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# Diversification history of *Adesmia* ser. *psoraleoides* (Leguminosae): Evolutionary processes and the colonization of the southern Brazilian highland grasslands

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## ABSTRACT

A molecular phylogeny is used to analyze the diversification history of *Adesmia* ser. *psoraleoides*, and its implications for understanding the historical assembly of the grasslands in the highlands of southern Brazil. All species of *A. ser. psoraleoides* were sampled, including multiple accessions for each species, plus representative species of the rest of *Adesmia* covering its geographic distribution. Phylogenetic analyses were based on nuclear and plastid DNA sequences, and a plastid *matK* phylogeny was dated. *A. ser. psoraleoides* is a well-supported monophyletic group, nested within the series *bicolores*, *muricatae*, *subnuda*, *longisetae* and *candidae*. The stem of *A. ser. psoraleoides* is c. 11 Mya, but most extant species diversified c. 3–5 Mya, suggesting that the flora of the subtropical grasslands of southern Brazil was assembled recently, co-incident with the expansion of other tropical grassland systems globally.

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## 1. Introduction

Analyses of phylogenetic trees integrated with developing knowledge of the timing and nature of climatic and geological changes have been providing new perspectives on the evolutionary history of biomes (Byrne et al., 2008). In the Neotropics, such studies have provided new insights into the historical assembly of the floras of Amazonia (e.g., Pennington and Dick, 2004), the savannas of the Brazilian cerrados (Simon et al., 2009) and seasonally dry tropical forests (e.g., Särkinen et al., 2011). This paper presents the first phylogenetic biogeographic study of the “Campos de Cima da Serra” (CCS grasslands), focusing on a clade of Leguminosae, *Adesmia* ser. *psoraleoides*, with the goal of illuminating the history of this poorly known biome.

The CCS is a subtropical grassland located in the highlands of southern Brazil. The CCS was often considered as a northern extension of the more southerly and low altitude temperate pampas grasslands (“Campos Sulinos” – southern grasslands), but Iganci et al. (2011) made a strong case that the CCS is a distinct subtropical formation

with an unappreciated level of plant species endemism as high as 25%. A principal goal of this study is to make the first investigation of the age of origin of the CCS grasslands by evaluating the age of origin of species of *A. ser. psoraleoides*, which are confined to this area. We test the hypothesis that the CCS are ecologically young, as is the case for other C4 grasslands globally (Cerling et al., 1997; Beerling and Osborne, 2006; Stromberg, 2011) and the neotropical savannas (“cerrados”) that are situated further north in Brazil (Pennington et al., 2006; Simon et al., 2009; Simon and Pennington, 2012).

The geographical affinities of the CCS flora are still poorly known. Relationships of the CCS flora with the Andes have been suggested by several workers and explored floristically (Rambo, 1951, 1953a,b, 1956; Smith, 1962; Safford, 1999, 2007; Iganci et al., 2011), but no phylogenetic studies have been done thus far. Around one-third of the genera found in the southern Brazilian flora as a whole are related to lineages assumed to be temperate Austral–Antarctic and Andean in origin (Rambo, 1951, 1953a,b, 1956; Smith, 1962; Safford, 1999, 2007; Waechter, 2002; Iganci et al., 2011). Many authors have assumed that eastern South American species in these groups are secondary radiations of Andean and Austral–Antarctic lineages (Rambo, 1953a,b; Safford, 1999, 2007; Waechter, 2002). One aim of this paper is to test this scenario in a phylogenetic context for *Adesmia*.

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*Adesmia* comprises around 240 species, and is formally subdivided into two subgenera (*Adesmia* subgen. *Adesmia* and *Adesmia* subgen. *Acanthadesmia* Burkart) and 43 series based on morphology (Burkart, 1967; Lewis et al., 2005). Most species are concentrated along the Andes spreading in temperate areas from the north of Peru to “Tierra del Fuego”, the southern tip of South America, and it is the most species-rich genus of Leguminosae in temperate South America (Burkart, 1967). The northeast limit of *Adesmia* is in the CCS. Almost all 11 species of *A. ser. psoraleoides* Burkart are endemic to the CCS, growing mainly in patches of grassland and rock outcrops amongst forests dominated by *Araucaria angustifolia* (Bertol.) Kuntze (Miotto and Leitão Filho, 1993; Miotto and Waechter, 1996; Iganci and Miotto, 2011). Given the high level of endemism in the CCS and the occurrence of diversity in other biomes, including the Andes and more southern grasslands, *A. ser. psoraleoides* makes an excellent study group to investigate the biogeographic affinities of the CCS flora and its diversification.

Species of *A. ser. psoraleoides* are unarmed, perennial herbs or shrubs that in common with other *Adesmia* species have decorative yellow flowers with brownish nectar guides and fruits that are a plane hemicaspedium with arillate seeds (Burkart, 1967; Miotto and Leitão Filho, 1993) (Fig. 1). Such morphological characters and others have collectively been used to circumscribe the series, but they are not synapomorphies because all are shared with other *Adesmia* species (Burkart, 1967). One aim of this study – a key prerequisite of the subsequent biogeographic analysis – is to evaluate the monophyly of *A. ser. psoraleoides* and how it relates to other species of *Adesmia* from other geographic areas.

Here we present a phylogenetic analysis of the diversification history of *A. ser. psoraleoides*, providing a time frame for the diversification of major clades. We address the following questions: (1) Is *A. ser. psoraleoides* a monophyletic group and when did this group originate?; (2) What is the sister group of *A. ser. psoraleoides* and where is it from?; and finally (3) What can be inferred from the species diversification pattern of *A. ser. psoraleoides* in terms of the evolutionary history of the CCS? Species diversification pattern is analyzed based on a time-calibrated, densely sampled plastid and nuclear ribosomal internal transcribed spacer (ITS) phylogeny of *A. ser. psoraleoides*. A further 35 species of *Adesmia* (from all main geographic areas where the genus occurs) are included as outgroups to fully analyze the sister relationships of the ingroup.

## 2. Material and methods

### 2.1. Taxon sampling

In total, 53 accessions of *A. ser. psoraleoides* were sampled, representing all the 11 species of the series and completely covering its geographic range. In addition, further accessions of 35 southern South American *Adesmia* species were included. These samples were chosen across the geographic distribution of the genus. Twenty-one accessions representing all five remaining genera of the wider *Adesmia* clade of the

Dalbergioid legumes were used as outgroups. Plant material was mostly sampled from the wild, and from cultivated specimens obtained from seeds collected in the wild or held in national collections. All collections plus georeferenced herbarium records for *A. ser. psoraleoides* were mapped using DIVA-GIS (Hijmans et al., 2004) to indicate the most species rich areas.

### 2.2. DNA extraction, amplifications and sequencing

Total DNA was isolated from 0.3 g of silica gel-dried leaf tissue using a modified CTAB extraction method (Doyle and Doyle, 1987). The molecular markers analyzed were the nuclear ribosomal 5.8S and flanking internal transcribed spacers – ITS1 and ITS2 (Delgado-Salinas et al., 1999), and three chloroplast (cpDNA) regions: the *trnK* intron including *matK* (Lavin et al., 2000, 2001; Wojciechowski et al., 2004), *rbcl* (Olmstead et al., 1992; Kajita et al., 2001) and *rps16* (Oxelman et al., 1997). Polymerase chain reaction (PCR) was conducted with a reaction volume of 25 µL containing ~20 ng of DNA template, 1× buffer, 2 µM MgCl<sub>2</sub>, 1 µM of each dNTP, 10 pmol of each primer and 1 U of *Taq* DNA Polymerase (CenBiot, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil). For ITS amplifications, 1 µL of DMSO, and for *matK* amplifications, 1 µL of BSA were also added. PCR conditions were as follows: ITS: 95 °C for 4 min, 35 cycles of 30 s at 94 °C, 30 s at 58 °C and 2 min at 72 °C, followed by a final extension of 7 min at 72 °C; *matK*: 95 °C for 4 min, 35 cycles of 30 s at 94 °C, 30 s at 50 °C and 2 min at 72 °C, followed by a final extension of 7 min at 72 °C; *rbcl*: 95 °C for 2 min, 30 cycles of 30 s at 95 °C, 30 s at 53 °C and 1:30 min at 72 °C, followed by a final extension of 7 min at 72 °C; *rps16*: 95 °C for 4 min, 35 cycles of 30 s at 94 °C, 30 s at 50 °C and 2 min at 72 °C, followed by a final extension of 7 min at 72 °C. PCR products were cleaned using exonuclease I and shrimp alkaline phosphatase (Exo/SAP) and sequenced using BigDye v.3.1 chemistry (Applied Biosystems, Warrington, UK). For the *matK* region, the samples were amplified in two parts and sequenced in eight reactions, using both the two outer and the two innermost primers for PCR amplification, and four additional internal primers for sequencing (see Lavin et al., 2000, 2001, for details). Consensus sequences were assembled using Sequencher v.3.1 (GeneCodes Corp., Ann Arbor, Michigan, USA), and imported to BioEdit (Hall, 1999) for manual alignment.

### 2.3. Phylogenetic analyses

All DNA regions were first analyzed independently (individual results in Table 1 and in S11), and the cpDNA regions were concatenated in a single matrix since resulting trees showed no incongruent clades. Due to problems in PCR and sequencing of some regions and for some of the accessions, the combined plastid matrix comprised 29 accessions for which all data for all regions were available. This combined plastid DNA matrix was analyzed and the resultant phylogenetic trees were compared with those arising from analysis of the ITS data.

**Table 1**

Summary of the lengths and indices of the resulting strict consensus tree in parsimony analyses of separated and combined datasets.

Data partition	No. of characters	No. of parsimony informative characters (%)	No. of most-parsimonious trees	Tree length	CI	RI
ITS	643	153 (23.8)	601	283	0.65	0.78
<i>matK</i>	2664	63 (2.3)	3869	261	0.73	0.47
<i>rps16</i>	421	20 (4.6)	3975	64	0.50	0.21
<i>rbcl</i>	639	79 (12.3)	3781	298	0.67	0.15
cpDNA combined matrix	3546	40(1.12)	5034	156	0.86	0.72
Global combined matrix	4178	90 (2.15)	432	248	0.86	0.84
<i>matK'</i>	1573	212 (13.4)	10,000	483	0.84	0.96

CI and RI are, respectively, the consistency and retention indices. *matK'*: analysis of *Adesmia* clade.

Because sampling across *Adesmia* and outgroups was widest in the *matK* dataset and because this locus has been used extensively for phylogenetic dating in legumes, a *matK*-only analysis was used to investigate molecular divergence time estimates.

Parsimony analyses were conducted using the program PAUP\* version 4.0b10 (Swofford, 2003). All the Maximum Parsimony (MP) analyses used a first step heuristic search with 1000 random addition replicates, tree bisection–reconnection (TBR) branch swapping and multrees on, with all character states unordered and equally weighted. A second stage search used all trees saved from the initial search, branch-swapped using TBR with multrees and steepest descent implemented, saving a maximum of 5000 most-parsimonious trees. Strict consensus trees were calculated from all most parsimonious trees. Branch support was evaluated using MP bootstrap analysis with 1000 bootstrap replicates of new heuristic searches (TBR branch swapping, multrees and steepest descent implemented). Bayesian analyses were performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). The best-fit models of sequence evolution were chosen using the Akaike Information Criterion (AIC) implemented in the program Modeltest (Posada and Crandall, 1998). The GTR + G nucleotide substitution model was chosen for *matK* and cpDNA analyses, and TIM + G for *ITS* analysis. Bayesian analyses were initiated from random starting trees, in two independent runs of 10,000,000 generations and four chains. In each run, trees were sampled every 100 generations, log-likelihood scores were compared for convergence, and the first 25% of trees were discarded as burn-in. Then, 50% majority-rule consensus and Bayesian posterior probabilities were generated for the resulting trees.

Divergence times were estimated for *matK* dataset using a Bayesian MCMC analysis conducted in BEAST version 1.6.1 package (Drummond and Rambaut, 2007) under the uncorrelated lognormal relaxed molecular clock approach. The nucleotide substitution model for *matK* matched that used in Bayesian analysis. Divergence times within the *Adesmia* clade were based on an initial higher level family-wide dated phylogenetic study of a large *matK* dataset, using 839 terminal taxa and 23 fossil constraints (Simon et al., 2009). In our study, three secondary calibration points were taken from dates inferred by Simon et al. (2009) and used for estimating divergence dates in BEAST using a normal distribution prior: the most recent common ancestor of *Adesmia* and *Chaetocalyx* (mean estimate of 40.9 Mya, standard deviation of 4.0); the most recent common ancestor of *Poiretia* and *Zornia* (mean

estimate of 23.3 Mya, standard deviation of 2.3); and the most recent common ancestor of *Nissolia* and *Chaetocalyx* (mean estimate of 12.3 Mya, standard deviation of 1.2). Three separate runs were set and 10,000,000 generations were performed, sampling every 100 generations. Results were accessed in Tracer version 1.4 (Drummond and Rambaut, 2007), and data from multiple runs were combined after exclusion of burn-in trees.

### 3. Results

#### 3.1. Nuclear data

The alignment of *ITS* sequences included 49 terminal taxa, and 643 characters, of which 153 (23.8%) were parsimony informative. The parsimony analysis yielded 601 equally most parsimonious trees of 283 steps, CI = 0.65 and RI = 0.78. The results are summarized in Table 1.

The *ITS* phylogenetic analysis suggests that *A. ser. psoraleoides* is monophyletic (Fig. 2) with high parsimony bootstrap support (100%) and Bayesian posterior probability (1.0). *Adesmia tristis* is placed as sister to all other species within the series (with low bootstrap support of 57.8% and Bayesian posterior probability of 0.93).

#### 3.2. Plastid data

The combined plastid matrix (*matK*, *rps16* and *rbcl*) with 29 terminal taxa included 3546 characters, of which 128 (3.6%) were parsimony informative (Table 1). The parsimony analysis yielded 5034 equally most parsimonious trees of 156 steps, CI = 0.86 and RI = 0.72. Both parsimony and Bayesian analyses resulted in similar topologies and the parsimony strict consensus tree is shown in Fig. 3. The monophyly of *A. ser. psoraleoides* is also well supported by the bootstrap (100%) and Bayesian posterior probability (1.0).

Another analysis was performed for the coding region of *matK*, where a wider sampling of the genus *Adesmia* was included, covering all its geographical range, plus samples from the other five genera of the wider *Adesmia* clade (Fig. 4). The matrix included 88 terminal taxa, and 1573 characters, of which 356 (22.6%) were parsimony informative. Parsimony analysis yielded 10,000 equally most parsimonious trees of 483 steps, CI = 0.84 and RI = 0.96. This analysis also confirmed

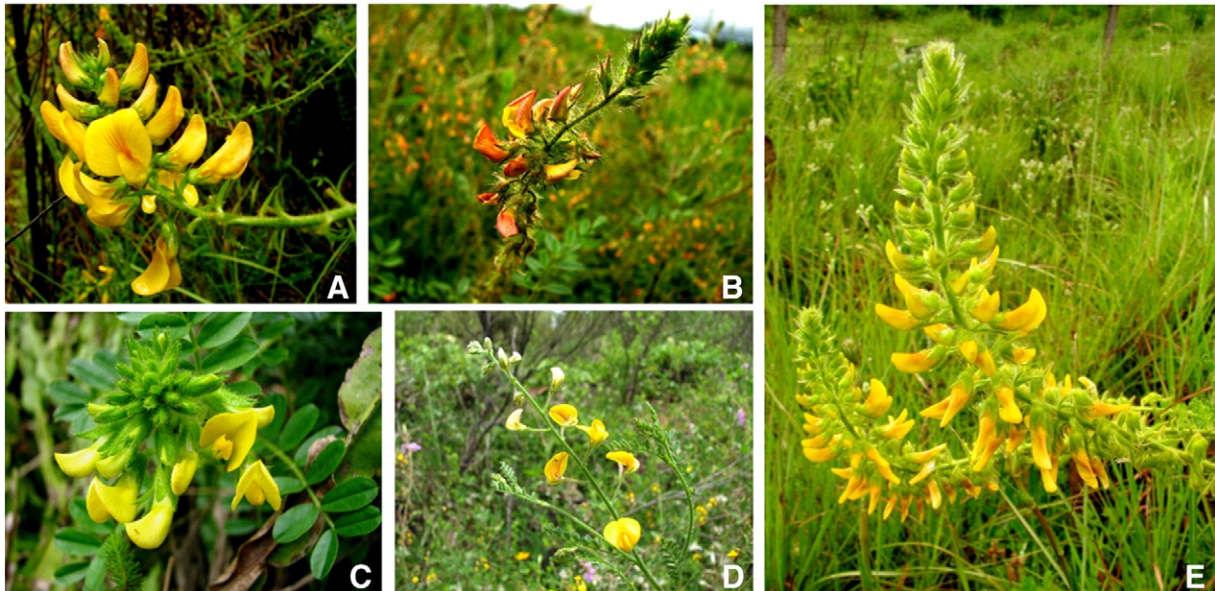


Fig. 1. Morphological diversity in *Adesmia ser. psoraleoides* endemic to the southern Brazilian subtropical grasslands. A. *Adesmia paranensis* Burkart; B. *Adesmia rocinhensis* Burkart; C. *Adesmia reitziana* Burkart; D. *Adesmia tristis* Vogel; E. *Adesmia psoraleoides* Burkart.



Fig. 2. Phylogeny of *Adesmia* ser. *psoraleoides* Burkart based on DNA sequences of the nuclear ribosomal 5.8S and flanking internal transcribed spacers (ITS1 and ITS2). One of the 601 most parsimonious trees. Numbers above and below nodes are bootstrap percentages and posterior probabilities from parsimony and Bayesian analysis respectively.

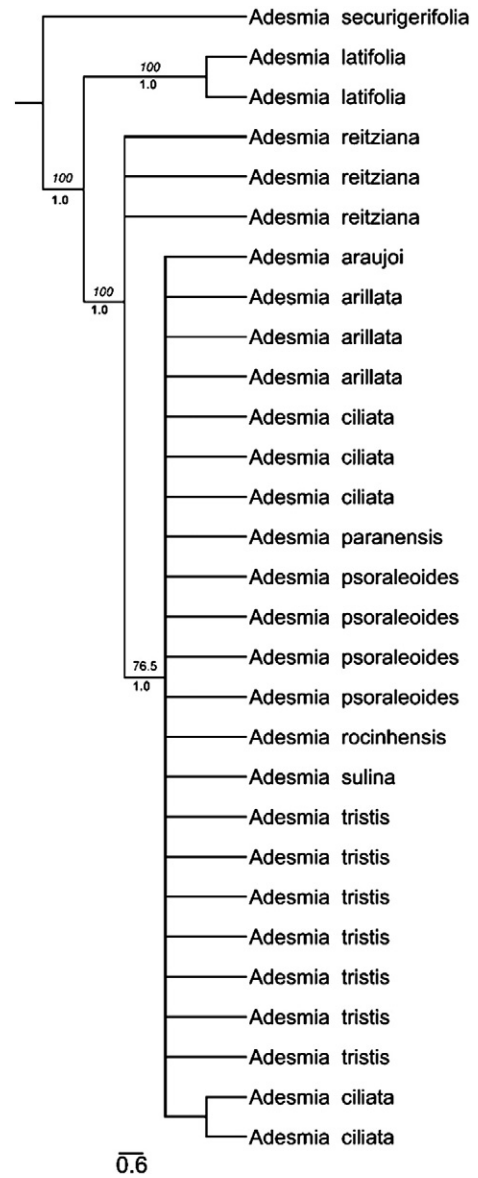


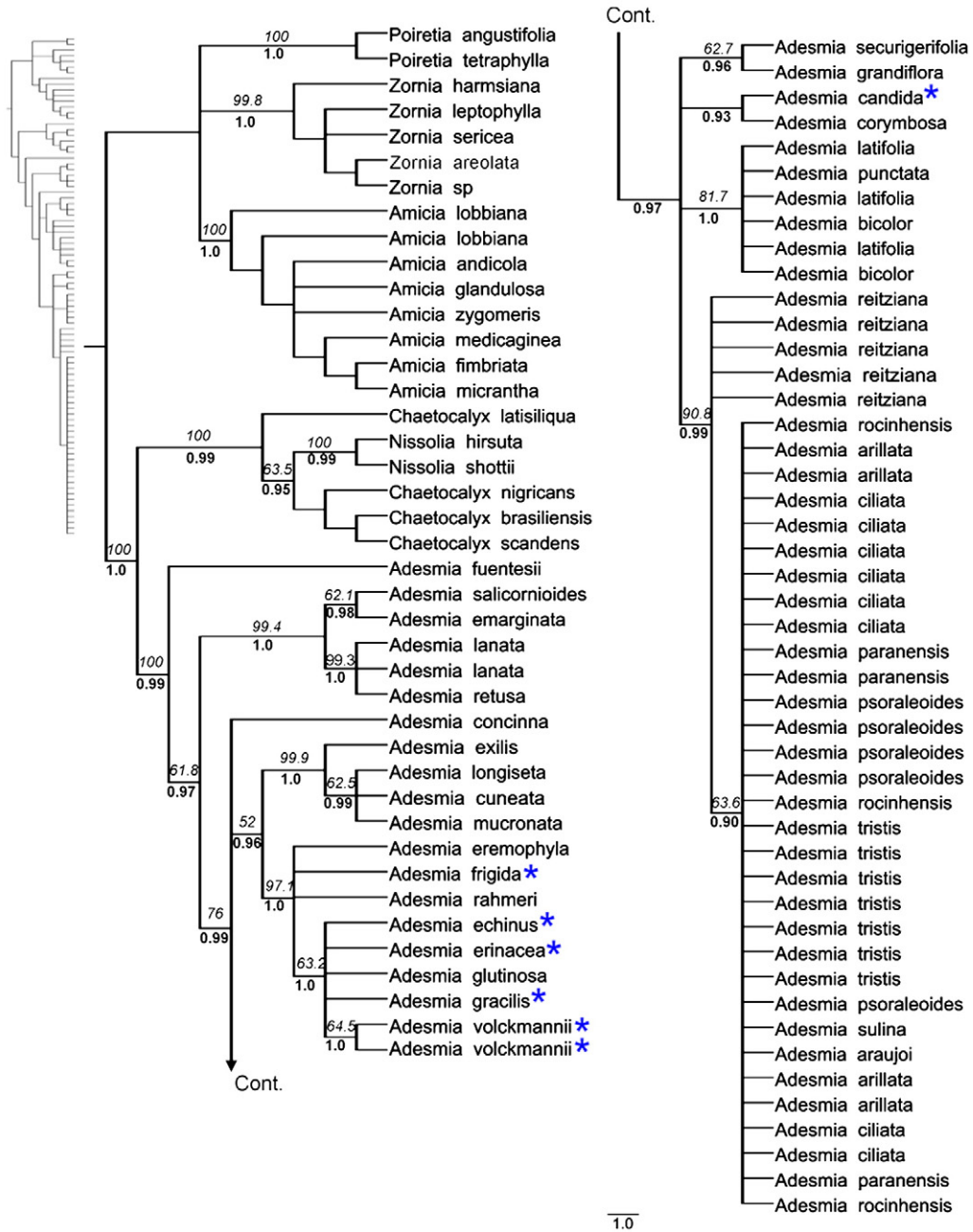
Fig. 3. Phylogeny of *Adesmia* ser. *psoraleoides* Burkart based on cpDNA sequences of the combined plastid markers *matK*, *rps16* and *rbcl*. Strict consensus tree of the 5034 most parsimonious trees. Numbers above and below nodes are bootstrap percentages and posterior probabilities from parsimony and Bayesian analyses respectively.

*A. ser. psoraleoides* as a monophyletic group well supported by both parsimony bootstrap analysis (90.8%) and by Bayesian posterior probability (0.99). In contrast to the *ITS* analysis, *Adesmia reitziana* was placed as sister to the other species within the series (bootstrap support of 76.5% and Bayesian posterior probability of 1.0), while *A. tristis* was placed between other species of the series.

4. Discussion

4.1. Phylogenetic relationships of *A. ser. psoraleoides*

The molecular phylogenetic analysis confirmed *A. ser. psoraleoides* as a monophyletic group (Figs. 2–4, SI3), agreeing with previous morphological and ecological circumscriptions, including the absence of stamens fused with the banner, and self-pollination in all species within the series (Burkart, 1967; Miotto and Leitão Filho, 1993; Tedesco et al., 2000; Iganci and Miotto, 2011). While characters used to circumscribe



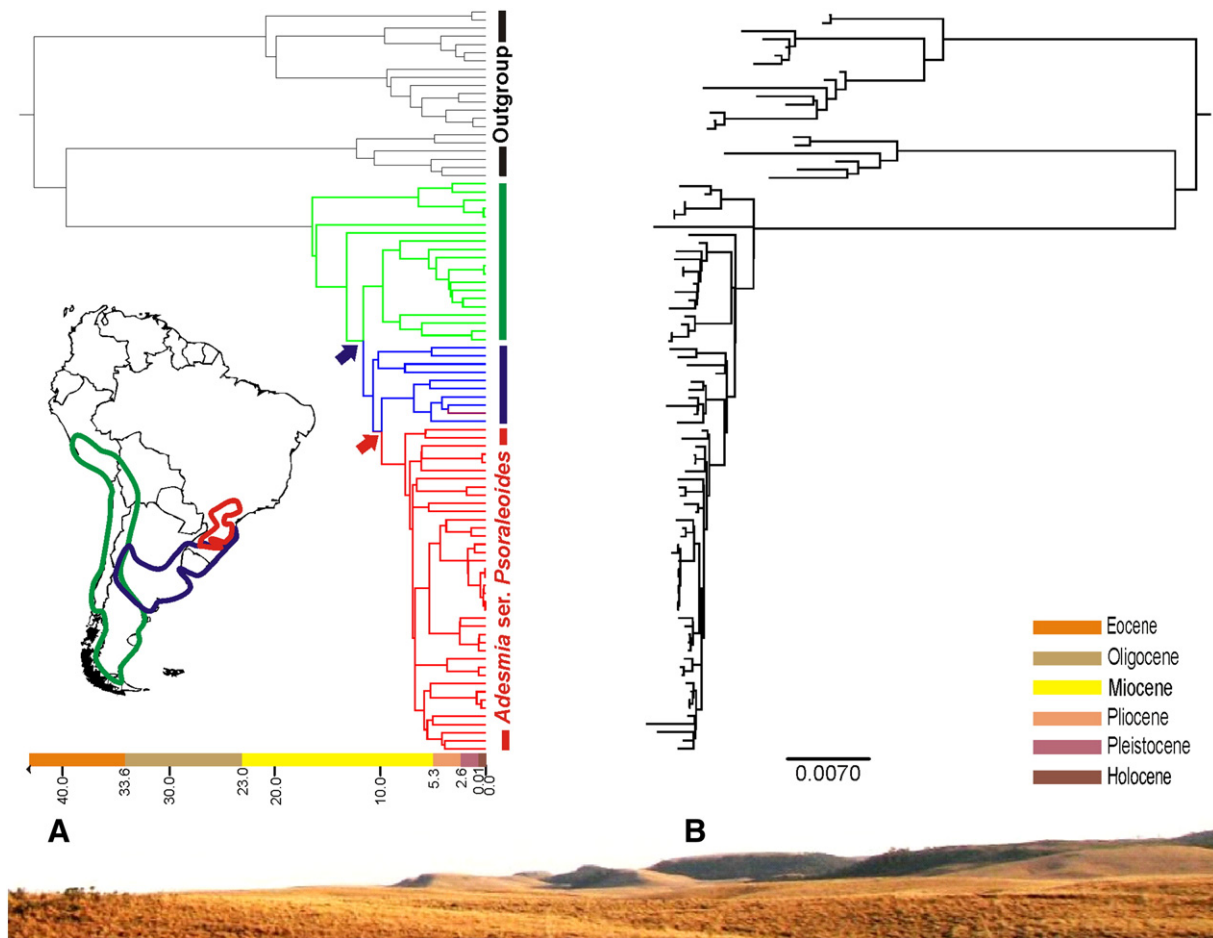
**Fig. 4.** Phylogeny of *Adesmia* ser. *psoraleoides* Burkart based on DNA sequences of the *matK*. Strict consensus tree of the 10,000 most parsimonious trees produced in parsimony analysis. Numbers above and below nodes are bootstrap percentages and posterior probabilities from parsimony and Bayesian analysis respectively. Species currently classified within the subgenus *Acanthadesmia* are indicated by a star (\*).

*A. ser. psoraleoides* are found in different lineages, when used together they can effectively characterize the series (Burkart, 1967; Miotto and Leitão Filho, 1993).

In comparing the *ITS* and cpDNA analyses, some incongruence was observed and for this reason we did not combine the datasets and analyze them simultaneously. However, the incongruence only concerns two species, and it does not affect our main conclusions. In the *ITS* analysis, *A. tristis*, a narrowly restricted species known only from a few small populations from the “Serra Geral” range in Santa Catarina, Brazil, is placed as sister to all other species within the series, though with relatively weak bootstrap support. *A. tristis* is widespread through the CCS, and the result from *ITS* is in agreement with its distinct morphological characters, which are exclusive for this species within *A. ser. psoraleoides* but are shared with other species of

*Adesmia*. In the combined cpDNA analysis, *A. tristis* is embedded with the other species of the series while *A. reitziana* is placed as sister to the remaining species of the series, though with weak bootstrap support (76.5%), but high Bayesian posterior probabilities (1.0).

The *matK* analysis places the monophyletic *A. ser. psoraleoides* in a polytomy with monophyletic groups representing *Adesmia ser. bicolores* Burkart, *A. ser. muricatae* Burkart, *A. ser. subnuda* Burkart, *A. ser. longisetae* Burkart and *A. ser. candidae* Burkart (Figs. 4–5, SI3). Species of these clades are spread over the lowland grasslands from the Pampas Domain in Argentina, Uruguay and Southern Brazil, and Patagonia in Argentina. Species of *A. ser. bicolores* are the most morphologically similar species to *A. ser. psoraleoides*, but differing in having stolons, and flowers with two stamens fused to the banner (Miotto and Leitão Filho, 1993).



**Fig. 5.** Divergence time estimates of *Adesmia*. A. Chronogram based on *matK* Bayesian dating analysis using an uncorrelated relaxed molecular clock; B. The correspondent phylogram. Arrows indicate the main diversification events discussed in the text. Colors in the phylogeny represent the geographic distribution of taxa, according to the map. See Supplementary information (SI2) for details on ancestral distribution methods.

The current classification of the genus *Adesmia* comprising two subgenera is not supported here. The subgenus *Adesmia* has been formally recognized by the absence of spines, while the subgenus *Acanthadesmia* has been circumscribed by the presence of spines. The spiny species of *Adesmia* are distributed over the tree and do not support the taxonomic classification into two subgenera (Fig. 4). Future studies are necessary to test the monophyly of the extant series within the genus (SI3).

#### 4.2. Biogeography

##### 4.2.1. Age of origin of the CCS grasslands

Previous considerations of the diversification of plant lineages in the southern Brazilian grasslands include the essentially descriptive analysis by Rambo (1953a,b), who considered the endemic taxa of the highland grasslands as the ancient plant lineages that colonized the area during the late Cretaceous at the time of the first South American Cenozoic glaciations (see also Miotto and Waechter, 1996). The estimation of diversification ages shows that *A. ser. psoraleoides* is much more recent with an estimated stem age of c. 11 Mya and crown age of c. 5–6 Mya (Fig. 5). The monophyly and recent origin of *A. ser. psoraleoides* are strikingly reminiscent of phylogenies of lineages endemic to the more northern tropical savannas of the Brazilian cerrados (Simon et al., 2009; Simon and Pennington, 2012). Numerous woody cerrado lineages have been shown to start to diversify less than 10 Mya, consistent with suggestions that C4 grasslands did not rise to dominance until 6–8 Mya (Cerling et al., 1997; Beerling and Osborne, 2006; Stromberg, 2011). Our study, showing a relatively recent diversification

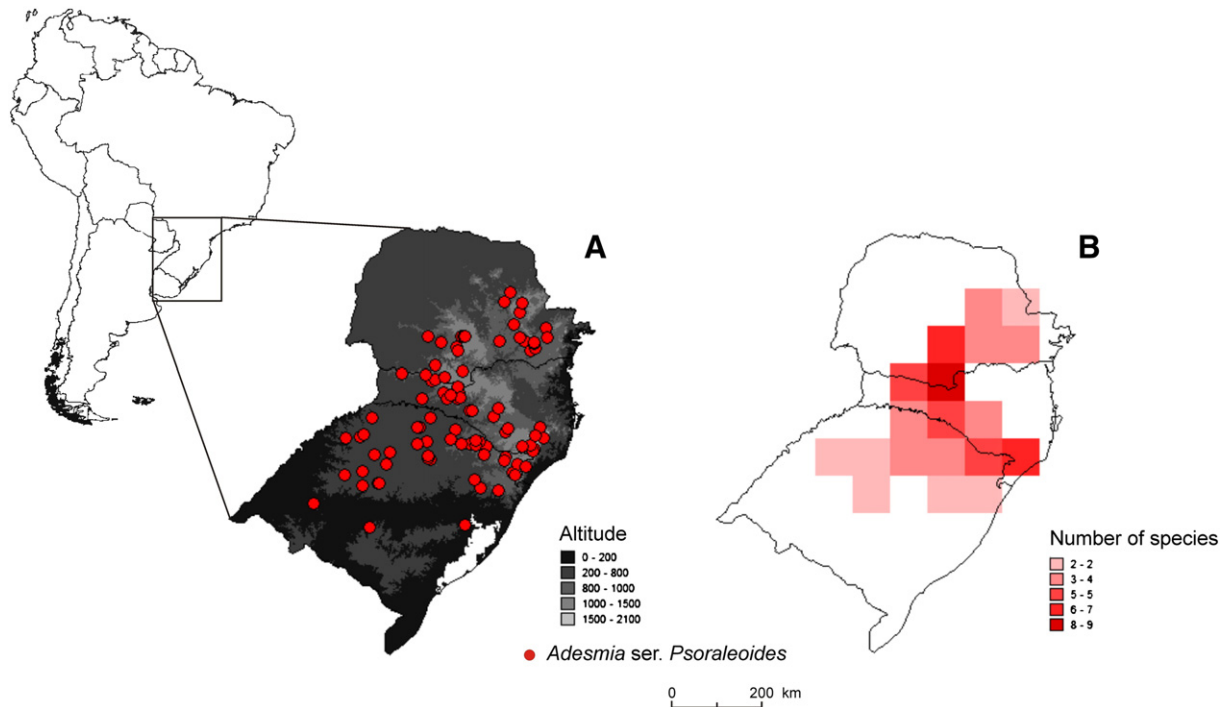
of *A. ser. psoraleoides*, a group endemic to the CCS, suggests that these distinctive subtropical grasslands have a recent origin in common with other tropical grassland systems.

##### 4.2.2. Historical assembly of the CCS grasslands

The time-calibrated phylogeny based on *matK* sequences shows a geographically structured diversification of *Adesmia* (Fig. 5). The basally divergent lineages of the genus are found in the arid and semi-arid formations of the Andes from Bolivia, through Chile to southernmost Argentinean Patagonia. During the middle Miocene, the estimated dates in our phylogeny (Fig. 5) indicate that *Adesmia* started its radiation in the Andes and southern South America. Subsequent expansion from this area may have been facilitated by the global cooling that began after the Mid-Miocene climatic optimum, around 15 Mya. Geological processes, such as the Andean uplift, and dry and cold climates, allowed colonization of areas further north, eventually allowing *Adesmia* to reach the southern Brazilian grasslands. This scenario is suggested by the geographically intermediate distribution of lineages (represented by blue clades in Fig. 5) that occur mostly in the lowland temperate grasslands (Pampas) from Argentina and Uruguay that reach their northern distribution in the southern Brazilian Pampas. A similar scenario of expansion from the Andes was described by Donato et al. (2003) for Coleoptera and Pérez-Losada et al. (2004) for freshwater crabs.

##### 4.2.3. Perspectives for future research and conservation of the CCS

Iganci et al. (2011) highlighted a series of taxa that have endemic species in the CCS, including *Mimosa* L., *Lathyrus* L. (Leguminosae), *Baccharis* L. (Asteraceae) and *Begonia* L. (Begoniaceae). Dated phylogenetic studies



**Fig. 6.** A. Geographic distribution of *Adesmia ser. psoraleoides* in Southern Brazil; B. The estimation of the richest areas in terms of species number, within the “Campos de Cima da Serra” – CCS.

of the kind described here that exhaustively sample these endemic species will be needed to determine if the results presented here for *A. ser. psoraleoides* – a young monophyletic radiation confined to the CCS – are general. If other groups of CCS endemic species are shown to be of a similar or younger age, it will be consistent with a recent geological origin of the CCS grasslands, coincident with the expansion of tropical grasslands worldwide.

*A. ser. psoraleoides* is representative of other taxa endemic to the CCS that also have related lineages in the southern Andes (Smith, 1962; Rambo, 1953a,b; Safford, 2007; Iganci et al., 2011). Such taxa need similar studies to the one described here to test for congruence in biogeographic histories. Examples include *Acaena* Mutis ex L., *Drimys* J.R. Forst. & G. Forst., *Fuchsia* L., *Griselinia* J.R. Forst. & G. Forst. and *Podocarpus* L'Hér. ex Pers., all of which have endemic species in both southern Brazil and the Andes. One taxon for which a phylogeny is available is *Butia* (Becc.) Becc. (Arecaceae), a genus which is widespread within southeastern South American grasslands including the CCS. It became disjunct from its sister, *Jubaea* Kunth, endemic to the southern Andes, around 14.5 Mya (Meerow et al., 2009), approximately congruent in time with the split between Andean and non-Andean *Adesmia* species (Fig. 5).

Given the Pleistocene age of divergence of some species within *A. ser. psoraleoides* (Fig. 5), it is possible that some speciation may have been driven by climate changes during glacial/interglacial periods. Paleorecords indicate that warmer interglacial climates favored the spread of forests over the CCS (Smith, 1962; Behling, 2002; Safford, 2007), and this may have isolated populations of grassland species promoting the allopatric diversification processes. Phylogenetic studies of other CCS endemic clades are necessary to evaluate if they too show Pleistocene species ages, but population genetic studies within widespread CCS species will be required to search for genetic signatures of recent climate-driven range expansion or areas of high genetic diversity that might represent areas where grasslands persisted during glacial periods.

The 11 species of *A. ser. psoraleoides* tend to occupy small patches of grasslands, rarely presenting large populations but almost all of them co-occur sympatrically, as shown in Fig. 6. The CCS has been highlighted

as harboring a large number of endemic plant species (Iganci et al., 2011) but it is highly threatened by land use and it is lacking protected areas. The effects of land use can be seen in the geographic distribution of *Adesmia rocinhensis*, which occurs in three disjunct populations, completely isolated from each other by patches of forest and agricultural areas, reflecting recent habitat fragmentation of a once more widespread CCS grassland. The grasslands that remain richest in species number of *A. ser. psoraleoides* are found in central Santa Catarina and Paraná states, in the localities known as the “Campos de Palmas” and “Água Doce”, as well as the eastern edge of the “Serra Geral” range in “Rio Grande do Sul” and “Santa Catarina” states. Conservation efforts should be directed to these species-rich areas to protect the endemic biodiversity of the grasslands of the CCS and their unique evolutionary history.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.sajb.2013.06.016>.

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