UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA

O gênero *Galactia* P. Browne (Leguminosae, Papilionoideae) no Brasil

Tese de doutorado

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Tese apresentada ao Programa de Pós-Graduação em Botânica da UFRGS como um dos requisitos para obtenção do título de Doutor em Ciências: Botânica

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RESUMO

O gênero Galactia P. Browne (Leguminosae, Papilionoideae) no Brasil

Autor: Guilherme Bordignon Ceolin
Orientadora: Drª Sílvia T. S. Miotto
Data da defesa: 11 de março de 2011

O objetivo desta tese foi fazer uma revisão do gênero *Galactia* P. Browne (Leguminosae, Papilionoideae) no Brasil, englobando diversos aspectos, tanto taxonômicos quanto ecológicos e de conservação. No entanto, esta tese está dividida em dois eixos temáticos principais, os quais se complementam e se suportam mutuamente: a primeira parte discute as práticas epistemológicas e metodológicas envolvidas no trabalho taxonômico, as quais dão sustentação às decisões taxonômicas desenvolvidas na segunda parte. O principal resultado desta tese é uma sinopse taxonômica, que mostra que o gênero Galactia no Brasil é composto por 26 táxons, dos quais dois são descritos pela primeira vez e um configura-se como registro novo.

ABSTRACT

The genus *Galactia* P. Browne (Leguminosae, Papilionodeae) in Brazil

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Date of public defense: 11 Mar 2011

The objective of this thesis was to review the genus *Galactia* P. Browne (Leguminosae, Papilionoideae) in Brazil, covering several aspects regarding taxonomy, ecology and conservation. This thesis is divided into two main themes, which complement and support each other: the first part discusses the epistemological and methodological practices involved in taxonomic labour, giving support to the taxonomic decisions developed in the second part. The main result of this thesis is a taxonomic synopsis, showing that *Galactia* in Brazil consists of 26 taxa, being two described for the first time and one appearing as a new record to the country.

Esta tese é dedicada a minha família; se cheguei até aqui foi por causa do apoio de vocês

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Após 30.744 horas trabalhadas, 1.300 exsicatas analisadas, quase 10.000 km rodados, milhares de picadas de mosquito e de reais gastos, 1 capítulo de livro, 7 artigos e nenhuma viagem ao Caribe, esta tese chega ao final. Escrever uma tese para muitos é um sofrimento sem tamanho; para mim foi bastante divertido, todavia não menos trabalhoso e desgastante. Ainda bem que pude contar com muitas pessoas que me ajudaram nesta empreitada. Por isso, meus agradecimentos vão para as seguintes pessoas, em ordem mais ou menos cronológica:

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A todos que tiveram algum papel importante na minha tese e que agora eu não lembro porque não anotei no dia; vocês também foram importantes mesmo que anônimos;

“(...) em vez de abalar sua teoria, o trabalho provava a Darwin que a variação estava em toda a parte. Dez anos antes ele pensava que a variação era uma exceção da natureza. (...) todas as partes ‘de cada uma das espécies’ estavam propensas a mudanças; quanto mais ele observava, tanto mais a estabilidade parecia uma ilusão. (...) todas aquelas variações tornaram-se um empecilho na sua tentativa de definir cada espécie com precisão. Onde as variedades terminavam e onde começavam as novas espécies? (...) aquela ‘variação desconcertante’ era uma benção duvidosa. (...) ‘Como um especulador, ela me é prazerosa, mas como sistemata, me é detestável’.

(...) Frequentemente ele descrevia espécies separadas, depois pensava melhor e reclassificava-as como variações de uma única espécie, em seguida mudava sua opinião, rasgava a descrição e começava tudo outra vez. E a cada vez ‘eu tenho rilhado meus dentes, amaldiçoado as espécies e me perguntado que pecado cometi para se tão punido’. Como as espécies de hoje eram as variedades de ontem, ele acabou por cortar o nó górdio simplesmente aglomerando as variantes em espécies reconhecidas. Nada disso importava. Não existiam formas absolutas, invariáveis e, portanto, uma classificação exata era, de todo modo, improvável.”

Trecho extraído de Desmond, A. & Moore, J. 2007. *Darwin – a vida de um evolucionista atormentado* (pp. 394 – 395). 5ª edição. São Paulo: Geração Editorial.

Prefácio

A tese a seguir representa o esforço de quase cinco anos trabalhando com o gênero *Galactia*. Inicialmente apenas a Região Sul do Brasil estava sendo contemplada; posteriormente, devido a um razoável número de imbróglios taxonômicos que surgiram com a identidade de alguns táxons, cuja área de ocorrência transcendia as fronteiras políticas da citada região, achou-se por bem expandir a área de abrangência para todo o território nacional, pois assim era possível englobar a distribuição geográfica completa de vários táxons e ter-se uma ideia melhor da variação intraespecífica dos mesmos.

Esta tese está dividida em seções temáticas, as quais dão suporte e convergem para a seção única que é de fato a tese a ser defendida. As seções temáticas, por sua vez, estão divididas em artigos a serem submetidos (ou já submetidos) a vários periódicos internacionais, bem como um capítulo de livro multiautoral em preparação. Por questão de otimização de espaço e de recursos, os artigos que não foram submetidos estão formatados de acordo com a revista a que se destinam apenas no que tange às referências e alguns outros detalhes de organização do texto. O restante das normas (e.g. espaçamento duplo) não foi seguido, pois representaria um desperdício demasiado de papel. No canto superior esquerdo está designado o nome do periódico pretendido, seguido pelo título, nome dos autores (sem dados de filiação e endereços), abstract e palavras-chave; posteriormente, segue o texto. Ao final, encontram-se as figuras, tabelas e legendas.

Apreciem a leitura

INTRODUÇÃO

Por que mais uma tese em taxonomia?

A espécie humana tem uma tendência inata a procurar padrões e a ordenar tudo o que a cerca, baseando-se em uma ordem que parece a mais lógica possível. Cada ser humano ordena seu dia-a-dia da maneira que lhe parece mais útil, cada pessoa tem seu próprio método de fazer sua lógica funcionar. Porém, isso só vale para coisas pontuais, pessoais, pois não precisamos (na maioria das vezes) que outras pessoas entendam como foram ordenados os talheres numa gaveta ou os livros numa estante. Como seres que vivem em sociedade, certos padrões devem ser entendidos e seguidos por todos, em um código único e universal. Vivendo em sociedade temos que seguir certas regras e convenções para que não se faça o caos, para que a sociedade não entre em colapso. Para isso, criamos códigos e símbolos que nos permitam representar a abstração com a qual muitas vezes nos deparamos ao tentar entender certos conceitos. Aliás, o próprio termo conceito já encerra uma ideia de limite, pois um conceito nada mais é do que uma definição (*de finire*: dar um fim, um limite). Ao conceituarmos coisas, nós encaixotamos a realidade em pacotes que nos permitem lidar com ela, que nos permitem transmitir ideias e nos fazermos entender. Esta capacidade nos permitiu a criação de várias formas de linguagens, sejam elas verbais ou não. Isso tem funcionado muito bem nos últimos milhares de anos, pois o desenvolvimento alcançado pela espécie humana não encontra paralelo em nenhuma outra forma de vida conhecida, seja ela viva ou extinta.

Talvez por ter esta natureza dita superior, nós temos uma tendência a acharmos que não fazemos parte da natureza, que somos seres a parte, que temos o papel de explorar suas riquezas em benefício próprio ou contemplar sua beleza. Para isso, criamos formas de tentar entender e dominar a natureza. Muitas pessoas inventam deuses e valem-se de mitos. A isso, dá-se o nome de religião. Outras pessoas partem de bases experimentais, racionais e tentam entender a natureza tentando extrair as verdades que ela tem para contar, baseadas em evidências fortes e (tanto quanto é possível) inequívocas. A isso, dá-se o nome de ciência. O cientista tenta definir, conceituar e encaixotar as partes da natureza, a fim de tentar entendê-la, subjugá-la e utilizá-la. Para isso, as diferentes ciências criaram suas próprias linguagens, que podem ser entendidas em qualquer parte do mundo, desde que se faça parte daquele grupo.

Na taxonomia, tudo o que foi posto acima se encaixa perfeitamente. Em nenhum outro ramo das Ciências Biológicas, tanto quanto eu consigo visualizar, a tarefa de dar limites para entidades é tão aplicada e discutida. Porém, na natureza raramente temos padrões estanques o bastante para que obtenhamos agrupamentos suficientemente definidos e inequívocos, que possam ser convertidos e codificados em um sistema universalmente compreendido. Qualquer tentativa de conceituar os seres vivos e dividi-los em agrupamentos é falha em algum momento. Sempre haverá organismos que apresentarão padrões intermediários ou que escapam completamente do sistema proposto. Na verdade, a natureza não se divide em classes, ela é muito mais um contínuo que reflete perfeitamente a relação de ancestralidade-descendência, a qual diz que todos os seres vivos derivam de um ancestral comum. Ainda assim, frequentemente conseguimos estabelecer classes com relativo sucesso e isso torna o trabalho do cientista incrivelmente mais fácil. Contudo, em maior ou menor grau todas as classes são frágeis e só funcionam se obedecermos a um período espaço-temporal limitado. Findo este período, tudo muda e faz-se necessário reorganizar as classes. Não por acaso que os estudos taxonômicos são ditos como sendo um trabalho sem fim, pois raras vezes se podem estudar todos os indivíduos de todas as populações, para que possamos quantificar toda a variação existente. Ainda assim, devido à dimensão temporal das espécies, corremos o risco de que logo se descubra um indivíduo que fuja ao padrão esperado. E realmente deve ser assim, pois, como postulou *Sir* Karl Popper (1959), o que garante a verdade do discurso científico é a condição de refutabilidade de suas generalizações. Em ciência, nunca podemos provar definitivamente que alguma generalização é verdadeira, nós só podemos provar com certeza que ela é falsa, bastando uma única observação que fuja ao padrão. Por isso, de tempos em tempos, torna-se imprescindível repetirmos os estudos taxonômicos, para que possamos confirmar ou refutar as hipóteses que o autor formulou ao trabalhar anteriormente com o grupo que está sendo revisado. De que hipóteses estamos falando? De espécies!

Uma espécie é uma hipótese e em sendo uma hipótese, é necessário testá-la. Vários autores já enfatizaram que a taxonomia é uma ciência guiada por hipóteses (e.g. Henderson 2005). O grande problema é que nem sempre os taxonomistas têm isso em mente quando se propõe a fazer a revisão de um grupo. Henderson (2005) advoga que se uma revisão ou monografia é produzida da maneira tradicional (a chamada Taxonomia Clássica), as espécies reconhecidas podem ser consideradas como hipóteses. Mas, geralmente não há o teste destas hipóteses. Elas precisarão esperar uma revisão futura do mesmo grupo, a qual pode demorar muitos anos (às vezes uma centena de anos) para acontecer. E se a nova revisão for realizada com a mesma abordagem subjetiva de outrora, sem qualquer teste explícito de hipóteses, na verdade ela não estará acrescentando nada novo, apenas propondo novas hipóteses. Por isso, não basta só refazermos o trabalho de outros autores, é necessário acrescentar algo novo, e não estou falando de novas espécies! Temos que melhorar a precisão científica e a previsibilidade do trabalho clássico, propondo novas ideias e métodos, movendo-nos da simples compilação de dados e descrição de padrões (e até isso às vezes ainda é mal feito) para o teste de hipóteses e a busca por novos paradigmas. Sim, pois, segundo Kuhn (1962), somente quando os paradigmas vigentes em uma ciência são desafiados e quebrados, sendo substituídos por outros, é que a ciência avança de fato. Isto aconteceu várias vezes na história da ciência e deve continuar acontecendo enquanto houver ciência organizada. E não há razão para que seja diferente na taxonomia alfa baseada em morfologia (*morphology-based alpha taxonomy* em inglês, abreviada como MOBAT). Porém, para que possamos começar a pensar a MOBAT em termos de formulações de hipóteses e quebras de paradigmas, temos que ter bem claro o conceito de nossa unidade básica de estudo, temos que estar seguros sobre o que é, afinal, uma espécie.

Uma breve história sobre a classificação e o conceito de espécie

Ao longo da história[1] humana, várias foram as formas de se classificarem os seres vivos, aqui falando exclusivamente de plantas. A forma mais usual e ainda recorrente de classificação é baseada no hábito (ervas, arbustos, árvores) ou na utilidade ao homem (alimentícias, venenosas, ornamentais). Esta forma prática de classificar as plantas surgiu junto com a agricultura, passou pelas grandes civilizações, atravessou a Idade Média até chegar a Lineu e seu *Species Plantarum* (1753). Frequentemente, dizemos que Lineu é o “Pai da Taxonomia”, pois ele popularizou o sistema binomial e criou um sistema organizado e lógico (ainda que artificial) para classificar as plantas. Com isso, Lineu fundou os pilares da sistemática formal, cuja lógica básica vigora inalterada até hoje: os organismos são classificados em agrupamentos hierárquicos cada vez mais abrangentes, sendo a espécie a menor unidade de classificação. O fato de a espécie ser a unidade básica de classificação é consenso entre todos os naturalistas e, em sendo um consenso, esperaríamos que a simples menção da palavra formasse uma imagem mental instantânea do que este conceito significa e que tal imagem fosse igual para todos. Ou seja, fosse um conceito universal. Na verdade, nada é mais fugidio que o conceito de espécie. Ou melhor dizendo, os conceitos de espécie.

Nos tempos de Lineu e dos primeiros taxonomistas no sentido estrito, não havia conflito, todos concordavam que as espécies tinham sido criadas por Deus na forma como as vemos hoje, não vindo de lugar nenhum nem dando origem a outras espécies. Tal noção de estase derivava basicamente das ideias de Platão de que tudo o que existe na natureza provém de um número limitado de tipos básicos, que existem em um plano diferente do plano material em que vivemos. Esta filosofia platônica, dita “essencialista”, foi aplicada por seu discípulo Aristóteles ao classificar os seres vivos. Para ele, os indivíduos de uma espécie não têm relação especial entre si, são apenas expressões do mesmo tipo,

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[1] Estou considerando como histórico o período iniciado a partir do surgimento da escrita

essência ou ideia (*eidos*) existentes em um plano metafísico. A variação é o resultado de manifestações imperfeitas do *eidos* de cada espécie. Porém, a partir da seminal obra de Darwin (1859), curiosamente (e resumidamente) intitulada "A Origem das Espécies"[2], muito tempo, dinheiro, papel e paciência tem sido gasto discutindo o chamado "Problema do Conceito de Espécie". Apesar disso, este problema está longe de terminar e mais de duas dúzias de conceitos novos e diferentes têm sido propostos nestes 150 anos, sem falar em alguns resquícios atuais das ideias platônicas. Ao designarmos tipos nomenclaturais como modelo para uma nova espécie que descrevemos, estamos nos valendo diretamente da filosofia essencialista platônica, pois escolhemos apenas uma amostra muito pequena da variação para representarmos o que se entende por aquela espécie. A designação de tipos é uma prática sobrevivente do chamado "Conceito Tipológico de Espécie" (CTE), um conceito que só se mantém devido à tradição (e talvez necessidade) de designarmos tais tipos. Nenhum taxonomista sério atualmente descreveria uma espécie nova baseado em pequenas diferenças, como os antigos faziam.

Um outro conceito bastante utilizado e que tem sobrevivido bravamente é o 'Conceito Biológico de Espécie' (CBE) (Mayr 1963). Tal conceito postula que espécies são grupos de populações naturais que cruzam entre si, mas são isoladas reprodutivamente de grupos similares. É um conceito coerente com a teoria evolutiva de Darwin-Wallace e que pode ser empiricamente testado, realizando-se cruzamentos intra e interespecíficos e testando-se a viabilidade dos híbridos. Basicamente, o que se quer evidenciar é se há fluxo gênico entre os indivíduos, dando-se especial atenção aos mecanismos que contribuem para mantê-lo constante dentro da espécie e impedi-lo entre grupos similares (mecanismos pré e pós-zigóticos de isolamento reprodutivo). É um conceito que se aplica a vários grupos de organismos e tem sido utilizado para dirimir dúvidas em complexos de espécies (p.ex. Teixeira & Ranga 2004), além de ser o mais popular dentre todos. Mas, existem exceções, como por exemplo organismos que se multiplicam por divisão binária ou espécies apomíticas. Tais exceções limitam a generalidade do conceito e o enfraquecem.

As limitações do CBE chamaram a atenção de vários pesquisadores, especialmente na década de 70, uma época em que todos estavam interessados em definir espécie levando em consideração apenas o fluxo gênico. Um destes pesquisadores foi Van Valen (1976) que propôs o que foi chamado "Conceito Ecológico de Espécie" (CEE). Este conceito postula que uma espécie é uma linhagem que ocupa uma zona adaptativa minimamente diferente de qualquer outra linhagem em sua distribuição e que evolui separadamente de todas as outras linhagens fora desta distribuição. Uma zona adaptativa seria a combinação de todas as variáveis ambientais sob as quais uma espécie ou população pode

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[2] Que a despeito do título, contribuiu para iniciar o caos, ao invés de solucioná-lo!

persistir. Tal definição é mais ou menos equivalente ao conceito de nicho ecológico hipervolumétrico (Hutchinson 1959). O principal mérito de Van Valen foi ter retomado a discussão sobre o papel da seleção natural como responsável por manter uma espécie separada da outra, já que cada uma ocupa uma zona adaptativa diferente. Um dos problemas deste conceito é a falta de um critério prático e objetivo para se estabelecer inequivocamente o que seja uma diferença mínima em uma zona adaptativa em relação à outra, pois muitos táxons são fenotipicamente plásticos o suficiente para ocuparem zonas adaptativas bastante extremas, sem necessariamente haver indivíduos ocupando os ambientes de transição. Por este motivo, o reconhecimento de espécies estaria fortemente atrelado a critérios subjetivos, sendo que cada pesquisador definiria uma linhagem como tendo mais ou menos espécies, de acordo com suas idiossincrasias (a velha dicotomia *Splitters vs. Lumpers*[3]).

Em 1973, Sneath & Sokal lançaram o agora clássico livro intitulado “Numerical Taxonomy”. Assim como Van Valen propôs o CEE para fazer um contraponto aos biólogos evolutivos que estavam reduzindo toda a questão taxonômica a fluxo gênico, Sneath & Sokal pretendiam contraporem-se ao *modus operandi* da “Velha Guarda”, cujas identificações e descrições de espécies eram baseadas totalmente na experiência pessoal do taxonomista, tornando os trabalhos taxonômicos altamente subjetivos. A principal ideia da obra de Sneath & Sokal é que os táxons deveriam ser reconhecidos levando-se em consideração a similaridade geral entre os indivíduos de uma espécie putativa e que estes indivíduos diferissem de outros indivíduos de outra espécie potencial. Mas, tal classificação de similaridades e dissimilaridades não deveria ser feita pelo taxonomista e sim por um programa de computador que pudesse avaliar as semelhanças e testar a significância estatística dos grupos formados, tornando a classificação totalmente objetiva. Ao pesquisador caberia reunir todas as informações (medidas, estados dos caracteres, tipo de ambiente, etc.) possíveis e que fossem úteis para construir um banco de dados que pudesse ser rodado em um programa específico. O resultado final da análise é um dendrograma (chamado *fenograma*, pois mede o grau de semelhança fenética entre indivíduos) agrupando os indivíduos mais semelhantes em ramos próximos e os diferentes em ramos mais distantes. Este modo de resolver o problema do reconhecimento de espécies muitas vezes é chamado de “Conceito Fenético de Espécie” (CFeE). Porém, estes fenogramas não necessariamente refletem similaridade genética ou proximidade evolutiva entre organismos, eles simplesmente nos informam o quanto dois grupos de indivíduos são mais ou menos similares. Estas limitações do método contribuíram para fazê-lo progressivamente cair em desuso, sendo substituído por um outro método,

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[3] Termos da língua inglesa sem equivalentes formais em Português, mas que servem para caracterizar os taxonomistas que tendem a separar (“splitters”) ou juntar táxons (“lumpers”).

este sim trazendo para a sistemática a história evolutiva dos grupos e causando uma verdadeira revolução no modo como encaramos a classificação dos organismos desde então.

Conforme vimos anteriormente, Lineu propôs um sistema que rompeu com o método de classificação baseado em formas de vida e utilidade para o ser humano e Darwin-Wallace propuseram uma alternativa plausível a ideia do fixismo na forma das espécies. Porém somente na década de 60 é que os dois conceitos (classificação por critérios universais e evolução por seleção natural) foram unidos por uma metodologia formal de classificação dos seres vivos. Tal metodologia foi chamada de 'cladística' e teve em Willi Hennig, um entomólogo alemão, seu principal expoente. Sua obra *Phylogenetic Systematics* (1966) foi a pedra fundamental da cladística, estabelecendo suas bases e metodologia de trabalho. A principal mudança introduzida pelo advento da cladística foi que não importa o quanto dois organismos sejam similares, o que importa para serem agrupados juntos é que eles compartilhem uma história evolutiva comum. Ou seja, derivem ambos de um mesmo ancestral (ou segundo o jargão da cladística, sejam *monofiléticos*). Assim como os feneticistas, os cladistas também montam bancos de dados a partir das características dos indivíduos, fazendo análises quali-quantitativas dos caracteres. Porém, o que se busca com estas análises é estabelecer a relação de ancestralidade-descendência destes organismos a partir de estados de caráter primitivos[4] e derivados. Um caráter no seu estado primitivo recebe o nome de *plesiomórfico*, enquanto que no seu estado derivado chama-se *apomórfico.* Os caracteres compartilhados por vários indivíduos em uma linhagem derivada são chamados *sinapomórficos*, e é isso que os cladistas buscam quando pretendem traçar a história evolutiva dos organismos. Assim, diferente da fenética, o que importa na cladística não é a similaridade geral entre todos os caracteres compartilhados pelos organismos, mas sim a similaridade entre os caracteres derivados que são compartilhados[5]. Somente os organismos monofiléticos são considerados e devem receber nomes formais, de acordo com os *rankings* taxonômicos clássicos (família, tribo, gênero, etc.). O mesmo vale para a categoria de espécie. Segundo algumas correntes de pensamento, deve-se considerar espécie como sendo uma linhagem de indivíduos que possuem uma relação hierárquica (ancestral-descendente) e que pode ser diagnosticada por um conjunto de caracteres únicos e exclusivos. Neste caso, não podemos chamar estes caracteres de sinapomorfias e sim de autapomorfias, pois são exclusivos de apenas uma linhagem, não tendo valor informativo para a reconstrução da filogenia de um grupo mais amplo que inclui várias linhagens. Esta definição é chamada de "Conceito Filogenético de Espécie" (CFiE).

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[4] Não estou emitindo qualquer juízo de valor ao usar a palavra "primitivo", de que organismos primitivos sejam melhores ou piores. Simplesmente, quero dizer que algo "primitivo" é algo que surgiu "primeiro".

[5] Para um maior esclarecimento e aprofundamento sobre pressupostos, teorias e métodos, sugiro a leitura da parte introdutória de Judd et al. 2009. *Sistemática vegetal – um enfoque filogenético*. Artmed: Porto Alegre.

Apesar de toda esta revolução na forma de encarar a classificação dos organismos ter acontecido nos últimos cinquenta anos, o conceito de espécie continuou sendo alvo de inúmeros debates acadêmicos e de inúmeras proposições novas sendo publicadas de tempos em tempos. Sempre que alguém descobria uma falha em algum conceito proposto, vinha outro e propunha algo com o intuito de melhorar o anterior e assim sucessivamente, inflacionando a lista de conceitos cada vez mais (Mayden 1997 discute mais de vinte conceitos!). Os taxonomistas acompanharam a virada do século XX com esta questão em aberto, a qual não é meramente uma questão de semântica, mas uma questão que permeia todos os campos da Biologia, pois a unidade básica da vida é a espécie.

O fim da controvérsia?

O debate sobre o problema do conceito de espécie vinha num crescente constante, até que em 2003 o geneticista e filósofo italiano naturalizado estadunidense Massimo Pigliucci publicou um artigo defendendo a ideia de que não existe uma única característica (ou conjunto de características) suficiente para definir todas as espécies de todos os tipos de organismos. Na verdade, existem alguns atributos que todas as espécies possuem, mas não sempre todos ao mesmo tempo. Tal definição é baseada em uma ideia do filósofo alemão Ludwig Wittgenstein, um estudioso da natureza da linguagem humana. Em sua obra, *Philosophical Investigation* (1953) ele propôs a ideia de que os humanos, por praticidade, se lançam no que ele chamou de “jogos de linguagem”, que é uma negociação social interativa do significado de alguns termos, mediante a interação contínua entre indivíduos. Como um meio prático para explicar o que isto quer dizer, Wittgenstein considerou como exemplo a definição de “jogo”. Por mais que se tente, é impossível definir tal termo com um conceito simples e que englobe todas as possibilidades do que se conhece por “jogo”. Coisas tão díspares como pôquer, xadrez, futebol, nado sincronizado e paciência são reconhecidas como tal, mas é impossível achar um conjunto de características que seja compartilhado por todos estes ao mesmo tempo. É mais fácil definir “jogo” dando-se um exemplo prático: “Isto e todas as coisas parecidas com isto são chamadas de jogo”. Na verdade, conceitos como este são definidos por conjuntos de características (*cluster concepts*), ou o que Wittgenstein chamou de “semelhança de família” (*family resemblance*), aludindo ao fato de que indivíduos de uma mesma família compartilham algumas características ou outras, mas nenhum atributo isolado identifica uma família como distinta da outra. E é exatamente o que Pigliucci (2003) sugere em seu artigo: que devemos encarar espécie (ou o conceito de espécie) como um conceito de semelhança de família, cujos representantes exibem algumas características fundamentais, como relações filogenéticas, similaridade genética, compatibilidade reprodutiva e papel ecológico. Estes atributos não estarão presentes sempre ao mesmo tempo em todos os tipos de organismos, mas irão ser mais ou menos relevantes dependendo do grupo em questão. Segundo Pigliucci, esta forma de encarar o problema exibe algumas vantagens práticas, sendo que a principal delas é que agora temos um conceito fluído sem ser arbitrário e flexível o suficiente para ser aplicável a uma variedade de casos biológicos reais, pondo um fim na interminável discussão sobre qual é o melhor conceito de espécie. Além disso, conceitos politéticos, como é o caso, nos forçam a sermos menos rígidos com as definições, permitindo uma maior apreciação de toda a variação existente nos organismos. Porém, quem pensa que a discussão terminou aqui, enganou-se. Mais lenha continuou sendo posta na fogueira, mostrando que Pigliucci estava equivocado ao dizer que nenhuma propriedade era sempre compartilhada por todas as espécies ao mesmo tempo. Que propriedade é esta é o que veremos a seguir.

Em artigo publicado em 2005, o herpetólogo estadunidense Kevin de Queiroz defende que existe uma característica que invariavelmente todas as espécies compartilham, que é o fato de serem linhagens metapopulacionais (ou segmentos de) evoluindo separadamente. Isto implica que todas as propriedades anteriormente usadas para definir espécies e que não se aplicavam a todos os organismos são muito mais contingentes do que necessárias para se reconhecer uma espécie. Isto é, as diferentes linhagens metapopulacionais vão adquirindo as diferentes propriedades (e.g. isolamento reprodutivo [Mayr 1942,], coalescência exclusiva de alelos [Baum & Shaw 1995], diagnostibilidade [Nixon & Wheeler 1990]) com o passar do tempo desde a separação entre elas. Como nem todas estarão no mesmo estágio ao mesmo tempo, diferentes métodos indicarão diferentes números de espécies. Assim, usando as palavras do autor: “esta proposta reconcilia conceitos e definições de espécie incompatíveis sem negar as propriedades que as tornam diferentes”. Por tudo isto, esta proposta é chamada de “Conceito Unificado de Espécie” (em inglês, *Unified Species Concept* [USC]). de Queiroz (2005) enfatiza em seu artigo que, sob o USC, o pressuposto de que o conceito ideal de espécie deveria ser vinculado ao método de descoberta deixa de existir, pois estes assuntos estão claramente separados. O conceito de espécie por si agora passa a ter uma conotação exclusivamente conceitual e teórica, relacionada à questão: “O que é uma espécie?” Já o método de descoberta tem uma conotação, como diz o próprio nome, metodológica, servindo para responder à pergunta: “Como nós reconhecemos um espécie na prática?” O fato de que todas as propriedades outrora utilizadas para definir uma espécie são contingentes muito mais do que suficientes faz com que os limites entre as espécies dependam do estágio de separação no qual duas linhagens metapopulacionais se encontram e do tipo de dados utilizados.

Tendo resolvido os problemas com a delimitação do nosso objeto de estudo, partiremos para outra discussão igualmente importante: os delineamentos experimentais e os métodos dos trabalhos taxonômicos.

Otimizando tempo, dinheiro e informação

É muito fácil acontecer de trabalhos taxonômicos terem uma relação custo/benefício ruim, com uma relação inversa acentuada entre recursos despendidos e baixa qualidade de informação adquirida. E isto é algo importante a ser pensado. Não podemos nos iludir e pensar que não devemos ponderar custos ao propormos um projeto de pesquisa, pois existe um abismo entre a ciência ideal e a ciência possível. A ciência também deve ser pragmática. Faz parte da responsabilidade dos pesquisadores produzirem conhecimento em prazos preferivelmente curtos e com gastos preferivelmente razoáveis. Ineficiência é incompatível com estas responsabilidades e, portanto, questionamentos sobre as práticas são eventualmente necessários. Por exemplo, quando os trabalhos taxonômicos são feitos em áreas limitadas por fronteiras políticas (e.g. inventário de um gênero para uma Região ou um Estado), a informação gerada normalmente é incompleta, pois só estamos analisando uma parte da distribuição do táxon estudado. Isso não permite muitas generalizações, pois só temos algumas páginas do livro, tornando a informação fragmentada, sem coesão, várias histórias estão sendo contadas ao mesmo tempo, mas sem um amálgama para uni-las. Isto diminui a probabilidade de que a informação seja extrapolada. Muito tempo e dinheiro será gasto com uma informação de uso limitado. Para balancear a equação, várias são as soluções possíveis. Por exemplo, é preferível diminuir a área amostral, mas amostrar uma região com fronteiras naturais, ao invés de políticas. Pode ser um bioma (Cerrado), uma serra (Serra do Espinhaço) ou mesmo um único morro (Morro da Igreja). O importante é que a área escolhida tenha uma característica o mais natural possível e que as perguntas feitas sejam adequadas. Isso melhora a chance de que a informação seja de boa qualidade, permitindo extrair conclusões mais amplas, que possam ser extrapoladas ou servirem de ponto de partida para outros experimentos. Informações de boa qualidade, sobretudo generalizáveis, é que tornam o saber científico útil. E informação de boa qualidade não necessariamente implica em sofisticação tecnológica nem em exaustivas coletas de dados. E este é outro ponto que pesa na equação custo-benefício.

Trabalhando mais com a cabeça do que com as pernas

Aqui no Brasil existe uma tendência a acreditar-se que o trabalho taxonômico só pode ser um bom trabalho se tiver exaustivas coletas em campo. E devido aos problemas logísticos que esta mentalidade acarreta a maioria dos trabalhos acaba caindo no que foi descrito anteriormente: revisões taxonômicas que ficam restritas a regiões politicamente delimitadas. Monografias completas de grupos para grandes áreas, como continentes, regiões fitogeográficas (e.g. Flora Neotropica) ou mesmo todo o planeta, raramente possuem pesquisadores brasileiros envolvidos. A maioria é coordenada por taxonomistas que trabalham para grandes centros como Royal Botanic Garden – Kew (Grã-Bretanha) ou Missouri Botanical Garden (EUA). Por que eles conseguem? Uma explicação recorrente é que estes centros habitam o chamado Primeiro Mundo, portanto, possuem mais dinheiro e mais tradição e, por isso, não podemos competir com eles. Há pouco tempo atrás, isto até poderia ser verdade. Mas atualmente, a situação da pesquisa brasileira é uma das melhores dos últimos anos, inclusive com a criação de linhas de financiamento exclusivamente para projetos taxonômicos (CNPq 2010). Ou seja, ainda que eu acredite que os grandes centros tenham realmente mais tradição (afinal, eles estão no ramo há bem mais tempo) e até mais financiamento, não há mais razão para darmos a velha desculpa de que nosso problema é falta de dinheiro. O fato é que dinheiro existe, o que falta são projetos bem delineados, em que a equação esforço/retorno opere com saldo positivo. Para que revisões taxonômicas para áreas muito amplas ou de grupos com grande número de táxons sejam viáveis, obrigatoriamente devemos restringir o esforço de coleta em campo. Querer atingir suficiência amostral para todos os táxons com coletas próprias torna o projeto impraticável, tanto em termos financeiros quanto temporais. A solução é dar mais valor aos herbários e à utilização de abordagens multidisciplinares.

O material depositado em herbários é uma rica e ainda inexplorada (eu diria até subestimada) fonte de informação, tanto taxonômica quanto ecológica, fenológica, biogeográfica, etc. Grandes revisões taxonômicas são baseadas quase que exclusivamente em material exsicatado (Henderson 2005). Então, quer dizer que as coletas em campo são totalmente desnecessárias? Absolutamente não! É importante coletar, pois as coletas permitem ao pesquisador conhecer a planta viva e observar nuances que se perdem na planta seca, mesmo que a etiqueta seja bem detalhada. O problema se dá quando a relação entre análise de coletas próprias e análise de material de herbário está invertida, favorecendo aquela e não esta. O material de herbário deve ser a principal fonte de informação taxonômica, deixando que as coletas sirvam como fonte auxiliar de informação. O que normalmente acontece é que as coletas próprias são a fonte primária de informação, deixando para os herbários a complementação. Algumas espécies são tão abundantes nos herbários e tão fáceis de reconhecer que torna desnecessária uma recoleta. Devemos direcionar os esforços de coleta para as espécies raras, para áreas inexploradas (e.g. Amazônia) ou para táxons que apresentem problemas de delimitação. E neste último caso, somente se as informações adquiridas estudando-se a espécie em seu hábitat natural forem imprescindíveis para a resolução do problema. E, muitas vezes, a simples mudança de foco metodológico pode resolver o problema nos complexos taxonômicos.

Por exemplo, taxonomistas brasileiros ainda exploram pouco as análises morfométricas. Talvez isso se deva ao fato de não existir uma tradição de ensino em estatística para taxonomistas, pois a mesma é vista como desnecessária para MOBAT. Tal fato não acontece com quem trabalha com taxonomia molecular, pois esta é notoriamente reconhecida pelo uso intensivo de análises estatísticas para testar hipóteses. Não por acaso, os poucos taxonomistas brasileiros que utilizam morfometria na taxonomia alfa, também são aqueles que trabalham com filogenias (e.g. Borba *et al.* 2002). Outra explicação para esta aversão à utilização de análises estatísticas talvez seja o fato que a MOBAT é uma ciência ainda muito baseada no respeito à autoridade dos antigos taxonomistas e a uma utilização equivocada do método científico.

Contra o método – Feyerabend revisitado

A maioria dos alfa-taxonomistas tem um cuidado extremo (medo?) de não contestar a autoridade dos ditos “figurões” dos grupos em que eles trabalham. Suponho que seja por este motivo que em MOBAT dê-se mais valor à descrição de espécies novas do que a sinonimização de espécies duvidosas. Métodos “frios e calculistas” como análises estatísticas podem mudar a configuração de alguns táxons ou reduzir à sinonímia algumas espécies. A principal queixa dos alfa-taxonomistas para com as filogenias é que elas muitas vezes extinguem táxons tidos como “carismáticos”. Este tipo de mentalidade centrada na autoridade foi abandonada já no século XVII, com a chamada Revolução Científica, cujos maiores expoentes foram Galileu Galilei, Isaac Newton e Francis Bacon. Bacon chegou a dizer que se quisermos compreender a natureza, devemos consultar a própria natureza e não os escritos de Aristóteles (Chalmers 1995). Mas, dirão alguns, os alfa-taxonomistas consultam a natureza, pois sempre baseiam suas conclusões nos dados obtidos através das coletas e revisão de materiais de herbário, ou seja, possuem um método. O que acontece, de fato, é a utilização equivocada do método indutivo. O método indutivo puro pressupõe ausência de hipóteses *a priori*: as conclusões são tiradas a partir dos dados sem interferência subjetiva do pesquisador. Os alfa-taxonomistas já partem de hipóteses pré-estabelecidas, que são as espécies já descritas e delimitadas por outros pesquisadores, muitas vezes com a interferência ostensiva da subjetividade. O resultado é que, na verdade, não há um método no sentido literal do termo, o qual prevê repetibilidade e teste independente dos resultados. E a MOBAT, como instância primeira no estudo de uma biodiversidade tão ameaçada atualmente, precisa muito de um método no sentido *ipsis literis* do termo. E tal método deve ter um embasamento teórico bem fundamentado para poder contemplar os pressupostos citados anteriormente. Aqui, discutirei um pouco os aspectos filosóficos e epistemológicos dos métodos em MOBAT, já que no artigo 1 desta tese eu reviso e discuto este tema do ponto de vista técnico, analisando e descrevendo as ferramentas para objetivamente delimitar uma espécie.

Discurso sobre o método (300 anos após Descartes)[6]

Já vimos como o método indutivo é mal aplicado na taxonomia devido à violação de seus pressupostos fundamentais. E mesmo que fosse corretamente aplicado na sua forma original, este não é o método mais indicado para alguns tipos de problema. E veremos por que. A ciência como instrumento de conhecimento do mundo tenta derivar leis e teorias que sejam as mais universais possíveis. O método indutivo parte da observação de um grande número de eventos para derivar estas leis. Na taxonomia temos um esboço do método indutivo, no sentido de que basicamente nosso trabalho é amostrar um grande número de indivíduos de uma espécie qualquer, analisar seus atributos e daí concluir que tais indivíduos pertencem a uma espécie ‘x’ já existente ou é uma espécie ainda não descrita. Nós decidimos isso a partir das similaridades ou diferenças entre os grupos. Quais similaridades e quais diferenças? É aí que reside o problema. Uma das principais críticas ao indutivismo “de raiz” é que ele supõe, além de um número muito grande de observações, uma ampla variedade de circunstâncias. Mas, o que vem a ser um grande número de observações e uma ampla variedade de circunstâncias? Para descobrir que o fogo queima, nem o indutivista mais radical colocaria a mão em uma vela acesa centenas de vezes para ter certeza disso. Nem precisaria testar esta hipótese em vários ambientes, com a luz acesa ou apagada, com chapéu ou sem, etc. O fogo sempre queima e sempre vai queimar, porque existe uma teoria subjacente explicando que reações do tipo exotérmica (com liberação de calor) como é a combustão, produzem sensação de queimadura em seres vivos com sistema nervoso. O indutivismo puro só se preocupa em responder a pergunta ‘o quê’ ao invés de responder também ‘por que’, não se preocupando em explicar o fenômeno observado. Ao se concentrar somente na descrição pura e simples, o indutivista corre o risco de que suas generalizações sejam falsificadas, bastando apenas uma única observação contrária (segundo a lógica de Popper, como já visto anteriormente). Portanto, na taxonomia não basta só dizer quantas espécies podem ser reconhecidas a partir de um grande grupo de indivíduos, temos que dizer por que motivos podemos afirmar isso. E este “porquê” não significa só elencar as diferenças morfológicas entre os grupos, significa justificar a escolha dos atributos amparando-se em um arcabouço teórico consistente. Explicar o princípio teórico pelo qual um fenômeno acontece não nos dá a certeza absoluta de que tal fenômeno sempre acontecerá, mas permite fazer previsões dos resultados, com probabilidade significativa de sucesso. O exemplo do fogo descrito acima ilustra bem este caso, pois a teoria nos diz que o fogo queima seres vivos; se um ser vivo colocar a mão no fogo, a probabilidade de se queimar é alta em todas as vezes. Em suma, este raciocínio é a base do método dedutivo.

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[6] Todos os conceitos e discussões sobre os métodos filosóficos da ciência basearam-se em Chalmers (1995) e foram aqui didaticamente simplificados. Para aprofundar o conhecimento, sugiro consultar a obra original.

Basicamente, o método dedutivo parte de premissas lógicas e verdadeiras, das quais se extrai uma conclusão igualmente lógica e verdadeira. Considerando-se as premissas do exemplo acima como sendo (A) o calor do fogo queima os seres vivos e (B) o sujeito que está pondo a mão no fogo é um ser vivo, a única conclusão lógica (C) é que o sujeito vai se queimar. Se A implica em B e B implica em C, logo A implica em C. Obviamente, convém testar se a conclusão realmente é verdadeira, pondo a mão no fogo pelo menos uma vez. A grande vantagem deste tipo de raciocínio comparado com o método indutivo é que ele nos dá uma ideia antecipada do resultado esperado de um experimento.

Utilizar o método dedutivo na escolha dos caracteres para reconhecer um táxon em MOBAT pode ser útil para diminuir a chance de selecionarmos um atributo pouco informativo. Por exemplo, se temos que (A) a espécie ‘x’ é reconhecida pelo formato da folha e (B) folhas são órgãos ambientalmente variáveis, logo (C) a espécie ‘x’ é ambientalmente variável. Reconheço que esta linha de raciocínio é um pouco forçada e que um dedutivista mais severo facilmente destruiria meu silogismo, mas a ideia ilustra bem o que eu estou tentando argumentar. Ao escolhermos um atributo para delimitar uma espécie, devemos refletir sobre o significado biológico do mesmo, se ele foi ou é importante atualmente para a manutenção da espécie enquanto linhagem evolutiva separada de outras linhagens ou se é meramente um reflexo da grande plasticidade ambiental que as plantas exibem. No exemplo acima, usei a folha para ilustrar o fato de os caracteres foliares normalmente exibirem uma grande amplitude de respostas ao ambiente, podendo variar novamente se a planta for cultivada em outro local, tendo pouco ou nenhum efeito sobre a manutenção da espécie enquanto entidade única. Utilizar características como densidade de tricomas, formato do ápice ou tamanho foliar como único critério para diferenciar duas espécies é perigoso, pois a chance desta generalização ser falseada rapidamente é grande. Por sua vez, utilizar caracteres que sabidamente têm implicações na manutenção da identidade da espécie enquanto linhagem evolutivamente separada de outras garante uma menor probabilidade desta generalização ser falseada (e.g. caracteres que garantam isolamento reprodutivo). Para dar embasamento teórico às nossas generalizações, atualmente contamos com um grande número de publicações sobre a função evolutiva e os padrões de expressão genética dos mais diferentes tipos de atributos. Por exemplo, no artigo 2 desta tese eu mostro que o padrão de *trade off*[7] existente entre o tamanho das flores e seu número pode ser útil na diferenciação taxonômica em alguns grupos similares de *Galactia*, pois este padrão tem um fundamento biológico bem estabelecido (Sargeant et al. 2007), aumentando as chances de ele ser bastante conservado dentro dos grupos.

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[7] Expressão inglesa que não tem um termo equivalente satisfatório em Português (uma tentativa recente tem sido traduzir o termo para “demanda conflitante”), mas se aplica em ocasiões nas quais o aumento de uma estrutura leva a diminuição da outra. No caso das plantas, o aumento no tamanho das flores leva a diminuição do seu número e vice versa.

Em resumo, o que eu quis mostrar é que em taxonomia não basta só coletar um grande número de indivíduos em diferentes situações, analisar se eles se encaixam no elenco de espécies descritas há vários anos por autores clássicos e elaborar chaves de identificação baseadas em quaisquer atributos que sejam razoavelmente diferentes dentro daquele grupo. Esta prática está mais para uma cama de Procusto[8] do que para uma empreitada científica, pois os dados podem ser analisados e manipulados das mais diferentes formas para se ajustarem à concepção que o taxonomista tem a respeito do grupo.

A monografia de ***Galactia*** *para o Brasil*

Com tudo isso em mente, propus-me a revisar o gênero *Galactia* P. Browne (Leguminosae, Papilionoideae) para o Brasil. A minha abordagem é essencialmente MOBAT, pois a principal deficiência dentro do grupo ainda é o correto reconhecimento das espécies, ou melhor dizendo, a correta designação dos indivíduos dentro das espécies já descritas. Baseei extensivamente minha abordagem em análise de material de herbários, os quais são abundantes e muito informativos (~ 1.300 exsicatas foram analisadas). Ainda assim, realizei algumas viagens de coleta, principalmente para regiões com grande diversidade de espécies (p.ex. regiões Sul e Centro-Oeste do Brasil). Esta metodologia de trabalho foi pensada para otimizar ao máximo os recursos limitados que eu dispunha, no intuito de atingir uma relação custo-benefício positiva que satisfatoriamente equacionasse tempo, dinheiro e informação de alta qualidade. Por isso, evitei conscientemente o uso de marcadores moleculares, pois a área de abrangência é bastante ampla, o número de táxons é grande e o tempo limitado. Uma abordagem combinada morfológica e molecular tornaria o trabalho dispendioso, tanto temporal quanto financeiramente. Além disso, também tinha a intenção de provar (principalmente para mim mesmo) que ainda é possível fazer um bom trabalho taxonômico sem recorrermos a *primers* e termocicladores. Apesar de parecer, não sou um "morfo-xiita tecnofóbico", pelo contrário, sou um entusiasta das revoluções que a biologia molecular vem nos proporcionando, gerando novos insights sobre as relações de parentesco entre os táxons, a evolução de caracteres, a biogeografia e até mesmo a identificação (e.g. *DNA Bar Coding*). Tanto isso é verdade que no artigo 3 utilizei uma abordagem combinada morfo-molecular para delimitar corretamente a identidade e as relações filogenéticas de dois táxons outrora considerados sinônimos e especular sobre os possíveis processos que os levaram à especiação.

O ponto de partida e centro gravitacional desta tese foi a monografia de *Galactia* para a América do Sul (Burkart 1971). Partindo do trabalho de Burkart, me preocupei em melhorar o que já

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[8] Procusto foi um personagem cruel da mitologia grega que obrigava seus prisioneiros a deitarem em uma cama. Se o indivíduo fosse menor que a cama, ele era esticado até que coubesse; se fosse maior, simplesmente ele tinha cortadas as partes que estivessem sobrando. Procusto foi morto por Teseu, o mesmo herói que matou o Minotauro.

havia sido feito, focando nos pontos obscuros e dando menor importância ao que já estava bem estabelecido. Desta forma, menos recursos foram gastos com táxons que não apresentavam problemas de delimitação (p.ex., *Galactia gracillima, G. benthamiana*, entre outras) e mais atenção foi dispensada com complexos taxonômicos (p.ex. *Galactia neesii-martii* e correlatos). Como eixo norteador deste trabalho, utilizei uma abordagem kuhniana, isto é, tentei seguir um novo paradigma, o qual serviu como suporte para atingir o objetivo principal desta tese, que é solucionar o problema do reconhecimento das espécies de *Galactia* para o Brasil. Este paradigma, apesar de bastante recente, vem sendo cada vez mais utilizado em trabalhos taxonômicos (Tobias *et al.* 2010, Padial *et al.* 2010) e está mais bem discutido e detalhado no artigo 1 desta tese, mas em linhas gerais corresponde ao seguinte:

> "Duas espécies que tenham reprodução sexuada não podem ser separadas e reconhecidas por qualquer tipo de diferença morfológica; esta diferença morfológica deve influenciar diretamente no isolamento reprodutivo da espécie estudada em relação a outras espécies similares".

Como a principal fonte de consulta do taxonomista é o material exsicatado, as diferenças a serem buscadas que supostamente influenciam o isolamento reprodutivo devem ser obrigatoriamente do tipo pré-zigóticas, uma vez que não é possível testar isolamento através de cruzamentos interespecíficos. Esta limitação pode ser contornada por diferentes tipos de análises, dependendo do tipo de hipótese a serem testadas. Muitas destas análises estão descritas nos artigos desta tese, enquanto outras ainda estão para serem descobertas. Com esta tese, espero estar contribuindo não só para a resolução dos problemas de identificação das espécies de *Galactia* no Brasil, mas também para incentivar novas abordagens e discussões sobre os paradigmas e métodos da MOBAT, pois é a partir de novas descobertas e de caminhos nunca antes tentados que a ciência avança.

DIVISÃO DA TESE

Este trabalho está dividido em três seções temáticas e uma seção única. As três primeiras são compostas por artigos independentes, mas que servem para justificar e convergem para o objetivo principal, que é a revisão de *Galactia* para o Brasil, o qual compõe a seção única e é por si só um resumo da tese e um artigo independente. O sumário abaixo ilustra esta divisão didaticamente:

Seção I – Revisão e métodos

1. Morphology-based alpha taxonomy in plants: review and critical analysis using the genus *Galactia* (Leguminosae) as a case study – *Taxon* (23 p.)

2. Flower size and number trade-off as a useful tool to recognize similar pairs of species in *Galactia* (Leguminosae) – *Brittonia* (10 p.)

Seção II - Tratamento taxonômico

3. Systematics and phylogenetic relationships with some insights on the divergence dates and speciation process in a controversial *Camptosema-Galactia* (Leguminosae) complex from southern South America – *Annals of Botany* (18 p.)

4. Paper 4 - Using an integrative non-molecular approach to increase the taxonomic resolution within the *Galactia neesii* (Leguminosae) complex – *Plant Systematics and Evolution* (22 p.)

5. Checking the status of infraspecific taxa within *Galactia jussiaeana* (Fabaceae) using Berg's correlation-pleiades hypothesis – *Phytotaxa* (21 p. – submetido a Phytotaxa)

6. O gênero *Galactia* P. Browne (Leguminosae, Papilionoideae) no Rio Grande do Sul – Capítulo de livro: *Leguminosas do Rio Grande do Sul* (9 p. – submetido)

Seção II –Autoecologia e conservação

7. Influence of fire in regeneration of *Galactia peduncularis* (Fabaceae) – implications for conservation of a Brazilian-Cerrado endemic – *Biodiverstiy and Conservation* (9 p.)

Seção única

8. The genus *Galactia* P. Browne (Leguminosae, Papilionoideae) in Brazil – *Systematic and Biodiversity* (25 p.)

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Seção I

Revisão e métodos

Taxon

Morphology-based alpha taxonomy in plants: review and critical analysis using the genus *Galactia* (Leguminosae) as a case study

Guilherme B. Ceolin
Sílvia T. S. Miotto

Abstract

Morphology-based taxonomic studies are very important as basic tools to assess the biodiversity on Earth. However, taxonomy is still lacking a universal and unequivocal method. The aim of this paper is to make a review and critical analysis on the practice of morphology-based alpha taxonomy in plants. We address the importance of using a suitable concept of species and discuss the advantages and limitations of usual methods employed to recognition of species. Furthermore, we discuss how taxonomic descriptions are made, suggesting simple methods to improve this task for *a posteriori* use of data in meta-analysis. *Galactia* is a good genus to use as case study because it is morphologically very plastic and illustrates many of the problems faced in alpha-taxonomy.

Key-words: speciation – taxonomic methods – integrative taxonomy – taxonomic keys – Diocleinae

Introduction

Species are the basic unit in ecology, evolution and biogeography. Taxonomic studies are fundamental to understanding and dealing with biodiversity, since high levels of richness and endemism are the main criteria for pointing out priority areas for conservation (Myers & al. 2000). In recent years, great advances have been done in molecular-based taxonomy (e.g. DNA Bar Coding), especially in elucidation of taxonomic complexes (Depypere & al. 2009) and discovery of cryptic species (Hebert & al. 2004, Whittall & al. 2004, Sotuyo & al. 2007). However, morphology-based alpha taxonomy (MOBAT, hereafter) can still be thought as the main and most well-established tool for primarily accessing biodiversity (Schlick-Steiner & al. 2007), because even apparently identical cryptic species can be differentiated using methods of morphometric statistics (Andrés-Sánchez & al. 2009). Moreover, molecular markers are not always informative enough to differentiate morphologically well established closely related species (Gemeinholzer & Bachmann 2005). Likewise, paleontological studies can not use (or rarely can, see Kim et al. 2004) molecular markers to infer the delimitation of extinct species, being essentially MOBAT (Padial et al. 2010).

Despite the recognized importance of MOBAT, its prestige and hence resources - including the number of taxonomists - has been gradually decreasing (Korf 2005, Agnarsson & Kuntner 2007, Schlick-Steiner & al. 2010). This decline may be reflecting the growing trend of new researchers prefer molecular characters to infer phylogeny and delimitation of species. This may happens because molecular characters are both more abundant and precise (only four possible states), whereas morphological characters often are scarce and frequently appear in intermediate states. In addition, as emphasized by Henderson (2005), most morphology-based taxonomic revisions or monographs do not follow a clear and explicit methodology. Thus, it is not possible to replicate and compare these studies. Such procedures make these kinds of study deeply subjective, reflecting the idiosyncrasies of the taxonomist more than actual delimitation and evolutionary history of the taxa. This situation makes no sense from a scientific viewpoint. Wiens (2001) stresses that good science requires clearly explained and reproducible methods and some taxonomic works extensively ignore this assumption, being common the absence of a Material & Methods section as well. Therefore, these works violate another assumption of science by failing to submit the methods, making impossible for other researchers to independently check the results in order to confirm or reject the conclusions (Popper 1959). For these reasons we believe that many researchers have been gradually abandoning MOBAT.

Many efforts have been done in order to try standardizing taxonomic practice, from Sneath & Sokal (1973), whose book is now a masterpiece of the taxonomic literature, to Henderson (2005), which proposes an explicit and functional methodology to be followed in monographs of plant groups. Henderson’s proposals consist of several stages, many of them already discussed in classic works such as application of statistical analysis. However, we understand that the greatest Henderson’s contribution was to emphasize that we must clearly define a species concept and follow it carefully before starting data collection. Defining the species concept to be used in our taxonomic revisions may be a great problem. There are over twenty proposed species concepts (Mayden 1997), but none is general enough to cover satisfactorily all the different sorts of organisms (Sites & Marshall 2003). This lack of consensus makes the number of species quite variable in many groups (Meudt & al. 2009). As conservation policies in general are based on number of species and degrees of endemism, this imbroglio is really a problem. Another remarkable Henderson’s point is that he argues we should think about the taxonomy as a hypothesis-driven science, and species as hypotheses to be tested. In other words, it is not enough to define and apply a species concept to the data; we also empirically must check if the species proposals do exist. Thinking the taxonomy in this way could reduce the nomenclatural chaos and instability in some difficult groups, making identifications more reliable for non-expert on such groups. This is the case of the genus we are studying here.

During the monograph of *Galactia* P. Browne (Leguminosae, Papilionoideae) in Brazil, we face a situation of total mess and confusion in delimitating several species. *Galactia* was firstly studied by Bentham (1859) and subsequently by Taubert (1894), but the main reference work is a MOBAT monograph of the genus to South America (Burkart 1971). Burkart accepts around 37 species as occurring in the continent, many of them described by the first time. Although comprehensive, the author admitted that his monograph is neither definitive nor complete because he examined few herbaria and the species show a wide morphological variation, mainly in traits like habit, hairiness and shape of leaflets. This variability makes the specimen identification a great challenge because many of the attributes used to identification are exactly those the author pointed as plastic. Another problem is the lack of explicit and coherent criteria to describing new taxa mainly at infraspecific level, what makes nomenclature equally complicated.

Our goal with this study is to shed some lights on the practice of MOBAT in plants, reviewing and trying to improve what is already being done by many previous authors. We shall use *Galactia* as a case study because it is a genus that illustrates many of the major problems faced by those using MOBAT. We shall address the importance of using a suitable concept of species and how we can link the theories underlying the species concept with the best traits for the recognition of taxa. We also discuss the advantages and limitations of usual methods employed to recognition of species. At last, we analyze how taxonomic descriptions are made, suggesting simple methods to improve this task for *a posteriori* use of data in meta-analysis.

Choosing the species concept

Before starting a taxonomic study, it is essential to establish a species concept. But this is not an easy task. The majority of species concepts are closely linked to the methods of species discovery (de Queiroz 2007), so that some of them cannot be applied in MOBAT. For example, those concepts involving purely genetic approaches (e.g. coalescence of alleles, Baum & Shaw 1995), are expensive and time-consuming, making difficult the revisions of large taxa and not applying extensively to paleobotany. A species concept to be functional in MOBAT should be as easily applicable and low cost as possible for allow the returning of the best conclusions. However, it must also be consistent with the actual evolutionary history of taxa, being possible to discern whether the differences in the attribute are due to speciation events or are merely morphological variation in widely plastic species. Therefore, the best species concept should equate evolutionary history – what actually is influential in maintaining two groups of individuals as separate species– with ease to find the boundaries of these species. In summary, the ideal species concept should link a good theoretical framework with a robust method of discovery, because if both are not properly linked, we could incur in substantial errors (Sites & Crandall 1997, Henderson 2005).

A major advance in the discussion of species concepts was the recognition that multiple rather than a unique and exclusive process contribute to unify the organisms into a species. This finding led Pigliucci (2003) to propose that the category "species" is best seen as a "family resemblance concept" or a "cluster concept". This idea was based on the concept of family resemblance groups proposed by the philosopher Ludwig Wittgenstein (1953). Wittgenstein argued that some sorts of words cannot be specified by a single concept or property that is necessary and sufficient to define them satisfactorily. He used the example of the word "game". We all recognize what a game is, but we cannot define it in terms of a unique and exclusive feature or set of features. Likewise, Pigliucci claims that the category "species" cannot be defined by means of a simple property shared by all species. However, de Queiroz (2005) disagrees with Pigliucci (2003) and argues that there is invariably a feature that all species share, which is the fact that they are separately evolving metapopulation (or segments of) lineages. This implies that all the previously used properties to define a species are more contingent than necessary to recognize it. That is, different metapopulation lineages acquire different properties (e.g. reproductive isolation [Mayr 1942], exclusive coalescence of alleles [Baum & Shaw 1995], diagnosability [Nixon & Wheeler 1990]) along the time since the splitting among them. Because not all the lineages are in the same stage at the same time, different methods indicate different numbers of species. So, in the author's words: "This proposal reconciles concepts and definitions of incompatible species without denying the properties that make them different." For all these reasons, this proposal is called "Unified Species Concept" (USC, hereafter). But how can we recognize a species by using this concept in practice since it does not provide ways to test the hypothesis of divergence among lineages?

de Queiroz (2005) emphasizes in his article that, under the USC, the assumption that the ideal species concept should be linked to the method of discovery does not exist at all, because these issues are clearly separated. The species concept per se is now replaced by a purely conceptual and theoretical connotation, regarding the question: "What is a species?" By the other hand, the method of discovery, ultimately, has just a methodological connotation and serves to answer the question: "How do we recognize a species in practice?” The fact that all properties formerly used to define a species are more contingent than sufficient makes the boundaries among species dependent on the stage of separation in which two metapopulation lineages are and the kinds of data used (Ross & al. 2010). And to do this, there are several possible methods to be employed.

Choosing the method

The methodology proposed by Henderson (2005) for herbarium-based taxonomic studies uses the Phylogenetic Species Concept (PSC, Nixon & Wheeler 1990), whose method of discovery is known as Population Aggregation Analysis (PAA, Davis & Nixon 1992). This concept defines species as the smallest aggregations of populations diagnosable by a unique combination of character states in comparable individuals. These populations are delimited by successive rounds of aggregation of populations, based on an analysis of character and traits distribution within and among such populations. Characters are attributes found in all comparable individuals within a terminal lineage. That is, the attributes appearing at a frequency of 100%. In turn, traits are those attributes not universally distributed among comparable individuals within such a lineage, that is, they appear at frequencies lower than 100%.

One of the advantages pointed out by Henderson in using this approach is that it is one of the few that explicitly link the species concept with the method of discovery and can be used in herbarium-based taxonomy, which is a common practice in MOBAT. Among the disadvantages, we point the fact that the characters are more qualitative than quantitative. It is not surprising because the very definition of the concept emphasizes unique combination of character states and Henderson himself recognizes this. However, he cautioned that quantitative data should be used as independent tests for the qualitative groups. The fact is that we have not always well-defined groups by unique qualitative combination of character states. According to USC, there is nothing preventing metapopulation lineages to behave as a species, with no gene flow among them, but which are not splitted long enough one each other to acquire unique characters. Often, significant differences in mean values of attributes may be important to prevent gene flow among metapopulations, especially in animal-pollinated plants (Schiestl & Schlüter 2009). Moreover, speciation is not always accompanied by obvious morphological differences, as in the case of cryptic species (Bickford & al. 2006). Therefore, it is not possible to include all the speciation mechanisms in an approach that emphasizes only qualitative attributes. In addition, even Davis and Nixon (1992) identified two faults in the PAA: (1) unless many characters are sampled, the true number of species may be overlooked and (2) unless many samples are analyzed, the true number of species may be overestimated (because the risk of considering traits as characters). The distinction between characters and traits in PAA is another problem. There is not an objective method to distinguish between them. Some traits may be widely distributed in populations, which make them look like characters. However, as soon as we increase the sampling, this traits show that are actually polymorphic. Wiens (1999) discusses the problem of polymorphism in attributes and suggests that more than "fixed" characters, we could examine differences in frequencies of character states, considering a confidence interval of 95%, for example. This approach seems to be feasible and scientifically correct, according to Popper's (1959) falsificationism: we can never prove that something is totally true, we can only surely prove something is false, just one example different from the pattern is enough. So, what is the ideal method to discovering a species?

It is likely that there is no such a method, given the diversity of the Tree of Life. But we could follow some general guidelines. For example, it is very important we use different kinds of data, both qualitative and quantitative, to independently validate taxonomic conclusions (Sites & Marshall 2004, Leache & al 2009, Schlick-Steiner & al. 2010). Different kinds of data imply the use of multivariate analysis, such as Principal Coordinates Analysis (PCoA), Principal Component Analysis (PCA) and Cluster Analysis (CA). It is not the scope of this paper to discuss the advantages and disadvantages of different methods (for details about the methods, see Hair & al. 1998, Henderson 2006, and also Palmer: http://ordination.okstate.edu/index.html), but we stressing that some care is crucial in using multivariate analysis. The most important concern at all is that we must to statistically test the significance of the clusters (in the case of CA) or the stability of the axes (in the case of ordinations). This is the Achilles' heel of the studies using morphometric analysis.

Generally, taxonomic studies that use multivariate approaches do not test the statistical significance for clusters or the stability of the axes in ordinations (e.g. Ames & al. 2008; Oliveira & al. 2008; Pedersen 2010) Thus, it becomes dangerous to draw conclusions from these results. Henderson (2006) emphasizes that multivariate analysis are primarily exploratory, that is, they reduce the complexity in a way that we can deal with it in practice, not being a way to test hypotheses. Statistically, hypotheses can only be accepted or rejected within a confidence interval, which is the estimated probability of that result has not been generated at random. The most used multivariate analysis tests do not do this. In this manner, Henderson recommends Discriminant Analysis (DA), because it uses a MANOVA test (Multivariate Analysis of Variance) to check whether there are significant differences between group centroids. If these differences do exist, then DA works on discrimination and classification. Despite being a reliable and widely used approach (Battaglia & Patterson 2001, Binns & al. 2002, Boyd 2002), it requires several steps. First, we need have *a priori* classification of the groups, which is achieved through exploratory methods. These groups are used to run the MANOVA. If there were significant differences among groups, we proceed to the steps of classification and discrimination. Indeed, DA is better configured like a group of methods, rather than a single. This is time-consuming. It is possible to join together in one single analysis the steps of groups delineating and hypothesis testing. This can be achieved through bootstrapping, just as we infer the branches support in phylogenetic analysis. Pillar (1999a, b) developed a bootstrap-based approach to test the significance of the clusters and the stability of axes in ordinations. Thus, we have in a single step both the boundaries of groups and the estimation of confidence that these groups differ from the random.

Now, we can see how applying in practice the previously discussed concepts and methods.

Case study: genus Galactia

The genus *Galactia* P. Browne (Leguminosae, Papilionoideae) is pantropical and has about 55-60 species, whose diversity center is located in continental America and Caribbean Islands (~50 spp.). It grows primarily in open areas, being common in savannas, grasslands and rocky fields and rare in forests. Due to the wide morphological variability and the relatively small number of diagnostic characters, the taxonomy of the group is quite confusing and there is disagreement about the identity of several species. In addition, previous phylogenies based on AFLP markers (Sede & al. 2008) and ITS, trnL-F, and morphology (Sede & al. 2009), did not resolve the relationships between *Galactia* and its closely relative genus *Camptosema*, pointing both as non-monophyletic. Our research group is ongoing testing new molecular markers and new approaches in order to resolve the phylogeny of *Galactia*, but here we strictly shall discuss solutions to improve the correct identification of taxa using MOBAT. We believe that such solutions can be extrapolated and serve as a model for other plant groups that have similar problems.

One of the main problems of the already done revisions of *Galactia* (e.g. Burkart 1971) was the lack of an explicit species concept. It is possible say that the authors used a mix of the Linnean typological and morphological (Cronquist 1978) concepts. On the one hand, some species are well-defined and easily recognizable, bearing seemingly "fixed" characters. On the other hand, there are some species with fuzzy boundaries, receiving either one or other name, depending on who identifies them. The recognition of such species is mainly based on labile attributes such as size, shape and hairiness of the leaflets. These attributes are better defined as traits instead of characters (Davis & Nixon 1992) and, therefore, they are not suitable for taxonomic recognition. So, to assign good attributes to distinguish *Galactia* species, we first should explicitly state the species concept to be used.

We agree with de Queiroz (2005) which defines species as separately evolving metapopulation lineages. These lineages are joined by gene flow (Templeton 1989) in organisms with sexual reproduction. We also agree with Van Valen (1976), who postulates that a species is a lineage occupying an adaptive zone minimally different from any other lineage and evolving separately from all other lineages outside this distribution. An adaptive zone would be a combination of all environmental factors under which a species or population can persist; this is roughly equivalent to the concept of ecological hipervolumetric niche (Hutchinson 1957). Recent studies have demonstrated the importance of including niche modeling in the species recognition (Raxworthy & al. 2007, Apodaca & Rissler 2007) and according to Kozak & al. (2008), environmental factors may be crucial in the processes of speciation. We must also bear in mind that a species should be readily recognizable, preferably by unambiguously macro-morphological attributes. Combining these premises, we have a polythetic species concept or cluster concept (sensu Pigliucci 2003), in which various contingent properties will be used to delimit the metapopulation lineage which we shall consider species and give a name follow the rules of International Code of Botanical Nomenclature (ICBN). Thus, a species of *Galactia* is a separately evolving metapopulation lineage reproductively isolated from others, occupying a unique adaptive zone (i.e. niche), and recognizable by unique attributes. Hereafter, we shall discuss the theoretical assumptions as well as the pros and cons of using each of these properties, giving practical examples of their application. We shall discuss methods to objectively delimiting species from these properties as well.

There are several processes by which two lineages may become evolutionarily splitted, either in allopatry, sympatry or parapatry (reviewed in Rieseberg & Willis 2007). Two lineages are kept separate only if there is no gene flow between them. The simplest way to prevent gene flow is geographical separation, i.e., individuals of two lineages can not mate due to being separated by a barrier, either a mountain, a river or two patches of forest. For instance, topographic variation in Andes Mountains is the primary reason by which many metapopulation lineages have evolved separately, making this area a biodiversity hot spot to several taxa (Hughes & Eastwood 2006). Another barrier is the differential adaptation of marginal populations in the borders of a lineage to an inhospitable environment (Antonovics 2006). Moreover, autopolyploidization events in plants can quickly give rise to separate lineages within a population (Ramsey & Schemske 1998).

It is very difficult to study the processes by which two lineages become species using only MOBAT and it is not our intention. We are seeking patterns that emerge from these processes and allow us to define and recognize species, for example, the mechanisms of reproductive isolation.

Infering reproductive isolation among lineages

Many of the MOBAT studies are widely based on herbaria material, which makes it impossible to test reproductive compatibility. However, morphology, particularly of the reproductive organs, can be used to infer isolation. Animal-pollinated plants bear several mechanisms to optimize the pollen capture within the species and reduce or even prevent the pollen deposition from other species. Some species within the same genus exhibit floral morphologies quite different according to pollinator

(Ramsey & al. 2003, Kephart & Theiss 2004) and, in more drastic cases, the same species shows different floral morphs depending on occur either in sympatry or allopatry with closely related species (character displacement; Breedlove 1969). Therefore, a practical way to studying reproductive isolation is to find significant differences in reproductive organs. For example, *Galactia decumbens* and *Galactia boavista* are two species often misidentified by non-specialists, due to a wide interspecific similarity, coupled with a broad intraspecific variability. Burkart (1971) used leaf traits in his taxonomic key to distinguish the species, just one of the more plastic attributes. Ceolin & Miotto (2011a) showed that in some species of *Galactia* there is a flower size and number trade-off in floral rachis, which results in differences in floral aggregation. By being very conservative and widely distributed among Angiosperms (Sargent et al. 2007), this feature is useful as taxonomic character. Furthermore, differences in floral arrangement have adaptive value because they increase the reproductive success (Kudos & Harder 2005). This trade-off is clearly present in *G. boavista* (lax inflorescence, with large flowers) and *G. decumbens* (contracted inflorescence, with small flowers), and, therefore, is a good attribute to correctly recognize these species.

This previous case illustrates the fact that we must do a critical analysis on the attributes we choose to recognize species. Recent articles have emphasized that we need to choose traits that play a key role in speciation process (Padial & al. 2010). For example, if we are using reproductive isolation as a criterion for separating species, the more suitable characters to be taken into account are those directly contributing to reproductive isolation (Tobias & al. 2010). In other words, we must look for causation rather than correlation. A correlation occurs when two phenomena are present together, but one is not the cause of another. The opposite is causation: the two phenomena appear together and one causes the other. Why is this important? Because the correlations can be spurious, i.e., they can be caused by a third phenomenon that influences together the two correlated variables. At the moment of this third phenomenon stops, the correlation no longer exists. An example of spurious correlation which is prone to induce to error is when we use certain vegetative characters (mainly foliar) in taxonomic keys. Variation in vegetative characters is primarily caused by environmental stimuli, while variation in reproductive one is caused by selective pressure of pollinators (Chalcoff & al. 2008). This implies that vegetative attributes have a greater morphological plasticity within species. By using characters such as leaf shape or density of trichomes to identify two putative species, we risk that the causes of these differences are merely the environmental conditions where the plant grows, not the intrinsic process of speciation. Thus, what we actually have is a correlation between morphological differences and the supposed process of speciation, but the cause of these differences is the environment not a distinct evolutionary process. Furthermore, consistent differences in reproductive morphology are strong indications of barriers preventing gene flow between the two putative species, even if the vegetative morphology is very similar. In this case, the evidences indicate that there were selection due to pollination pressure on floral traits, while the vegetative morphology converged (or remained stable, in case of sister species) to explore the same environment. Unlike correlation, here we have a process of causation, in which the morphological differences are caused by evolutionary divergence among lineages. These traits have adaptive value and are more likely to be conserved throughout the distribution range, allowing greater confidence in a taxonomic key. We ought to pay the attention to the fact that the size of floral pieces can also be variable along environmental gradients, especially precipitation (Lambrecht & Dawson, 2007). We therefore recommend that whenever necessary to use quantitative traits, we only must to conclude based on appropriate statistical tests and a representative sample size, covering the full range of the species.

Testing ecological differences among groups

As we have seen before, the Unified Species Concept postulates that the only property invariably present in all species is that they are separately evolving metapopulation lineages. However, two metapopulation lineages not always accumulate sufficient morphological differences to be readily recognized as a species, despite actually being isolated lineages. A valid attempt to test the separation among lineages is the niche modeling, assuming that divergences in adaptive zones are contingent features among lineages. This assumption had already been proposed by Van Valen (1976), who considered a species as a lineage occupying an adaptive zone minimally different from any other lineage in its distribution. This adaptive zone can be roughly interpreted as the ecological niche occupied by lineage. In order to test the hypothesis that two groups are divergent lineages, and therefore potentially different species, we can compare if the groups show significant differences in ecological features extracted from their occurrence points. This approach is currently widely used as a complementary tool in taxonomic studies, especially for animals (Raxworthy & al. 2007, Apodaca & Rissler 2007, Ross & al. 2009; review and perspectives in Kozak & al. 2008), but still rarely used for plants. Ceolin & Miotto (2011b) used niche differentiation to delimit potentially divergent lineages within the taxonomic complex *G. neesii* and allies; ecological lineages were later tested to morphological congruence. However, this approach was somewhat different from the standard approach used by previous authors. Normally, the searching of divergent lineages is made through molecular markers, and subsequently tested for ecological and/or morphological congruence. Ceolin & Miotto (2011b) did not use molecular markers; they inferred divergent lineages directly from ecological data through a cluster analysis with a sharpness test of groups (according to Pillar, 1999a).

This study demonstrated that it is possible to satisfactorily resolve difficult taxonomic complexes combining both ecological and morphological data without using molecular markers.

Just as we must be careful when we use differences in floral traits to infer reproductive isolation, we should also be cautious when we use niche modeling to infer boundaries among lineages. We must avoid two situations. First, we must bear in mind that a vicariance event may occur when an environmental change in the geographic space between two subpopulations of a species occurs more rapidly than the adaptation to new ecological conditions, fragmenting the original population (Wiens 2004). Thereafter, these two lineages are likely to differ one each other, become distinct species (classic example of allopatric speciation). However, their ecological requirements and therefore their niches remain the same. This general tendency of species to retain their ecological niches over time is called niche conservatism (Wiens & Graham 2005). This phenomenon would imply that a niche analysis used to infer divergence among lineages result in false negative, that is, two putative species appear identical as they share the same ecological requirements. A simple change in thinking can be enough to solve this problem. Rather than test whether the differences are significance between lineages, we need look for regions where the lineages are absent, due to inhospitable conditions. If these regions are separating the lineages, then one can infer that they are possibly allopatric. Although difficult to confirm the absence of a taxon, we can use some programs of ecological modeling (eg MAXENT; Phillips & al. 2006) that quantify the likelihood of presence from known starting points. Probabilities below a stipulated threshold can be considered absences. Kozak & Wiens (2006) counted as no occurrence the probabilities values below the 10th percentile. But another problem arises. If we are testing whether these two groups are geographically isolated, we must be sure that the possibility of gene flow between populations is low enough to ensure the differential evolution of these two lineages. If small morphological differences between lineages are detected, a method to infer the role of distance in these differences is to make a spatial autocorrelation analysis.

This kind of analysis measures the similarity between samples as a function of spatial distance (Diniz-Filho al. 2003). In other words, this analysis assumes that two spatially close objects tend to be more similar to each other than expected by chance. The most common measure of spatial autocorrelation is Moran's I index. This index ranges from -1 (totally negative spatial correlation) to +1 (totally positive spatial correlation), with zero indicating that space has no influence on the characteristics of an object. An index I negative means that two objects are more different than expected for a given distance and a positive index means that the space is the main variable explaining the differences between objects. For taxonomic purposes, these values can be interpreted as an inference of reproductive isolation. Between two individuals or groups of individuals geographically close, a negative spatial autocorrelation index means that these groups are more different than expected for that geographical distance. It may indicate a possible barrier limiting gene flow between these groups. Negative spatial autocorrelation can also occur if two groups are geographically separated by a distance large enough to be expected that they were different and they are not. This result combined with niche modeling showing inhospitable areas separating two groups of individuals may be indicative of allopatric speciation with niche conservatism. On the other hand, positive spatial autocorrelation among individuals or groups of geographically close individuals may be an indication that these groups are interconnected by gene flow. Thus, analysis of spatial autocorrelation can be very useful in taxonomic works, although it be still an underestimated kind of analysis. However, as we pointed out earlier, even in tests of spatial autocorrelation, not any kind of difference can be used to infer reproductive isolation. The differences should be on features that restrict gene flow between lineages, usually reproductive characters. Ceolin & Miotto (2011c), studying two varieties within *G. jussiaeana*, found that the only significant differences between the taxa were related to leaf traits with no influences on gene flow. Both varieties occupy the same niches and occur sympatrically in some areas. An analysis of spatial autocorrelation, although not used in that study, most likely would return a negative I index, since individuals were more different than expected by chance, even when close one each other. However, these differences were related to leaf traits and not to floral pieces. In short, the leaves were significantly different among taxa, but not the flowers, which implied in absence of barriers to gene flow.

Improving taxonomic descriptions in MOBAT

In MOBAT, morphological data are traditionally acquired either from herbaria collections or from *in situ* collections. The specimens are qualitatively and quantitatively analyzed in search of features that allow a subsequent recognition of the taxon under study. The main outcome of this morphological analysis is a detailed species description, which is a complement to taxonomic key. Typically, quantitative attributes (e.g. dimensions of leaves and flowers) appear in descriptions as ranges (e.g., "length of the leaflet (9) 11 – 15"); the number in brackets indicates an unusual value. This way of describing a species, despite being the most used in MOBAT, presents many practical problems and has been widely criticized. According to Henderson (2005, p.458) “(...) ranges are one of the least informative summary statistics, providing only extreme values with no indication of their distribution between the extremes”. Alternatively, he suggests using means, medians, coefficients of variation and sample sizes in descriptions of quantitative attributes, because they allow a greater knowledge of the data being analyzed. On his web page devoted to systematics of the palm genus *Geonoma* (http://www.nybg.org/botany/geonoma/), Henderson describes his methods and proposes the use of a measure of central tendency (mean) and two measures of dispersion (coefficient of variation and range) in descriptions of taxa, as well inform the sample size. He prefers the coefficient of variation instead of standard deviation because it allows comparing the relative amount of variation in each taxon and each variable.

Although there is no doubt that summary statistics are more informative than just the range, some improvements can be implemented with the intention of a better use of this data in further analysis. For example, suppose a researcher is interested in knowing the dimensions of the leaves of the species X to do an analysis based on functional-types. The researcher can measure himself the leaves of several individuals to know their dimensions. However, although reliable, this methodology is feasible only when there are few species of easy access. Contrariwise, in case of a large-scale study, it is faster and less costly access the informations through literature. For this, taxonomic revisions are great sources of data, because they usually have very detailed descriptions. Thus, the taxonomic descriptions that have statistical summaries are extremely useful because many programs are able to run some common statistical tests from parameters. An example is the software PAST (Hammer & al. 2001) that can runs *t* and F tests from parameters such as mean, variance and sample size. So, thinking strictly in optimizing the time, standard deviation (SD) is better than coefficient of variation (CV), because variance is the squared root of standard deviation (variance = $\sqrt{\sigma}$); in turn, to estimate the variance from the coefficient of variation is a little less practical, since CV = SD/mean. Undoubtedly, both are simple operations that can be automated with a few lines of command in common softwares, but using the standard deviation has an additional advantage: integers (e.g. 12±3) are more easily understood than percentages (e.g. mean = 12, CV = 25%).

Although important, the discussion about the need for quantitative descriptions being a statistic summary instead of a range is recent and, therefore, most of the MOBAT studies bring ranges rather than summaries. So that we can use satisfactorily this information, we are proposing a simple method to extract parameters from a range. Our reasoning is that in describing the dispersion a given attribute as a range, we are assuming that the attribute can take any value within the range because these measures are continuous. For example, if the leaf length of a given species ranges from 8 to 13 cm, we are saying that any value between 8 and 13 cm is possible (8, 8.03, 8.1, 8.2.., 13). Mathematically speaking, the number of values between any intervals tends to infinite, but practically speaking, the amount of values will depend on the scale and precision required. So, in order to extract statistical parameters from ranges, all we need to do is randomize a certain amount of values within the range;

subsequently, we can calculate any statistical parameter from these values (e.g. mean, variance, standard deviation) and perform statistical tests as well (e.g. *t*-test, ANOVA).

To evaluate our predictions, we used a modified morphometric matrix (14 attributes, 30 individuals) of *G. jussiaeana* (Ceolin & Miotto 2011c) to compare the statistical parameters estimated from ranges against those obtained by direct measurement. For each attribute, we take the minimum and maximum values and generate a vector of same size as the actual sample (30 values) with random values within this range. This vector with random values was compared against the actual values by a *t*-test to verify if the means between observed and simulated values were different. We repeated this operation a thousand times for each attribute and calculate the average values of *P*. We used a chi-square test to check whether the frequency of significant and non-significant *P* values were the same. We performed all analysis in R (R Development Core Team 2011; more detail in supplementary material). The results showed that only three of 14 attributes had the simulated values significantly different from those observed by direct measurement (table 1). The chi-square test showed that this difference in frequency is significant (χ^2 = 4.5714, df = 1, *P* = 0.033). This result means that it is feasible and relatively safe to estimate values of attributes from ranges, allowing the use of these values in meta-analysis. To generate these random values, it is possible to use the R function available in the supplementary material of this article, but both Excel® (Microsoft) and Calc (Open Office) spreadsheets can also be used to generate random numbers from a range.

Concluding remarks

The human brain automatically and naturally tends to look for patterns in everything that happens and it sees (Huettel & al. 2002). It is of the human nature to categorize everything into well-defined boundaries as it helps to deal with abstractions and complex concepts. For example, it is much simpler to deal with the immense Earth's biodiversity by "packing" the different kinds of organisms into taxonomic units in different orders of complexity, from the lower (species) to the largest unit (kingdom). This search of boundaries is that makes taxonomy a difficult task. Delimiting species using only MOBAT is often almost impossible since, as already emphasized by Padial & al. (2010), morphological characters usually are more continuous than discrete, so that limits can only be established from a given criterion.

As seen in this review, the first and one of the most important criteria is the choice of a species concept. The species concept is responsible for where we should cut-off one species in a continuous universe. The most promising species concept being used is the Unified Species Concept (USC), precisely because it takes into account that different evolutionary lineages (ie species) acquire different properties during the time since divergence. This concept adapts to any type of organisms and allows that different methods are used to recognize a species. Choosing the most appropriate method for recognizing a species is the second most important criterion in a taxonomic study.

It is not possible to know which property was crucial for the formation of a lineage, so we ought to test several lines of evidence to confirm the identity of a given species, an approach called “integrative taxonomy” (sensu Dayrat 2005). Although most studies employing integrative taxonomy have used molecular data, we show that in MOBAT is possible and desirable to use other sorts of analysis (e.g. niche modeling) to support taxonomic decisions, especially in solving species complex. In addition, we emphasize that the morphological characters used to separate two species should have some influence in the process of speciation, because it guarantees a higher reliability to the character.

Despite losing space to more modern techniques, the practice of MOBAT is still the most practical method to explore the biodiversity. In addition, well-conducted taxonomic studies can be priceless source of data for various kinds of meta-analysis (e.g. Duarte et al. 2007). Therefore, we hope we have successfully showed with this review that is possible to conduct seriously taxonomic studies based exclusively on non-molecular data, following a methodology based on a solid theoretical framework. Approaching MOBAT in this way brings several advantages. The use of a standardized protocol diminish the importance of the "taxonomist’s experience". This enhances the formation of a taxonomist and helps to increase its number. Furthermore, reproducible and comparable methodologies would certainly help a lot to reduce the nomenclatural confusion still occurring in several groups of organisms.

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Table 1: Summary of the analysis testing simulated values from a range against directly observed measurements. Left column shows the attributes extracted from Ceolin & Miotto (2010c), with the range between brackets (in mm). Right column shows the average *P* values extracted from 1,000 *t*-tests comparing the equality of means between simulated and observed.

Attribute (range)	**Average *P* value**
Petiole (9 – 30)	**0.0025**
Length Middle Leaflet (30 – 73)	0.568
Stipule (2 – 4)	0.435
Stipel (1 – 3)	0.724
Inflorescence (11 – 45)	0.537
Pedicel (1 – 4)	0.278
Downer Lobe Calix (3 – 7)	0.541
Standard Length (8 – 16)	0.118
Standard Width (4 – 10)	0.513
Wing Length (7 – 13)	0.310
Wing width (3 – 4)	**0.00090**
Keel Length (8 – 14)	0.279
Keel Width (3 – 5)	**0.0040**
Bracteole (1 – 3)	0.624

Supplementary material

R commands to run the simulation tests

```
range.test = function(observed,min,max) {
p.resu = vector()
for(i in 1:1000) {
aleat = round(runif(length(observed), min=minimum, max=maximum),0)
p.resu[i] = t.test(observed, aleat)$p.value
}
mean(p.resu)
}
```

Brittonia

Flower size and number trade-off as a useful tool to recognize similar pairs of species in *Galactia* (Leguminosae)

GUILHERME BORDIGNON CEOLIN & SÍLVIA TERESINHA SFOGGIA MIOTTO

Abstract

Size and flower number trade-off is a widely spread trait in Angiosperms. It is a way to compensate the constraints in resource allocation on reproductive structures. This propriety makes this trait a valuable tool to use as taxonomic discriminative character, because it is likely to be conservative and heritable within the species. In this paper, we utilize some pairs of similar species of *Galactia* P. Browne (Leguminosae) to show that size and flower number trade-off could be a useful tool to recognize similar pairs of species.

Key-words: trade-off – Fabaceae – Diocleinae – taxonomic theory – reproductive isolation

Introduction

Historically, species have been described from morphological characters, but recently there has been an increasing use of molecular characters, with proposals such as DNA Barcoding (e.g. Hebert et al., 2003). However, many authors exhort that we use a multidisciplinary strategy, integrating multiple sources of evidence for increasing reliability in the recognition of species, an approach called integrative taxonomy (Dayrate, 2005; Padial et al., 2010). This approach requires that we take into account all the possible characters for species recognition, such as ecological, behavioural, molecular, and also morphological (e.g. Leache et al., 2009). Thus, the search for informative morphological characters really became a major concern.

The progressive abandonment of morphology-based alpha taxonomy (MOBAT, hereafter) can have many causes, since the fact that molecular characters are "fashionable", but also that morphological traits are more labile and less abundant than the molecular ones. Padial et al. (2010) point out that many morphological characters are continuous rather than discrete, which difficult to be sure about the fixation of a given state of character without requiring a large number of samples. In addition, there is always a degree of subjectivity when establishing a state of character. However, these authors acknowledge some advantages in using morphological characters, like the speed with which individuals can be assigned as belonging to a given species, and the possibility they can be applied both to extant and extinct species. Moreover, Tobias et al. (2010) pointed out that molecular analysis still require technologies that are not available to the reality of many researchers in some tropical countries, where most biodiversity resides. They also argue that morphological analysis that consider evolutionarily independent characters through the whole phenotypic spectrum can generate a better view of genomic differentiation than relatively few regions of DNA. Added to this, there is a growing tendency to consider as taxonomic character only those morphological traits that play a key role in the process of speciation (Padial et al., 2010). Tobias et al. (2010) state that if we are using reproductive isolation as a criterion for separating species, the characters more suitable to be taken into account are those characters that directly contribute to reproductive isolation.

In animal-pollinated plants, the floral display has a great influence on the reproductive success (Worley et al., 2000). It has already been shown that the number and size of the flowers displayed in an inflorescence has remarkable effect on pollinator visitation rates and the total seed production (Bell, 1985). Following this reasoning, one would expect to find that evolutionary pressures would act to increase these two attributes, but given the limited nature of resources, flower size and number trade-offs are quite common among Angioperms (Caruso, 2004). Although some studies have failed to demonstrate this pattern (e.g. Ashmann & Majestic, 2006), Sargeant et al. (2007) proved that there is really a trade-off between the size and number of flowers, in a study using phylogenetically independent contrasts in a large number of Angiosperms.

In this paper, we investigate whether the flower size and number trade-off could be a useful tool to separate some morphologically very similar pairs of species in *Galactia* P. Browne (Leguminosae). Several species of *Galactia* are too difficult to recognize correctly, because they show a large morphological plasticity combined with a lack of taxonomically informative characters. In our view, flower size and number trade-off includes two important features to be taken into account when seeking taxonomic markers: it has direct influence on reproductive success and it is of wide occurrence among flowering plants. Furthermore, if we were able to prove this taxonomic value, we hope that this attribute can be used as an additional marker in other groups of species with similar troubles in taxonomic identification.

Material & Methods

We used pairs of the morphologically similar species *G. boavista* - *G.decumbens* and *G. peduncularis* - *G. heringeri* to test our hypothesis. In general, the four species are very similar among themselves; however, the first group can be separated from the second due to unifoliated leaves, while the remaining group has trifoliate leaves. Within each group, the identification is more difficult, so that we decided to test the usefulness of flower size and number trade-off as taxonomic marker. We selected herbarium specimens with a state of preservation as best as possible, that is, containing the largest number of flowers. We counted either complete flowers or evidence thereof, such as buds and calyx without petals. To calculate the density, we divided the number of flowers for the length of the rachis, so the floral density is given by number of flowers per mm (NF/mm). For flower size, we used the length of standard (in mm) because it is the largest petal in papilionaceous flowers. We always measured the largest flowers in the inflorescence (1 – 3 per exsicatae), in order to be sure we were measuring only fully developed flowers. Altogether we quantified 24 exsicatae. We rehydrated the flowers to allow full expansion of the standard and we measured them under a stereomicroscope with graph paper (accuracy 0.1 mm). Our previous experience with the genus *Galactia* showed that there is not a drastic reduction in the dimensions of floral pieces in dry compared with fresh material, ensuring that the measures are not underestimated. To demonstrate the occurrence of the flower size number trade-off, we ran an Ordinary Least Squares Regression, using respectively density and flower size as independent and dependent variable. The floral density was log-transformed so that its frequency distribution fitted a normal curve.

Results

The regression results showed that there is a significant negative correlation between flower size and density in the floral rachis, that is, the smaller the flowers, the more contracted the inflorescence (R^2: -0.78456, P <0.0001, figure 1) . This character became possible to separate unequivocally *G. boavista* of *G. decumbens* and *G. peduncularis* of *G. heringeri*.

Discussion

Our results showed that is possible to distinguish clearly between pairs of similar species of *Galactia* using flower size and number trade-off, making this pattern a reliable taxonomic marker for these species pairs.

The existence of a size and number trade off in an inflorescence is an expected pattern because resources are limited (Caruso, 2004) and ought to be divided hierarchically within the plant: firstly for vegetative parts, then to reproductive parts and lastly within them (Weiss, 2005). Therefore, the existence of large and abundant flowers at the same time is unlikely, even bring obvious benefits for plant reproduction. Although theoretically expected, some studies have failed to confirm the existence of this trade-off (e.g. Ashman & Majestic, 2006). However, in a wide study, Sargeant *et al.* (2007)

proved that this trade off does exist in Angiosperms in general. Furthermore, the authors raised a number of possible explanations for the apparent failure in detecting this pattern in some taxa.

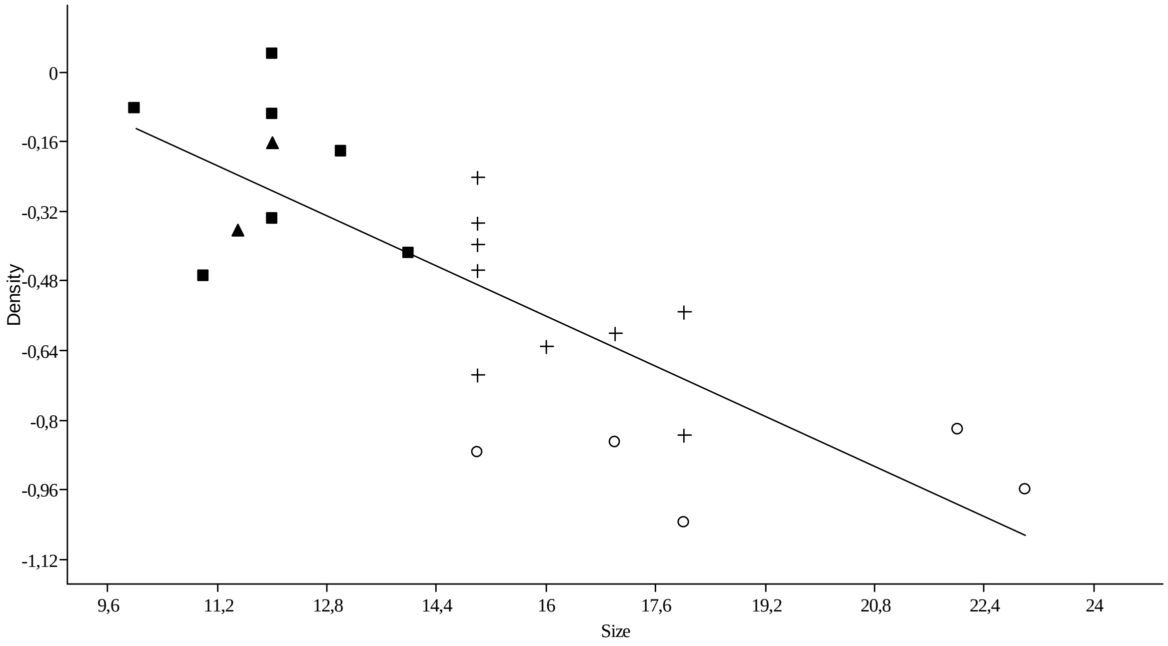

Figure 1: Size and number flower trade-off among some pairs of similar species of *Galactia*. Cross: *G. boavista;* square: *G. decumbens*; circle: *G. peduncularis*; triangle: *G. heringeri* (R^2: -0.78456, $P < 0.0001$).

One reason would be the complexity of these attributes, the number and size of flowers being influenced by a series of ecological and historical factors. Another explanation may be that closely related species are phylogenetically more similar in their ecological requirements and life history due to shared ancestry, which can mask some patterns among related attributes. This second hypothesis seems to exert a minor influence as an explanation for the failure to detect this trade-off, since our study found this pattern even in species of the same genus without examining the influence of phylogeny. Furthermore, Sargeant *et al.* (2007) were unable to confirm the pattern in the genera *Collinsia* (Plantaginaceae) and *Narcissus* (Amaryllidaceae), even correcting for phylogeny. The main driver for the acquisition and maintenance of this trade off in our species seems to be in fact ecological and historical factors. In our study, we analyzed pairs of morphologically very similar species that grow in similar environments and often occur sympatrically. On the one hand, the overlap in the characteristics often leads to misleading identification, but, on the other hand, it may be a possible starting point to understand the occurrence of this trade-off and speculate on their mechanisms of speciation.

One of the best known and largely confirmed ecological theories is the limiting similarity, which stated that two morphologically similar species only coexist in the same habitat if there is a limit to this similarity (MacArthur & Levins, 1967; Weiher et al., 1998; Stubbs & Wilson, 2004). Otherwise, if the two species are exactly alike in their requirements, there may be exclusion of the less competitive species. Some authors suggest that limiting similarity among individuals of the same species plays an important role in events of sympatric speciation (Doebeli & Dickmann, 1999; Svanbäck & Bolnick, 2007). Within the same species, competition among individuals is highest, because they are almost equal. Therefore, natural selection would tend to act more strongly on individuals whose variations were more different from the average population, favouring the more extreme phenotypes and eliminating intermediaries. This process is called disruptive selection (Bolnick, 2004). Over time, there would be a discontinuity between the extreme phenotypes large enough to decrease gene flow between the divergent populations, enabling the arising of two new species. In our study, the dissimilar architecture of inflorescences caused by the flower size and number trade off would be responsible for maintaining reproductive isolation between taxa, allowing their coexistence. Several authors have shown that differences in pollination mechanisms are responsible for isolation and coexistence of sympatric species (Armbruster et al., 1994; Kephart & Theiss, 2004; Zjhra 2008). Although phylogenetic studies within the genus *Galactia* are ongoing and are still not possible say that these species are sister-groups, it is plausible that this process was responsible for the emergence of these species. How can we explain that vegetative attributes have diverged less than reproductive attributes, if they are primarily responsible for acquiring the resources and, therefore, by the underlying competition?

The analyzed species of *Galactia* grow in open environments, usually grasslands with dystrophic, shallow and rocky soils, suffering both nutritional and water stress. Therefore, it is not surprising that these species show a vegetative similarity greater than expected by chance among them, since functional convergence is a common feature in communities where the main forces influencing the structure are environmental filters instead of competition (Weiher & Keddy, 1995). Meinzer (2003) highlights that the existence of a marked convergence in strategies regarding economy of carbon and water by plants does not mean that the identity of the species has no importance; rather, the presence of unique and species-specific attributes, mainly related to reproduction and metabolism of secondary compounds, is the rule not the exception. Moreover, even Burkart (1971) in his monograph for *Galactia* in South America, drew attention to the remarkable morphological plasticity of the genus, especially in the growth habit and leaf traits, such as shape and hairiness. This plasticity exclusively could be sufficient to helping in alleviating the intraspecific competition, because it would allow individuals to adjust their strategies depending on the intensity of competition. Based on this reasoning, it is not difficult to imagine a scenario where some individuals show variations in a kind of attributes which enable them to explore alternative resources in a better way. Consequently, this new "phenotype" increases the survival chance of those individuals. Thus, natural selection tends to favour those individuals who, in addition to this positive change, have attributes in the reproductive organs that would decrease the gene flow with the original population, increasing dominance of the alternative phenotype. Although speculative, this scenario is quite plausible, because there are an increasingly amount of data in the literature demonstrating the role of phenotypic plasticity as a promoter of population divergence and speciation (Price et al., 2003; West-Eberhard, 2005; Pfennig & McGee 2010).

In a recent review, Pfennig et al. (2010) suggested a mechanism by which phenotypic plasticity may promote speciation. They pointed out that the occurrence of polyphenism, that is, divergent, environmentally triggered, resource-use phenotypes within a population is a critical early stage of speciation in some models. These alternative and better adapted phenotypes often show parallel evolution of several traits that promote assortative mating within the polyphenic population, establishing a reproductive isolation with the original population. Levin (2009) points out that one of the most common mechanisms that promote reproductive isolation between core and marginal populations are changes in the flowering season. In *Galactia* there are few species with different flowering periods, which generally occurs from October to March, with peaks in November and December (corresponding to the Southern Hemisphere spring and summer). The species we studied here are not exception, so that this is not the dominant mode of reproductive isolation among species. What ensures reproductive isolation in most species of *Galactia* seems to be in fact the floral display. In our particular case, the flower size and number trade off between large and scarce flowers on the one hand and small and aggregated flowers on the other hand. It is not possible to know exactly how these phenotypes have evolved, but it is well documented in the literature that simple genetic and developmental changes may cause very evident effects in the architecture of inflorescences (Bradley et al., 1997; Prusinkiewicz et al., 2007; Kellogg 2007).

The main outcome of an often long taxonomic work is an identification key, which allows anyone to securely recognize a species within a given group. However, this is not always true because there are still no universal criteria for choice of characters to be used in such keys. In this fashion, taxonomy becomes a subjective work, because choose of the characters is driven by the preferences of the taxonomist, instead of objective criteria. However, some authors have stressing that not any attributes can be used for species identification (Padial et al., 2010; Tobias et al., 2010). The attributes to be used in taxonomic works must have some influence in maintaining the boundary between the species. In sexually-reproducing plants, the best attributes are those related to reproductive organs, such as flowers and inflorescences. In the case of floral display, several studies have shown that the architecture of inflorescences have considerable influence in pollinator behaviour and, ultimately, contribute to maintaining reproductive isolation between closely related species (Wyatt, 1982; Worley et al., 2000; Kudo & Harder, 2005; Ishii et al., 2008; Zjhra 2008). Furthermore, several studies have shown that the reproductive organs are more canalized and less influenced by changes in external environment than the vegetative ones (Fenster & Galloway, 1997; Brock & Weinig, 2007). This feature underpins that reproductive traits should be preferentially used in taxonomic keys.

The main conclusion of this study is that the flower size and number trade off is sufficiently reliable to be used as an additional taxonomic marker in pairs of similar species of *Galactia*. Although the flower size and number trade-off has not been confirmed in all the taxa, it was proved to be of widely occurrence among the Angiosperms, making it a useful tool to be used in other taxa as an additional taxonomic marker.

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Seção II

Tratamento taxonômico

Annals of Botany

Systematics and phylogenetic relationships with some insights on the divergence dates and speciation process in a controversial *Camptosema-Galactia* (Leguminosae) complex from southern South America

Guilherme B. Ceolin, João R. V. Iganci, Silvia T. S. Miotto & José F. M. Valls

- *Background and aims: Galactia marginalis* and *G. pretiosa* (Leguminosae) are very similar species that were early treated as synonyms, being identified chiefly by the length of peduncles. However, this trait is ambiguous in some individuals, which causes misidentification. Overmore, previous phylogenetic studies placed *G. marginalis* into the same group of *Camptosema rubicundum*, but they did not test their phylogenetic relationship with *G. pretiosa*. Thus, our goals were (1) provide alternative characters to safely distinguish *G. marginalis* and *G. pretiosa*, (2) highlight the phylogenetic relationships among these three taxa and (3) gain some insights on the speciation process among these species through a dated phylogeny.
- *Methods:* we carried out both a maximum likelihood (ML) and Bayesian analysis (BA) of nuclear ITS to access the phylogenetic relationships and the dates of divergences among the taxa. We also performed a multivariate analysis of morphological traits seeking additional useful characters to recognize *G. marginalis* and *G. pretiosa*.
- *Key results:* the ML- and BA-based phylogenies showed that the three taxa are merged together in a clade whose divergence occurred ca. 5.11 (1.76 – 8.46) mya; *G. pretiosa* and *G. marginalis* are sister groups one each other and have diverged ca. 4.67 (1.65 – 7.72) mya; *C. rubicundum* is sister of them. The morphometric analysis showed that these two species can be safely recognized by a suite of characters, but not only the peduncle length. We also provided some insights into the speciation process of this group.
- *Conclusions:* our main conclusion is that *G. marginalis* and *G. pretiosa* are surely distinct and consistent species, easily recognized by several characters. Because these two species are sister-groups of *C. rubicundum*, we stress the urgency of a large and comprehensive phylogenetic study concerning *Galactia* and *Camptosema* in order to improve our knowledge into the relationships within this group.

Key-words: molecular dating – Fabaceae – Diocleinae – speciation – Pampas – Bayesian analysis

INTRODUCTION

The genus *Galactia* P. Browne (Leguminosae, Papilionoideae) belongs to tribe Phaseoleae, subtribe Diocleinae and includes perennial herbs, subshrubs and shrubs, erect, prostrate or voluble, widely spread in pantropical regions and growing preferentially in open areas, as grasslands and savannas. The taxonomy of this genus is very difficult and inaccurate, because the criteria to species recognition rely extensively on characters that are know to be labile, such as hairiness, habit and shape of leaflets. Furthermore, the phylogenetic relationships between Galactia and the very closely related genus *Camptosema* are still not well resolved. Few phylogenetic studies have been done until now and all of them have shown that both genera are non-monophyletic (Queiroz et al. 2003; Sede et al. 2008, 2009).

The Sede's et al. (2009) phylogeny, based on *trn*L-F, ITS and morphology, showed that *G. marginalis* Benth is sister group of *Camptosema rubicundum* Hook & Arn with high support (85% jackknife value). The later species is the type species of the genus and its morphology is very different from *Galactia*. Although surprising, this result just corroborated what Sede et al. (2008) have already found on a previous AFLP-based phylogeny, but with low support (53% bootstrap value).

Galactia marginalis is a perennial species widely distributed in the whole Americas, occurring disjunct in southern South America and southwestern United States (Burkart 1971). Because of differences in length of inflorescences, sessile in some individuals and large in others, Chodat & Hassler (1904) described *Galactia marginalis* var. *pedunculata* and later, Hassler (1919) changed the status of this variety to subspecies. However, Burkart (1949) stated that the differences between the taxa were consistent enough to accept the specific rank, naming it *Galactia pretiosa* Burkart. Thus, individuals bearing sessile inflorescences with peduncle up to 15mm are called *G. marginalis* and individuals bearing large inflorescence with peduncle longer than 15 mm are called *G. pretiosa*. During the revision of the genus in Brazil, we find some specimens with both large and sessile inflorescence. In addition, other characters like habit and shape of the leaflets also appeared very plastic, causing misidentification.

The main aim of our study is shed some light on the taxonomy and the phylogenetic relationships within this controversial taxonomic complex from southern South America. We wonder whether *G. pretiosa* and *G. marginalis* actually are two good species; if so, we wonder whether they are morphologically striking similar because they share a common evolutive history or merely by convergence. Additionally, our secondary aims were provide alternative characters to safely distinguish

G. marginalis and *G. pretiosa*, and gain some insights through a dated phylogeny on the process by which the species within the *C. rubicundum – G. marginalis/pretiosa* complex has becoming divergent taxa.

MATERIAL & METHODS

Phylogenetic and divergence dates analysis

We based our study extensively on Sede's et al. (2009) phylogeny. However, from the three sources of characters used by that study, we used only the ITS marker because morphological characters have proved to be highly homoplasic and the *trn*L-F was scarcely informative, providing low-resolved trees. Furthermore, the number of informative characters of ITS alone was higher than *trn*L-F and morphology together. Thus, we extracted the ITS data from GenBank for both the ingroup and outrgroup taxa, except *G. pretiosa* (Supplementary material), from which we extracted total genomic DNA from herbarium vouchers or silica-gel dried material using the CTAB method with addition of 3% β-mercaptoethanol (Doyle & Doyle 1990).

We amplified the ribosomal ITS region (ITS1 – 5.8S – ITS2) using primers 92 + 75 and ITS3 + 75 (Desfeux and Lejeune 1996). To run the polymerase chain reaction (PCR), we prepared 1.1 μl of template DNA (30–100 ng), 2.5 μl of reaction buffer 10x, 0.75 μl of MgCl2 (50 mM), 0.5 μl of dNTP (10 mM), 0.25 μl of Taq DNA polymerase (5 U/μl), 0.5 μl of each primer, 2.5 μl of DMSO (96%) and H_2O until a final volume of 25 μl. We carried out DNA amplifications using the "Hot Start" PCR method: 94°C for 5 min, 72°C for 6 min; 35 cycles at 94°C for 45 s, 58°C for 1 min, 72°C for 1 min and 30 s; with a final extension at 72°C for 10 min. Before sequencing, we purified the amplified material with enzymatic pre-treatment (Shrimp alkaline phosphatase and exonuclease I, Amersham Biosciences).

We firstly aligned the sequences using ClustalW option in BioEdit software (Tom Hall, North Carolina State University, USA); later, we edited the matrix by hand to correct some equivocal alignments. With the aligned matrix, we ran a maximum-likelihood analysis (ML) using PHYML (Guindon et al. 2005). PHYML is a software that estimates ML phylogenies from alignments of nucleotide or amino acid sequences, implementing the heuristic described by Guindon & Gascuel (2003). We used the default parameters of software and estimated the support values for nodes on the ML tree with 999 bootstrap replicates.

We used BEAST 1.6.0 (Drummond & Rambaut 2007) to estimate the phylogenetic trees and divergence times. The tree was calibrated using age estimates for three nodes extracted from Lavin et al. (2005): *Miletia – Philenoptera* (26.1±2.0 mya), *Platisciamus – Phaseolus* (39.7±2.0 mya) e *Apios – Phaseolus* (27.8±1.6 mya). We ran a chain of 10,000,000 generations sampling every 1,000 generations using Yule speciation model, a relaxed clock uncorrelated lognormal and assuming a general time-reversible model, with invariable sites and among-site rate heterogeneity (GTR+I+Γ). We assessed adequate sampling and likelihood stability using TRACER 1.5 (Rambaut & Drummond 2007) and two thousand trees (20%) were discarded as burn in to generate the final tree.

Morphometric analysis

For morphometric analysis, we evaluated specimens deposited at the ICN, HBR and MBM herbaria (acronym according to Holmgren & Holmgren, 2007), totalizing 123 individuals (60 of *G. pretiosa* and 63 of *G. marginalis*). We scored 11 qualitative discrete characters from each individual (table 1) and performed a permuted cluster analysis (Ward's method, Eucliden Distance, 1,000 permutations) to evidence how many groups actually there were in the data set. Later, we used a multivariate two-group permutation test as a cross-validation of the permuted cluster analysis in order to confirm whether the groups were significant. Two-group permutation uses Mahalanobis distance and tests the equality of means by (5,000 permutations in our case). This test is an option for standard Discriminant Analysis (DA), in which the equality of means between two groups is tested by a multivariate analogue of the *t* test, called Hotelling's T-squared. The permutation test is an alternative to the Hotelling test when the assumptions of multivariate normality and equal covariance matrices are violated. Lastly, we analyzed carefully all the specimens within either cluster seeking exclusive characters to be used in taxonomic identification. All the analyses were done in PAST (Hammer et al. 2001).

RESULTS

Phylogenetic analysis and divergence dates

The maximum likelihood analysis confirmed the monophyly of the clade formed by *Galactia marginalis-pretiosa* and *Camptosema rubicundum* with high bootstrap support (98%). Within this clade, *G. pretiosa* proved to be monophyletic with high bootstrap support (85%). However, the maximum likelihood was not able to show if *G. marginalis* is sister group of *G. pretiosa* or *C. rubicundum* because of the low resolution within this clade (Fig. 1). On the other hand, the Bayesian analysis confirmed that *C. rubicundum* clade is the sister group of *Galactia marginalis-pretiosa* and also showed that *G. marginalis* and *G. pretiosa* are in fact sister groups, which is consistent with the very similar morphology of these species (Fig. 2).

The molecular dating showed that the major evolutionary events in this group occurred in the Miocene and Pliocene (~ 23 – 2 mya). The first divergence occurred ca. 17 (23 – 11) mya and separated two distinct lineages: the group *G. marginalis-G. pretiosa-C. rubicundum* on the one hand, and the remaining *Galactia* and *Camptosema* to another hand. Later, there was a splitting event between *C. rubicundum* and *G. marginalis-G. pretiosa* at ca. 5.11 (1.76 - 8.46) mya and finally *G. pretiosa* and *G. marginalis* became independent species ca. 4.67 (1.65 - 7.72) mya.

Morphometric analysis

The cluster analysis resulted in two groups with 100% bootstrap support (Figure 3), which were corroborated by the Two-groups Permutation (P <0.0002), although some individuals have been placed inversely to the predominant species within that cluster. This happened because we have previously named each specimen, based on the ambiguous character "peduncle length" (some specimens have the two states). This led to a priori misidentification and wrong placement in the cluster analysis. To avoid this error, we recommend the use of a suite of characters instead of only one, in a stepwise manner. Table 2 summarizes the steps required for the correct recognition of either species, in order of priority. The petiole is certainly the most important and reliable character, as always showed well-defined states for each species: present and measurable in all specimens of *G. marginalis* and absent in all specimens of *G. pretiosa.*

DISCUSSION

Systematics and phylogenetic relationships

Our analysis supported the hypothesis previously proposed by Burkart (1949) that *G. pretiosa* and *G. marginalis* are different species. Molecular analysis showed that these taxa in fact share a common ancestor, but that currently follow different evolutionary histories. However, unlike Burkart (1949, 1971), we put the primary attribute to distinguish these species is the length of the petiole instead of peduncle because the later presents both states in some individuals of *G. pretiosa*. Moreover, the geographical distribution of both species is predominantly disjunct, with a narrow contact zone about the latitudes 29ºS and 30ºS. Moreover, the molecular analysis showed that the group *G. marginalis-G. pretiosa* is sister of *C. rubicundum*, confirming the results previously shown in Sede et al. (2008, 2009). This is counterintuitive, since *Camptosema* is morphologically quite different from *Galactia*, mainly due to adaptations for ornithophyly. Some hypotheses may explain this apparent paradox.

One of the most remarkable features in all attempts to reconstruct the phylogenetic relationships between *Camptosema* and *Galactia* was that both genera have always appeared as non-monophyletic (Queiroz 1999, Queiroz et al. 2003; Sede et al. 2008, 2009; our study). Many species of *Camptosema* often appear nested into clades with several species of *Galactia*. For example, Queiroz (1999) and Queiroz et al. (2003) based their phylogeny on morphological data and found that *C. scarlatinum* always grouped within the group of *Galactia*, mainly due to characters of the inflorescence. Further studies using molecular markers (Sede et al. 2009; our study) showed that *C. scarlatinum* is sister group of a clade encompassing *G. neesii* and related species. This result is consistent, because several features of *C. scarlatinum* differ from standard *Camptosema*, such as corymb-like inflorescences, campanulate calyx, subsessile ovary and chromosome number 2n = 20 (Sede et al. 2003; 2006). The explanation for successive mistakes in the circumscription of these groups may be related to the different pollination syndromes between *Galactia* (mellitophyly) and *Camptosema* (ornithophily). Queiroz (1999) hypothesized that *Camptosema* may actually be an artificial grouping of different ornitophilous lineages within the subtribe Diocleinae. Corroborating this hypothesis, Queiroz et al. (2003) showed that the exclusion of traits regarding bird pollination radically changes the phylogenetic relationships among several species of *Galactia* and *Camptosema*, making inconclusive their actual evolutionary histories. Moreover, many authors recognize that changes in pollination system within a group can be source of taxonomic problems (Crisp 1994, Bruneau 1997). There are several examples of phylogenetically close related groups that show shifts in pollination system, either from bees to hummingbirds or vice versa (Whittall and Hodges 2007; Tripp and Manos 2008). Therefore, it is quite plausible that the hypothesis of Queiroz (1999) is correct and that the clade formed by *C. rubicundum* - *G.marginalis/pretiosa* is a closely related group, despite being morphologically so different. Having a dated phylogeny with the approximate dates of divergence among the taxa of this group allows us to speculate on some possible processes that gave rise to these species.

Divergence dates and insights on speciation processes

The main ecological events in tropical regions in the Late Miocene were forest retraction to riparian areas and spreading of grasslands and savannas due to a combination of climatic and disturbance factors that reduced the tree cover, mainly changes in rainfall patterns and fire regime (Osborne 2008, Edwards et al. 2010). Consequently to expansion of grasslands, there were major changes in faunal composition, with an increasing number of species adapted to living in savannas such as large grazers, also in southern South America (Webb 1978, Cerling et al.1997). Thus, the main cause driving the divergent evolution between *C. rubicundum* and *G. marginalis* might be the increasing pressure of grazing. The former species grows in the floodplains of the Parana, Uruguay and Rio de la Plata river basins, twining on trees of their associated riparian forests. Eventually, this species can also be found in dry and open grasslands, growing among grasses and forbs. Open grasslands a priori were not suitable environments for *C. rubicundum* because grazing would prevent the advancement of the individuals into the area. However, several studies have suggesting that one reason for success of many invasive species can be phenotypic plasticity (Sultan 2001, Richards et al. 2005). *Camptosema rubicundum* might have been beneficed by this mechanism to circumvent this limitation and colonize this new environment. Phenotypic plasticity can be defined as the ability of different genotypes express different phenotypes for a trait, i.e., it refers to the potential of specific traits to respond to different environments. (Richards et al. 2006). This plasticity can affect the reproductive success of an organism and contribute to its evolution by natural selection. However, in order to successful occurrence of this process, the different reaction norms for a particular trait must be present in the genotype. Some key attributes that make *G. marginalis* different from *C. rubicundum*, such as protected (sessile) inflorescences, small leaves and height, prostrate and stoloniferous growth, are classic adaptations to grazing (Landsberg et al.1999, Diaz et al. 2007) and all of them are common to several others species of both *Galactia* and *Camptosema*. Even other distinctive attributes not primarily associated with grazing, such as unifoliolate leaves and mellitophylous pollination, also appeared several times within the group. However, these same traits may have played an important role in the colonization and maintenance of this emerging lineage in a new environment.

Eastwood et al. (2008) showed that all of the unfioliolate *Lupinus* species (tribe Genisteae) both in North and South American show striking ecological similarities. Both groups grow in lowland, subtropical and fire-prone grasslands/Cerrado (Brazilian savannoid biome). They suggest that unifoliated leaves may confer adaptive advantages to promote rapid growth in fire-prone habitats. The only a priori difference between environments where *Lupinus* and *G. marginalis* grow is the presence of fire and grazing, respectively. However, the ability to rapidly resprout after a disturbance, whatever if fire or grazing, is a crucial attribute for survival of any species (McIntyre et al. 1999). Furthermore, fire played an important role in spreading of grasslands in the Miocene (Osborne 2008) and might have been an additional disturbance to be overcome. Concerning the shifts in pollination syndrome, the melittophily may have prevailed over ornithophyly because of the growing trend of the individuals in open grasslands show prostrate habit and protected inflorescences, which would complicate the visitation of hummingbirds. Thus, those individuals bearing mutations favoring floral visitation by bees were likely to be favored by natural selection and became dominant. Moreover, this shift in pollination syndrome may have been the cutoff point for the establishment of new species, because it prevented gene flow among individuals in grasslands and forest. Cases of populations that colonize previously inhospitable environments and establish some kind of reproductive isolation with the parental populations are common in the literature, this phenomenon being well-documented in plants colonizing heavy metal-contaminated soils (Antonovics 1968, Antonovics 2006).

In short, divergence processes among populations driven by disturbance, the most likely being grazing in our case, are well-documented and neither of the morphological differences between *C. rubicundum* e *G. marginalis* demanded complex genetic alterations; all of them were already part of the standard genotypic reaction norms of the species. Corroborating this affirmation, we can mention species that bear both unifoliolate and trifoliolate leaves (*C. coriaceum* (Nees & Mart.) Benth.) or short and large inflorescences (*G. benthamiana* Mich.) at the same specimen. The same processes may be suggested regarding speciation between *G. marginalis* and *G. pretiosa*, however, in response to different selective pressures.

The geographic distribution of *Galactia pretiosa*, unlike its sister species, is related to grasslands in the highlands of the southern Brazilian Plateau (details about the grasslands of south Brazilian Plateau in Overbeck et al. 2007); the only contact zone between these two species occurs in granite hills of the region close to the city of Porto Alegre (30°51'S), the capital of the southermost Brazilian Sate, Rio Grande do Sul. This may be an indication that the main process of divergence between these species occurred at that site, whereas these granite hills behaved as islands during the successive events of marine transgression and regression. Carreño et al. (1999) showed that at the boundary of Miocene – Pliocene (6 – 5 mya) the entire area around these granite hills were covered by an ocean of at least 200 m depth. At approximately 4 mya a period of marine regression that peaked in the late Pliocene began (ca. 2 mya). This cycles of marine regression and transgression continued occurring at regular intervals throughout the Quaternary (Tomazzelli 2006) and several authors attribute the large number of endemic species in these granite hills to the isolation caused by these events (Rambo1954; Setubal et al. 2010). The first marine regression in the early Pliocene may have allowed some populations of *G. marginalis* colonize these hills and later expanding their range towards the Plateau, perhaps taking advantage of a climatic optimum in the Middle Pliocene (Haywood et al. 2000). The subsequent marine transgressions and regressions probably isolated these populations and completed the speciation. This process is feasible, because our analysis showed that the range of the dates of divergence between the taxa are within this epoch.

The most remarkable features in the distinctive characters between *G. pretiosa* and *G. marginalis* are the trade offs between the first two attributes shown in Table 3: if stoloniferous plants, then large petiole (*G. marginalis*); if erect, then absent petiole (*G. pretiosa*). Understanding the causes underlying this pattern can help us to gain some insights on the microscale processes that drove the speciation of these taxa.

Elongation of internodes and petioles is one of the typical responses to shading (Hutchings and de Kroon 1994) and plants capable of producing long structures are best competitor than small and less plastic plants (Weinig 2000). Thus, one would expect a positive correlation in elongation of both attributes in order to maximize light capture. However, finite resources often results in life-history trade offs (Roff 2000). Furthermore, the influence of responses of these two structures on the fitness of a species depends on the selection regime under which the plant is growing, whether in competition or disturbance (Weijschede et al. 2008). Thus, for *G. marginalis*, intense grazing selected individuals with smaller structures and stoloniferous habit, since the vertical growth is not advantageous because the loss of biomass outweigh the costs of its production (Dorn et al. 2000). Although stoloniferous growth and shorter internodes improve the chances of survival under grazing, they hinder the capture of light. Therefore, the species retained the ancestral state of large petioles, since it is the main spacer organ in stoloniferous species, rising the leaves above the herbaceous layer to capture light (Huber et al. 1998). Moreover, from the late Pliocene, during the probable expansion of *G. pretiosa* to the Plateau, there was a gradual cooling of the planet (Ravelo et al. 2004). This cooling was probably more intense on the highlands because of the altitude, and may have influenced an increasing in the number of caespitose and rosette-forming plants, because this is a common trait in high altitude grasslands (Safford 1999). A larger number of caespitose plants increases the height of the herbaceous layer and consequently the vertical competition for light. In this case, a greater vertical elongation of the internodes confers adaptive advantages to individuals that are sufficiently plastic in this trait. As elongation of petioles does not affect the performance under competition (Wejschede et al. 2008), the petiole may have been removed to optimize resource allocation. Moreover, even if the disturbances have been frequent and intense in the South Brazilian Plateau during the Pliocene and Pleistocene, a higher number of species of tussock and rosette-forming plants may protects against these events those individuals that are able to survive near these species. Currently, it is not uncommon to find individuals of *G. pretiosa* entangled among grass tussocks and *Eryngium* spp. (Apiaceae), a rosette-forming species, which helps to support our hypothesis. Therefore, even with frequent disturbances, light competition in the grasslands on the south Brazilian Plateau might have been the main factor driving the morphological divergence between *G. marginalis* and *G. pretiosa*.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Our main conclusion is that *G. marginalis* and *G. pretiosa* are two sister but independent taxa which also share an evolutionary history with *C. rubicundum*. The climatic changes that take place at the Middle Miocene onwards may be the causes of the emergence of these species. However, further studies with a larger number of both taxa and molecular markers are urgently in order to resolve the relationships between *Camptosema*, *Galactia* and other closely related groups, since all the phylogenies carried out to date have shown that many taxa within this group are not monophyletic. In addition, other analyses are also needed to enlighten the evolution of these taxa and even of the subtribe Diocleinae. Phylogeographical studies are crucial to test our hypotheses about the speciation of this group and to explain the processes that led to the disjunct distribution of *G. marginalis*; studies on the evolution of characters are needed to elucidate the process of divergence in the morphology of individuals and the difference in chromosome number within the clade as well (*C. rubicundum* 2n = 22; *G. marginalis* and *G. pretiosa* 2n = 20).

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Table 1. List of characters and states used in morphometric analysis of the *G. marginalis* – *G. pretiosa* group.

Attribute	**States**
Habit	[0] prostrate; [1] erect
Stoloniferous growing	[0] absent; [1] present
Stem lignification	[0] absent; [1] present
Number of branches	[0] 1-2 branches; [1] > 2 branches
Texture of leaves	[0] cartaceous; [1] coriaceous
Leaf orientation	[0] normal; [1] conduplicate
Petiole	[0] < 1 mm; [1] > 1 mm
Trichomes in leaves	[0] absent; [1] present
Marginal nervure	[0] absent; [1] present
Number of flowers	[0] 1 – 4 flowers; [1] > 4 flowers
Peduncle	[0] < 15 mm; [1] > 15 mm; [2] both states

Table 2. Useful characters to recognize *G. marginalis* and *G. pretiosa*, in order of importance.

	G. marginalis	*G. pretiosa*
1º - Petiole	Present and conspicuous	Absent
2º - Stoloniferous growth	Present	Absent
3º - Peduncle length	Up to 15 mm	Larger than 15 mm (but some specimens show simultaneously both states of character)
4º - Geographic distribution	Predominantly in grasslands of southern South America (Pampas biome)	Predominantly in the highlands of the Southern Brazilian Plateau (Atlantic Rainforest biome)

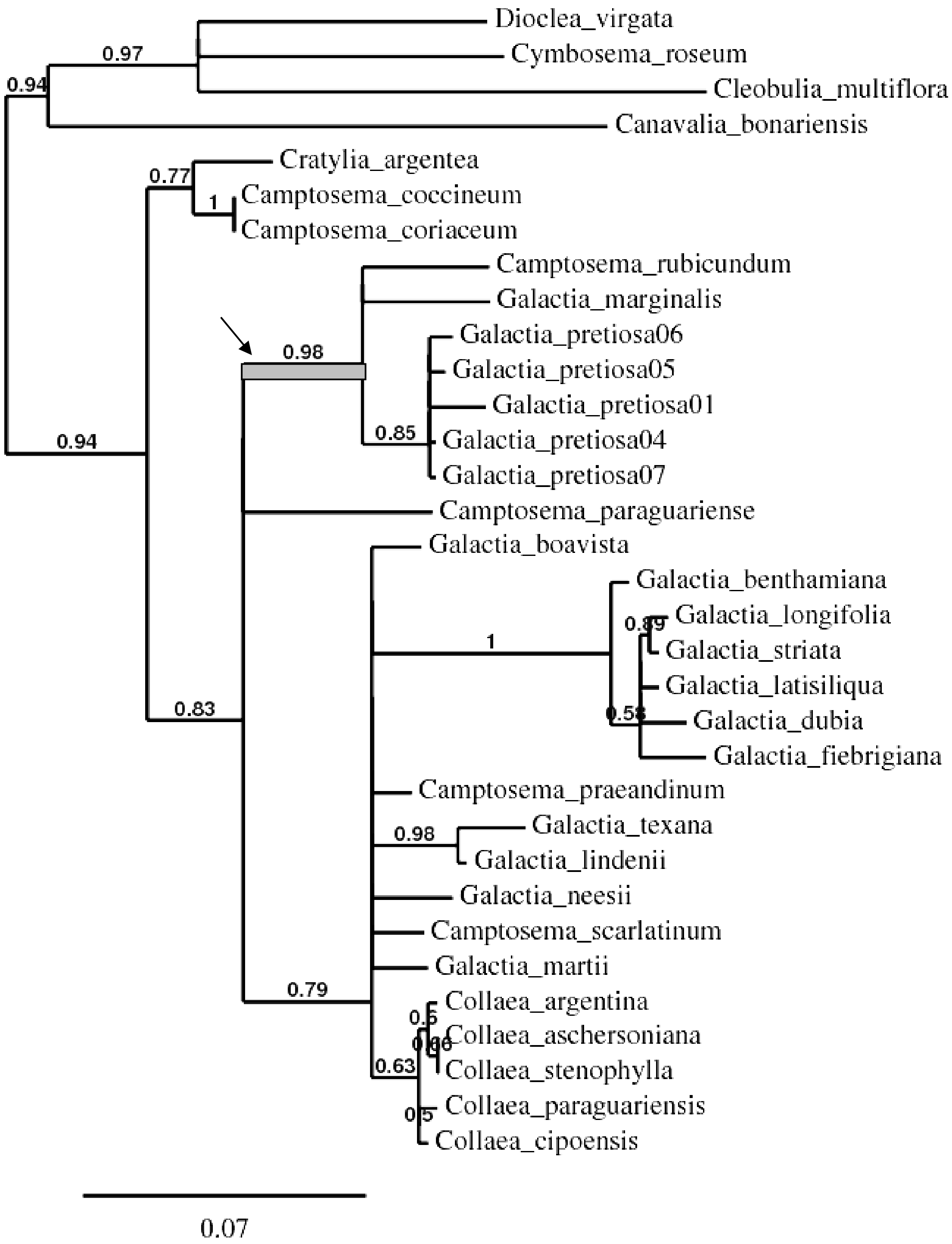

Figure 1. Maximum likelihood phylogeny derived from ITS sequences showing the relationships of some groups within the subtribe Diocleinae (emphasis in *Galactia* and *Camptosema*). Our group of interest is highlighted by an arrow and a thicker pale-gray bar. Numbers above branches are bootstrap values.

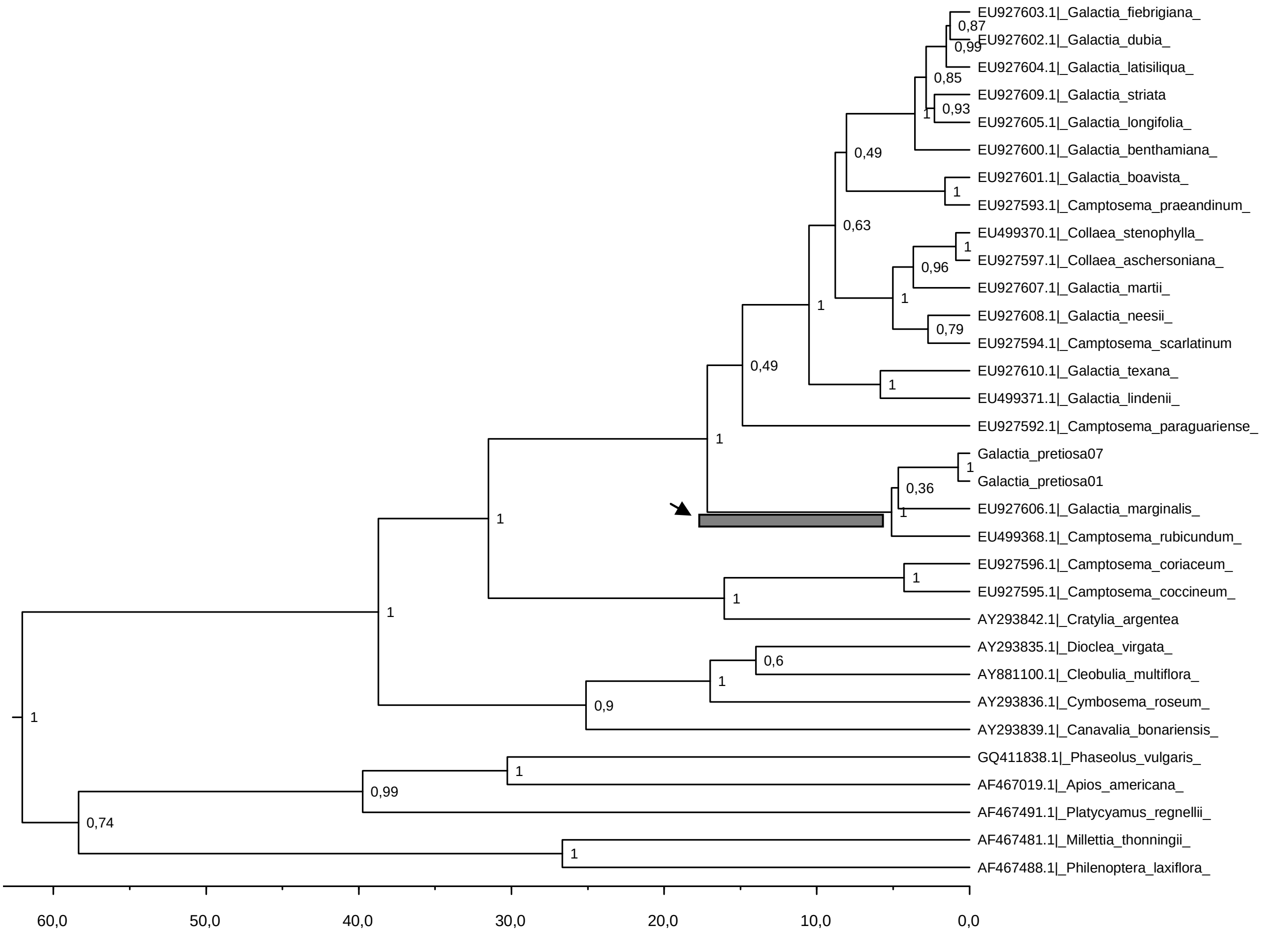

Figure 2. The 50% majority rule consensus phylogenetic tree and chronogram (in million years) obtained by Bayesian analysis of ITS sequence data for some groups of the subtribe Diocleinae (emphasis *Galactia* and *Camptosema*). Our group of interest is highlighted by an arrow and a thicker pale-gray bar. The number between the branches are posterior probabilities.

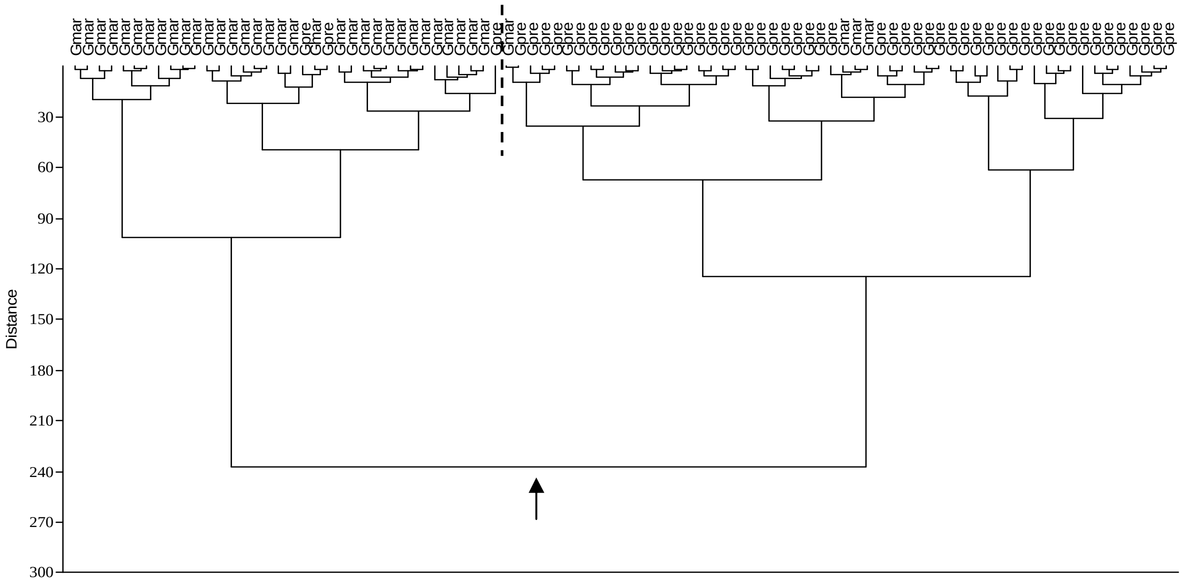

Figure 3. Permuted cluster analysis of the morphological data showing the classification of individuals either in *G. marginalis* or in *G. pretiosa* (Ward's method, Euclidean distance, 1,000 permutations). The arrow in the first branching indicates 100% bootstrap value; all of the other branches had bootstrap values below 50%. Dashed line indicates the boundary between the two species.

Plant Systematics and Evolution

Using an integrative non-molecular approach to increase the taxonomic resolution within the *Galactia neesii* (Leguminosae) complex

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Abstract

The aim of this paper is to solve the taxonomic complex formed by *G. neesii*, *G. martii*, their infraspecific taxa and other similar species, totalizing eight names. This group grows from southern Uruguay to northeastern Brazil and the morphology of individuals gradually changes along this latitudinal gradient, being possible identify many morphotypes, which were assigned by previous authors either as species or varieties. We combined ecological and morphometrical approach to increase the resolution within this complex. Firstly, we used permuted cluster analysis of ecological data to test the significance of the eight-group hypothesis. Later, the significant groups found by the cluster analysis were tested a posteriori with non-parametric MANOVA to discover congruent morphological groups. Our results showed that from the eight previously hypothesized groups, only three were both ecologically and morphologically significant and could represent separately evolving lineages. The necessary nomenclatural changes are provided in this paper, as well as a short taxonomic description of the taxa.

Key-words: integrative taxonomy – Diocleinae – ecologic speciation – taxonomic methodology - Leguminosae

Introduction

Species are the basic biological units and their correct identification is fundamental in fields such as ecology, evolution, conservation biology and even pest management (Wiens 2007, Light et al. 2008). However, the task of delimiting species is frequently quite complicated, because there are over twenty species concepts (Mayden 1997) and a fairly large number of methods for species recognition (Sites & Marshall 2004). Fortunately, currently there seems to be a growing consensus among systematists and evolutionists that a species is a separately evolving population or metapopulation lineage (de Queiroz, 2005, 2007), which acquires several properties along its evolutionary timeframe (e.g. reproductive isolation, ecological niche, diagnostic characters). These properties are contingent criteria for delimiting species. Even so, the recognition of these lineages is still difficult for several groups that have experienced recent divergence, since it is less likely that these groups have all or even many of the properties quoted above (Ross et al. 2009). To deal with this problem, many authors have proposed the use of multiple sources of evidence to define divergent lineages (Leache et al 2009, Ross et al. 2009, Schlick-Steiner et al. 2010), in a combined approach termed “integrative taxonomy” (Fonseca et al. 2008, Gibbs 2009, Padial et al. 2009, 2010).

In integrated taxonomic studies, usually the discovery of potentially separate lineages is primarily achieved by use of molecular characters, and these lineages are subsequently tested for congruence with other data types, such as ecological, morphological and behavioral (Rissler & Apodaca 2007, Cuervo & Cadena 2010). The use of molecular characters has undeniably become taxonomic studies most consistent and have enabled as resolution of taxonomic complex as discovery of cryptic species (Burns et al. 2008, Leache et al. 2009). However, the morphology-based alpha taxonomy (MOBAT, hereafter) can still be seen as the main and most well-established tool for primarily accessing biodiversity (Schlick-Steiner et al. 2007). It presents some advantages, such as being applicable to fossil records and allows rapid identification of taxa with almost no financial cost. Moreover, molecular markers not always are informative enough to differentiate morphologically well-established closely related species (Gemeinholzer & Bachmann 2005).

One of the main disadvantages of using morphological characters is its continuous nature rather than discrete (Padial et al. 2010). This feature of morphological attributes complicates the establishment of precise boundaries in some groups and often makes the species delimitation a subjective endeavor. However, we can avoid these problems by using of statistic morphometrical analysis, which has shown good performance even for further identification of cryptic taxa discovered by molecular markers (Sanchez-Andres et al. 2009, Favret 2009, Newmaster & Ragupathy 2009). Thus, an integrated taxonomic approach exclusively using non-molecular characters is perfectly plausible and may be a quickly and inexpensively alternative for resolution of taxonomic complexes.

In this article, we will use a combined morphological and ecological approach to shed some light on the taxonomic complex formed by *Galactia neesii* DC. and related species (Leguminosae, Papilionoideae). This complex comprises primarily four species (*G. neesii, G. martii, G. crassifolia* and *G. stereophylla*) and different varieties within them (*G. martii* var. *acuta, G. neesii* var. *australis, G. neesii* var. *macropoda*), totalizing seven taxa. The main reference for identification of these taxa is the Burkart’s (1971) monograph of *Galactia* to South America. Even so, the correct delimitation of the taxa listed above is very complicated, since the criteria for recognition are fuzzy and based on combinations of widely labile traits, with no discrete unique characters. Moreover, even Burkart suspected that some individuals would be better defined if placed in new varieties. He wrote a new name in some exsicatae (*G. martii* var. *volubilis*), but unfortunately he deceased in 1976 and did not conclude the description of this new taxon, which is actually a *nomem nudum*. However, in our analysis we will consider all the eight taxa (the former seven plus this non described variety) to test the hypothesis that these groups are evolutionarily independent lineages, which have cohesion in their morphological and ecological characteristics. We will provide the necessary taxonomic changes, as well as a brief description of each taxon.

Material and methods

We selected 151 individuals across the entire geographic distribution of the complex, from Uruguay to the northeast of Brazil. The examined exsicatae are deposited in the following herbaria: BAB, CEN, CTES, HEPH, HUEFS, IBGE, ICN, MBM, NYBG, SI, SP, PAMG, UEC, UFMT (acronyms according to Holmgren & Holmgren 2007). For the identification of individuals, we used the name on the exsicatae label when present and for that individuals identified only as *Galactia* sp., we tentatively identified following the Burkart's (1971) taxonomic key. Actually, this identification was not employed in order to test a posteriori the boundaries of these taxa; it was made just to serve as a nomenclatural reference. For the analysis, we sequentially numbered the specimens in ascending order and this number were the only identification they received. We employed this methodology because, as stated above, the taxa of this complex are recognized for qualitative traits such as hairiness, texture and shape of the leaflets. The states of these characters are not species-specific; on the contrary, they are largely plastic, sometimes within the same specimen. Therefore, we only considered quantitative traits. Altogether, we measured 16 characters, which are summarized in Table 1.

Our experience with *Galactia* shows that there is no drastic reduction in trait size between fresh and dry specimens, so that morphometric analysis based on dried material are convenient for this group. We always selected the largest structure by individual, to be sure that we sampled only fully developed traits. Thus, we made only one measurement per individual. We used this methodology to decrease the number of missing data per individual, especially in floral pieces, because the flowers of most specimens were scarce or with missing parts (especially standards and wings). We rehydrated reproductive traits to allow full expansion of its parts and measured under a stereomicroscope using graph paper (0.1 mm precision). We used a ruler to measure vegetative structures (1 mm precision). We discarded fruits and seeds because they are absent in most individuals. We considered only the measures of central leaflet for further analysis, since the dimensions of lateral leaflets are strongly correlated with the central leaflet (Regression analysis, $r^2 = 0.95$, $P < 0.001$).

We used the software DIVA-GIS (Hijmanns et al., 2005a) to map all specimens using geographic coordinates, either extracted directly from the herbarium sheets or from gazetteers. From every georeferenced point, we extracted altitude and 19 bioclimatic variables provided by the database Worldclim (Hijmans et al., 2005b). Worldclim bioclimatic variables are georeferenced layers containing biologically relevant information about rainfall and temperature. The altitudes and bioclimatic data for each item were standardized to zero mean and normalized to unitary standard deviation due to differences in units (e.g. °C, m, mm).

Because we are only using non-molecular data, we primarily used ecological data to infer the possibly divergent lineages. We performed a bootstrapped cluster analysis (Ward method, 1,000 iterations, $\alpha = 0.1$) with ecological data in order to test the sharpness of the eight-group partition hypothesis, according to Pillar's (1999) method. We performed this analysis to test the hypothesis that the eight taxa proposed by Burkart (1971) actually are different ecological lineages. After that, we tested the significantly different ecologic groups for morphological coherence. For this, we edited the morphologic matrix using Kruskal-Wallis test for equality of means to remove from the analysis those attributes that were not significantly different among the clusters (11 attributes remained in the final matrix). Later, to test the consistency of these morphological groups, we used non-parametric MANOVA (NPMANOVA hereafter, Anderson 2001) with Bonferroni correction (10,000 permutations, Euclidean distance). We used NPMANOVA because the final matrix did not fit the assumption of multivariate normality even after several transformations (eg, log, square-root).

Lastly, we performed a Principal Component Analysis (PCA) with the morphological matrix to extract the attributes that most influenced the variability within the group. This ordination served as a guide for preparation of taxonomic descriptions used for further taxa recognition. As an additional source of useful characters for identification, we also used some data from Worldclim to characterize the abiotic conditions where the taxa grow.

Results

Cluster analysis of ecological data supported only three of the supposed eight groups (figure 1). The NPMANOVA of morphological data confirmed that these groups are also morphologically coherent (table 2). We named the clusters as 1, 2 and 3 and we will refer to them this way hereafter. The map (figure 2) shows the geographical distribution in South America and table 3 presents some abiotic characteristic parameters of each cluster.

Cluster 1 has a southern distribution, growing predominantly in Uruguay, southern Paraguay, northeastern Argentina (provinces of Misiones and Corrientes), and in the southernmost Brazilian state of Rio Grande do Sul (RS). We can roughly say that cluster 1 shows a geographical distribution entirely within the lowland grasslands known as South American Pampas, generally below 800 m a.s.l. In turn, the cluster 2 inhabits almost exclusively the north of Minas Gerais and south-central Bahia, in areas where the average annual rainfall is below 1000 mm, with altitudes ranging from 500 to 1200 m a.s.l. Finally, cluster 3 is the most widely distributed, inhabiting from Cerrado (Brazilian savannoid biome) to the highlands of the southern states of Brazil, a region known as the Araucaria Plateau due to the outstanding presence the conifer *Araucaria angustifolia* (Bert.) Kuntze.

The PCA showed that most of the morphological variation within the complex was satisfactorily synthesized in first two axes (axis 1: 66.1%; axis 2: 17.4%). In axis 1, most important attributes were inflorescence length (99% correlation with the axis) and petiole (44%). On Axis 2 there was a greater contribution of the leaflets length (95% correlation with the axis), followed by bracteoles (62%) and lower calyx lobe (60%). Other attributes, such as leaflet width, stipules and stipels contributed almost uniformly, with around 55% of correlation with axis In general, the PCA did not show sharp and well-defined groups (Figure 3), mainly because the continuous morphological variation of the cluster 3 within the sample space. However, this result is not surprising, given the widely geographic distribution of this cluster. In turn, there is a subtle and little overlapping gradient between clusters 1 and 2, which reflects the differences in geographic distribution between them.

Discussion

The accurate identification of divergent lineages (i.e. species) is a constant challenge for taxonomists, particularly when lineages had not time to accumulate sufficient morphological differences to allow unambiguous recognition (Raxworthy et al. 2007). These “cryptic” species may have subtle and overlapping differences in morphology, with character states not yet fixed (Wiens & Servedio 2000). On the one hand, speciation events with no fixed morphological differences are a problem because it hinders the instant recognition of different species within a taxonomic complex. But, on the other hand, is a very exciting field for new discoveries. In this paper, we address both points. The complex *G. neesii* bears certain very evident and constant characters like trifoliolate and usually conduplicated and pendulous leaves, large and showy flowers, which are strongly congested at the apex of the floral rachis, forming an almost umbellate inflorescence. We can say that these attributes are fixed within the complex, because they allow quick and unequivocal recognition, being not shared with any other taxon within the genus. However, whereas it is quite easy to establish the boundaries of this group over other groups, the large morphological variability within the complex makes any attempt to promptly assign an individual to one of the described names an exhausting and often frustrating task. According to Dayrat (2005), the creation of names without regard to intraspecific variability is not only the major source of synonyms but also a methodological error. Furthermore, he disagrees with the argument that species names are hypotheses and hence all names are valid. If names are hypothesis, their assumptions must be at least rigorously tested based on a consistent theoretical framework. The current confusion within the *G. neesii* complex largely stems from the problems pointed out by Dayrat (2005), especially the lack of explicit criteria for considering a group of specimens as sufficiently different to earn a new name. Our goal was to find clusters of individuals that were both ecologically and morphologically congruent within them, but significantly different from other. Seeking congruence between different attributes is a way of increasing the reliability of the conclusions concerning species boundaries (Shaffer & Thomson 2007, Wiens 2007) and only consistently congruent groups must be considered taxa.

Unlike other studies whose primary source to infer possible lineage differences were molecular markers, our starting point were ecological differences. The use of ecological analysis in this way can be an efficient manner to infer primarily divergent lineages without using molecular markers. There is a plethora of examples in the literature demonstrating that many speciation events were primarily driven by ecological divergence (Schluter 2001, 2009, McKinnon et al. 2004; Nosil & Rundle 2005; Funk et al. 2006) and that niche modeling can be an important tool for the species recognition in taxonomic complexes (Raxworthy et al. 2007, Rissler & Apodaca 2007, Ross et al. 2009). The standard methodology to infer isolation of lineages by using ecological niche modeling utilize associations between environmental variables and known species' occurrence localities to determine abiotic conditions within which populations can be maintained (Guisan & Thuiller 2005, Raxworthy et al. 2007). In our study, we did not use this default analysis, because our goal was not to validate lineages discovered by other means. Instead, we use ecological data to test the hypothesis that the *G. neesii* complex was actually composed by that previously named eight taxa. For this, the Pillar's (1999) method appears to be more appropriated.

Pillar (1999) proposed this method to deal with the problem of how many groups actually exist in a data set, since cluster analysis will always show groups, even in data set with no clear structure of groups. This analysis uses bootstrap to calculate the probability that a hypothesis of partition in a given number of groups (in our case, eight groups) is sharp or fuzzy. However, by using this test, we must pay attention to some details when interpreting the results. Unlike standard statistical tests, values above a stated threshold (α) support the sharpness of the groups. This is due to the fact that the null hypothesis is that the groups do exist in the data set. In other words, the null hypothesis is that the groups are sharp. Thus, values below the threshold reject the null hypothesis and accept the alternative hypothesis that the groups are fuzzy. Moreover, values above the threshold may indicate not only that the groups are sharp, but also that the sample size is too small. According to Pillar (1999), the sufficiency of the sample size can be assessed by examining the stability of the probabilities along a series of different sample sizes, as we did in figure 1. The hypothesis for partition level in 2 and 3 groups have stabilized above the threshold with very small sample sizes, which strongly indicates that this group structure does exist within the data. It is extremely important that we have a robust estimate of the sharpness of taxonomic groups, because this is a critical step largely overlooked in studies that use multivariate analysis.

Several current studies that use multivariate analysis to infer species boundaries do not explicitly test the reliability of the groups (Oliveira et al. 2008, Estrella et al, 2009, Pedersen 2010). Thus, it is dangerous to draw conclusions from these results. Henderson (2006) emphasizes that multivariate analysis are primarily exploratory, that is, they reduce the complexity in a way that we can deal with it in practice, but do not allow us to test hypotheses. Statistically, hypotheses can be only accepted or rejected within a confidence interval, which is the estimated probability of that result has not been generated at random. The most commonly used multivariate analysis (e.g. PCA and cluster analysis) do not test confidence intervals, they just grouped individuals according to their similarities. By contrast, Pillar’s (1999) method while clustering individuals into groups, provides the statistical support to them, hence being straightforward and less time-consuming.

With the groups revealed through the ecological analysis, our next step was to verify the morphological congruence within them. The NPMANOVA confirmed that the three groups were also morphologically congruent and the PCA showed that differences were in both vegetative and reproductive traits. Although the differences are statistically significant, it is noteworthy that PCA was not able to show a very evident morphological gradient among the groups. This may be an indication that the divergence process among these lineages occurred subtly and strongly influenced by phytogeographic and ecological factors. In fact, when we analyze the distribution map of clusters (Fig. 1), we note that clusters 1 and 2 grow in areas in the southern and northern ranges of geographic distribution of the cluster 3, respectively. This type of geographical distribution of clusters is consistent with parapatric speciation models, in which different species can arise from marginal populations in the boundaries of a widespread taxon (Turelli et al. 2001). The fact that these populations maintain contact zones does not represent an obstacle to the emergence of divergent evolutionary lineages, since there are several convincing examples of speciation with gene flow (Nosil 2008, Mila et al. 2009). This is especially true for cluster 2, because there is still a significant contact zone with cluster 3 in the northern of the Brazilian state of Minas Gerais. Theoretically, this contact zone would be sufficient to maintain gene flow between the two clusters and prevent the occurrence of a speciation event, but some factors can contribute to the divergent evolution of these two clusters.

Table 3 shows that the annual rainfall in most localities where the cluster 2 grows weakly exceeds 1000 mm. Thus, these populations suffer a hydric stress higher than suffered by the surrounding populations of the cluster 3. Cases of divergent selection and adaptation of marginal populations into new and often more hostile environments are not scarce in the literature, being the evolution of plants growing on heavy metal-contaminated soils one of the best known examples (Antonovics 2006). Several authors have shown that in heterogeneous landscapes, restricted gene flow may occur by natural selection acting on traits that increase fitness when environmental conditions differ between sites (Rundle & Nosil 2005, Hoekstra 2006, Niemiller et al. 2008). Parallel to this, a growing number of studies have shown that this selection may promote phenotypic differentiation along ecological gradients between parapatric populations even with gene flow (Ogden & Thorpe 2002, Petren et al. 2005, Nosil et al. 2008). In fact, statistical analysis in the scores of the reproductive attributes of PCA between clusters 2 and 3 shows that there are significant differences between them, especially in size of floral traits (inflorescence, keel and standard; results not shown). These differences may be sufficient to reduce gene flow between populations of the two clusters. Schluter et al. (2009) discuss and detail the genetic mechanisms underlying the emergence of reproductive isolation mediated by divergent ecological selection, especially selection against migrants and assortative mating.

On the other hand, the situation of cluster 1 is somewhat different, since effectively does not exist a contact zone with the cluster 3. Although the populations occurring in the Araucaria Plateau have been classified as belonging to cluster 3 and are geographically very close to cluster 1, both clusters are actually isolated because cluster 3 inhabit the high altitude grassland existing within the Araucaria forest, usually above 800 m a.s.l. These forest areas in the slopes of the Plateau serves as a barrier preventing contact between them, since most species of *Galactia* are obligatorily heliophile, growing only in sunny places and avoiding the shadow of forests (Burkart 1971). The most likely hypothesis for the isolation of these two lineages is that there was fragmentation of a ancient continuous population due to the ongoing advance of forests on the grasslands, which has been occurring since the early Holocene (Behling et al. 2005). The populations of the lowlands continued their expansion towards the south, suffering selective pressures and evolving adaptive traits to support the different conditions of temperature and precipitation existing in the Pampas, acquiring a clearly differentiated morphology.

Taxonomic treatment

Our results showed that only three of the eight previously accepted taxa bear a set of morphological and ecological characteristics sufficiently consistent and robust to be considered independent evolutionary lineages, and, therefore, taxa. Below we provide the nomenclatural changes necessary to elevate the clusters to a category of taxon, with a brief description of the diagnostic features that allow their recognition (values are shown as mean ± standard deviation). Valid names in bold, synonyms in italics.

Galactia australis (Malme) Ceolin & Miotto, **stat. nov.** (Lectotype: Malme, Iter Regn. II-508 y 508 a., S).

Basionym: *Galactia neesii* DC. var. *australis* Malme, Arkiv f. Bot. 23, A (13): 35. 1931.

Diagnostic characteristics: the main diagnostic characteristic of this taxon is the distribution restricted to the lowlands of the southern South American Pampas. Morphologically, the most conspicuous feature is the average length/width ratio of leaflet about 1 (1.47±0.29), making several leaflets almost orbicular, with rounded base and apex. Furthermore, length of inflorescences 87.3±33.6 mm, standard 9.13±2.2 mm and keels 13.2±0.9 mm.

This taxon is derived from individuals classified as belonging to cluster 1, since their morphology and geographic distribution corresponds to *G. neesii* var. *australis*, especially in the width and leathery texture of the leaflets. Although we did not examine the varietal type, we included two samples of the typical region (Porto Alegre, about 30°S, 51°W), which fit the original description and support our decision. Moreover, Burkart (1971) reported specimens of the State of Santa Catarina (neighboring state of Rio Grande do Sul) on his examined material. These specimens are from populations that inhabit the Araucaria Plateau and were classified in our analysis as belonging to cluster 3; therefore, we exclude these populations of the *G. australis* circumscription. In fact, these individuals are easily assigned as different chiefly because of the narrower, acute and less leathery leaves.

Galactia lineata Ceolin & Miotto., **sp. nov.**

Holotype: BA, Abaíra, 13º22'S 41º49'W, 1180 m, rocky grassland, sandy soil, W. Ganev 2898, 27/01/1994, fl. (NYBG).

Foliola angusta vel linearis apex foliolis acutus vel subacutus 42 – 90 mm longa × 6 – 38 mm lata; vexillum 12 – 20 mm longum;racemi axillares 37 – 135 mm longi; carina 11 – 13 mm longa; habitat aridibus regionis in Minas Gerais et Bahia brasiliensis provinciae.

Diagnostic characteristics: the main feature is its geographical distribution in semiarid regions (Caatinga biome) of north Minas Gerais and central-southern Bahia. Morphologically, we can recognize this species by the usually very narrow leaflets, sometimes almost linear (length/width ratio 4.7±3.1 leaflet). Length of inflorescences 78.4±28.9 mm, standard 14.4±2.3 mm and keel 12.9±0.4 mm.

According to Burkart (1971), *G. martii* var. *martii* is mainly characterized by narrow, linear and oblong leaflets, occurring from the central region of Minas Gerais (MG; around the capital, Belo Horizonte) to the center-southern region of Bahia (BA). Our analysis showed that in the drier areas of northern MG and central-southern BA there is a well-defined group of individuals, which are different from the individuals that grow in central MG. This group bears very narrow leaflets, fitting the description of *G. martii* var. *martii*. However, the holotype of this taxon is from most central region of MG (city of São João del Rey). In fact, some species from this region, especially in areas with iron-rich soils (regionally called *canga*), have long and narrow leaflets. Nevertheless, because the northern regions of MG and central-south BA have a lower rainfall, we maintain the individuals from these sites as different evolutionary lineage. Therefore, here we are describing a new species, whose circumscription encompasses only those leaflets showing narrow and long leaflets that grow in semiarid regions of MG and BA, excluding those that grow in more central areas of MG.

Galactia neesii DC., Prodrom. Syst. Nat. Regn. Veg. 2: 238. 1825 (Isotype: Pohl 3402, BR!).
Galactia neesii DC. var. *macropoda* (Cowan) Burkart, Darwiniana 16: 732. 1971. **Type:** Braga & Lange 164 (SI!).
Galactia martii var. *martii* DC., Syst. Nat. 2: 238. 1825. **Type:** Martius (M 12722).
Galactia martii DC. var. *acuta* Burkart, Darwiniana 16: 738. 1971. **Type:** Pedersen 5181 (SI!).
Galactia stereophylla Harms, Repert. Spec. Nov. 19: 17. 1923. **Type**: Glaziou 20900 (F!, photo).
Galactia crassifolia (Benth.) Taub., Die Natürl. Pflanzenfam. 3: 368. 1894. **Type:** Pohl 2895,(W!, photo).

Diagnostic characteristics: the geographical distribution of this taxon fits almost entirely the distribution of Cerrado (Brazilian savannoid biome), with some populations occurring in the highlands of southern Brazil. Morphologically, this taxon has leaflets 68.6±20.2 × 27.5±14.9 mm (legth × width); length of inflorescences 103.3±43.3 mm, standard 15.7±2.8 mm and keels 14.2±0.7 mm.

Due to its wide distribution, this taxon is also the most morphologically variable. Previous authors have described many taxa and varieties within it in an attempt to deal with this morphological variation. However, these morphological variations generally are not geographically consistent and regard only vegetative attributes, usually habit, pubescence and shape of leaves. According to several authors, subspecies or varieties to be valid should possess geographically coherent morphological variations which are more than simple variations in vegetative traits (Pennington 1969, Bennett et al. 2008). Otherwise, it is more appropriate to accept only a single widely polymorphic taxon, as we did here. Despite being largely plastic and bear most of the traits overlapping with those of other lineages, this taxon can be considered reproductively consistent, because the size of keel pieces are quite conservative within it (low standard deviation) and significantly different from other species (Kruskal-Wallis test, pair-wise comparisons, *G. neesii* vs. *G. australis*, $P = 0.04$; *G. neesii* vs. *G. lineata*, $P = 0.005$).

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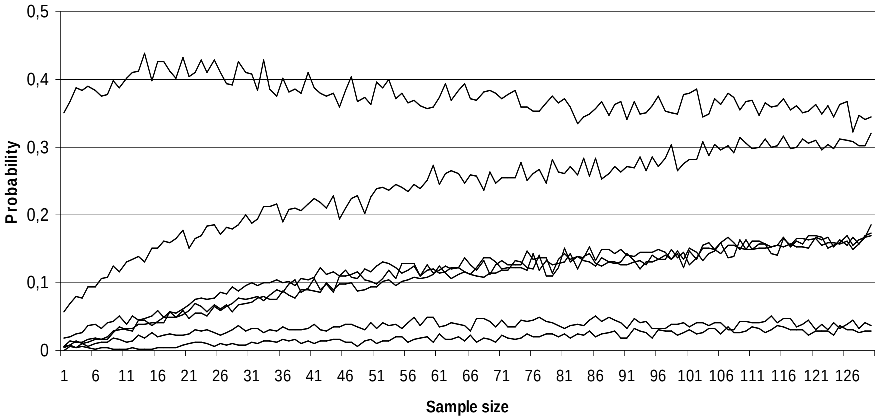

Figure 1. Stability of the probabilities of partition depending on the sample size. The curves show the successive partition hypothesis into groups, from 2 (uppermost curve) to 8 groups (lowest curve). The hypothesis of partition into 2 and 3 groups are the best supported even with small sample sizes (< 10 specimens), because the curves stabilized early above the threshold of sharpness.

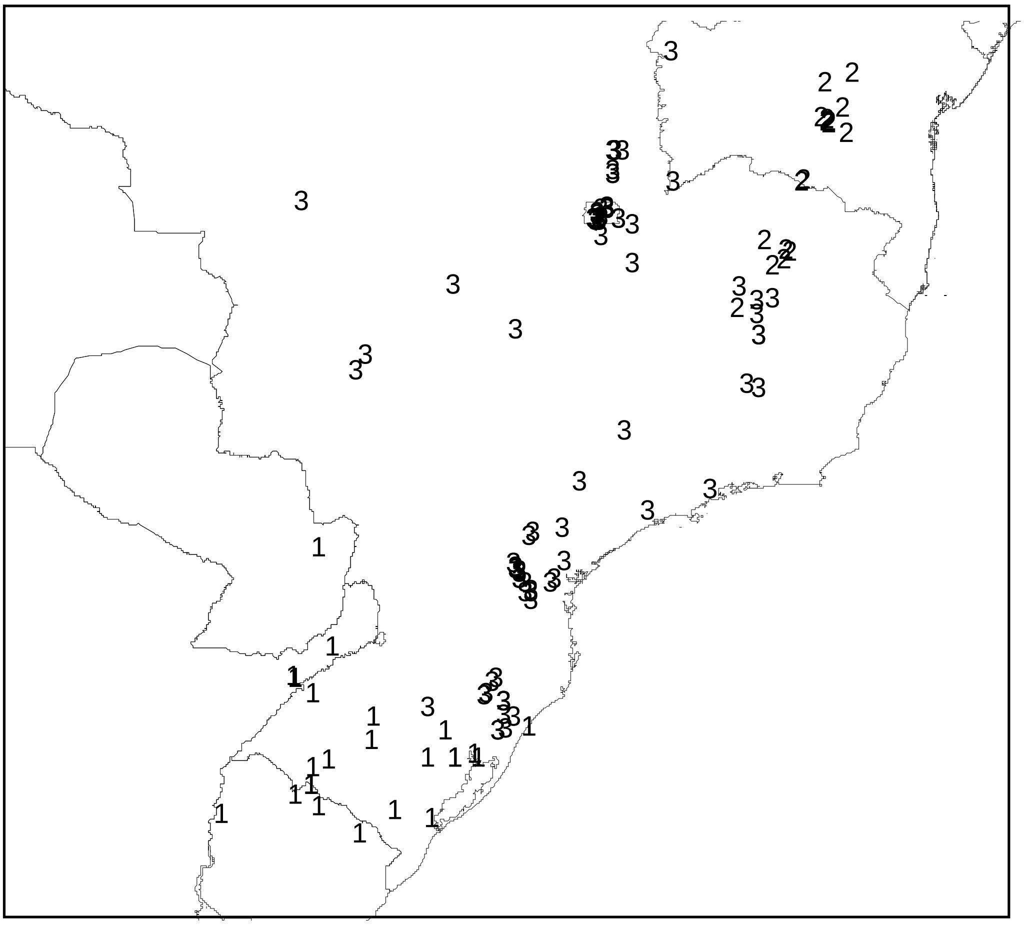

Figure 2. Map showing the distribution of the clusters in South America.

Table 1. List of the quantitative attributes measured in specimens of *G. neesii* complex. The sign '*' indicates that attributes whose means were different among cluster and were used to NPMANOVA

Attributes (mm)
Petiole*
Middle Leaflet (length* and width*)
Stipules*
Stipels*
Inflorescence*
Pedicels*
Calix (upper, lower* and lateral lobe)
Standard (length* and width)
Wing (length and width)
Keel (length* and width)
Bract
Bracteole*

Table 2. Non-parametric MANOVA testing for differences in morphological traits of the clusters 1, 2 and 3 (Bonferroni correction; 10,000 permutations; Euclidean distance).

	Cluster 1	Cluster 2	Cluster 3
Cluster 1	_		
Cluster 2	**0.006**	_	
Cluster 3	**0.003**	**0.03**	_

Table 3. Summary of altitude (m) and precipitation (mm) parameters where the populations of the clusters grow.

	Altitude			**Precipitation**		
	Cluster 1	*Cluster 2*	*Cluster 3*	*Cluster 1*	*Cluster 2*	*Cluster 3*
Min	6	542	399	1230	655	1212
Max	439	1320	1320	1761	1100	2100
Mean	152.115	972.278	964.54	1467.85	940.278	1560.92
SD	116.725	203.449	181.183	144.377	107.46	196.711

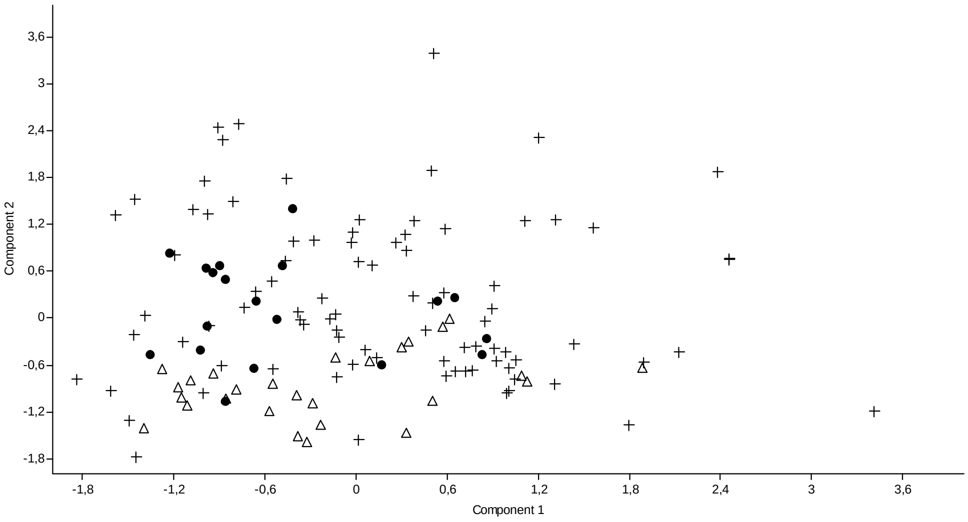

Figure 3. PCA plot summarizing morphologic variation within the clusters 1 (triangle), cluster 2 (solid circle) e cluster 3 (cross).

Checking the status of infraspecific taxa within *Galactia jussiaeana* (Fabaceae) using Berg's correlation-pleiades hypothesis

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Abstract

Berg's correlation-pleiades hypothesis proposes that phenotypic variation between plant vegetative and reproductive traits is decoupled (i.e. varies independently) due to different selective pressures: pollinators in reproductive traits and environment in vegetative traits. We used this hypothesis to check if the intraspecific leaf variation in *Galactia jussiaeana* is accompanied by an equal variation in floral traits. If so, we might be facing a process of divergence between the two lineages, since differences in floral morphology could restrict gene flow due to a mismatch with pollinators. Our results showed that there was no correlation between the variation in vegetative and reproductive traits in *G. jussiaeana* var. *angustifolia*, demonstrating that both are genetically uncoupled and respond to different selective pressures. There were also no significant differences in reproductive structures between the alternative and the typical variety. For these reasons, we propose the synonymization of *G. jussiaeana* var. *angustifolia* under *G. jussiaeana*.

Key words: alpha taxonomy, floral traits, variety, subspecies, Leguminosae, taxonomic theory.

Introduction

Species are the basic units for biogeographic, ecological, evolutionary, and conservation studies; thus, methods for delimiting species are necessary to achieve greater rigor and objectivity in scientific research. Some attempts to delimit species have achieved relative success, such as the use of small sequences of mitochondrial DNA for animals (DNA Barcode; Hebert *et al.* 2004). There is not yet a sequence working as a DNA barcode for all plant lineages, although several candidates have been tested (Cowan *et al.* 2006; Hollingsworth *et al.* 2009). Despite efforts to discover these DNA barcode sequences, morphology is still the main source of characters for description and recognition of plant species; hence, the discovery of methods to employ in morphological taxonomy must be objective and robust.

Some authors point out that taxonomy is a hypothesis-driven science (Henderson 2005), so an explicit methodology to test hypotheses should be applied. Currently, the method most used to test differences among groups of individuals is multivariate analysis (e.g. cluster analysis, PCA, MANOVA; Henderson 2006; Pedersen 2010). These tests quantify the amount of trait similarities and differences for clustering or splitting the individuals into two or more species. Yet how much "difference" is necessary to recognize two groups of individuals as different species? What kind of traits we should consider? How could we recognize subspecies or varieties?

According to de Queiroz (2005), species are metapopulation lineages (or segments of) evolving separately. These lineages are joined together by gene flow in organisms with sexual reproduction (Templeton 1989); thus, one lineage only evolves separately from another one when gene flow is either very low or near zero. This reproductive isolation is either prezygotic, which impedes mating or fertilization, or postzygotic, which acts against the hybrid offspring; in plants, however, the first kind of barrier contributes to isolation more than the second one (Rieseberg & Willis 2007). The most important early-acting mechanisms of reproductive isolation are prepollination barriers, which can be achieved by pollinator isolation (Rieseberg & Willis 2007). Therefore, one way to recognize separately evolving lineages is to focus on attributes that prevent pollination among groups but allow pollination within groups.

Another point to consider is the level of plasticity in the attribute, since the most desired characteristic in a putative attribute for a taxonomic key is constancy along the entire range of a taxon. We could conclude from this reasoning that not just any kind of attributes serve to infer specific boundaries among groups of individuals. For instance, if we are trying to infer reproductive isolation based on morphological traits, we cannot choose any sort of trait; we must use traits that (presumably) influence reproductive isolation and show low plasticity. It is typical for vegetative traits to vary widely along the geographical distribution of a species because they respond more strongly to environmental heterogeneity among sites (Sultan & Bazzaz 1993). By contrast, reproductive traits have to be conservative within taxa in order to guarantee the integrity of reproduction within groups and maintain isolation from other groups. Therefore, reproductive traits are thought to be more canalized (Brock & Weinig 2007) and independent of changes in environment and in other characters (Fenster & Galloway 1997). If floral traits closely accompanied variation in vegetative traits, there would probably be a disruption in the reproductive function of widely dispersed species (Conner & Via 1993).

Berg (1960) proposed that vegetative and reproductive traits show independence in their variations in plants with specialized pollination systems. She hypothesized that plants whose floral dimensions fit the size of their pollinators are more effectively pollinated because they reduce the chance of pollen being deposited in inappropriate places on pollinators (e.g. parts that would not touch the stigma of another flower). Individuals whose floral variation is tightly linked to the wide variation exhibited by leaves would suffer an overall decrease in fitness, since an increased number of flowers with unusual shapes complicate effective pollination. This would lead to a stabilizing selection on flower size and a decoupled phenotypic variation between vegetative and reproductive traits (Conner & Sterling 1996; van Kleunen *et al.* 2008). Thus, plants with specialized pollination need to show a strong correlation among their reproductive characters, but not with vegetative ones. Berg called this pattern of covariation the correlation-pleiades hypothesis. Several studies have tested the hypothesis, with mostly positive results. Some studies fully confirmed the correlation-pleiades hypothesis (Conner & Via 1993; Conner & Sterling 1996). Armbruster *et al.* (1999), however, found that correlation patterns within and between floral and vegetative traits tend to be species-specific and usually more complicated than proposed by Berg (1960), and are not easily predicted from pollination ecology. Nevertheless, Berg’s hypothesis was not entirely rejected. Many of its predictions have been confirmed; for example, species with specialized pollination show lower variation for floral traits than for vegetative ones (Hansen et al. 2007; Meng *et al.* 2008).

Galactia jussiaeana Kunth (1824: 55) is an erect and trifoliolate subshrub, inhabiting open grasslands and savannas in northern South America, Central America, and some Caribbean Islands (Burkart 1971). It is easily recognizable by the occurrence of axillary and congested inflorescences, generally smaller than the subtended leaf. Due to its wide distribution, the species possesses great variability in leaf attributes such as size, shape, and hairiness. Kunth (1824) considered some individuals with narrow leaflets as part of another species, called *G. angustifolia* (1824: 56; from the Latin *angustus* = narrow; *folia* = leaf). In turn, Burkart (1971) reasoned that the narrowness of the leaflets could not support the ranking of species but only the status of variety. Therefore, *G. jussiaeana* now has two varieties: *G. jussiaeana* var. *jussiaeana* Kunth (wide leaflets, 13-30 mm) and *G. jussiaeana* var. *angustifolia* (Kunth) Burkart (1971: 714; narrow leaflets, 8-13 mm). *Galactia jussiaeana* var. *angustifolia* has a distribution apparently restricted to Venezuelan savannas and the Guyana Shield, but is completely within the range of the typical variety. The few studies on pollination of *G. jussiaeana* showed that it belongs to a group of small-flowered Faboideae which presented a non-specialized pollination and a great diversity of bee pollinators (Queiroz 1996). It is visited not only by small bees such as *Melipona quinquefasciata* Lepeletier (1836: 418), *Trigonisca* spp., *Partamona* spp., *Euglossa cordata* (Linnaeus) (1758: 575) and *Apis melifera* Linnaeus (1758: 576), but also by large bees such as *Bombus atratus* Franklin (1913: 118) and *Centris* sp. (Queiroz 1996; Lorenzon *et al.* 2003; Albuquerque *et al.* 2007).

Galactia jussiaeana has a somewhat generalized pollination and is widespread in Americas; consequently, one could expect, based on Berg's prediction, that its reproductive traits do not maintain morphological integrity along the entire range of species, being labile as the leaflets are. Thus, our aim was to check whether reproductive traits tightly accompanied the variation in vegetative traits. If correlation between variation in vegetative and reproductive traits does exist, the differences in floral morphology could restrict gene flow due to a mismatch with pollinators. These differences might explain why the two varieties maintain differences in leaf morphology even occurring in sympatry; thus, we might be facing a process of divergence between the two lineages. However, if there is no correlation between vegetative and reproductive traits, other factors may be responsible for differences in leaf morphology. For this reason, we checked for differences in the ecological requirements between the two taxa as well (e.g. precipitation, temperature, altitude, seasonality). Depending on the results, it could be necessary to change the taxonomic status of *G. jussiaeana* var. *angustifolia* by either resurrecting Kunth's proposal (*G. angustifolia*) or synonymizing the variety under *G. jussiaeana*.

Material and methods

For morphometric analysis, we evaluated specimens deposited at the BAB, NYBG, and UFMT herbaria (acronyms according to Thiers 2010). Our previous experience with the genus *Galactia* has shown that reduction in traits size from fresh to dry plants is not significant, so morphometric analyses based on herbarium material are suitable. We always selected the larger structure per individual to be sure that fully developed structures were sampled. Thus, we made only one measurement per individual. We used this methodology to decrease the number of missing data, because many specimens showed scarcity of flowers or lack of some floral pieces (especially standards and wings). We measured flowers under a stereomicroscope, using graph paper after rehydration and full expansion of the floral pieces (0.1 mm precision). We used a ruler to measure vegetative structures (1 mm precision). We discarded fruits and seeds because they are absent in most individuals. Altogether, we measured 16 complete individuals of *G. jussieaeana* var. *angustifolia* and 17 complete individuals of *G. jussiaeana* var. *jussiaeana.* We considered only the measures of central leaflet for further analysis, since the dimensions of lateral leaflets are strongly correlated with the central leaflet ($R^2 = 0.913$, $P < 0.001$). Tables 1 and 2 summarize which attributes were measured and to which class they belong (vegetative or reproductive).

First, we compared the means of traits to test for significant differences between taxa with a permutation *t*-test (5,000 iterations, $\alpha < 0.05$). This test is similar to the Student *t*-test and was chosen because some data (e.g. petiole length) were strongly asymmetric even after transformation, such as logarithmic and square root. Second, we calculated a correlation matrix (Tables 1 and 2) for all attributes in both taxa using a permuted Spearman correlation (10,000 iterations, $\alpha < 0.05$). Then, we extracted the mean correlation coefficient from isolated vegetative, isolated reproductive, and the interaction between vegetative and reproductive traits. We evaluated the heterogeneity between groups through one-way ANOVA with Tukey's HSD test for paired difference in means, since the correlation coefficient means passed the normality (Shapiro-Wilk's test) and homoscedasticity tests (Levene's test). Finally, we calculated the coefficients of variation (CV) for each attribute and compared the means of the log-transformed vegetative and reproductive traits through one-way ANOVA ($\alpha < 0.05$).

We used the software DIVA-GIS (Hijmans *et al.* 2005a) to map all analyzed individuals using geographic coordinates extracted either directly from herbarium labels or from gazetteers using the description of localities (Fig. 1). For each georeferenced point, we obtained the altitude and 19 environmental variables provided by the database WorldClim (Hijmans *et al.* 2005b). The WorldClim environmental variables are georeferenced layers with biologically relevant information of rainfall and temperature. The altitudes and bioclimatic data for each item were standardized to zero mean and normalized to unitary standard deviation due to differences in units (e.g. ºC, m, mm). We utilized Principal Component Analysis (PCA) to extract the environmental variables that best summarized heterogeneity in the distribution of individuals. We used the Jolliffe cut-off value as an indicator of how many axes were significant (Jolliffe 1986). We then extracted and used the values of the most significant axes in summarizing the environmental variation as latent variables. We ran a second PCA with the morphological data and extracted the values of the first significant axes for use as latent variables as well. We utilized morphological (dependent) and environmental (independent) latent variables in multiple regressions to assess whether it was possible to explain the morphological heterogeneity between taxa through climate variables. Because PCA is not a method for testing hypotheses, we used a two-group permutation test to check for possible differences in ecological requirements between the two taxa. This test uses Mahalanobis distance and tests the equality of means by permutation (5,000 iterations). It is an alternative to standard discriminant analysis, in which Hotelling's T-squared, a multivariate analogue of the t test, is utilized to test the equality of means between two groups. The two-group permutation test is useful as an alternative to the Hotelling test when the assumptions of multivariate normality and equal covariance matrices are violated. We performed all tests in PAST v. 2.00 (Hammer et al. 2001).

Results

The results of *t* tests showed no significant differences in vegetative and floral characters between the two taxa, except length (P < 0.05) and width of leaflets (P < 0.001). In Table 3, we summarize the results of correlation analyses and their associated ANOVA tests. For *G. jussiaeana* var. *angustifolia*, the mean coefficients of correlation (mcc) were very low both in vegetative traits (mcc = -0.06) and interclass (mcc = 0.08), while the reproductive attributes presented moderate correlation (mcc = 0.27). ANOVA with Tukey’s pairwise comparison showed that the mcc of reproductive traits was different from vegetative and interclass mcc, which, in turn, were not different from each other. For *G. jussiaeana* var. *jussiaeana*, we found that the correlation among reproductive traits was slightly higher than *G. jussiaeana* var. *angustifolia* (mc = 0.29); in addition, there was a very low interclass correlation (mcc = -0.05), and the mcc of vegetative traits was greater than in the alternative variety (mcc = 0.12). However, ANOVA and Tukey’s tests showed that all of the mcc were different from each other in *G. jussiaeana* var. *jussiaeana*. Furthermore, although *G. jussiaeana* var. *jussiaeana* showed a higher mmc in vegetative traits compared with *G. jussiaeana* var. *angustifolia*, this difference was not significant (*t*-test, *P* = 0.17).

The mean CVs for vegetative traits (0.32) in *G. jussiaeana* var. *jussiaeana* were higher than for floral traits (0.14), and this difference was significant (One-Way ANOVA, $F_{1,11}$ = 13.03, *P* = 0.004). For *G. jussiaeana* var. *angustifolia*, we obtained similar results (CV vegetative = 0.25; CV floral = 0.12; One-Way ANOVA, $F_{1,11}$ = 30.5, *P* < 0.001).

The first two PCA axes with environmental variables were significant (Jolliffe cut-off value > 0.7) and accounted for approximately 65% of the variation in data (40.09% in the first axis and 24.94% in the second axis). The two gradients shown by PCA were related to temperature (axis 1) and precipitation (axis 2). The PCA with morphological variables also returned two significant axes: the first axis was largely dominated by the width of leaflets (91% correlation), and the second axis by the length of leaflets (84% correlation), confirming the *t*-test results. In spite of the environmental PCA showing that there was an environmental gradient dominated by temperature and precipitation, the two-group permutation test showed no differences in ecological requirements between the two taxa ($P = 0.411$). This result to some extent was reflected by the multiple regressions, because the tests with environmental axes 1 and 2 (independent variable) and morphological axes 1 and 2 (dependent variables) explained neither the variation in the width of leaflets (morphological PCA, axis 1, adjusted $R^2 = -0.05$, $F = 0.18$, $P = 0.83$) nor in the length of leaflets (morphological PCA, axis 2, adjusted $R^2 = -0.01$, $F = 0.71$, $P = 0.49$). Thus, other factors may be responsible for differences in leaf traits variability.

Discussion

Our results showed that the only traits that differed significantly between the two taxa were the length and width of leaflets. We also found that variation in leaf traits of *G. jussiaeana* var. *angustifolia* were not accompanied by parallel variation in floral traits. Furthermore, the low mean correlation coefficient of vegetative traits showed that these characters were independent of each other, whereas floral traits showed a higher mean correlation coefficient, indicating greater integration among floral pieces. The coefficients of variation pointed to the same conclusions, since they were significantly lower for reproductive traits than for vegetative ones in both taxa. These results show that vegetative traits are more plastic than reproductive ones, supporting Berg's hypothesis that vegetative and reproductive traits are uncoupled, i.e. belong to different modules of the plant and respond to different selective agents (Berg 1960; Pérez-Barrales *et al.* 2009). In addition, we did not find significant variation in ecological requirements between the taxa, thus showing that they share the same niche. These results have direct consequences on the acceptance of infraspecific ranks within *G. jussiaeana*.

Currently, we face two contrasting situations regarding the species concept discussion. On the one hand, there are almost no divergences among taxonomists on the species concept per se, due to increasing use of the Unified Species Concept (De Queiroz 2005; Leaché *et al.* 2009). On the other hand, the use of taxa below the species rank is still controversial and far from a consensus practice. Subspecies and varieties are usually defined as subsets of a species with ecological, geographical, and (presumably) phylogenetic integrity (Cron *et al.* 2007). While the use of infraspecific ranks is important in order to recognize useful variations for conservation, they are often used without clear criteria. Hamilton & Reichard (1992) found that the variety ranking is often defined without any additional extra-morphological criteria (e.g. geographical or ecological distinctness) and superimposed with the ranking subspecies. Luckow (1995) and later Henderson (2005), under the Phylogenetic Species Concept, propose to use the subspecific ranking in lineages that differ in means of quantitative traits and are associated with geographic or ecological cohesion. Pennington (1969) did not recognize any infraspecific taxa within *Vavaea amicorum* Benth. (1843: 212; Meliaceae), primarily because different variants showed no geographical correlation, occupied the same habitat, and were recognized from single variations in vegetative traits. An identical position was taken by Bennett *et al.* (2008) upon recognizing just a single polymorphic species in *Strobilanthes echinata* Nees (1832: 85; Acanthaceae), reducing 19 taxa to synonymy. Clearly, there has been a growing trend among authors to designate infraspecific rankings only when morphological variation in taxa is followed by structural changes on other extra-morphological features.

For *G. jussiaeana*, we found that the differences between the taxa are related only to vegetative traits and that geographic structure and environmental requirements between them are not different either. Therefore, it is more parsimonious to consider *G. jussiaeana* as a polymorphic species instead of splitting it into two varieties. Nevertheless, why do variations in leaf traits persist even with apparently no barriers to gene flow among individuals?

Phenotypic plasticity is attributable to two main causes: (a) natural selection by abiotic and/or biotic factors, and (b) stochastic events such as genetic drift and founder effect (Chalcoff *et al.* 2008). Givnish (1987) points out that the width of leaves tends to decrease as irradiance and altitude increase and as precipitation and soil fertility decrease. In our work, neither altitude nor rainfall explained stenophylly of the alternative variety within *G. jussiaeana*. Irradiance is a difficult parameter to infer based on herbaria material because the information contained in exsicatae labels did not establish adequate accuracy concerning lighting conditions where individuals are growing. Thus, any attempt to deduce environmental conditions based on exsicatae labels can be highly biased. However, it is unlikely that irradiance plays a significant role in stenophylly in some specimens, since for other biomes with high irradiance such as Caatinga (Northeast Brazilian semi-arid vegetation) and Cerrado (Brazilian savanna), there are no records of plants with narrow leaflets (Lewis 1987; Queiroz 2009).

Another explanation for stenophylly could be low soil fertility, since the Venezuelan savannas where narrow-leafed individuals typically grow present acidic and low fertility soils with phosphorus (P) deficiency and high concentrations of aluminium (Al; Izaguirre-Mayoral et al. 2002; Mora & Toro 2007). However, the distribution map of varieties (Fig. 1) does not show a large separation between the taxa, showing that they grow very close and theoretically share the same soil conditions. Furthermore, the hypothesis of stenophylly caused by low fertile soils does not explain again the lack of records of the alternative variety in the Brazilian Cerrado, where P deficiency and high concentrations of Al are also present (Goodland & Pollard 1973). In short, the environmental factors tested in our study were not able to explain the leaf dimorphism found within *G. jussiaeana*. Thus, genetic drift or founder effect could be invoked as possible processes responsible for leaf dimorphism within this species, but the data we have collected in this study do not allow us to go further and explain how stenophylly has appeared and remained. Nevertheless, there are some processes that could explain the floral conservatism in *G. jussiaeana*.

Berg's (1960) hypothesis predicted that floral traits should show little variation because pollination would be more effective if the size of the flowers fits the size of pollinators. Thus, selection would act against large variation in floral traits among individuals, favouring integration of different floral traits within the individual. However, this selection would be stronger in plants with specialized pollination systems than in plants with generalist systems. *Galactia jussiaeana* is thought to possess a strictly non-specialized pollination system because it is visited both by large and small bees (Queiroz 1996); this feature is a general trend in small-flowered Faboideae plants (Herrera 2001). Viewed from Berg's assumption, one would expect that reproductive traits in *G. jussiaeana* would be more labile in their morphology than the results we have found.

A possible explanation for this apparent fault in Berg's hypothesis would be the conservatism of the floral morphology in Faboideae species. Some evidence indicates that the characteristic pattern of symmetry in papilionaceous flowers results from a surprisingly uniform ontogeny (Tucker 2003). Thus, developmental constraints *per se* could be an explanation of the low variation in flowers of *G. jussiaeana*. Yet in analyzing the question from a phylogenetic perspective, we found that there are several examples of drastic variation in floral morphology driven by pollinator pressure in closely related taxa. For instance, all of the phylogenetic studies performed in subtribe Diocleinae (where *Galactia* is placed) showed that several shifts in pollination syndromes occurred along the subtribe, either from bees to hummingbirds or vice-versa, even in species within the same genus (Queiroz et al. 2003; Queiroz, 2008; Sede et al. 2008, 2009). These results indicate that the pressure exerted by pollinators is strong enough to surpass ontogenetic conservatism and promote pollination shifts in paplionaceous flowers, even those involving coordinated changes in several attributes as is required in ornithophily (e.g. flower color, depth of corolla, volume of nectar). Several studies have demonstrated that shifts in size of pollinators are often accompanied by differences in size of flowers (Johnson & Steiner 2000; Herrera 2005), but we found that the geographic distribution of all bees which visit *G. jussiaeana* encompasses the entire geographic distribution of the species (Camargo & Pedro 1992; Pedro & Camargo 2003; Camargo & Pedro 2008; Natural History Museum *Bombus* Database 2010). Therefore, we hypothesize that the uniformity in flowers of *G. jussiaeana* might be attributable to pollinator pressures (or, in this case, the lack thereof). Thus, even though this species is generalist, the constancy in pollinators helps to maintain the integrity of its reproductive system along its entire distribution.

Since Berg (1960) first proposed her hypothesis, numerous studies have tested it and have either fully (e.g. Conner & Sterling 1996; van Kleunen *et al.* 2008) or partially corroborated its assumptions (e.g. Armbruster *et al.* 1999; Hansen *et al.* 2007), but have never totally refuted it. Thus, Berg's hypothesis is useful to check if variations in vegetative traits are coupled with parallel variation in reproductive traits, or if the variations in these traits are independent of one another. In the first case, the coordinated variation between these two sets of traits may reflect a process of lineage divergence and reproductive isolation. In the second case, the unparalleled variation between traits may reflect plastic responses of vegetative modules of the plant to a given environmental condition without any consequence to the species' reproductive integrity. Checking for independence in variation between the different modules of a plant is especially useful to test for reproductive integrity in widely dispersed species, which are prone to show morphological variation along their geographical range. The use of this approach to search for coupled divergences in floral and leaf morphology could be a reliable and robust methodology to test if two lineages are in a process of speciation or are in fact two already established species. In this paper, we used this approach to check the consistency of infraspecific division within *G. jussiaeana* and concluded that it is a polymorphic species instead of two taxa. We provide below the new proposed taxonomic status and a short description of *G. jussiaeana* including the new range of leaflet sizes.

Taxonomic treatment

Galactia jussiaeana Kunth (1824: 55). Type: Bonpland 853 (P).
= *Galactia jussiaeana* var. *angustifolia* (Kunth) Burkart (1971: 714). Type: von Humboldt (type photo F 2384!).

≡ *Galactia angustifolia* Kunth (1824: 56).

≡ Collaea angustifolia (Kunth) Bentham (1837: 65).

= *Galactia camporum* Sprague (1905: 430). Type: Sprague 166 (NYBG, GH, US).

SUBSHRUB erect, branched, 0.2–1 m, pubescent, STIPULES and STIPELS 1–3 mm, PETIOLE 7–46 mm, LEAVES pinnate-trifoliolate, LEAFLETS chartaceous to coriaceous, 30–73 mm length × 5–60 mm width, elliptical, oval to narrow-oblong, pilose, RACEME smaller or the same length of the subtended leaf, 11–45 mm long, densiflorous, with flowers along all the axis, FLOWERS pink to lilac, 8–16 mm, BRACTS and BRACTEOLES 1–3 mm.

Acknowledgements

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Table 1: Correlation table of *G. jussiaeana* var. *angustifolia*. Vegetative traits: Pet: petiole length; LLe: leaf length; WLe: leaf width; Sti: stipule length; Spe: stipele length; Inf: inflorescence length. Reproductive traits: Ped: pedicel length; LCal: calyx length; LSt: standard length; LWi: wing length; LKe: keel length; Br: bracteole length. *: P<0.05; **: P<0.01; ***: P<0.001.

Table 2: Correlation table of *G. jussiaeana* var. *jussiaeana*. Vegetative traits: Pet: petiole length; LLe: leaf length; WLe: leaf width; Sti: stipule length; Spe: stipele length; Inf: inflorescence length. Reproductive traits: Ped: pedicel length; LCal: calyx length; LSt: standard length; LWi: wing length; LKe: keel length; Br: bracteole length. *: P<0.05; **: P<0.01; ***: P<0.001.

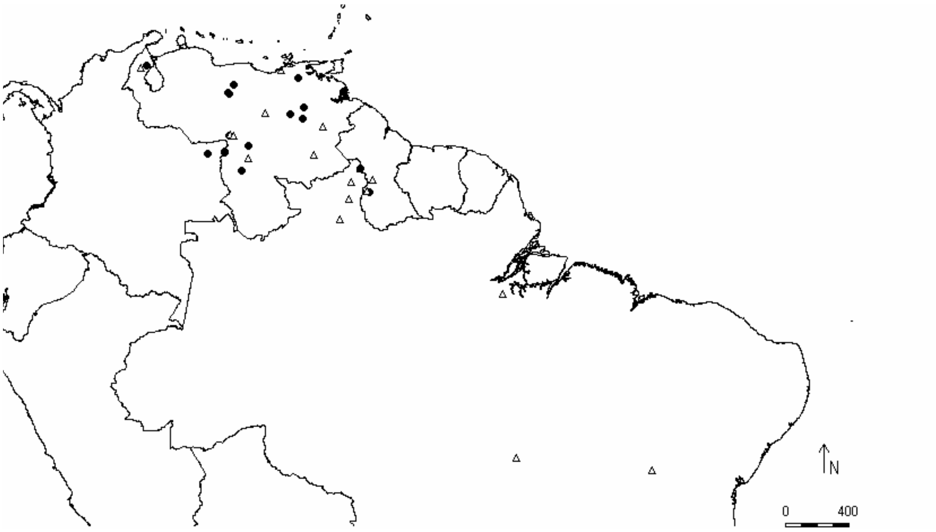
Table 3: Mean correlation coefficients values for isolated vegetative (V), isolated reproductive (R), and the interaction between vegetative and reproductive traits (V × R) in the two taxa. Overall heterogeneity was tested with ANOVA and differences between pairs were tested with post-hoc Tukey's HSD test (superscripts). Means with different letters differs at $P < 0.05$. The numbers between brackets indicate sample size.

Figure 1: Distributional map of the taxa within *Galactia jussiaeana*. Open triangles: *G. jussiaeana* var. *jussiaeana*. Solid circles: *G. jussiaeana* var. *angustifolia*.

	Pet	**LL**	**WL**	**Sti**	**Spe**	**Inf**	**Ped**	**LCal**	**LSt**	**Lwi**	**Lke**	**Br**
Pet	1											
LL	0.18	1										
WL	0.43	-0.14	1									
Sti	0.00	-0.51	-0.15	1								
Spe	-0.23	0.20	-0.16	0.14	1							
Inf	-0.24	-0.17	0.14	-0.26	-0.18	1						
Ped	0.06	0.18	-0.19	0.00	-0.08	0.16	1					
LCal	0.38	-0.20	0.25	0.16	-0.19	0.36	0.25	1				
LSt	0.31	-0.47	0.22	0.42	-0.05	-0.17	0.12	0.62*	1			
Lwi	0.37	-0.13	0.38	0.15	-0.16	0.04	-0.08	0.72**	0.73***	1		
Lke	0.26	-0.14	0.27	0.58*	-0.15	-0.32	0.00	0.41	0.69**	0.72***	1	
Br	0.32	0.08	0.38	-0.37	-0.16	0.35	0.00	0.23	-0.11	0.02	-0.21	1

	Pet	**LLe**	**WLe**	**Sti**	**Spe**	**Inf**	**Ped**	**LCal**	**LSt**	**LWi**	**LKe**	**Br**
Pet	1											
LL	0.48	1										
WL	0.46	0.19	1									
Sti	0.10	-0.07	0.16	1								
Spe	0.18	0.41	-0.14	-0.33	1							
Inf	-0.05	-0.12	0.46	0.42	-0.28	1						
Ped	0.05	0.34	-0.16	-0.07	0.04	-0.35	1					
LCal	-0.10	-0.15	-0.23	0.09	-0.45	-0.07	0.43	1				
LSt	-0.02	0.31	-0.12	-0.24	-0.06	0.00	0.45	0.35	1			
Lwi	-0.20	0.04	-0.24	0.00	0.07	-0.08	0.28	0.29	0.43	1		
Lke	-0.49 *	-0.15	-0.43	-0.14	0.03	0.08	0.21	0.16	0.55 *	0.76 ***	1	
Br	0.28	0.11	0.06	0.08	0.00	0.18	0.14	-0.08	0.36	-0.015	0.18	1

	Correlation			
	V	**R**	**V × R**	***P* ANOVA**
G. j. angustifolia	-0.06^{a}	0.27^{b}	0.08^{a}	0.005
	(15)	(15)	(36)	
G. j. jussiaeana	0.12^{a}	0.29^{b}	-0.05^{c}	> 0.001
	(15)	(15)	(36)	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Capítulo de Livro – *Leguminosas do Rio Grande do Sul*

Galactia P. Browne

BURKART. A. 1971. El género *Galactia* (Legum-Phaseoleae) en Sudamérica con especial referencia a la Argentina y paises vecinos. *Darwiniana 16:* 663-796.

Ervas a **subarbustos** prostrados, decumbentes, volúveis a eretos. **Folhas** digitado-trifolioladas, pinado-trifolioladas ou unifolioladas, pecioladas ou sésseis. **Estípulas** e **estipelas** geralmente presentes. **Pseudorpseudorracemos** nodosos. **Brácteas** e **bractéolas** pequenas. **Cálice** 4-laciniado, campanulado, pubescente. **Corola** violácea, azulada, púrpura a rosada. **Estandarte** obovado a orbicular, glabro ou pubescente no ápice. **Alas** e **peças da carena** glabras. **Androceu** diadelfo a pseudomonadelfo. **Legume** séssil a raramente estipitado, mesmo assim com estípite breve, deiscência elástica, plurisseminado, com tecido celulósico entre as sementes (falsos septos).

O gênero é pantropical com 55-60 espécies, destas aproximadamente 30 ocorrem no Brasil primariamente em áreas abertas, sendo comum em savanas, pradarias e campos rupestres e raro em florestas. No Rio Grande do Sul ocorrem 11 táxons.

A taxonomia do grupo é bastante confusa devido à ampla variabilidade morfológica e ao relativamente pouco número de caracteres diagnósticos, havendo divergências quanto à identidade de algumas espécies dentro de complexos taxonômicos. Além disso, as filogenias prévias do grupo baseadas em AFLP (Sede *et al*. 2008) e nos marcadores ITS, *trn*L-F e morfologia (Sede *et al.* 2009), não resolveram as relações de parentesco entre *Galactia* e o gênero próximo *Camptosema*, apontando os mesmos como não-monofiléticos. Portanto, alguns táxons considerados neste trabalho podem futuramente sofrer modificações na sua circunscrição, à medida que novos estudos forem sendo finalizados e novas evidências taxonômicas surgirem.

Chave para as espécies de *Galactia* do Rio Grande do Sul

1. Folhas unifolioladas.. 2
    2. Inflorescências paucifloras, com 1-3 flores por pedúnculo... 3
        3. Folhas com pecíolo... 7. *G. marginalis*
        3’. Folhas sem pecíolo.. 10. *G. pretiosa*
    2’. Inflorescências plurifloras, com mais de 4 flores por pedúnculo.. 4

4. Pecíolos (8)11-62(80) mm de comprimento; plantas prostradas, geralmente volúveis.. 2. *G. benthamiana*

4’. Pecíolos até 7 mm de comprimento; plantas prostradas a semieretas, nunca volúveis.. 5

5. Estandarte 13-15 mm de comprimento; inflorescência subumbeliforme, congestiflora.. 4. *G. dimorphophylla (indivíduos jovens)*

5’. Estandarte 15-20 mm de comprimento; inflorescência com flores da metade para o ápice da ráquis, laxiflora.. 3. *G. boavista*

1’. Folhas trifolioladas. 6

6. Folhas do ápice da planta sempre pinado-trifolioladas, com ráquis foliar conspícua, maior que 5 mm de comprimento................. 7

7. Todas as folhas sempre pinado-trifolioladas; folíolos membranáceos, ligeiramente pubescentes.. 11. *G. striata*

7’. Folhas sempre pinado-trifolioladas no ápice e geralmente unifolioladas na base da planta; folíolos coriáceos a cartáceos, tomentosos.. 8

8. Estandarte 13-15 mm e pecíolo 2-7 mm comprimento..... 4. *G. dimorphophylla*

8’. Estandarte 10-12 mm e pecíolo 10-22 mm comprimento......... 6. *G. latisiliqua*

6’. Folhas digitado-trifolioladas, com ráquis foliar curta, menor que 5 mm de comprimento..... 9

9. Plantas prostradas a decumbentes, nunca volúveis.. 10

10. Folíolos largo-elípticos, orbiculares a sub-orbiculares, ápice arredondado; peças da quilha 10–13 mm, habita o bioma Pampa......................... 1. *G. australis*

10’. Folíolos elípticos, ápice agudo; peças da quilha 14–18 mm; habita os Campos da Cima da Serra .... 9. *G. neesii*

9’. Plantas volúveis... 11

11. Inflorescências sésseis.. 5. *G. gracillima*

11’. Inflorescências pedunculadas.. 6. *G. martioides*

1. *Galactia australis* (Malme) Ceolin & Miotto, Pl Syst Evol xx. Xxxx (submetido)

Subarbustos prostrados a ascendentes, não volúveis, até 0.8 m de comprimento. **Folhas** digitado-trifolioladas, às vezes com ráquis breve até 5 mm de comprimento. **Pecíolo** (29)33-76 mm de comprimento, pubescente. **Folíolos** frequentemente conduplicados e pêndulos, coriáceos, (15)30-65 x (15)25-40 mm, largo-elípticos, orbiculares a suborbiculares; ápice arredondado a obtuso; base arredondada a atenuada. **Estípulas** 4-8 mm de comprimento, lanceoladas, pubescentes. **Estipelas** 1,5-6 mm de comprimento, subuladas, finamente pubescentes. **Pseudorracemos** axilares, subcapituliformes, plurifloros. **Brácteas** 1,5-5 mm de comprimento, subuladas, tomentosas. **Bractéolas** 2-5 mm de comprimento, lanceoladas, pubescentes. **Cálice** 6-11(13) mm comprimento, tomentoso. **Corola** púrpura, lilás a rosada, glabra a esparsamente pubescente no ápice. **Estandarte** orbicular, 12-16(18) x 9-16 mm. **Alas** 12-16(18) x 4-6(8) mm. **Peças da carena** 10-13 x 5-7 mm. **Legumes** (20)25-46 x 5-8 mm, pubescentes. **Sementes** oblíquas, ovóides, 3-5 mm de diâmetro, castanhas ou marmoreadas com manchas negras.

Material selecionado: Porto Alegre, *R. Setubal & A. Mello 171*, 02/XI/2005, fl. (ICN).

Distribuição geográfica: Argentina, Uruguai, Paraguai, Brasil: RS, ocorrendo no bioma Pampa.

Floração e frutificação: setembro a março.

Observações ecológicas: habita campos em áreas planas ou barrancos, ocorrendo em solos geralmente pedregosos e rasos.

Comentários gerais: esta espécie é resultado da revisão taxonômica do complexo *G. neesii* (Ceolin & Miotto 2011), na qual foi proposto o reconhecimento de *G. australis* como uma espécie válida a partir de *G. neesii* var. *australis*.

2. *Galactia benthamiana* Mich., Symbol. Flor. Brasil Central, Videnskabel. Medelelser f. Naturhist. Forening. Kjöbenhavu 7: 81. 1875.

Ervas prostradas a volúveis, de 0,2-1 m de comprimento. **Folhas** unifolioladas e pecioladas. **Pecíolo** (8)11-62(80) mm de comprimento, pubescente. **Folíolos** ovados a elípticos, (36)40-112 x 14-34(46) mm, ápice arredondado a obtuso, base cordada a raramente arredondada. **Estípulas** 2-6 mm de comprimento, pubescentes. **Estipelas** na base do peciólulo, subuladas, pubescentes. **Pseudorracemos** pedunculados ou sésseis, com 2 a 3 flores por nó. **Brácteas** com 3-5 mm, lanceoladas a subuladas, pubescentes. **Bractéolas** lanceoladas a subuladas, pubescentes. **Cálice** 7-12 mm comprimento, tomentoso. **Corola** lilás a azulada, glabra, mais longa que o cálice. **Estandarte** 8 x 14-17 mm, orbicular a obovado, glabro. **Alas** 4-6 x (9)12-15 mm. **Peças da carena** 3-5 x (7)10-15 mm. **Legumes** (30)36-48 x 4-6 mm, tomentoso. **Sementes** ovóides, obliquamente dispostas, 2-4 mm diâmetro, castanhas, às vezes marmoreadas de negro.

Material selecionado: Santiago, RS-168 para Bossoroca, Km-18, 17/XII/2008, fr., *G.B. Ceolin 160* (ICN).

Distribuição geográfica: Argentina, Uruguai, Brasil: SC, PR, SP e MG. No RS, é frequente nas Missões e Campanha.

Floração e frutificação: de novembro a março.

Observações ecológicas: ocorre principalmente em solos pedregosos.

Comentários gerais: pouco abundante, podendo ser considerada de ocasional a rara; possui longas raízes, às vezes engrossadas.

3. *Galactia boavista* (Vell.) Burkart, Darwiniana 16: 783-788. 1971.

Subarbustos prostrados ou eretos, até 1,2 m de comprimento. **Folhas** unifolioladas, sésseis ou com pecíolo até 4 mm de comprimento. **Folíolos** (45)50-150(180) x 14-110 mm, ovados, obovados a elípticos, ápice obtuso a truncado, às vezes emarginado, base aguda a cuneada. **Estípulas** 2-6(8) mm de comprimento, pubescentes. **Estipelas** ausentes. **Pseudorracemos** eretos, com flores da metade para o ápice, plurifloro, laxifloro. **Brácteas** 2-5 mm de comprimento, lanceoladas, tomentosas. **Bractéolas** 2-5 mm, lanceoladas a subuladas, pubescentes. **Cálice** 35-45 mm de comprimento, tomentoso. **Corola** lilás, violácea a azulada, quase o dobro do tamanho do cálice. **Estandarte** 8-15 x 9-17 mm, orbicular a obovado, ápice às vezes pubescente. **Alas** 4-5 x 12-17 mm, glabras ou levemente pubescentes. **Peças da carena** 4-6 x 11-14 mm, glabras. **Legume** 20-50 x 4-8 mm, densamente tomentoso. **Sementes** arredondadas, ovóides a prismáticas, 4-5 mm de diâmetro, castanhas a avermelhadas.

Material selecionado: Cambará do Sul, 13/XII/1980, fl., *D. Falkenberg s.n.* (ICN59437).

Distribuição geográfica: Argentina, Brasil: SC, PR, SP, MG, MS. No RS, está restrita aos Campos de Cima da Serra.

Floração e frutificação: setembro a março.

Observações ecológicas: ocorre em campo, em solos rasos e pedregosos.

Comentários gerais: primeiro registro da espécie para o RS, pois até então, pensava-se que seu limite sul era Santa Catarina. Esta espécie apresenta xilopódio, que a torna resistente ao fogo.

4. *Galactia dimorphophylla* Fortunato, Sede & Luckow, Brittonia 60: 350. 2008.

Subarbustos até 0.5 m de altura. **Folhas** pinado-trifolioladas no ápice da planta, ráquis 3-6 mm de comprimento; unifolioladas no meio e na base; em indivíduos jovens normalmente todas unifolioladas. **Pecíolo** em ambos os tipos de folhas 2-7 mm comprimento, pubescentes. **Folíolos** 27-120 x 20-60 mm, coriáceos a cartáceos, elípticos a ovados, tomentosos, ápice obtuso a emarginado, base às vezes cordada. **Estípulas** 1-10 mm, lanceoladas, pubescentes. **Estipelas** 1-3 mm, setáceas. **Pseudorracemos** axilares, pedunculados, subumbeliformes, plurifloros, flores congestas. **Brácteas** e **bractéolas** 4-5 mm, lanceoladas a lineares, pilosas. **Cálice** 11-14 mm comprimento, tomentoso. **Corola** azulada a lilás. **Estandarte** 13-15 x 7-10 mm, ovado a obovado, glabro. **Alas** e **peças da carena** ambas

12-15 x 4-5 mm, glabras. **Legumes** 27-45 x 4-6 mm, velutinos. **Sementes** 3-4 mm diâmetro, subelipsoide a obovoide, marrom-avermelhadas a marrons.

Material selecionado: RS, Giruá, K. Hagelund (ICN).

Distribuição geográfica: Argentina, Uruguai e Brasil: no RS, na Campanha tanto no oeste (fronteira com a Argentina) quanto no sul (fronteira com o Uruguai).

Floração e frutificação: janeiro a abril.

Observações ecológicas: cresce nos campos, em barrancos e encostas rochosas.

Comentários gerais: esta espécie foi recentemente descrita para o nordeste da Argentina (Províncias de Corrientes e Misiones) e o norte do Uruguai (Departamentos de Tacuarembó e Rivera), na fronteira com o Rio Grande do Sul. Todavia, os autores da espécie comentaram que seria muito provável a ocorrência desta espécie no Rio Grande do Sul. Algumas exsicatas não identificadas depositadas no herbário ICN ajustam-se à descrição desta espécie; portanto, este é o primeiro registro desta espécie para o Brasil, confirmando a suspeita dos autores. A principal característica de *G. dimorphophylla* são as folhas trifolioladas e unifolioladas no mesmo indivíduo, sendo que nos indivíduos jovens todas as folhas são unifolioladas. Por isso, na chave acima, há duas entradas que chegam nesta espécie, dependendo de o indivíduo analisado apresentar um ou três folíolos.

5. *Galactia gracillima* Benth., Fl. Bras. 15 (1): 142. 1862.

Trepadeiras volúveis, atingindo de 0,5-1 m de comprimento. **Folhas** digitado-trifolioladas. **Pecíolos** (5)10-37(39) mm de comprimento, pubescentes. **Folíolos** sub-iguais, (9)11-70(73) x (1)2-5 mm, oblongo-lanceolados a lanceolados, ápice agudo a obtuso, curto-mucronado; base aguda a atenuada. **Estípulas** 1-5 mm de comprimento, lanceoladas a subuladas, pubescentes. **Estipelas** 1 mm de comprimento, subuladas, pubescentes. **Pseudorracemos** axilares, sésseis, 1-3 flores. **Brácteas** 1-2 mm de comprimento, subuladas, pubescentes. **Bractéolas** 1-3 mm de comprimento, subuladas, pubescentes. **Cálice** 6-9 mm comprimento, pubescente. **Corola** lilás ou rosada. **Estandarte** (9)10-16 x 4-10 mm, obovado a elíptico. **Alas** (7)8-13(15) x 3-4 mm, glabras. **Peças da carena** 8-13 x 2-3 mm, glabras. **Legume** 21-30(32) x 2-4 mm, tomentoso. **Sementes** 1,3-2,2 mm de diâmetro, globosas, assimétricas, negras e lisas.

Material selecionado: São Francisco de Assis, *E. Freitas 648,* 27/02/2009, fl. (ICN).

Distribuição geográfica: Argentina, Uruguai, Brasil: RS, SC, PR, SP, MG, MS, GO. No RS, ocorre nos Campos de Cima da Serra, na Campanha, nas Missões, na Serra do Sudeste e na Depressão Central.

Floração e frutificação: outubro a fevereiro.

Observações ecológicas: ocorre em campo, normalmente enrolando-se em gramíneas cespitosas ou entre touceiras de *Eryngium* spp.

Comentários gerais: muitos autores mencionam em suas descrições o caráter frágil da espécie, com ramos muito tenros e finos, inclusive utilizando este critério como diagnósticos em chaves de identificação. Porém, alguns indivíduos crescendo plenamente expostos ao sol, em solos rasos e pedregosos podem apresentar a base lenhosa e certo grau de crescimento secundário do caule. Além disso, podem ter folíolos bastante coriáceos, rígidos e pilosos. Portanto, recomenda-se cautela ao se usar a aparência frágil e tenra como critério de separação em chaves dicotômicas, pois este depende do ambiente em que o espécime encontra-se.

6. ***Galactia latisiliqua*** Desv., Annal. Scienc. Nat. 9: 414. 1826.

Subarbustos eretos a decumbentes, ramificado desde a base, com o ápice às vezes volúvel, até 1 m de altura. **Folhas** pinado-trifolioladas ráquis 7 mm de comprimento. **Pecíolo** (8)10-22 mm de comprimento, tomentoso. **Folíolos** (18)21-42 x 12-23(25) mm, coriáceos, com indumento velutino, elípticos a ovados; ápice emarginado a obtuso; base sub-cordada a cordada. **Estípulas** 2 mm de comprimento, lanceoladas, densamente pubescentes. **Estipelas** subuladas, pubescentes. **Pseudorracemos** axilares, pedunculado, flores dispostas em nós a partir da metade apical, 2 ou 3 por nó. **Brácteas** e **bractéolas** 2-3 mm de comprimento, pubescentes, lanceoladas. **Cálice** 7-9 mm comprimento, tomentoso. **Corola** violácea, glabra. **Estandarte** 10-12 x 8-9 mm, obovado. **Alas** 8-10 x 3-4 mm. **Peças da carena** 8-10 x 3-5 mm, Legume 25-38(42) x 4-6 mm, densamente pubescente. **Sementes** globosas, ovóides a achatadas lateralmente, inseridas obliquamente, 2-4 mm de diâmetro, amareladas, castanhas a avermelhadas, levemente marmoreadas de negro.

Material selecionado: entre Unistalda e São Borja, BR-287, km-447, 19/II/2009, fl., *M. Grings, 559* (ICN).

Distribuição geográfica: Espécie amplamente distribuída na América do Sul, desde as Guianas até o centro da Argentina e Uruguai. No Brasil, ocorre em praticamente todo o território nacional, porém na Região Sul é espécie bastante rara, não havendo registro para SC. Há poucas coletas para o RS, todas restritas às regiões de fronteira com a Argentina.

Floração e frutificação: setembro a fevereiro.

Observações ecológicas: habita solos secos e pedregosos

7. ***Galactia. marginalis*** Benth., De Legum. Gen. Annal. Mus. Vindob. 2 (2): 126. 1838.

Ervas a subarbustos não volúveis, eretos a decumbentes, simples ou ramificados, até 0,55 m altura. **Folhas** unifolioladas, glabras. **Pecíolo** até 10 mm comprimento. **Folíolos** não raro conduplicados, coriáceos, (17)25-115(130) x (3)5-15(17) mm, sub-orbiculares, elípticos, oblongos a lanceolados, ápice agudo a levemente truncado, base aguda, cuneada a decurrente. **Estípulas** 1-3 mm comprimento, lanceoladas a subuladas, pubescentes. **Estipelas** subuladas, pubescentes. **Pseudorracemos** axilares, sésseis a subsésseis, paucifloros, com 1-3 flores. **Brácteas** e **bractéolas** 2 mm de comprimento, lanceoladas, pubescentes. **Cálice** 6-7 mm comprimento, indumento viloso. **Corola** purpúrea, lilás, rosada a azul, glabra a esparsamente pubescente. **Estandarte** 11-13 x 6-8 mm, orbicular a obovado. **Alas** 10-13(15) x 2-5 mm. **Peças da carena** (10)11-13 x 2-5 mm. **Legumes** (22)25-42(44) x 4-6 mm, pubescentes. **Sementes** ovóides, assimétricas, 2-4 mm diâmetro, castanhas a negras, marmoreadas.

Material selecionado: Rio Pardo, 20/X/2007, fl.fr., *G.B. Ceolin 127* (ICN).

Distribuição geográfica: apresenta curiosa distribuição disjunta: sudoeste dos Estados Unidos e América do Sul, sendo encontrada na metade norte da Argentina e em todo território do Uruguai. No Brasil, ocorre no RS, quase que exclusivamente restrita ao bioma Pampa.

Floração e frutificação: entre outubro e fevereiro.

Observações ecológicas: habita solos secos e pedregosos, freqüentemente encontrada entre gramíneas cespitosas e *Eryngium* spp. (Apiaceae).

Comentários gerais: esta espécie pode formar grandes populações devido ao seu sistema de ramificação através de eixos plagiotrópicos subterrâneos. Além disso, por possuir xilopódio, é resistente a queimadas. Indivíduos desta espécie crescendo em locais sujeitos a pastejo e/ou fogo regular são muito diferentes quando comparados aos indivíduos que habitam áreas sem distúrbio; os primeiros sendo geralmente menores, prostrados, com folíolos quase orbiculares e coriáceos.

8. ***Galactia martioides*** Burkart, Darwiniana 16: 742. 1971.

Ervas volúveis, até 1 m comprimento. **Folhas** digitado-trifolioladas, pilosas. **Pecíolo** 18-51(55) mm comprimento. **Folíolos** geralmente conduplicados e pêndulos, coriáceos, (35)50-94 x (7)9-25 mm, estreito-oblongos a lanceolados, ápice agudo, base aguda a atenuada. **Estípulas** (2)5-9 mm comprimento, subuladas, pubescentes. **Estipelas** 2 mm, setáceas. **Pseudorracemos** axilares, pedunculados, subcapituliformes, plurifloros. **Brácteas** e **bractéolas** 5 mm de comprimento, lanceoladas, pubescentes. **Cálice** 10-17 mm de comprimento, tomentoso. **Corola** purpúrea a violácea, glabra. **Estandarte** 15-20 x 12-18 mm, obovado. **Alas** 15-18 x 6-9 mm. **Peças da carena** 15-18 x 6-9 mm. **Legumes** 35-40 x 4-5 mm, pubescentes. **Sementes** 2-3 mm diâmetro, castanhas.

Material selecionado: Itaara, Reserva do Ibicuí Mirim, 05/III/2009, fl.fr., *L.C.P. Lima & A. Pairet, 549* (ICN).

Distribuição geográfica: ocorre na Argentina e Brasil. No Brasil, até o momento, só há registro para o RS, nas Missões e Depressão Central.

Floração e frutificação: janeiro a abril.

Observações ecológicas: campos graminosos, em áreas de barranco.

Comentários gerais: apesar de bastante restrita, esta espécie forma grandes populações onde ocorre.

9. *Galactia neesii* DC., Prodromus Systematis Naturalis Regni Vegetabilis 2: 238. 1825.

Subarbustos prostrados a ascendentes, não volúveis, até 1 m de comprimento. **Folhas** digitado-trifolioladas, às vezes com ráquis breve até 5 mm de comprimento. **Pecíolo** (29)33-85 mm de comprimento, pubescente. **Folíolos** frequentemente conduplicados e pêndulos, cartáceos a coriáceos, (25)32-65 x (10)12-40 mm, elípticos; ápice agudo; base aguda a atenuada. **Estípulas** 2-8 mm de comprimento, lanceoladas, pubescentes. **Estipelas** 1-4 mm de comprimento, subuladas, finamente pubescentes. **Pseudorracemos** axilares, subcapituliformes, plurifloros. **Brácteas** 1,5-5 mm de comprimento, subuladas, tomentosas. **Bractéolas** 2-5 mm de comprimento, lanceoladas, pubescentes. **Cálice** 6-11(14) mm comprimento, tomentoso. **Corola** púrpura, lilás a rosada, glabra a esparsamente pubescente no ápice. **Estandarte** orbicular, 12-19(20) x 9-18 mm. **Alas** 12-18(20) x 4-6(8) mm. **Peças da carena** 14-16(18) x 5-8 mm. **Legumes** (20)25-46 x 5-7 mm, pubescentes. **Sementes** oblíquas, ovóides, 3-4 mm de diâmetro, castanhas ou marmoreadas com manchas negras.

Material selecionado: São Francisco de Paula, BR-116, 24/XI/2006, fl., *G.B. Ceolin & S.T.S. Miotto 122* (ICN).

Distribuição geográfica: Brasil: BA, DF GO, MG, MS, MT, SP, PR, SC. No RS, ocorre nos Campos de Cima da Serra.

Floração e frutificação: outubro a fevereiro.

Observações ecológicas: habita campos em áreas planas ou barrancos, ocorrendo em solos geralmente pedregosos e rasos.

Comentários gerais: esta espécie geralmente forma grandes populações, pois apresenta crescimento vegetativo bastante vigoroso através de órgãos subterrâneos, rapidamente cobrindo a superfície onde se encontra. Sua circunscrição foi alterada recentemente em trabalho realizado com o complexo taxonômico formado por *G. neesii* e espécies afins (Ceolin & Miotto 2011).

10. *Galactia pretiosa* Burkart, Darwiniana 9: 93. 1949.

Ervas eretas, não volúveis, podendo atingir até 0,6 m altura. **Folhas** unifolioladas, glabras. **Pecíolo** ausente. **Folíolos** frequentemente conduplicados, coriáceos, (25)55-115(130) x (3)5-15(17) mm, oblongos a lanceolados, ápice agudo a levemente truncado, base cuneada a decurrente, **Estípulas** 1-3 mm comprimento, subuladas. **Estipelas** subuladas. **Pseudorracemos** axilares, paucifloros, com 1-3 flores, pedúnculo 15-102 mm comprimento. **Brácteas** 2 mm de comprimento, pubescentes. **Bractéolas** 2 mm de comprimento, subuladas. **Cálice** 6-7 mm de comprimento, indumento viloso. **Corola** purpúrea, lilás, rosada a azul, glabra a esparsamente pubescente. **Estandarte** 11-13 x 6-8 mm orbicular a obovado. **Alas** 10-13(15) x 2-5 mm. **Peças da carena** (10)11-13 x 2-5 mm. **Legumes** (22)25-42(44) x 4-6 mm, pubescentes. **Sementes** ovóides, assimétricas, 2-4 mm diâmetro, castanhas a negras, marmoreadas.

Material selecionado: São Francisco de Paula, ERS 484, 25/XI/2004, fl., *R. Lüdtke, 292* (ICN).

Distribuição geográfica: Paraguai, Argentina (Misiones), Brasil: SC, PR, SP, MS, MG. No RS, habita os Campos de Cima da Serra e os morros graníticos no entorno de Porto Alegre, não havendo registros para a Campanha.

Floração e frutificação: outubro a março.

Observações ecológicas: cresce tanto em campos limpos quanto barrancos, geralmente em solos rasos e pedregosos.

Comentários gerais: em algumas chaves dicotômicas, o passo que separa *G. pretiosa* de *G. marginalis*, espécie bastante próxima, é a presença de inflorescências pedunculadas na primeira e ausência de pedúnculo na segunda. Entretanto, este caráter não é inequívoco, pois muitos indivíduos de *G. pretiosa* apresentam ao mesmo tempo tanto inflorescências sésseis quanto pedunculados. O reconhecimento seguro destas duas espécies pode ser feito porque *G. pretiosa* não tem nem pecíolo, nem apresenta crescimento plagiotrópico subterrâneo, que são características sempre presentes em *G. marginalis.* Além disso, suas áreas de ocorrência em grande parte não se sobrepõem, pois *G. marginalis* ocorre em maior abundância nas Campanhas sul e oeste e *G. pretiosa* nos Campos de Cima da Serra. A única região onde elas ocorrem juntas é entre os paralelos 29ºS a 31ºS, nos morros graníticos da região de Porto Alegre.

11. *Galactia striata* (Jacq.) Urban., Symb. Antill. 2(2): 320. 1900.

Trepadeira volúvel, de 0,6 a 4 m de comprimento. **Folhas** pinado-trifolioladas, ráquis 8-11(14) mm de comprimento. **Pecíolos** 22(25)-47 mm de comprimento, pubescentes. **Folíolos** (38)40-65(67) x (18)-30(34) mm, elípticos a ovados, textura cartácea a membranácea, ligeiramente pubescentes na face abaxial, ápice obtuso a agudo, base arredondada. **Estípulas** 2-5 mm de comprimento, lanceoladas pubescentes a tomentosas. **Pseudorracemos** axilares, eretos, flores dispostas em nós ao longo da ráquis, com 2-4 flores por nó. **Brácteas** 1-2 mm de comprimento, ovadas a lanceoladas, pubescentes. **Bractéolas** 2-3 mm de comprimento, lanceoladas, pubescentes. **Cálice** 4-10 mm de comprimento, indumento viloso. **Corola** lilás a rosada, com base e dorso do estandarte verde-claros. **Estandarte** 10-15 x 5-7 mm, orbicular a obovado, glabro a finamente pubescente no ápice. **Alas** 10-14 x 2-3 mm, glabras. **Peças da carena** 10-12 mm de comprimento, glabras. **Legume** 45-80 x 5-10 mm, tomentoso. **Sementes** achatadas lateralmente, 4-7 mm de diâmetro, ovóides, reniformes a levemente discóides, inseridas obliquamente, avermelhadas a castanhas, marmoreadas.

Material selecionado: Itaqui, 500 m da ponte Uruguaiana-Itaqui, 13/I/2002, fl., *S . T. S. Miotto 2020* (ICN).

Distribuição geográfica: Ocupa uma extensa área nos trópicos americanos, desde o sudeste dos Estados Unidos (Flórida) até a Argentina (Entre Rios). Não é citada para Santa Catarina. Para o Paraná, os registros de herbário são antigos e restritos ao antigo Parque Nacional das Sete Quedas, hoje transformado na Hidrelétrica Binacional de Itaipu, em Guaíra e arredores. No RS, há registros pontuais para a Fronteira Oeste.

Floração e frutificação: setembro a abril.

Observações ecológicas: é a espécie de *Galactia* mais associada a ambientes florestais, ocorrendo principalmente em florestas de galeria.

Comentários gerais: espécie há muito utilizada como forrageira, tendo já sido realizados diversos trabalhos de melhoramento, existindo, inclusive, uma cultivar lançada (cv. Yarana).

Seção III

Autoecologia e conservação

Influence of fire in regeneration of *Galactia peduncularis* (Fabaceae) – implications for conservation of a Brazilian-Cerrado endemic

Guilherme B. Ceolin, Ernestino S. G. Guarino & José F. M. Valls

Abstract

Because fire has considerable potential to influence the structure and composition of vegetation, especially in seasonal tropical savannas and because there are frequent observations from collectors in exsicatae that *G. peduncularis* resprouted after fire, we evaluated how fire affects the regeneration of this species and what are the impacts for its management and conservation. We placed four plots in a *ca.* 30-day-old-burned site and four plots in an unburned site and evaluated the total number of individuals, the number of branches per individual and the soil cover in each plot. We found 372 of *G. peduncularis* in located at the burned site and only 39 in the unburned site. The average number of branches per individual was 1.82 for the burned site and 1.33 for the unburned site. The soil cover was higher in the unburned than in the burned site and linear regression showed a negative correlation between the number of individuals and the soil cover. We concluded that fire is a key factor in the regeneration and persistence of *G. peduncularis*, maily because periodic burning alleviates the competition with dominant grasses and increases the availability of nutrients.

Key-words: Cerrado – Neotropic – Leguminosae – savanna conservation – reserve management

Introduction

Fire has considerable potential to influence the structure and composition of vegetation, especially in seasonal tropical savannas. The savannoid Brazilian biome known as Cerrado is considered a fire-related type of vegetation (Eiten 1972; Soares 1990). The savannas are modified by fire originated by both natural and anthropogenic causes (Sarmiento 1984), and many tropical savannas are currently maintained by periodic anthropogenic fires (Walker 1981). Many species exhibit adaptations to survive to the periodic disturbance, notably showing a high ability to resprout after burning (Medeiros and Miranda, 2008), mainly due to the development of fire-resistent underground organs, which store water and nutrients (Rizzini and Heringer, 1962), and for forming an evergreen protective mantle over apical buds (Coutinho, 1990).

In spite of the recognized influence of fire in savannoid biomes around the world and the existence of several studies about this issue, it is still controversial if fire is beneficial or harmful to Cerrado vegetation. Some studies demonstrate that frequent fire gradually decreases the resprouting ability of plants by killing the shoots, an effect known as top-kill (Miranda and Sato, 2005). This effect is especially harmful to trees because the regrowth of individuals that suffered top-killing is expensive in terms of nutrients (Hoffman 1998). Depending on how often fires occur tree species may not have time to form a bark thick enough to protect the cambium, or be tall enough to escape from the zone of high temperatures, which is around 0.5 m (Medeiros and Miranda, 2005, Medeiros and Miranda, 2008). On the other hand, some studies show that the absence of fire can promote an increase in woodiness and height of vegetation in savannas, as well as the invasion of some exotic species. Swaine et al. (1992) reported that 32 years of fire protection led to the invasion from forest species, including exotic timber species in a savanna, in Ghana. This increased abundance of taller and woody plants with the exclusion of fire may preclude the existence of light-dependent herbaceous species, leading their populations to local extinction along the time. In addition, some studies link the occurrence of fire to increased rates of flowering and seed germination of some species (Stone, 1951; Coutinho, 1980; Parker and Kelly, 1989). Fire also increased the rates of speciation in some plant lineages in Cerrado during the late Miocene and early Pliocene (Simon et al. 2009).

Galactia peduncularis (Benth.) Taub. (Fabaceae) is a heliophyte subshrub to herbaceous plant, apparently restricted to Cerrado of Central Brazil; it dwells in especially dry, wet and rocky grasslands (Bentham 1859, Burkart 1971). In herbaria sheets, there are frequent observations from collectors that the species resprouted after fire. Furthermore, there are evidences of adaptations to fire in its morphology, like an enlarged xylopodium. Thus, we expect a correlation among fire and the frequency and abundance of *G. peduncularis.* For this reason, we evaluated how fire affects the regeneration of *G. peduncularis* and what are the impacts for the management and conservation of this species. Our hypothesis is that fire enables the species populations to grow because it reduces the competition with the grasses that are otherwise dominant.

Methodology

We carried out the work at Parque Estadual da Serra de Caldas Novas (PESCAN hereafter) at the end of the dry season in October 2002. PESCAN is located between the towns of Caldas Novas and Rio Quente, in the southeastern state of Goiás, Brazil (17º43'56" S, 48°40'0" W). We allocated eight 25 x 4 m plots in an area of rocky grassland. Four plots were placed in a *ca.* 30-day-old-burned site and four plots in an unburned site. A road between these sites worked like a firebreak. We set the plots at least 5 meters from the road in order to avoid its effects (e.g. different temperature, rainfall runoff and transport of seeds).

In the eight plots, we measured the total number of individuals of *G. peduncularis*, the number of branches per individual and the soil cover by the method of frame pins. This method uses a wooden frame, which has ten metal pins arranged perpendicularly to the soil and parallely to each other (Kent and Coker, 1994). In each plot, we performed ten measurements. We used the t-test to compare the differences in the number of individuals and the soil cover between the burned and unburned areas, and the Mann-Whitney (U) test to evaluate the differences in the number of branches per individuals between the treatments. We also performed a linear regression to test the correlation between the number of individuals and the soil cover. We ran all the statistical tests in the PAST software v. 1.89 (Hammer et al. 2001).

Results

We found 411 individuals of *G. peduncularis*, of which 372 were located at the burned site and only 39 in the unburned site ($t = 8.976$, $P < 0.001$, figure 1a). The soil cover was higher in the unburned (79.5%) than in the burned site (43.75%; $t = 10.062$, $P < 0.001$, figure 1b). The average number of branches per individual was 1.82 for the burned site and 1.33 for the unburned site (Mann-Whitney test $U = 5391$, $P = 0.008$, figure 1c). Linear regression showed a negative correlation between the number of individuals and the soil cover ($R^2 = 0.928$, $P < 0.001$, figure 2).

Discussion

Both the higher number of individuals and new branches in the burned site indicate that fire positively influences the abundance and the competitive ability of *G. peduncularis*. The increase in density of individuals and the number of new branches seems to be related to soil cover, since the lower the soil cover, the greater the number of individuals and new branches. A possible explanation for this pattern is that fire promotes a decrease in competition and an increase in available nutrients.

Long-time unburned savannas in regions that experienced rainy season with high precipitation (*ca.* 1500 mm.year^{-1}) are prone to accumulate a very large amount of biomass from grasses that grew in the rainy season and died in the dry season (Moreira 2000). This is the case of Brazilian Cerrado. Several authors have shown that disturbance maintains the diversity in highly productive grassland because they reduce the biomass of dominant grasses, reducing the number of competitors (Grime, 1973, Collins et al. 1999). Overall accumulation of biomass prevents access to light to geophytes and limits the space available. Coates *et al.* (2006) showed that populations of the Australian orchid

Prasophyllum correctum increase their frequency and abundance in fire-impacted areas due to changes in the levels of shoot competition with dominant grasses. Thus, the increased number of new branches showed by *G. peduncularis* after fire seems to be very important to outcompete the dominant grasses because this strategy enables plants to recover immediately the ground (Hanes 1971).

Fire can also change the energy flows because it mobilizes mineral nutrients (mainly N and P) otherwise stranded in dead biomass, making them available again for plants (Medina and Silva, 1990, Gimeno-García et al. 2000). However, the nutrient losses caused by rainfall erosion can be high after fire (Thomas et al. 1999). One of the suggested benefits of a resprouting strategy is the capacity of quick absorption of the released nutrients (Romanya et al. 2001), allowing that plants can easily retain them after the disturbance. Again, *G. pecuncularis* seems to fit into this response pattern because it exhibits a great ability to resprout after fire. Furthermore, Sardans *et al.* (2006), working in Mediterranean-basin, stressed that resprouting capacity should be especially important in nutrient-poor environments, specially where there is a great risk of post-fire nutrient losses caused by torrential rainfalls. This is the case of Mediterranean environments, but also is the case of Cerrado, where the soils are generally poor and the rainfalls could be heavy (Ratter *et al.* 1997).

We can say that the cleanse promoted by fire in the soil cover allows underground-dormant individuals of *G. peduncularis* to access the light and its high potential for resprouting after disturbance capacitates not only a rapid space occupancy but also the absorption of the increased input of available nutrient.

Regarding the controversy if fire is beneficial or harmful to Cerrado vegetation and linking this to the conservation of *G. peduncularis*, we can say that depends on what one wants to conserve. It is well established that periodic fires is limiting to the survival of some non-tolerant species, especially trees, favoring many species of herbs and shrubs with shorter life cycles (Moreira, 2000; Medeiros and Miranda, 2008). Vegetation tends to become denser with total abolition of fire, preventing the survival of species whose life cycle depends on the disturbance, such as the species we are studying. In addition, large accumulations of dry biomass can fuel large natural fires, such as those caused by lightning (Ramos-Neto and Pivello, 2000). Such fires can be more devastating to the biota than planned and controlled burns. Hence, how is possible to combine the conservation of *G. peduncularis* and other fire-dependent species with the conservation of non-tolerant tree species?

A feasible solution would be the use of different and controlled fire regimes, periodically alternating burned and unburned areas, or yet, through well spaced burning along the time. Andrade (2002) observed that *G. peduncularis* forms a viable seed bank in the soil, especially in areas subjected to biennial burning. Theoretically, a biannual fire incidence could ensure the continuity of populations of *G. peduncularis*. However, this frequency of fire may not be enough for some plants to reach height or diameters enabling the escape from the harmful effects of fire, since individuals with less than 1 m tall and 5 cm in stem diameter are subject to high mortality rates (Medeiros and Miranda, 2005). The best solution, therefore, would be controlled burning in limited areas and with adequate and different time spacing, depending on the type of vegetation to be conserved. In the case of Parks in Cerrado, where the main goal is to conserve as many physiognomies and consequently the greatest biodiversity possible, methodologies to proceed periodical burns must be embodied in management plans, like it is done in other parks and reserves in savannoid regions around the world (Saxon, 1984, Parsons et al., 1986, Australian National Parks and Wildlife Service, 1991, Russell-Smith, 1997). Some studies have shown that biodiversity in some Australian grassland reserves has declined as a result of non-implementation of planned disturbances, especially fire (Morgan, 1995, Lunt and Morgan, 2002). Similar responses have occurred in northern Europe and North America due to the abandonment of traditional management (Willems et al., 2001; Wotavová et al., 2004).

In PESCAN, the rocky shallow soil where *G. peduncularis* grows is not reliable *per se* to expansion of a very taller savanna, so a biennial fire regime could be sufficient to remove the accumulated biomass and stimulate sprouting and germination of the seed bank. Elsewhere in the park, where the soil is deeper and the possibility of sustaining a savanna with tallest vegetation is higher, fire must be avoided or used with great control and in very large time intervals, depending on the vegetation dynamics and on the characteristics of each site.

We conclude that fire is a key factor in the regeneration and persistence of *G. peduncularis*, not only because periodic burning alleviates the competition with dominant grasses but also because increases the availability of nutrients. Some areas to be burned periodically must be included in management plans of parks and reserves, so that optimal conditions for the survival of *G. peduncularis* are ensured, without affecting other areas where is desiderable to maintain a more closed physiognomy.

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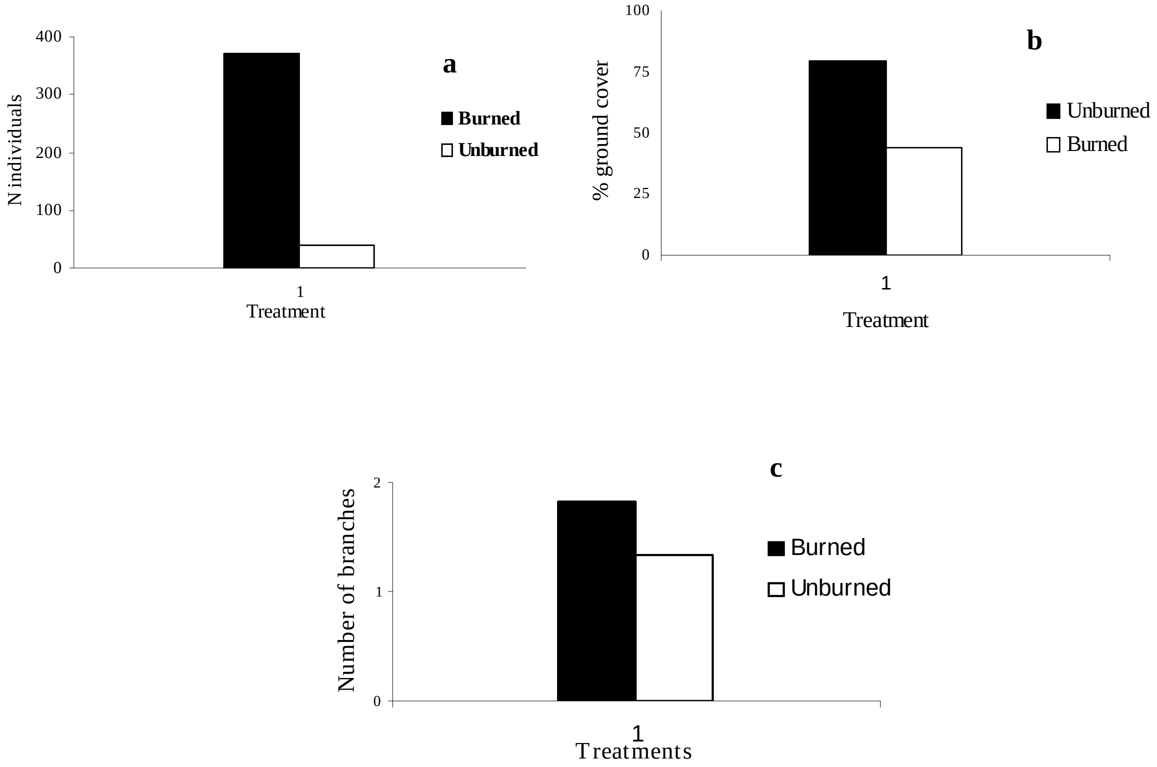

Figure 1: a) Number of individuals in burned and unburned sites (t = 8,9759, p = 0,00010687); b) Percentage of soil cover in burned and unburned sites (t = 10,062, p<0.0001); c) Number of branches in plants of *G. peduncularis* growing in burned and unburned sites (U = 5391, p = 0.00836).

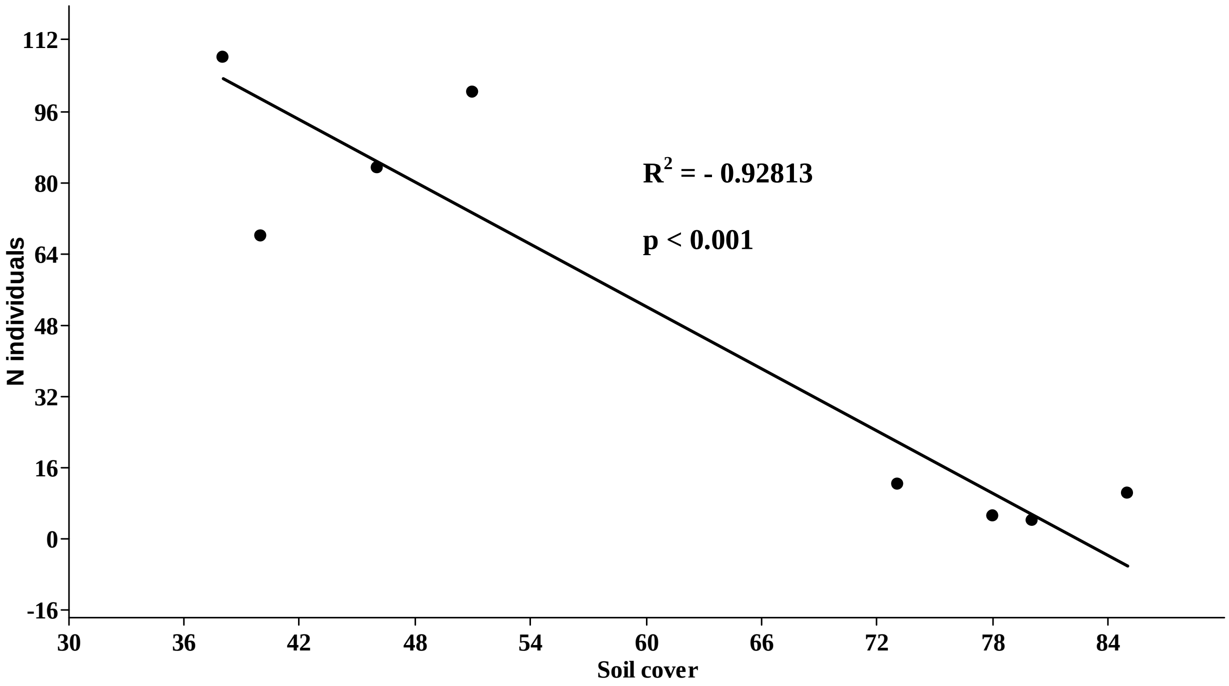

Figure 2: Linear regression showing the relationship between the percentage of soil cover and number of individuals of *Galactia peduncularis* in burned and unburned plots.

Seção Única

O gênero *Galactia* P. Browne (Leguminosae, Papilionoideae) no Brasil

Systematic and Biodiversity

The genus *Galactia* P. Browne (Leguminosae, Papilionoideae) in Brazil

GUILHERME B. CEOLIN, SÍLVIA T. S. MIOTTO & JOSÉ F. M. VALLS

The aim of this paper is to make a synopsis about the knowledge of the genus *Galactia* P. Browne (Leguminosae, Papilionoideae) in Brazil. This synopsis addresses several aspects of the genus, as taxonomy, ecology, economical potential and conservation. This paper have a taxonomic key, description of one new species, new synonymizations, some illustrations, a brief description just stressing the diagnostic characters and a brief commentary about each species, concerning the distribution and some noteworthy ecological and economical characteristic.

Key-words: Diocleinae – Phaseolae – Neotropic – Fabaceae – species concept – economic potential

Introduction

Galactia is a pantropical genus growing in all continents except Europe and Polar Zones. It was described by Patrick Browne, who named the genus due to the latescent sap in some species (from Greek, *galáktos* = milk). Despite being widely distributed and have their centre of richness in the American continent, there are few published studies concerning *Galactia*. In Neotropical regions, we can cite Beyra-Matos et al. (2005), who studied the genus for Cuba, and also Torres et al. (1983) for Venezuela. For Brazil, there are few taxonomic studies of *Galactia* since the publication of the Flora Brasiliensis (Bentham 1859). Even the Burkart's (1971) monograph for the whole South America often has incomplete information for most of the country; the author himself admits to not having achieved full success in the task, because several regions remained underestimated, as most areas of the Central and Eastern Brazil.

This synopsis of *Galactia* to Brazil is part of the doctoral thesis of GB Ceolin and arose from the growing need to deal with the large amount of specimens deposited in Brazilian herbaria. Most of these collections result from the efforts of STS Miotto and JFM Valls, who have been collecting specimens of *Galactia* along all the country since the late 1970s, as part of a broader study of Brazilian Leguminosae. This study was needed for a long time, because the taxonomy of *Galactia* is quite complicated and there are several reasons for this.

First of all, it is not possible to define *Galactia* by a single and striking autopomorphic feature; instead, we recognize it by eliminating other closely genera, in a stepwise manner. Firstly, we ought to look at the calyx: it should have 4 lobes, because the uppermost lobe results from the fusion of the two vexilar lobes usually found in other legume genera. If so, we need to look at the flower colour; in *Galactia* the corolla is never yellow, being pink, purple and lilac the most common colours, rarely red. Late, we should analyze the inflorescences, which in

Galactia are nodose pseudoracemes. Finally, the ovary in *Galactia* must to be sessile; just one species bear a small stipite in ovarium (*G. marginalis*) but it is never larger than the half of the ovary length. However, even resorting to this suite of characters, there is no warranty of safe recognition of *Galactia*, chiefly because there is not still a comprehensive phylogeny of the whole genus. The current published phylogenies are punctual and regionally limited efforts mainly concerned to solve specific problems (Sede *et al.* 2008, 2009; Ceolin *et al.* 2011a). The absence of an improved phylogeny makes the correct boundaries of the genus still undefined, conflicting with other closely related genera as *Collaea* DC. and *Camptosema* Hook & Arn. It also causes controversies on the actual number of species within *Galactia*, which varies around 60±5, depending on the author (Burkart 1943, 1952, 1971; Lewis et al. 2005; Fortunato et al. 2008; Sede et al. 2008, 2009).

In addition, the boundaries among species are also far from a consensus. In his monograph, Burkart (1971) wrote that the species of *Galactia* are often difficult to recognize because of the widely morphologic variation in habit, shape and hairiness of the leaflets. In parallel with this extensive plasticity of vegetative attributes, the floral traits of *Galactia* are homogeneous and monotonous, so that taxonomically informative characters are scant. This sum of factors makes the taxonomy of *Galactia* a chaotic endeavour, both in micro (boundaries among species) and macroscale (circumscription of the genus).

This synopsis addresses several aspects of the genus, as taxonomy, ecology, economical potential and conservation. We provide a taxonomic key, description of one new species, new synonymizations, some illustrations, a brief description stressing the diagnostic characters and a brief commentary about each species, concerning the distribution and some noteworthy ecological and economical characteristic. However, we are aware that this synopsis is not complete, because as we previously pointed out, we shall achieve a satisfactory resolution of the genus only when a comprehensive phylogeny is published. Still, we hope to have contributed to improving the understanding of the genus and that this study could be the starting point for future efforts aiming to solving taxonomic problems still unresolved.

Material and methods

We based our study extensively in bibliographic data and dry specimens form the following herbaria (acronyms according to Holmgren & Holmgren 2007): BA, BHCB, C, CEN, CGMS, CTES, ESA, F, FLOR, HAS, HB, HBR, HEPH, HISA, HUEFS, IAC, IBGE, ICN, LP, MBM, NYBG, P, PACA, PAMG, PEL, SI, SMDB, SP, UB, UEC, UFMT, UPCB, US, VIC. We complemented the herbaria data through some field trips, which followed some criteria in order to improve the collection efforts. Thus, we collected preferentially either rare or taxonomically difficult species, in areas with great specific richness (both Southern and Central-western Brazil), and in the months of the southern hemisphere spring and summer, in which occur the flowering and fructification of most species. Altogether, we analyzed ca. 1,300 specimens. All herbarium vouchers were deposited in the ICN herbarium.

We defined the species boundaries within *Galactia* through the Unified Species Concept (USC; de Queiroz 2005), which defines a species as separately evolving metapopulations (or segments of) lineages that show several contingents characteristics over the time (e.g. diagnosability, exclusive ecological niche, allele coalescence). Two lineages only can evolve separately if gene flow between them is absent, what is achieved in sexual lineages by reproductive isolation. As we are not using molecular markers, it is impossible infer reproductive isolation directly. Furthermore, our study is essentially herbarium-based, so that it is also not possible proceed with cross experiments. Thus, reproductive isolation only can be indirectly inferred. Basically, three processes could be responsible for reproductive isolation: habitat separation (exclusive niche, geographic barrier), exclusivity of pollinators (divergent floral morphology, non-synchronized flowering) and polyploidization events (additional details on speciation process and criteria to recognize species in Ceolin & Miotto 2011b). However, concerning the later process, currently there are no evidences of this kind of event in *Galactia*, since all the chromosome counts have showed the constant number 2n = 20 (Sede et. al. 2003, 2006). Therefore, we followed the two first criteria in recognizing the taxa and used them to elaborate the identification key, describe new species, and propose the synonymizations. Burkart (1971) placed the *Galactia* species into three sections, largely based on floral traits as hairiness of the standards, flower size and color and characteristics of stamens and ovary. Because *Galactia* appeared as non-monophyletic in all of the phylogenetic studies (Queiroz et al. 2003, Sede et al. 2008, 2009) and the traits are viewed as homoplasic we are not considering the Burkart's infrageneric classifications in this synopsis.

We extracted the information about phenology, habitat, geographic distribution, vernacular names, and economic importance from bibliography, exsiccatae labels and personal observation in fieldtrips. The geographic distribution will be given by biomes and also by State (between brackets). In order to be concise, we will use the State abbreviation. Both the mean of abbreviations and a map of the Brazilian biomes in appendix 1. Valid names appear in bold and synonymous in italics.

Results

Taxonomic key

In order to be didactic, we divided the keys into two possible pathways concerning the number of leaflets. If the specimen bears just one leaflet, go to the key I; if the specimen bears three leaflets go to the key II. The species assign with the Latin expression *pro parte* have two pathways, because they show an ambiguous state of character in that attribute.

Key I – One-leaflet

1. Inflorescences larger than the subtended leaf

2. Habit voluble, at least the apex; petiole conspicuous, 5 – 80 mm long

…………………………………………………………………………….. *G. benthamiana* (*pro parte)*

2’. Habit prostrate to erect, not voluble; petiole absent or very short, up to 8 mm long

3. Leaflets glabrous, bluish-green; inflorescences terminal, rarely axillary

....*G. glaucescens* (*pro parte*)

3’. Leaflets pilose, yellow-green; inflorescences axillary

4. Inflorescences pauciflorous (1 – 5 flowers).. *G. pretiosa*

4’. Inflorescences pluriflorous (> 10 flowers)

5. Subshrub to shrubs erect, from 0.2 to 1.5 m height

6. Base of leaflet heart-shaped, almost completely surrounding the stem (amplexicaulous).. *G. cordifolia* **sp. nov.**

6’. Base of leaflet rounded to truncate, never amplexicaulous.................................... *G. douradensis*

5’. Herbs to subshrubs prostrate to suberects, up to 1 m long

7. Inflorescences subumbeliforms; flowers aggregated at the apex..... *G. dimorphophylla (pro parte)*

7’. Inflorescences pseudoracemes; flowers not aggregated at the apex

8. Standards 15 – 20 mm long; inflorescence lax…………... *G. boavista*

8’. Standards 10 – 14 mm long; inflorescence contracted……………......................... *G. decumbens*

1’. Inflorescence smaller, equal or rarely larger than subtended leaf

9. Plants voluble... *G. benthamiana* (*pro parte*)

9’ Plants prostrate to erect, but not voluble

10. Inflorescences pluriflorous (>10 flowers); flowers densely aggregated along the rachis

.... *G. grewiaefolia*

10’. Inflorescences pauciflorous (1 – 5 flowers), lax

11. Plant growing in Cerrado biome, in Central Brazil; petiole absent; flowers 20 mm long

.... *G. weddelliana*

11’. Plants growing in Pampa biome, in southern Brazil; petiole always present; flowers 13 – 19 mm long.. *G. marginalis*

Key II – Three-leaflets

1. Inflorescences larger than the subtended leaf

2. Petiole large and conspicuous, longer than 7 mm long

3. Flowers scattered along the entire rachis, inflorescences pseudorracemes

4. Plants totally volubles or at least the apex

5. Leaflets membranaceous, sparsaly pubescents; plants growing in forest.......................... *G. striata*

5’. Leaflets chartaceous to coriaceous, densely pilose; plants growing in open areas..... *G. latisiliqua*

4’. Plants erect to prostrate, but not voluble

6. Endemic to Caatinga biome; leaflets obovate.. *G. remansoana*

6’. Endemic to Cerrado biome; leaflets oblong, eliptic to lanceolate

7. Flowers 20 – 27 mm long; stem flattened ……….. *G. peduncularis*

7’. Flowers 11 – 15 mm long; stem cylindrical

8. Inflorescences sessile and peduncled on the same specimen; habit erect …................... *G. hoehnei*

8’. Inflorescences always peduncled; habit prostrate to decumbent................................ *G. heringeri*

3’. Flowers aggregated at the apex; inflorescences like an umbel or corymb

9. Habit prostrate to erect; if voluble, then restrict to Chapada dos Veadeiros (Brazilian State of Goiás); widely spread in Brazil

10. Restrict either to South-American Pampas or to Caatinga biome; keel 11 – 13 mm long;

11. Growing in South-American Pampas; leaflet length/width ratio ca. 1.5:1 .... *G. australis*

11’. Growing in the semiarid regions of Minas Gerais and Bahia States; leaflet length/width ratio ca. 4.5:1 .... *G. lineata*

10’. Growing in Cerrado and in the highlands of the southern Brazil; keel ca. 14 mm long ………………….. *G. neesii*

9’. Habit voluble; apparently restrict to central region of Rio Grande do Sul State....... *G. martioides*

2’. Petiole short, sometimes absent, up to 7 mm long

12. Leaflet glabrous, bluish-green; inflorescences terminal, rarely axillary................. *G. glaucescens*

12'. Leaflets pilose, yellow-green; inflorescences axillary

13. Leaflet lanose.. *G. lamprophylla*

13'. Leaflet sericeous or velutinous, non-lanose .... *G. eriosematoides*

14. Inflorescence pseudorracemes; flowers lax……..………....................................... *G. dimorpha*

14'. Inflorescences subumbeliform; flowers aggregated…..………… *G. dimorphophylla* (*pro parte*)

1'. Inflorescences smaller, equal or rarely longer than the subtended leaf

15. Leaves pinate-trifoliolate; flowers abundant, densely aggregated in the rachis…..... *G. jussiaeana*

15'. Leaves digitate-trifoliolate; flowers scarce (1 – 3); rachis absent........................... *G. gracillima*

Descriptive synopsis of **Galactia** *in Brazil (in alphabetical order)*

1. **G. australis** (Malme) Ceolin & Miotto, Plant Syst. Evol. (submitted paper). **Type:** Malme, Iter Regn. II-508 y 508 a. (S).

Diagnostic characteristics: herbaceous, habit prostrate; trifoliolate leaves; leaflets almost orbicular (length/width leaflet ratio ~1), with rounded base and apex; length of keels 13.2±0.9 mm; endemic to lowlands of the southern South American Pampas.

Voucher: RS, Porto Alegre, R. Setubal & A. Mello 171, 02 Nov 2005, fl. (ICN).

Commentary: new status derived from *G. neesii* var. *australis*. This species inhabits the Pampa biome, growing in grasslands; rocky and shallow soils; plain and hillsides; it is able to form large population that quickly cover the surface where the species grow due to its rhizomae. Its geographic distribution encompasses Argentina, Paraguay, Uruguay and Brazil (RS). Flowering and fructification from October to February. Arechavaleta (1901) points that the infusion of its roots is used as aphrodisiac in popular medicine. Illustrations in Burkart (1971, pp. 733 – 734).

2. **G. benthamiana** Mich., Videnskabel. Medelelser f. Naturhist. Forening. Kjöbenhavu 7: 81. 1875. **Type**: Warming 2857 (C!, photo).
Fig. 7

Diagnostic characteristics: herbaceous voluble, at least the apex; leaves unifoliolate; petiole present;

base of leaflets heart-shaped; inflorescences sessile or large.

Voucher: RS, Santiago, G.B. Ceolin 160, 17 Dez 2008, fr., (ICN).

Commentary: this species inhabits the Pampa, Mata Atlântica and Cerrado biomes, growing in grasslands; rocky soils; plain and steep relief; it has large and sometimes thick roots; scant. It occurs in Argentina, Uruguay and Brazil (RS, SC, PR, SP e MG). Flowering and fructification from November to March.

3. **G. boavista** (Vell.) Burkart. Darwiniana 16: 783-788. 1971. **Type:** Vellozo, Icones 7: 115.

Fig. 8

Diagnostic characteristics: herbs to subshrubs prostrate or decumbent, rarely erect; leaves unifoliolate; petiole short, sometimes absent; base of leaflets acute or attenuated; inflorescences large, longer than the subtended leaf; flowers 15 – 20 mm long, lax.

Voucher: PR, Balsa Nova, 25°27’”S 49°46’W, G. B. Ceolin 131, 09 Jan 2008, fr. (ICN).

Commentary: this species inhabits the Pampa, Mata Atlântica and Cerrado biomes, growing in grasslands; shallow and rocky soils; plain and steep relief; it is fire-resistant due to xylopodium. Its geographic distribution encompasses Paraguay, Argentina and Brazil (RS, SC, PR, SP, MG, MS). Flowering and fructification from September to March.

4. **G. cordifolia** Ceolin & Miotto, **sp. nov**.

Fig. 17 and 18

Type: Brazil, MS, Ribas do Rio Pardo, 20º38'56"S 53º39'20"W, 376 m, A. Pott & V. J. Pott 14941, 24 Feb 2008, fl. fr. (Holotype CGMS; isotype ICN).

A Galactia glaucescens *differt foliis unifoliolatis cum basis folioliorum subampexicaulis cordatis, coriaceis, petioli desunt, racemi in parte distale 150 mm long., carina 14 long. × 5 – 6 mm lat.*

Diagnostic characteristics: subshrub erect ca. 0.5 m height; leaves unifoliolate, glabrous, coriaceous 60 – 90 × 70 – 80 mm; base of leaflets heart-shaped, almost completely surrounding the stem (amplexicaulou); petiole absent; inflorescences erect and terminal, ca. 150 mm long, pluriflorous; pedicele 1 – 3 mm long; standards pilose 14 × 12 – 13 mm; wings 11 – 13 × 5 – 6 mm and keels 14 × 5 – 6 mm; fruits 41 × 8 mm.

Additional specimens: MS, Ribas do Rio Pardo, A. Krapovickas & C. L. Cristóbal 34375, 25 Jan 1979, fl.fr. (CTES, SI).

Commentary: this species looks like *G. glaucescens* due to erect habit and terminal inflorescences, however, is different from it because of unifoliolate leaves, with a heart-shaped base and flowers larger and aggregated. This species inhabits the Cerrado biome, growing in open grasslands and arboreal areas (Cerradão). Flowering and fructification from December to March.

5. **G. decumbens** (Benth.) Hoehne, Rev. Mus. Paulista 10: 695. 1918. **Type:** Clausen 106, ser. 2442, (NYBG, photo).

G. decumbens (Benth.) Hoehne f. *suberecta* Burkart, Darwiniana 16: 790. 1971. **Type:** Irwin 25440 (NYBG). **syn. nov.**

G. bullata var. *bullata* (Benth.) Taub., Die Nat. Pflanzenfam. 3 (3): 368: 1894. **Type:** Gardner 3663. **syn. nov.**

Galactia bullata var. *magnifolia* Burkart, Darwiniana 16: 767. 1971. **Type:** Irwin 25154 (SI!). **syn. nov.**

Fig. 10

Voucher: MT, Chapada dos Guimarães, 15°28'S 55°48'W, G. B. Ceolin 170, 15 Jan 2009, fl. (ICN).

Diagnostic characteristics: herbs prostrate to ascendant, sometimes erect; leaves unifoliolate; inflorescences longer than the subtended leaf; small flowers (10 – 14 mm), aggregated at the apex, with vinaceous strips in the outside surface of standards.

Commentary: the characteristics of the type of *Galactia bullata* var. *magnifolia* fit almost perfectly the characteristics of *G. decumbens*, due to unifoliolate leaves, large inflorescences, and flowers congested with vinaceous strips on the outside surface of standards; its main difference is the erect habit and the number and size of flowers. These differences contemplate the criteria used in this study to infer reproductive isolation, since differences in flower size and density are characteristics able to influence the behaviour of pollinators and prevent gene flow (Worley et al. 2000). However, as far as we could observe, it was not possible to find any specimen in our fieldtrips, even in the region where the type was collected. In addition, there are no other exsicatae of *Galactia bullata* in herbaria, except the type, and even Burkart admitted that he saw no material of *G. bullata* var. *bullata*. So, due to lack of further evidence, the most parsimonious attitude we can take is to regard *G. bullata* as an aberrant form of *G. decumbens* and propose its synonymization. Additionally, Burkart (1971) described *G. decumbens* f. *suberecta* due to the ascendant habit of some individuals. Habit is a very plastic attribute in *Galactia*, so that does not make sense to assign valid taxonomic status to each different states of this character; thus, we are synonymizing this taxon as well. *Galactia decumbens* inhabits the Cerrado biome, growing in open grasslands, shrublands, forest edges, slopes and rock outcrops; 600 – 1250 m above sea level (a.s.l.); clay and sandy soils. It is a Brazilian endemic (SP, MG, GO, MS, MT, DF). Flowering and fructification from November to May.

6. **G. dimorpha** Burkart, Darwiniana 16: 766. 1971. **Type:** Saint-Hilaire 661 (P!).

Fig. 11

Diagnostic characteristics: shrub erect, up to 1.5 m height; leaves pinate-trifoliolate, petiole absent; inflorescences large, flowers lax.

Voucher: GO, Rio Verde, 17°53'S 50°58'W, G. B. Ceolin 165, 10 Jan 2009, fl. (ICN).

Commentary: this species inhabits the Cerrado biome, growing in grasslands; 700 – 900 m a.s.l.; clay and sandy soils; plain and hillsides; common but small populations. It occurs in Bolivia and Brazil (MS, MT, GO, MG). Flowering and fructification from August to May.

7. **G. dimorphophylla** Fortunato, Sede & Luckow, Brittonia 60: 350. 2008. **Type:** Fortunato & Luckow 7552 (BAB!).

Diagnostic characteristics: subshrub up to 0.5 m height; leaves pinate-trifoliolate in the apex and unifoliolate in the base as well as in young individuals; inflorescences subcapituliform; flowers aggregated; it is endemic to RS.

Voucher: RS, Giruá, K. Hagelund (ICN).

Commentary: this species was recently described to northwestern Argentina and northern Uruguay, closely adjacent to Brazilian borderlands. The authors of this species hypothesized the occurrence of this species in Brazil, which we are confirming here. This species inhabits the Pampa biome, growing in grasslands and brushlands; laterite soils; rocky slopes and hillsides. In Brazil, is endemic to RS. Flowering and fructification from January to April. Ilustrations in Fortunato et al. (2008).

8. **G. douradensis** Taub., Bot. Jahrbüch. 21; 438. 1896. **Type:** Ule 2864 (P!)

Diagnostic characteristics: shrub up to 1.5 m height; leaves unifoliolate, petiole absent; inflorescence longer than the subtended leaf; flowers aggregated with vinaceous strips in the outside surface of standards.

Voucher: GO, Caldas Novas, 17°44'S 48°41'S, G. B. Ceolin 166, 10 Jan 2009, fl. (ICN).

Commentary: this species inhabits the Cerrado biome, growing in shrublands; soils with red clay and sand; plain relieves; generally abundant. It is a Brazilian endemic (GO, MT). Flowering and fructification from November to May. Illustration in Burkart (1971, p. 782).

9. **G. eriosematoides** Harms, Bot. Jarbüch 33, Beiblat 72: 27. 1903. **Type:** Glaziou 20902 (F!, photo).

Fig. 14

Diagnostic characteristics: shrub up 1.3 m height; leaves pinate-trifoliolate, basal leaves sometimes unifoliolate; petiole absent; inflorescence longer than the subtended leaf, flowers aggregated, with vinaceous strips in the outside surface of standards.

Voucher: GO, Caldas Novas, 17°44'S 48°41'S, G. B. Ceolin 167, 11 Jan 2009, fl.fr. (ICN).

Commentary: this species inhabits the Cerrado biome, growing in shrublands; 500 – 1000 m a.s.l.; soils with red clay and sand; plain relieves. Flowering and fructification from December to May.

10. **G. glaucescens** Kunth, Nova Genera et Spec. Plant. 6: 431. 1823. **Type:** von Humboldt s.n. (F 2388!, photo).

G. glaucescens Kunth var. *lancifolia* (Benth.) Burkart, Darwiniana 16: 771. 1971. **Type:** Gardner 3664 (F!, photo). **syn. nov.**

G. glaucescens Kunth var. *obtusa* (Benth.) Burkart, Darwiniana 16: 771. 1971. **Type:** Schüch 148 (US). **syn. nov.**

Fig. 15

Diagnostic characteristics: subshrub erect; leaves pinate-trifoliolate or rarely unifoliolate, bluish-green, glabrous; petiole absent or very short (up to 7 mm long); inflorescences lignose, terminal, rarely axillary; flowers often with the the outside surface of standards greenish, with vinaceous strips.

Voucher: MT, Pontes e Lacerda, 15°38'S 58°46'W, G. B. Ceolin 172, 19 Jan 2009, fr. (ICN).

Commentary: Burkart (1971) established the taxa *G. glaucescens* var. *lancifolia* and *G. glaucescens* var. *obtuse*, changing the specific status of *G. lancifolia* and *G. obtuse*. The former is recognized by the narrower leaves and the later because of its unifoliolate leaves, rather than three. According to some authors (Cron et al. 2007; Bennett et al. 2008), infraspecific taxa should be accepted only if they have morphological variations in a set of attributes. Furthermore, this variation should be correlated with the area of occurrence of the taxon (geographic coherence). This is not the case, because just the leaflets showed some variation; all the other traits (mainly the reproductive ones) were constant. The occurrence of individuals with single leaflet at the base of the plant or even fully unifoliated specimens is common within usually trifoliolated species of *Galactia*; additionally, sometimes some species have individuals with more than three leaflets (4-5 leaflets). For instance, in the very *G. glaucescens*, the specimen *Coradin 2185* (CEN) bears five leaflets. Therefore, this kind of intraspecific variation is common in *Galactia* and is not sufficient to maintain the status of these varieties within *G. glaucescens*. This species inhabits a wide sort of biomes and environments; it grows close to moist areas, as riparian forests and floodplains, however, is eventually found in grassy or arboreal savannas, dry grasslands, anthropogenic grasslands and shrublands; 150 – 1100 m a.s.l.; serpentine, clay, rocky and sandy soils; abundant, forming great populations. It occurs in Venezuela, Ecuador, Colombia, Bolivia, Paraguay and Brazil (MA, BA, TO, RO, MT, MS, GO, MG). Flowering and fructification almost in every month. One of the vernacular name of this species is “cascaveleira”, in allusion of its use in popular medicine against rattlesnake venom (from Portuguese, *cascavel* = rattlesnake). Dal Belo et al. (2008) tested this popular knowledge and showed that ethanolic extract of *Galactia glauscescens* leaves does prevent the neuromuscular paralysis induced by rattlesnake venom on mouse phrenic nerve-diaphragm preparation.

11. **G. gracillima** Benth., Flora Brasiliensis 15: 142. 1859. **Type:** Saint-Hillaire C2-1326 (P).

Fig. 3

Diagnostic characteristics: herb voluble; leaves digitate-trifoliolate, with petiole; flowers sessile, axillary.

Voucher: SC, Campos Novos, 27°29”S 51°20’W, G. B. Ceolin 133, 12 Jan 2008, fl. (ICN).

Commentary: some taxonomic keys use the fragile appearance of this species, with very soft and thin branches, as diagnostic character. However, some individuals growing fully exposed to sunlight, in shallow and stony soils may have a woody base and a certain degree of secondary growth of the stem. Furthermore, they may have very coriaceous leaflets. Therefore, we did not recommend the using of these traits as diagnostic of the species because their characteristics depend on the environment where the specimen is growing. This species inhabits the Pampa, Mata Atlantica and Cerrado biomes, growing grasslands, twining on other herbs; clay, sandy and rocky soils; It is resistant to drought, grazing and fire because of underground xylopodium. It occurs in Paraguay, Argentina, Uruguay and Brazil (DF, GO, SP, PR, SC, RS). Flowering and fructification from July to March.

12. **G. grewiaefolia** (Benth.) Taub., Die Nat. Pflanzenfam. 3: 368. 1894. **Type:** Riedel 2487 (F!, photo; isotype W!).

G. irwinii Cowan, Bolet. Soc. Venezol. Cienc. Nat. 22: 284. 1961. **Type:** Irwin 2381 (US!). **syn. nov.**

Diagnostic characteristics: herbs or subshurbs prostrate to erect; leaves unifoliolate, petiole almost absent; inflorescences smaller ore qual to the subtended leaf; flowers densely aggregated.

Voucher: MS, Campo Grande, 20º31'S 54º13'W, G. A. Damasceno-Junior 4770, 22 Dez 2006, fl. (CGMS).

Commentary: Burkart (1971) stated that *G. grewiaefolia* and *G. irwinii* appear morphologically closely related species. In fact, at first glance, the type of *G. irwinii* really looks like a specimen of *G. grewaefolia*, mainly due to dense white pubescence on the adaxial surface of leaflets. However, the main feature to distinguish among them is the inflorescences of *G. irwinii*, which are longer than the subtended leaves and floriferous only in its distal third. Here, the situation is the same as when we proposed the synonymy of *G. bullata* under *G. decumbens*: both species are similar in most of its attributes, but have differences that might confer reproductive isolation. The problem in accepting these taxa relies in the fact that there are few collections, in most cases only the holotype. Thus, it is not possible to know surely if they are only isolated cases of individual anomalies. Most specimens of *G. grewiaefolia* have inflorescence smaller than the subtended leaf and flowers densely aggregated along the rachis. However, many species of *Galactia* have short and long inflorescences in the same individual and the number of flowers may vary simply due to the age of the inflorescence. In short, there are many causes for this sort of variation and only one individual is not sufficient to decide the case. Therefore, we are proposing here the synonymization of *G. irwinii* under *G. grewiaefolia*. We chose this species rather than *G. douradensis*, which also shares the single leaflet and the large inflorescence, due to the similarity of the leaflets and the fact that the synonymous species occur in Minas Gerais State, while the excluded species grows in Goias and Mato Grosso. *Galactia grewiaefolia* inhabits the Cerrado biome, growing in grassy, shruby and arboreal savannas; sandy, clay and rocky soils; plain and steep relieves; abundant. It occurs in Paraguay and Brazil (MT, MS, DF, GO, MG, SP). Flowering and fructification from September to May.

13. **G. heringeri** Burkart, Darwiniana 16: 772. 1971. **Type:** Heringer 8262 (SI!)

Fig. 16

Diagnostic characteristics: herbs prostrate to decumbent; leaves pinate-trifoliolate, petiole present; inflorescences with aggregated flowers.

Voucher: DF, Brasília, G. P. Silva & G. Abdala 4237, 04 Mar 2000, fl.fr. (CEN).

Commentary: this species inhabits the Cerrado biome, growing generally in dry grasslands and eventually in moist ones; clay or rocky soils; fire-resistant. It is a Brazilian endemic (DF, GO, MG). Flowering and fructifications from November to March.

14. **G. hoehnei** Burkart, Darwiniana 16: 763. 1971.

Diagnostic characteristics: subshrub erect; stem cylindrical; leaves digitate-trifoliolate, leaflets linear, rigid; inflorescences axillary, often sessile and peduncled in same specimen; flowers small, aggregated.

Voucher: MT, Diamantino, 14º17'S 56º30'W, V. C. Souza 16915, 22 May 1997, fl. (UFMT).

Commentary: this species inhabits the Cerrado biome, growing in grasslands and forest edges; sandy and rocky soils; uncommon; fire-resistant. It is a Brazilian endemic (MT, GO, MG). Flowering and fructification from September to May. Vernacular name is “verga-teso”. Illustration in Burkart (1971, p. 765).

15. **G. jussiaeana** Kunth., Nova Gen. et Spec. Plant. 6: 336. 1823. **Type:** Bonpland 853 (P).

G. jussiaeana Kunth var. *angustifolia* (Kunth) Burkart, Darwiniana 16: 714. 1971. **Type:** von Humboldt (F 2384!, photo).

Fig. 12

Diagnostic characteristics: subshrub erect; leaves pinate-trifoliolate; inflorescences with densely aggregated flowers, smaller than the subtended leaf.

Voucher: MT, São Félix do Araguaia, 11º33’S 51º13’W, V. C. Souza 14746, 21 Mar 1997, fl. (UFMT).

Commentary: *Galactia jussiaeana* var. *angustifolia* was synonymized with *G. jussiaeana* by Ceolin & Miotto (2011c), who showed through a morphometrical study that the only difference between these two taxa was the width of the leaflets. This species inhabits the biomes Amazonia, Cerrado and Caatinga, growing in open grasslands, shrublands, forest edges, gallery forests and anthropogenic grasslands, often with periodic droughts; sandy and rocky soils; abundant. Its geographic distribution encompasses some Caribbean Islands and countries from Central America, Venezuela, Colombia, Surinam, Guyana, Brazil (RR, AP, PA, MT, MS, MA, PI, CE, BA). Flowering and fructification in every months.

16. **G. lamprophylla** Harms, Bot. Jahrb. Syst. 33, Beibl. 72: 28. 1903. **Type:** Glaziou 20898 (F!, photo).

Diagnostic characteristics: subshrub erect ca. 0.3 m height; leaves pinate-trifoliolates; petiole almost absent; leaflets large 70 – 110 mm × 30 – 70 mm, densely pilose, lanose; inflorescences larger than the subtended leaf; flowers ca. 17 mm long, lax.

Voucher: DF, Brasília, 15°55'S 48°11'W, G. P. Silva 937, 02 Mar 1992, fl.fr. (CEN).

Commentary: this species inhabits the Cerrado biome, growing in dry and moist grasslands; clay and sandy soils; steep relief; rare. It is a Brazilian endemic (GO, DF, MG). Flowering and fructification from July to March.

17. **G. latisiliqua** Desv., Annal. Scienc. Nat. 9: 414. 1826. **Type:** Dombey 861 (P).

G. latisiliqua Desv. var. *chacoensis* Burkart, Darwiniana 16: 691. 1971. **Type:** Burkart 13116 (SI). **syn. nov.**

G. latisiliqua Desv. var. *orbicularis* Burkart, Darwiniana 16: 693. 1971. **Type:** Burkart13346 (SI). **syn. nov.**

Fig. 1

Diagnostic characteristics: subshurb erect or prostrate, apex voluble; leaves pinate-trifoliolate, coriaceous, pilose; inflorescences with lax and small (7 – 12 mm long) flowers.

Voucher: RS, Unistalda, M. Grings 559, 19 Feb 2009, fl.fr. (ICN).

Commentary: the varieties described by Burkart (1971) for this species do not fit our criteria to recognition of infraspecific taxa, hence being synonymized. *Galactia latisiliqua* inhabits almost all the Brazilian biomes, growing in dry grasslands and forest edges; 100 – 1200 m a.s.l.; clay and rocky soils; steep relief; common. It is of widely occurrence in South America, from Guianas to Argentina, Uruguay and Brazil (MS, PR, RS, SP, RJ, ES, MG, BA). Flowering and fructification from September to February.

18. **G. lineata** Ceolin & Miotto, Plant Syst. Evol. (submitted paper). **Type:** Ganev 2898 (NYBG).

Diagnostic characteristics: leaflets narrow 42 – 90 × 6 – 38 mm; inflorescences 37 – 135 mm long; standard 12 – 20 mm long; keel 11 – 13 mm long; endemic to semiarid regions of Northern Minas Gerais and Central-Southern Bahia.

Voucher: BA, Mucugê, 13º13S 04º12'W, L. Coradin 4515, 23 Aug 1981, fl. (CEN).

Commentary: this species was segregated from the *G. neesii* complex and inhabits the Caatinga biome, growing in dry and rocky grasslands; 500 – 1300 m a.s.l.; sandy and rocky soils. The collection *G.P. Silva 3199* (CEN) has evidences on stem of flowers of the holoparasite *Pilostyles* cf. *ulei* (Apodanthaceae), which is a common parasite of legumes. It is a Brazilian endemic (BA, MG). Flowering and fructification from November to March. Ilustrations in Lewis (1987).

19. **G. marginalis** Benth., Comm. Legum. Gen. 62. 1837. **Type:** Drummond 145 (US!, photo).

Fig. 2

Diagnostic characteristics: herbs stoloniferous-prostrate to erect; leaves unifoliolate; petiole present; inflorescence pauciflorous, subsessile, peduncle up to 15 mm long.

Voucher: RS, Rio Pardo, 30º12´S 52º28´W, G. B. Ceolin 127, 20 Out 2007, fl.fr. (ICN).

Commentary: this species is resistant to grazing and burning and is able to form large populations due to its xylopodium and stoloniferous growing through underground plagiotropic branchs. It inhabits the Pampa biome, growing in open grasslands, often among tussocks of grasses and moist grasslands; dry, shallow and rocky soils; common. Its geographic distribution is disjunct: south-western USA and southern South America, encompassing Argentina, Uruguay and Brazil (RS). Flowering and fructification from October to February.

20. **G. martioides** Burkart, Darwiniana 16: 742. 1971. **Type:** van de Venne (SI 7483!).

Fig. 5

Diagnostic characteristics: herb voluble; leaves digitate-trifoliolate; petiole present; leaflets 34 – 94 × 7 – 25 mm; inflorescences subumbeliform; flowers 16 – 20 mm long.

Voucher: RS, Itaara, 05 Mar 2009, fl.fr., *L.C.P. Lima & A. Pairet, 549* (ICN).

Commentary: this species inhabits the Pampa biome, growing in open grasslands, twining on grasses and other herbs; sandy and clay soils; steep relief; although restrict, this species forms great populations. It occurs in Argentina and Brazil (RS). Flowering and fructification from January to April.

21. **Galactia neesii** DC., Prodrom. Syst. Nat. Regn. Veg. 2: 238. 1825. **Type:** Pohl 3402 (BR!).

Galactia neesii DC. var. *macropoda* (Cowan) Burkart, Darwiniana 16: 732. 1971. **Type:** Braga & Lange 164 (SI!).

Galactia martii var. *martii* DC., Syst. Nat. 2: 238. 1825. **Type:** Martius (M 12722).

Galactia martii DC. var. *acuta* Burkart, Darwiniana 16: 738. 1971. **Type:** Pedersen 5181 (SI!).

Galactia stereophylla Harms, Repert. Spec. Nov. 19: 17. 1923. **Type**: Glaziou 20900 (F!, photo).

Galactia crassifolia (Benth.) Taub., Die Natürl. Pflanzenfam. 3: 368. 1894. **Type:** Pohl 2895,(W!, photo).

Fig. 4

Diagnostic characteristics: herbs to subshrubs prostrate to erect, only those individuals growing in Chapada dos Veadeiros (GO) have voluble habit; keel ca. 14 mm long.

Voucher: SC, Capão Alto, 28º08'S 50º38'W, G. B. Ceolin 134, 12 Jan 2008, fr. (ICN).

Commentary: the current circumscription of *G. neesii* results from the review of the taxonomic complex formed by *G. neesii*, *G. martii*, and allies performed by Ceolin & Miotto (2011d). This species inhabits the Cerrado biome and the altitude grasslands interspersed within forests of *Araucaria angustifolia* (Bertol.) Kuntze (Araucariaceae) in the highlands of southern Brazil (Mata Atlantica biome), usually growing in dry fields, 300 – 1300 m a.s.l.; sandy, clay and stony soil; abundant. Its geographical distribution includes Argentina, Paraguay and Brazil (SC, PR, SP, MG, MS, MT, DF,GO, BA). Flowering and fruiting from October to March.

22. **G. peduncularis** (Benth.) Taub., Bot. Jahrbüch. 21: 439. 1896. **Type:** Pohl 2071 (W!).

G. nana Burkart, Darwiniana 16: 775. 1971. **Type:** Irwin 25232 (NYBG!). **syn. nov.**

Fig. 13

Diagnostic characteristics: subshrub erect 0.3 – 0.6 m height; leaves pinate-trifoliolate, rigid, coriaceous; inflorescences large 90 – 270 mm long; flowers lax, 20 – 27 mm long.

Voucher: DF, Brasília, 15º48'S 47º48'W, G. P. Silva 5481, 29 Set 2001, fl.fr. (CEN).

Commentary: as with *G. bullata* and *G. irwin*, we are here synonymizing *G. nana* because the single specimen collected is the type and because the general appearance of this species is very similar to *G. peduncularis*, mainly due to trifoliolate, coriaceous leaves, and long inflorescences, with large and lax flowers (~ 20 mm). This species inhabits the Cerrado biome, growing in dry and moist grasslands; 900 – 1100 m a.s.l.; clay, sandy, shallow soils, often with rock outcrops; steep relief; abundant. It is a Brazilian endemic (DF, GO, MG). Ceolin et al. (2011e) showed that fire has a crucial role in its life-cycle, because its abundance significantly increases after burning. Flowering and fructification from September to November. Vernacular name is “chapéu-de-sol”.

23. **G. pretiosa** Burkart, Darwiniana 9: 93. 1949. **Type:** Hatschbach 1126 (SI!).

G. pretiosa Burkart var. *trifoliolata* (Hassl.) Burkart, Darwiniana 16: 753. 1971. **Type:** Fiebrig 754 (SI!).

Fig. 6

Diagnostic characteristics: herb erect, with no stoloniferous growing; leaves unifoliolate (rarely trifoliolate); petiole absent; inflorescences pauciflorous (1 – 3 flowers); flowers lax; peduncle larger than 15 mm long.

Voucher: PR, Mangueirinha, 26°09'S 52°11'W, G. B. Ceolin 113, 18 Dez 2006, fl.fr. (ICN).

Commentary: Burkart (1971) described *Galactia pretiosa* var. *trifoliolata* to include individuals with three leaflets instead of the usual one. As we emphasized earlier, individuals bearing differences in the number of leaflets unless accompanied by differences in other attributes do not necessarily represent independent evolutionary lineages and have no valid taxonomic status. This species inhabits the Cerrado biome and the altitude grasslands of the Mata Atlantica biome in southern Brazil, growing in open fields and hillsides; 300 – 1200 m asl; clay, shallow and rocky soils; common. Its geographical distribution includes Paraguay, Argentina and Brazil (RS, SC, PR, MS, MT, SP, MG, BA). Flowering and fruiting from October to March.

24. **G. remansoana** Harms, Bot. Jahrbüch.42: 216. 1908. **Type:** Ule 7375 (F!, photo).

Diagnostic characteristics: herb prostrate, pilose; leaves pinate-trifoliolate, leaflets suborbicular to obovate; endemic to Caatinga biome.

Voucher: BA, Xique-Xique, L. P. de Queiroz 12165, 11 Mar 2006, fl.fr. (HUEFS); MG, Mocambinho, M. Brandão 20310, 21 Jan 1992, fl.fr. (PAMG).

Commentary: this species inhabits the Caatinga biome, growing in scrublands; 400 – 600 m a.s.l.; sandy or rocky soils, sometimes with arenite outcrops. It is a Brazilian endemic (BA, CE, PE, MG); the specimen *M. Brandão 20310* (PAMG) from the city of Mocambinho is the first record of this species to Minas Gerais State, which it is not surprising since that city is also within the Caatinga biome. Flowering and fructification from January to April. Vernacular names are “feijão-bravo” and “jitirana-preta”.

25. **G. striata** (Jacq.) Urban., Symbolae Antillanae 2: 320. 1900. **Type:** Bertero (F 33424!, photo).

G. striata (Jacq.) Urban. var. *tenuiflora* (Klein ex Willd.) Burkart, Darwiniana 16: 721. 1971. **Type:** Klein (F 2397!, photo). **syn. nov.**

Fig. 9

Diagnostic characteristics: vines; leaves pinate-trifoliolate; petiole present; leaflets almost totally glabrous, membranaceous; inflorescences sessile to peduncled; it is the unique species of *Galactia* who is able to growing in forests.

Voucher: MS, Nioaque, 21º26'S 57º02W, L. C. P. Lima 427, 23 Set 2008, fl.fr. (ICN).

Commentary: we are synonymizing *G. striata* var. *tenuiflora* under *G. striata* due to the lack of consistence in criteria used to describe this infraspecific taxon, mainly regarding both vegetative and reproductive labile traits. This species is intensively cultivated as forage, even existing a commercial cultivar (cv. Yarana); for this reason, it is not possible to be sure where it is native or introduced and which are its original ecological preferences, because it is widely distributed for the entire american continent. However, there are evidences suggesting that it originally grows in the riparian forests of western Brazil. Flowering and fructification in every months.

26. **G. weddelliana** Benth., Flora Brasiliensis 15: 142. 1859. **Type:** Weddell 2979 (P).

Diagnostic characteristics: subshrub erect; leaves unifoliolate; inflorescence sessile, pauciflorous (up to 5 flowers); flowers 20 mm long, lax.

Voucher: MT, Campo Verde, G. Hatschbach 63532, 19 Out 1995, fl. (MBM).

Commentary: this species inhabits the Cerrado biome, growing in open grasslands, often after burning; clay and sandy soils; rare. It is a Brazilian endemic (MT). Flowering and fructification from September to February. Illustrations in Burkart (1971).

Excluded species

Burkart (1971) cited the occurrence of *G. dubia* to the Brazilian States of Ceará and Maranhão, which represents an outstanding disjunction in its geographic distribution in South América since that this species typically grows in Bolivia and northern Argentina (Salta and Jujuy provinces). The main distinctive characteristic of *G. dubia* is axillary and sessile inflorescences. For this reason, we suspect that Burkart may have confounded this species with some specimens of *G. striata* bearing reduced inflorescences (former assigned as *G. striata* var. *tenuiflora*), which occurred in the referred Brazilian States. Thus, we are excluding out *G. dubia* of this revision, because it was not possible to confirm unequivocally if this species grows in Brazil.

Concluding remarks and future perspectives

We found that the genus *Galactia* in Brazil is represented by 26 taxa, from which one has been described here, another has been recorded to Brazil for the first time and six have never been illustrated before. We synonymized 11 formerly accepted taxa because the criteria employed to their acceptance appear inconsistent under the species concept we used in this study. Despite being comprehensive, we acknowledge that our synopsis is tentative and must to be continuously improved, because the genus is remarkably non-monophyletic and have an evolutionary history still unclear. In short, this synopsis ought to be viewed as a starting point rather than a complete work, whose main goal was helping in the recognition of the species belonging to genus.

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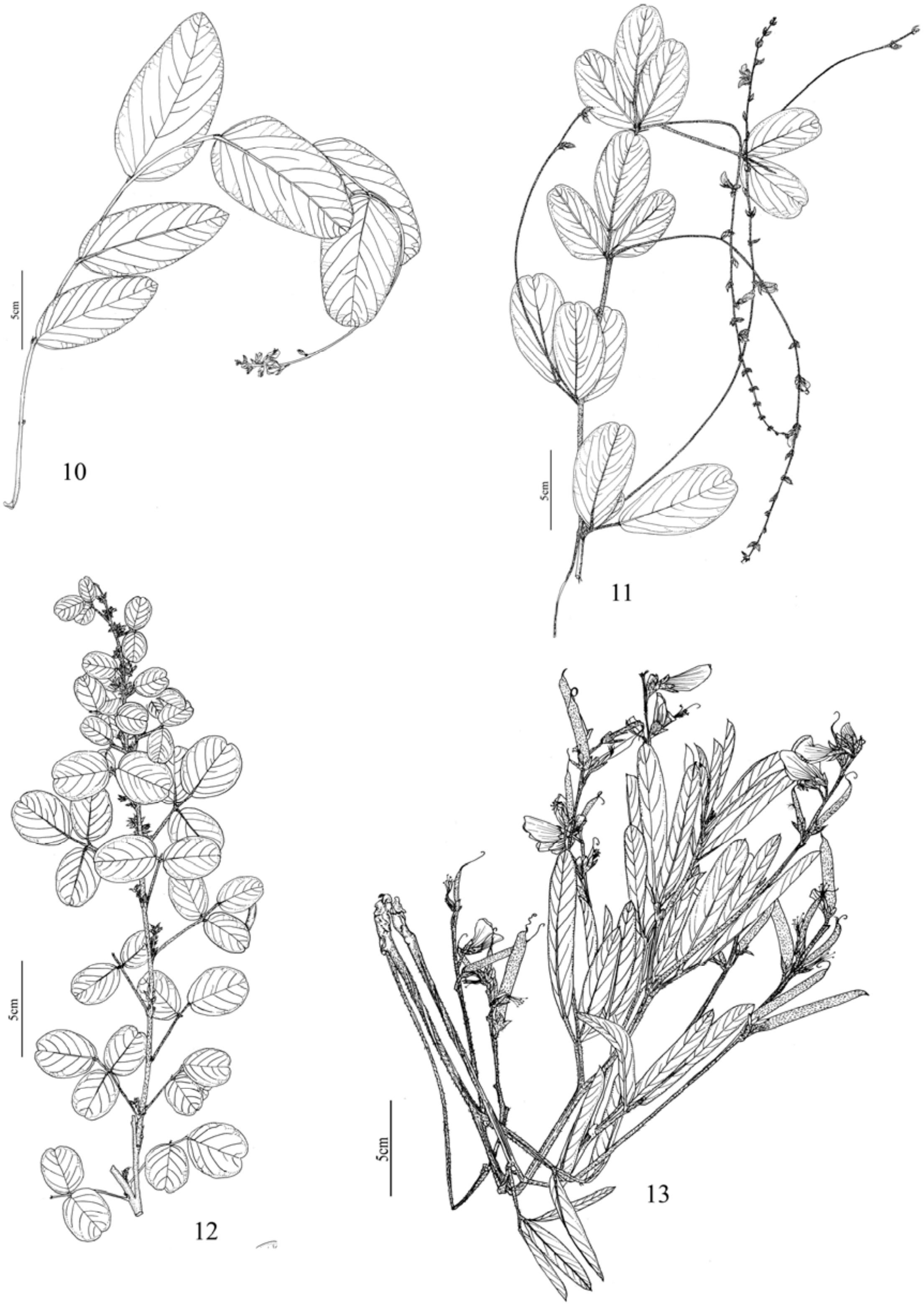
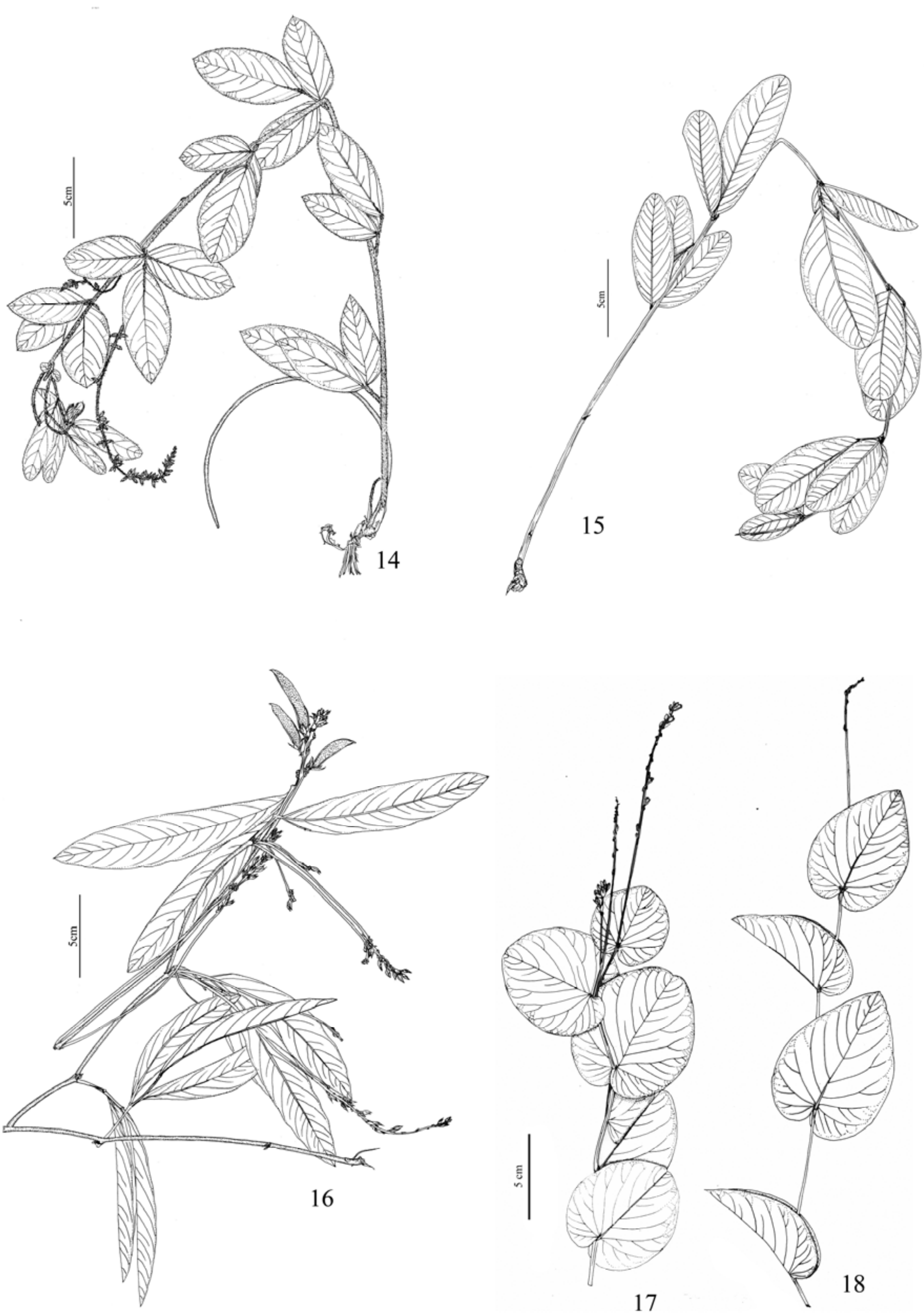
Figures 1–4: 1) *G. latisiliqua* 2) *G. marginalis* 3) *G. gracillima* 4) *G. neesii*

Figures 5–8: 5) *G. martioides* 6) *G. pretiosa* 7) *G. benthamiana* 8) *G. boavista* 9) *G. striata*



Figures 10–13: 10) *G. decumbens* 11) *G. dimorpha* 12) *G. jussiaeana* 13) *G. peduncularis*



Figures 14–18: 14) *G. eriosematoides* 15) *G. glaucescens* 16) *G. heringeri* 17) and 18) *G. cordifolia*

Appendix 1

Appendix 1

a) Map of Brazilian biomes

From: Brazilian Institute of Geography and Statistics (IBGE). Freely available in: http://www.ibge.gov.br/home/presidencia/noticias/noticia_visualiza.php?id_noticia=169

b) Abbreviations of Brazilian States (in alphabetical order)

Acre (AC); Alagoas (AL); Amapá (AP); Amazonas (AM); Bahia (BA); Ceará (CE); Distrito Federal (DF); Goiás (GO); Espírito Santo (ES); Maranhão (MA); Mato Grosso (MT); Mato Grosso do Sul (MS); Minas Gerais (MG); Pará (PA); Paraíba (PB); Paraná (PR); Pernambuco (PE); Piauí (PI); Rio de Janeiro (RJ); Rio Grande do Norte (RN); Rio Grande do Sul (RS); Rondônia (RO); Roraima (RR); São Paulo (SP); Santa Catarina (SC); Sergipe (SE); Tocantins (TO).

Posfácio

Como dito no prefácio, o objetivo desta tese foi pôr ordem ao caos que se configurava a taxonomia de *Galactia* para o Brasil. Todavia, como dito por Burkart (1971), "*no creo haber logrado ser completo*", pois muitos dos problemas responsáveis pela desordem dentro do gênero não foram sanados neste trabalho; para isso, é fundamental que se obtenha uma filogenia realmente abrangente de *Galactia*, a qual permita a elucidação de sua história evolutiva e das relações com gêneros próximos.

Por outro lado, "(...) *espero que esta actualización llegue a constituir un paso adelante*"; mais do que um passo adiante, esta tese deve ser encarada como um ponto de partida, um passo inicial, no entendimento deste gênero ao mesmo tempo tão desafiador e fascinante quanto enlouquecedor. A principal contribuição desta tese, por assim dizer, reside no fato de que, dos *ca.* de 40 nomes até então aceitos dentro do gênero como ocorrendo para o país, entre espécies, variedades e formas, só 26 restaram como válidos; destes, três são inéditos (ou novas espécies ou novos registros). Estes 26 táxons foram propostos e podem ser reconhecidos com base em critérios objetivos que foram amplamente discutidos e ponderados nesta tese; estes critérios levaram a elaboração de uma chave taxonômica a mais clara e não-ambígua quanto possível para um gênero com tanta plasticidade morfológica e tão poucos caracteres taxonomicamente informativos.

Em resumo, esta tese tem duas utilidades principais: a primeira é para quem busca uma discussão sobre a prática taxonômica tradicional e novas soluções para problemas semelhantes aos enfrentados nesta tese (seção I); a segunda é para quem pretende trabalhar com o gênero *Galactia* e precisa identificar seus táxons ou obter informações a seu respeito (seções II, III e seção única).

Para finalizar, falando em primeira pessoa agora, gostaria de dizer que realizar este trabalho foi recompensador, pois a satisfação de vencer um desafio tão difícil é inigualável. Ainda que muitas coisas não tenham sido resolvidas, espero ter contribuído também para encorajar outros pesquisadores que igualmente trabalham com grupos difíceis a vencer seus desafios. Somente assim é que poderemos compreender e preservar toda a diversidade destes organismos eucariontes autotróficos dependentes de luz (vulgo plantas) cuja evolução vem acontecendo desde muito mais de meio bilhão de anos.