Molecular phylogeny and diversification history of *Prosopis* (Fabaceae: Mimosoideae)

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The genus *Prosopis* is an important member of arid and semiarid environments around the world. To study *Prosopis* diversification and evolution, a combined approach including molecular phylogeny, molecular dating, and character optimization analysis was applied. Phylogenetic relationships were inferred from five different molecular markers (*mat*K-*trn*K, *trn*L-*trn*F, *trn*S-*psb*C, G3pdh, NIA). Taxon sampling involved a total of 30 *Prosopis* species that represented all Sections and Series and the complete geographical range of the genus. The results suggest that *Prosopis* is not a natural group. Molecular dating analysis indicates that the divergence between Section Strombocarpa and Section Algarobia plus Section Monilicarpa occurred in the Oligocene, contrasting with a much recent diversification (Late Miocene) within each of these groups. The diversification of the group formed by species of Series Chilenses, Pallidae, and Ruscifoliae is inferred to have started in the Pliocene, showing a high diversification rate. The moment of diversification within the major lineages of American species of *Prosopis* is coincident with the spreading of arid areas in the Americas, suggesting a climatic control for diversification of the group. Optimization of habitat parameters suggests an ancient occupation of arid environments by *Prosopis* species. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, **93**, 621–640.

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INTRODUCTION

The genus *Prosopis* L. (Fabaceae) has approximately 45 species, which are important members of arid and semiarid environments (Fig. 1). Some of these species are characteristic of the driest regions in the world. For example, *Prosopis tamarugo* F. Philippi, is one of the few tree species capable of surviving in the extremely arid climate of the Atacama desert in Northern Chile. Other species are distinctive of the large deserts of North America (Tamaulipas, Sonora, Chiuahua) and of arid and semiarid regions of South America (Monte, Patagonia, Puna, and Chaco). However, only a few representatives of the genus,

such as *Prosopis africana* (Guill., Perr., & Rich.) Taubert, are partially distributed in subhumid tropical or subtropical regions. From a taxonomic point of view, the genus is divided into five Sections, based mainly on the presence and type of armature and shoot structure (Burkart, 1976). The two main Sections (Algarobia and Strombocarpa) are native to North and South America and include approximately 90% of all the species of the genus. Section Monilicarpa comprises only one species endemic to central Argentina. Sections Anonychium and Prosopis have exclusively Old World representatives. *Prosopis africana*, the only species of Section Anonychium, is native to the Soudano-Guinean zone and neighbouring areas of Africa, from Senegal in the west to Sudan and Kenya in the east (Pasiecznik *et al.*, 2001).

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Figure 1. Natural distribution of *Prosopis* after Pasiecznik *et al*. (2001). Number of native species are indicated for each region.

Section Prosopis comprises three species that are native to North Africa and Asia, stretching east to India, north to Georgia and Turkmenistan, and west to Algeria along the North African coast (Pasiecznik *et al*., 2001).

Different studies have evaluated the relationships among *Prosopis* species (Saidman & Vilardi, 1987, 1993; Saidman *et al*., 1996, 1998; Ramírez *et al*., 1999; Bessega, Saidman & Vilardi, 2005; Bessega, Vilardi & Saidman, 2006). However, these generally involved phenetic analyses and included species of only some of the Series and Sections of the genus. In addition, none of these studies have evaluated the nature of the *Prosopis* generic limits as either outgroups were not included (Ramírez *et al*., 1999; Bessega *et al*., 2005), or these were distantly related to *Prosopis* species (Bessega *et al*., 2006). The only cladistic analysis performed (Bessega *et al*., 2006) suggested that most of the groups taxonomically recognized are not monophyletic.

Several ideas were proposed about the timing of *Prosopis* diversification (Pasiecznik *et al*., 2001). Burkart (1976), based on the presence of the genus in both New and Old World, suggested a late Cretaceous or Early Tertiary origin for the genus, previous to the formation of the Atlantic Ocean. Following this idea, Ramírez *et al*. (1999) suggested that the genus would have originated 130 Mya. However, this time frame is strongly contradicted by both palaeonthological evidence (Herendeen, Crepet & Dilcher, 1992) and molecular dating (Lavin, Herendeen &

Wojciechowski, 2005) because both support the notion that the subfamily *Mimosoideae* originated between 42–50 Mya. Burkart & Simpson (1977) also considered that *Prosopis* is an old genus that diverged early into several principal lineages but that, within some of these lineages, more recent episodes of expansion and isolation produced further speciation. To date, no attempt has been made to evaluate the divergence times within *Prosopis* using molecular dating.

The observation of hybrid formation between some species of Section Algarobia, together with their high morphological similarity and partially overlapping geographical distributions, led to the consideration that this group is a syngameon (Palacios & Bravo, 1981). However, recent molecular studies have supported the biological meaning of the specific limits within this group (Saidman & Vilardi, 1987; Burghardt, 1995; Bessega *et al*., 2000) and indicate the possible existence of isolating mechanisms that restrict the introgression between its species. Previous cladistic analysis (Bessega *et al*., 2006) suggested that these species do not form a monophyletic group. Nonetheless, in that analysis, taxon sampling of non-hybridizing species within Algarobia was relatively scarce. A more complete taxon sampling within Algarobia would give the opportunity to study the timing and evolution of isolating barriers within this group.

During recent years, different studies have supported the hypothesis that dry-adapted taxa in different regions of the world diverged concomitantly with the expansion of arid environments. That is the case

for *Phylica* (Richardson *et al.*, 2001), *Ruchoideae* (Klak, Reeves & Hedderson, 2003), and *Ehrharta* (Verboom, Linder & Stock, 2003) in South Africa, *Tiquillia* (Moore & Jansen, 2006) and *Agave* (Good-Avila *et al.*, 2006) in North America, and *Rheum* (Wang, Yang & Liu, 2005) in East Asia. Evidence supporting this hypothesis is in all cases a temporal relationship between the increase of arid environments and the diversification of these groups. Due to the affinity of *Prosopis* with arid environments, this is a promising hypothesis to be tested in this genus.

OBJECTIVES AND HYPOTHESIS

The present study aimed to investigate the main patterns of *Prosopis* diversification. We are particularly interested in answering the following questions. (1) Which are the relationships among the main lineages within *Prosopis*? (2) When did *Prosopis* diversification occur? (3) Has the spreading of arid environments driven *Prosopis* evolution, as suggested for other arid-adapted taxa? To answer these questions, a combined approach was applied that included molecular phylogeny, molecular dating, and character optimization analyses.

MATERIAL AND METHODS

MOLECULAR TECHNIQUES

Five different gene regions were sequenced including two nuclear markers: coding and noncoding sequences of the glyceraldehyde-3-phosphate dehydrogenase gene (G3pdh), one intron of the nitrate reductase gene (NIA) and three chloroplast markers: partial sequences of *trn*S-*psb*C, *trn*K-*mat*K and *trn*L-*trn*F regions.

Total DNA was extracted from five day old cotyledons or silica dried leaves and/or stems with the DNeasy Plant Mini Kit (QIAGEN). All polymerase chain reaction reactions were conducted in a total volume of 50 μL, containing 80 ng of DNA template, $1 \times$ Taq-Buffer, 2.5 mM MgCl$_2$, 0.25 U Taq polymerase, 0.2 mM of each DNTP and 0.04 μM of each primer. The intergen between the *psb*C and *trn*S genes was amplified using the primers *psb*C and *trn*S (Demesure, Sodzi & Petit, 1995) together with a new internal primer *psb*Cint (5′-CGTTCTTGCCAAGGCTGTAT-3′). Since a preliminary survey indicated that most of the variation among sequences was located in the first 500 bp from the 3′ end region of *trn*S gene, only this region was subsequently sequenced for the rest of the species. G3pdh region was amplified with primers GPDX7F and GPDXR9 (Strand, Leebens-Mack & Milligan, 1997) and a newly designed primer G3pdhintF (5′-GACTGGAGGGGTGGAAGAG-3′). NIA intron was amplified with a degenerate primer

pair: NIA3F-NIA3R (Howarth & Baum, 2002) and sequenced with a nondegenerate pair: NIAproF (5′-GAACCAGCAGTTGTTCATCAT-3′) and NIAproR (5′-ACTGGTGCTGGTGTTTTTGG-3′). *trn*L intron was amplified and sequenced using the primers *c*, *d*, *e* and *f* (Taberlet *et al.*, 1991). Partial sequences of *trn*K intron were amplified with Ac283R and *trn*K 3914F primers (Miller & Bayer, 2001). Partial sequences of *mat*K were amplified and sequenced with primers 685F and 1265R (Lavin *et al.*, 2000). To amplify the region *trn*S-*psb*C, *trn*K-*mat*K and *trn*L-*trn*F, the following cycling profile was used: 35 cycles of 1 min at 94 °C, 1 min at 56 °C, and 1.5 min at 72 °C. In the case of G3pdh and specific NIA amplification, the cycling profile was the same, except that annealing temperature for these regions was set to 52 °C. For the amplification of NIA region with degenerate primers, a stepdown programme was used that included ten cycles in which melting temperature decreased 1 °C per cycle starting from 55 °C. Twenty additional cycles were performed with 30 s at 94 °C, 30 s at 45 °C, and 40 s at 72 °C. In all cases, the programme started with one cycle at 94 °C for 4 min and finished with 7 min at 72 °C.

PHYLOGENETIC ANALYSIS

Thirty species of *Prosopis* were included in this analysis. Taxon sampling within *Prosopis* was planned to include representatives from the entire geographical range of the genus (Appendix 1). Moreover, taxonomic diversity of the genus was totally represented as species of all Sections and Series were included. Three different analyses that involved different combinations of taxa and molecular markers were performed. A first analysis was performed comprising *trn*L-*trn*F and *trn*K-*mat*K (two-marker analysis) sequences of 80 legume species downloaded from GenBank (see Supplementary material) plus five *Prosopis* species that represented each Section of the genus. In the second analysis (three-marker analysis), G3pdh, NIA, and *trn*S-*psb*C sequences from all the sampled species of *Prosopis* plus *Prosopidastrum angusticarpum*, *Acacia caven*, *Xerocladia viridiramis*, and *Lotus japonicus* were analysed in a total evidence context (Kluge, 1989). As more than one sequence of each species was sampled in Algarobia clade, the total number of terminals was 41. This was due to the high level of shared polymorphism found in previous analyses within this group (Saidman & Vilardi, 1987; Bessega *et al.*, 2000). A third analysis (five-marker analysis) involved a combination of the first two datasets.

Sequences were edited with Bioedit software (Hall, 1999) and aligned with Clustal X (Thompson *et al.*, 1997) with a posteriori minor manual changes. Par-

simony based searches were performed using the TNT program (Goloboff, Farris & Nixon, 2003). Characters and state transformations were considered as equally weighted. Gaps were alternatively treated as a fifth state, missing entries or as separate characters Simmons & Ochoterena (2000). Since the results were very similar, only those based on fifth state coding are shown and discussed. Within *trn*L-*trn*F region, a deletion of approximately 300 bp was found in some Mimosoid species. Individual gaps representing this deletion were treated as missing data. As a model-based analysis alternative to the parsimony analysis, we also conducted a Bayesian analysis. Search strategies in parsimony and Bayesian analysis are indicated in Appendix 2.

ESTIMATING DIVERGENCE TIMES

To estimate divergence times, G3pdh and *trn*S sequences were concatenated and analysed together. Simultaneous analysis of gene sequences from multiple loci was performed because the penalized likelihood method used for age estimation (see below) is prone to errors when dealing with zero length branches or very short branches (Sanderson, 2003). The congruence between both datasets in branch lengths was evaluated by the likelihood ratio test described by Lewis (1998) and Xiang *et al*. (2005). The statistics of this test is $-2 [\ln L - (\ln L_1 + \ln L_2)]$, where L_1 is the likelihood of the tree obtained from the first gene, L_2 is the likelihood of the tree obtained from the second gene and L is the likelihood obtained from the combined analysis of both genes. The value of this statistic was compared to a χ^2 distribution with degrees of freedom equal to the sum of $2N + 3 - P$, where N is the number of terminals and P is the number of free parameters of the model used in the likelihood calculation. NIA sequences were not included because it was not possible to obtain the sequence of this region from *P. angusticarpum*, one of the taxa that defines the unique fixed node in the calibration step. Furthermore, *X. viridiramis* was not included because only a part of G3pdh was possible to be sequenced. Branch lengths were estimated by Maximum Likelihood using PAUP* (Swofford, 2002). The evolutionary model was chosen with the hierarchical likelihood ratio test as implemented in Modeltest, version 3.06 (Posada & Crandall, 1998). Penalized likelihood implemented in the R8s software (Sanderson, 2003) was used for age estimation using the Powell algorithm and defining smoothing parameter values with a cross validation procedure (Sanderson, 2002).

Divergence times were derived from molecular data combining two calibration points. The time obtained in Lavin *et al*. (2005) for the divergence between one

species of Section Algarobia (*Prosopis pallida*) and one species of genus *Prosopidastrum* (*Prosopidastrum mexicanum*) was used to fix the node subtending *Prosopidastrum* and the American species of *Prosopis* (ASP). To take into account the error associated with the estimation of Lavin *et al*. (2005), we repeated the analysis considering the mean ± SD. A second calibration point was used to give minimum age to the ASP divergence. In this case, this fossil corresponds to pollen grains obtained from Early Oligocene sediments of British Columbia, Canada (Piel, 1971). As this Epoch extends from 28.4 and 33.7 Mya, the former age was used to define the minimum age. Since the sister group of ASP + *Xerocladia* clade was not clearly defined in our phylogenetic analysis, two different topologies were considered, one of them corresponding to the results of the three-marker analysis and the other corresponding to the two-marker analysis. Error in age estimates was evaluated by bootstrapping following Sanderson (2002).

DIVERSIFICATION ANALYSIS

To study the possible changes in diversification rate over time a lineages through time (LTT) analysis (Nee *et al*., 1995) was performed. LTT analysis was repeatedly performed with extreme values of divergence times obtained in the penalized likelihood analysis. The lack of resolution in the mesquite clade (see Results) was considered in two ways for this analysis: (1) as a hard polytomy with all the lineages diverging simultaneously and (2) as if the diversification occurred at a constant rate. Consequently, PHYLOGEN program, version 1.1 (Rambaut, 2002), was used to simulate trees under a Yule Model of diversification (Yule, 1924) with the final number of species equal to the present in the mesquite group. Subsequently, this subtree was grafted in the original tree, replacing the polytomy in the mesquite clade. This was repeated for 100 different simulated subtrees. As the general pattern obtained was the same, LTT of one randomly chosen tree is shown. To examine the effects of missing species in LTT analysis, these were placed in the most likely position on the tree on the grounds of taxonomic treatment (Burkart, 1976) in accordnace with Barraclough & Vogler (2002). To compare divergence rates within *Prosopis* with those obtained in other plant genera, per lineage net diversification rate (NDR) *sensu* Coyne & Orr (2004) was calculated. As in LTT analysis, missing species were included in their most likely place on the tree.

ESTIMATION OF ANCESTRAL HABITAT

To study the historical relationship of *Prosopis* with arid environments, maximum and minimum value of humidity index (HI) were gathered for the natural

Table 1. Statistics of the different phylogenetic analyses based on parsimony

	Two-marker analysis	Three-marker analysis	Five-marker analysis
Number of MPT	224	9	4680
Length	5079	1552	6710
Number of characters	4031 (1444–2587)†	1764 (648–701–415)*	5795
Number of informative characters	1357 (609–748)†	391 (193–183–15)*	1458
CI/RI	0.496/0.655	0.695/0.846	0.535/0.700

*Statistics for G3pdh, NIA and *trn*S-*psb*C characters, respectively.

†Statistics for *trn*L-*trn*F and *trn*K-*mat*K characters, respectively.

CI, consistency index (excluding uninformative characters); MPT, most parsimonious trees; RI, retention index.

geographical range of the species of *Prosopis* included in the phylogenetic analysis plus *X. viridiramis*. HI is defined as the ratio of precipitation to potential evapotranspiration (UNEP, 1992). Species distributions were defined from the bibliography (Ross, 1975; Burkart, 1976; Rzedowski, 1988; Roig, 1993) and from herbarium specimens (BAFC, SI, LIL, CTES). Climate data for each species (see Supplementary material) were extracted from FAO World Climate Database using the New LocClim software (FAO, 2005). Regions were classified as a function of HI values following UNEP (1992): 0.05 < Hyper-Arid; 0.05–0.20 Arid; 0.20–0.50 Semi-Arid; 0.50–0.65 Dry-Subhumid; > 0.65 Humid. The phrase 'arid environments' is used here to collectively represent hyperarid, arid and semiarid classes. Maximum and Minimum of HI (MaHI and MiHI, respectively) were independently optimized on the tree as in the MinMax coding (Hardy & Linder, 2005). Once these values were optimized, they were used to delimit the total range of HI for each ancestral node. We considered that MinMax coding is the most suitable for this character because maximum drought tolerance might be partial or totally decoupled from maximum humidity tolerance. MaHI and MiHI were optimized using the implementation in TNT (Goloboff *et al.*, 2003) of Farris (1970) optimization to deal with continuous characters (Goloboff, Mattoni & Quinteros, 2006). In this case, linear changes are minimized along each branch. To evaluate how uncertainty in the state of the root can affect the results, the analysis was repeated with two extreme values: hyperarid and humid. In addition, the effect of topological uncertainty within the mesquite clade (see Results) in the results was evaluated by considering 10 000 different resolutions of this clade. A strict association of a particular node with arid environments was determined when the value of MaHI was lower than the limit between humid and arid environments (HI < 0.50). A nonstrict association with arid environments corresponded to a value of MiHI lower and MaHI higher than this limit. No association with arid

environment occurred when MiHI value was higher than this limit.

RESULTS

PHYLOGENETIC RESULTS

Two-marker analysis
Statistics of the different phylogenetic analyses based on parsimony are shown in Table 1. Strict consensus suggests that *Prosopis* is not monophyletic (Fig. 2). Forcing the monophyly of the genus gave a tree nine steps longer than the optimum (Table 2). ASP formed a highly supported clade (BS = 4; J = 95) with *X. viridiramis* (subsequently named ASP + *Xerocladia*). ASP did not appear as monophyletic because *X. viridiramis* formed a monophyletic group with *P. tamarugo*. However, this clade is not well supported and (BS = 1; J = 66) and forcing the monophyly of ASP gave a tree only one step longer than the optimum. *Prosopis cineraria* appeared as sister group of this clade though with low support (BS = 1; J < 50). *Prosopis nigra* of Section Algarobia formed a strongly supported group (BS = 5; J = 98) with *Prosopis argentina* of Section Monilicarpa. Bayesian analysis gave identical results concerning the lack of monophyly of *Prosopis* and the relationships among APS. By contrast, *P. cineraria* does not appear forming a monophyletic clade with ASP + *Xerocladia*. Posterior probabilities for the groups found in the Bayesian analysis are given in Figure 2.

Three-marker and five-marker analyses
Strict consensus obtained in this analysis agreed with two-marker analysis in rejecting *Prosopis* monophyly (Fig. 3). Forcing the monophyly of the genus gave a tree six steps longer than the optimum (Table 2). As in two-marker analysis, ASP formed a monophyletic group with *X. viridiramis* (APS + *Xerocladia* clade), though with lower support (BS = 1; J = 64). In addition, forcing the monophyly of the ASP gave a tree only one steps longer than the optimum. Two
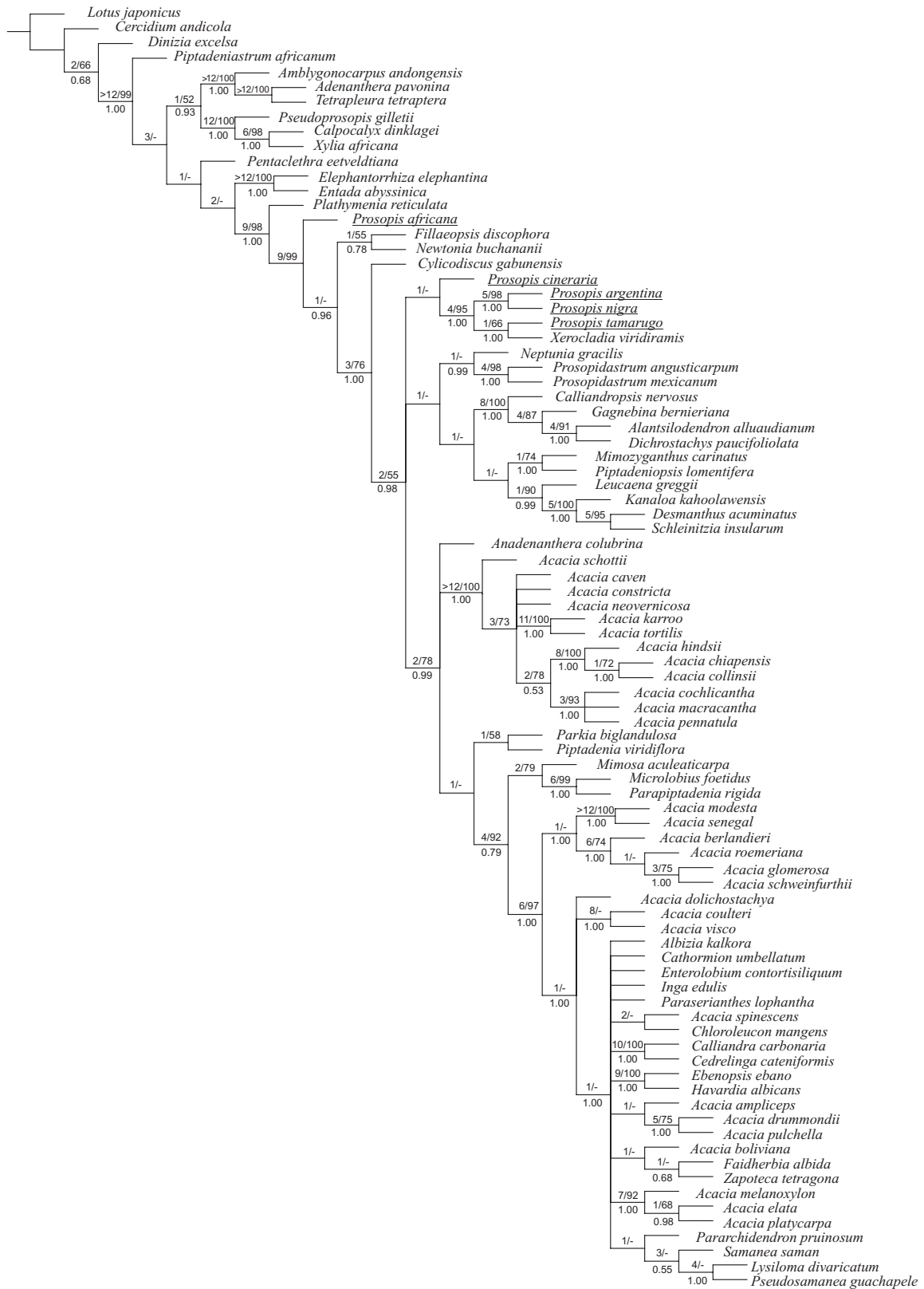
Figure 2. Strict consensus of the 224 optimal trees based two-marker analysis (*trn*L-*trn*F, *trn*K-*mat*K) with parsimony as optimality criteria. Absolute Bremer support (left) and jackknifing values over 50% (right) are indicated above branches. Posterior probabilities estimated in Bayesian analysis are indicated below branches. *Prosopis* species are underlined.

Table 2. Extra steps obtained in searches conducted with positive constrains for some groupings not appearing in most parsimonious trees

	Two-marker analysis (5079)	Three-marker analysis (1552)	Five-marker analysis (6710)
Section Strombocarpa + *Prosopis cineraria*	6	63	68
Prosopis + Prosopidastrum	14	3	14
Prosopis + Xerocladia	4	5	5
Genus Prosopis	9	6	11
Section Algarobia	NA	10	10
OSP + *Xerocladia*	8	6	11
OSP	4	5	11
ASP	1	1	3

In parentheses: optimal lengths in unconstrained searches. ASP, Americans species of *Prosopis*; NA, not applicable; OSP, Old World species of *Prosopis*.

subclades were recognized in ASP + *Xerocladia* clade. One of them is formed by species of Section Strombocarpa plus *X. viridiramis* whereas the other (subsequently named Algarobia *s.l.*) is formed by species of Section Algarobia plus *P. argentina* of Section Monilicarpa (*BS* = 6; *J* = 100). Section Algarobia appeared as paraphyletic because *P. argentina* formed a well supported clade (*BS* = 5; *J* = 100) with species of Series Humiles, Sericanthae, and Denudantes of Section Algarobia (*Prosopis kuntzei–P. argentina* clade). Forcing the monophyly of Section Algarobia gave a tree ten steps longer. Series Denudantes (represented here by *Prosopis denudans*, *Prosopis ruizleali* and *Prosopis castellanosii*) appeared as monophyletic but Series Sericanthae (*P. kuntzei* and *Prosopis sericantha*) would be paraphyletic. The sister group of the *P. kuntzei–P. argentina* clade was a very well supported clade (*BS* = 6, *J* = 100) formed by species of series Chilenses, Pallidae, and Ruscifoliae (mesquite clade). However, the relationships among the species of this clade are unclear because the strict consensus is highly unresolved and individuals of the same species in most of the cases did not form monophyletic groups. Section Strombocarpa was monophyletic with *X. viridiramis* as its sister clade. Within this section, two groups were well supported. One of them corresponded to Series Cavenicarpae (*Prosopis ferox* and *P. tamarugo*) whereas the other was formed by two North American species of Series Strombocarpae: *Prosopis pubescens* and *Prosopis palmeri*. Bayesian analysis showed very similar results (Fig. 3). The

main difference was related with the sister group of APS + *Xerocladia*. In this analysis, the sister group was formed by *A. caven* and *P. angusticarpum*.

The results obtained in the five-marker analysis were very concordant with those obtained in the other two analyses (see Supplementary material) and the support increased in several relevant nodes: ASP + *Xerocladia* clade (BS = 9; *J* = 96); Strombocarpa clade (BS = 2; *J* = 83); Strombocarpa + *X. viridiramis* clade (BS = 2; *J* = 77). However, differing from the two-marker analysis, *P. cineraria* did not appear as sister group of APS + *Xerocladia*. Strict consensus indicated identical relationships within APS + *Xerocladia* clade than those given by the three-marker analysis, except that the groupings within the mesquite clade are less resolved.

MOLECULAR DATING

The optimal model chosen by the hierarchical likelihood ratio test was Hasegawa–Kishino–Yano plus Gamma (HKY + Γ). A likelihood ratio test significantly rejected the hypothesis of evolutionary rate constancy for the molecular regions analysed (Clock constrained –lnL = 4394.64; unconstrained –lnL = 4363.50; d.f. = 39; *P* < 0.05). Therefore, divergence times were estimated in a penalized likelihood approach that does not assume rate constancy. To study the possible effect of short branches within mesquite clade in the age estimated for the other nodes outside this group, an analysis leaving only one
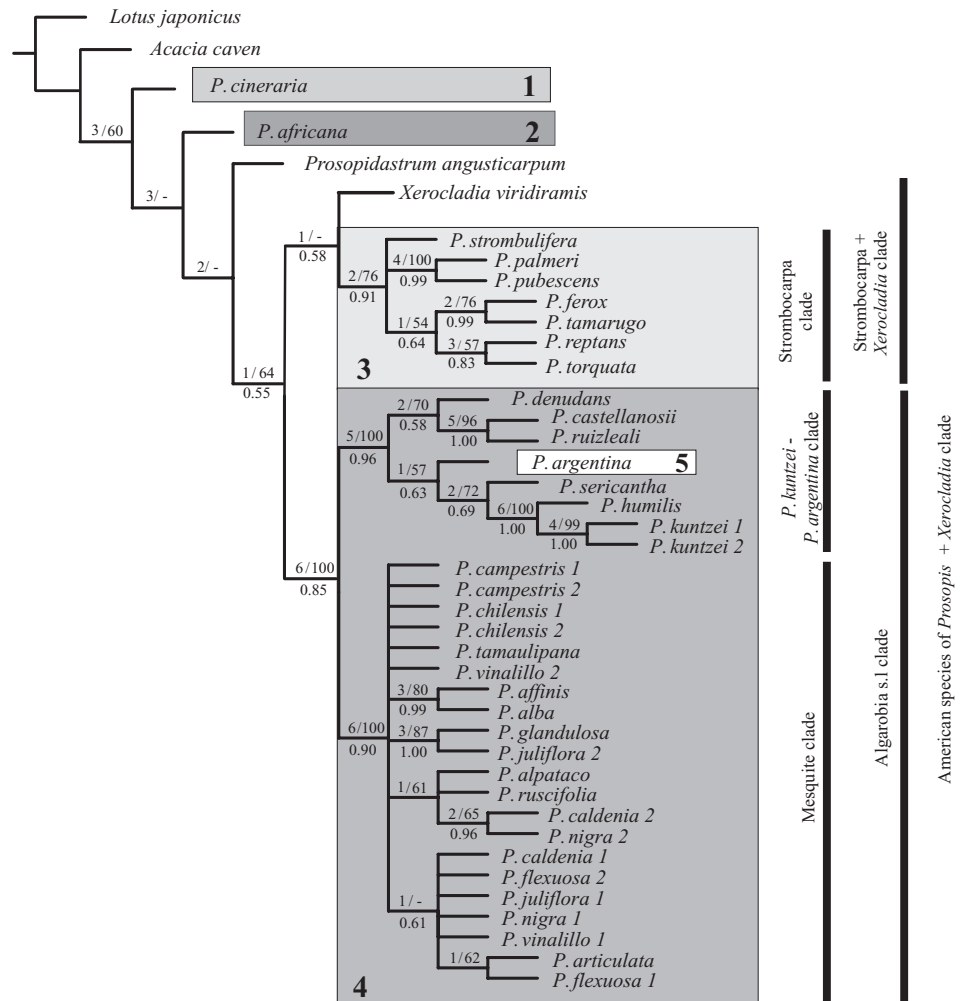
Figure 3. Strict consensus of the nine optimal trees obtained in the three-marker analysis (*trn*S-*psb*C, G3pdh, NIA) with parsimony as optimality criteria. Absolute Bremer support (left) and jackknifing values over 50% (right) are indicated above branches. Posterior probabilities estimated in Bayesian analysis are indicated below branches. Rectangles delimit each Section: 1, Prosopis; 2, Anonychium; 3, Strombocarpa; 4, Algarobia; 5, Monilicarpa. Black bars indicate the clades whose names are used throughout the manuscript.

terminal was performed. The results differ only slightly (data not shown).

The common and most relevant pattern observed in the different analyses was (Fig. 4; Table 3): (1) an ancient divergence between the two main lineages of ASP within the Oligocene; (2) a more recent divergence, starting in the late Miocene, within Strombocarpa and Algarobia *s.l.*; and (3) a divergence among mesquite species starting in the Pliocene.

DIVERSIFICATION ANALYSIS

Phylogenetic analyses indicated that Old World species of *Prosopis* are not closely related with the ASP. This was true for *P. africana* in all the analyses and for *P. cineraria* in two out of three analyses.

Taking this into account, the study of diversification and climate affinity evolution was restricted to the ASP + *Xerocladia* clade.

Lineage through time analysis clearly suggests an increase in the diversification rate of the ASP group from the Late Miocene (Fig. 5A, B) until the present. Within this period, a more detailed examination suggests two different moments of rate acceleration: one in the Late Miocene and the other in the Pliocene. This general pattern is neither affected by the inclusion of missing species in the analysis (Fig. 5B), nor by the different topologies used to derive the chronograms (data not shown). The NDR estimated for different clades within *Prosopis* and for other taxa is shown in Table 4.
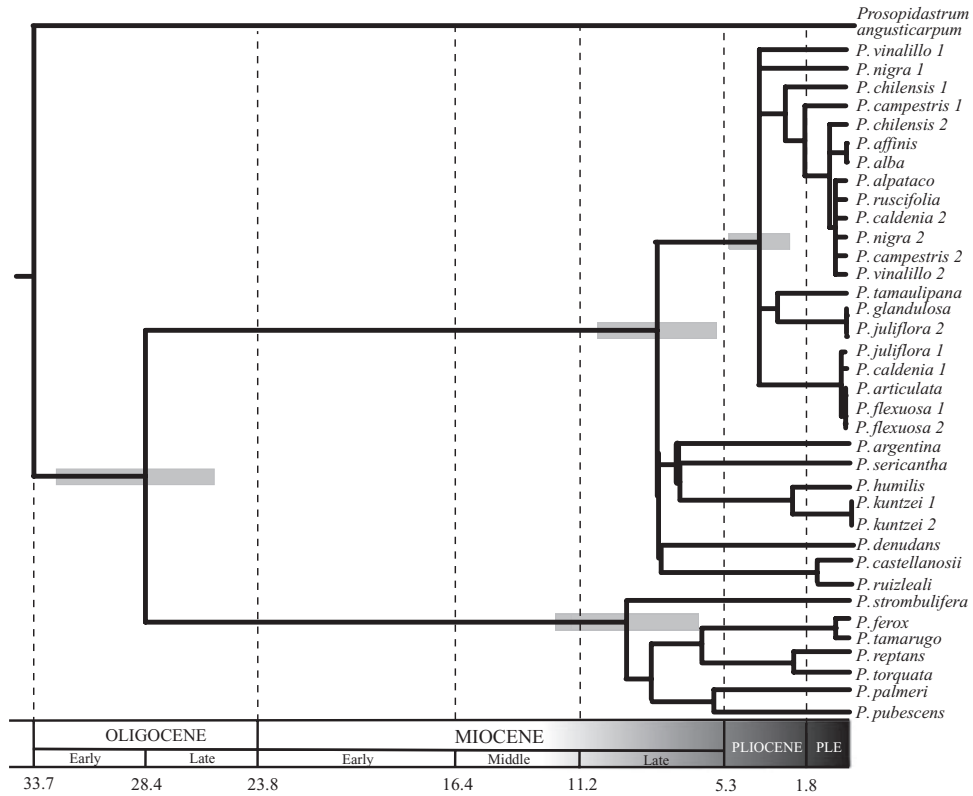
Figure 4. Chronogram resulting from penalized likelihood analysis. The topology corresponds with one of the most parsimonious trees obtained in three-marker analysis (Fig. 3) and calibrated according to divergence times derived from Lavin *et al.* (2005). The approximate temporal spreading of arid zones in America is shown over the timescale with darker zones representing the moment of its maximum historical extension. Grey bars over the nodes represent 95% confidence interval for node ages obtained by bootstrap techniques. PLE, Pleistocene.

Table 3. Divergence times (Mya) obtained in the penalized likelihood analysis

	Outgroup placement	
MRCA	Three-marker analysis	Two-marker analysis
ASP + *Xerocladia*	28.96 (26.25–31.68)	29.37 (27.39–33.05)
Algarobia *s.l.*	7.89 (7.15–8.62)	7.96 (7.58–9.14)
Mesquites	3.65 (3.31–3.99)	3.69 (3.38–4.08)
Strombocarpa	9.21 (8.35–10.07)	9.31 (9.18–11.07)

Ages estimated fixing the node subtending ASP + *Xerocladia* clade and *Prosopidastrum* to 33.2 Mya following the mean value obtained in Lavin *et al.* (2005). Ages estimated considering the mean ± one standard deviation are given in parentheses. When the topology corresponded to the two-marker analysis the node subtending ASP + *Xerocladia* node was constrained to a minimum age of 28.4 Mya following the fossil evidence.
ASP, American species of *Prosopis*; MRCA, most recent common ancestor.

ANCESTRAL CLIMATE ESTIMATION

Optimization analysis unambiguously indicated that the range of the most recent common ancestor (MRCA) of the clade formed by the ASP plus *X. viridiramis* extended to semiarid or more xeric conditions (Fig. 6). A strict association of this node with arid environments was obtained for all the reconstructions when the state of the root was defined as hyperarid and, in some of the reconstructions, when the state of the root was defined as humid. In the case of Strombocarpa clade, the different assignments of states to
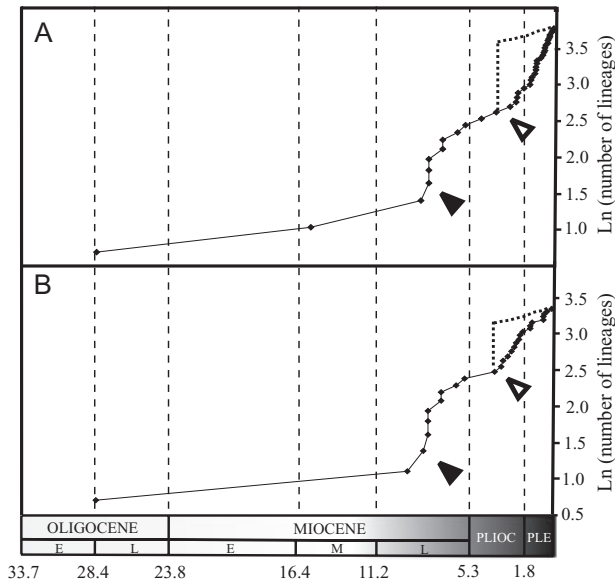
Figure 5. Lineages-through-time plot (LTT) derived from the chronogram of Fig. 4. A, LTT including missing species. B, LTT with species included in the three-marker analysis. Dotted lines represent LTT when the polytomy in the mesquite clade is considered as hard. Black arrowheads indicate the beginning of the diversification within Algarobia *s.l.* and Strombocarpa clades. Empty arrowheads indicate the start of mesquite diversification. E, Early; M, Medium; L, Late; PLIOC, Pliocene; PLE, Pleistocene. The approximate temporal spreading of arid zones in America is shown over the timescale with darker zones representing the moment of its maximum historical extension.

the root did not affect the results as, in all cases, its MRCA appear to be restricted to arid environments. The same result was obtained for the MRCA of *X. viridiramis* + Strombocarpa clade. In the case of the MRCA of Algarobia *s.l.*, *P. kuntzei–P. argentina* and mesquite clades, optimal reconstructions included in some cases ranges that fell completely within arid conditions whereas, in others, the ranges extended from arid to humid conditions.

DISCUSSION

SYSTEMATICS

The phylogenetic analysis performed in the present study is the first to include species of all the Sections and Series of the genus and intends to test *Prosopis* monophyly by incorporating several species of related genera. The results of this analysis suggest that *Prosopis* would not be monophyletic. ASP appear to be more related with *X. viridiramis*, the only species of this southern Africa genus, than with Old World representatives of *Prosopis*. A close rela-

tionship among *Prosopis* species and *X. viridiramis* has been previously indicated by Luckow *et al*. (2004). However, considering that only species of Section Algarobia were included in that analysis, a closer relationship of ASP with *X. viridiramis* than with Old World *Prosopis* species is a novel and interesting result. *Prosopis* monophyly was also rejected due to the position of *P. africana* and, in some of the analyses, the position of *P. cineraria*. It is suggestive that many of the characters used to define the genus by Burkart (1976) would be, as stated by him, primitive within the whole subfamily (symplesiomorphic). If this is confirmed, the idea of *Prosopis* as natural group might also be questioned at morphological grounds.

A closer relationship among species of the American sections of *Prosopis* (Strombocarpa, Algarobia, and Monilicarpa) than with species of the Old World sections agrees with the subgeneric classification proposed by (Guinet & Bessedik, 1984) based mainly on palynological evidence. Particularly, they included all the ASP in the subgenus Neoprosopis, whereas the other two subgenera corresponded to Section Prosopis and Anonychium, the two Old World sections considered in Burkart (1976). However, the correspondence between the groups obtained in our analyses and the Subgenus Neoprosopis is not strict because ASP formed a monophyletic group with *X. viridiramis*. This close relationship among American Sections of *Prosopis* contrasts with the idea stated by Burkart (1976) that Section Strombocarpa is closely related to Section Prosopis because species of both Sections present comparatively smaller legumes and share the ability to spread by means of rootsuckers.

Species of Section Algarobia and *P. argentina* formed a highly supported clade (Algarobia *s.l.*) that is subsequently divided into two highly supported clades. One of them corresponded to species of Series Pallidae, Chilenses, and Ruscifoliae (mesquite clade), whereas the other corresponded to species of series Sericanthae, Humiles, and Denudantes plus *P. argentina* of Section Monilicarpa (*P. kuntzei–P. argentina* clade). This latter clade, endemic to southern South America, includes all the subaphyllous and aphyllous species sampled for the phylogenetic analysis. This result suggests that this condition might have originated only once in *Prosopis* history.

The results obtained in the present study are consistent with the only previous cladistic analysis involving *Prosopis* species (Bessega *et al*., 2006) in showing that the Section Algarobia is not monophyletic, and that *P. argentina* (Section Monilicarpa) is close to species of Section Algarobia. However, several disagreements between these two studies can be observed. Within Section Algarobia, the clade formed

Table 4. Comparison of net diversification rates (NDR) estimated within *Prosopis* with those obtained for other plant taxa

	NDR	Life forms
Prosopis		
Mesquite clade	0.58–0.72*	Trees†
Prosopis kuntzei–Prosopis argentina clade	0.16–0.21*	Trees and shrubs
Strombocarpa clade	0.27–0.37*	Trees and shrubs
ASP + *Xerocladia* clade	0.12–0.14*	Trees and shrubs
Others taxa		
Inga[1]	0.50	Trees
Lupinus[2]	1.92–3.84	Postrate herbs to treelet
Ruschioideae[3]	0.77–1.75	Stone plants to tree-like shrubs
Clarkia, section Peripetasma[4]	0.62	Herbs
Gentianella[5]	1.7–3.2	Herbs and small shrubs
Valeriana[6]	0.8–1.3	Herbs
Yucca[7]	0.21–0.27	Succulent rossete plants to arborescent
Ehrharta[8]	0.12–0.57	Herbs
Gaertnera[9]	0.71–0.83	Shrubs and small trees
Hawaiian silverwoods[10]	0.55	Vines and shrubs to trees
Agave[7]	0.51	Succulent rossete plants to arborescent

*Range of rates values estimated with times derived from the different calibration strategies.
†95% of the species are trees or present arboreal forms.
[1]Richardson *et al*. (2001); [2]Hughes & Eastwood (2006); [3]Klak *et al*. (2003); [4]Hey (1992); [5]Von Hagen & Kadereit (2001); [6]Bell & Donoghue (2005); [7]Good-Avila *et al*. (2006); [8]Verboom *et al*. (2003); [9]Malcomber (2002); [10]Baldwin & Sanderson (1998).
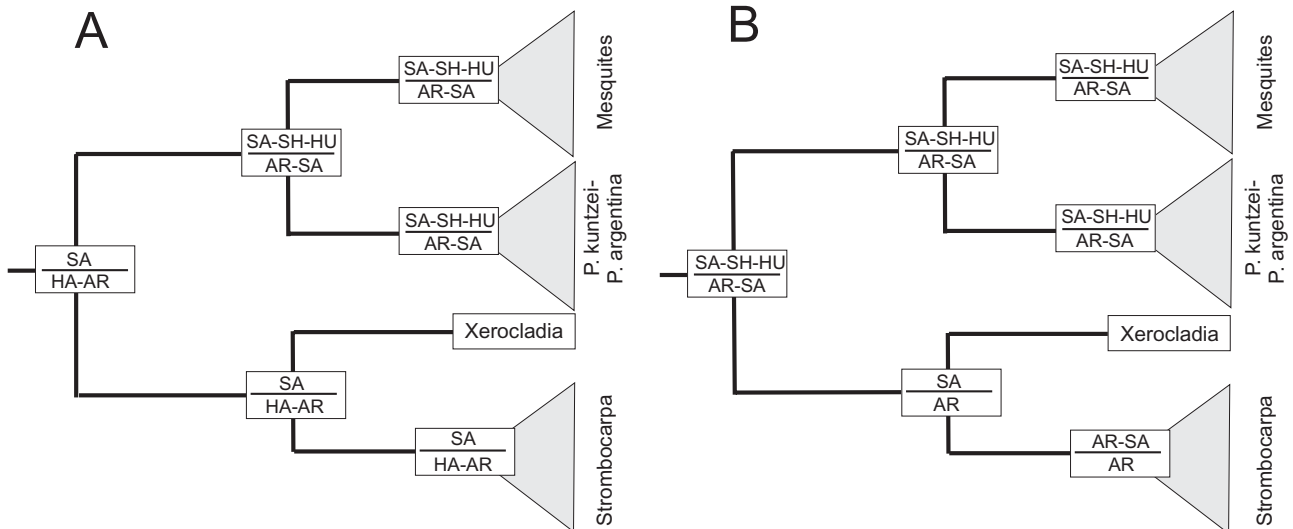ASP, Americans species of *Prosopis*.

Figure 6. Reconstruction of ancestral states of relevant nodes for maximum (above) and minimum (below) humidity index (MaHI and MiHI, respectively). Instead of HI values, classes that represent each value are shown. A, optimization result when the state of the root was defined as hyperarid. B, optimization result when the state of the root was defined as humid. The presence of ambiguities in the optimization step and/or the different possible resolutions within the mesquite clade produced, in some nodes, more than one optimal value for MiHI and MaHI.

by species of Series Ruscifoliae, Chilenses, and Pallidae (mesquite clade) is not monophyletic in the analysis of Bessega *et al.* (2006). A similar situation is observed in Section Strombocarpa, which is monophyltic in our analysis (Fig. 3) but not in that of Bessega *et al.* (2006). In addition, in the present analysis, we found that American *Prosopis* species are more closely related among themselves than with Old World *Prosopis* species, whereas in the analysis of Bessega *et al.* (2006), *P. cineraria*, an Old World species, appears intermingled with New World species. The groupings obtained in our analysis are generally more in agreement with previous analyses (Ramírez *et al.*, 1999; Bessega *et al.*, 2005), and with traditional taxonomy (Fig. 3). Although several ad hoc explanations could be given to account for the disagreement between these two analyses, it is possible that the wider sampling of species and molecular-markers performed in the present study might be the best explanation for these differences.

MOLECULAR DATING AND FOSSIL RECORD

Molecular dating analysis suggests that the divergence between the two main groups within ASP + *Xerocladia* clade is remarkably ancient (26.2–33 Mya) considering that the diversification of the extant members of the whole subfamily Mimosoideae would have occurred during the last 40 Myr (Lavin *et al.*, 2005). However, most of the process of diversification within each of the major clades occurred more recently, in the Late Miocene. This contrast between early divergence of the main groups and late divergence within these groups had already been established on the grounds of morphological evidence (Burkart & Simpson, 1977; Pasiecznik *et al.*, 2001).

The number of fossil remains assigned to *Prosopis* is particularly abundant (Catalano *et al*. unpubl. data). However, as none of the fossils have been analysed in a phylogenetic context and some of the descriptions are poorly detailed, their assignation to *Prosopis* and its subgroups is, in some cases, very tentative. The oldest fossil that could be attributed to the ASP clade (Guinet & Bessedik, 1984), which was used as one of the calibration points in our molecular dating analysis, belongs to pollen grains (*Prosopis quesneli*) of the early Oligocene from British Columbia, Canada (Piel, 1971). A similar age has been estimated for fruit remains (*Prosopis lazarii*) found in the palaeoflora of Puebla, Mexico (Magallon-Puebla & Ceballos-Ferriz, 1994). Nevertheless, the affinity of this fossil with extant groups within the genus is uncertain. Recently, Anzótegui & Herbst (2004) have described, based on leaflets remains, a new fossil species of *Prosopis* from the Middle Miocene of Argentina that appears to be related with extant members of Section Strombocarpa. The age of this fossil is compatible with the times estimated in the molecular dating analysis as both indicate that the divergence between the to major groups of *Prosopis* occurred before the middle Miocene. A more detailed comparison of divergence times will require the combined inclusion of fossil and extant species in a phylogenetic analysis.

MESQUITES DIVERSIFICATION

The results obtained in the present study strongly support a close relationship of species of Series Chilenses, Pallidae, Ruscifoliae (mesquite clade). Interestingly, many of these species were supposed to form a syngameon (Palacios & Bravo, 1981) because of their ability to hybridize naturally, their high morphological similarity and partially overlapping geographical distributions. However, several recent analyses suggest that hybrid formation does not produce significant gene flow among these species. First, molecular studies showed that, in spite of their high genetic similarities, populations formed groupings coincident with their specific origin (Palacios & Bravo, 1981; Saidman & Vilardi, 1987; Burghardt, 1995; Bessega *et al.*, 2000). This was true even for populations of different species that were sympatric. In addition, Bessega *et al.* (2000) showed that sympatric species are not genetically more similar than allopatric ones. Second, the only analysis of hybrid viability available to date (Naranjo, Poggio & Enus Zeiger, 1984) suggests that, at least for some species combinations, hybrids are partially or totally sterile. In the light of our results, the high genetic similarity among these species might now be explained by their recent divergence (< 4 Mya) without the need to invoke introgression among them.

The average per lineage diversification rate estimated for the mesquite group is comparable to other known rapid plant radiations (Table 4). In particular, the diversification rate estimated for this group is found to be higher than in *Inga*, another genus from the same subfamily that also comprises tree species. It is possible that this rapid and recent radiation of mesquites might be the cause of the lack of resolution obtained in the mesquite clade in the present study. A similar explanation for the lack of resolution has been proposed in diverse plant genera (Hodges & Arnold, 1995; Baldwin & Sanderson, 1998; Richardson *et al.*, 2001; Hughes & Eastwood, 2006).

EVOLUTION OF REPRODUCTIVE ISOLATING MECHANISM WITHIN ALGAROBIA

Molecular dating and phylogenetic results obtained in the current study provides an improved insight into

the evolution of isolating mechanism within the genus. In the case of the mesquite clade, as previously indicated, gene flow among its species would be highly restricted. This suggests the existence of isolating mechanism(s) that prevent introgression among them. The existence of hybrid between these species in nature indicates that prezygotic reproductive isolation barriers are probably weak (Palacios & Bravo, 1981) and stresses the potential importance of postzygotic isolating mechanisms within this group. The observation that hybrids between mesquite species are generally found in disturbed environments (Palacios & Bravo, 1981; Verga, 1995) supports the idea that hybrids are not intrinsically inviable, but that their development is limited by the lack of suitable environmental conditions (ecological inviability; Coyne & Orr, 2004). Evidence for a second postzygotic isolating barrier has been obtained by Naranjo *et al.* (1984) who observed diminished fertility in some hybrid combinations. Given the times derived from molecular dating, these postzygotic isolating mechanisms would have developed in less than 4 Myr, the total span for the diversification of this group (Table 3).

The phylogenetic results suggest that the ability to hybridize is not a characteristic extended throughout the Algarobia *s.l.* group. Indeed, the species of the two main clades found within this group (Fig. 3) present strikingly different behaviour. Whereas, as previously indicated, mesquites do frequently hybridize, hybrid formation between species of the *P. kuntzei–P. argentina* clade has not been documented. Although species of the *P. kuntzei–P. argentina* clade might have developed isolating mechanisms that prevent hybrid formation, we consider that the reduced level of sympatry between most of these species is a sufficient explanation for the difference. Furthermore, no hybrids have been observed between mesquite species and species of *P. kuntzei–P. argentina* clade even if in this case they frequently appear in sympatry. This suggests the development of another barrier either intrinsic postzygotic or prezygotic in less than 8 Myr (Table 3), the estimated divergence time between these two clades.

ANCESTRAL CLIMATE RECONSTRUCTION

During recent years, optimization techniques have been increasingly used to infer ancestral ecological conditions (Verboom *et al.*, 2003; Graham *et al.*, 2004; Hardy & Linder, 2005; Schrire, Lavin & Lewis, 2005). In the present study, an optimization analysis was performed to evaluate the historical association of *Prosopis* with arid environments. In particular, we were interested in defining since when *Prosopis* species have occupied this kind of environment. The

analysis performed suggested an ancient association of *Prosopis* with arid conditions (Fig. 6), already established before the splitting of the two main groups within ASP + *Xerocladia* clade (26.7–33 Mya). This result disagrees with Roig (1993) who proposed that extant species of *Prosopis* occupying arid regions in America have originated from ancestral lineages of this genus which were restricted to wetter regions. Palaeoenvironmental data suggest that, although the development of large arid areas in America started in the late Miocene (see below), there were local arid conditions in North America (Axelrod, 1979a) and South America (Volkheimer, 1971; Jordan, Schlunegger & Cardozo, 2001) during much of the Tertiary. It is possible therefore that ancestral lineages of *Prosopis* had occupied these sites until the later spreading of arid conditions.

DIVERSIFICATION PATTERN AND CAUSES

One of the predictions derived from the hypothesis of aridity-driven diversification of *Prosopis* was a temporal correlation between the spreading of arid areas in the Americas and the diversification of the genus. Molecular clock dating showed that, except for the splitting between the two main groups, the divergence among extant lineages within ASP clade would have occurred in the Late Miocene or more recently, coincident with the estimated spreading of arid environments in America. Although with distinctive characteristics in each case, the development of arid environments occur simultaneously in North and South America starting in the Late Miocene and continuing in the Pliocene and Pleistocene. In North America, the development of regional arid areas is thought to have started in the late Miocene to Pliocene (Graham, 1999; Riddle & Hafner, 2006) associated with the uplift of the plateaus in Western North America. The Sonora desert, where some species of the genus are present today, would have developed from 15 to 8 Mya (van Devender, 2000). This process of aridification continued until the Pleistocene, reaching a considerable distribution in the interglacials (Axelrod, 1979a,b). In South America, the spreading of large areas of semiarid-arid conditions possibly started in the late Miocene concomitantly with the uplift of the Andes in the Quechua distrophic phase when it reached half of its present elevation (Gregory-Wodzicki, 2000) and started to act effectively as a barrier to moisture laden winds. During the 'Diaguita' diastrophic Phase, the final uplift of the Pampean Mountain Range and the Central Andes produced a rain-shadow effect that resulted in the extremely xeric conditions existing at present on the areas located between them (Pascual, Ortiz-Jaureguizar & Prado, 1996; Alberdi. Bonadonna

& Ortiz-Jaureguizar, 1997). These climatic changes would have produced the establishment of the biomes where *Prosopis* is established today (Axelrod, Kalin Arroyo & Raven, 1991; Pascual *et al*., 1996).

The possible correlation of the ASP diversification with the expansion of arid environments is also supported by the analysis of the temporal changes of diversification rates. LTT analysis indicates a clear increase in diversification rates from the late Miocene until the present compared to previous times. When this pattern is analysed in detail, two different shifts towards higher rates may be distinguished. The first of those occurred in the late Miocene and is coincident with the start of the divergence within each of the two major lineages of the ASP. The second shift appears to be associated with the start of mesquites divergence in the Pliocene and is in agreement with the high diversification rate estimated for this group (Table 4). A wrong assignment of missing taxa could have potentially affected the conclusion derived from this analysis. However, as most of the species not sampled belong to Series Chilensis, Ruscifoliae, and Pallidae, which formed the highly supported mesquite clade, a wrong assignment seems to be improbable.

The combined assessment of the results obtained in the present study is in agreement with the hypothesis that *Prosopis* evolved and diversified concomitantly with the spreading of arid areas in the Americas. This result is coincident with those obtained for other dry-lover plant groups distributed almost exclusively in North America, which is suggested to have undergone a parallel radiation in the last 10 Myr (Good-Avila *et al*., 2006). To our knowledge, no previous study found this pattern for a group with mainly South American distribution, as is the case of *Prosopis*. Consequently, the results of the present study suggest an extension of this parallel radiation to a continental scale.

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SUPPLEMENTARY MATERIAL

The following material is available for this article online:

Figure S1. Strict consensus of 4680 most parsimonious trees obtained in the five-marker analysis (*trn*S-*psb*C, G3pdh, NIA, *trn*L-*trn*F, *trn*K-*mat*K).
Table S1. Sequences downloaded from GenBank.
Table S2. Maximum and minimum humidity index for species included in the ancestral climate estimation analysis.

This material is available as part of the online article from:
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APPENDIX 1

List of species sequenced in the present study, indicating the Section and Series to which they belong, voucher specimen, the natural distribution and accession numbers

Species	Section–Series	Voucher specimen/seed bank accesion	Natural distribution	GenBank Accesion numbers G3pdh	trnS-psbC	NIA	trnL-trnF	trnK/matK
Prosopis cineraria (L.) Druce	Prosopis	DANIDA N° 01089/82	AF	EF165221	EF165309	EF165269	EF165292	EF165287/ EF165248
Prosopis africana (Guill., Perr., & Rich.) Taubert	Anonychium	HDRA N°63	AF	EF165209	EF165297	EF165253	EF165291	EF165286/ EF165251
Prosopis strombulifera (Lam.) Bentham	Strombocarpa–Strombocarpae	Hunziker & Gamerro 11359 (SI)	SA	EF165241	EF165327	EF165281	–	–
Prosopis reptans Bentham	Strombocarpa–Strombocarpae	Saidman 36 (BAFC)	SA, NA	EF165237	EF165333	–	–	–
Prosopis torquata (Cavanilles ex Lagasca)	Strombocarpa–Strombocarpae	Hunziker 9733 (SI)	SA	EF165244	EF165330	–	–	–
Prosopis pubescens Bentham	Strombocarpa–Strombocarpae	Evans N° 15 (USDA-USA)	NA	EF165236	EF165323	–	–	–
Prosopis palmeri S. Watson	Strombocarpa–Strombocarpae	–	NA	EF165234	EF165322	EF165278	–	–
Prosopis ferox Grisebach	Strombocarpa–Cavenicarpae	Hunziker *et al.* 10451 (SI)	SA	EF165223	EF165311	–	–	–
Prosopis tamarugo F. Philippi	Strombocarpa–Cavenicarpae	DANIDA N° 01215/83	SA	EF165242	EF165328	–	EF165293	EF165289/ EF165250
Prosopis argentina Burkart	Monilicarpa	BNG Prosopis N° 15-88.	SA	EF165212	EF165300	EF165261	EF165290	EF165288/ EF165249
Prosopis sericantha Gilles ex Hooker & Arnott	Algarobia–Sericanthae	Saidman & Vilardi 961 (BAFC)	SA	EF165240	EF165326	EF165280	–	–
Prosopis kuntzei Harms	Algarobia–Sericanthae	Saidman & Vilardi 516 (BAFC); Saidman & Vilardi 521 (BAFC)	SA	EF165230 EF165231	EF165318 EF165319	EF165276 EF165277	–	–
Prosopis ruscifolia Grisebach	Algarobia–Ruscifoliae	Saidman & Vilardi 420 (BAFC)	SA	EF165239	EF165325	EF165257	–	–
Prosopis vinalillo Stuckert	Algarobia–Ruscifoliae	Saidman & Vilardi 505 (BAFC); Saidman & Vilardi 511 (BAFC)	SA	EF165245 EF165246	EF165331 EF165332	EF165283 EF165284	–	–
Prosopis denudans Bentham	Algarobia–Denudantes	Catalano 33 (BAFC)	SA	EF165222	EF165310	EF165270	–	–
Prosopis ruizleali Burkart	Algarobia–Denudantes	Catalano 13 (BAFC)	SA	EF165238	EF165324	EF165279	–	–

Species	Section	Region	Voucher (BNG)					
Prosopis castellanosii Burkart	Algarobia–Denudantes	SA	Catalano 8 (BAFC)	EF165218	EF165306	EF165267	–	–
Prosopis humilis Gilles ex Hooker & Arnott	Algarobia–Humiles	SA	Catalano 1 (BAFC)	EF165227	EF165315	EF165275	–	–
Prosopis campestris Griesebach	Algarobia–Pallidae	SA	Catalano 18 (BAFC); Catalano 20 (BAFC)	EF165216 EF165217	EF165304 EF165305	EF165265 EF165266	–	–
Prosopis affinis Sprengel	Algarobia–Pallidae	SA	Saidman *et al.* 526 (BAFC)	EF165208	EF165296	EF165256	–	–
Prosopis articulata S. Watson	Algarobia–Pallidae	NA	–	EF165213	EF165301	EF165262	–	–
Prosopis tamaulipana Burkart	Algarobia–Pallidae	NA	–	EF165243	EF165329	EF165282	–	–
Prosopis chilensis (Molina) Struntz emend. Burkart	Algarobia–Chilenses	SA	Saidman & Vilardi 911 (BAFC); Saidman & Vilardi 922 (BAFC)	EF165219 EF165220	EF165307 EF165308	EF165268 EF165285	–	–
Prosopis juliflora (Swartz) DC	Algarobia–Chilenses	NA,CA,SA	Hunziker 10039 (SI); Hunziker 10040 (SI)	EF165228 EF165229	EF165316 EF165317	EF165258 EF165274	–	–
Prosopis nigra (Grisebach) Hieronymus	Algarobia–Chilenses	SA	Saidman & Vilardi 435 (BAFC); Saidman 315 (BAFC)	EF165232 EF165233	EF165320 EF165321	EF165259 EF165260	–	–
Prosopis caldenia Burkart	Algarobia–Chilenses	SA	Saidman & Vilardi 350 (BAFC); Saidman & Vilardi 138 (BAFC)	EF165214 EF165215	EF165302 EF165303	EF165263 EF165264	–	–
Prosopis flexuosa DC	Algarobia–Chilenses	SA	Saidman & Vilardi 302 (BAFC); Catalano 10 (BAFC)	EF165224 EF165225	EF165312 EF165313	EF165271 EF165272	–	–
Prosopis glandulosa Torrey	Algarobia–Chilenses	NA	Evans N° 5 (USDA-USA)	EF165226	EF165314	EF165273	–	–
Prosopis alpataco R.A. Philippi	Algarobia–Chilenses	SA	Catalano 34 (BAFC)	EF165211	EF165299	EF165255	–	–
Prosopis alba Grisebach	Algarobia–Chilenses	SA	Saidman & Vilardi 413 (BAFC)	EF165210	EF165298	EF165254	–	–
Prosopidastrum angusticarpum Palacios & Hoc	NA	SA	Peter 244 (LP)	EF165235	EF165295	–	–	–EF165252
Xerocladia viridiramis Taub.	NA	AF	Germishuizen 8244 (PRE)	EF585492	EF585493	–	Supp. data	Supp. data
Acacia caven (Mol.) Molina	NA	SA	Saidman *et al.* 789 (BAFC)	EF165207	EF165294	EF165206	Supp. data	Supp. data
Lotus japonicus L.	NA	SA	–	EF165247	Supp. data	–	Supp. data	Supp. data

BNG *Prosopis*, Banco Nacional de Germoplasma de *Prosopis* (Cordoba, Argentina); DANIDA, Danida Forest Seed Centre, Denmark; GRS-USDA-USA, United States Department of Agriculture; HDRA, Henry Doubleday Research Association, United Kindom; SI, Darwinion Herbarium, San Isidro, Argentina; BAFC, Herbarium of Facultad de Ciencias Exactas y Naturales UBA, Buenos Aires, Argentina; AF, Africa; AS, Asia; CA, Central America; NA, North America; SA, South America; Supp. data, accession number included in Supplementary material (Table S1).

APPENDIX 2

Search strategies in parsimony and Bayesian analyses

In three-marker analysis, parsimony searches involved 100 Random Addition Sequences (RAS) followed by ten cycles of Tree Drifting. In the two-marker and five-marker analyses, the strategy involved 200 RAS followed by Tree Drifting and Random Sectorial Searches (Goloboff, 1999). These two algorithms intend to overcome the problem of local optima during tree searches. In Tree Drifting, suboptimal solutions are accepted with a certain probability in different moments of the swapping whereas, in Sectorial Searches, reduced datasets are created for different parts of the tree which are analysed by tree-bisection-reconnection (TBR). Then, the best tree for each sector is replaced in the original tree. In all cases, trees found using these strategies were used as starting trees for a final round of TBR keeping a maximum of 10 000 trees. Constrained searches forcing groups not found in the most parsimonious trees were run with the same settings.

Support was evaluated with jackknifing procedure with an independent character removal probability of 0.36. For each jackknifing replicate, search strategy was the same than previously described except that the number of RAS was decreased to 10. Absolute Bremer Support (Bremer, 1994) was calculated saving up to 6000 suboptimal trees in successive steps up to trees 12 steps longer than the optimal length.

Bayesian phylogenetic analyses were performed with the Metropolis-coupled Markov chain Monte Carlo algorithm implemented in MrBayes, version 3.1.2 (Ronquist & Huelsenbeck, 2005). The optimal model was selected with Modeltest (Posada & Crandall, 1998) and the values of each parameter were determined during each run. The analysis involved two independent runs of 2×10^6 generations sampled every 100 generations after a burnin period of 0.50×10^6 generations. Each run involved three heated chains. Convergence was assessed evaluating likelihood plots and standard deviation of the split frequencies.