozenda P, Roth I., eds. *Encyclopedia of Plant Anatomy*. Gebrüder Bornstraeger. Berlin.
- Rowe NP, Speck T. 1996. Biomechanical characteristics of the ontogeny and growth habit of the tropical liana *Condylocarpon guianense* (Apocynaceae). *International Journal of Plant Sciences* 157: 406-417.
- Rowe N, Isnard S, Speck T. 2004. Diversity of mechanical architectures in climbing plants: an evolutionary perspective. *Journal of Plant Growth and Regulation* 23: 108-128.
- Rowe NP, Isnard S, Gallenmüller F, Speck T. 2006. Diversity of mechanical architectures in climbing plants: an ecological perspective. In: Herrel A, Rowe NP, Speck T. *Biomechanics and Ecology*. Dekker.
- Santiago LS, Pasquini SC, De Guzman ME. 2015. Physiological implications of the liana growth form. In: Schnitzer, SA, Bongers F, Burnham R, Putz FE, eds. *Ecology of Lianas*. Wiley-Blackwell Publishers, Oxford.
- Schenck H 1892. Beiträge zur Biologie und Anatomie der Lianen, im Besonderen der in Brasilien einheimischen Arten. I. Theil. Beiträge zur Biologie der Lianen. In: Schimper AFW. *Botanische Mittheilungen aus den Tropen*. Gustav Fisher. Jena.
- Schenck H. 1893. Beiträge zur Biologie und Anatomie der Lianen im Besonderen der in Brasilien einheimischen Arten. II. Theil. Beiträge zur Anatomie der Lianen. In: Schimper AFW. *Botanische Mittheilungen aus den Tropen*. Gustav

Fisher. Jena.

Schnitzer SA, Bongers F, Burnham RJ, Putz FE. 2015. Ecology of lianas. Wiley Brackwell, West Sussex.

Silvestro D, Cascales-Minana B, Bacon CD, Antonelli A. 2015. Revisiting the origin and diversification of vascular plants through a comprehensive Bayesian analysis of the fossil record. *New Phytologist* 207: 425-436.

Stevens GC. 1987. Lianas as structural parasites: the *Bursera simaruba* example. *Ecology* 68: 77-81.

Tamaio N, Vieira RC, Angyalossy V. Origin of successive cambia on stem in three species of Menispermaceae. *Revista Brasileira de Botânica* 32: 839-848.

Van Thieghem Ph. 1884. Traité de Botanique. Librairie F. Savy, Paris.

Zimmermann MH, Jeje AA. 1981. Vessel-length distribution in stems of some American woody plants. *Canadian Journal of Botany* 59: 1882-1892.

GENERAL CONCLUSION

General conclusions

The evolution of the lianescent habit imprints an enormous overall modification in the *bauplan* of a plant. Here we used three different lineages of vascular plants and different approaches to investigate how the vascular system has evolved in lineages where lianas are present. Bignoniaceae (Lamiales) within the rosids to investigate the secondary xylem, Malpighiaceae (Malpighiales) within the asterids to investigate the ontogeny and evolution of the stem, and the tracheophytes as a whole to investigate the secondary phloem.

In **Chapter 1**, we analyzed the wood anatomy of 85% of the genera of Bignoniaceae and encountered that evolution has taken different routes, even opposite, within Bignoniaceae. Classical linear theories of wood evolution suggest, for instance, that rays evolved from heterocellular to homocellular, and while this pattern was encountered for some lineages, the opposite pattern was found too, especially in lineages evolving the lianescent habit. The evolution of lianas greatly impacted the family, and lianescent lineages such as Bignoniaceae and Tecomeae s.s. are the ones where cambial variants are encountered, wide vessels, vessel dimorphism, wide heterocellular rays and perforated ray cells. Occupation of more seasonal climate regimes has also played an important role in the evolution of Bignoniaceae, and ring porous woods with helical thickening were present in trees and lianas growing at higher latitudes, in the geographic limits of distribution of the family. However, although these aspects promoted change in many anatomical features of Bignoniaceae, major clades are still quite homogeneous anatomically, and at least 9 anatomical synapomorphies could be delimited for Bignoniaceae, showing the importance of more broad studies of this type for other plant families.

In **Chapter 2**, we used phylogenetically controlled pairs of lianas and their closest self-supporting species, sampling monilophytes (ferns), gymnosperms (Ephedraceae), angiosperms magnoliids, monocots, rosids and asterids. We found that overall, lianas have wider sieve elements, with wider sieve pores, aspects that contribute to a more efficient conductive system. Similarly to the xylem, it maintained the taller and heterocellular rays typical of lianas, which has been related to an increased flexibility for the stems to climb and a better vertical conduction of solutes, respectively. Self-supporting plants whose most recent common ancestor is known to be a liana were shown to preserve lianescent features, such as the presence of cambial variants and high and heterocellular rays, a phenomenon here interpreted as phylogenetic inertia. Plants where two phloem types co-occur exhibit important dissimilarities in these phloem types, with the variant phloem having wider sieve elements – more efficient in solute conduction –, indicating an specialization for conduction, while the regular phloem has tiny sieve tubes, but abundant parenchyma, probably indicating specialization for storage. Overall, besides from differences in the dimensions of sieve tubes and ray height and composition, all other phloem features of lianescent species conserve the phloem

anatomy correspondent to the taxa to which they belong. This evidences that while there has been convergent evolution in lianas towards more efficient photosynthate conduction, the phloem still carries a high phylogenetic signal.

In **Chapter 3**, we investigated the stem anatomy of Malpigiaceae, selecting 12 genera in which we encountered cambial variants to study in detail. Ontogenetic analyses allowed us to delimit 7 different types of cambial variants. When data from ontogeny were mapped onto the phylogeny of Malpigiaceae we could detect 8 independent evolutions of the cambial variants in the family. The cambial variants of Malpigiaceae are: (i) Interxylary phloem, present in *Dicella* (ii) Interxylary cambia, present in several taxa, being the main cambial variant in *Stigmaphyllon* and *Banisteriospsis nummifera* (iii) Phloem wedges furrowing the xylem, present in *Alicia*, *Callaeum*, *Diplopterys*, *Flabellaria*, *Heteropterys*, *Mascagnia*, *Mezia*, *Niedenzuella*, *Peixotoa* and *Stigmaphyllon*. Fissured xylem of 3 different types, some (iv) without inner xylem partition, as in *Diplopterys*, some with (v) inner xylem partition, as in *Alicia* and *Callaeum* and some (vi) without complete inner xylem partition, as *Flabellaria* and *Mezia*, and (vii) Asymmetrical stems, apparently exclusively in *Heteropterys* subsect. *Aptychia*, in which some species also develop interxylary cambia. Overall we can conclude that several cambial variants in Malpigiaceae share common stages of development, while some have almost identical cambial variants, but deriving from different ontogenetic trajectories. Also, although quite diverse, cambial variants are generally conserved within the genera and sister genera, even in groups that have undergone intercontinental disjunctions. Further studies in Malpigiaceae can now explore what is the impact of these different cambial variants in the process of diversification in the group.

Studies of anatomy within a phylogenetic framework allow to better understand the sequence of changes anatomy has undergone within evolutionary time and to raise hypothesis on what are the mechanisms leading to anatomical change over time.