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Tese de Doutorado

Estrutura filogenética e funcional de comunidades vegetais a partir de ecologia reprodutiva: padrões espaciais e temporais.

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Porto Alegre, Maio de 2015

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"Andar com fé eu vou, que a fé não costuma falhar"

Gilberto Gil

Resumo

Nas últimas décadas estudos em ecologia de comunidades incorporaram a perspectiva evolutiva para entender como as comunidades se organizam no espaço e no tempo. A similaridade evolutiva entre espécies é considerada fundamental para o entendimento da estruturação das comunidades e dos padrões de coexistência das espécies. O avanço de ferramentas analíticas e computacionais propiciariam a reconstrução de filogenias descrevendo com maior precisão as relações de parentesco entre espécies, o que levou ao desenvolvimento da área de ecologia filogenética. A hipótese de que espécies filogeneticamente próximas são similares em relação às suas características ecológicas foi inicialmente utilizada para interpretar quais processos poderiam estar determinando a organização das comunidades. Porém, a abordagem estritamente filogenética tem sido questionada pela falta de correlação com a estrutura funcional das comunidades. A integração das abordagens filogenética e funcional tem demonstrado uma complementaridade na explicação dos padrões de coexistência das espécies. Dentre as características funcionais, atributos reprodutivos são fundamentais para sobrevivência das espécies, influenciando a dispersão e a colonização de novos ambientes. Esta tese avaliou diferentes métodos de reconstrução de filogenias buscando primeiramente obter relações filogenéticas mais precisas entre espécies para que em conjunto com atributos reprodutivos fosse avaliada a organização das comunidades no espaço e no tempo. No primeiro capítulo foi avaliado o efeito de filogenias moleculares, pseudocronogramas e filogenias topológicas em medidas de diversidade e dispersão filogenética alfa e beta. A influência das diferentes filogenias nas medidas de dispersão e diversidade filogenética variaram de acordo com a composição das unidades amostrais. A correção dos procedimentos de reconstrução filogenética no programa *Phylocom* aumentou a similaridade com filogenias moleculares sem perda de poder estatístico, dependendo da medida de dispersão e diversidade avaliadas. Em

uma situação de deficiência de dados moleculares, pseudocronogramas gerados pelo programa *Phylocom* são uma boa escolha especialmente para o cálculo do índice de parentesco líquido. No segundo capítulo foi avaliado a variação na composição de espécies, filogenética e funcional de plantas trepadeiras no processo de avanço da floresta sobre o campo. Foi analisada a associação de características de dispersão dos frutos e filogenia em um gradiente de manchas florestais de diferentes áreas e isolamento em relação a floresta contínua circundante. Foi encontrado um padrão aninhado na composição de espécies, indicando que manchas florestais menores são subconjuntos de manchas maiores que por sua vez são subconjuntos da floresta contínua. A área e o isolamento da mancha florestal são fatores determinantes do padrão de organização das comunidades, criando um filtro ambiental selecionando a composição de espécies. Além disso, as comunidades não são organizadas de acordo com as relações filogenéticas das espécies, mas atributos de dispersão como o tipo de fruto e a síndrome de dispersão mostraram uma associação com o gradiente de área e isolamento, sendo determinantes para colonização dos diferentes tipos de manchas florestais. No terceiro capítulo foi avaliado a conservação filogenética na fenologia reprodutiva de uma comunidade de trepadeiras e analisado se havia uma associação entre a composição de espécies em floração e frutificação e variáveis climáticas, mediada pela relação filogenética entre as espécies. Foi observado um baixo sinal filogenético nos picos de floração e frutificação, mostrando que espécies filogeneticamente próximas diferem em seus períodos fenológicos. Porém, espécies de um mesmo clado em escalas filogenéticas amplas ou finas, responderam de maneira similar à mesma variável climática. A integração das abordagens filogenética e funcional, demonstraram uma complementaridade permitindo compreender de uma maneira mais completa como as comunidades se estruturam no espaço e no tempo.

Palavras-chave: Filogenia, Resolução filogenética, Sinal filogenético, Diversidade filogenética, Fenologia, Trepadeiras, Lianas, Floresta com Araucária, Manchas florestais, Síndromes de dispersão.

Abstract

In the last decades, community ecology studies incorporated an evolutionary perspective to understand how communities are organized in space and time. The similarity between species is fundamental for understanding the structure of communities and species coexistence patterns. The advance of analytical and computational tools enabled the reconstruction of reliable phylogenies describing with more precision the relationships among species, which has led to the development of community phylogenetics area. The hypothesis that closely related species are similar in relation to their ecological traits was initially used to interpret what processes could be determining the organization of communities. However, a strictly phylogenetic approach has been doubted due to the lack of correlation with the functional structure of communities. The integration of phylogenetic and functional approaches demonstrated a complementarity for explaining species coexistence patterns. Among functional attributes, reproductive traits are essential for species survival, influencing dispersion and colonization of new habitats. This thesis evaluated different phylogenetic reconstruction methods seeking to mainly obtain a more precise phylogenetic relationship between species. This, in addition to reproductive traits, were used to evaluate communities structuring in space and time. In the first chapter, we evaluated the effects of molecular phylogenies, pseudo-chronograms and topological phylogenies in metrics of alpha and beta phylogenetic diversity and dispersion. The relative influence of different phylogenetic reconstruction techniques on phylogenetic community metrics varied among sampling units. Correcting the pseudo-chronogram construction workflow in *Phylocom* enhances the similarity with molecular phylogenies, with no significant loss of statistical power depending on the phylogenetic community metric. In a situation of deficient genetic sequence data, pseudo-chronograms are a good choice, especially for the net relatedness index metric. In the second

chapter, we assessed the variation of species composition, phylogenetic clades and functional traits of climbing plants along the process of forest expansion over grassland. We analyzed the association of dispersal traits and phylogeny along the gradient of forest patches of increasing sizes and isolation to the surrounding continuous forest. We found a nested pattern on species composition, indicating that smaller patch categories are subsets of larger ones and forest sites. Patch area and isolation play an important role in forest structure, generating an environmental filtering on species composition. Moreover, communities are not organized according to phylogenetic relationships. However, dispersal traits like fruit type and dispersal syndrome showed an association with the patch size and isolation gradient, being determinants of forest patches colonization. In the third chapter, we evaluated the phylogenetic conservatism in the reproductive phenology of a climbing plant community. We assessed if there was an association between the composition of flowering and fruit-bearing species and climatic variables, mediated by phylogenetic relationships. We observed low phylogenetic signal for flowering and fruiting peaks, showing that phylogenetically close species have different phenological periods. However, species of the same clade in larger or finer scales responded similarly to the same climatic variable. The integration of phylogenetic and functional approaches demonstrated a complementarity, allowing a better understanding of how communities are structured in space and time.

Keywords: Phylogeny, Phylogenetic resolution, Phylogenetic signal, phylogenetic diversity, Phenology, Climbing plants, Lianas, Araucaria forest, Forest patches, dispersal syndromes.

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Introdução

Estruturação de comunidades e a abordagem filogenética

No último século, estudos de ecologia de comunidades tem focado na descrição da diversidade de espécies ao longo do espaço e do tempo. O interesse em compreender como as comunidades se estruturam e diferentes espécies coexistem, levou ao entendimento de que a similaridade entre espécies é uma 'peça' fundamental para entender qual(is) fatores podem estar determinando a montagem das comunidades. Em uma comunidade vegetal, espécies coexistentes interagem baseado em suas similaridades e diferenças fenotípicas, que são determinadas a partir de sua história evolutiva (Webb et al. 2002). Espécies aparentadas tenderiam a serem mais similares ecologicamente devido à sua ancestralidade comum (Harvey & Pagel 1991), conservando suas características ao longo da evolução, um padrão chamado de sinal filogenético (Blomberg et al. 2003). Devido à presença de sinal filogenético, espécies aparentadas tendem a explorar e se estabelecer em ambientes similares onde condições ideais para seu desenvolvimento estão disponíveis, possibilitando sua coexistência (Donoghue 2008). Esta similaridade entre espécies aparentadas em relação ao seu nicho ecológico (condições bióticas e abióticas que possibilitam a espécie persistir; Holt 2009), ou seja, a tendência de reterem as características de seus nichos ao longo do tempo, tem sido chamada de conservação filogenética de nicho (Wiens & Graham 2005). Por outro lado, espécies aparentadas podem experimentar fortes interações competitivas devido à sua similaridade ecológica (Darwin 1859), o que pode limitar sua coexistência e dirigir espécies filogeneticamente próximas a uma divergência de suas características ecológicas, criando uma diferenciação de nicho (Cavender-Bares et al. 2009).

Estudos pioneiros na investigação da estruturação de comunidades com base na similaridade das espécies, utilizaram a medida da razão espécie/gênero para inferir se

comunidades eram formadas por espécies distantes ou proximamente relacionadas (p.ex., Jaccard 1926; Elton 1946). Essa abordagem foi o primeiro passo para se investigar qual o papel de interações bióticas ou abióticas na organização de comunidades. Com base na hipótese de conservação filogenética de nicho, a coexistência de espécies filogeneticamente distantes (dispersão filogenética) seria um indicativo de competição entre espécies ecologicamente similares (limitação de similaridade; MacArthur & Levins 1967), enquanto a coexistência de espécies filogeneticamente próximas (agrupamento filogenético) seria um indicativo da atuação de filtros ambientais selecionando espécies ecologicamente similares (Keddy 1992). Webb et al. (2002) reuniram essas hipóteses de estruturação filogenética de comunidades e juntamente com a proposta de uma nova medida de diversidade e dispersão filogenética (Webb 2000), criaram um arcabouço teórico relacionando os padrões filogenéticos e fenotípicos de coexistência de espécies com os processos de limitação de similaridade e filtros ambientais. A partir desse trabalho a área de ecologia filogenética se desenvolveu rapidamente (Mouquet et al. 2012) e novas teorias foram propostas, questionando e expandindo a discussão acerca dos processos estruturadores das comunidades (p.ex., Mayfield & Levine 2010; Gerhold et al. 2015).

Juntamente com o trabalho de Webb et al. (2002), o desenvolvimento da área de ecologia filogenética se deu em grande parte ao desenvolvimento das ferramentas computacionais *Phylomatic* e *Phylocom* (Webb & Donoghue 2005; Webb et al. 2008) para a reconstrução de filogenias. Utilizando esses programas qualquer usuário sem conhecimentos em taxonomia filogenética é capaz de reconstruir uma filogenia, fornecendo uma simples lista de espécies com suas respectivas famílias. Além da facilidade de utilização, a filogenia resultante possui estimativas (em milhões de anos) dos comprimentos dos ramos ligando as espécies, calculadas através de estimativas de idade para parte dos clados de angiospermas proposto por Wikström et al. (2001). Clados não datados são estimados através de um algoritmo chamado BLADJ (do

inglês *Branch Length Adjustment*) que simplesmente particiona a idade entre dois nós datados, de acordo com a quantidade de nós não datados entre eles. Devido a esse procedimento, filogenias geradas através desses programas são chamadas de pseudocronogramas. Outra interessante vantagem dos programas *Phylomatic* e *Phylocom* é que o usuário pode editar a árvore de referência das angiospermas utilizada para reconstrução da filogenia, que tem resolução até o nível de família, incorporando resolução dentro das famílias seguindo trabalhos de taxonomia filogenética publicados.

Com a transição do sistema de classificação filogenética de APG II (APG 2003) para APG III (APG 2009) os programas *Phylocom/Phylomatic* atualizaram a árvore de referência, mas aparentemente não atualizaram a sintaxe e nomenclatura dos nós dessa árvore para corresponderem aos nomes dos nós das idades propostas por Wikström et al. (2001) utilizados para estimativa dos comprimentos dos ramos. Gastauer and Meira-Neto (2013) evidenciaram esse problema, mostrando que os resultados de medidas de estrutura filogenética de comunidades utilizando essas árvores apresentaram superestimativa dos resultados, podendo causar interpretações contrárias acerca dos processos estruturadores das comunidades. Durante meu doutorado notei que os programas apresentavam outros problemas além do que foi evidenciado por Gastauer and Meira-Neto (2013). Percebi que algumas filogenias reconstruídas através programas *Phylocom/Phylomatic* geravam superestimativas dos valores de ramos que não correspondiam aos valores gerados quando se datava toda a filogenia das angiospermas. Intrigado com esse fato, descobri que se tratava de uma abordagem errônea proposta no manual do programa e que uma inversão na ordem dos procedimentos de datação e geração da filogenia contendo apenas as espécies desejadas resolvia problema. Entretanto, essa solução implica em uma correção manual da filogenia não ultramétrica que o programa passa a gerar. Além disso, a árvore de referência não é atualizada desde 2012 e estimativas

mais recentes de idades dos clados, como proposto por Bell et al. (2010), poderiam ser utilizadas.

Estudos recentes mostram que a resolução filogenética, ou seja, a quantidade de politomias (quando um nó produz mais que duas linhagens descendentes) afeta os resultados de diferentes medidas filogenéticas (Swenson 2009; Davies et al. 2012; Münkemüller et al. 2012; Seger et al. 2013; Molina-Venegas & Roquet 2014). Com o desenvolvimento de programas computacionais de interface simples para a reconstrução de filogenias moleculares (p.ex., Silvestro & Michalak 2012; Pearse & Purvis 2013) juntamente com a maior disponibilidade de sequências genéticas de diferentes marcadores moleculares em bancos de dados públicos (*Genbank*; Benson et al. 2013), a possibilidade de reconstrução de filogenias com melhor resolução em diferentes níveis taxonômicos se tornou possível. Os estudos de Cadotte et al. (2008) e Kress et al. (2009), foram pioneiros ao utilizar marcadores disponíveis no *Genbank* e sequenciamento molecular *in situ*, respectivamente, para reconstrução de filogenias contendo diversas famílias botânicas. O estudo de Kress et al. (2009) foi importante no sentido de salientar que filogenias com baixa resolução geradas através dos programas *Phylocom/Phylomatic* podem causar um alto erro tipo I e II em comparação com filogenias moleculares mais precisas. Entretanto, a reconstrução de filogenias moleculares requer um conhecimento não usual a muitos ecólogos, além do desafio da utilização de diferentes programas nas etapas de reconstrução. Visto essas dificuldades, Pearse and Purvis (2013) desenvolveram um programa chamado *phyloGenerator*, reunindo os diversos programas utilizados em reconstrução de filogenias, com uma interface simples buscando atrair o público leigo sem conhecimento em taxonomia filogenética.

Com essa nova ferramenta disponível e a correção dos procedimentos de reconstrução de filogenias nos programas *Phylocom/Phylomatic*, não se sabe os efeitos dessas diferentes filogenias nas medidas mais utilizadas em ecologia filogenética de comunidades. Com esse

questionamento desenvolvi o primeiro capítulo da tese, comparando o efeito de filogenias moleculares, pseudocronogramas gerados pelos programas *Phylocom/Phylomatic* e filogenias topológicas (sem estimativas de valores de ramos) em diferentes medidas de diversidade e dispersão filogenética alfa e beta. Além disso, comparei dentro de e entre cada tipo de filogenia qual o efeito de diferentes estimativas de ramos e resoluções (politomias) em diferentes níveis taxonômicos. Com o desenvolvimento desse capítulo pude escolher qual método é mais apropriado para construção de árvores filogenéticas, utilizados no demais capítulos da tese.

Integrando filogenia e funcionalidade no espaço e no tempo

Recentemente, a hipótese de que espécies filogeneticamente próximas são ecologicamente similares tem sido questionada como critério para o uso exclusivo de uma abordagem filogenética na estruturação de comunidades (Losos 2011; Mouquet et al. 2012). Isso parte do princípio de que em uma comunidade composta por um grande número de espécies, a distribuição filogenética de atributos pode ser uma complexa mistura de conservação e convergência (Webb et al. 2002). Portanto, um mesmo processo estruturador de comunidades, como por exemplo a limitação de similaridade, pode estar ocorrendo tanto se a comunidade apresentar uma agregação ou divergência filogenética (Kraft & Ackerly 2010; Mayfield & Levine 2010). Estudos tem demonstrado essa falta de correlação entre medidas de dispersão filogenética e funcional, questionando o uso restrito de uma abordagem filogenética (Swenson 2013).

Ao investigar a estrutura funcional de comunidades, a escolha de qual característica ecológica de uma espécie de planta deve-se utilizar é em grande parte fundamentada pela hipótese permeando o estudo, em que por exemplo, característica foliares podem estar relacionadas a gradientes hídricos ou luminosos. O uso de características ecológicas das espécies deve ser baseado no pressuposto de que as características avaliadas são de fato

características de nicho, que influenciam a sobrevivência das espécies envolvidas (Cooper et al. 2010). Desta maneira, características reprodutivas como as características de flores, frutos, sementes e a fenologia da floração e frutificação são extremamente importantes, pois estão diretamente relacionadas ao *fitness* (dispersão, colonização, estabelecimento, sobrevivência, crescimento e reprodução) de cada espécie.

Características de frutos são importantes do ponto de vista do sucesso de dispersão, influenciando potencialmente os padrões de fluxo gênico e a estrutura genética intra e interpopulacional (Willson & Traveset 2000; Jordano et al. 2006). Adaptações a agentes dispersores (vento e animais) são esperadas, visto que, sob o ponto de vista das plantas, todo o processo que otimize seus eventos reprodutivos e aumente a possibilidade de perpetuação da espécie é vantajoso. O processo de dispersão de sementes influencia diretamente a estrutura espacial de populações em pequenas e amplas escalas espaciais (Nathan & Muller-Landau 2000), dependendo das características dos frutos que influenciam dispersões a curta ou longa distância. Nesse sentido, processos evolutivos e de dispersão são reconhecidos como fundamentais na formação de comunidades locais (Leibold et al. 2004; Graham & Fine 2008). Dentro desse contexto, busquei no segundo capítulo da tese integrar as abordagens filogenética e funcional, avaliando a associação de características de dispersão dos frutos (síndromes de dispersão e tamanho/peso) e filogenia na estrutura de comunidades de trepadeiras em manchas florestais inseridas em uma matriz campestre. Além de integrar filogenia e funcionalidade, explorei possíveis padrões no espaço de acordo com características estruturais das manchas florestais no contexto da paisagem. O interesse surgiu a partir dos estudos de Duarte et al. (2006a; 2006b; 2007) e Duarte (2011) que avaliaram padrões composicionais, filogenéticos e de atributos de frutos zoocóricos em plantas lenhosas jovens na mesma área de estudo, com o enfoque no processo de avanço da floresta sobre o campo. Como o avanço da floresta sobre o campo é um processo em que a floresta substitui a vegetação campestre mais antiga e

dominante (Veldman et al. 2015) ao invés da floresta retornar ao seu estado 'original' como nos processos sucessionais, nada se sabe sobre o papel das plantas trepadeiras nesse contexto. Nesse capítulo considerei além da influência da área das manchas florestais (significando diferentes estágios de desenvolvimento) na estruturação das comunidades, o seu grau de isolamento para as outras manchas e a floresta contínua. Dessa maneira, avaliei se a composição de espécies, filogenética e funcional poderia estar estruturada ao longo desse gradiente de área e isolamento, identificando quais clados ou características das espécies poderiam estar associadas a diferentes níveis do gradiente. Além disso, busquei avaliar se a composição de espécies, filogenética e funcional se modifica ao longo do gradiente de área, apresentando um padrão aninhado, ou seja, manchas florestais menores seriam subconjuntos de manchas florestais maiores, que por sua vez seriam subconjuntos da floresta contínua.

Além das características dos frutos, a fenologia da floração e frutificação das espécies, ou seja, o período do ano em que estão reprodutivamente ativas são determinantes no processo de dispersão e consequente fluxo gênico. Muitos organismos podem mostrar uma conservação temporal de nicho estando ativos, reprodutivos ou presentes em uma região durante os períodos do dia ou do ano em que há um dado conjunto de condições ambientais (Wiens et al. 2010). Estudos fenológicos têm demonstrado uma forte relação entre fenologia e condições climáticas, como temperatura e pluviosidade (Fenner 1998). Entretanto, outros mecanismos podem estar envolvidos, como por exemplo, a restrição do período de floração pelo nicho temporal de seus polinizadores (Levin 2006) ou uma sazonalidade na frutificação coincidindo com o influxo de frugívoros migratórios (Thompson & Willson 1979; Loiselle & Blake 1991). Além de fatores bióticos e abióticos, a fenologia pode estar sendo expressada devido a fatores evolutivos, com espécies filogeneticamente próximas apresentando padrões fenológicos similares (p.ex., Kochmer & Handel 1986; Smith-Ramirez & Armesto 1994; Staggemeier et al. 2010). Essa estruturação filogenética no tempo tem sido recentemente abordada através da medida de sinal

filogenético ao nível de comunidade, buscando identificar se espécies filogeneticamente próximas possuem picos de floração ou frutificação similares. Porém, a presença de sinal filogenético pode depender do clado avaliado, o que não é perceptível quando analisado ao nível de comunidade (Seger et al. 2013). Sintetizar o período fenológico de uma espécie utilizando o pico da fenofase, impossibilita a avaliação das interações das diversas espécies de uma comunidade que podem estar apresentando suas fenofases com diferentes intensidades em um determinado mês. Outra questão importante é que um clado de determinado nível taxonômico (p.ex. ordem ou família) pode não apresentar picos de floração ou frutificação similares (baixo sinal filogenético), mas ainda assim responderem de forma semelhante ao mesmo estímulo ambiental (p.ex., pluviosidade, comprimento do dia, temperatura). Com o foco na estruturação filogenética no tempo, desenvolvi o terceiro capítulo da tese, abordando a influência da filogenia na fenologia da comunidade trepadeiras em uma floresta com Araucária ao longo de dois anos. Comparei os resultados obtidos através da medida de sinal filogenético na floração e frutificação com uma nova abordagem de avaliação da estrutura filogenética temporal. Através dessa nova abordagem pude avaliar se a relação da fenologia com o clima é mediada pelas relações filogenéticas entre as espécies e com isso inferir se há uma conservação filogenética nas respostas ao gradiente climático local.

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Capítulo 1. Disentangling the effects of phylogenetic tree reconstruction techniques on phylogenetic community metrics[1]

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Abstract

In the last decade, the development of community phylogenetics has been promoted by the improvement of phylogenetic reconstruction techniques. Most studies in this field have relied on phylogenies reconstructed using the software Phylocom/Phylomatic which is based on a partially resolved supertree to generate a pseudo-chronogram using published age estimates. However, this approach has some limitations mainly due to branch length estimates and the lack of phylogenetic resolution on terminal nodes. With more accessible technology and available databases of genetic sequences, studies are starting to use phylogenies based on molecular data enhancing phylogenies accuracy. In this study, we tested the effects of different phylogenetic reconstruction approaches in ecology on different phylogenetic alpha and beta diversity and dispersion metrics. For this, we compared topological trees, pseudo-chronograms and molecular phylogenies with different ages, branch length estimates and resolution levels, and discussed the pros and cons of each technique. Within each phylogeny type, branch length estimates accounted for most of the variation in the results for almost all metrics. The relative influence of different phylogenetic reconstruction techniques on phylogenetic community metrics varied among sampling units, demonstrating that biases themselves may differentially emerge as the phylogenetic composition of sampling units varies. Correcting the pseudo-chronogram construction workflow in Phylocom enhances the similarity with molecular phylogenies, with no significant loss of statistical power depending on the phylogenetic community metric. In a situation of deficient genetic sequence data, pseudo-chronograms are a good choice, especially for NRI metric. Even with the need for background knowledge, the reconstruction of well resolved and standardized molecular phylogenies is an important task for the development of community phylogenetics.

Keywords: Phylocom; Phylomatic; phyloGenerator; Phylogenetic resolution; Branch length estimate; Molecular phylogeny; Community phylogenetics; alpha diversity; beta diversity.

Running head: Phylogenies and community metrics

Introduction

The integration of phylogenetic information into community ecology has given researchers a new lens through which we can view the ecological processes underlying community assembly (Webb et al. 2002). Phylogenies have been used in community ecology to provide novel information regarding species coexistence at different spatial scales, from local to regional (e.g. Swenson et al. 2006), up to continental or even global perspectives (e.g. Hawkins et al. 2014). The use of phylogenetic information as a proxy for similarity in these studies is grounded in the comparative methods literature and Darwin's original discussion of the propensity of allied forms to compete more intensely (Darwin 1859; Felsenstein 1985; Harvey & Pagel 1991), but it is important to test this assumption when possible to permit more robust inferences (Losos 2011; Mouquet et al. 2012).

During the last decade, different approaches and tools have been developed to facilitate phylogenetically-informed research conducted by non-phylogeneticists (e.g. Crozier et al. 2005; Webb & Donoghue 2005; Beaulieu et al. 2012). Many of the early phylogenetic analyses of community structure relied on cladograms (i.e., diagrams depicting relationships among groups, but without branch length estimation) (Crozier 1997). Pairwise phylogenetic distances between species were then estimated by counting the nodes separating pairs of species or setting branch lengths between nodes to one (Webb 2000). Those phylogenies were often reconstructed following taxonomic hierarchies (Crozier et al. 2005), classifying species in pre-determined major groups (e.g., orders, families, tribes, etc.) or following published topologies, a type of supertree approach, based on phylogenetic hypotheses like the APG II (APG 2003) with a family level resolution. However, when using these types of phylogenies, well sampled clades inflates the distance in relation to poorly sampled clades by solely an artifact of sampling rather than real evolutionary distance (i.e., as most sampled the clade is, higher the number of

branches), and results between communities are comparable only if they are a subset of the same reference phylogeny (Webb 2000).

These issues were greatly mitigated when branch lengths started to be estimated with software's easily accessible for ecologists, such as Phylocom/Phylomatic software (Webb & Donoghue 2005; Webb et al. 2008). Phylocom (with the bundled Phylomatic software) reconstructs phylogenies using a supertree based on a previous phylogenetic hypothesis (e.g. APG 2009 for angiosperm plants) with clade age estimates derived from branch lengths generated using a fossil node date and non-parametric rate smoothing (Wikström et al. 2001), giving an estimate of divergence time between taxa. The advantage of Phylocom from the perspective of ecologists is its simplicity to use, allowing any non-phylogeneticist to reconstruct their own phylogeny by providing a simple species list. In the last decade, many studies have been based on phylogenies reconstructed by Phylocom using different phylogenetic hypotheses (APG 2003; Davies et al. 2004; APG 2009) and Wikström's node age estimates (Wikström et al. 2001). Recently, alternative age estimates have been published (Bell et al. 2010) and, when compared with the more widely used Wikström's age estimates, different results were obtained (Seger et al. 2013). Another important point is that phylogenies generated by Phylocom usually resolve taxa relationships up to family level and, only occasionally, present genera resolution. This means that generic and species relationships in the phylogenetic trees are treated as polytomies (i.e., when a single node produce more than two descendant lineages). Phylogenetic tree resolution may have strong effects on the statistical results in community phylogenetics (Swenson 2009; Davies et al. 2012; Seger et al. 2013; Molina-Venegas & Roquet 2014), depending on whether polytomies are present close to the tips or to the root of the tree.

In recent years, phylogenetic ecology studies have started to estimate species relatedness by reconstructing phylogenies of communities based on molecular data (e.g. Cavender-Bares

et al. 2004; Kress et al. 2009). This is possible due to the increasing availability of sequences for different organisms and genetic markers in online databases (e.g. GenBank; Benson et al. 2013). The advantage of this approach to reconstructing community phylogenies is that the resolution is higher in comparison to the most used methods applied in community phylogenetics, being able to resolve species level depending on the data available. The effect of molecular trees on phylogenetic dispersion metrics results may change our inferences regarding the dominant ecological mechanisms underlying community structure when compared to phylogenies generated by Phylocom (Kress et al. 2009; Pei et al. 2011).

The use of molecular trees in ecological studies is promising, but taking such an approach requires a stronger understanding of molecular phylogenetic methods that is unusual for most ecologists and this is especially true given that phylogenetic literature is large with constant new methodologies and concepts being published (e.g. Roquet et al. 2013). To fill this gap a new software for molecular tree reconstruction called phyloGenerator (Pearse & Purvis 2013) was developed. However, the degree to which phylogenies like those produced by something like phyloGenerator affect phylogenetic community metrics and its differences for the most employed methods by now is unknown.

In this paper, our aim is to compare different types of phylogenetic trees that an ecologist may reconstruct and test their effects on some commonly employed alpha and beta phylogenetic diversity and dispersion metrics. For this, we reconstructed different phylogenetic trees through Phylocom and phyloGenerator software, applying different branch length estimates and resolutions on terminal nodes. We addressed the following questions: 1 – How similar are molecular trees with other types of phylogenies? 2 – Which properties (e.g., resolution level and branch length estimates) within each type of phylogeny affect the phylogenetic diversity and dispersion metrics tested? 3 – Which phylogenetic properties between different types of phylogeny are more important during the steps of decision when

reconstructing phylogenies? We discuss the pros and cons of each phylogenetic reconstruction technique and compare different workflows among the different methods.

Material and Methods

Study Site

To compare these different types of phylogenies, we utilized data from tree communities of an *Araucaria* forest at the National Forest of São Francisco de Paula – ICMBIO (29º25'S, 50º24'W), Rio Grande do Sul State, Brazil. Sampling units were composed by 18 blocks containing nine quadrats of 100 m^2 (totaling 1.62 ha) at six forest sites containing three blocks each. In each quadrat, we measured and identified all trees with a diameter at breast height ≥ 5 cm and height ≥ 4 m. We found 76 angiosperms species, two gymnosperms and two ferns, but used for the analysis only angiosperms since gymnosperms and ferns might lead to biased phylogenetic overdispersion patterns due to their distant relatedness. We sampled 2,394 individuals, belonging to 26 families and 51 genera, with an average of 30.2 species per block (ranging from 24 to 39) and 130 individuals per block (ranging from 92 to 160). There is a great richness and abundance of Myrtaceae (22 spp., 10 genera and 632 individuals) and Lauraceae (8, 4, and 340) families, which allows measuring the effect of different resolutions close to the phylogeny tips.

Phylogenetic reconstructions

We reconstructed 16 phylogenetic trees (Supplementary material Appendix 1) belonging to three major groups named Topological, BLADJ (Branch Length Adjustment algorithm) and Molecular. In each group, we reconstructed two types of trees according to the branch length estimations and each type had two subtypes according to the resolution of terminal nodes (scheme in Figure 1). Topological trees had no information regarding branch lengths, so there

is no measurement of divergence time between nodes. Pairwise phylogenetic distances between species were calculated as the number of nodes separating them plus one, i.e. a genus with two species will have species distance equal to two (sum of branches connecting them) with one node separating them. In this group, phylogenies were reconstructed using a angiosperm supertree based on the phylogenetic hypothesis of APG III (APG 2009) to the order level and resolving relationships among families according to the Angiosperm Phylogeny Website (Stevens 2001). In this "megatree" (megatree R20120829; available at https://github.com/camwebb/tree-of-trees/blob/master/megatrees/R20120829.new) we removed single nodes and intra-familial resolution, so it resolves species relationships to a family level. Webb (2000) stated that using topological trees for the comparison of phylogenetic structure results between communities is valid only if the communities are a subset of the same reference phylogeny and the solution for this issue would be the use of a single phylogeny of all extant plants as the reference phylogeny. Based on such premise, two types of Topological trees were reconstructed according to its branch length calculation. In the first type, all sampled species were inserted into the angiosperm megatree and phylogenetic distances were computed considering all nodes separating them (hereafter 'Entire'). In Entire trees terminal nodes were represented by genera. In the second type (hereafter 'Pruned'), species were inserted into the megatree and pruned from it, resulting in a phylogeny with only the sampled species where only the basal nodes connecting species were retained, and hence many intermediate nodes connecting species were not considered for computing phylogenetic species distances. To generate the Pruned trees we used the bundled Phylomatic 2 software in the Phylocom 4.2 software (Webb & Donoghue 2005) to insert and prune the sampled species from the megatree. To reconstruct the Entire trees we used the function *match.phylo.comm* of package *picante* v. 1.6-2 (Kembel et al. 2010) in the R Statistical Environment (R Core Team 2014) to make a phylogeny with only the sampled species.

We applied two levels of resolution on terminal nodes within Pruned and Entire trees: *family level*, where genera and species were represented by polytomies; and *genus level*, where we combined the megatree information with a more precise intra-familial resolution to genera level for 20 families (84.2% of species) and thus, only species relationships within each genus were represented by polytomies. These families' phylogenies were reconstructed based on recent studies that we consider as being the best available hypotheses of relationships within these families (references in Supplementary material Appendix 2).

In the BLADJ group, phylogenies have their branch lengths estimated in millions of years, so there is a measurement of divergence time between nodes. As the group name states, we used the BLADJ algorithm of branch length adjustment in the Phylocom 4.2 software (Webb et al. 2008). Phylocom assigns published dated nodes given by the user into a topological megatree, in this case the same megatree used in the Topological group. The remaining undated nodes are estimated evenly between dated nodes by the BLADJ algorithm, to minimize variance in branch lengths, producing a pseudo-chronogram. With its bundled software, Phylomatic 2 (Webb & Donoghue 2005), the species given by the user are inserted into the megatree as terminals according to their taxonomy and then, the megatree is pruned resulting in a tree with only the supplied species.

Phylocom user's guide mentions that the phylogenetic tree should be dated after inserting the species and pruning the large phylogeny to only contain the supplied species to avoid the resulting phylogeny from being non-ultrametric. This can have a significant impact on branch lengths from one phylogeny to the next, because if we prune species first the resulting phylogeny will not contain many intermediate nodes that contain age information. The consequence is that the nodes estimated using BLADJ might not have the same age as if we have dated the whole megatree first and pruned the species later. This problem will manifest itself to different degrees in different systems because it is dependent on the clade composition

of the assemblage. To address this issue, we reconstructed two types of phylogenies within this group, named ‘Entire’ and ‘Pruned’ in the same way as the Topological group. In the Entire trees, we dated the megatree first using BLADJ and pruned the species later, and the phylogenies contained branch length information provided by all aged nodes. In this type, the resulting phylogeny is not ultrametric at first and we 'ultrametricized' it by hand by extending branches appropriately. In Pruned trees, we pruned the species from the megatree first, losing many nodes with age information, and dated the resulting phylogeny later using BLADJ. Within these two types, we dated the phylogenies using the clade age estimates of Wikström et al. (2001) and Bell et al. (2010), resulting in four different phylogenies (scheme in Figure 1). The criteria to choose the clades age estimates provided by these studies were: 1) the clades of Wikström’s/Bell’s phylogeny that matched Phylocom’s megatree topology, 2) the ages of orders and clades of superior hierarchy where the topologies did not match (i.e. clades relationships are in a different order/sequence), but only if some missing sub-clades (e.g. families) were not placed in a different clade and the absence of any sub-clade in discordance with megatree’s topology and 3) in clades below order's hierarchy, we also considered the node age when two clades diverge and each one contain all families regardless of its order/sequence. Using these criteria, we selected 250 Bell’s ages and 262 Wikström’s ages. Comparing our Wikström’s ages selection with the 175 ages provided by Phylocom, we selected 129 family ages while Phylocom provides 120, but 16 of these ages are outdated due to changes in family’s circumscription and 7 ages above the family level did not match the APG III (APG 2009) topology. To complement the megatree, we added recent phylogenetic hypotheses for the Monilophytes and Gymnosperms (Lehtonen 2011; Burleigh et al. 2012) with age estimates from Magallón et al. (2013). The two age estimates and their respective megatrees with node names matching the ages’ names for using in Phylocom are available in Supplementary Material Appendix 3. In these four phylogenies, we applied the same resolution levels on

terminal nodes (family and genus) of the Topological group explained before. In the genus level trees we also added the clade age estimates available within some of the 20 families added to the megatree (references in Supplementary material Appendix 2), as long as they were younger than the age of the immediate upper clade according to Bell and Wikström age estimates.

The Molecular group of phylogenies had their branch lengths proportional to the rate of evolution of the used markers (loci) and can be transformed to be proportional to divergence time. To reconstruct molecular phylogenies we used the phyloGenerator v.1.2. software (Pearse & Purvis 2013), a recent automated phylogeny generation tool that enables researchers to produce phylogenies using different tools through a simple workflow. For this, we chose some of the most widely used nuclear and chloroplastidial genetic markers for plants (ITS1/5.8S/ITS2, rbcL, matK, trnL-trnF spacer, trnL intron, psbA-trnH spacer, psbA gene, and NADH dehydrogenase subunit F) containing coding and non-coding regions, which are used to resolve species relationships at higher and lower taxonomic levels. phyloGenerator downloaded species sequences automatically from GenBank (consulted at April 2014; Benson et al. 2013) and we found sequences for 42 of 76 sampled species. For genera without sequence data (16 genera), we used data of congeneric relatives always seeking for species with the same life form, that occur geographically close to the sampled area, and of the same taxonomic tribe. The effect of using a congeneric relative sequence is minimal since they were not used together with a species of its same genus (Cadotte & Strauss 2011). For two sampled genera, the only sequence available at GenBank did not match the markers of its close relative(s) within family, because of it we chose other congeneric species following the same criteria explained above for which their markers match, improving tree reconstruction (Cadotte et al. 2009). The 15 species without sequence were merged manually in the resulting phylogenies, splitting them halfway along their congener branch with sequence data, and positioning it as a polytomy at

the genus node if there was more than one congener or at the family node if their position was uncertain.

phyloGenerator allows aligning sequences using different software. We opted to standardize the procedure using only the MAFFT v6.847b software (Katoh & Toh 2008) integrated into phyloGenerator. Reviewing the alignments, we found that some markers presented a bad alignment. Therefore, we aligned through the MAFFT online software (available at http://mafft.cbrc.jp/alignment/server/) outside phyloGenerator, which found and corrected 10 species sequences that were reverse complements. We used the Q-INS-i alignment strategy for ITS1/5.8S/ITS2, which takes into account a secondary structure information of RNA, and the E-INS-i strategy for the other markers. Using Mesquite 2.75 software (Maddison & Maddison 2011) we checked and excluded some misaligned species sequences and manually trimmed the alignment. In the next step, the alignments were inserted into the phyloGenerator, concatenated in a supermatrix and the phylogeny was reconstructed using a maximum-likelihood approach (ML) through the RAxML 7.3.0 software (Stamatakis 2006). We defined the ML searches to 1000 times, estimating a bootstrap support value for each node. In the ML approach, we used two different backbone constraint trees, one to family level and other to genus level, both reconstructed through Phylocom software using Bell's age estimates (both "Entire" type trees). The constraint trees were used to limit the software searches to trees compatible to its topology, reducing the artifact of patchy dataset (Roquet et al. 2013). Therefore, both family and genus level trees resolved the polytomies of their respective constraint trees exclusively by molecular data, resolving almost all species level relationships. The resulting phylogenies had their branch lengths proportional to the rate of evolution of the used markers (hereafter "Undated" type). In phyloGenerator we also transformed branch lengths to be proportional to time through rate smoothing, setting the root age to one (hereafter "Dated" type) using the PATHd8 software (Britton et al. 2007). In both approaches we defined

Nymphaea odorata Aiton (Nymphaeaceae), as the outgroup and pruned it prior to statistical analyses (Cadotte & Strauss 2011).

Data Analysis

To evaluate the effect of these distinct phylogenetic trees we chose the most frequently used alpha and beta phylogenetic diversity and dispersion metrics that are sensitive to variation on deeper or terminal nodes of the phylogenetic tree. To measure phylogenetic relatedness within communities (alpha diversity), we employed the abundance weighted mean pairwise distance (MPD) between all individuals in each community and the mean nearest taxon distance (MNTD) that calculates the mean distance separating each individual in the community from its closest relative (Webb 2000). For both metrics we calculated their respective standardized effect size (SES) metrics, the net relatedness index (NRI) and the nearest taxon index (NTI), which give a measurement of phylogenetic dispersion (clustering or overdispersion) of each community (Webb et al. 2002). We also measured the presence-absence weighted Faith's Index of phylogenetic diversity (hereafter FI; Faith 1992), which sums the branch lengths separating all species in a community expressing a proportion of this sum compared to the total branch lengths of the species pool, and its SES metric of phylogenetic dispersion (hereafter ses.FI). To calculate the phylogenetic dispersion metrics we used the *phylogeny.pool* null model, which gives equal probability to all species in the phylogeny to be included in the null communities. To evaluate the phylogenetic relatedness among communities (beta diversity), we employed the abundance weighted pairwise dissimilarity (D_{pw}') and nearest neighbor dissimilarity (D_{nn}') metrics (Swenson et al. 2011). The D_{pw}' calculates the mean of the average phylogenetic distance of each individual in a community in relation to all individuals in another community, while D_{nn}' calculates the mean of the phylogenetic distance of each individual in a community with its nearest closest relative

in another community. For both metrics, we calculated their respective SES metrics of phylogenetic dispersion (hereafter ses.D_{pw}' and ses.D_{nn}') using a null model implemented by Swenson (2014) that shuffles tip names across the phylogeny 999 times maintaining the spatial structure of species in the system. Comparing all metrics, MPD, NRI, D_{pw}' and ses.D_{pw}' are sensitive to variation at deeper nodes of the phylogenetic tree; MNTD, NTI, D_{nn}' and ses.D_{nn}' are sensitive to terminal nodes; and for FI and ses.FI the branch lengths are important. For all metrics we used the 18 blocks as sampling units. All metrics were calculated using the package *picante* v. 1.6-2 (Kembel et al. 2010) in the R Statistical Environment (R Core Team 2014).

To test the correlation and similarity of phylogenetic dispersion metrics between phylogenies we used simple linear regressions and nested ANOVAs (restricting permutations within sampling units) between the molecular dated tree with family constraint resolution level (hereafter "best-tree") and all other trees. The "best-tree" was considered the phylogeny that presented the "best" hypothesis of relationships within families, since it resolves the uncertainty of some genera relationships without any constraint. In this comparison, we also evaluated the over or underestimation of metrics' results (error type I and II) forcing the regression lines to cross the origin through data centering and evaluating their slope values (Swenson 2009), where values greater or lower than one (perfect correlation with the best-tree) denote a type I or II error, respectively. To test the effect of branch length estimation and resolution level within each group of trees (Topological, BLADJ and Molecular), we performed ANOVAs with a split plot design (Crawley 2012a). ANOVA significance was evaluated using permutation tests (10,000 permutations; Pillar & Orlóci 1996) restricting permutations of sampling units within nesting groups in bundles (further details in Supplementary material Appendix 4). The 18 blocks were taken as sampling units for alpha diversity/dispersion metrics and 153 pairwise comparisons between blocks were taken as sampling units for beta diversity/dispersion metrics. Within the BLADJ group, we also tested

the effect of clade age estimates of Bell and Wikström. To test the effect of the three different groups of phylogenetic trees, we selected one tree of each group considered to express the highest amount of information with credibility (Topological-Entire-Family level; BLADJ-Entire/Bell-Genus level; Molecular-Dated-Family constraint) and used the ANOVA design explained before. To identify which phylogeny property (type of tree, branch length estimation and tree resolution) is more important when deciding for a phylogenetic tree reconstruction, we performed linear mixed-effect models (LME; Crawley 2012b) between each property of all trees and the phylogenetic diversity and dispersion metrics, and calculated a linear regression of the LME results with their respective diversity/dispersion metric results. To allow the comparison among different phylogenetic trees, which species distances are represented in different units, when evaluating the phylogenetic diversity metrics, we standardized the phylogenetic trees modifying their branch lengths for species distance varying between zero and one. ANOVAs were performed using MULTIV 3.27b statistical software (by V. Pillar; available at http://ecoqua.ecologia.ufrgs.br/software) and the LMEs through the package *nlme* v.3.1-117 (Pinheiro et al. 2014) in the R Statistical Environment (R Core Team 2014).

Results

We calculated the correlation (Figure 2 and Supplementary material Appendix 5) and the over/underestimation of phylogenetic dispersion results (regression slopes; Figure 2, Supplementary material Appendix 5 and 6) of all reconstructed phylogenies with the "best-tree". The Molecular-Dated-Genus constraint phylogeny presented the highest correlation with the "best-tree", followed by BLADJ-Entire and Molecular-Undated phylogenies. The best-tree results were most correlated with the Molecular-Undated phylogenies for NTI and ses.D_{nn}' metrics, while NRI and ses.D_{pw}' results were more correlated with BLADJ-Entire phylogenies and ses.FI results with BLADJ-Entire-Genus level phylogenies. A few phylogenies showed a

trend towards type I error (Supplementary material Appendix 6) but most slope values were closer to one. The majority of phylogenies showed a bias towards random phylogenetic structure (type II error). The phylogenies with a slope close to one (within 0.95 and 1.05) were more represented by BLADJ-Entire and Molecular-Dated-Genus level phylogenies. Pruned type phylogenies presented the highest type II error with an exception for BLADJ-Family level for NTI metric (Supplementary material Appendix 6).

The effect of each factor (branch length estimation and resolution level) on the phylogenetic metrics within each tree group showed greater significant results for phylogenetic diversity metrics compared to dispersion metrics (Table 1). The branch length estimates were responsible for the highly significant effects in almost all metrics. In the Topological group, the branch length estimation (Entire versus Pruned trees) strongly affected NRI and ses.D_{pw}' metrics and did not differ for only the NTI and ses.FI metrics. The resolution level did not differ for only NRI, NTI and ses.D_{nn}', but the significant effects were low. In the BLADJ group, the age estimates of Bell and Wikström only influenced D_{pw}', explaining little of the variation observed. Entire and Pruned phylogenies differed in almost all metrics with an exception for NRI, which was not affected by any of the tested factors. In metrics where the resolution level did not differ, it affected results variation indirectly through an interaction with branch length estimates. In the Molecular group, branch length estimates (Dated and Undated) significantly differed only in phylogenetic diversity metrics, and within dispersion metrics, only the ses.D_{pw}' presented weak significant results for resolution level and its interaction with branch length estimates. Comparing the "best-tree" of each group, there was a significant difference in all metrics with a high effect in phylogenetic diversity metrics compared to dispersion metrics.

The linear mixed-effect models (LME) differed in their results depending on the phylogenetic metric and the phylogenies properties influence in each metric was heterogeneous among sampling units. These results suggest that the importance of phylogenies properties

varies according to the phylogenetic composition of sampling units. In phylogenetic diversity metrics the branch length estimation accounted for the most results variation in MPD and D_{pw}' metrics in all sampling units, showing a trend for decreasing diversity values with an increase of its importance (Supplementary material Appendix 7). For MNTD the tree type accounted for most results variation in all sampling units while for FI and D_{nn}' the tree type shares the explanation of the observed variation with branch lengths estimation. In all diversity metrics, the resolution level explained little of results variation within each sampling unit. For alpha dispersion metrics, the tree type accounted for most of the variation in a few sampling units, which tended to be overdispersed (Figure 3). The branch lengths estimation explained the variation in NRI and ses.D_{pw}' results in most sampling units, with a slight tendency for increasing its importance in sampling units leading to clustering for NRI metric. For NTI and ses.FI metrics, branch length estimates and resolution level explained most variation in almost the same amount of sampling units, with a trend for increasing their importance at sampling units with clustering results for ses.FI and NTI, respectively. The ses.D_{nn}' was the only metric which the resolution level accounted for most of the observed variation in several sampling units.

Discussion

We stand in a moment where advanced molecular techniques are being integrated in community phylogenetics, enabling the reconstruction of more robust inferences of phylogenetic trees. Our results demonstrate that correcting the workflow of phylogeny reconstruction by Phylocom and updating node clades estimates, enhances the similarity with Molecular phylogenies. Furthermore, different branch length estimates presented the highly significant effects within each type of phylogeny tested. In general, the importance of different

phylogeny types, branch length estimates and resolution levels varies between phylogenetic community metrics depending on the phylogenetic composition of sampling units.

The comparison of the "best-tree" results with the other reconstructed phylogenies revealed that BLADJ-Entire and Molecular-Undated phylogenies presented the highest correlations and the lowest type I and II errors. Kress et al. (2009) and Pei et al. (2011) also compared pseudo-chronograms reconstructed in Phylocom software with molecular trees and found that the lack of resolution of Phylocom trees lead to a type II error of finding non-significant phylogenetic structure. Apparently, Kress et al. (2009) used a Phylocom Pruned type tree, which can present different estimates of undated nodes by BLADJ algorithm compared to the Entire type trees, resulting in long branch lengths particularly close to the root, depending on the species clades composing the phylogeny and the species pool size. The Pruned type phylogenies of both Topological and BLADJ groups were the most dissimilar to the best-tree" and branch length estimates was the phylogenetic property that most explained results variation (Table 1), highlighting the difference between Pruned and Entire tree types. It is difficult to estimate how many studies may have inaccurately reconstructed phylogenies due to this problem because of the lack or simplified information about phylogenetic reconstruction in most manuscripts. The more accurate way of phylogenetic reconstruction obtained here, using Phylocom first (dating the tree) and then using Phylomatic to insert/prune species, requires extra work for correcting the non-ultrametric resulting tree. Future studies should seek a solution to this issue, implementing a routine that facilitates the reconstruction of Entire type phylogenies of large species pool by Phylocom/Phylomatic.

The split plot design ANOVAs results presented more significant and stronger effects of phylogenetic properties on diversity metrics compared to dispersion metrics, showing that in dispersion metrics randomizations probably attenuate the loss of statistical power (Swenson 2009). An interesting ANOVA result is the similarity of Bell and Wikström age estimates used

in BLADJ trees in the majority of tested metrics. This result stresses that, even though Wikström age estimates were calculated using a single fossil calibration point compared to the 36 used by Bell, their estimates were not significantly different. This similarity is dependent of which node age estimates of each study (Bell and Wikström) were selected by our criteria. The difference of Wikström's ages available with Phylocom software compared to our selection would probably show a significant strong difference in comparison to Bell's ages. This is because Gastauer and Meira-Neto (2013) showed that the corrections they made in some inconsistencies on the syntax and/or nomenclature between deeper nodes and the Wikström's ages file provided by Phylocom, created a significant difference in some phylogenetic community metrics they tested. Recently, Hawkins et al. (2014) proposed that Davies et al. (2004) age estimates were more reliable compared to Bell's ages by simply considering how much each age estimates matched the fossil record of their sampled families. The reliability of the most used age estimates is an issue that remains to be statistically tested.

The ANOVA results within the Molecular group showed no difference between Dated and Undated trees for phylogenetic dispersion metrics and significant differences for phylogenetic diversity metrics. The similarity of Undated and Dated molecular trees has been shown by Cadotte et al. (2008), which also found significant correlations with a BLADJ type tree in relation to a phylogenetic diversity metric similar to Faith's index (Faith 1992). However, the use of Undated Molecular trees needs some caution, mainly due to long branches in some species, created by poor quality sequence alignment, which will cause an extreme variation in root-to-tip distances (Pearse & Purvis 2013).

Another important factor in Molecular trees is the amount of missing data, which can generate dichotomous basal nodes with a branch of zero length due to the use of a constraint tree. The different resolution levels generated by whether a constraint tree is used at the family or genus level in the Molecular group, showed significant weak effects in a few metrics.

Nevertheless, the use of constraint trees to family or genus level has a great impact at tree topology and can introduce some bias if some relationships within families or genera are not well resolved. In our case, in the constraint tree to genus level we used the hypothesis of Myrteae tribe of Myrtaceae family following Lucas et al. (2007), while the phylogenies reconstructed using a family level constraint tree showed different genera relationships as suggested by Murillo-A et al. (2013). An alternative is to use a constraint tree to order level, since APG III (APG 2009) resolves clades relationships to this level, as done by Erickson et al. (2014) with families' relationships being resolved by molecular data.

The LME results showed that the effect of phylogenies properties (type of tree, branch length estimates and resolution level) is different among phylogenetic community metrics and heterogeneous among sampling units. These results show that, depending on the phylogenetic composition of sampling units, a certain phylogenetic property is more important and this prevents to find any general pattern of increasing or decreasing of metrics results when comparing the phylogenies. Evaluating the studies that compared molecular with Phylocom phylogenies, Molina-Venegas and Roquet (2014) found heterogeneous results when using pseudo-chronograms reconstructed with Wikström ages, depending on the tested metrics and the dataset size. Kress et al. (2009) also found heterogeneous results among sampling units (habitats) for NRI and NTI, and Pei et al. (2011) found the same pattern for NTI, but a general trend of higher NRI values in molecular phylogenies. The LME results showed that the resolution level is the most important step of decision when calculating ses.D_{nn}' and it shares the importance with branch length estimates when calculating NTI, both metrics sensitive to variation in terminal nodes. Molina-Venegas and Roquet (2014) proposed that resolving terminal nodes based on published phylogenies does not necessarily improve the accuracy of phylogenetic structure metrics. Our results showed that resolving terminal nodes had different effects depending on the phylogenetic metric (Table 1), generally enhancing the similarity of

BLADJ-Entire trees with Molecular phylogenies (Figure 2 and Supplementary material Appendix 5). Swenson (2009) showed that polytomies at deeper nodes substantially reduced the statistical power to detect non-random phylogenetic community structure, while Molina-Venegas and Roquet (2014) demonstrated that the 'stemminess' of branch lengths close to the root also creates the same statistical pattern. Our phylogenies do not have polytomies in basal nodes, but BLADJ-Pruned trees presented some long branch lengths close to the root compared to BLADJ-Entire trees, showing that not only the 'stemminess' close to the root affects phylogenetic community metrics results, but also the long branches can have a negative effect. During the development of phylogenetic community ecology, studies relied heavily on an understanding of the phylogenetic hypothesis for plants that is still under development, specially between and within large plant clades. Specifically, many studies relied on phylogenetic hypothesis with a large amount of polytomies at deeper nodes (e.g. APG 2003; Davies et al. 2004) and even the APG III (APG 2009) hypothesis presents some degree of uncertainty. In the last few years new phylogenetic hypotheses have been published (Smith et al. 2011; Soltis et al. 2011; Zanne et al. 2014) resolving polytomies at different taxonomic levels, but their distinct proposals of clades relationships (e.g. monocots position) and age estimates highlight the need for a consolidated hypothesis, unifying this new information available.

Phylogenetic reconstruction using molecular techniques is a promising approach for ecophylogenetics that will help the standardization of species relationships for ecological inference. However, the reconstruction of molecular phylogenies still has some limitations to be overcome. One of the basic issues is the deficiency of genetic sequences especially for Neotropical plant species, which will not appear in global databases without a refocused effort on field botany and systematics. The quantity of missing data directly influences the reconstruction of a reliable phylogeny (Roquet et al. 2013). Obtaining sequence data for all

species of a community from GenBank is probably not possible, but it does not preclude the reconstruction of a molecular phylogeny with a genus level resolution that likely will produce robust inferences. Further, an interesting issue pointed by Erickson et al. (2014) is that large molecular phylogenies improve estimates of branch length among species, compared to using smaller phylogenies containing only the species presented in the community being evaluated, further reinforcing the need for large well-sampled molecular phylogenies. Certainly, with more accessible technology and cost-effective gene sequencing, in a short amount of time molecular phylogenies will be a common reality for ecologists.

Lastly, it is important to note that in order to facilitate the use of phylogenetic information in ecology we must have methods and software that is accessible to ecologists and reliable when a collaboration with a molecular phylogeneticist is not possible. The simple approach that Phylocom provides for tree reconstruction has been a tremendous service to ecology and recent tools like phyloGenerator are also great improvements, but there are more ways for an ecologist to unknowingly make large important errors along the way. Importantly, our results show that when Phylocom phylogenies are reconstructed with care, the generated pseudo-chronograms can yield the same results as molecular phylogenies in some phylogenetic community metrics. Even with the limitations mentioned, we believe that the reconstruction of well resolved and molecular phylogenies is a necessary and important task for the development of community phylogenetics.

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Table 1: Split plot design ANOVAs results (regression coefficients) of alpha and beta phylogenetic diversity/dispersion metrics; **BL** – Branch Length estimates; **Res** – Resolution level; ***P* ≤ 0.05 and > 0.01; ****P* ≤ 0.01. Bold values are statistically significant. For details of alpha and beta phylogenetic community metrics see Material and Methods. For details of permutation tests see Supplementary material Appendix 4.

Tree groups	Factors	MPD	NRI	MNTD	NTI	FI	ses.FI	D_{pw}'	ses.D_{pw}'	D_{nn}'	ses.D_{nn}'
	BL	**0.81****	**0.36****	**0.73****	0.06	**0.47****	<0.01	**0.88****	**0.6****	**0.11****	**0.03****
Topological	**Res**	**0.03****	<0.01	**<0.01****	<0.01	**0.08****	**0.03****	**0.04****	**<0.01****	**0.01****	<0.01
	BL x Res	**0.01***	<0.01	<0.01	**0.02****	<0.01	<0.01	**0.01****	<0.01	**<0.01***	**<0.01****
	Age	0.01	<0.01	<0.01	<0.01	0.02	<0.01	**0.01****	<0.01	<0.01	<0.01
	BL	**0.71****	0.04	**0.26****	**0.1****	**0.46****	**0.19****	**0.84****	**0.03****	**0.09****	**0.02****
	Age x BL	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
BLADJ	**Res**	<0.01	<0.01	<0.01	**0.03****	<0.01	**0.04****	**<0.01****	**<0.01****	**<0.01****	<0.01
	Age x Res	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	**<0.01***	**<0.01****	**<0.01****	**<0.01***
	BL x Res	**<0.01****	<0.01	**0.12****	<0.01	**0.06****	**0.01****	<0.01	<0.01	**<0.01****	**<0.01****
	Age x BL x Res	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	**<0.01***	<0.01	<0.01	<0.01
	BL	**0.94****	0.03	**0.65****	0.02	**0.81****	<0.01	**0.97****	<0.01	**0.29****	<0.01
Molecular	**Res**	**<0.01****	<0.01	<0.01	<0.01	**<0.01****	<0.01	**<0.01****	**<0.01****	**<0.01****	<0.01
	BL x Res	**<0.01****	<0.01	<0.01	<0.01	<0.01	<0.01	**<0.01****	**<0.01****	<0.01	<0.01
"Best-trees"	**Tree**	**0.92****	**0.13****	**0.73****	**0.02***	**0.83****	**0.04****	**0.97****	**0.28****	**0.32****	**<0.01****

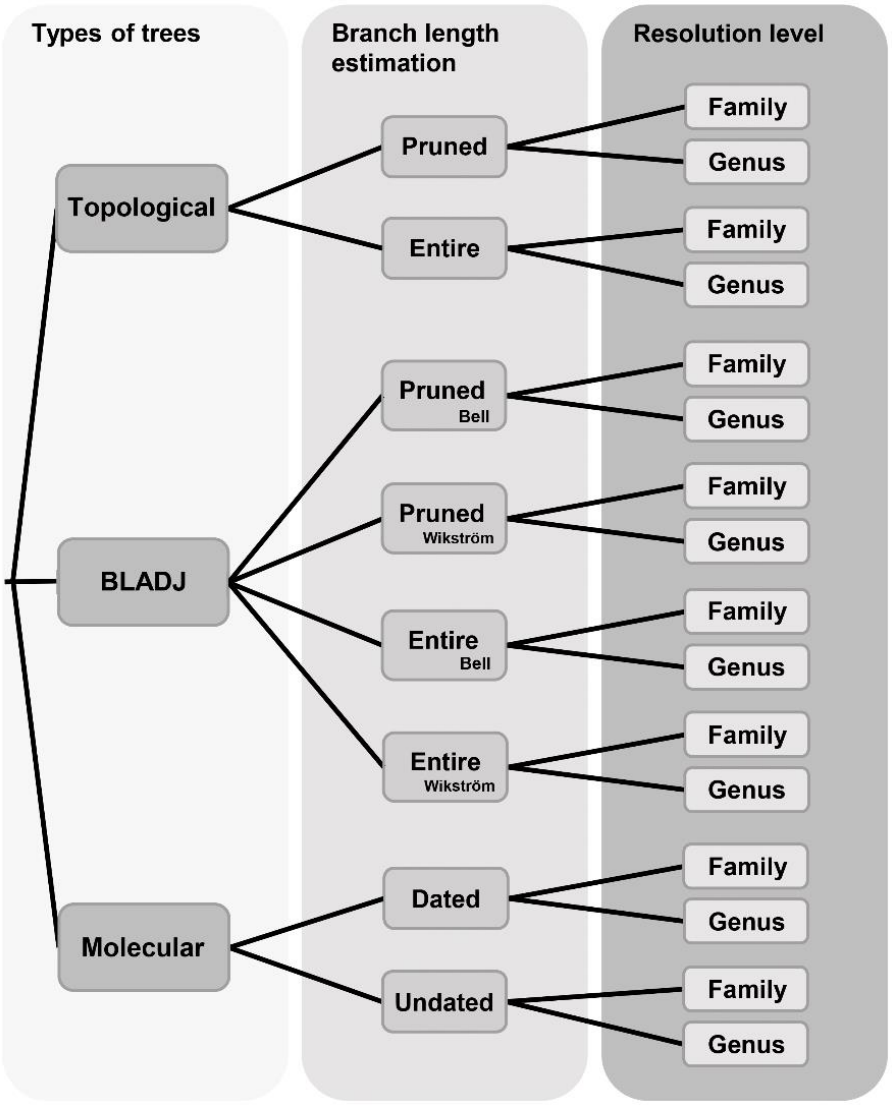

Figure 1: Scheme illustrating all 16 phylogenetic trees reconstructed and evaluated in this study, with their respective properties and levels.

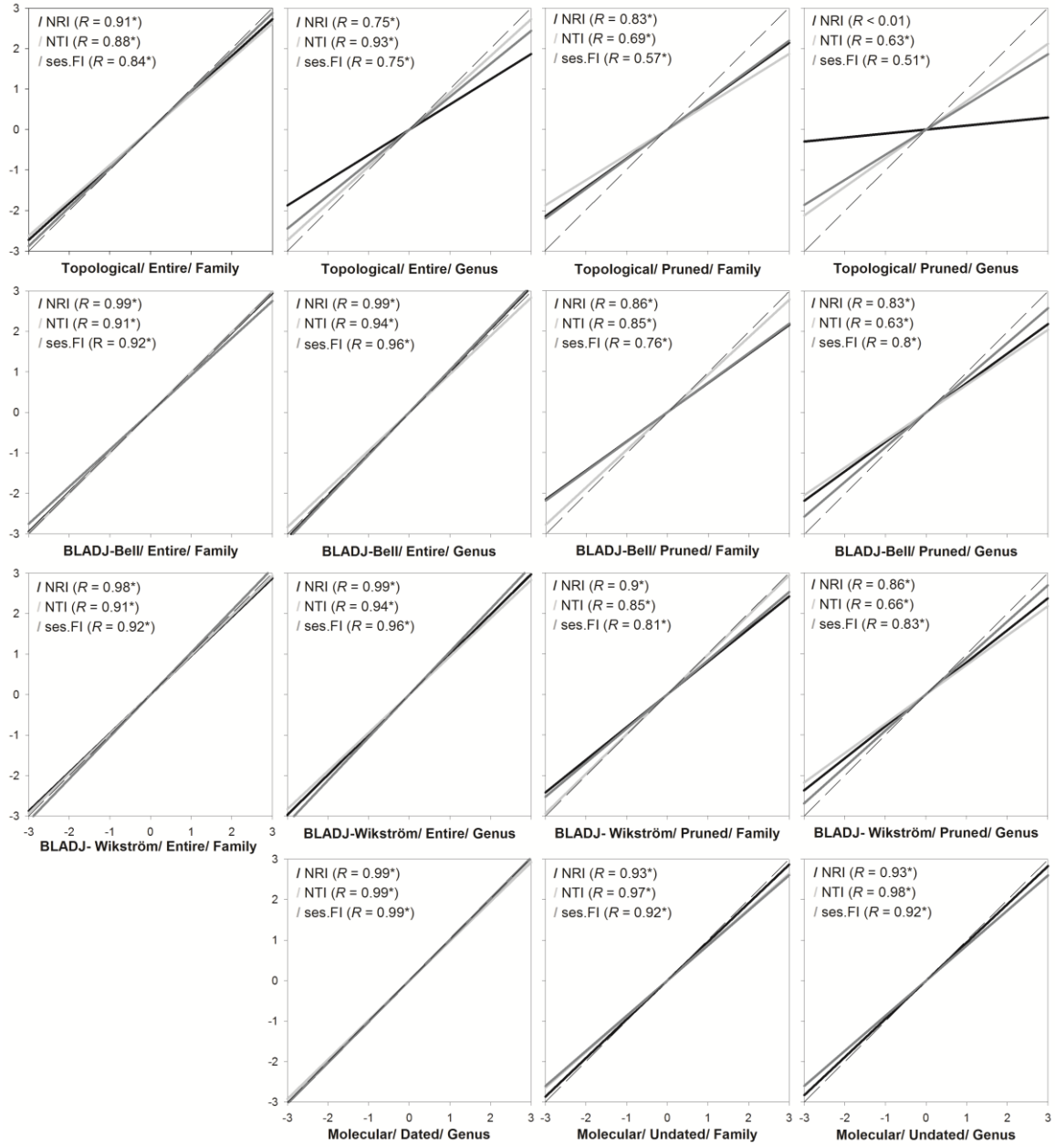

Figure 2: Simple linear regressions of phylogenetic alpha dispersion results between the molecular dated phylogeny with family constraint resolution ("best-tree") and all other reconstructed phylogenies. Regression lines were forced to cross the origin through a data centering. Dashed lines represent the expected relationship of a perfect 1:1 correlation (slope = 1) with the "best-tree". Regression lines over or under the dashed line (slopes > 1 or < 1) represent false positives or false negatives results (type I or II error). Positive/negative values of phylogenetic diversity metrics represent phylogenetic clustering/overdispersion. *$P \leq 0.05$. For the slope values of regression lines see the Supplementary material Appendix 6.

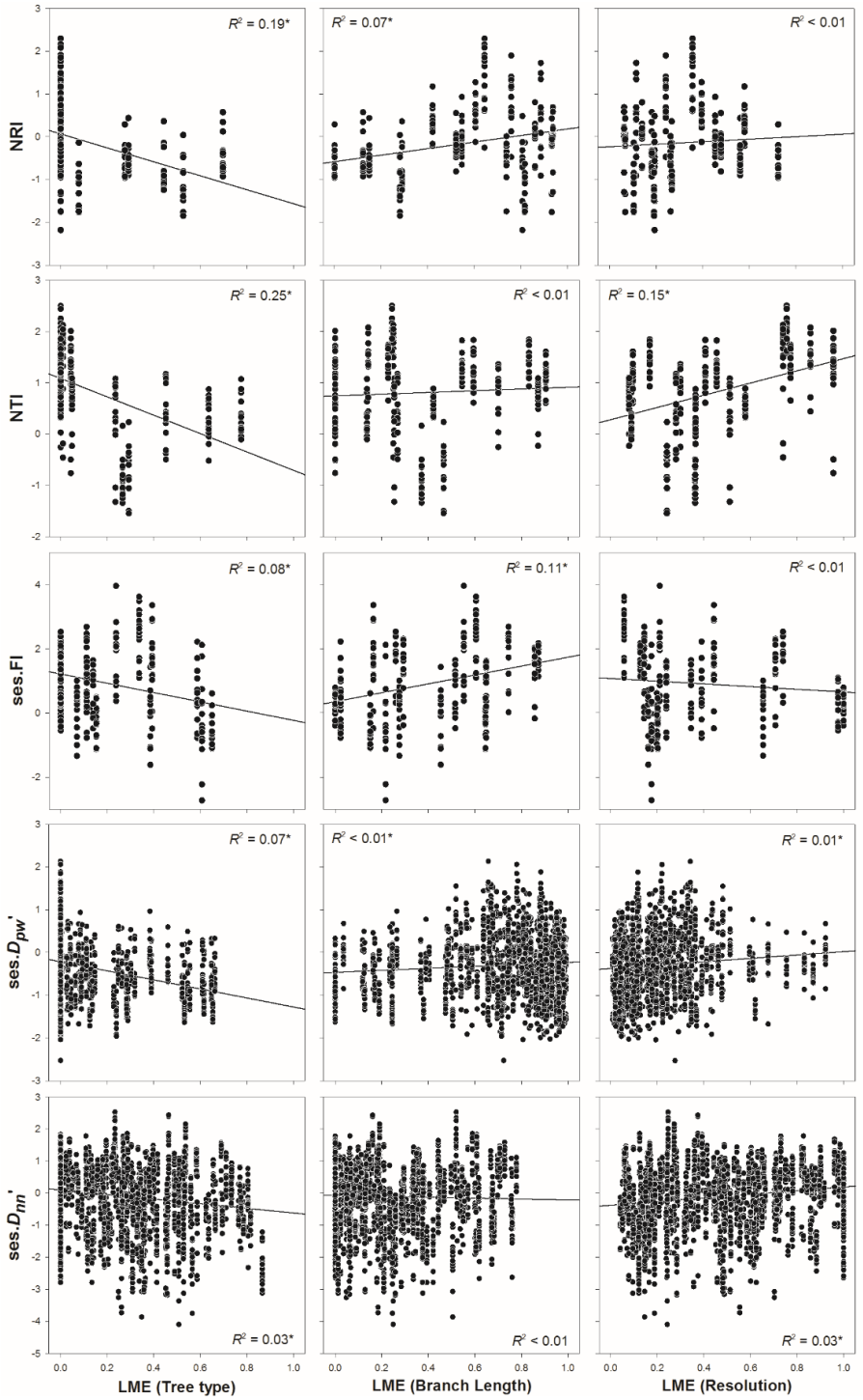

Figure 3: Scatter plots of a linear regression between the Linear Mixed-Effect models results (regression coefficients) of all trees for each tested factor (type of tree, branch length estimation and resolution level) and their respective phylogenetic alpha and beta dispersion results. Positive/negative values of phylogenetic diversity metrics represent phylogenetic clustering/overdispersion. *$P \leq 0.01$.

Appendix 1. The 16 different types of phylogenetic trees compared in this study. Molecular tree types also contain bootstrap support values as node names.

Topological/ Entire/ Family level

((annona_rugulosa:7,(hennecartia_omphalandra:3,((cinnamomum_amoenum:1,cinnamomum_glaziovii:1)cinnamomum:1,cryptocarya_aschersoniana:2,nectandra_megapotamica:2,(ocotea_elegans:1,ocotea_porosa:1,ocotea_puberula:1,ocotea_pulchella:1)ocotea:1)lauraceae:2):3):3,(roupala_montana:5,((((prunus_myrtifolia:5,inga_virescens:5)nitrogen_fixing_clade:1,(maytenus_evonymoides:4,((lamanonia_ternata:2,weinmannia_paulliniifolia:2)cunoniaceae:4,((banara_parviflora:2,(casearia_decandra:1,casearia_obliqua:1)casearia:1,xylosma_pseudosalzmannii:2)salicaceae:4,(sapium_glandulosum:2,(sebastiania_brasiliensis:1,sebastiania_commersoniana:1)sebastiania:1,stillingia_oppositifolia:2)euphorbiaceae:2)malpighiales:1):1):1):2,((luehea_divaricata:8,(lithrea_brasiliensis:5,((allophylus_edulis:2,cupania_vernalis:2,matayba_elaeagnoides:2)sapindaceae:1,((cedrela_fissilis:2,trichilia_elegans:2)meliaceae:1,(pilocarpus_pennatifolius:2,(zanthoxylum_astrigerum:1,zanthoxylum_kleinii:1,zanthoxylum_rhoifolium:1)zanthoxylum:1)rutaceae:1):1):1):3):3,((blepharocalyx_salicifolius:2,calyptranthes_concinna:2,(campomanesia_rhombea:1,campomanesia_xanthocarpa:1)campomanesia:1,(eugenia_involucrata:1,eugenia_oeidocarpa:1,eugenia_subterminalis:1,eugenia_uruguayensis:1)eugenia:1,(myrceugenia_cucullata:1,myrceugenia_foveolata:1,myrceugenia_mesomischa:1,myrceugenia_miersiana:1,myrceugenia_myrcioides:1,myrceugenia_oxysepala:1)myrceugenia:1,(myrcia_lajeana:1,myrcia_oligantha:1)myrcia:1,(myrcianthes_gigantea:1,myrcianthes_pungens:1)myrcianthes:1,(myrciaria_delicatula:1,myrciaria_floribunda:1)myrciaria:1,myrrhinium_atropurpureum:2,siphoneugena_reitzii:2)myrtaceae:2,miconia_cinerascens:4):4)malvidae:1):3,(((myrsine_coriacea:1,myrsine_lorentziana:1)myrsine:4,(laplacea_acutifolia:3,(symplocos_uniflora:1,symplocos_tetrandra:1)symplocos:3):1):3,(((coutarea_hexandra:2,rudgea_parquioides:2)rubiaceae:2,(duranta_vestita:13,(brunfelsia_cuneifolia:2,solanum_sanctaecatharinae:2)solanaceae:3):1):2,((citronella_gongonha:4,(ilex_brevicuspis:1,ilex_dumosa:1,ilex_microdonta:1,ilex_paraguariensis:1)aquifoliaceae:3)aquifoliales:1,(baccharis_dentata:2,dasyphyllum_spinescens:2,vernonanthura_discolor:2)asteraceae:7)campanulidae:1)lamiidae_and_campanulidae:1)ericales_and_others:5)pentapetalae:3):4);

Topological/ Entire/ Genus level

((annona_rugulosa:17,(hennecartia_omphalandra:7,(cryptocarya_aschersoniana:5,((cinnamomum_amoenum:1,cinnamomum_glaziovii:1)cinnamomum:4,((ocotea_elegans:1,ocotea_porosa:1,ocotea_puberula:1,ocotea_pulchella:1)ocotea:1,nectandra_megapotamica:4):2):9):3):3):3,(roupala_montana:11,((((prunus_myrtifolia:10,inga_virescens:28)nitrogen_fixing_clade:1,(maytenus_evonymoides:14,((lamanonia_ternata:3,weinmannia_paulliniifolia:6):7,((banara_parviflora:2,(casearia_decandra:1,casearia_obliqua:1)casearia:1,xylosma_pseudosalzmannii:2)salicaceae:4,((stillingia_oppositifolia:2,sapium_glandulosum:2):2,(sebastiania_brasiliensis:1,sebastiania_commersoniana:1)sebastiania:4):12)malpighiales:1):1):1):2,((luehea_divaricata:12,(lithrea_brasiliensis:5,(((matayba_elaeagnoides:3,cupania_vernalis:3):9,allophylus_edulis:11):8,((cedrela_fissilis:7,trichilia_elegans:9)meliaceae:1,(pilocarpus_pennatifolius:2,(zanthoxylum_astrigerum:1,zanthoxylum_kleinii:1,zanthoxylum_rhoifolium:1)zanthoxylum:1)rutaceae:1):1):1):3):3,((((((siphoneugena_reitzii:4,(myrciaria_delicatula:1,myrciaria_floribunda:1)myrciaria:1)pliniagroup:2,(calyptranthes_concinna:3,(myrcia_lajeana:1,myrcia_oligantha:1)myrcia:1)myrciagroup:1):1,(myrceugenia_cucullata:1,myrceugenia_foveolata:1,myrceugenia_mesomischa:1,myrceugenia_miersiana:1,myrceugenia_myrcioides:1,myrceugenia_oxysepala:1)myrceugenia:2):1,(((campomanesia_rhombea:1,campomanesia_xanthocarpa:1)campomanesia:2,myrrhinium_atropurpureum:1):3,((eugenia_involucrata:1,eugenia_oeidocarpa:1,eugenia_subterminalis:1,eugenia_uruguayensis:1)eugenia:1,(myrcianthes_gigantea:1,myrcianthes_pungens:1)myrcianthes:1)eugeniagroup:1):2):1,blepharocalyx_salicifolius:1):9,miconia_cinerascens:17):4)malvidae:1):3,((myrsine_coriacea:1,myrsine_lorentziana:1)myrsine:14,(laplacea_acutifolia:3,(symplocos_uniflora:1,symplocos_tetrandra:1)symplocos:3):1):3,(((rudgea_parquioides:14,coutarea_hexandra:10)rubiaceae:2,(duranta_vestita:16,(solanum_sanctaecatharinae:11,brunfelsia_cuneifolia:4):5):1):2,((citronella_gongonha:4,(ilex_brevicuspis:1,ilex_dumosa:1,ilex_microdonta:1,ilex_paraguariensis:1)aquifoliaceae:3)aquifoliales:1,(dasyphyllum_spinescens:6,(

vernonanthura_discolor:16,baccharis_dentata:19):8)asteraceae:7)campanulidae:1)lamiidae_and_campanulidae:1)ericales_and_others:5)pentapetalae:3):4);

Topological/ Pruned/ Family level

(((((((lithrea_brasiliensis:1,(((cedrela_fissilis:1,trichilia_elegans:1)meliaceae:1,(pilocarpus_pennatifolius:1,(zanthoxylum_astrigerum:1,zanthoxylum_kleinii:1,zanthoxylum_rhoifolium:1)zanthoxylum:1)rutaceae:1):1,(allophylus_edulis:1,cupania_vernalis:1,matayba_elaeagnoides:1)sapindaceae:1):1):1,luehea_divaricata:1):1,(miconia_cinerascens:1,(blepharocalyx_salicifolius:1,calyptranthes_concinna:1,(campomanesia_rhombea:1,campomanesia_xanthocarpa:1)campomanesia:1,(eugenia_involucrata:1,eugenia_oeidocarpa:1,eugenia_subterminalis:1,eugenia_uruguayensis:1)eugenia:1,(myrceugenia_cucullata:1,myrceugenia_foveolata:1,myrceugenia_mesomischa:1,myrceugenia_miersiana:1,myrceugenia_myrcioides:1,myrceugenia_oxysepala:1)myrceugenia:1,(myrcia_lajeana:1,myrcia_oligantha:1)myrcia:1,(myrcianthes_gigantea:1,myrcianthes_pungens:1)myrcianthes:1,(myrciaria_delicatula:1,myrciaria_floribunda:1)myrciaria:1,myrrhinium_atropurpureum:1,siphoneugena_reitzii:1)myrtaceae:1):1)malvidae:1,((maytenus_evonymoides:1,((lamanonia_ternata:1,weinmannia_paulliniifolia:1)cunoniaceae:1,((sapium_glandulosum:1,(sebastiania_brasiliensis:1,sebastiania_commersoniana:1)sebastiania:1,stillingia_oppositifolia:1)euphorbiaceae:1,(banara_parviflora:1,(casearia_decandra:1,casearia_obliqua:1)casearia:1,xylosma_pseudosalzmannii:1)salicaceae:1)malpighiales:1):1):1,(inga_virescens:1,prunus_myrtifolia:1)nitrogen_fixing_clade:1):1):1,(((((ilex_brevicuspis:1,ilex_dumosa:1,ilex_microdonta:1,ilex_paraguariensis:1)ilex:1,citronella_gongonha:1)aquifoliales:1,(baccharis_dentata:1,dasyphyllum_spinescens:1,vernonanthura_discolor:1)asteraceae:1)campanulidae:1,((coutarea_hexandra:1,rudgea_parquioides:1)rubiaceae:1,((brunfelsia_cuneifolia:1,solanum_sanctaecatharinae:1)solanaceae:1,duranta_vestita:1):1):1)lamiidae_and_campanulidae:1,((myrsine_coriacea:1,myrsine_lorentziana:1)myrsine:1,((symplocos_tetrandra:1,symplocos_uniflora:1)symplocos:1,laplacea_acutifolia:1):1):1)ericales_and_others:1)pentapetalae:1,roupala_montana:1):1,(annona_rugulosa:1,(((cinnamomum_amoenum:1,cinnamomum_glaziovii:1)cinnamomum:1,cryptocarya_aschersoniana:1,nectandra_megapotamica:1,(ocotea_elegans:1,ocotea_porosa:1,ocotea_puberula:1,ocotea_pulchella:1)ocotea:1)lauraceae:1,hennecartia_omphalandra:1):1):1):1;

Topological/ Pruned/ Genus level

(((((((lithrea_brasiliensis:1,(((cedrela_fissilis:1,trichilia_elegans:1)meliaceae:1,(pilocarpus_pennatifolius:1,(zanthoxylum_astrigerum:1,zanthoxylum_kleinii:1,zanthoxylum_rhoifolium:1)zanthoxylum:1)rutaceae:1):1,(allophylus_edulis:1,(cupania_vernalis:1,matayba_elaeagnoides:1):1):1):1):1,luehea_divaricata:1):1,(miconia_cinerascens:1,(blepharocalyx_salicifolius:1,((((calyptranthes_concinna:1,(myrcia_lajeana:1,myrcia_oligantha:1)myrcia:1)myrciagroup:1,((myrciaria_delicatula:1,myrciaria_floribunda:1)myrciaria:1,siphoneugena_reitzii:1)pliniagroup:1):1,(myrceugenia_cucullata:1,myrceugenia_foveolata:1,myrceugenia_mesomischa:1,myrceugenia_miersiana:1,myrceugenia_myrcioides:1,myrceugenia_oxysepala:1)myrceugenia:1):1,(((campomanesia_rhombea:1,campomanesia_xanthocarpa:1)campomanesia:1,myrrhinium_atropurpureum:1):1,((eugenia_involucrata:1,eugenia_oeidocarpa:1,eugenia_subterminalis:1,eugenia_uruguayensis:1)eugenia:1,(myrcianthes_gigantea:1,myrcianthes_pungens:1)myrcianthes:1)eugeniagroup:1):1):1):1):1)malvidae:1,((maytenus_evonymoides:1,((lamanonia_ternata:1,weinmannia_paulliniifolia:1):1,(((sapium_glandulosum:1,stillingia_oppositifolia:1):1,(sebastiania_brasiliensis:1,sebastiania_commersoniana:1)sebastiania:1):1,(banara_parviflora:1,(casearia_decandra:1,casearia_obliqua:1)casearia:1,xylosma_pseudosalzmannii:1)salicaceae:1)malpighiales:1):1):1,(inga_virescens:1,prunus_myrtifolia:1)nitrogen_fixing_clade:1):1):1,(((((ilex_brevicuspis:1,ilex_dumosa:1,ilex_microdonta:1,ilex_paraguariensis:1)ilex:1,citronella_gongonha:1)aquifoliales:1,((baccharis_dentata:1,vernonanthura_discolor:1):1,dasyphyllum_spinescens:1)asteraceae:1)campanulidae:1,((coutarea_hexandra:1,rudgea_parquioides:1)rubiaceae:1,((brunfelsia_cuneifolia:1,solanum_sanctaecatharinae:1):1,duranta_vestita:1):1):1)lamiidae_and_campanulidae:1,((myrsine_coriacea:1,myrsine_lorentziana:1)myrsine:1,((symplocos_tetrandra:1,symplocos_uniflora:1)symplocos:1,laplacea_acutifolia:1):1):1)ericales_and_others:1)pentapetalae:1,roupala_montana:1):1,(annona_rugulosa:1,((((cinnamomum_amoenum:1,cinnamomum_glaziovii:1)cinnamomum:1,(nectandra_megapotamica:1,(ocotea_elegans:1,ocotea_porosa:1,ocotea_puberula:1,ocotea_pulchella:1)ocotea:1):1):1,cryptocarya_aschersoniana:1):1,hennecartia_omphalandra:1):1):1):244.12001;

BLADJ/ Entire – Bell ages/ Family level

(((((((lithrea_brasiliensis:67.5,(((cedrela_fissilis:38.0,trichilia_elegans:38.0)meliaceae:7.5,(pilocarpus_pennatifolius:40.0,(zanthoxylum_astrigerum:20.0,zanthoxylum_kleinii:20.0,zanthoxylum_rhoifolium:20.0)zanthoxylum:20.0)rutaceae:5.5):5.5,(allophylus_edulis:42.0,cupania_vernalis:42.0,matayba_elaeagnoides:42.0)sapindaceae:9.)bell0929:16.5):24.699997,luehea_divaricata:92.2):12.466667,(miconia_cinerascens:75.666667,(blepharocalyx_salicifolius:46.0,calyptranthes_concinna:46.0,(campomanesia_rhombea:23.0,campomanesia_xanthocarpa:23.0)campomanesia:23.0,(eugenia_involucrata:23.0,eugenia_oeidocarpa:23.0,eugenia_subterminalis:23.0,eugenia_uruguayensis:23.0)eugenia:23.0,(myrceugenia_cucullata:23.0,myrceugenia_foveolata:23.0,myrceugenia_mesomischa:23.0,myrceugenia_miersiana:23.0,myrceugenia_myrcioides:23.0,myrceugenia_oxysepala:23.0)myrceugenia:23.0,(myrcia_lajeana:23.0,myrcia_oligantha:23.0)myrcia:23.0,(myrcianthes_gigantea:23.0,myrcianthes_pungens:23.0)myrcianthes:23.0,(myrciaria_delicatula:23.0,myrciaria_floribunda:23.0)myrciaria:23.0,myrrhinium_atropurpureum:46.0,siphoneugena_reitzii:46.0)myrtaceae:29.666664):29.0)malvidae:5.833336,((maytenus_evonymoides:100.0,((lamanonia_ternata:29.0,weinmannia_paulliniifolia:29.0)cunoniaceae:68.0,((sapium_glandulosum:30.666666,(sebastiania_brasiliensis:15.333334,sebastiania_commersoniana:15.333334)sebastiania:15.333334,stillingia_oppositifolia:30.666668)euphorbiaceae:61.333332,(banara_parviflora:63.0,(casearia_decandra:31.5,casearia_obliqua:31.5)casearia:31.5,xylosma_pseudosalzmannii:63.0)salicaceae:29.0)malpighiales:5.0):3.0):3.0,(inga_virescens:99.0,prunus_myrtifolia:99.0)nitrogen_fixing_clade:4.0):7.50):10.5,(((((ilex_brevicuspis:22.5,ilex_dumosa:22.5,ilex_microdonta:22.5,ilex_paraguariensis:22.5)ilex:64.5,citronella_gongonha:87.0)aquifoliales:13.0,(baccharis_dentata:43.0,dasyphyllum_spinescens:43.0,vernonanthura_discolor:43.0)asteraceae:57.0)campanulidae:8.0,((coutarea_hexandra:57.0,rudgea_parquioides:57.0)rubiaceae:37.666664,((brunfelsia_cuneifolia:38.0,solanum_sanctaecatharinae:38.0)solanaceae:47.333336,duranta_vestita:85.333336):9.333328):13.333333)lamiidae_and_campanulidae:2.0,((myrsine_coriacea:61.0,myrsine_lorentziana:61.0)myrsine:12.75,((symplocos_tetrandra:20.194445,symplocos_uniflora:20.194445)symplocos:46.972221,laplacea_acutifolia:67.166664):6.583336):36.25)ericales_and_others:11.0)pentapetalae:7.333328,roupala_montana:128.333328):39.00002,(annona_rugulosa:120.5,(((cinnamomum_amoenum:6.5,cinnamomum_glaziovii:6.5)cinnamomum:6.5,cryptocarya_aschersoniana:13.0,nectandra_megapotamica:13.0,(ocotea_elegans:6.5,ocotea_porosa:6.5,ocotea_puberula:6.5,ocotea_pulchella:6.5)ocotea:6.5)lauraceae:54.0,hennecartia_omphalandra:67.0):53.50):46.833349):243.586666;

BLADJ/ Entire – Bell ages/ Genus level

(((((((lithrea_brasiliensis:67.5,(((cedrela_fissilis:38.0,trichilia_elegans:38.0)meliaceae:7.5,(pilocarpus_pennatifolius:40.0,(zanthoxylum_astrigerum:20.0,zanthoxylum_kleinii:20.0,zanthoxylum_rhoifolium:20.0)zanthoxylum:20.0)rutaceae:5.5):5.5,(allophylus_edulis:26.526316,(cupania_vernalis:5.157894,matayba_elaeagnoides:5.157894):21.368422):24.473686)bell0929:16.5):24.699997,luehea_divaricata:92.199997):12.466667,(miconia_cinerascens:87.5,(blepharocalyx_salicifolius:27.2,((((calyptranthes_concinna:12.75,(myrcia_lajeana:6.375,myrcia_oligantha:6.375)myrcia:6.375)myrciagroup:4.25,((myrciaria_delicatula:5.1,myrciaria_floribunda:5.1)myrciaria:5.1,siphoneugena_reitzii:10.2)pliniagroup:6.8):3.4,(myrceugenia_cucullata:6.8,myrceugenia_foveolata:6.8,myrceugenia_mesomischa:6.8,myrceugenia_miersiana:6.8,myrceugenia_myrcioides:6.8,myrceugenia_oxysepala:6.8)myrceugenia:13.6):3.4,(((campomanesia_rhombea:1.7,campomanesia_xanthocarpa:1.7)campomanesia:5.1,myrrhinium_atropurpureum:6.8):10.2,((eugenia_involucrata:4.25,eugenia_oeidocarpa:4.25,eugenia_subterminalis:4.25,eugenia_uruguayensis:4.25)eugenia:4.25,(myrcianthes_gigantea:4.25,myrcianthes_pungens:4.25)myrcianthes:4.25)eugeniagroup:8.5):6.8):3.400002):60.299999):17.166664)malvidae:5.833336,((maytenus_evonymoides:100.0,((lamanonia_ternata:19.333334,weinmannia_paulliniifolia:19.333334):77.666666,(((sapium_glandulosum:12.321428,stillingia_oppositifolia:12.321428):20.535715,(sebastiania_brasiliensis:6.571429,sebastiania_commersoniana:6.571455)sebastiania:26.285715):59.142857,(banara_parviflora:63.0,(casearia_decandra:31.5,casearia_obliqua:31.5)casearia:31.5,xylosma_pseudosalzmannii:63.0)salicaceae:29.0)malpighiales:5.0):3.0):3.0,(inga_virescens:99.0,prunus_myrtifolia:99.0)nitrogen_fixing_clade:4.0):7.5):10.5,(((((ilex_brevicuspis:22.5,ilex_dumosa:22.5,ilex_microdonta:22.5,ilex_paraguariensis:22.5)ilex:64.5,citronella_gongonha:87.0)aquifoliales:13.0,((baccharis_dentata:32.882355,vernonanthura_discolor:32.882355):10.117648,dasyphyllum_spinescens:43.0)asteraceae:57.0)campanulidae:8.0,((coutarea_hexandra:57.0,rudgea_parquioides:57.0)rubiaceae:37.666666,((brunfelsia_c

uneifolia:34.2,solanum_sanctaecatharinae:34.2):51.133387,duranta_vestita:85.333387):9.333328):13.333333)lamiidae_and_campanulidae:2.0,((myrsine_coriacea:3.683366,myrsine_lorentziana:3.683366)myrsine:77.716667,((symplocos_tetrandra:15.783378,symplocos_uniflora:15.783378)symplocos:56.483337,laplacea_acutifolia:72.26668):9.133331):28.599995)ericales_and_others:11.0)pentapetalae:7.333328,roupala_montana:128.333328):38.999979,(annona_rugulosa:120.5,((((cinnamomum_amoenum:3.353594,cinnamomum_glaziovii:3.353620)cinnamomum:23.475159,(nectandra_megapotamica:22.701250,(ocotea_elegans:11.350669,ocotea_porosa:11.350669,ocotea_puberula:11.350669,ocotea_pulchella:11.350669)ocotea:11.350625):4.127500):48.783627,cryptocarya_aschersoniana:75.612381):24.054283,hennecartia_omphalandra:99.666664):20.833340):46.833311):243.586666;

BLADJ/ Pruned – Bell ages/ Family level

(((((((lithrea_brasiliensis:66.666664,(((cedrela_fissilis:38.0,trichilia_elegans:38.0)meliaceae:7.5,(pilocarpus_pennatifolius:40.0,(zanthoxylum_astrigerum:20.0,zanthoxylum_kleinii:20.0,zanthoxylum_rhoifolium:20.0)zanthoxylum:20.0)rutaceae:5.5):5.5,(allophylus_edulis:42.0,cupania_vernalis:42.0,matayba_elaeagnoides:42.0)sapindaceae:9.0)bell0929:15.666664):15.666664,luehea_divaricata:82.333328):15.666672,(miconia_cinerascens:73.5,(blepharocalyx_salicifolius:49.0,calyptranthes_concinna:49.0,(campomanesia_rhombea:24.5,campomanesia_xanthocarpa:24.5)campomanesia:24.5,(eugenia_involucrata:24.5,eugenia_oeidocarpa:24.5,eugenia_subterminalis:24.5,eugenia_uruguayensis:24.5)eugenia:24.5,(myrceugenia_cucullata:24.50,myrceugenia_foveolata:24.5,myrceugenia_mesomischa:24.5,myrceugenia_miersiana:24.5,myrceugenia_myrcioides:24.5,myrceugenia_oxysepala:24.5)myrceugenia:24.5,(myrcia_lajeana:24.5,myrcia_oligantha:24.5)myrcia:24.5,(myrcianthes_gigantea:24.5,myrcianthes_pungens:24.5)myrcianthes:24.5,(myrciaria_delicatula:24.5,myrciaria_floribunda:24.5)myrciaria:24.5,myrrhinium_atropurpureum:49.0,siphoneugena_reitzii:49.0)myrtaceae:24.5):24.5)malvidae:15.666664,((maytenus_evonymoides:101.555557,((lamanonia_ternata:29.0,weinmannia_paulliniifolia:29.0)cunoniaceae:67.777779,((sapium_glandulosum:61.333332,(sebastiania_brasiliensis:30.666666,sebastiania_commersoniana:30.666666)sebastiania:30.666666,stillingia_oppositifolia:61.333332)euphorbiaceae:30.666666,(banara_parviflora:63.0,(casearia_decandra:31.5,casearia_obliqua:31.5)casearia:31.5,xylosma_pseudosalzmannii:63.0)salicaceae:29.0)malpighiales:4.777779):4.777779):4.777779,(inga_virescens:99.0,prunus_myrtifolia:99.0)nitrogen_fixing_clade:7.333336):7.333328):7.333333,(((((ilex_brevicuspis:43.5,ilex_dumosa:43.5,ilex_microdonta:43.5,ilex_paraguariensis:43.5)ilex:43.5,citronella_gongonha:87.0)aquifoliales:13.0,(baccharis_dentata:43.0,dasyphyllum_spinescens:43.0,vernonanthura_discolor:43.0)asteraceae:57.0)campanulidae:8.0,((coutarea_hexandra:57.0,rudgea_parquioides:57.0)rubiaceae:25.5,((brunfelsia_cuneifolia:38.0,solanum_sanctaecatharinae:38.0)solanaceae:22.25,duranta_vestita:60.25):22.25):25.5)lamiidae_and_campanulidae:2.0,((myrsine_coriacea:41.25,myrsine_lorentziana:41.25)myrsine:41.25,((symplocos_tetrandra:27.5,symplocos_uniflora:27.5)symplocos:27.5,laplacea_acutifolia:55.0):27.5):27.5)ericales_and_others:11.0)pentapetalae:96.639999,roupala_montana:217.639999):96.639999,(annona_rugulosa:213.853333,(((cinnamomum_amoenum:6.5,cinnamomum_glaziovii:6.5)cinnamomum:6.5,cryptocarya_aschersoniana:13.0,nectandra_megapotamica:13.0,(ocotea_elegans:6.5,ocotea_porosa:6.5,ocotea_puberula:6.5,ocotea_pulchella:6.5)ocotea:6.5)lauraceae:100.426666,hennecartia_omphalandra:113.426666):100.426666):100.426666):96.640007;

BLADJ/ Pruned – Bell ages/ Genus level

(((((((lithrea_brasiliensis:66.666664,(((cedrela_fissilis:38.0,trichilia_elegans:38.0)meliaceae:7.5,(pilocarpus_pennatifolius:40.0,(zanthoxylum_astrigerum:20.0,zanthoxylum_kleinii:20.0,zanthoxylum_rhoifolium:20.0)zanthoxylum:20.0)rutaceae:5.5):5.5,(allophylus_edulis:34.0,(cupania_vernalis:17.0,matayba_elaeagnoides:17.0):17.0):17.0)bell0929:15.666664):15.666664,luehea_divaricata:82.333328):15.666672,(miconia_cinerascens:85.75,(blepharocalyx_salicifolius:73.5,((((calyptranthes_concinna:24.5,(myrcia_lajeana:12.25,myrcia_oligantha:12.25)myrcia:12.25)myrciagroup:12.25,((myrciaria_delicatula:12.25,myrciaria_floribunda:12.25)myrciaria:12.25,siphoneugena_reitzii:24.5)pliniagroup:12.25):12.25,(myrceugenia_cucullata:24.5,myrceugenia_foveolata:24.5,myrceugenia_mesomischa:24.5,myrceugenia_miersiana:24.5,myrceugenia_myrcioides:24.5,myrceugenia_oxysepala:24.5)myrceugenia:24.5):12.25,(((campomanesia_rhombea:15.3125,campomanesia_xanthocarpa:15.3125)campomanesia:15.3125,myrrhinium_atropurpureum:30.625):15.3125,((eugenia_involucrata:15.3125,eugenia_oeidocarpa:

15.3125,eugenia_subterminalis:15.3125,eugenia_uruguayensis:15.3125)eugenia:15.3125,(myrcianthes_gigantea:15.3125,myrcianthes_pungens:15.3125)myrcianthes:15.3125)eugeniagroup:15.3125):15.3125):12.25):12.25):12.25)malvidae:15.666664,((maytenus_evonymoides:101.555557,((lamanonia_ternata:48.388889,weinmannia_paulliniifolia:48.388889):48.388889,(((sapium_glandulosum:30.666666,stillingia_oppositifolia:30.666666):30.666666,(sebastiania_brasiliensis:30.666666,sebastiania_commersoniana:30.666666)sebastiania:30.666666):30.666666,(banara_parviflora:63.0,(casearia_decandra:31.5,casearia_obliqua:31.5)casearia:31.5,xylosma_pseudosalzmannii:63.0)salicaceae:29.0)malpighiales:4.777779):4.777779):4.777779,(inga_virescens:99.0,prunus_myrtifolia:99.0)nitrogen_fixing_clade:7.333336):7.333328):7.333333,(((((ilex_brevicuspis:43.5,ilex_dumosa:43.5,ilex_microdonta:43.5,ilex_paraguariensis:43.5)ilex:43.5,citronella_gongonha:87.0)aquifoliales:13.0,((baccharis_dentata:21.5,vernonanthura_discolor:21.5):21.5,dasyphyllum_spinescens:43.0)asteraceae:57.0)campanulidae:8.0,((coutarea_hexandra:57.0,rudgea_parquioides:57.0)rubiaceae:25.5,((brunfelsia_cuneifolia:27.5,solanum_sanctaecatharinae:27.5):27.5,duranta_vestita:55.0):27.5):25.5)lamiidae_and_campanulidae:2.0,((myrsine_coriacea:41.25,myrsine_lorentziana:41.25)myrsine:41.25,((symplocos_tetrandra:27.5,symplocos_uniflora:27.5)symplocos:27.5,laplacea_acutifolia:55.0):27.5):27.5)ericales_and_others:11.0)pentapetalae:96.639999,roupala_montana:217.639999):96.639999,(annona_rugulosa:269.382843,((((cinnamomum_amoenum:67.345711,cinnamomum_glaziovii:67.345711)cinnamomum:67.345711,(nectandra_megapotamica:89.794289,(ocotea_elegans:44.897144,ocotea_porosa:44.897144,ocotea_puberula:44.897144,ocotea_pulchella:44.897144)ocotea:44.897144):44.897133):44.897156,cryptocarya_aschersoniana:179.588577):44.897141,hennecartia_omphalandra:224.485718):44.897125):44.897156):96.640007;

BLADJ/ Entire – Wikström ages/ Family level

(((((((lithrea_brasiliensis:57.666668,(((cedrela_fissilis:30.0,trichilia_elegans:30.0)meliaceae:17.5,(pilocarpus_pennatifolius:39.0,(zanthoxylum_astrigerum:19.5,zanthoxylum_kleinii:19.5,zanthoxylum_rhoifolium:19.5)zanthoxylum:19.5)rutaceae:8.5):8.5,(allophylus_edulis:36.0,cupania_vernalis:36.0,matayba_elaeagnoides:36.0)sapindaceae:20.0)wik_136:1.666668):36.333332,luehea_divaricata:94.0):14.333336,(miconia_cinerascens:74.666666,(blepharocalyx_salicifolius:45.333332,calyptranthes_concinna:45.333336,(campomanesia_rhombea:22.666668,campomanesia_xanthocarpa:22.666668)campomanesia:22.666668,(eugenia_involucrata:22.666668,eugenia_oeidocarpa:22.666668,eugenia_subterminalis:22.666668,eugenia_uruguayensis:22.666668)eugenia:22.666668,(myrceugenia_cucullata:22.666668,myrceugenia_foveolata:22.666668,myrceugenia_mesomischa:22.666668,myrceugenia_miersiana:22.666668,myrceugenia_myrcioides:22.666668,myrceugenia_oxysepala:22.666668)myrceugenia:22.666668,(myrcia_lajeana:22.666668,myrcia_oligantha:22.666668)myrcia:22.666668,(myrcianthes_gigantea:22.666668,myrcianthes_pungens:22.666668)myrcianthes:22.666668,(myrciaria_delicatula:22.666668,myrciaria_floribunda:22.666668)myrciaria:22.666668,myrrhinium_atropurpureum:45.333336,siphoneugena_reitzii:45.333336)myrtaceae:29.333330):33.666676)malvids:4.333328,((maytenus_evonymoides:94.0,((lamanonia_ternata:42.0,weinmannia_paulliniifolia:42.0)cunoniaceae:49.0,((sapium_glandulosum:27.0,(sebastiania_brasiliensis:13.5,sebastiania_commersoniana:13.5)sebastiania:13.5,stillingia_oppositifolia:27.0)euphorbiaceae:54.0,(banara_parviflora:40.0,(casearia_decandra:20.0,casearia_obliqua:20.0)casearia:20.0,xylosma_pseudosalzmannii:40.0)salicaceae:41.0)malpighiales:10.0)wik_21:3.0)wik_20:4.0,(inga_virescens:94.0,prunus_myrtifolia:94.0)wik_81:4.0)wik_19:14.666664):12.333333,(((((ilex_brevicuspis:24.5,ilex_dumosa:24.5,ilex_microdonta:24.5,ilex_paraguariensis:24.5)ilex:72.5,citronella_gongonha:97.0)aquifoliales:10.0,(baccharis_dentata:44.0,dasyphyllum_spinescens:44.0,vernonanthura_discolor:44.0)asteraceae:63.0)campanulids:5.0,((coutarea_hexandra:47.333332,rudgea_parquioides:47.333305)rubiaceae:50.0,((brunfelsia_cuneifolia:41.0,solanum_sanctaecatharinae:41.0)solanaceae:46.666664,duranta_vestita:87.666664):9.666672):14.666667)wik_213:2.166664,((myrsine_coriacea:49.0,myrsine_lorentziana:49.0)myrsine:25.125,((symplocos_tetrandra:19.854166,symplocos_uniflora:19.854170)symplocos:46.989582,laplacea_acutifolia:66.843750):7.281250):40.041664):10.833339)pentapetalae:19.0,roupala_montana:144.0)wik_7:17.666672,(annona_rugulosa:131.000000,(((cinnamomum_amoenum:17.0,cinnamomum_glaziovii:17.0)cinnamomum:17.0,cryptocarya_aschersoniana:34.0,nectandra_megapotamica:34.0,(ocotea_elegans:17.0,ocotea_porosa:17.0,ocotea_puberula:17.0,ocotea_pulchella:17.0)ocotea:17.0)lauraceae:50.333343,hennecartia_omphalandra:84.333343):46.666660):30.666672):249.253342;

BLADJ/ Entire – Wikström ages/ Genus level

(((((((lithrea_brasiliensis:57.666668,(((cedrela_fissilis:30.0,trichilia_elegans:30.0)meliaceae:17.50,(pilocarpus_pennatifolius:39.0,(zanthoxylum_astrigerum:19.5,zanthoxylum_kleinii:19.5,zanthoxylum_rhoifolium:19.5)zanthoxylum:19.5)rutaceae:8.5):8.5,(allophylus_edulis:22.736841,(cupania_vernalis:4.421052,matayba_elaeagnoides:4.421052):18.315786):33.263157)wik_136:1.666668):36.333332,luehea_divaricata:94.0):14.333336,(miconia_cinerascens:87.0,(blepharocalyx_salicifolius:27.200001,(((((calyptranthes_concinna:12.75,(myrcia_lajeana:6.375,myrcia_oligantha:6.375)myrcia:6.375)myrciagroup:4.25,((myrciaria_delicatula:5.1,myrciaria_floribunda:5.1)myrciaria:5.1,siphoneugena_reitzii:10.200001)pliniagroup:6.800001):3.4,(myrceugenia_cucullata:6.800006,myrceugenia_foveolata:6.800006,myrceugenia_mesomischa:6.800006,myrceugenia_miersiana:6.800006,myrceugenia_myrcioides:6.800006,myrceugenia_oxysepala:6.800006)myrceugenia:13.6):3.4,(((campomanesia_rhombea:1.7,campomanesia_xanthocarpa:1.7)campomanesia:5.1,myrrhinium_atropurpureum:6.8):10.200001,((eugenia_involucrata:4.25,eugenia_oeidocarpa:4.25,eugenia_subterminalis:4.25,eugenia_uruguayensis:4.25)eugenia:4.25,(myrcianthes_gigantea:4.25,myrcianthes_pungens:4.25)myrcianthes:4.25)eugeniagroup:8.5):6.8):3.400002):59.799999):21.333336)malvids:4.333328,((maytenus_evonymoides:94.0,((lamanonia_ternata:28.0,weinmannia_paulliniifolia:28.0):63.0,(((sapium_glandulosum:10.848214,stillingia_oppositifolia:10.848214):18.080357,(sebastiania_brasiliensis:5.785714,sebastiania_commersoniana:5.785728)sebastiania:23.142859):52.071423,(banara_parviflora:40.0,(casearia_decandra:20.0,casearia_obliqua:20.0)casearia:20.0,xylosma_pseudosalzmannii:40.0)salicaceae:41.0)malpighiales:10.0)wik_21:3.0)wik_20:4.0,(inga_virescens:94.0,prunus_myrtifolia:94.0)wik_81:4.0)wik_19:14.666664):12.333333,(((((ilex_brevicuspis:24.5,ilex_dumosa:24.5,ilex_microdonta:24.5,ilex_paraguariensis:24.5)ilex:72.5,citronella_gongonha:97.0)aquifoliales:10.0,((baccharis_dentata:33.647060,vernonanthura_discolor:33.647060):10.352941,dasyphyllum_spinescens:44.0)asteraceae:63.0)campanulids:5.0,((coutarea_hexandra:68.464287,rudgea_parquioides:68.464287)rubiaceae:28.869049,((brunfelsia_cuneifolia:36.900002,solanum_sanctaecatharinae:36.900002):50.766663,duranta_vestita:87.666664):9.666672):14.666667)wik_213:2.166664,((myrsine_coriacea:3.683335,myrsine_lorentziana:3.683335)myrsine:82.516663,((symplocos_tetrandra:16.4,symplocos_uniflora:16.4)symplocos:59.499992,laplacea_acutifolia:75.899994):10.300003):27.966667):10.833339)pentapetalae:19.0,roupala_montana:144.0)wik_7:17.666672,(annona_rugulosa:131.0,((((cinnamomum_amoenum:3.353590,cinnamomum_glaziovii:3.353590)cinnamomum:23.475159,(nectandra_megapotamica:22.701250,(ocotea_elegans:11.350638,ocotea_porosa:11.350638,ocotea_puberula:11.350638,ocotea_pulchella:11.350638)ocotea:11.350625):4.1275):47.259819,cryptocarya_aschersoniana:74.088570):22.911430,hennecartia_omphalandra:97.0):34.0):30.666672):249.253342;

BLADJ/ Pruned – Wikström ages/ Family level

(((((((lithrea_brasiliensis:69.875000,(((cedrela_fissilis:30.0,trichilia_elegans:30.0)meliaceae:17.5,(pilocarpus_pennatifolius:39.0,(zanthoxylum_astrigerum:19.5,zanthoxylum_kleinii:19.5,zanthoxylum_rhoifolium:19.5)zanthoxylum:19.5)rutaceae:8.5):8.5,(allophylus_edulis:36.0,cupania_vernalis:36.0,matayba_elaeagnoides:36.0)sapindaceae:20.0)wik_136:13.875):13.875,luehea_divaricata:83.75):13.875,(miconia_cinerascens:73.21875,(blepharocalyx_salicifolius:48.8125,calyptranthes_concinna:48.8125,(campomanesia_rhombea:24.40625,campomanesia_xanthocarpa:24.40625)campomanesia:24.40625,(eugenia_involucrata:24.40625,eugenia_oeidocarpa:24.40625,eugenia_subterminalis:24.40625,eugenia_uruguayensis:24.40625)eugenia:24.40625,(myrceugenia_cucullata:24.40625,myrceugenia_foveolata:24.40625,myrceugenia_mesomischa:24.40625,myrceugenia_miersiana:24.40625,myrceugenia_myrcioides:24.40625,myrceugenia_oxysepala:24.40625)myrceugenia:24.40625,(myrcia_lajeana:24.40625,myrcia_oligantha:24.40625)myrcia:24.40625,(myrcianthes_gigantea:24.40625,myrcianthes_pungens:24.40625)myrcianthes:24.40625,(myrciaria_delicatula:24.40625,myrciaria_floribunda:24.40625)myrciaria:24.40625,myrrhinium_atropurpureum:48.8125,siphoneugena_reitzii:48.8125)myrtaceae:24.40625):24.40625)malvids:13.875,((maytenus_evonymoides:94.0,((lamanonia_ternata:42.0,weinmannia_paulliniifolia:42.0)cunoniaceae:49.0,((sapium_glandulosum:54.0,(sebastiania_brasiliensis:27.0,sebastiania_commersoniana:27.0)sebastiania:27.0,stillingia_oppositifolia:54.0)euphorbiaceae:27.0,(banara_parviflora:40.0,(casearia_decandra:20.0,casearia_obliqua:20.0)casearia:20.0,xylosma_pseudosalzmannii:40.0)salicaceae:41.0)malpighiales:10.0)wik_21:3.0)wik_20:4.0,(inga_virescens:94.0,prunus_myrtifolia:94.0)wik_81:4.0)wik_19:13.5):13.5,(((((ilex_brevicuspis:48.5,ilex_dumosa:48.5,ilex_microdonta:48.5,ilex_paraguariensis:48.5)ilex:48.5,citronella_gongonha:97.0)aquifoliales:10.0,(baccharis_dentata:44.0,dasyphyllum_spinescens:44.0,vernonanthura_discolor:44.0)asteraceae:63.0)campanulids:5.0,((coutarea_hexandra:44.166668,rudgea_parquioides:44.166668)rubiaceae:44.166668,((brunfelsia_cuneifolia:41.0,solanum_sanctaecatharinae:41.0)solanaceae:23.666664,duranta_vestita:64.666664):23.666672):23.666666)wik_213:6.5,((myrsine_coriacea:44.4375,myrsine_lorentziana:44.4375)myrsine:44.4375,((symplocos_tetrandra:

29.625,symplocos_uniflora:29.625)symplocos:29.625,laplacea_acutifolia:59.25):29.625):29.625):6.5)pentapetalae:19.0,roupala_montana:144.0)wik_7:133.460022,(annona_rugulosa:196.306686,(((cinnamomum_amoenum:17.0,cinnamomum_glaziovii:17.0)cinnamomum:17.0,cryptocarya_aschersoniana:34.0,nectandra_megapotamica:34.0,(ocotea_elegans:17.0,ocotea_porosa:17.0,ocotea_puberula:17.0,ocotea_pulchella:17.0)ocotea:17.0)lauraceae:81.153343,hennecartia_omphalandra:115.153343):81.153343):81.153336):133.460007;

BLADJ/ Pruned – Wikström ages/ Genus level

(((((((lithrea_brasiliensis:69.875,(((cedrela_fissilis:30.0,trichilia_elegans:30.0)meliaceae:17.5,(pilocarpus_pennatifolius:39.0,(zanthoxylum_astrigerum:19.5,zanthoxylum_kleinii:19.5,zanthoxylum_rhoifolium:19.5)zanthoxylum:19.5)rutaceae:8.5):8.5,(allophylus_edulis:37.333332,(cupania_vernalis:18.666666,matayba_elaeagnoides:18.666666):18.666666):18.666666)wik_136:13.875):13.875,luehea_divaricata:83.75):13.875,(miconia_cinerascens:85.421875,(blepharocalyx_salicifolius:73.21875,(((((calyptranthes_concinna:24.40625,(myrcia_lajeana:12.203125,myrcia_oligantha:12.203125)myrcia:12.203125)myrciagroup:12.203125,((myrciaria_delicatula:12.203125,myrciaria_floribunda:12.203125)myrciaria:12.203125,siphoneugena_reitzii:24.40625)pliniagroup:12.203125):12.203125,(myrceugenia_cucullata:24.40625,myrceugenia_foveolata:24.40625,myrceugenia_mesomischa:24.40625,myrceugenia_miersiana:24.40625,myrceugenia_myrcioides:24.40625,myrceugenia_oxysepala:24.40625)myrceugenia:24.40625):12.203125,(((campomanesia_rhombea:15.253906,campomanesia_xanthocarpa:15.253906)campomanesia:15.253906,myrrhinium_atropurpureum:30.507813):15.253906,((eugenia_involucrata:15.253906,eugenia_oeidocarpa:15.253906,eugenia_subterminalis:15.253906,eugenia_uruguayensis:15.253906)eugenia:15.253906,(myrcianthes_gigantea:15.253906,myrcianthes_pungens:15.253906)myrcianthes:15.253906)eugeniagroup:15.253906):15.253906):12.203125):12.203125):12.203125)malvids:13.875,((maytenus_evonymoides:94.0,((lamanonia_ternata:45.5,weinmannia_paulliniifolia:45.5):45.5,(((sapium_glandulosum:27.0,stillingia_oppositifolia:27.0):27.0,(sebastiania_brasiliensis:27.0,sebastiania_commersoniana:27.0)sebastiania:27.0):27.0,(banara_parviflora:40.0,(casearia_decandra:20.0,casearia_obliqua:20.0)casearia:20.0,xylosma_pseudosalzmannii:40.0)salicaceae:41.0)malpighiales:10.0)wik_21:3.0)wik_20:4.0,(inga_virescens:94.0,prunus_myrtifolia:94.0)wik_81:4.0)wik_19:13.5):13.5,(((((ilex_brevicuspis:48.5,ilex_dumosa:48.5,ilex_microdonta:48.5,ilex_paraguariensis:48.5)ilex:48.5,citronella_gongonha:97.0)aquifoliales:10.0,((baccharis_dentata:22.0,vernonanthura_discolor:22.0):22.0,dasyphyllum_spinescens:44.0)asteraceae:63.0)campanulids:5.0,((coutarea_hexandra:42.0,rudgea_parquioides:42.0)rubiaceae:42.0,((brunfelsia_cuneifolia:28.0,solanum_sanctaecatharinae:28.0):28.0,duranta_vestita:56.0):28.0):28.0)wik_213:6.5,((myrsine_coriacea:44.4375,myrsine_lorentziana:44.4375)myrsine:44.4375,((symplocos_tetrandra:29.625,symplocos_uniflora:29.625)symplocos:29.625,laplacea_acutifolia:59.25):29.625):29.625):6.5)pentapetalae:19.0,roupala_montana:144.0)wik_7:133.460022,(annona_rugulosa:237.822876,((((cinnamomum_amoenum:59.455719,cinnamomum_glaziovii:59.455719)cinnamomum:59.455719,(nectandra_megapotamica:79.274292,(ocotea_elegans:39.637146,ocotea_porosa:39.637146,ocotea_puberula:39.637146,ocotea_pulchella:39.637146)ocotea:39.637146):39.637146):39.637146,cryptocarya_aschersoniana:158.548584):39.637146,hennecartia_omphalandra:198.185730):39.637146):39.637146):133.460007;

Molecular/ Dated/ Family constraint

((((((((((cupania_vernalis:0.02659,matayba_elaeagnoides:0.02659)88.00:0.05473,allophylus_edulis:0.08132)100.00:0.16114,((cedrela_fissilis:0.09220,trichilia_elegans:0.09220)100.00:0.06578,(pilocarpus_pennatifolius:0.08273,(zanthoxylum_rhoifolium:0.04137,zanthoxylum_astrigerum:0.04137,zanthoxylum_kleinii:0.04137):0.04137)100.00:0.07525)100.00:0.08448)100.00:0.01485,lithrea_brasiliensis:0.25731)100.00:0.06356,luehea_divaricata:0.32087)100.00:0.01252,(miconia_cinerascens:0.15135,((((siphoneugena_reitzii:0.02995,((myrcianthes_gigantea:0.00787,myrcianthes_pungens:0.00787)100.00:0.01649,(eugenia_involucrata:0.01050,eugenia_uruguayensis:0.01050,eugenia_oeidocarpa:0.01050,eugenia_subterminalis:0.01050)100.00:0.01386)95.00:0.00559)76.00:0.00000,((calyptranthes_concinna:0.01221,(myrciaria_floribunda:0.006105,myrciaria_delicatula:0.006105):0.006105)86.00:0.00722,(blepharocalyx_salicifolius:0.01743,(myrceugenia_oxysepala:0.00531,myrceugenia_foveolata:0.00531,myrceugenia_mesomischa:0.00531,(myrceugenia_myrcioides:0.00525,(myrceugenia_cucullata:0.00525,myrceugenia_miersiana:0.00525)96.00:0.00000)87.00:0.00006)100.00:0.01213)49.00:0.00199)32.00:0.01052)57.00:0.00200,(myrcia_oligantha:0.015975,myrcia_lajeana:0.015975):0.015975)52.00:0.00421,(campoman

esia_rhombea:0.018075,campomanesia_xanthocarpa:0.018075):0.018075,myrrhinium_atropurpureum:0.03615)100.00:0.11520)100.00:0.18204)100.00:0.02600,((maytenus_evonymoides:0.31668,((lamanonia_ternata:0.05260,weinmannia_paulliniifolia:0.05260)100.00:0.26179,(((sebastiania_brasiliensis:0.0299,sebastiania_commersoniana:0.0299):0.0299,(stillingia_oppositifolia:0.03275,sapium_glandulosum:0.03275)97.00:0.02704)100.00:0.19314,((banara_parviflora:0.06983,xylosma_pseudosalzmannii:0.06983)100.00:0.09212,(casearia_decandra:0.080975,casearia_obliqua:0.080975):0.080975)100.00:0.09098)100.00:0.06146)100.00:0.00229)100.00:0.03898,(inga_virescens:0.33159,prunus_myrtifolia:0.33159)100.00:0.02407)100.00:0.00373)100.00:0.04105,((((((ilex_paraguariensis:0.03241,ilex_dumosa:0.03241)82.00:0.00542,(ilex_brevicuspis:0.00787,ilex_microdonta:0.00787)100.00:0.02995)100.00:0.16095,citronella_gongonha:0.19877)100.00:0.05865,(dasyphyllum_spinescens:0.23673,(vernonanthura_discolor:0.22045,baccharis_dentata:0.22045)92.00:0.01628)100.00:0.02069)100.00:0.04928,((coutarea_hexandra:0.22833,rudgea_parquioides:0.22833)100.00:0.07027,((brunfelsia_cuneifolia:0.09026,solanum_sanctaecatharinae:0.09026)100.00:0.16770,duranta_vestita:0.25795)100.00:0.04064)100.00:0.00811)100.00:0.01777,((myrsine_coriacea:0.0968,myrsine_lorentziana:0.0968):0.0968,((symplocos_uniflora:0.06316,symplocos_tetrandra:0.06316):0.06316,laplacea_acutifolia:0.12632)100.00:0.06728)100.00:0.13087)100.00:0.07596)100.00:0.10651,roupala_montana:0.50694)100.00:0.04390,(annona_rugulosa:0.27279,((((cinnamomum_amoenum:0.01095,cinnamomum_glaziovii:0.01095)91.00:0.03503,((nectandra_megapotamica:0.02130,(ocotea_puberula:0.01666,ocotea_pulchella:0.01666)78.00:0.00464)79.00:0.01036,ocotea_elegans:0.03166,ocotea_porosa:0.03166)79.00:0.01432)100.00:0.14050,cryptocarya_aschersoniana:0.18648)100.00:0.02230,hennecartia_omphalandra:0.20878)100.00:0.06400)100.00:0.27805)100.00:0.44916,nymphaea_odorata:1.00000):1.00000;

Molecular/ Dated/ Genus constraint

(((((((lithrea_brasiliensis:0.25758,(((cedrela_fissilis:0.09225,trichilia_elegans:0.09225)100.00:0.06597,(pilocarpus_pennatifolius:0.08276,(zanthoxylum_rhoifolium:0.04138,zanthoxylum_astrigerum:0.04138,zanthoxylum_kleinii:0.04138):0.04138)100.00:0.07546)100.00:0.08451,(allophylus_edulis:0.08125,(cupania_vernalis:0.02651,matayba_elaeagnoides:0.02651)100.00:0.05475)100.00:0.16148)100.00:0.01486)100.00:0.06387,luehea_divaricata:0.32145)100.00:0.01022,(miconia_cinerascens:0.14050,(blepharocalyx_salicifolius:0.03148,myrrhinium_atropurpureum:0.03148,((((calyptranthes_concinna:0.01030,(myrcia_oligantha:0.00515,myrcia_lajeana:0.00515):0.00515)100.00:0.00654,((myrciaria_floribunda:0.00842,myrciaria_delicatula:0.00842):0.00842,siphoneugena_reitzii:0.01684)100.00:0.00000)100.00:0.00226,((myrceugenia_myrcioides:0.00532,(myrceugenia_cucullata:0.00532,myrceugenia_miersiana:0.00532)97.00:0.00000)92.00:0.00029,myrceugenia_oxysepala:0.00561,myrceugenia_foveolata:0.00561,myrceugenia_mesomischa:0.00561)100.00:0.01348)100.00:0.00954,((campomanesia_xanthocarpa:0.014315,campomanesia_rhombea:0.014315):0.014315,((eugenia_involucrata:0.01146,eugenia_uruguayensis:0.01146,eugenia_oeidocarpa:0.01146,eugenia_subterminalis:0.01146)100.00:0.01435,(myrcianthes_gigantea:0.00891,myrcianthes_pungens:0.00891)100.00:0.01690)100.00:0.00282)100.00:0.00000)100.00:0.00285)100.00:0.10901)100.00:0.19118)100.00:0.02646,((maytenus_evonymoides:0.31693,((lamanonia_ternata:0.05266,weinmannia_paulliniifolia:0.05266)100.00:0.26195,(((sapium_glandulosum:0.03287,stillingia_oppositifolia:0.03287)100.00:0.02701,(sebastiania_brasiliensis:0.02994,sebastiania_commersoniana:0.02994):0.02994)100.00:0.19337,((banara_parviflora:0.07002,xylosma_pseudosalzmannii:0.07002)100.00:0.09232,(casearia_decandra:0.081175,casearia_obliqua:0.081175):0.081175)100.00:0.09090)100.00:0.06137)100.00:0.00231)100.00:0.03909,(inga_virescens:0.33218,prunus_myrtifolia:0.33218)100.00:0.02384)100.00:0.00212)100.00:0.04174,((((((ilex_paraguariensis:0.03241,ilex_dumosa:0.03241)51.00:0.00544,(ilex_brevicuspis:0.00787,ilex_microdonta:0.00787)100.00:0.02998)100.00:0.16114,citronella_gongonha:0.19898)100.00:0.05906,((baccharis_dentata:0.22118,vernonanthura_discolor:0.22118)100.00:0.01632,dasyphyllum_spinescens:0.23750)100.00:0.02054)100.00:0.04943,((coutarea_hexandra:0.22894,rudgea_parquioides:0.22894)100.00:0.07037,((brunfelsia_cuneifolia:0.09040,solanum_sanctaecatharinae:0.09040)100.00:0.16817,duranta_vestita:0.25857)100.00:0.04074)100.00:0.00817)100.00:0.01778,((myrsine_coriacea:0.096915,myrsine_lorentziana:0.096915):0.096915,((symplocos_uniflora:0.063255,symplocos_tetrandra:0.063255):0.063255,laplacea_acutifolia:0.12651)100.00:0.06732)100.00:0.13143)100.00:0.07461)100.00:0.10673,roupala_montana:0.50661)100.00:0.04381,(annona_rugulosa:0.27315,((((cinnamomum_amoenum:0.01099,cinnamomum_glaziovii:0.01099)100.00:0.03492,(nectandra_megapotamica:0.02980,(ocotea_elegans:0.02980,ocotea_porosa:0.02980,(ocotea_pulchella:0.01667,ocotea_puberula:0.01667)100.00:0.01314)100.00:0.00000)100.00:0.01611)100.00:0.14070,cryptocarya_aschersoniana:0.18661)100.00:0.02245,hennecartia_omphalandra:0.20906)100.00:0.06409)100.00:0.27726)100.00:0.44958,nymphaea_odorata:1.00000):1.00000;

Molecular/ Undated/ Family constraint

((((((((((cupania_vernalis:0.02111,matayba_elaeagnoides:0.00480)100.00:0.01060,allophylus_edulis:0.07178)100.00:0.11041,((cedrela_fissilis:0.05130,trichilia_elegans:0.03861)100.00:0.03042,(pilocarpus_pennatifolius:0.02760,(zanthoxylum_rhoifolium:0.026505,zanthoxylum_astrigerum:0.026505,zanthoxylum_kleinii:0.026505):0.026505)100.00:0.03843)100.00:0.01726)100.00:0.00865,lithrea_brasiliensis:0.11601)100.00:0.03544,luehea_divaricata:0.12030)100.00:0.02239,(miconia_cinerascens:0.26470,((((siphoneugena_reitzii:0.01037,((myrcianthes_gigantea:0.00661,myrcianthes_pungens:0.00120)100.00:0.00528,(eugenia_involucrata:0.00575,eugenia_uruguayensis:0.00575,eugenia_oeidocarpa:0.00575,eugenia_subterminalis:0.00575)100.00:0.00956)95.00:0.00961)76.00:0.00257,((calyptranthes_concinna:0.00486,(myrciaria_floribunda:0.003605,myrciaria_delicatula:0.003605):0.003605)86.00:0.00186,(blepharocalyx_salicifolius:0.00994,(myrceugenia_oxysepala:0.00137,myrceugenia_foveolata:0.00137,myrceugenia_mesomischa:0.00137,(myrceugenia_myrcioides:0.00038,(myrceugenia_cucullata:0.00282,myrceugenia_miersiana:0.00354)96.00:0.00066)87.00:0.00053)100.00:0.00558)49.00:0.00175)32.00:0.00000)57.00:0.00216,(myrcia_oligantha:0.000995,myrcia_lajeana:0.000995):0.000995)52.00:0.00287,(campomanesia_rhombea:0.00409,campomanesia_xanthocarpa:0.00409):0.00409,myrrhinium_atropurpureum:0.00818)100.00:0.04254)100.00:0.07894)100.00:0.00798,((maytenus_evonymoides:0.16310,((lamanonia_ternata:0.01439,weinmannia_paulliniifolia:0.03697)100.00:0.07928,(((sebastiania_brasiliensis:0.0115,sebastiania_commersoniana:0.0115):0.0115,(stillingia_oppositifolia:0.01896,sapium_glandulosum:0.01308)97.00:0.01628)100.00:0.08198,((banara_parviflora:0.03789,xylosma_pseudosalzmannii:0.03012)100.00:0.05011,(casearia_decandra:0.03416,casearia_obliqua:0.03416):0.03416)100.00:0.05646)100.00:0.04602)100.00:0.00000)100.00:0.01845,(inga_virescens:0.17586,prunus_myrtifolia:0.14706)100.00:0.01412)100.00:0.01226)100.00:0.02397,((((((ilex_paraguariensis:0.01300,ilex_dumosa:0.01859)82.00:0.00816,(ilex_brevicuspis:0.00309,ilex_microdonta:0.00469)100.00:0.00922)100.00:0.08996,citronella_gongonha:0.05071)100.00:0.00000,(dasyphyllum_spinescens:0.07294,(vernonanthura_discolor:0.10991,baccharis_dentata:0.10486)92.00:0.02920)100.00:0.05775)100.00:0.00675,((coutarea_hexandra:0.07472,rudgea_parquioides:0.14769)100.00:0.05690,((brunfelsia_cuneifolia:0.05047,solanum_sanctaecatharinae:0.03754)100.00:0.08460,duranta_vestita:0.11979)100.00:0.00472)100.00:0.03170)100.00:0.01404,((myrsine_coriacea:0.07448,myrsine_lorentziana:0.07448):0.07448,((symplocos_uniflora:0.03242,symplocos_tetrandra:0.03242):0.03242,laplacea_acutifolia:0.05832)100.00:0.00555)100.00:0.04062)100.00:0.02854)100.00:0.05435,roupala_montana:0.12045)100.00:0.03958,(annona_rugulosa:0.16173,((((cinnamomum_amoenum:0.00721,cinnamomum_glaziovii:0.00356)91.00:0.00818,((nectandra_megapotamica:0.00405,(ocotea_puberula:0.00980,ocotea_pulchella:0.00654)78.00:0.00546)79.00:0.00227,ocotea_elegans:0.02394,ocotea_porosa:0.02394)79.00:0.01147)100.00:0.06955,cryptocarya_aschersoniana:0.08395)100.00:0.01325,hennecartia_omphalandra:0.08517)100.00:0.02766)100.00:0.03038)100.00:0.22670,nymphaea_odorata:0.00000):1.00000;

Molecular/ Undated/ Genus constraint

(((((((lithrea_brasiliensis:0.11446,(((cedrela_fissilis:0.05065,trichilia_elegans:0.03808)100.00:0.02979,(pilocarpus_pennatifolius:0.02723,(zanthoxylum_rhoifolium:0.026155,zanthoxylum_astrigerum:0.026155,zanthoxylum_kleinii:0.026155):0.026155)100.00:0.03821)100.00:0.01715,(allophylus_edulis:0.07073,(cupania_vernalis:0.02080,matayba_elaeagnoides:0.00473)100.00:0.01047)100.00:0.10880)100.00:0.00854)100.00:0.03512,luehea_divaricata:0.11869)100.00:0.02216,(miconia_cinerascens:0.26181,(blepharocalyx_salicifolius:0.00978,myrrhinium_atropurpureum:0.00978,((((calyptranthes_concinna:0.00655,(myrcia_oligantha:0.00177,myrcia_lajeana:0.00177):0.00177)100.00:0.00052,((myrciaria_floribunda:0.00415,myrciaria_delicatula:0.00415):0.00415,siphoneugena_reitzii:0.01246)100.00:0.00053)100.00:0.00113,((myrceugenia_myrcioides:0.00038,(myrceugenia_cucullata:0.00279,myrceugenia_miersiana:0.00350)97.00:0.00065)92.00:0.00077,myrceugenia_oxysepala:0.00112,myrceugenia_foveolata:0.00112,myrceugenia_mesomischa:0.00112)100.00:0.00652)100.00:0.00000,((campomanesia_rhombea:0.00538,campomanesia_xanthocarpa:0.00538):0.00538,((eugenia_involucrata:0.00644,eugenia_uruguayensis:0.00644,eugenia_oeidocarpa:0.00644,eugenia_subterminalis:0.00644)100.00:0.00968,(myrcianthes_gigantea:0.00738,myrcianthes_pungens:0.00129)100.00:0.00542)100.00:0.01046)100.00:0.00072)100.00:0.00188)100.00:0.03849)100.00:0.08143)100.00:0.00772,((maytenus_evonymoides:0.16101,((lamanonia_ternata:0.01419,weinmannia_paulliniifolia:0.03644)100.00:0.07817,(((sapium_glandulosum:0.01289,stillingia_oppositifolia

:0.01867)100.00:0.01605,(sebastiania_brasiliensis:0.011335,sebastiania_commersoniana:0.011335):0.011335)100.00:0.08079,((banara_parviflora:0.03747,xylosma_pseudosalzmannii:0.02978)100.00:0.04958,(casearia_decandra:0.033725,casearia_obliqua:0.033725):0.033725)100.00:0.05570)100.00:0.04537)100.00:0.00000)100.00:0.01822,(inga_virescens:0.17342,prunus_myrtifolia:0.14551)100.00:0.01393)100.00:0.01210)100.00:0.02366,((((ilex_paraguariensis:0.01284,ilex_dumosa:0.01835)51.00:0.00806,(ilex_brevicuspis:0.00305,ilex_microdonta:0.00463)100.00:0.00910)100.00:0.08872,citronella_gongonha:0.05026)100.00:0.00000,((baccharis_dentata:0.10418,vernonanthura_discolor:0.10830)100.00:0.02896,dasyphyllum_spinescens:0.07193)100.00:0.05717)100.00:0.00668,((coutarea_hexandra:0.07375,rudgea_parquioides:0.14614)100.00:0.05620,((brunfelsia_cuneifolia:0.04981,solanum_sanctaecatharinae:0.03706)100.00:0.08359,duranta_vestita:0.11842)100.00:0.00468)100.00:0.03127)100.00:0.01391,((myrsine_coriacea:0.073415,myrsine_lorentziana:0.073415):0.073415,((symplocos_uniflora:0.03198,symplocos_tetrandra:0.03198):0.03198,laplacea_acutifolia:0.05757)100.00:0.00551)100.00:0.03991)100.00:0.02819)100.00:0.05374,roupala_montana:0.11895)100.00:0.03899,(annona_rugulosa:0.15946,((((cinnamomum_amoenum:0.00690,cinnamomum_glaziovii:0.00370)100.00:0.00892,(nectandra_megapotamica:0.00449,(ocotea_elegans:0.02486,ocotea_porosa:0.02486,(ocotea_pulchella:0.00617,ocotea_puberula:0.00989)100.00:0.00608)100.00:0.00000)100.00:0.01168)100.00:0.06868,cryptocarya_aschersoniana:0.08282)100.00:0.01315,hennecartia_omphalandra:0.08401)100.00:0.02731)100.00:0.02993)100.00:0.22370,nymphaea_odorata:0.0000):1.0000;

Appendix 2. References of studies used to construct the phylogenetic trees of 20 families and references of intra-familial and families' age estimates combined with Bell *et al*. (2010) and Wikström *et al*. (2001) age estimates.

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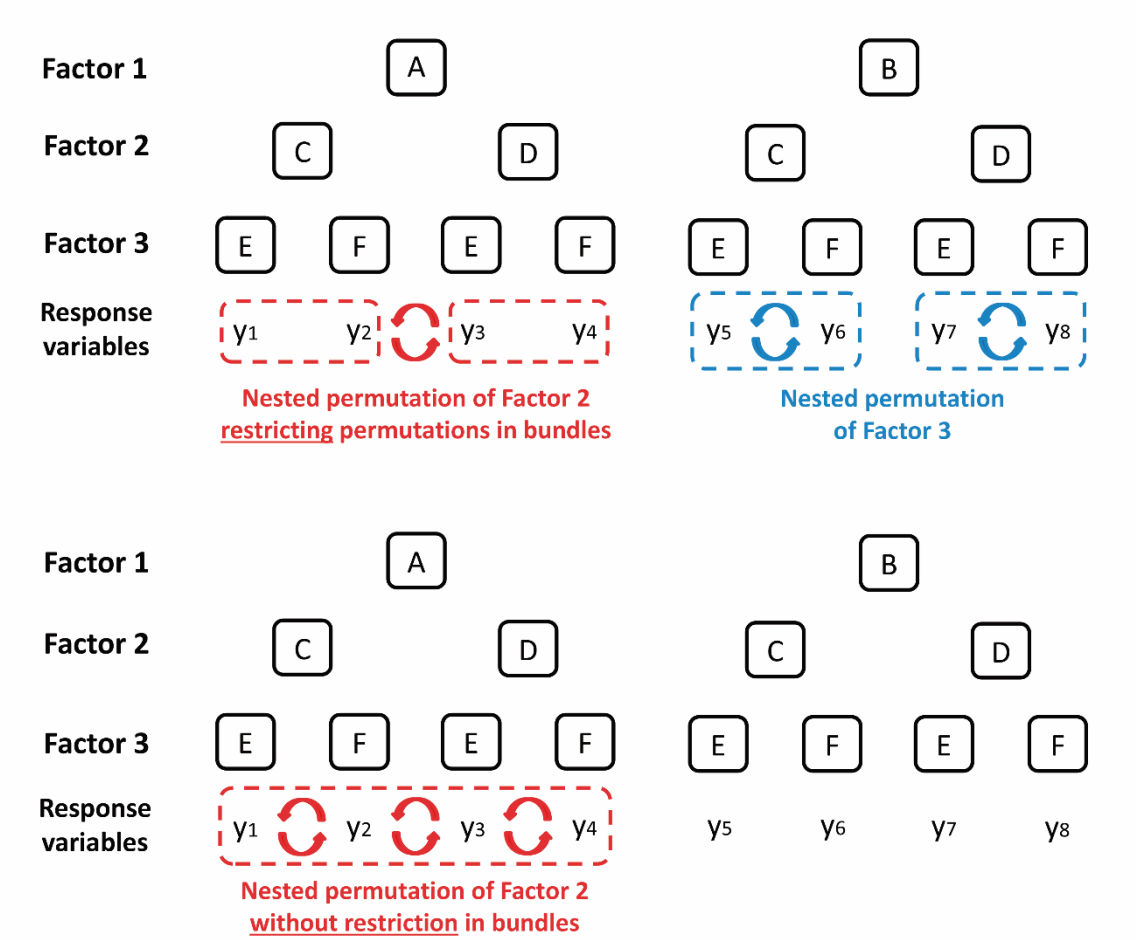

Appendix 4. Example of an ANOVA with split plot design restricting permutations of sampling units within nesting groups, with or without restricting permutation in bundles. The third factor is permuted without restriction bundles, since it does not have replicates. The ANOVAs with permutation tests were calculated using the using MULTIV 3.27b statistical software (Pillar 1997; available at http://ecoqua.ecologia.ufrgs.br/software).

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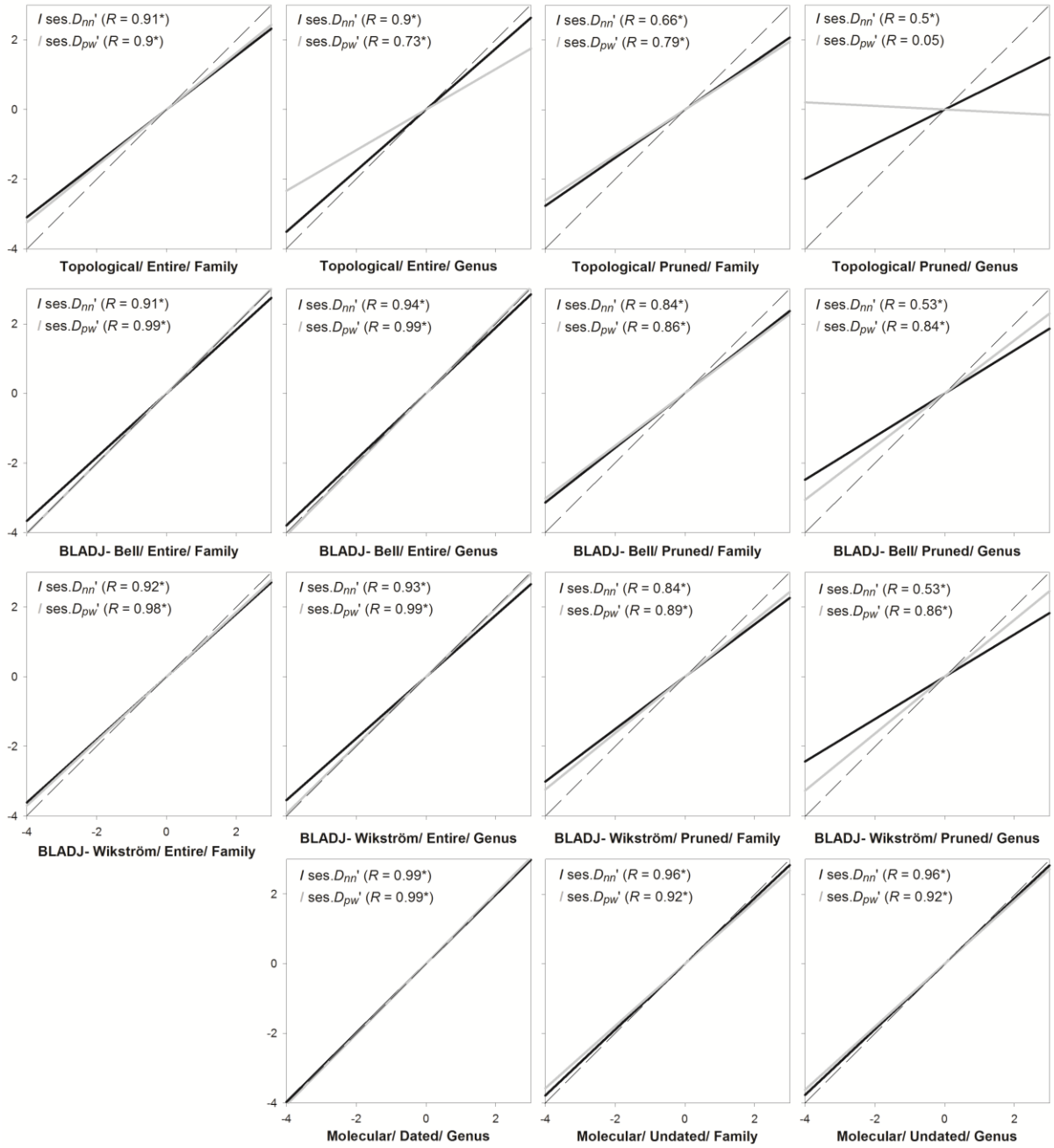
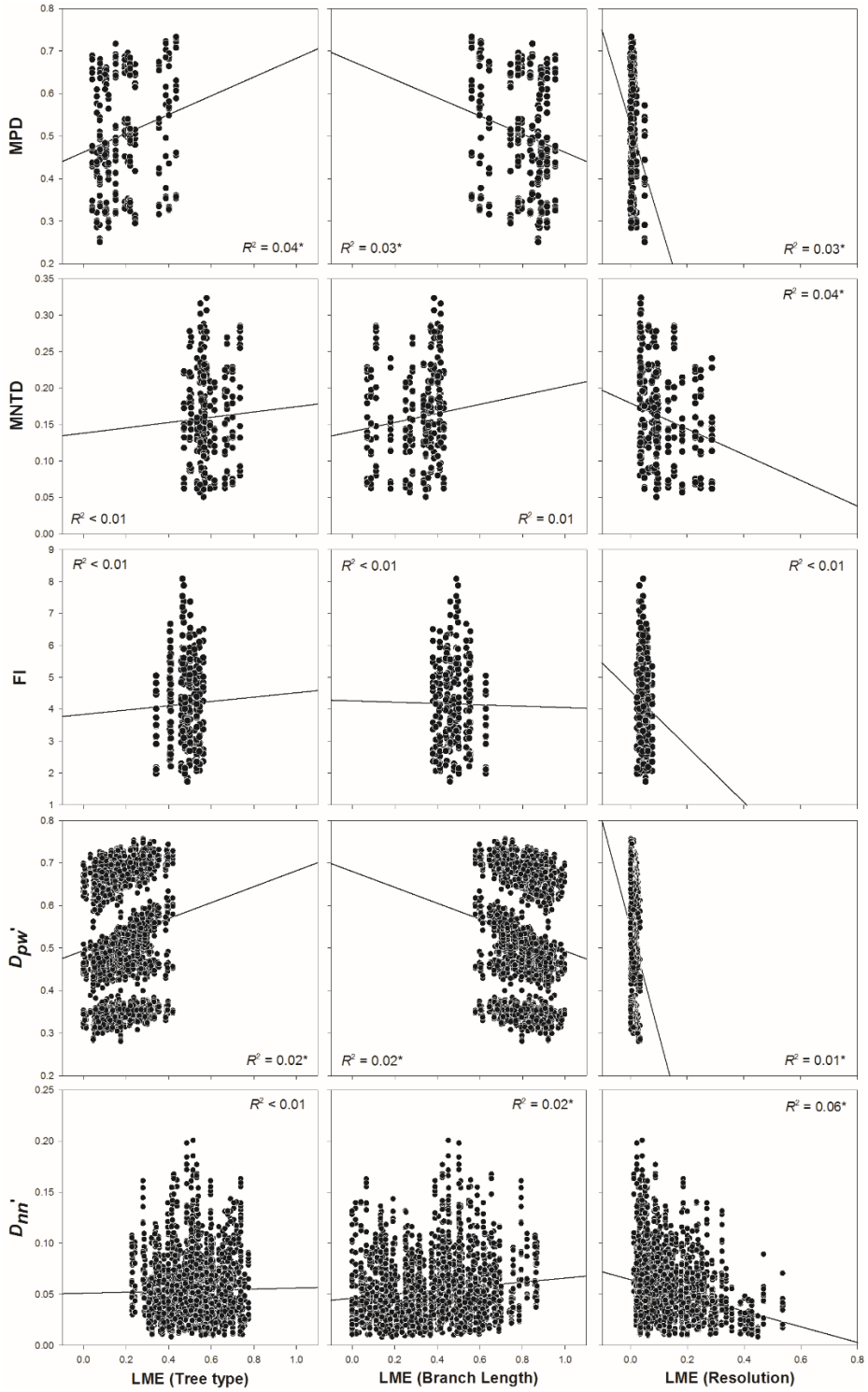


Appendix 5. Simple linear regressions of phylogenetic beta dispersion results between the molecular dated phylogeny with family constraint resolution ("best-tree") and all other constructed phylogenies. Regression lines were forced to cross the origin through a data centering. Dashed lines represent the expected relationship of a perfect 1:1 correlation (slope = 1) with the "best-tree". Regression lines over or under the dashed line (slopes > 1 or < 1) represent false positives or false negatives results (type I or II error). Positive/negative values of phylogenetic diversity metrics represent phylogenetic clustering/overdispersion. *$P \leq 0.05$.

Appendix 6. Slopes values of simple linear regression between the molecular dated phylogeny with a family constraint resolution ("best-tree") and all other constructed phylogenies, for alpha and beta phylogenetic dispersion metrics. **NS**: Non-Significant regression results. For details of alpha and beta phylogenetic community metrics see Material and Methods.

			NRI	NTI	ses.FI	ses.D_{pw}'	ses.D_{nn}'
Topological	**Entire**	**Family**	0.908	0.874	0.960	0.810	0.774
		Genus	0.623	0.910	0.813	0.583	0.878
	Pruned	**Family**	0.714	0.623	0.732	0.652	0.690
		Genus	0.098^{NS}	0.706	0.620	-0.052^{NS}	0.498
BLADJ-Bell	**Entire**	**Family**	0.983	0.995	0.917	1.002	0.915
		Genus	1.032	0.944	1.047	1.017	0.948
	Pruned	**Family**	0.719	0.928	0.728	0.757	0.786
		Genus	0.728	0.681	0.857	0.763	0.620
BLADJ-Wikström	**Entire**	**Family**	0.958	0.976	1.040	0.925	0.903
		Genus	0.99	0.943	1.057	0.984	0.886
	Pruned	**Family**	0.808	0.980	0.843	0.81	0.753
		Genus	0.791	0.725	0.897	0.818	0.609
Molecular	**Dated**	**Genus**	1.012	0.975	1.009	1.014	0.993
	Undated	**Family**	0.956	0.881	0.868	0.893	0.945
		Genus	0.943	0.868	0.866	0.904	0.941



Appendix 7. Scatter plots of a linear regression between the Linear Mixed-Effect models results (regression coefficients) of all trees for each tested factor (type of tree, branch length estimation and resolution level) and their respective phylogenetic alpha and beta diversity results. *$P \leq 0.01$.

Capítulo 2. Phylogenetic and functional nestedness in climbing plant assemblages in woody patches advancing over Campos grassland[2]

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Abstract

Question: We assessed the variation of species composition, phylogenetic clades and functional composition of climbing plants along the process of forest expansion over grassland. We hypothesized that the phylogenetic and functional composition of vegetation patches show a nested pattern towards the surrounding continuous forest. Furthermore, the variation in species composition among patches is expected to be determined by the dispersion of major phylogenetic lineages, associated to the frequency of species traits related to dispersal and climbing, and mostly explained by patch size and isolation.

Location: Subtropical grassland surrounded by *Araucaria* forest, Southern Brazil (29°28'S, 50°13' W).

Method: We recorded the composition and abundance of climbing plant species along the gradient of forest patches of increasing sizes and distance to the surrounding continuous forest. We employed the treeNODF method to estimate whether forest patch size cause nestedness of species, phylogenetic and functional composition. We performed analysis of principal coordinates of phylogenetic structure (PCPS) to analyze the distribution of phylogenetic clades across the patches and the forest sites. Each PCPS was tested for the association with patch area and isolation using linear models, and for the correlation to species traits (dispersal traits and climbing methods).

Results: We found a nested pattern on species composition, indicating that smaller patch categories are subsets of larger ones and forest sites. Moreover, there was a habitat filtering on species composition mediated by patch size and isolation. Species with plumed diaspores were associated with single-tree patches while winged and fleshy diaspores with large patches and the continuous forest.

Conclusion: Patch area and isolation play an important role on the distribution of climbing plants during the advance of woody vegetation over grassland. Our findings highlight that

taking into account a phylogenetic and functional approach on nestedness analysis, exploring the patterns of phylogeny-weighted species composition and integrating it with patch properties, improves our knowledge of the colonization and forest expansion dynamics.

Nomenclature: APG (2009)

Keywords: *Araucaria* forest; nucleation; lianas; vines; forest patches; patch isolation; patch size; climbing mechanisms; dispersal syndromes; PCPS.

Running head: Climbing plants and the forest expansion over grassland

Introduction

The dynamics of *Araucaria* forest expansion over the *Campos* grasslands in southern Brazil highlands is affected by events such as climate change during the Holocene and, more recently, disturbances by fire and grazing (Oliveira & Pillar 2004; Behling & Pillar 2007). In this scenario, forest expansion occurs via edge dynamics or via nucleation, which involves the establishment of woody species in the grassland matrix through nurse plants or nurse rock outcrops (Duarte et al. 2006a; Carlucci et al. 2011a; Carlucci et al. 2011b). As woody pioneer species establish on grassland and change environmental conditions, they facilitate the establishment of species less tolerant to the harsh environmental conditions of grasslands (Duarte et al. 2006b). Nucleation leads to the development of forest patches of different sizes and is well documented for woody saplings species, showing the increase of zoochoric species along the process (Duarte et al. 2007). Furthermore, Duarte (2011) has observed that such process involves some degree of phylogenetic filtering of angiosperm deeper nodes, which tend to get restricted to more developed forest patches.

The flow and arrival of species in a forest patch can be influenced by the distance and area of nearby habitats (degree of isolation), namely other patches and/or the continuous forest (Fahrig 2013). Therefore, patch isolation and plant species traits relevant for dispersal and establishment (e.g., dispersal syndrome, plant architecture) are likely to play an important role in the development of forest patches (Piessens et al. 2005; Kooyman et al. 2013). In this context, the distribution of forest patches into a grassland matrix creates a range of possibilities for dispersal of other plant types, like climbing species. Climbing plants are important forest components, presenting mixed positive and negative effects on forest dynamics. They compete with trees and shrubs for sunlight, nutrients and water, and can strongly influence host growth and survival (Toledo-Aceves 2015). On the other hand, climbing plants may alter forest structure by sealing forest edges and covering forest canopy, changing the forest interior

microclimate and diminishing edge effects (Campbell et al. 2015). Moreover, climbing plants provide food resources for the fauna, such as fruits, nectar and highly nutritional leaves (Kazda 2015; Michel et al. 2015), which may attract seed dispersers and in this way affect plant succession.

The plasticity and the lack of self-supporting in most part of climbing plants life cycle, enable an active habitat prospection and a quick adaptation to environmental changes and perturbation compared to trees and shrubs (Rowe & Speck 2015). Climbing plants show a variety of mechanisms to ascend the forest canopy due to their lack of self-supporting structures (Hegarty 1991; Rowe & Speck 2015). The climbing mechanism determines the species' range of suitable supports and accordingly, species distribution can be influenced by the successional stage or disturbance regime of the forest (Hegarty & Caballé 1991; Schnitzer & Bongers 2002). Further, climbing plants can be classified as an early successional guild of pioneer species with high diversity and abundance in young forests (DeWalt et al. 2000).

Studies in tropical forests reported a positive relationship between habitat disturbance and climbing plants abundance or distribution (e.g., Laurance et al. 2001; Arroyo-Rodríguez & Toledo-Aceves 2009; Schnitzer & Bongers 2011). Some studies evaluating the successional patterns of climbing plants in post-agricultural areas of tropical forests, found evidences of a species turnover with the highest species richness occurring in early and intermediate stages of succession (e.g., DeWalt et al. 2000; Letcher & Chazdon 2009; reviewed in Letcher 2015). However, there was no clear pattern in relation to climbing methods over succession. Besides that, little is known about phylogenetic patterns of climbing plants along forest succession (Letcher 2010; Roeder et al. 2014). It is noteworthy, that forest development can occur by a different process than forest recovery and no study by now evaluated climbing plants in the process of forest expansion over another ancient vegetation type, like the old-growth grasslands (Veldman et al. 2015).

In this study, we assessed the variation on composition of species, phylogenetic clades and functional traits of climbing plants throughout the process of forest expansion towards grassland. We recorded species composition and abundance along the gradient of forest patches of increasing sizes and isolation, in a grassland matrix surrounded by forest. We hypothesized that the species, phylogenetic and functional composition of vegetation patches show a nested pattern from patches of increasing size towards the surrounding continuous forest. Moreover, we expect a variation in species composition among patches determined by the dispersion of major phylogenetic lineages, associated to the frequency of species traits related to dispersal and climbing, and mostly explained by patch size and isolation.

Methods

Study area

The study was conducted at Pró-Mata Research and Nature Conservation Center (CPCN Pró-Mata, 29°29'S, 50°12' W) in the Northeastern plateau (at ca. 900 m a.s.l.) of Rio Grande do Sul State, Southern Brazil (Fig. 1). The regional climate is characterized as subtropical rainy, with precipitation uniformly distributed throughout the year. The annual mean rainfall reaches 2,252 mm and the annual mean temperature is 14.4 ºC with the occurrence of negative temperatures from April to September, occasional frosts and rare events of snow (National Institute of Meteorology – INMET). The CPCN Pró-Mata is located at a region characterized by the contact of three vegetation types: highland *Campos* grassland, *Araucaria* forest and Atlantic forest. The study site was located in a grassland area (ca. 78 ha; Fig. 1) surrounded by *Araucaria* forest, with forest patches of different sizes and development stages (Duarte et al. 2006b). This grassland area is free of cattle grazing and until this study 4the last fire event dates of 1993 (Oliveira & Pillar 2004), allowing the establishment of woody species and the development of forest patches as well as the continuous forest edge expansion.

Data sampling

We surveyed for climbing plants during January 2012 and 2013 in four sites into the continuous forest surrounding the grassland matrix and 25 forest patches in the grassland matrix. Forest patches differed in size, developmental stage and vegetation structure (more details in Duarte et al. 2006b), and were composed by 12 single-tree patches (around 30 m^2), seven small patches (206 – 738 m^2) and six large patches (1,702 – 5,904 m^2). According to patch size/category (as a proxy of forest development), we sampled a proportional number of 5x5 m plots distributed along the longest patches' axis (single-tree patches = 1; small patches = 2; large patches = 4). In single-tree patches the plot was centered in the tree. At each of the four continuous forest sites, we surveyed three plots along the forest edge and three plots 100 m into the forest. These plots were arranged parallel to the forest edges and distant 50 m from each other. A summary of sampling effort on each category can be seen in Table 1.

Single-tree patches were characterized by only one tree ≥ 5 m, free of contact to neighbor trees and a shrub stratum not connected to another forest patch. The majority of single-tree patches were of the species *Araucaria angustifolia* (Bertol.) Kuntze, excepting one that was of the species *Mimosa scabrella* Benth. Small patches were defined as a group of woody plants (few adult trees) forming an open canopy with few shrub/treelet individuals and grassland species in the understory. Large patches were defined by higher tree abundance and a closed canopy, with a defined shrub stratum and no grassland species in the understory.

We identified to the species level all climbing plant individuals that were rooted inside the plots. Species were classified according to its climbing mechanisms, dispersal syndrome and diaspore types (size/weight classes) using specialized literature (Seger et al. 2013; Seger & Hartz 2014) and personal observation. Climbing mechanisms were classified according to Hegarty (1991) into stem twiner, petiole twiner, tendril (that coil), adhesive tendril (terminal

adhesive pads), clasp tendril, hook/spine (also aculeus or trichomes that prevent slipping), scrambler and adherent roots. Many species combine different climbing mechanisms (Hegarty 1991) making them difficult to be correctly classified, so in those cases we considered the species as having more than one climbing mechanism. Dispersal syndromes were classified according to van der Pijl (1982) into anemochory, zoochory or barochory. Anemochoric species were also classified as pterochoric (winged diaspore) and pogonochoric (plumed diaspore). Diaspores types were classified according to a proxy of their size and weight. Zoochoric species were classified in three size classes: fleshy small – diaspore size ratio (DSR; average of length plus diameter) < 1 cm; fleshy medium – DSR ≥ 1 cm and < 5 cm; and fleshy big – DSR ≥ 5 cm. Pogonochoric species were classified according to their pappus length into: plumed small – pappus < 1 cm; and plumed big – pappus ≥ 1 cm. Pterochoric species were classified according to a proxy of their size and weight into: winged small – wing < 1 cm; winged big – wing ≥ 1 cm; and winged membranaceous – light wings without size limit.

Phylogenetic and functional trees

To obtain the phylogenetic affinities among species we reconstructed a molecular phylogeny for all sampled species (newick format in Appendix S1). For this, we downloaded species sequences of nine widely used genetic markers available at GenBank (Benson et al. 2013). The phylogeny was reconstructed using a maximum-likelihood approach with estimated bootstrap support values for each node (for detailed information of phylogenetic reconstruction workflow, see the Appendix S1). The branch lengths were transformed to be proportional to divergence time through maximum likelihood approach with discrete rate variation (Paradis 2013). Thereafter we calculated a matrix of phylogenetic distances among pairs of terminal taxa.

To assess the functional affinities among species we constructed five functional trees (newick format in Appendix S2). One tree was based only on the climbing mechanisms, another only on the dispersal syndromes and the other three combined dispersal syndromes with diaspore types, climbing mechanisms with dispersal syndromes, and a combination of all traits. For this, we constructed functional dendrograms using as cluster method the unweighted pair-group method with arithmetic averages (UPGMA). As resemblance measurement between species, we used a modification of Gower's distance (Pavoine et al. 2009). For all functional trees we obtained high values of cophenetic correlation (between 0.98 and 1). The modified Gower's distance was calculated through the package *ade4* 1.5-2 (Dray & Dufour 2007) in the R Statistical Environment (R Foundation for Statistical Computing, Vienna, AT; http://www.R-project.org).

Landscape metrics

To measure the isolation of forest patches to other patches in the grassland matrix and to the continuous forest, we first mapped all forest patches in the study site with the aid of satellite image (Google Earth© 2013; available at http://www.google.com/earth/), totaling 42 patches. We then used the Proximity Index (PXfg; Gustafson & Parker 1992) for inferring patches' isolation (high values indicating low isolation). The PXfg is computed based on patches area and the nearest-neighbor distance for each patch within a specified search distance. The search distance will determine the neighbor forest patches that will be included in the calculation and reflects the potential range of dispersal events of the studied species. We set up a distance threshold of 668 m, which is half of the largest distance among continuous forest edges. With this criterion, we assumed that all climbing plants diaspores can potentially reach any forest patch within this distance, which is sufficient for any diaspore dispersing from the continuous forest to reach the center of the grassland matrix. The Proximity Index was calculated through

the V-LATE 2.0 beta (Vector-based Landscape Analysis Tools Extension; available at https://sites.google.com/site/largvlate/gis-tools/v-late) in the ArcGIS 10.3 software (ESRI; http://www.arcgis.com/features/). For patches' values of PXfg, area and distance to the continuous forest, see the Appendix S3.

Data analysis

We used treeNODF method (Melo et al. 2014), to test whether phylogenetic and functional composition of small as well as single-tree patches are nested subsets of large patches, which by its turn would be subsets of continuous forest sites. treeNODF is an extension of the NODF index (Nestedness based on Overlap and Decreasing Fill; Almeida-Neto et al. 2008) used to assess nestedness of species composition. NODF evaluates whether species present in species-poor sites constitute proper subsets of those ones present at species-rich sites (Almeida-Neto et al. 2008). The treeNODF evaluates nestedness among objects (patches) taking into account the resemblance among the descriptor variables (species) expressed as a phylogenetic/functional tree. It is the joint contribution of species and phylogenetic (or functional) composition to nestedness (treeNODF) and as such can be partitioned in a component due to species composition (S.fraction) and a component due to phylogenetic (or functional) resemblance (topoNODF; simply the subtraction of S.fraction from treeNODF).
Sampling effort was not uniform among patches and forest sites. In order to avoid the effect of sampling effort on diversity estimates (species richness, functional and phylogenetic diversities), we constructed a procedure that randomly draws one plot of 5x5 m of each of the four site categories (single-tree patches, small patches, large patches and forest sites) to calculate nestedness. A common use of NODF is to reorder rows and columns of a matrix according to decreasing species richness (for rows) and species frequencies (for columns). A disadvantage of this approach is the weak link between the observed nestedness pattern and a

plausible inferred mechanism (Melo et al. 2014). We opted to explicitly include our hypothesis in the test by ordering rows of our four-rows matrix (with one randomly draw plot of each site category) according to decreasing area. As our hypotheses regard ordering of rows of the matrix, we devised a permutation test that performed all possible permutations of rows (24 combinations), calculated the nestedness metrics for each of the 24 permuted matrices and obtained a mean expected value. It should be noted that this permutation test is conservative as only a 24 permuted matrices are possible. Accordingly, we repeated the subsampling procedure and the nestedness calculation for the observed and the 24 permuted matrices 9999 times, and expressed statistical significance as the proportion of cases in which the average (over 24 values) statistic generated by permuting the four-rows matrices were higher than the observed statistic ([cases+1]/[9999+1]).

To evaluate the relationship of phylogenetic clades along the different sites we performed a principal coordinates of phylogenetic structure (PCPS; Duarte 2011). First, we calculated the phylogenetic weights of taxa through the fuzzy weighting method developed by Pillar and Duarte (2010). The method transforms the pairwise phylogenetic distance matrix into dissimilarities ranging from zero to one and standardize it by unit column totals. This procedure weights taxa by its phylogenetic relationship resulting in a phylogenetic belonging of each taxon to each other, that reflects the amount of evolutionary history shared between a given taxon and all other taxa in the data set. Next we used this standardized matrix of weights and the matrix of species abundance by site (matrix **W**) transformed by square root, to generate a matrix of phylogeny-weighted taxon composition for each site (matrix **P**), which expresses the representativeness of different lineages across the sites (Duarte et al. 2014). Finally, we performed a principal coordinates analysis, based on square root transformed Bray–Curtis dissimilarities, on matrix **P** to obtain the PCPS, the resulting eigenvectors describing an orthogonal phylogenetic gradient in the data set. PCPS with the highest eigenvalues describe

wide phylogenetic gradients related to the deepest nodes in the phylogenetic trees like superorders or orders and as the eigenvalues decrease, finer taxonomic relationships are described (Duarte et al. 2014).

To evaluate the influence of the environmental gradient on species assembly in the sampled sites, we tested the association of each PCPS with the site properties (site categories and isolation) through linear models (LM). Before this procedure, we tested all possible models containing site categories and/or isolation (log-transformed) with each PCPS containing at least 5% of explanation on matrix **P**. We selected the best model based on Akaike's criterion (AIC; Burnham & Anderson 2002) that presented the lower AIC value compared to a null model, in which the response variable is explained by its median and variance. The best model of each PCPS was tested through LM using two null models (Duarte et al. unpubl.), one that shuffles tip names across the phylogeny (*taxa shuffle*) and the other that shuffles the sites across the environmental gradient (*site shuffle*). The *site shuffle* tests the combined association between species and phylogenetic composition and the environmental gradient, while the *taxa shuffle* null model evaluates the association between phylogenetic composition (species composition is kept unchanged) and the environmental gradient. If both tests return significant p values, the association between species distribution across sites and the environmental gradient is mediated by the phylogenetic relationships among species. On the other hand, if only site shuffle returns a significant p-value, species composition is related to the environmental gradient, but such association is independent of the phylogenetic relationships among species.

To evaluate which traits were related to the patch size/isolation gradient, we calculated a matrix of community weighted mean trait value (CWM; Garnier et al. 2004), using matrix **W** and a binary matrix containing each species trait. The CWM calculates the average of trait values in each community weighted by the relative abundances of each species. We performed

a correlation of each trait of CWM with each PCPS for which the AIC model selection presented better models than the null model tested.

All analyses were performed in the R Statistical Environment (R Foundation for Statistical Computing, Vienna, AT; http://www.R-project.org). The treeNODF analysis was calculated using the package *CommEcol* v. 1.6.0 (Melo et al. 2014). The PCPS and LM (function *pcps.sig*) analyses were performed using the package *PCPS* v.1.0.2 (Debastiani & Duarte 2014). The model selections based on AIC criterion were performed in the package *MuMIn* v. 1.13.4. The CWM was calculated using the package *SYNCSA* v. 1.3.2 (Debastiani & Pillar 2012).

Results

We surveyed 921 individuals belonging to 46 species, 30 genera and 17 families (Appendix S4). The richest families were Asteraceae (16 species), Apocynaceae (6) and Bignoniaceae (4), comprising 56.5% of the total richness. Seven genera contained two or more species, comprising 50% of species, which *Mikania* Willd., with 11 species, was the richest one. Five species comprised 60.8% of total abundance. The most common climbing mechanism was stem twiner, followed by tendril and scrambler (Appendix 5). There was a predominance of anemochoric dispersal syndrome (33 species and 746 individuals), followed by zoochoric (12 and 174). Within anemochory, there was a majority of pogonochory (24 species and 655 individuals) than pterochory (9 and 91). Within pogonochory, small diaspores were more common than big diaspores while within pterochory, membranaceous diaspores were more common, followed by big diaspores (Appendix 5). Comparing zoochoric species, fleshy small diaspores were the most common, followed by fleshy medium. Regarding site categories, single-tree patches had the lowest average species richness and abundance per plot (Table 1). Small and large patches were very similar on the average richness per plot, but small patches

showed the highest average abundance per plot. Single-tree and small patches presented two exclusive species each, while big patches and continuous forest present six and 11 exclusive species respectively. Clasp tendril and barochory were the only traits restricted to only one site category (continuous forest). Only six species were presented in all site categories (Appendix S4).

The effect of site categories (patches area) on nestedness was significant for the phylogenetic and two out of the five functional diversities (Table 2). Functional nestedness was significant for the combination of dispersal syndromes and diaspores types, and the combination of all traits. The results indicate that diversities in the smaller patch categories are subsets of larger ones and forest sites. These significant results for treeNODF, however, represent the joint effect of species and phylogenetic/functional diversities, since neither the S.fraction nor the topo.NODF presented significant results (Table 2).

The principal coordinates analysis performed on matrix **P**, generated 26 PCPS (eigenvectors). The first four PCPS axes contained each at least 5% of total variation in matrix **P** and jointly explained 63.9% of the total variation. These four PCPS were submitted to a model selection based on AIC with the site properties (site category and isolation) and only the first two PCPS presented one or more models with AIC values lower than the null model (Table 3). For the first PCPS, a single model was important and included the isolation variable. For the second PCPS all tested models presented AIC values lower than the null model and the best one included the variable site category. With the selected models for each PCPS, we performed the LM analyses that presented significant results only for the *site shuffle* null model. The isolation explained 12.7 % of variation in PCPS1, while site categories explained 48% of variation in PCPS2 (Table 3). In PCPS2, single-tree patches significantly differed from other site categories ($P < 0.001$). These results show that for both PCPS there is an influence of predictor variables on species assembly independently of phylogeny.

Finally, we correlated the first and second PCPS, used in the ordination scatter plot, with the CWM (for correlation results see Appendix S6). For the first PCPS, four traits were significantly correlated (Fig. 2), ranging from r = -0.39 to 0.76. Within these traits plumed small diaspores presented the highest correlation (r = 0.76), being related to more isolated sites where Asteraceae species are better represented (Fig. 2). In the second PCPS nine of the 21 traits were significantly correlated, ranging from r = -0.75 to 0.62. Within these traits the highest positive correlations were presented by plumed big diaspores and pogonochory (r = 0.62 and 0.61), showing an association with single-tree patches mainly represented by Apocynaceae species. The highest negative correlations were shown by hook/spine and fleshy small diaspores (r = -0.75 and -0.59), being associated with large patches and forest sites.

Discussion

This study demonstrates that there is a habitat filtering on species composition throughout the process of Araucaria forest expansion over grassland, and this is mediated by patch area and isolation. Moreover, our results partially agree with our previous expectation showing a nested pattern along the patch size gradient, but explained by a joint effect of species and phylogenetic/functional diversities. Further, dispersal syndromes and diaspores types were more structured in the patch size/isolation gradient than climbing methods.

The nested pattern along the patch size gradient demonstrate that the forest expansion over another ancient vegetation type like old-growth grasslands, presents the opposite of turnover patterns found during the succession of tropical forests in post-agricultural areas (Yuan et al. 2009; Letcher 2015). This pattern indicates a tendency that some species occur in all stages of succession and that some others are restricted to large patches and/or forest sites. Climbing plants have a higher tree fall surviving, and some long-lived species establish early and persist throughout the forest development (DeWalt et al. 2000). Furthermore, the high

number of species exclusive to continuous forest sites could be an indicative of species that only stablish and grow in shaded environments, like the differentiation of early and late-successional species as found by Letcher (2015).

In fragmented landscapes, a phylogenetic nested pattern occurs if dispersal ability is a strongly conserved trait in the phylogeny (Melo et al. 2014). In this study, where forest patches are not the outcome of fragmentation and forest development occurs by processes different of post-disturbance recovery, the significant results of treeNODF highlights that specific phylogenetic lineages spread over the patch size gradient are responsible for colonizing patches at initial stage of development. The PCPS analysis clarified which phylogenetic clades, and their association with functional traits, were responsible for the colonization of patches at initial development stages, showing the relationship of some clades with site categories and site isolation (Fig. 2). It should be noted that the PCPS did not present a significant association of phylogenetic composition and the patches size/isolation gradient, but it showed which lineages and traits were more abundant in the gradient. Pogonochoric species were prevalent in single-tree patches, with small plumed diaspores characteristic of Asteraceae family reaching sites more isolated, while big plumed diaspores were associated with less isolated sites, mostly due to species of Apocynaceae family. The low weight of pogonochoric diaspores enhances their capacity of long distance dispersal compared to pterochoric diaspores, which showed a correlation with larger and less isolated sites. Associated with their long distance dispersal, the abundance of pogonochoric species in single-tree patches could be explained by the fact that the anemochoric syndrome is prevalent in the area but also in climbing species in the Neotropics (Gallagher & Leishman 2012).

Letcher and Chazdon (2012) found an association of abiotic dispersal syndromes with early successional stages but no trend in zoochoric species, while DeWalt et al. (2003) found no trend on zoochory and anemochory of lianas across a chronosequence in Panama. In contrast

with the mentioned studies, our results showed the association of zoochoric species, particularly small fleshy diaspores, with large patches and forest sites, agreeing with Duarte et al. (2006b), which found the increase of zoochoric sapling species along the patch size gradient. In a study with frugivorous birds in the study area, Hartz et al. (2012) showed that migratory species occurred more frequently in forest interior and in the forest patches, while resident species were more frequent in forest edges and forest patches. The correlation of zoochoric species with most forest and large patch sites, possibly shows that in large patches the abundance of climbing plants may help restore the light environment, microclimatic conditions, and vegetation structure that forest-interior frugivorous birds prefer (Michel et al. 2015), but also present well developed edges that could be visited by resident birds. The low abundance and the absence of zoochoric species in most single-tree patches may indicate that most frugivorous species are specialized to well-developed forest environments.

Not only the dispersal capacity determines climbing plants distribution, but also the availability of suitable supports influences each climbing mechanism. Trellis (young plants and small-diameter branches) density, higher on forest edges and treefall gaps, facilitate the access to the canopy, since few species are capable to climb supports with more than 10-20 cm of diameter (Putz 1980, 1984; Putz & Holbrook 1991). In this way, each climbing method has support limitations that could direct its occurrence and abundance to particular vegetation stands (Hegarty & Caballé 1991). In our study, the vegetation structure completely changes along the patch size gradient from single-tree patches to patches of increasing size and to the continuous forest. Thus, we should expect that the climbing mechanisms would increase in diversity and abundance following the patch size gradient and presenting a nested pattern. Our results do not confirm this expectation, since there was a functional nestedness when evaluating climbing mechanisms and dispersal traits together, instead of when evaluating climbing mechanisms alone.

Besides the accessibility of suitable supports, the high light availability at forest edges and treefall gaps seems to determine the high density and diversity of climbing plants on these habitats (DeWalt et al. 2000; Londré & Schnitzer 2006). In this study, small patches represent an intermediate stage of forest development with an open canopy and an understory with variable height that sometimes reaches trees canopy, as opposed to single-tree patches. In forests with a well-developed understory, climbing plants use trellis as a support and reduce the dependence on lower branches of trees for reaching the canopy (Campbell & Newbery 1993). Moreover, the abundance of shrub/treelet in the low strata of vegetation patches may account for a greater richness and abundance of climbing plants due to the increasing of trellis availability (Garbin et al. 2012). In this study, the high light availability and trellis density might explain the high species abundance in small patches compared to other site categories, and this could explain why we did not find a nested pattern on climbing mechanisms distribution over the patch size gradient. The height of the lowest extremities of the host-tree, i.e. crown depth, was shown as one of many determinants of climbing plants occupation (Balfour & Bond 1993; Campbell & Newbery 1993). Interestingly, single-tree patches did not bear climbing plants in their crowns. This fact is possibly explained by the dominance of *A. angustifolia* species that always presents a shallow crown, and an insufficient understory height to provide it a bridge.

The present study showed that the distribution of climbing plants in the process of forest expansion over grassland is structured by phylogeny and dispersal traits. The patterns found, shows that the differentiation of vegetation structure through the patch size gradient and the patches isolation, influence the distribution of specific lineages and traits. Our findings highlight that taking into account a phylogenetic and functional approach on nestedness analysis, exploring the patterns of phylogeny-weighted species composition and integrating it

with patches properties, improves our knowledge of the colonization and forest expansion dynamics.

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Table 1. Description of site categories in relation to sampling effort, species richness and abundance at Pró-Mata Research and Nature Conservation Center, RS, Brazil.

	Sites	Plots	Total area (ha)	Species		Individuals	
				N°	Average per plot (range)	N°	Average per plot (range)
Forest	4	24	0.06	33	5.92 (2 - 10)	336	14 (2 - 29)
Large patches	6	24	0.06	28	5.29 (2 - 10)	300	12.5 (5 - 35)
Small patches	7	14	0.035	20	5.15 (2 - 8)	217	15.5 (5 - 25)
Single-tree patches	12	12	0.03	12	2.33 (1 - 4)	68	5.67 (2 - 12)

Table 2. Phylogenetic, functional and species nestedness results according to site categories ordering, under the constructed permutation test with 9999 permutations. Significant results highlighted in bold. CM – Climbing Methods; DS – Dispersal Syndromes; DT – Diaspore Types.

	treeNODF	***P***	**S.fraction**	***P***	**topoNODF**	***P***
Phylogenetic	60.56	**0.04**	32.72	0.091	27.84	0.327
Functional (CM)	52.64	0.181	32.41	0.096	20.24	0.476
Functional (DS)	58.21	0.116	32.41	0.095	25.8	0.384
Functional (DS+DT)	62.15	**0.048**	32.51	0.095	29.64	0.321
Functional (CM+DS)	56.31	0.108	32.55	0.096	23.8	0.407
Functional (CM+DS+DT)	57.27	**0.048**	32.51	0.092	24.76	0.385

Table 3. Results of models selection based on Akaike's information criterion (AIC) between the first two PCPS and the landscape properties (area and isolation), and the linear model results of models with lower AIC values compared to the null model. The significance of linear model was evaluated via two null models, one that shuffles tip names across the phylogeny and the other that shuffles the sites across the environmental gradient.

Models	Information-based model selection					R^2 Adj.	Permutation test		
	Log likelihood	K	AICc	ΔAICc	w_i		F-value	P site shuffle	P taxa shuffle
PCPS 1									
Isolation	20.05	3	-33.0	0	0.594	0.13	4.78	0.035	0.141
Null (Mean + σ^2)	17.68	2	-30.9	2.19	0.199				
Site category + Isolation	23.49	6	-30.8	2.26	0.191				
Site category	19.33	5	-25.8	7.26	0.016				
PCPS 2									
Site category	32.84	5	-52.8	0	0.80	0.48	8.92	< 0.001	0.155
Site category + Isolation	33.09	6	-50.0	2.83	0.195	0.46	6.64	0.001	0.18
Isolation	24.53	3	-42.0	10.79	0.004	0.11	4.25	0.048	0.242
Null (Mean + σ^2)	22.41	2	-40.3	12.49	0.002				

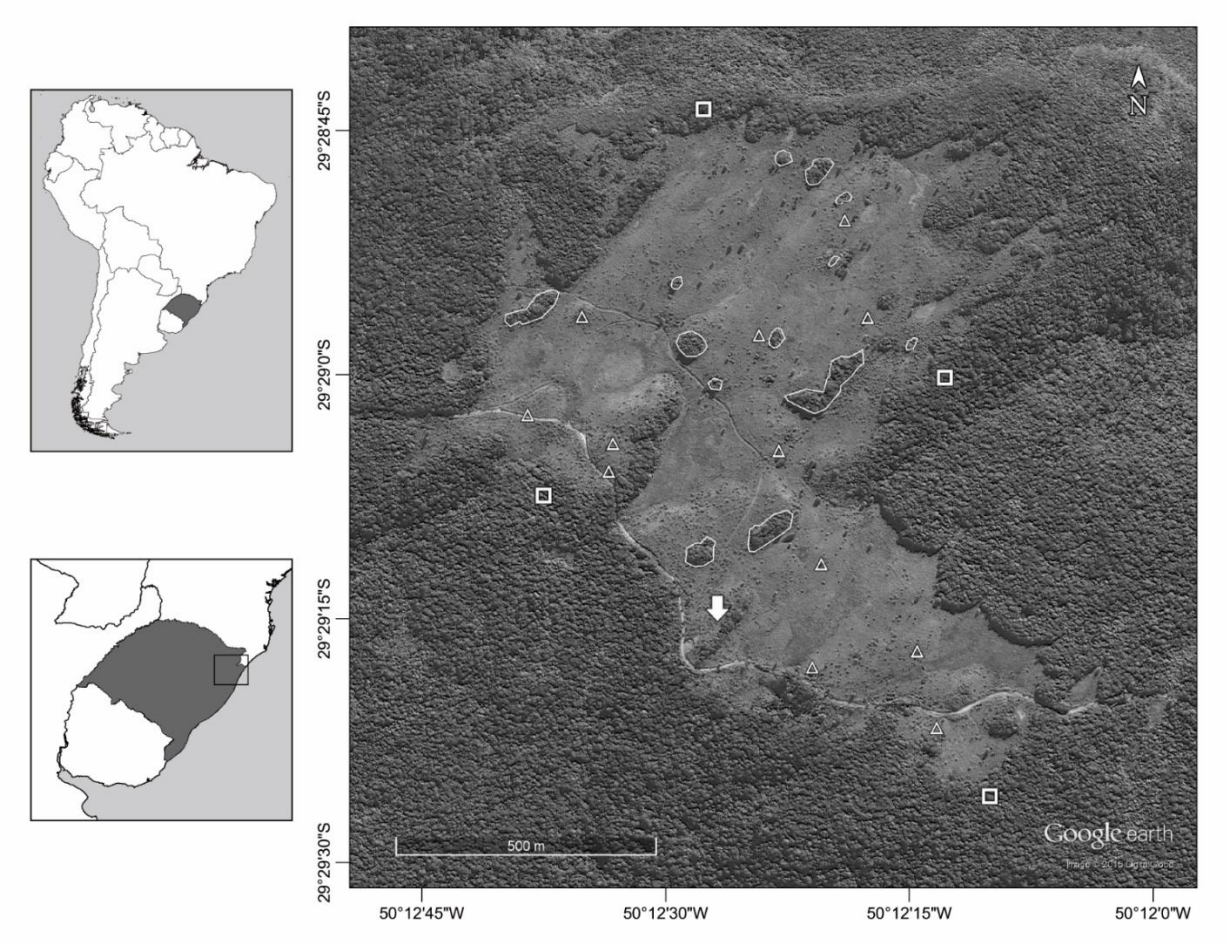

Fig. 1. Location of Pró-Mata Research and Nature Conservation Center, RS, Brazil (29°28'S, 50°13' W) and a satellite image (obtained from Google Earth Pro© 2015) showing the sampled forest patches (white circumscribed) within the grassland area. Triangles represent single-tree patches and squares represent the forest sites. The arrow points an example of a forest patch not surveyed but used in calculations of the proximity index.

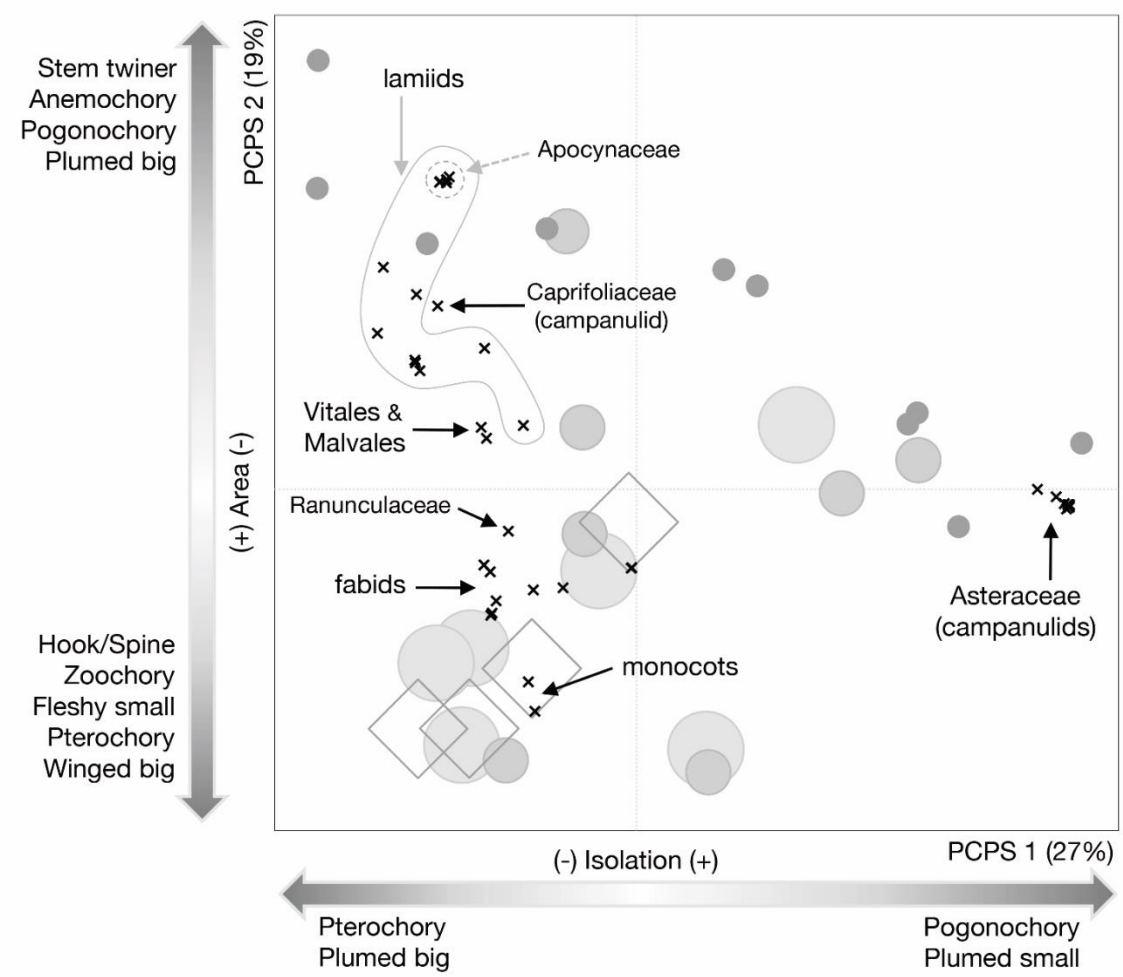

Fig. 2. Scatter diagram of the first two principal coordinates of phylogenetic structure (PCPS) of climbing plants species occurring along the sites size/isolation gradient of forest expansion over grassland. Single-tree, small and large patches are represented by circles of different sizes, and continuous forest sites by grey squares. The "x" symbols represent species with an indication of their phylogenetic clades. Significant properties of forest patches through linear models and traits correlated with each PCPS are specified above and below arrows.

Appendix S1. Phylogenetic tree and molecular phylogeny reconstruction workflow. The phylogenetic tree contains as node names the bootstrap support values.

Phylogenetic tree in newick format

(((((((Forsteronia_glabrescens:0.06612188334,Mandevilla_atroviolacea:0.06612188334)100:0.1097685196,((Oxypetalum_mosenii:0.05950939414,Peplonia_axillaris:0.05950939414)60:0.006456351912,(Jobinia_connivens:0.04612726094,Orthosia_scoparia:0.04612726094)94:0.01983848512)100:0.1099246569)100:0.3494738919,(Manettia_verticillata:0.3126575509,Galium_hypocarpium:0.3126575509)100:0.2127067439)100:0.08596634081,((Amphilophium_crucigerum:0.08260961354,(Tanaecium_selloi:0.06513355775,(Dolichandra_unguiscati:0.032566778875,Dolichandra_uncata:0.032566778875):0.032566778875)70:0.01747605579)100:0.5254697011,(Convolvulus_crenatifolius:0.5273161936,(Solanum_flaccidum:0.2636580968,Solanum_inodorum:0.26365809688):0.2636580968)100:0.08076312095)100:0.00325132109)100:0.06983043097,((Mutisia_speciosa:0.1909311961,(Piptocarpha_ramboi:0.1754924132,(Baccharis_anomala:0.1661243199,(Pentacalia_desiderabilis:0.1565380735,(Calea_serrata:0.05888012744,(Mikania_burchellii:0.02944006372,Mikania_campanulata:0.02944006372,Mikania_glomerata:0.02944006372,Mikania_hirsutissima:0.02944006372,Mikania_involucrata:0.02944006372,Mikania_laevigata:0.02944006372,Mikania_orleansensis:0.02944006372,Mikania_paranensis:0.02944006372,Mikania_smaragdina:0.02944006372,Mikania_sp:0.02944006372,Mikania_ternata:0.02944006372):0.029440006372)100:0.09765794601)75:0.009586246446)100:0.009368093282)100:0.01543878287)100:0.4748835851,Valeriana_scandens:0.6658147811)100:0.01534628551)100:0.08595672617,(((((Cayaponia_palmata:0.006520905634,Cayaponia_pilosa:0.006520905634)100:0.659431773,(Rubus_sellowii:0.33297633935,Rubus_erythrocladus:0.33297633935):0.33297633935)100:0.005972515247,((Heteropterys_aenea:0.03325233952,Heteropterys_intermedia:0.03325233952)100:0.5208609652,((Passiflora_caerulea:0.2607474288,Passiflora_organensis:0.2607474288)100:0.2601001233,Anchietea_pyrifolia:0.5208475521)100:0.03326575257)100:0.1178118892)100:0.03915511622,Fuchsia_regia:0.7110803101)100:0.0156130001,Cissus_striata:0.7266933102)100:0.0404244826)100:0.1487037193,Clematis_bonariensis:0.9158215121)100:0.08417848792,(Dioscorea_multiflora:0.6550253496,Smilax_cognata:0.6550253496)100:0.3449746504):0.1481660659;

Molecular phylogeny reconstruction

The phylogenetic tree was reconstructed using molecular sequences available online at GenBank (accessed in January 2015; Benson et al. 2013). For this, we downloaded sequences of nuclear (ITS1 and ITS2) and chloroplastidial markers (rbcL, matK, rps16, trnL-trnF spacer, trnL intron, psbA-trnH spacer and NADH dehydrogenase subunit F) containing coding and non-coding regions, known to resolve species relationships at higher and lower taxonomic levels. At GenBank we checked for species names synonyms and found sequences for 16 of 46 sampled species. For 18 genera without sequence data, we used sequences of congeneric relatives always looking for climbing species that occur geographically close to the sampled area and of the same taxonomic tribe. Within these genera, we used sequences of only one

species and the 12 remaining species without sequence were manually merged in the resulting phylogeny, splitting them halfway along their congener branch with sequence data or positioning it as a polytomy at the genus node if there was more than one congener.

To align the sequences we used the MAFFT online software (available at http://mafft.cbrc.jp/alignment/server/), using the option to adjust direction according to the first sequence for highly divergent taxa, which found and corrected some sequences that were reverse complements. In MAFFT online software we used the Q-INS-i alignment strategy (Katoh & Toh 2008) for the markers ITS1, ITS2, rps16, psbA-trnH spacer, trnL-trnF spacer, and trnL intron, which takes into account a secondary structure information of RNA. For rbcL, matK and NADH dehydrogenase subunit F we used the E-INS-i alignment strategy. Using Mesquite 2.75 software (Maddison & Maddison 2011) we checked and excluded some misaligned species sequences, and manually trimmed alignments tips. After this treatment the alignments were concatenated in a supermatrix using the software FASconCAT (Kück & Meusemann 2010).

The phylogenetic tree was reconstructed using a maximum-likelihood approach (ML) through the raxmlGUI 1.3.1 software (Silvestro & Michalak 2012). We chose the GTR+GAMMA+I evolutionary model and set partitions for each marker. We defined the ML searches to 1,000 times and estimated a bootstrap support value for each node. We chose *Nymphaea alba* L. (Nymphaeaceae), an early divergent angiosperm species, as the outgroup and used a backbone constraint tree reconstructed using the Phylocom/Phylomatic software (Webb & Donoghue 2005; Webb et al. 2008). The constraint tree was used to limit the software searches to trees compatible to its topology, reducing the artifact of patchy dataset (Roquet et al. 2013). The constraint tree followed the phylogenetic hypothesis for angiosperm plants of APG III (APG 2009) to the order level and resolving relationships among families according to the Angiosperm Phylogeny Website (Stevens 2001). For this, we used the “megatree” R20120829

(available at https://github.com/camwebb/tree-of-trees/blob/master/megatrees/R20120829.new) and removed single nodes and intra-familial resolution, so it resolves species relationships to a family level (relationships within families are represented as polytomies). The branch lengths of the constraint tree were defined using the clade age estimates proposed by Bell et al. (2010).

The resulting phylogeny had their branch lengths proportional to the rate of evolution of the used markers and we transformed it to be proportional to divergence time through rate smoothing, setting the root age to one. For this, we used the maximum likelihood approach with discrete rate variation (Paradis 2013), setting the smoothing parameter (lambda) to one. The molecular dating with maximum likelihood approach was calculated through the function *chronos* of the package *ape* v.3.2, (Paradis et al. 2004) in the R Statistical Environment (R Core Team 2015).

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Appendix S2. Functional trees in newick format.

Climbing methods

(cissus_striata:0.5,((solanum_inodorum:0,(clematis_bonariensis:0,solanum_flaccidum:0):0):0.5,((dioscorea_multiflora:0.3535533906,(anchietea_pyrifolia:0,(manettia_verticillata:0,(heteropterys_intermedia:0,(heteropterys_aenea:0,(convolvulus_crenatifolius:0,(valeriana_scandens:0,(mikania_ternata:0,(mikania_sp:0,(mikania_smaragdina:0,(mikania_paranensis:0,(mikania_orleansensis:0,(mikania_laevigata:0,(mikania_involucrata:0,(mikania_hirsutissima:0,(mikania_glomerata:0,(mikania_campanulata:0,(mikania_burchellii:0,(peplonia_axillaris:0,(oxypetalum_mosenii:0,(orthosia_scoparia:0,(mandevilla_atroviolacea:0,(forsteronia_glabrescens:0,jobinia_connivens:0):0):0):0):0):0):0):0):0):0):0):0):0):0):0):0):0):0):0):0):0):0.3535533906):0.1458094448,((smilax_cognata:0.3535533906,(passiflora_organensis:0,(passiflora_caerulea:0,(cayaponia_pilosa:0,(cayaponia_palmata:0,(tanaecium_selloi:0,(mutisia_speciosa:0,amphilophium_crucigerum:0):0):0):0):0):0):0.3535533906):0.1441528167,((dolichandra_uncata:0,dolichandra_unguiscati:0):0.4885310363,((rubus_erythrocladus:0,rubus_sellowii:0):0.3626692072,(pentacalia_desiderabilis:0.3535533906,(galium_hypocarpium:0,(fuchsia_regia:0,(piptocarpha_ramboi:0,(baccharis_anomala:0,calea_serrata:0):0):0):0):0.3535533906):0.009115816645):0.1258618291):0.009175170954):0.001656628089):0.0006371646496):0);

Dispersal syndromes

((cissus_striata:0,(solanum_inodorum:0,(solanum_flaccidum:0,(smilax_cognata:0,(galium_hypocarpium:0,(rubus_sellowii:0,(rubus_erythrocladus:0,(passiflora_organensis:0,(passiflora_caerulea:0,(fuchsia_regia:0,(cayaponia_palmata:0,cayaponia_pilosa:0):0):0):0):0):0):0):0):0):0):0):0.5,(convolvulus_crenatifolius:0.5,((clematis_bonariensis:0,(valeriana_scandens:0,(piptocarpha_ramboi:0,(pentacalia_desiderabilis:0,(mutisia_speciosa:0,(mikania_ternata:0,(mikania_sp:0,(mikania_smaragdina:0,(mikania_paranensis:0,(mikania_orleansensis:0,(mikania_laevigata:0,(mikania_involucrata:0,(mikania_hirsutissima:0,(mikania_glomerata:0,(mikania_campanulata:0,(mikania_burchellii:0,(calea_serrata:0,(baccharis_anomala:0,(peplonia_axillaris:0,(oxypetalum_mosenii:0,(orthosia_scoparia:0,(mandevilla_atroviolacea:0,(forsteronia_glabrescens:0,jobinia_connivens:0):0):0):0):0):0):0):0):0):0):0):0):0):0):0):0):0):0):0):0):0):0):0.4082482905,(anchietea_pyrifolia:0,(manettia_verticillata:0,(heteropterys_intermedia:0,(heteropterys_aenea:0,(dioscorea_multiflora:0,(dolichandra_unguiscati:0,(dolichandra_uncata:0,(amphilophium_crucigerum:0,tanaecium_selloi:0):0):0):0):0):0):0):0):0.4082482905):0.09175170954):0);

Dispersal syndromes + Diaspore types

(((solanum_flaccidum:0,(rubus_sellowii:0,(rubus_erythrocladus:0,(passiflora_organensis:0,(fuchsia_regia:0,(cayaponia_palmata:0,cayaponia_pilosa:0):0):0):0):0):0):0.3535533906,(passiflora_caerulea:0.3535533906,(cissus_striata:0,(solanum_inodorum:0,(galium_hypocarpium:0,smilax_cognata:0):0):0):0.3535533906):0):0.1464466094,(convolvulus_crenatifolius:0.5,(((heteropterys_aenea:0,heteropterys_intermedia:0):0.3535533906,((anchietea_pyrifolia:0,(dolichandra_unguiscati:0,(dolichandra_uncata:0,(amphilophium_crucigerum:0,tanaecium_selloi:0):0):0):0):0.3535533906,(dioscorea_multiflora:0,manettia_verticillata:0):0.3535533906):0):0.102882074,((clematis_bonariensis:0,(mutisia_speciosa:0,(peplonia_axillaris:0,(oxypetalum_mosenii:0,(orthosia_scoparia:0,(mandevilla_atroviolacea:0,(forsteronia_glabrescens:0,jobinia_connivens:0):0):0):0):0):0):0):0.3535533906,(valeriana_scandens:0,(piptocarpha_ramboi:0,(pentacalia_desiderabilis:0,(mikania_ternata:0,(mikania_sp:0,(mikania_smaragdina:0,(mikania_paranensis:0,(mikania_orleansensis:0,(mikania_laevigata:0,(mikania_involucrata:0,(mikania_hirsutissima:0,(mikania_glomerata:0,(mikania_campanulata:0,(mikania_burchellii:0,(baccharis_anomala:0,calea_serrata:0):0):0):0):0):0):0):0):0):0):0):0):0):0):0.3535533906):0.102882074):0.04356453541):0);

Climbing methods + Dispersal syndromes

((cissus_striata:0.3535533906,((solanum_flaccidum:0,solanum_inodorum:0):0.3535533906,((smilax_cognata:0.25,(passiflora_organensis:0,(passiflora_caerulea:0,(cayaponia_palmata:0,cayaponia_pilosa:0):0):0):0.25):0.09706556499,((fuchsia_regia:0,galium_hypocarpium:0):0.25,(rubus_erythrocladus:0,rubus_sellowii:0):0.25):0.0970

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Climbing methods + Dispersal syndromes + Diaspore types

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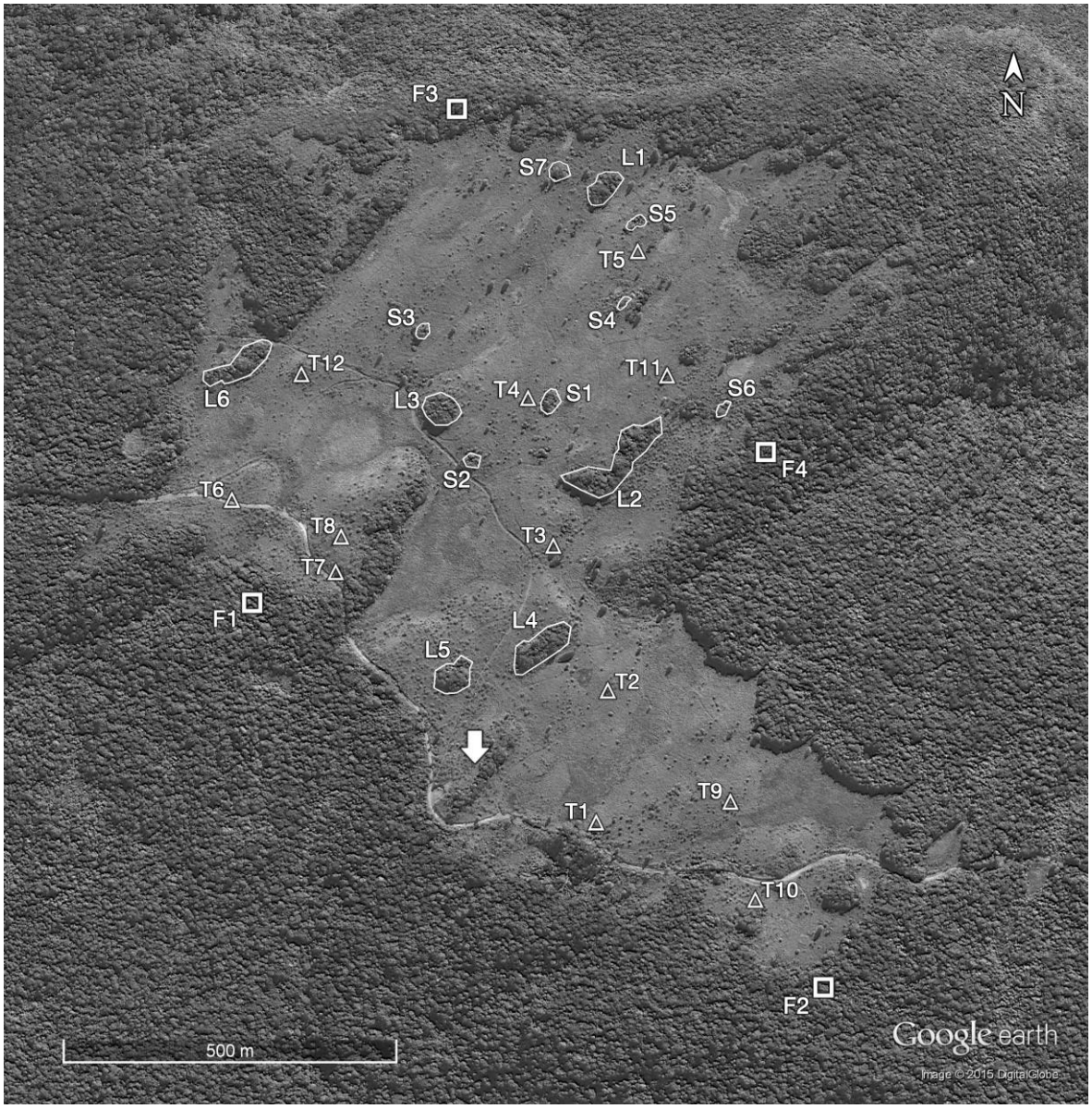
Appendix S3. Table with the values of the area (m^2), distance to the continuous forest (m) and the Proximity Index of each surveyed patch, and the map providing their location at Pró-Mata Research and Nature Conservation Center, RS, Brazil. T – Single-tree patches (triangles in the map); S – Small patches; L – Large patches; F – Forest sites (squares in the map). The arrow points an example of a forest patch not surveyed but used in calculations of the proximity index.

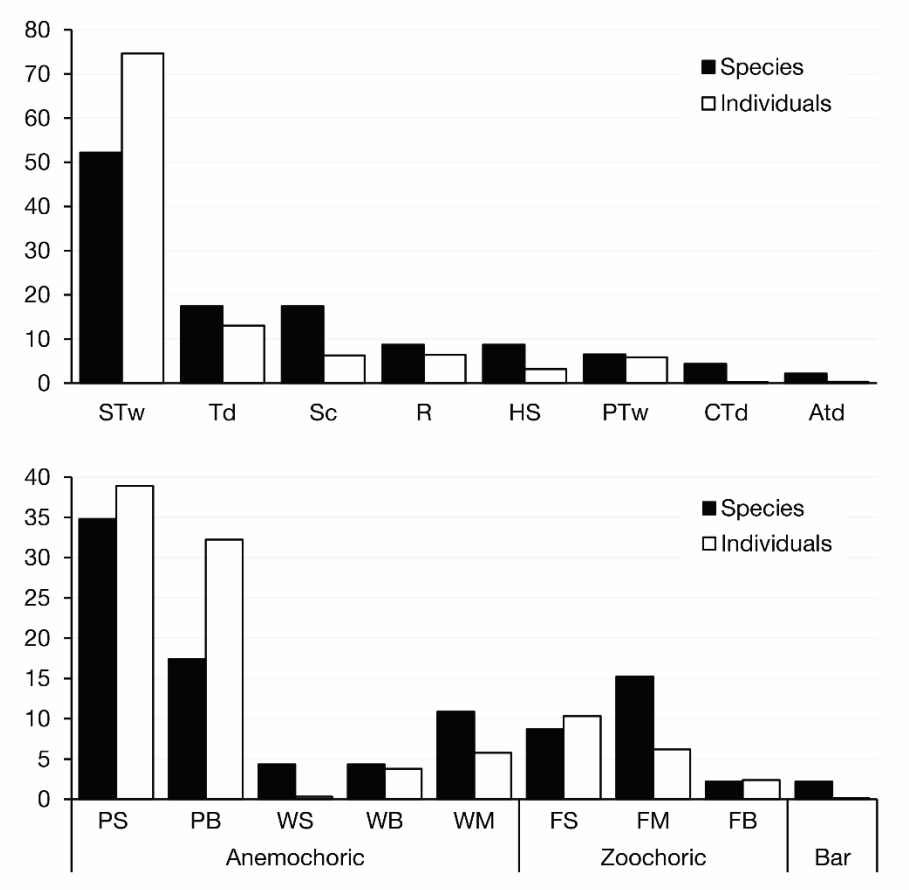
Patch	Area (m^2)	Forest distance (m)	Proximity Index
T 1	25	32.64	1257.21
T 2	25	110.37	187.54
T 3	25	67.65	335.41
T 4	25	255.36	74.28
T 5	25	124.65	172.41
T 6	25	53.39	563.59
T 7	25	19.64	3266.60
T 8	25	72.53	370.70
T 9	25	86.92	265.69
T 10	25	14.07	6072.30
T 11	25	120.46	207.60
T 12	25	60.05	424.14
S 1	206.93	215.54	87.85
S 2	308.01	231.36	78.12
S 3	355.94	141.97	116.99
S 4	409.09	174.80	127.65
S 5	442.04	91.73	255.34
S 6	669.58	24.92	2084.36
S 7	739.50	33.53	1223.98
L 1	1706.03	47.69	693.14
L 2	1981.33	66.38	403.25
L 3	2220.25	212.28	76.10
L 4	3076.39	58.70	435.41
L 5	3521.99	43.35	829.73
L 6	5904.74	11.00	9860.47


F3
N
S7
L1
S5
T5
S3
S4
T12
T11
L6
L3
T4
S1
S6
F4
S2
L2
T6
T8
T3
T7
F1
L4
L5
T2
T9
T1
T10
F2
500 m
Google earth
Image © 2015 DigitalGlobe

Appendix S4. Sampled species with their respective climbing mechanisms, dispersal syndrome, diaspore type and abundance within site categories at Pró-Mata Research and Nature Conservation Center, RS, Brazil. Climbing Mechanism (CM): STw – Stem Twiner; PTw – Petiole Twiner; Td – Tendril; CTd – Clasp Tendril; ATd – Adhesive Tendril; Sc – Scrambler; HS – Hook/Spine; R – Adherent Roots. Dispersal Syndromes (DS): A – Anemochoric; B – Barochoric; Z – Zoochoric; Pg – Pogonochoric; Pt – Pterochoric. Diaspore Types (DT): FS – Fleshy Small; FM – Fleshy Medium; FB – Fleshy Big; PS – Plumed Small; PB – Plumed Big; WS – Winged Small; WB – Winged Big; WM – Winged Membranaceous.

Family/Species	**CM**	**DS**	**DT**	**Abundance**			
				Single-tree	**Small**	**Large**	**Forest**
APOCYNACEAE							
Forsteronia glabrescens Müll. Arg.	STw	A (Pg)	PB	0	0	0	6
Jobinia connivens (Hook. & Arn.) Malme	STw	A (Pg)	PB	0	0	2	0
Mandevilla atroviolacea (Stadelm.) Woodson	STw	A (Pg)	PB	0	0	1	0
Orthosia scoparia (Nutt.) Liede & Meve	STw	A (Pg)	PB	23	61	65	49
Oxypetalum mosenii (Malme) Malme	STw	A (Pg)	PB	0	0	1	0
Peplonia axillaris (Vell.) Fontella & Rapini	STw	A (Pg)	PB	2	21	6	49
ASTERACEAE							
Baccharis anomala DC.	Sc	A (Pg)	PS	4	0	0	0
Calea serrata Less.	Sc	A (Pg)	PS	0	5	1	1
Mikania burchellii Baker	STw	A (Pg)	PS	0	11	23	12
Mikania campanulata Gardner	STw	A (Pg)	PS	2	1	4	0
Mikania glomerata Spreng.	STw	A (Pg)	PS	0	0	1	0
Mikania hirsutissima DC.	STw	A (Pg)	PS	0	4	0	0
Mikania involucrata Hook. & Arn.	STw	A (Pg)	PS	0	0	0	1
Mikania laevigata Sch. Bip. ex Baker	STw	A (Pg)	PS	0	0	2	3
Mikania orleansensis Hieron.	STw	A (Pg)	PS	0	3	4	4
Mikania paranensis Dusén	STw	A (Pg)	PS	18	61	58	55
Mikania smaragdina Dusén ex Malme	STw	A (Pg)	PS	0	1	15	15
Mikania ternata (Vell.) B.L.Rob.	STw	A (Pg)	PS	0	1	0	7
Mikania sp.	STw	A (Pg)	PS	0	0	1	0
Mutisia speciosa Aiton ex Hook.	Td	A (Pg)	PB	8	0	0	0
Pentacalia desiderabilis (Vell.) Cuatrec.	Sc/R	A (Pg)	PS	2	8	10	10

Piptocarpha ramboi G. Lom. Sm	Sc	A (Pg)	PS	0	0	2	5
BIGNONIACEAE							
Amphilophium crucigerum (L.) L.G.Lohmann	Td	A (Pt)	WM	0	0	0	5
Dolichandra uncata (Andrews) L.G.Lohmann	CTd/R	A (Pt)	WM	0	0	0	1
Dolichandra unguis-cati (L.) L.G.Lohmann	CTd/R	A (Pt)	WM	0	0	0	1
Tanaecium selloi (Spreng.) L.G.Lohmann	Td	A (Pt)	WM	0	0	18	1
CAPRIFOLIACEAE							
Valeriana scandens L.	STw	A (Pg)	PS	0	0	0	2
CONVOLVULACEAE							
Convolvulus crenatifolius Ruiz & Pav.	STw	B	-	0	0	0	4
CUCURBITACEAE							
Cayaponia palmata Cogn.	Td	Z	FM	0	3	4	2
Cayaponia pilosa (Vell.) Cogn.	Td	Z	FM	1	4	10	6
DIOSCOREACEAE							
Dioscorea multiflora Mart. ex Griseb.	STw/HS	A (Pt)	WS	0	0	1	0
MALPIGHIACEAE							
Heteropterys aenea Gris.	STw	A (Pt)	WB	0	5	0	1
Heteropterys cf. *intermedia* (A. Juss.) Griseb.	STw	A (Pt)	WB	0	3	10	16
ONAGRACEAE							
Fuchsia regia (Vell.) Munz	Sc	Z	FM	1	0	0	3
PASSIFLORACEAE							
Passiflora caerulea L.	Td	Z	FB	0	10	10	2
Passiflora organensis Gardner	Td	Z	FM	0	0	0	12
RANUNCULACEAE							
Clematis bonariensis Juss. ex DC.	PTw	A (Pg)	PB	0	0	0	1
ROSACEAE							
Rubus erythrocladus Mart.	Sc/HS	Z	FM	0	0	0	2
Rubus sellowii Cham. & Schltdl.	Sc/HS	Z	FM	0	2	0	0
RUBIACEAE							
Galium hypocarpium (L.) Endl. ex Griseb.	Sc	Z	FS	0	4	11	7
Manettia glaziovii Wernham	STw	A (Pt)	WS	0	0	0	2
SMILACACEAE							
Smilax cognata Kunth	Td/HS	Z	FS	0	4	9	11
SOLANACEAE							
Solanum flaccidum Vell.	PTw	Z	FM	5	0	2	0
Solanum inodorum Vell.	PTw	Z	FS	1	5	25	15
VIOLACEAE							
Anchietea pyrifolia A. St.-Hil.	STw	A (Pt)	WM	0	0	2	25
VITACEAE							
Cissus striata Ruiz & Pav.	ATd	Z	FS	1	0	2	0



Appendix S5. The relative richness and abundance of climbing mechanisms and diaspore types at Pró-Mata Research and Nature Conservation Center, RS, Brazil. Climbing Mechanisms: STw – Stem Twiner; PTw – Petiole Twiner; Td – Tendril; CTd – Clasp Tendril; ATd – Adhesive Tendril; Sc – Scrambler; HS – Hook/Spine; R – Adherent Roots. Diaspore Types (DT): PS – Plumed Small; PB – Plumed Big; WS – Winged Small; WB – Winged Big; WM – Winged Membranaceous; FS – Fleshy Small; FM – Fleshy Medium; FB – Fleshy Big; Bar – Barochory.

Appendix S6. Correlation results of each community weighted means traits and the first two principal coordinates of phylogenetic structure (PCPS). Correlations significance was evaluated using permutation tests (10,000 permutations) using MULTIV 3.27b statistical software (by V. Pillar; available at http://ecoqua.ecologia.ufrgs.br/software). For details about climbing mechanisms, dispersal syndromes and diaspore types see the Material and Methods section in the main manuscript.

	PCPS1		**PCPS2**	
	r	***P***	***r***	***P***
Stem twiner	-0.213	0.292	0.391	0.045
Petiole twiner	-0.172	0.392	-0.009	0.965
Tendril	0.244	0.235	-0.264	0.185
Adhesive tendril	-0.352	0.074	0.216	0.317
Clasp tendril	-0.210	0.308	-0.285	0.168
Scrambler	0.235	0.237	-0.343	0.081
Hook/Spine	-0.284	0.144	-0.750	< 0.001
Adherent roots	0.173	0.408	-0.260	0.198
Zoochory	-0.236	0.238	-0.454	0.016
Barochory	-0.212	0.293	-0.235	0.370
Anemochory	0.245	0.217	0.462	0.015
Pogonochory	0.383	0.050	0.608	0.001
Pterochory	-0.390	0.043	-0.499	0.007
Fleshy small	-0.124	0.541	-0.590	0.001
Fleshy medium	-0.212	0.290	0.064	0.754
Fleshy big	0.053	0.795	-0.260	0.195
Plumed small	0.760	< 0.001	-0.257	0.194
Plumed big	-0.412	0.035	0.619	< 0.001
Winged small	-0.257	0.208	-0.292	0.158
Winged big	-0.345	0.071	-0.473	0.011
Winged membranaceous	-0.286	0.146	-0.349	0.068

Capítulo 3. Phylogenetic and climatic determinants of phenological processes in climbing plant assemblages[3]

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[3] Artigo formatado segundo as regras do periódico *Oikos*.

Climate has long been recognized as an important factor shaping plant phenology. Seasonal climatic variables can exert a strong influence on flowering and fruiting timing. Besides environmental cues, evolutionary history might be a strong determinant of phenological periods, with phylogenetically close species showing similar phenological activities. Under such scenario, phenological responses to temporal climatic gradients would be likely phylogenetically conserved in co-occurring plant species. In this study, we assessed phylogenetic conservatism in the reproductive phenology of a climbing plant community, evaluating if there is an association between the composition of flowering and/or fruit-bearing species and temporal climatic gradients, and to what extent such association is mediated by the phylogenetic relationships among species. For this, we estimated phylogenetic signal in flowering and fruiting peaks and used principal coordinates of phylogenetic structure (PCPS analysis) to test if phylogenetically closely flowering and/or fruit-bearing species respond similarly to climatic gradients. We observed low phylogenetic signal for flowering and fruiting peaks. On the other hand, PCPS analysis showed that flowering species from asterid and rosid clades responded similarly to daylength and the temperature of two month lag. For fruiting phenology, PCPS showed association only with lagged climatic variables, highlighting the effect of past events on triggering the fruiting periods. In this case, within major clades different lineages did not respond equally for the same climatic variable compared to flowering. The influence of shared evolutionary history on the association between phenological processes and temporal gradients varied across phylogenetic lineages of climbing plants, and depended on the climatic variable considered. Moreover, through the PCPS analysis we showed that event tough phenological patterns present a low phylogenetic signal, considering the co-occurrence of species in phenological activity reveals the importance of phylogenetic relatedness in the association of phenology and climate.

Keywords: Lianas; vines; phylogenetic signal; PCPS; model selection; temporal autocorrelation; Flowering; Fruiting.

Running head: Phylogenetic patterns on phenology

Introduction

The reproductive phenology of plants is a key trait directly influencing species fitness (Rathcke & Lacey 1985). Plant phenology and their association with climate have been widely studied over the last decades. Long-term studies show that climate changes can lead to shifts in plant phenology (Wright & Calderón 2006; Cleland et al. 2007; Willis et al. 2008). Seasonal climatic variables like daylength, temperature and rainfall have been recognized as rulers of phenological activity in many plant communities (e.g., van Schaik et al. 1993; Smith-Ramirez & Armesto 1994). For instance, in localities with a marked dry season, rainfall may determine plant phenology (Opler et al. 1976; Borchert 1983; but see Zimmerman et al. 2007); however, at higher latitudes daylength and temperature play a significant role (van Schaik et al. 1993; Ting et al. 2008). Furthermore, even in 'aseasonal' tropical sites with no dry period or great changes in temperature, a small variation in daylength can regulate plant phenology (Wright & van Schaik 1994; Morellato et al. 2000; Borchert et al. 2005).

Besides the influence of climate, phenological activity may also be phylogenetically conserved with close relatives presenting similar phenological patterns (e.g., Kochmer & Handel 1986; Smith-Ramirez & Armesto 1994; Staggemeier et al. 2010). Recently, studies demonstrated different degrees of phylogenetic signal on flowering and fruiting peaks of communities (e.g., Davies et al. 2013; Seger et al. 2013; CaraDonna & Inouye 2015; Du et al. 2015). Moreover, taxonomic families can show different flowering times (Kochmer & Handel 1986), presenting phylogenetically conserved phenological periods (Staggemeier et al. 2010), which may not be detected when evaluating the whole community composed of diverse clades (Seger et al. 2013). These evolutionary trends are integrated on the debate of the advantageous or deleterious effects of temporal partitioning on flowering and fruiting peaks of close relatives, as a strategy for avoiding competition for pollinators and dispersers (Levin 2006; Morales & Traveset 2008).

Different plant life forms can show distinct flowering periods (Kochmer & Handel 1986; Du et al. 2015). Although most phenological studies worldwide worked with tree or herb species, few focused on climbing plants communities (e.g., Putz & Windsor 1987; Ibarra-Manriquez et al. 1991; Opler et al. 1991; Morellato & Leitão-Filho 1996). Climbing plants compete with trees for resources above and below ground (Toledo-Aceves 2015), actively placing themselves in the most productive position directly exposed to sunlight (Hegarty & Caballé 1991). In localities with a marked dry season, climbing plants show discrete flowering and fruiting patterns compared to trees (Ibarra-Manriquez et al. 1991; Morellato & Leitão-Filho 1996). These authors observed that climbing plants are more active during the dry season when canopy trees lose their foliage, which enhances sunlight access, facilitates seed dispersal and avoids competition for pollinators.

As different families vary in their presentation of a phylogenetically conserved period of phenological activity (Seger et al. 2013), clades of different taxonomic levels could respond differentially to environmental cues. Moreover, specific clades may respond similarly to common climatic variables but may not present similar phenophase peaks (low phylogenetic signal). In this study, we aimed to unveil if the phylogenetic relationships among species mediate the association of species composition of flowering and fruiting with climatic gradients. We recorded the abundance of climbing plants over two years in a subtropical Araucaria forest in South Brazil, and calculated the phylogenetic signal in flowering, fruiting and time of diaspore development. Then, we used the principal coordinates of phylogenetic structure (PCPS analysis; Duarte 2011) to identify which phylogenetic clades may be structured along climatic gradients. We hypothesize that in both flowering and fruiting, phylogenetic clades may respond similarly to environmental cues even if they present distinct phenological peaks.

Material and Methods

Study area

The phenological study was conducted at the National Forest of São Francisco de Paula – ICMBio (FLONA-SFP), a federal conservation unit of sustainable use category in Southern Brazil (29°25'24''S, 50°23'13" W). FLONA-SFP is composed of a mosaic landscape of Araucaria forest remnants together with ecologically-managed *Araucaria angustifolia* (Bertol.) Kuntze, *Eucalyptus* spp. and *Pinus* spp. plantations and a small area of unmanaged *Campos* grassland. It covers 1,606 ha, where 36.6 % is covered by Araucaria forest remnants, ranging in altitude from 600 to 923 m a.s.l. (Seger & Hartz 2014). The regional climate is subtropical rainy, with precipitation uniformly distributed throughout the year. Photoperiod differs 3 h 47 min between the longest and shortest days. Historical data (1930-60) shows an annual mean rainfall of 2,252 mm and a mean month temperature ranging between 10.2 and 19.1 ºC, with the occurrence of negative temperatures from April to September, occasional frosts and rare events of snow (National Institute of Meteorology – INMET).

Phenological and environmental data

The flowering and fruiting phenology of climbing plant species was recorded monthly between September 2007 and August 2009, along 13.5 km of trails inside and at the edges of Araucaria forest remnants, in planting areas of *A. angustifolia* and at the edge of the Araucaria forest and *Campos* grassland ecotone. The number of individuals of each species bearing open flowers and ripe diaspores was recorded with the aid of binoculars, and fallen flowers or ripe fruits were considered as indicators of phenological activity when direct observation was not possible. We recorded 78 species belonging to 27 families and 43 genera, which both flowered and fruited during the study (species list in Appendix 1). This represents 76% of total climbing species reported for FLONA-SFP (Seger & Hartz 2014).

The climatic data was obtained from two sources. Since FLONA-SFP does not have an automated climatological station, we obtained data of mean temperature (T) and potential evapotranspiration (PET) from the climatological station of Cambará do Sul municipality (National Institute of Meteorology – INMET), which is at a similar altitude, around 47 km far from the study site. Rainfall (R) was measured *in situ* (ICMBio 2010, unpublished data). Besides T, PET and R we calculated the historical rainfall (Rh; 2003 to 2009) and the monthly mean daylength (D). Historical mean temperature was not used due to its high correlation with actual temperature. For climatic variables graphics see Appendix 2. As a biotic variable we also used the monthly leaf fall (LF) of tree community (estimated during 2008-2009; G.D.S. Seger unpublished data) measured by the Fournier's activity index (Fournier 1974), which shows the percentage of canopy expressing the phenophase. LF shows the year period with most sunlight reaching the understory, which may be important for climbing species that do not reach the canopy. For each climatic variable we also tested the effects of their monthly lags up to three months before the phenological event, totaling 23 variables.

Phylogenetic reconstruction

To estimate the phylogenetic relationships among species we reconstructed a molecular phylogeny (newick format in Appendix 3) using nine widely used genetic markers downloaded from GenBank (accessed in April 2015; Benson et al. 2013). The phylogenetic reconstruction was performed using a maximum-likelihood approach with estimated bootstrap support values for each node (for details about phylogenetic reconstruction workflow, see the Appendix 3). The resulting phylogeny had their branch lengths transformed to be proportional to divergence time through maximum likelihood approach with a discrete model of substitution rate variation (Paradis 2013). Then, we calculated the phylogenetic distances among pairs of terminal taxa.

Data analyses

To assess the phylogenetic signal (PS) in flowering and fruiting we applied the Blomberg's K Statistic (Blomberg et al. 2003). For this, we converted the dates of observation into days of the year and rescaled from 0° to 360°, where one degree is approximately equivalent to one day of the year. Then, we used the abundance of individuals of each species flowering or fruiting to calculate the mean angle representing the peak of the phenophase (Appendix 1). If more than one year of full observations was available, we calculated the mean of mean angles. In some species with one or two individuals that flowered in all months, we considered the peak as the month with most open flowers. The K statistic assesses whether the measured traits deviates from a Brownian evolutionary model, where a K value = 1 indicates a perfect fit with the model. A K value > 0 shows that phylogenetically related species have similar traits more than expected under a Brownian model (strong PS), which means a phylogenetic conservatism. A K value < 1 indicates that close relatives resemble each other less than expected under a Brownian model (weak PS), which could be caused by adaptive evolution independent of phylogeny (e.g., convergence; Blomberg et al. 2003). We also tested the time of diaspore development (TDD) for phylogenetic signal, expecting that if phylogenetically related species present similar TDD and flowering peaks, the fruiting phenology would be determined by the flowering event. The TDD was calculated in a linear scale as the difference in days between the peak of flowering and the following fruiting (Appendix 1). If a species presented two complete flowering and fruiting events within the two years records, TDD was calculated as the mean of these separate events. For two species that produced one diaspore but flowering was spread in several months, we could follow which flowering event generated the diaspore and could calculate the correct TDD.

To evaluate the relationship of phylogenetic clades along the months we performed a principal coordinates of phylogenetic structure (PCPS; Duarte 2011). First, the method

generate a matrix of phylogeny-weighted taxon composition for each site (in our case represented by months) called matrix P. The matrix P is obtained by matrix multiplication of the matrix of species abundance by month transformed by square root and the pairwise phylogenetic distance matrix, transformed into dissimilarities (ranging from zero to one) and standardized by marginal total through the fuzzy weighting method developed by Pillar and Duarte (2010). Matrix P is submitted to a principal coordinates analysis (PCoA), based on square root transformed Bray–Curtis dissimilarities, to obtain the PCPS, the eigenvectors describing an orthogonal phylogenetic gradient in the data set. Labelling species according to their clades (e.g., orders or families) in the PCPS ordination scatterplot allows the identification of the representativeness of different lineages across the temporal gradient. PCPS with the highest eigenvalues describe wide phylogenetic gradients related to the deepest nodes in the phylogenetic trees like superorders or orders and as the eigenvalues decrease, finer taxonomic scales are described (Duarte et al. 2014).

To evaluate if there is an association of climatic variables and the phenology along months, and if this association is due to species or phylogenetic composition, we tested the relation of each PCPS containing at least 5% of explanation on matrix P with different climatic models, through linear models (LM; see Appendix 4 for all tested models). The climatic models were constructed *a priori*, based on reliable expectations about climate influences in phenology. The predictors were tested for multicollinearity (correlation results in Appendix 4), avoiding the inclusion of high correlated predictors in the same model. We identified the best-fitting models based on Akaike's Information Criterion (AIC; Burnham & Anderson 2002). We selected the models that presented lower AIC values compared to a null model, in which the response variable is explained by its median and variance. To test if the association between the species distribution over time is mediated or not by phylogenetic relationships, we tested the significance of each best model through an LM using two null models (Duarte et al.

unpubl.), one that shuffles tip names across the phylogeny (*taxa shuffle*) and another that shuffles the sites across the environmental gradient (*site shuffle*). The *taxa shuffle* tests the association between phylogenetic composition (species composition is kept unchanged) and the climatic gradient, while the *site shuffle* null model evaluates the combined association between species and phylogenetic composition and the climatic gradient. If both null models show significant results, the phylogenetic relationships among species mediates the association between species distribution across months (phenology) and the climatic gradient. If only *site shuffle* returns a significant result, the association between phenology and the climate gradient is due to species composition, independently of phylogenetic relationships among species.

An important issue when dealing with temporal data is that sampling units are in a circular scale, so there is no start or endpoint to the time of year. As an example, the circular difference between January and October is three months, instead of ten months in a linear scale. Therefore, depending on the seasonality of the climatic variable, the climatic gradient is likely to be temporally structured. Considering climatic variables as predictors to the phylogenetic structure of species over months, creates the possibility of some degree of temporal autocorrelation in the residual variation of response variables (PCPS). To control for temporal autocorrelation presented in some linear models, we employed an approach commonly used in spatial analysis, the principal coordinates of neighbourhood matrices analyses (PCNM; Borcard et al. 1992). For this, we transformed the mean date of each field trip in each month to a circular scale represented by angles, which one degree is approximately equivalent to one day of the year. Then we calculated a geodesic distance between all sampling units (months). This distance matrix was used for calculating the PCNM, truncating at the maximum distance between sampling units (0.48; based on a circle with radius equal to one) using a minimum spanning tree criterion (Rangel et al. 2006). The PCNM analyses generated 16 orthogonal temporal filters whereas PCNM with higher eigenvalues represent broad temporal filters and

PCNM with lower eigenvalues represent finer temporal gradients. If the best climatic models presented temporal autocorrelation in their residuals, we employed a model selection of the PCNMs based on AIC, testing within the best-fitting models the PCNM that controls for autocorrelation. To evaluate temporal autocorrelation we employed the Moran's *I* correlation statistic. If the best-fitting models presented temporal autocorrelation, they were recalculated in the LM including the selected PCNM in of the AIC selection.

Most tests were performed in the R Statistical Environment (R Foundation for Statistical Computing, Vienna, AT; http://www.R-project.org). The K statistic with randomization tests was calculated with the package *phytools* 0.4-05. The PCPS and LM (function *pcps.sig*) analyses were performed using the package *PCPS* v.1.0.2 (Debastiani & Duarte 2014). The model selections based on AIC criterion were performed in the package *MuMIn* v. 1.13.4. Circular scale transformation and geodesic distances were performed with the package *circular* v. 0.4-7. PCNM analyses were performed in the package *Vegan* v.2.2-1. The Moran's *I* correlation statistic was performed using the software SAM 4.0 (Rangel et al. 2006).

Results

The flowering concentrates at the end of spring to late summer, with a higher number of species within November and April and the highest abundance in October (Figure 1). The fruiting presented two peaks of richness and abundance of species (Figure 1), a lower peak in the transition of spring to summer (November to January) and a higher peak in the transition of autumn to winter (April to July). In the evaluation of phenophase peaks, most species presented their peak of flowering from October to February (67 % of spp.), while the fruiting peaks concentrated from April to June (47 % of spp.) and between November and January (30 % of spp.). These results show that some species have disproportional abundance and that evaluating the phenology using different methods can give discrepant but complementary

results. The K statistic showed significant but low phylogenetic signal results for flowering (K = 0.28; *P* =0.001), fruiting (K = 0.17; *P* = 0.014) and TDD (K = 0.48; *P* = 0.001). These results show that close relatives resemble each other less than expected under Brownian model.

The PCPS analysis generated 23 eigenvectors for both flowering and fruiting. For flowering the first four PCPS axes contained each at least 5% of total variation in matrix P and accumulated 75 % of total variation, while for fruiting there were four PCPS accumulating 67 % of total variation. These PCPS were submitted to a model selection based on AIC criterion with the constructed climatic models (Appendix 5) and we selected the models with a ΔAICc lower and close to the threshold value of two. For flowering, all the selected models presented significant results for the *site.shuffle* null model, but only the first and fourth PCPS presented significant results for the *taxa.shuffle* null model (results in the Appendix 6). For fruiting, only the models with the second PCPS did not present significant results in both null models (results in the Appendix 7). When controlling for autocorrelation with the inclusion of the PCNM axis in the climatic models, the results' significance of both null models did not change, with an exception for the second PCPS of fruiting that showed significant results. Only the third PCPS of flowering could not be controlled for temporal autocorrelation with the inclusion of any PCNM axis in the models, while the fourth PCPS of fruiting did not present temporal autocorrelation in the regression residuals and the inclusion of PCNM was not necessary. We selected for each PCPS the best model according to their LM R^2_{adj} results, with an exception for the first PCPS of flowering and fruiting. In this case, we selected the simplest model containing one predictor, which was present in all selected and tested models (Table 1).

For flowering the first and fourth PCPS showed significant results for both null models, indicating that the phylogenetic relationships among species mediate the association between species distribution across months and the climatic gradient. The first PCPS exhibited a clear separation of asterids from the monocots, Ranunculales and rosids clades (Figure 2), showing

that the flowering of asterids increase in months with low daylength, and the flowering of monocots, Ranunculales and rosids clades increase in months with high daylengths. The fourth PCPS presented a positive association with the temperature of two month lag, showing it as an important trigger for flowering. An interesting issue is that within asterids and rosids not all clades presented all their species with the same trend for this variable. For fruiting the best models of all PCPS presented significant results for both null models, indicating a phylogenetic organization of species along the climatic gradient over months. Furthermore, all PCPS showed an association with lagged climatic variables, highlighting the effect of past events on triggering the fruiting periods. The first PCPS showed a positive association with the temperature of three month lag, separating monocots from rosids, no clear trend in lamiids and a separation within campanulids of the Asteraceae and Caprifoliaceae families (Figure 3). The second PCPS was positively associated with the historical rainfall of two and three month lags, separating campanulids and Ranunculales from monocots and most rosids (Figure 3). The third and fourth PCPS of fruiting showed a negative association with the daylength of one month lag and the temperature of one month lag respectively (Appendix 8). The third PCPS only grouped the species of Ranunculaceae, while it separated clades within lamiids, campanulids and rosids. The fourth PCPS showed a separation between lamiids and most campanulids.

Discussion

Recently, studies started to incorporate phylogenetic methods in the explanation of phenological patterns (e.g., Staggemeier et al. 2010; Seger et al. 2013; Du et al. 2015) finding different degrees of phylogenetic conservatism in plant phenology. Our results show a low phylogenetic signal (PS) in flowering and fruiting peaks of climbing plants, but an evidence that species of the same clade on different taxonomic levels respond similarly to the environmental gradient, depending on the climatic variable.

In a plant community, species can differentially respond to environmental cues presenting a mixture of phenological patterns. Lessard-Therrien et al. (2014) and Davies et al. (2013) compared flowering phenology of plant communities in different localities and altitude ranges, suggesting a conservatism in responses to abiotic cues instead of flowering time. Lessard-Therrien et al. (2014) suggested that the presence of phylogenetic signal locally but not across sites was an evidence to conservatism in responses to environmental cues that vary across sites. Our results using the PCPS approach also suggests a phylogenetic conservatism in responses to local climatic gradients. The PCPS demonstrated to be a promising method for disentangling the different responses of phylogenetic clades to climatic gradients. The PCPS brought a new perspective not covered by phylogenetic signal methods, showing at which taxonomic level may be occurring a conserved response to a determined abiotic factor, regardless if the community phenological times are not phylogenetically conserved.

The flowering showed a low PS agreeing with previous results found in the same area but evaluating different life forms together (Seger et al. 2013). The low PS is an evidence that phylogenetically related species are not similar in their flowering peaks, which could be an evidence for the hypothesis of lower competition for shared pollinators (Morales & Traveset 2008). Furthermore, this low PS is similar to results found by Du et al. (2015) at the same latitude in the Northern hemisphere, but the latitudinal gradient suggested by the authors remain to be tested in the Neotropics. Compared to trees (G.D.S. Seger unpublished data), climbing plants showed the same pattern of lower species number in the winter, but presented a delay of one month for the main peak and the beginning of the increase of flowering species. The PCPS results showed an association of flowering with daylength and the temperature of two month lags. These variables were determinant of flowering periods of climbing plants in previous studies in Araucaria forests in South Brazil (Marques et al. 2004; Liebsch & Mikich 2009), despite their low species number evaluated. An important point is that the PCPS analysis

showed a clear opposing effect of daylength between larger clades (Ranunculales, monocots and rosids versus asterids). However, there is a mixed effect within some lower level clades for temperature of two month lags (Figure 2). This shows a conservatism in the response of larger clades for daylength, requiring higher or lower levels of daylength for flowering. Moreover, the temperature of two month lags may act as an important trigger for flowering development, presenting consistent patterns within some clades, and showing that some species need low temperatures to initiate flowering process (Henderson et al. 2003).

The fruiting peaks present a low phylogenetic signal, demonstrating that phylogenetically close species tend to not overlap their fruiting periods. The low phylogenetic signal found for the TDD and flowering peaks do not confirm our hypothesis that flowering time would be regulating fruiting period. Fruiting is expected to occur in the best period for germination (Primack 1987) and seed dispersal, matching lower canopy foliage for wind-dispersed species (van Schaik et al. 1993) and the highest availability of frugivores for zoochoric species (Fenner 1998). We evaluated the whole community pattern of fruiting, but the bimodal peak pattern found for both species and individuals number is driven by anemochoric instead of zoochoric species (Appendix 9). Most climbing species evaluated in the area present anemochoric dispersal syndrome, a common fact for climbing plants in the Neotropics (Gallagher & Leishman 2012). Leaf fall of one month lag was the second best model for the fourth PCPS of fruiting. However, it is complicated to determine if the leaf fall effect on fruiting is through facilitation of wind diaspores dispersal or through an increase of sunlight in the understory that enhances fruit production. In middle latitudes, the seasonality of daylength and temperature confine resources in a limited period, which may restrict fruiting to occur before winter (Fenner 1998). For fleshy fruits, fruiting peaks occur after summer solstice in temperate zones of Southern hemisphere, being strongly determined by temperature (Ting et al. 2008). Marques et al. (2004) and Liebsch and Mikich (2009) found an effect of temperature and daylength in

fruiting of Araucaria forest sites in Southern Brazil. Our results showed this same overall pattern for temperature and daylength but interestingly, rainfall and historic rainfall were present in most best models selected by AIC criterion (Appendices 6 and 7), even not presenting a seasonal pattern. The PCPS presented less clear patterns in large clades (rosids versus asterids) in comparison with flowering (Figure 3 and Appendix 8).

This study brings a new approach for evaluating phylogenetic patterns in phenological responses to environmental cues. We found a phylogenetic conservatism in responses of plant clades to local climatic gradients showing that different climatic variable can act in distinct taxonomic levels. A future direction would be to test if this conserved response to environmental cues of specific clades remains over broad spatial scales or if this is restricted to sites presenting similar climatic patterns. Moreover, understanding evolutionary patterns on plant phenology and their association with climate will be useful to predict how plant clades may respond under a scenario of climate change.

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Table 1. Linear model results of the best models for each PCPS of flowering and fruiting. Models without PCNM did not present temporal autocorrelation in the regression residuals. The tested models for PCPS3 of flowering could not be controlled for temporal autocorrelation with the inclusion of any PCNM. R – Rainfall; Rh – Historic Rainfall; D – Daylength; T – Temperature. Variables with a number preceding the lag indicate the respective monthly lag.

Models		**Permutation test**		
	$R^2_{Adj.}$	**F-value**	$P_{site\ shuffle}$	$P_{taxa\ shuffle}$
Flowering				
PCPS1				
D + PCNM1	0.84	62.47	0.001	0.05
PCPS2				
R 1lag + T 1lag + PCNM3	0.71	20.12	0.001	0.149
PCPS3				
-	-	-	-	-
PCPS4				
T 2lag + PCNM2	0.56	15.87	0.001	0.02
Fruiting				
PCPS1				
T 3lag + PCNM6	0.71	29.48	0.001	0.023
PCPS2				
Rh 2lag + Rh 3lag + PCNM1	0.55	10.45	0.001	0.046
PCPS3				
D 1lag + PCNM3	0.63	20.68	0.001	0.025
PCPS4				
T 1lag	0.33	12.29	0.004	0.049

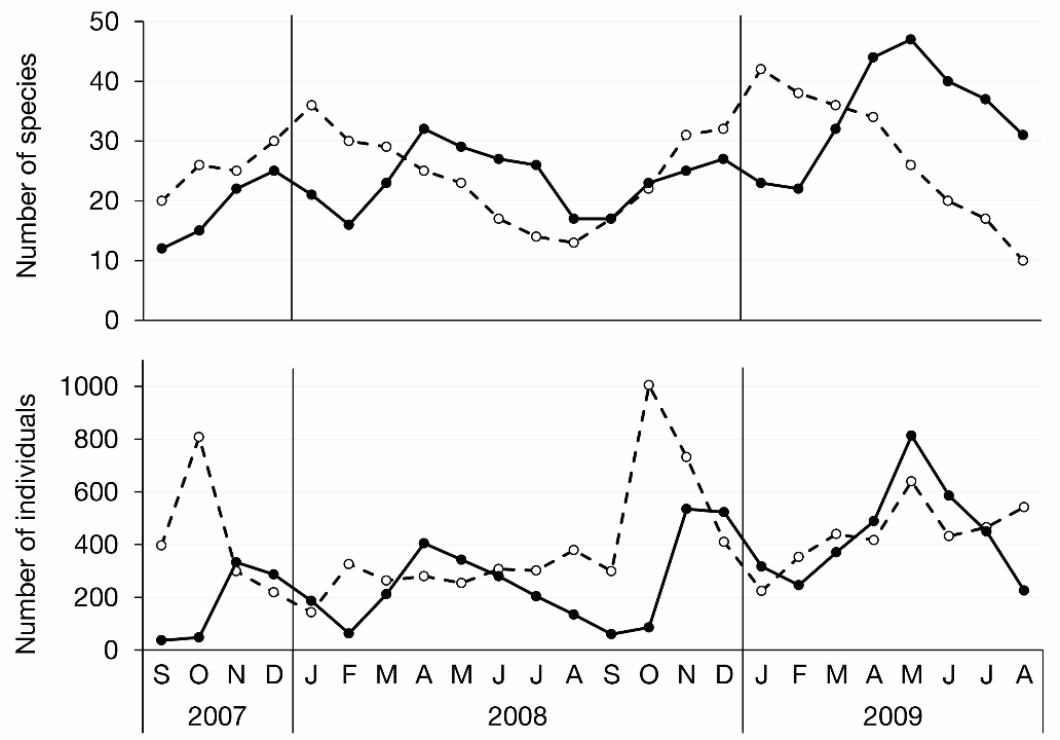


Figure 1. Phenological patterns of climbing plants represented by the number of species and individuals flowering and fruiting over two years record. Dashed lines represent flowering and solid lines represent fruiting.

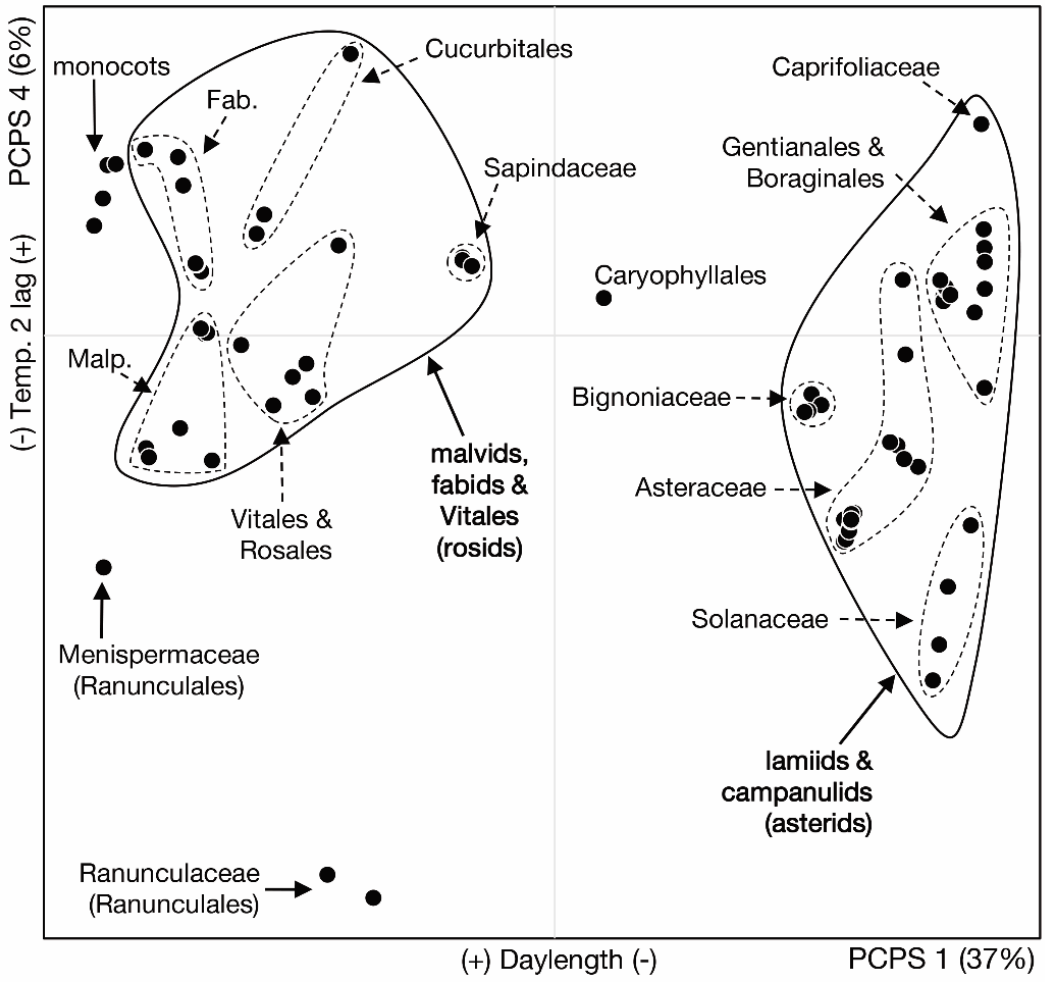


Figure 2. Scatter diagram of the first and fourth PCPS of flowering climbing plants. Full circles represent species grouped in monophyletic clades delimited by dashed and solid lines. Significant climatic variables through linear models are specified for each PCPS.

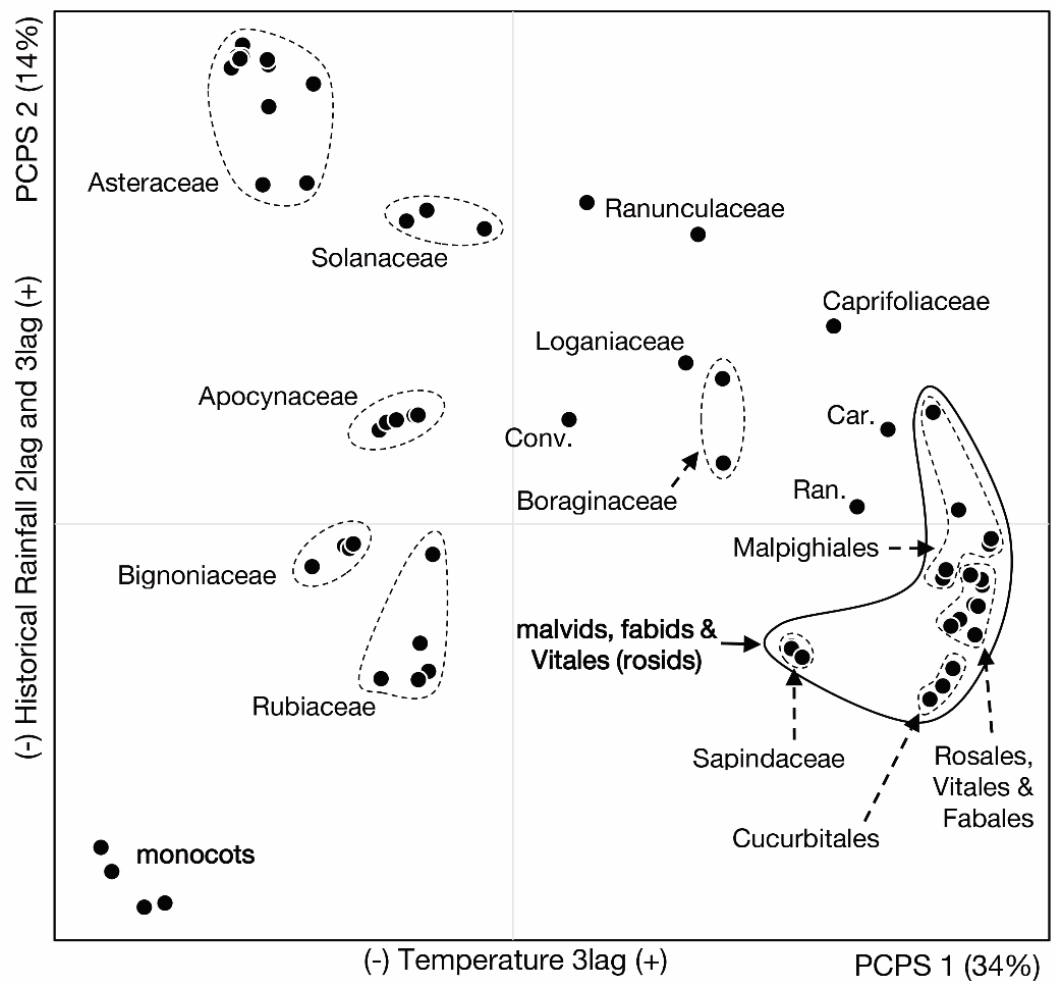


Figure 3. Scatter diagram of the first and second PCPS of fruit-bearing climbing plants. Full circles represent species grouped in monophyletic clades delimited by dashed and solid lines. Significant climatic variables through linear models are specified for each PCPS. Fab. – Fabales; Ran. – Ranunculales; Car. – Caryophyllales; Conv. – Convolvulaceae.

Appendix 1. Species list and their respective peaks of flowering and fruiting as mean angles, and the time of diaspore development (TDD).

Family/Species	Flowering (mean angle)	Fruiting (mean angle)	TDD (days)
ALSTROEMERIACEAE			
Bomarea edulis (Tussac) Herb.	356.5	120	113.6
APOCYNACEAE			
Orthosia scoparia (Nutt.) Liede & Meve	166.74	323.38	158.8
Orthosia urceolata E. Fourn.	196.52	328.97	134
Orthosia virgata (Poir.) E. Fourn.	91.52	315.62	219.1
Oxypetalum mosenii (Malme) Malme	69.06	146.92	78.8
Oxypetalum pannosum Decne.	30.15	99	63.1
Oxypetalum pedicellatum Decne.	206.38	115	99
Oxypetalum wightianum Hook. & Arn.	51.47	133.58	82.7
ASTERACEAE			
Baccharis anomala DC.	96.06	134.68	39.3
Baccharis trinervis (Lam.) Pers.	166.29	254.99	68.3
Calea pinnatifida (R.Br.) Less.	312.4	16.71	65.2
Lepidaploa balansae (Chodat) H.Rob.	193.52	249.38	58.5
Mikania burchellii Baker	284.75	325.62	41.4
Mikania campanulata Gardner	144.18	175.88	32.2
Mikania cordifolia (L.f.) Willd.	95.29	167.24	72.9
Mikania hirsutissima DC.	274.79	321.06	46.9
Mikania involucrata Hook. & Arn.	280.76	314.91	34.6
Mikania laevigata Sch. Bip. ex Baker	265.24	316	55
Mikania micrantha Kunth	129.47	161.75	32.7
Mikania oreophila Ritter & Miotto	97.52	145.02	49.9
Mikania orleansensis Hieron.	269.48	314.97	46.1
Mikania paranensis Dusén	93.32	145.93	53.8
Mikania parodii Cabrera	62.9	96.5	34.2
Mikania ternata (Vell.) B.L.Rob.	84.16	125.72	44.7
Mutisia campanulata Less.	263.65	337	74.4
Mutisia speciosa Aiton ex Hook.	339.37	16.11	37.6
Piptocarpha ramboi G.Lom.Sm.	40.66	132.07	92.7
BEGONIACEAE			
Begonia fruticosa A. DC.	132.23	310.74	167.9
BIGNONIACEAE			
Amphilophium crucigerum (L.) L.G.Lohmann	357.12	271.44	278.1
Dolichandra uncata (Andrews) L.G.Lohmann	318.69	234	282.9
Dolichandra unguis-cati (L.) L.G.Lohmann	320.33	167.03	209.7
Tanaecium selloi (Spreng.) L.G.Lohmann	8	181	175.4

BORAGINACEAE			
Tournefortia breviflora DC.	356.6	69.5	56
Tournefortia paniculata Cham.	359.97	54.16	55
CANNABACEAE			
Celtis iguanaea (Jacq.) Sarg.	311.5	130	181
CAPRIFOLIACEAE			
Valeriana scandens L.	78.26	88.04	10
CONVOLVULACEAE			
Convolvulus crenatifolius Ruiz & Pav.	10	118.23	67.9
CUCURBITACEAE			
Apodanthera laciniosa (Schltdl.) Cogn.	38.59	84.66	46.4
Cayaponia diversifolia Cogn.	12	89.35	78.5
Cayaponia palmata Cogn.	19.68	96.47	62.8
Cayaponia pilosa (Vell.) Cogn.	2.76	64.2	78.4
DIOSCOREACEAE			
Dioscorea multiflora Mart. ex Griseb.	338.7	135.95	160.1
Dioscorea subhastata Vell.	338.19	107	126
EUPHORBIACEAE			
Tragia volubilis L.	323.21	50.03	82.9
FABACEAE			
Canavalia bonariensis Lindl.	16.3	171.4	155.6
Dalbergia frutescens (Vell.) Britton	349.14	175.8	189.1
Lathyrus nervosus Lam.	308	8	60.8
Piptadenia affinis Burkart	31.41	132.42	102.9
Senegalia nitidifolia (Speg.) Seigler & Ebinger	8	137.35	131.1
Senegalia velutina (DC.) Seigler & Ebinger	1.71	129.46	129.6
LOGANIACEAE			
Strychnos brasiliensis Mart.	338.7	156.2	179.9
MALPIGHIACEAE			
Heteropterys aenea Gris.	14.69	109.5	96.2
Heteropterys intermedia (A. Juss.) Griseb.	39.1	153.15	115.9
Tetrapterys phlomoides (Spreng.) Nied.	3.5	84	81.8
MENISPERMACEAE			
Cissampelos pareira L.	1.65	90.9	91.2
PASSIFLORACEAE			
Passiflora actinia Hook.	320.53	67.33	111.2
Passiflora caerulea L.	291.3	341	49.9
PHYTOLACCACEAE			
Seguieria americana L.	48.55	157.05	109.8
RANUNCULACEAE			
Clematis bonariensis Juss. ex DC.	47.04	145.05	67.8
Clematis dioica L.	291.87	347.55	56.5
ROSACEAE			
Rubus erythrocladus Mart.	297.74	4.31	63.7
Rubus sellowii Cham. & Schltdl.	55.87	111.8	56.7

RUBIACEAE			
Manettia paraguariensis Chodat	28.08	180.24	153.1
Manettia pubescens Chamisso & Schlechtendal	219.54	3.86	166.6
Manettia tweedieana K.Schum.	72.03	211.63	128.7
Manettia verticillata Wernham	66.19	156.02	91.6
Galium hypocarpium (L.) Endl. ex Griseb.	307	20.88	74.8
SAPINDACEAE			
Serjania cf. *laruotteana* Cambess.	45.79	218.96	168.6
Serjania meridionalis Cambess.	50.27	179.41	134.7
Urvillea ulmacea Kunth	178.07	265.5	87.2
SMILACACEAE			
Smilax cognata Kunth	339.57	314.15	338.4
SOLANACEAE			
Solanum flaccidum Vell.	30.17	103.44	74.2
Solanum inodorum Vell.	229.27	351.84	122.9
Solanum laxum Spreng.	261.17	345.3	81.6
TROPAEOLACEAE			
Tropaeolum pentaphyllum Lam.	295.57	350.11	50.4
VIOLACEAE			
Anchietea pyrifolia A. St.-Hil.	296.31	342.3	46.8
VITACEAE			
Cissus striata Ruiz & Pav.	11.25	96.85	86.4
Cissus verticillata (L.) Nicolson & C.E.Jarvis	30.11	128.91	100.4

Appendix 2. The historical (Fig. 1) and actual (Fig. 2) climatic variables for the study area. The historical rainfall was calculated from 2003 to 2009, while the historical mean temperature was calculated from January 2004 to July 2010.

Figure 1

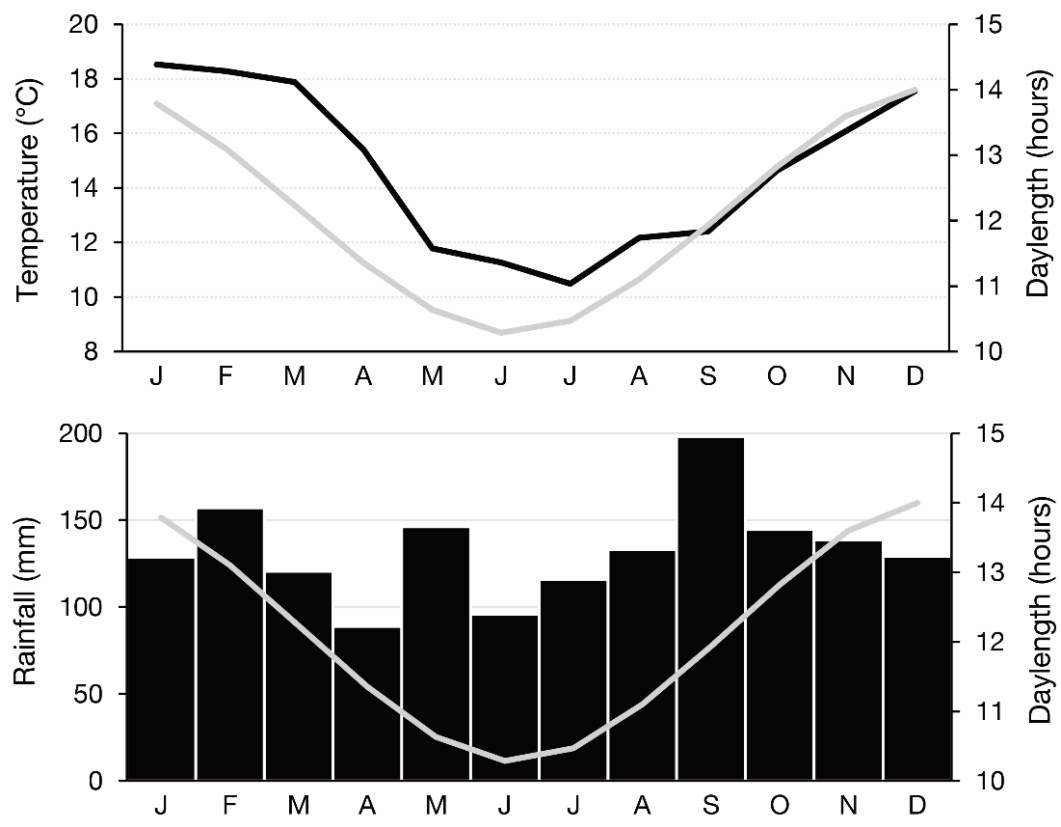
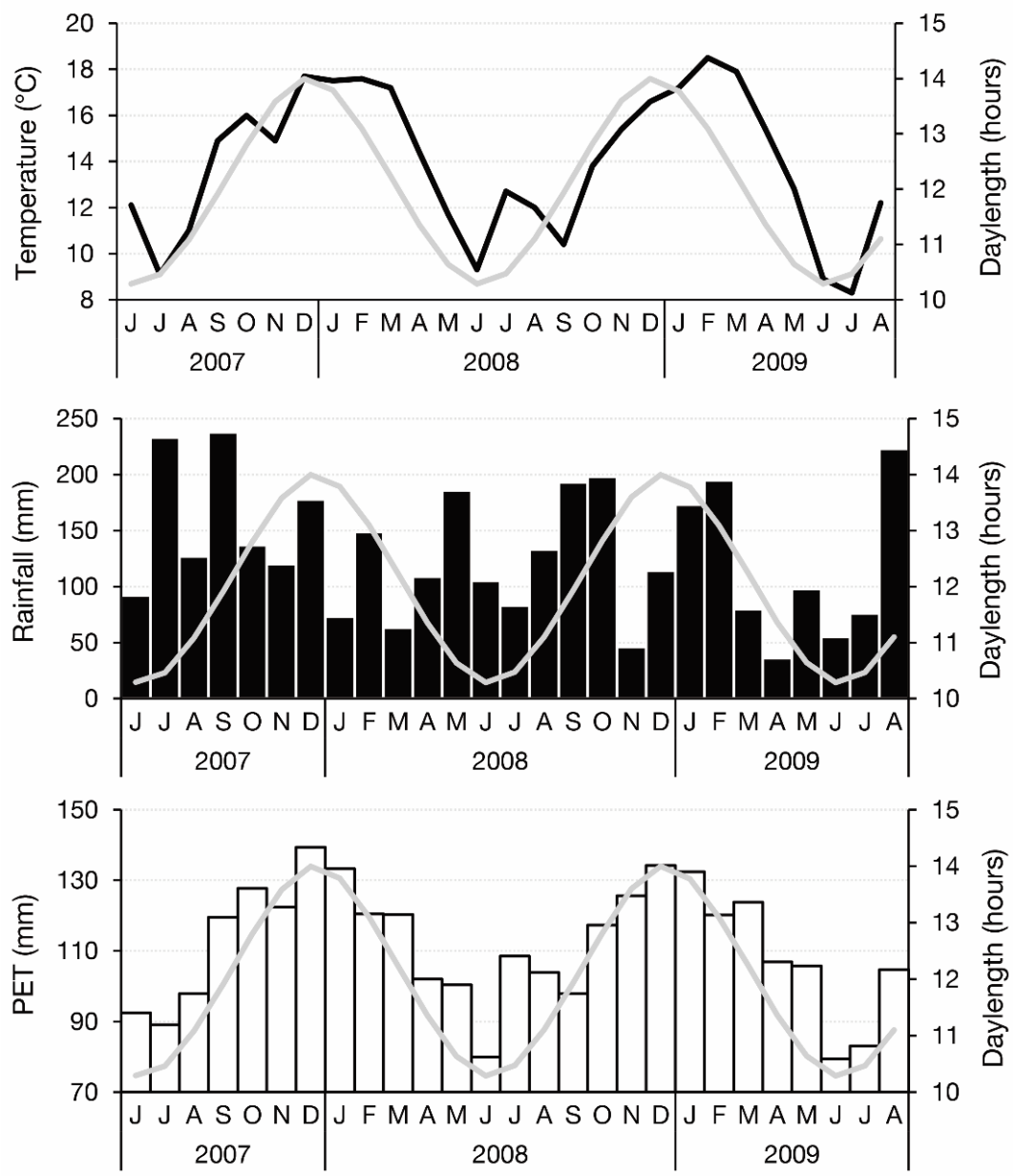


Figure 2


Temperature (°C)
Daylength (hours)
Rainfall (mm)
PET (mm)
J J A S O N D J F M A M J J A S O N D J F M A M J J A
2007
2008
2009

Appendix 3. Phylogenetic tree in newick format and the detailed molecular phylogeny reconstruction workflow. The phylogenetic tree node names are bootstrap support values.

Phylogenetic tree

(((bomarea_edulis:0.4809246559,smilax_cognata:0.4809246559)100:0.1118463212,(dioscorea_multiflora:0.29638548855,dioscorea_subhastata:0.29638548855):0.29638548855)100:0.4072290229,((((((tournefortia_brevifl ora:0.2911188781,tournefortia_paniculata:0.2911188781):0.2911188781,(((((orthosia_virgata:0.05429532378,(orthosia_scoparia:0.02798473689,orthosia_urceolata:0.02798473689)90:0.02631058688)100:0.01600375719,((oxypetalum_mosenii:0.01423620749,oxypetalum_wightianum:0.01423620749)100:0.01695587146,oxypetalum _pannosum:0.03119207895,oxypetalum_pedicellatum:0.03119207895)100:0.03910700201)100:0.2798316827, strychnos_brasiliensis:0.3501307637)100:0.1502304984,((manettia_paraguariensis:0.1688075344,manettia_pub escens:0.1688075344,manettia_tweedieana:0.1688075344,manettia_verticillata:0.1688075344):0.1688075344,g alium_hypocarpium:0.3376150688)100:0.1627461933)100:0.07487209516,((amphilophium_crucigerum:0.070 80736784,((dolichandra_unguiscati:0.01780890586,dolichandra_uncata:0.01780890586)100:0.03991107679,ta naecium_selloi:0.05771998264)64:0.01308738519)100:0.4998097333,(convolvulus_crenatifolius:0.462352453 6,(solanum_laxum:0.2311762268,solanum_flaccidum:0.2311762268,solanum_inodorum:0.2311762268):0.2311 762268)100:0.1082646475)100:0.004616256099)44:0.007004398965)100:0.07394040917,(((mutisia_campanul ata:0.08970709625,mutisia_speciosa:0.08970709625):0.08970709625,((lepidaploa_balansae:0.06221239444,pi ptocarpha_ramboi:0.06221239444)100:0.1107570162,((baccharis_trinervis:0.0806111257,baccharis_anomala:0 .0806111257):0.0806111257,(calea_serrata:0.07417790001,(mikania_burchellii:0.05251934304,mikania_camp anulata:0.05251934304,mikania_cordifolia:0.05251934304,mikania_hirsutissima:0.05251934304,mikania_invo lucrata:0.05251934304,mikania_laevigata:0.05251934304,mikania_micrantha:0.05251934304,mikania_oreophi la:0.05251934304,mikania_orleansensis:0.05251934304,mikania_paranensis:0.05251934304,mikania_parodii:0 .05251934304,mikania_ternata:0.05251934304)100:0.02165855697)100:0.08704435141)100:0.01174715927)1 00:0.006444781772)100:0.4514749485,valeriana_scandens:0.630889141)100:0.02528902442)100:0.07319053 595,seguieria_americana:0.7293687014)100:0.03006410809,((((((begonia_fruticosa:0.4222460966,(apodanther a_laciniosa:0.1695446985,(cayaponia_diversifolia:0.00680695567,cayaponia_palmata:0.00680695567,cayaponi a_pilosa:0.00680695567)100:0.1627377428)100:0.2527013981)100:0.2260038419,(celtis_iguanaea:0.5430620 219,(rubus_erythrocladus:0.27153101095,rubus_sellowii:0.27153101095):0.27153101095)100:0.1051879166)1 00:0.0003514717407,((dalbergia_frutescens:0.4373551942,(canavalia_bonariensis:0.2465116805,lathyrus_nerv osus:0.2465116805)100:0.1908435137)84:0.06364053262,(piptadenia_affinis:0.08295631272,(senegalia_nitidif olia:0.04147815636,senegalia_velutina:0.04147815636):0.04147815636)100:0.4180394141)100:0.1476056834)100:0.03197073047,(((passiflora_actinia:0.05357795372,passiflora_caerulea:0.05357795372)100:0.436906629 7,anchietea_pyrifolia:0.4904845834)100:0.04077972971,(tragia_volubilis:0.4915892633,((heteropterys_aenea: 0.03112788984,heteropterys_intermedia:0.03112788984)100:0.01894330209,tetrapterys_phlomoides:0.050071 19192)100:0.4415180714)50:0.0396750498)100:0.1493078276)100:0.01105941392,(((serjania_meridionalis:0. 044336475365,serjania_sp:0.044336475365):0.044336475365,urvillea_ulmacea:0.08867295073)100:0.550228 9163,tropaeolum_pentaphyllum:0.638901867)100:0.0527296876)100:0.03923160723,(cissus_striata:0.2751092 704,cissus_verticillata:0.2751092704)100:0.4557538915)100:0.02856964757)100:0.1147286198,(cissampelos_ pareira:0.6819842737,(clematis_bonariensis:0.34099213685,clematis_dioica:0.34099213685):0.34099213685)1 00:0.1921771555)100:0.1258385708):0.1460446621;

Molecular phylogeny reconstruction

The phylogenetic tree was reconstructed using molecular sequences available online at GenBank (accessed in April 2015; Benson et al. 2013). For this, we downloaded sequences of

nuclear (ITS1 and ITS2) and chloroplastidial markers (rbcL, matK, rps16, trnL-trnF spacer, trnL intron, psbA-trnH spacer and NADH dehydrogenase subunit F) containing coding and non-coding regions, known to resolve species relationships at higher and lower taxonomic levels. At GenBank we checked for species names synonyms and found sequences for 38 of 78 sampled species. For 17 genera without sequence data, we used sequences of congeneric relatives always looking for climbing species of the same taxonomic tribe and that occur geographically close to the sampled area. Within these genera, we used sequences of only one species and the 24 remaining species without sequence were manually merged in the resulting phylogeny, splitting them halfway along their congener branch with sequence data or positioning it as a polytomy at the genus node if there was more than one congener.

The sequences were aligned through the MAFFT online software (available at http://mafft.cbrc.jp/alignment/server/), using the option to adjust direction according to the first sequence for highly divergent taxa, which found and corrected some sequences that were reverse complements. In MAFFT we used the E-INS-i alignment strategy (Katoh & Toh 2008) for rbcL, matK and NADH dehydrogenase subunit F, and the Q-INS-i alignment strategy, which takes into account a secondary structure information of RNA, for the other markers. The alignments tips were manually trimmed using the Mesquite 2.75 software (Maddison & Maddison 2011) and some misaligned species sequences were excluded. After this treatment the alignments were concatenated in a supermatrix using the software FASconCAT (Kück & Meusemann 2010).

The phylogenetic tree of the climbing plant community was reconstructed using a maximum-likelihood algorithm (ML) in the raxmlGUI 1.3.1 software (Silvestro & Michalak 2012). The supermatrix was partitioned in each marker and we set the GTR+Γ+I evolutionary model for the whole supermatrix. We applied 1,000 ML searches and a rapid bootstrap analysis, estimating a support value for each node. We chose *Nymphaea alba* L. (Nymphaeaceae), an

early divergent angiosperm species, as the outgroup. To reduce the artifact of patchy dataset, we used a backbone constraint tree (Roquet et al. 2013) to constraint the ML searches to its topology. The constraint tree had a family level resolution, so the polytomies were resolved solely by molecular data. Therefore, the relationships within and between genera were unconstrained. The constraint tree was reconstructed using the Phylocom/Phylomatic software (Webb & Donoghue 2005; Webb et al. 2008), following the phylogenetic hypothesis for angiosperms of APG III (APG 2009) to the order level and resolving relationships among families according to the Angiosperm Phylogeny Website (Stevens 2001). For this, we used the "megatree" R20120829 (available at https://github.com/camwebb/tree-of-trees/blob/master/megatrees/R20120829.new) and removed intra-familial resolution, so relationships within families were represented as polytomies. The branch lengths of the constraint tree were estimated using the clade age estimates of Bell et al. (2010).

The resulting phylogeny had their branch lengths proportional to the rate of evolution of the used markers and we transformed it to be proportional to divergence time using a ML approach. The root age was set to one value and the discrete model of substitution rate variation was applied (Paradis 2013), setting the smoothing parameter (lambda) to one. This substitution model makes categories of branches characterized by different rates variation. The molecular dating with ML was calculated using the function *chronos* of the package *ape* v.3.2, (Paradis et al. 2004) in the R Statistical Environment (R Core Team 2015).

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Appendix 4. Results of Spearman correlations (lower diagonal) and their respective significant values (upper diagonal) of all tested climatic variables. D – Daylength; PET – Potential evapotranspiration; T – Temperature; R – Rainfall; Rh – Historic Rainfall; LF – Leaf Fall. Variables with a number preceding the lag indicate the respective monthly lag.

	D	**D 1lag**	**D 2lag**	**D 3lag**	**PET**	**PET 1lag**	**PET 2lag**	**PET 3lag**	**T**	**T 1lag**	**T 2lag**	**T 3lag**	**R**	**R 1lag**	**R 2lag**	**R 3lag**	**Rh**	**Rh 1lag**	**Rh 2lag**	**Rh 3lag**	**LF**	**LF 1lag**	**LF 2lag**
D	0	< 0.01	0.01	0.99	< 0.01	< 0.01	0.06	0.71	< 0.01	0.02	0.85	0.03	0.45	0.10	0.08	0.06	0.17	0.02	0.01	0.02	< 0.01	0.02	0.57
D 1lag	0.87	0	< 0.01	0.01	< 0.01	< 0.01	< 0.01	0.07	< 0.01	< 0.01	0.02	0.98	0.85	0.28	0.26	0.10	0.83	0.18	0.02	0.01	< 0.01	< 0.01	0.02
D 2lag	0.50	0.87	0	< 0.01	0.02	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.02	0.26	0.81	0.74	0.32	0.31	0.83	0.18	0.02	< 0.01	< 0.01	< 0.01
D 3lag	0.00	0.50	0.87	0	0.77	0.01	< 0.01	< 0.01	0.05	< 0.01	< 0.01	< 0.01	0.06	0.47	0.60	0.87	0.03	0.32	0.82	0.17	0.21	< 0.01	< 0.01
PET	0.89	0.80	0.49	0.06	0	< 0.01	0.10	0.93	< 0.01	0.01	0.89	0.09	0.43	0.15	0.05	< 0.01	0.18	0.10	0.02	0.02	< 0.01	0.01	0.71
PET 1lag	0.73	0.90	0.81	0.51	0.64	0	< 0.01	0.07	< 0.01	< 0.01	0.01	0.93	0.49	0.32	0.36	0.05	0.92	0.18	0.09	0.01	< 0.01	< 0.01	0.01
PET 2lag	0.38	0.74	0.90	0.81	0.35	0.67	0	< 0.01	< 0.01	< 0.01	< 0.01	0.02	0.35	0.90	0.90	0.50	0.63	0.92	0.19	0.10	0.04	< 0.01	< 0.01
PET 3lag	-0.08	0.38	0.74	0.90	-0.02	0.37	0.64	0	0.15	< 0.01	< 0.01	< 0.01	0.05	0.78	0.33	0.80	< 0.01	0.60	0.83	0.25	0.40	0.03	< 0.01
T	0.80	0.89	0.75	0.41	0.90	0.77	0.57	0.30	0	< 0.01	0.14	0.73	0.78	0.19	0.06	0.04	0.53	0.14	0.05	0.03	< 0.01	< 0.01	0.10
T 1lag	0.48	0.80	0.90	0.76	0.49	0.91	0.79	0.59	0.72	0	< 0.01	0.15	0.15	0.60	0.45	0.06	0.47	0.53	0.13	0.04	0.01	< 0.01	< 0.01
T 2lag	0.04	0.49	0.80	0.90	0.03	0.52	0.90	0.77	0.31	0.75	0	< 0.01	0.11	0.39	0.65	0.63	0.09	0.47	0.54	0.14	0.44	0.01	< 0.01
T 3lag	-0.45	0.01	0.46	0.78	-0.36	0.02	0.47	0.88	-0.08	0.30	0.71	0	0.05	0.28	0.13	0.36	0.01	0.07	0.36	0.74	0.32	0.44	0.01
R	0.16	-0.04	-0.24	-0.39	0.17	-0.15	-0.20	-0.41	0.06	-0.30	-0.33	-0.41	0	0.88	0.59	0.77	< 0.01	0.81	0.13	0.69	0.59	0.72	0.24
R 1lag	0.35	0.23	0.05	-0.16	0.30	0.21	-0.03	-0.06	0.28	0.11	-0.18	-0.23	0.03	0	0.64	0.90	0.46	< 0.01	0.63	0.26	0.57	0.52	0.58
R 2lag	0.36	0.24	0.07	-0.11	0.40	0.19	0.03	-0.21	0.39	0.16	-0.10	-0.32	-0.12	0.10	0	0.98	0.59	0.48	< 0.01	0.78	0.15	0.69	0.75
R 3lag	0.39	0.35	0.21	0.03	0.58	0.41	0.14	-0.05	0.43	0.38	0.10	-0.19	-0.06	-0.03	-0.01	0	0.91	0.59	0.52	< 0.01	0.23	0.13	0.73
Rh	0.29	0.05	-0.22	-0.44	0.28	-0.02	-0.10	-0.59	0.14	-0.16	-0.35	-0.56	0.68	0.16	0.11	0.02	0	0.79	0.73	0.60	0.59	0.42	0.40
Rh 1lag	0.46	0.29	0.05	-0.22	0.34	0.28	-0.02	-0.11	0.31	0.13	-0.16	-0.38	0.05	0.72	0.15	0.12	0.06	0	0.80	0.73	0.20	0.60	0.41
Rh 2lag	0.53	0.46	0.29	0.05	0.48	0.35	0.28	-0.04	0.41	0.32	0.13	-0.20	-0.32	0.10	0.61	0.14	-0.08	0.06	0	0.79	0.10	0.19	0.59
Rh 3lag	0.45	0.53	0.46	0.29	0.48	0.49	0.34	0.25	0.43	0.43	0.31	0.07	0.09	-0.24	-0.06	0.59	-0.11	-0.08	0.06	0	0.31	0.10	0.18
LF	-0.70	-0.73	-0.57	-0.27	-0.73	-0.59	-0.43	-0.18	-0.82	-0.52	-0.17	0.21	-0.11	-0.12	-0.31	-0.25	-0.12	-0.28	-0.34	-0.22	0	0.01	0.83
LF 1lag	-0.47	-0.70	-0.73	-0.57	-0.53	-0.73	-0.60	-0.45	-0.71	-0.81	-0.53	-0.16	-0.07	-0.14	-0.09	-0.31	-0.17	-0.12	-0.28	-0.34	0.55	0	0.01
LF 2lag	-0.12	-0.47	-0.70	-0.73	-0.08	-0.54	-0.74	-0.62	-0.34	-0.71	-0.82	-0.54	0.25	-0.12	-0.07	-0.07	0.18	-0.17	-0.12	-0.28	0.05	0.55	0

Appendix 5. Constructed climatic models tested for flowering and fruiting phenology expressed as principal coordinates of phylogenetic structure (PCPS). R – Rainfall; Rh – Historic Rainfall; D – Daylength; T – Temperature; LF – Leaf fall; PET – Potential evapotranspiration. Variables with a number preceding the lag indicate the respective monthly lag.

Flowering (FL)

FL ~ R	FL ~ PET	FL ~ R + T 1lag
FL ~ R 1lag	FL ~ PET 1lag	FL ~ R + D 1lag
FL ~ R + R 1lag	FL ~ PET 2lag	FL ~ R + T 2lag
FL ~ Rh	FL ~ PET 3lag	FL ~ R + D 2lag
FL ~ Rh 1lag	FL ~ Rh + T	FL ~ R + T 3lag
FL ~ Rh + Rh 1lag	FL ~ Rh + D	FL ~ R + D 3lag
FL ~ D	FL ~ Rh + T 1lag	FL ~ R 1lag + T
FL ~ D 1lag	FL ~ Rh + D 1lag	FL ~ R 1lag + D
FL ~ D 2lag	FL ~ Rh + T 2lag	FL ~ R 1lag + T 1lag
FL ~ D 3lag	FL ~ Rh + D 2lag	FL ~ R 1lag + D 1lag
FL ~ T	FL ~ Rh + T 3lag	FL ~ R 1lag + T 2lag
FL ~ T 1lag	FL ~ Rh + D 3lag	FL ~ R 1lag + D 2lag
FL ~ T 2lag	FL ~ R + T	FL ~ R 1lag + T 3lag
FL ~ T 3lag	FL ~ R + D	FL ~ R 1lag + D 3lag

Fruiting (FR)

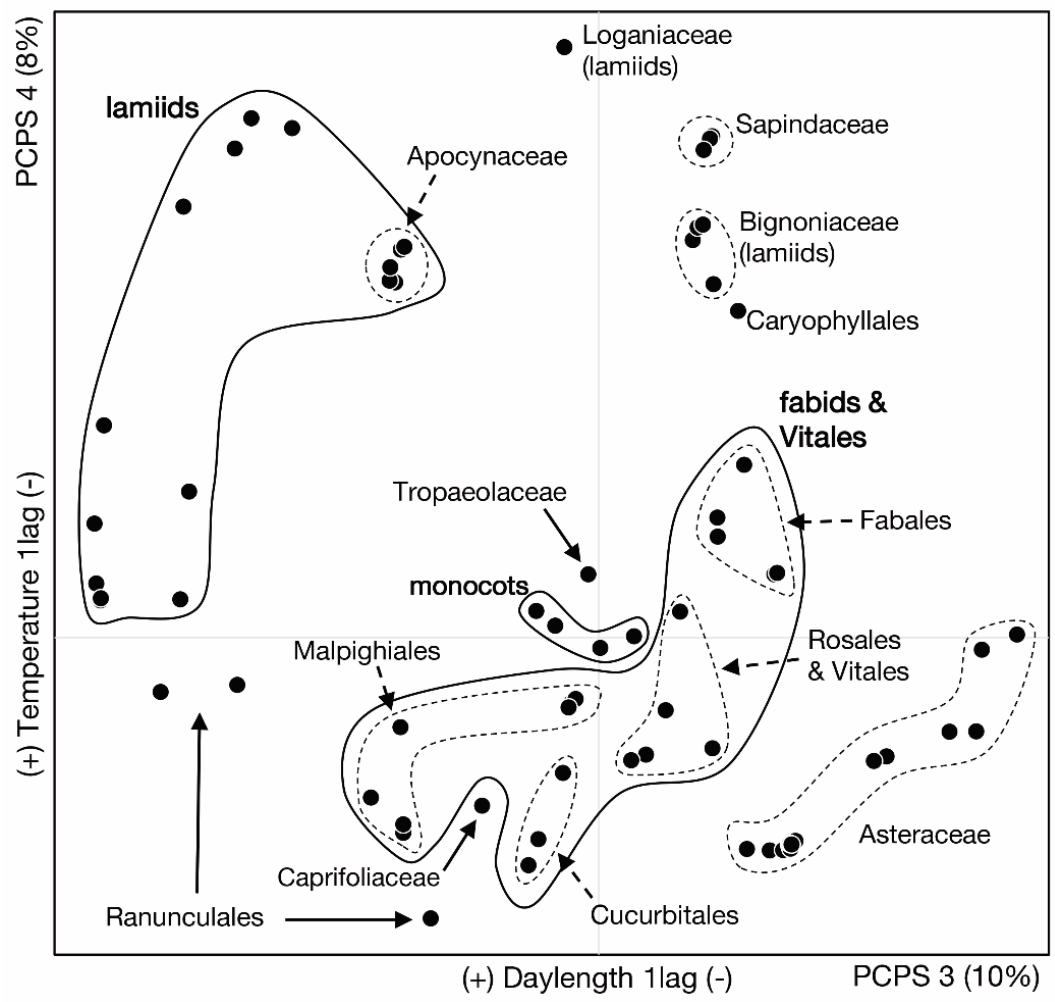
FR ~ T	FR ~ Rh 3lag	FL ~ R + T
FR ~ T 1lag	FR ~ Rh + Rh 1lag	FL ~ R + T 1lag
FR ~ T 2lag	FR ~ Rh 1lag + Rh 2lag	FL ~ R + T 2lag
FR ~ T 3lag	FR ~ Rh 1lag + Rh 2lag + Rh 3lag	FL ~ R + T 3lag
FR ~ D	FR ~ R	FR ~ R 1lag + T
FR ~ D 1lag	FR ~ R 1lag	FR ~ R 1lag + T 1lag
FR ~ D 2lag	FR ~ R 2lag	FR ~ R 1lag + T 2lag
FR ~ D 3lag	FR ~ R 3lag	FR ~ R 1lag + T 3lag
FR ~ PET	FR ~ R + R 1lag	FR ~ LF
FR ~ PET 1lag	FR ~ R 1lag + R 2lag	FR ~ LF 1lag
FR ~ PET 2lag	FR ~ R 1lag + R 2lag + R 3lag	FR ~ LF 2lag
FR ~ PET 3lag	FR ~ Rh + T	FR ~ LF + LF 1lag
FR ~ Rh	FR ~ Rh + T 1lag	FR ~ LF 1lag + LF 2lag
FR ~ Rh 1lag	FR ~ Rh + T 2lag	
FR ~ Rh 2lag	FR ~ Rh + T 3lag	

Appendix 6. Best models results of the model selection based on Akaike's information criterion (AIC) between the constructed climatic models and the four PCPS of flowering containing at least 5% of explanation on matrix P, and the linear model (LM) results of best models. In the model selection based on AIC the climatic models were compared to a null model in which the response variable is explained by its median and variance. The LM significance was evaluated by two null models, one that shuffles tip names across the phylogeny and the other that shuffles the sites across the environmental gradient. For details of models selection and null models interpretation, see Material and Methods section in the manuscript. R – Rainfall; Rh – Historic Rainfall; D – Daylength; T – Temperature; PET – Potential evapotranspiration. Variables with a number preceding the lag indicate the respective monthly lag.

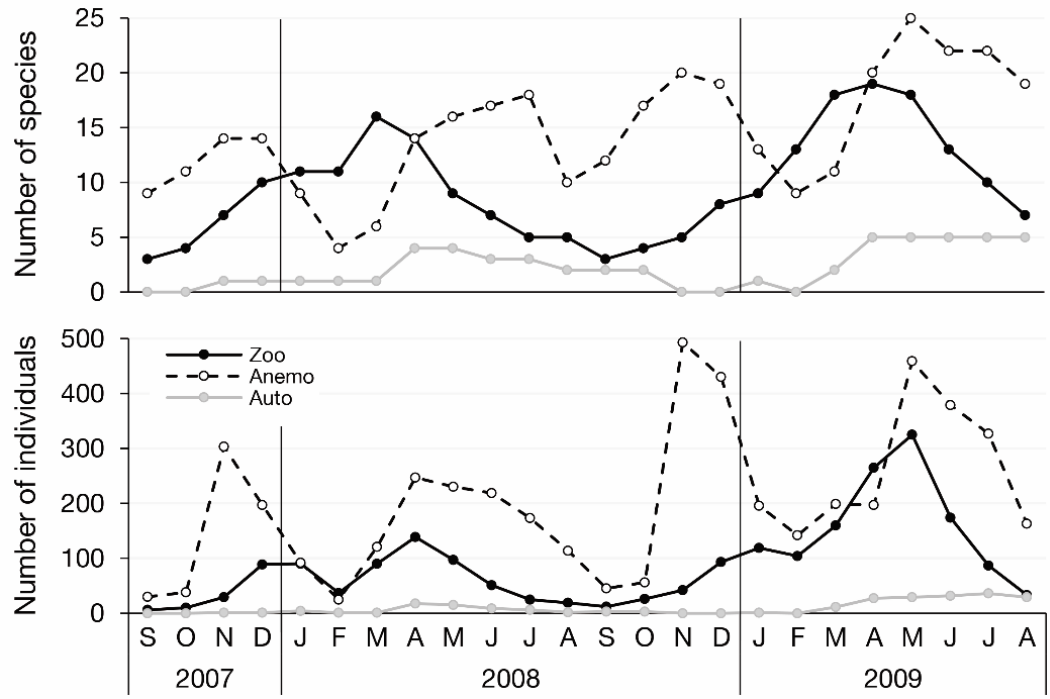
Models	**Information-based model selection**					$R^2_{Adj.}$	**Permutation test**		
	Log likelihood	**K**	**AICc**	**ΔAICc**	w_i		**F-value**	$P_{site\ shuffle}$	$P_{taxa\ shuffle}$
PCPS1									
Rh + D	36.87	4	-63.64	0.00	0.49	0.84	61.59	0.001	0.046
R 1lag + D	36.31	4	-62.51	1.13	0.28	0.83	58.27	0.001	0.047
D	34.40	3	-61.59	2.05	0.18	0.81	100.88	0.001	0.05
Null (Mean + σ^2)	13.75	2	-22.94	40.7	< 0.01				
PCPS2									
R 1lag1 + T 1lag	26.88	4	-43.66	0.00	0.17	0.31	6.10	0.006	0.478
Rh + T 1lag	26.34	4	-42.57	1.09	0.10	0.28	5.36	0.014	0.58
R 1 lag + T 2lag	26.16	4	-42.21	1.44	0.08	0.26	5.12	0.024	0.525
Rh lag 1	24.68	3	-42.16	1.50	0.08	0.21	6.96	0.019	0.165
Rh + Rh 1lag	25.81	4	-41.51	2.14	0.06	0.24	4.69	0.025	0.296
Null (Mean + σ^2)	21.38	2	-38.19	5.46	0.01				
PCPS3									
D 3lag	31.73	3	-56.27	0.00	0.16	0.26	9.24	0.007	0.223
D 2lag	31.55	3	-55.91	0.36	0.13	0.26	8.96	0.009	0.185
R 1lag + D 3lag	32.93	4	-55.75	0.52	0.12	0.31	6.07	0.006	0.206
Rh + D 3lag	32.87	4	-55.64	0.63	0.12	0.30	5.99	0.008	0.25
PET 3lag	31.36	3	-55.51	0.75	0.11	0.24	8.28	0.009	0.175
R + D 3lag	32.05	4	-53.99	2.28	0.05	0.25	4.80	0.018	0.26
Null (Mean + σ^2)	27.52	2	-50.48	5.79	< 0.01				
PCPS4									
T 2lag	45.52	3	-83.84	0.00	0.36	0.52	26.27	0.001	0.006
R 1lag + T 2lag	46.69	4	-83.28	0.56	0.28	0.55	14.78	0.001	0.009
Rh + T 2lag	45.92	4	-81.74	2.10	0.13	0.52	13.21	0.001	0.013
Null (Mean + σ^2)	36.09	2	-67.61	16.23	< 0.01				

Appendix 7. Best models results of the model selection based on Akaike's information criterion (AIC) between the constructed climatic models and the four PCPS of fruiting containing at least 5% of explanation on matrix P, and the linear model (LM) results of best models. In the model selection based on AIC the climatic models were compared to a null model in which the response variable is explained by its median and variance. The LM significance was evaluated by two null models, one that shuffles tip names across the phylogeny and the other that shuffles the sites across the environmental gradient. For details of model selection and null models interpretation, see Material and Methods section in the manuscript. R – Rainfall; Rh – Historic Rainfall; D – Daylength; T – Temperature; LF – Leaf fall. Variables with a number preceding the lag indicate the respective monthly lag; ^{NS} – Non-significant regression results.

Models	Information-based model selection					$R^2_{Adj.}$	Permutation test		
	Log likelihood	K	AICc	ΔAICc	w_i		F-value	$P_{site\ shuffle}$	$P_{taxa\ shuffle}$
PCPS1									
R 1lag + T 3lag	36.34	4	-62.58	0.00	0.58	0.76	36.61	0.001	0.008
Rh + T 3lag	35.40	4	-60.70	1.89	0.23	0.74	33.09	0.001	0.01
T 3lag	33.43	3	-59.66	2.93	0.13	0.70	55.49	0.001	0.016
Null (Mean + σ^2)	18.31	2	-32.07	30.52	< 0.01				
PCPS2									
Rh 3lag	30.51	3	-53.81	0.00	0.09	0.10^{NS}	3.52	0.073	0.251
Rh 2lag + Rh 3lag	31.90	4	-53.70	0.11	0.08	0.16^{NS}	3.19	0.053	0.305
Rh 2lag	30.09	3	-52.98	0.83	0.06	0.07^{NS}	2.66	0.11	0.342
Null (Mean + σ^2)	28.72	2	-52.87	0.94	0.06				
PCPS3									
D 1lag	43.57	3	-79.93	0.00	0.83	0.54	28.53	0.001	0.021
D	41.15	3	-75.09	4.84	0.07	0.44	19.30	0.001	0.024
Null (Mean + σ^2)	33.58	2	-62.61	17.32	< 0.01				
PCPS4									
T 1lag	40.48	3	-73.77	0.00	0.24	0.33	12.29	0.004	0.049
LF 1lag	39.61	3	-72.01	1.76	0.10	0.28	9.87	0.004	0.047
R + T 1lag	40.74	4	-71.38	2.39	0.07	0.31	6.23	0.014	0.064
Null (Mean + σ^2)	35.16	2	-65.74	8.02	< 0.01				



Appendix 8. Scatter diagram of the third and fourth principal coordinates of phylogenetic structure (PCPS) of climbing plants fructification. Full circles represent species grouped in monophyletic clades delimited by dashed and solid lines. Significant climatic variables through linear models are specified for each PCPS.



Appendix 9. Phenological patterns of climbing plants represented by the number of species and individuals of different dispersal syndromes fruiting over two years record. Zoo – Zoochoric species; Anemo – Anemochoric species; Auto – Autochoric species (including barochory). Classification of species dispersal syndromes followed (Seger & Hartz 2014).

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Conclusões

Na última década, a ecologia de comunidades incorporou a perspectiva evolutiva para a explicação dos processos estruturadores da coexistência entre espécies (Cavender-Bares et al. 2009; Mouquet et al. 2012). Em princípio, os mais variados estudos exploraram a estrutura de comunidades com uma visão estritamente filogenética, porém a integração com atributos funcionais passou de uma necessidade a uma realidade (Swenson 2013). Essa integração filogenética/funcional propiciou uma expansão na compreensão dos processos que influenciam a organização de comunidades. Processos antes reconhecidos por ocorrerem sob determinado padrão filogenético, como por exemplo a limitação de similaridade por competição ser um processo determinante em comunidades apresentando um padrão filogenético disperso ou aleatório, foram reinterpretados quando incorporada uma perspectiva funcional (Mayfield & Levine 2010; Godoy et al. 2014).

Conjuntamente com o avanço das técnicas computacionais e moleculares a área de ecologia filogenética/funcional se expandiu e novas abordagens se tornaram possíveis. Nesse contexto, a reconstrução de filogenias foi fortemente influenciada e técnicas até então restritas a filogeneticistas se tornaram acessíveis. Muitos estudos recentes utilizaram filogenias moleculares e mostraram que os métodos que vinham sendo utilizados poderiam levar a interpretações errôneas dos resultados (p.ex., Kress et al. 2009; Molina-Venegas & Roquet 2014). No primeiro capítulo da tese demonstrei que dependendo da medida de diversidade e dispersão utilizadas, o uso de filogenias moleculares não influencia os resultados comparado a filogenias geradas através dos programas *Phylomatic/Phylocom*, que são extensamente utilizados. Porém, pude demonstrar que da maneira que os programas *Phylomatic/Phylocom* vinham sendo utilizados, as filogenias geradas apresentavam erros nas estimativas dos valores dos ramos resultando em um alto erro do tipo II (subestimativa dos valores) quando

comparadas a filogenias moleculares. Esse resultado é preocupante já que é difícil estimar quantos estudos podem ter reconstruído filogenias com esse problema. O uso de filogenias moleculares é promissor, porém não é um procedimento simples e exige um conhecimento de técnicas moleculares não usuais a muitos ecólogos (Roquet et al. 2013). A disponibilidade de sequências moleculares em bancos de dados públicos (*Genbank*; Benson et al. 2013) ainda é pouca, mas não impede a reconstrução de filogenias com resolução ao nível de gênero para muitas famílias. Acredito que seja o momento de uma maior colaboração entre filogeneticistas, ecólogos e botânicos, não apenas para uma maior prospecção de espécies ainda não sequenciadas, mas também para uma maior aplicabilidade de técnicas moleculares no campo da ecologia filogenética e funcional.

Com o conhecimento obtido de reconstrução de filogenias moleculares, utilizei filogenias com maior resolução nos demais capítulos da tese. Meu enfoque foi em integrar as abordagens filogenética e funcional para avaliar a organização de comunidades no espaço e no tempo. No segundo capítulo avaliei a variação na composição de espécies, filogenética e funcional de plantas trepadeiras ao longo do processo de expansão da floresta sobre o campo. Abordei o quanto características de paisagem como a área e o isolamento de manchas florestais podem estar determinando a composição das comunidades. Como resultado encontrei que a área e o isolamento da mancha florestal são fatores determinantes do padrão de organização das comunidades, criando um filtro ambiental selecionando a composição de espécies. Além disso, as comunidades não são organizadas de acordo com as relações filogenéticas das espécies, mas atributos de dispersão como o tipo de fruto e a síndrome de dispersão mostraram uma associação com o gradiente de área e isolamento, sendo determinantes para colonização dos diferentes tipos de manchas florestais.

No terceiro capítulo, a integração entre filogenia e funcionalidade foi avaliada na escala temporal. Estudos têm explorado se espécies florescendo ou frutificando em uma comunidade

podem estar filogeneticamente estruturadas no tempo, ou seja, espécies filogeneticamente próximas apresentariam picos de atividade similares (p.ex., Staggemeier et al. 2010; Davies et al. 2013; Seger et al. 2013; Du et al. 2015). A relação entre fenologia e clima é a base de todos os eventos fenológicos (Fenner 1998), porém a história evolutiva pode estar mediando tanto o período fenológico como a sensibilidade fenológica a variações climáticas (CaraDonna & Inouye 2015). Portanto, clados filogeneticamente próximos ou distantes podem responder diferentemente a algum estímulo ambiental (p.ex. temperatura). Com isso, me questionei se espécies filogeneticamente próximas estariam respondendo de forma similar a alguma variável climática, independente de não apresentarem sinal filogenético nos seus picos de floração ou frutificação. Nesse capítulo a utilização de uma nova abordagem estatística permitiu confirmar essa hipótese, mostrando que amplos clados filogenéticos respondem de maneira similar à mesma variável climática. Além disso, a associação com algum estímulo ambiental pode estar atuando em uma escala filogenética mais fina como ao nível de família. Esses padrões diferiram entre a floração e frutificação, mostrando que possivelmente diferentes pressões evolutivas moldaram os períodos fenológicos nos diferentes clados. Essa abordagem permitiu uma nova visão de como não apenas variáveis abióticas como o clima podem estar estruturando temporalmente uma comunidade, mas também as relações filogenéticas podem ter papel fundamental nessa estruturação.

Destacado por Losos and Miles (1994), qualquer análise histórica só é precisa se a filogenia utilizada é correta e, consequentemente, é importante perceber que filogenias são hipóteses sobre padrões de descendência evolutiva. Acredito que nos próximos anos a precisão das filogenias aumentará consideravelmente e hipóteses filogenéticas se tornarão certezas concretas. A integração entre filogenia e funcionalidade já é uma realidade na área de ecologia de comunidades. Nessa tese pode-se perceber que ambas abordagens são complementares,

permitindo compreender de uma maneira mais completa como as comunidades se estruturam no espaço e no tempo.

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