

Phylogeny of *Prosopis* (Leguminosae) as shown by morphological and biochemical evidence

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Abstract. *Prosopis* L. is a rather primitive genus within the Leguminosae–Mimosoideae. This genus has been divided into five sections on the basis especially of the vegetative diversification of the spines. Three of the sections, *Algarobia*, *Monilicarpa* and *Strombocarpa*, are distributed in America. In order to elucidate systematic relationships between the American sections of *Prosopis*, a morphological and biochemical phylogeny were obtained. Twenty-two morphological characters were scored for 27 species of *Prosopis* and the outgroup taxon following polyacrilamide gel electrophoresis of seed storage proteins. The results obtained clearly prove that the section *Strombocarpa* is a natural taxon. The section *Algarobia*, on the other hand, seems to comprise an artificial grouping of species. Members of the series *Denudantes* appear isolated from the remaining species, therefore this taxon should be treated as a new section within *Prosopis*. The section *Algarobia* should be circumscribed to series *Chilenses*, *Ruscifoliae* and *Pallidae*, which are always united in a monophyletic clade. Finally, no evidence was found to confirm the existence of the section *Monilicarpa*.

Introduction

Prosopis L. is a rather primitive genus within the Leguminosae–Mimosoideae (Burkart 1976a). Burkart (1976a) based his hypothesis of *Prosopis* evolution on morphological characters, such as the simple pollen grains, the mostly free petals and the decandrous condition. Nevertheless, his hypothesis was not supported by further analysis. The genus includes trees or shrubs of different sizes, rarely subshrubs with spines or unarmed. A few species are sub-aphyllous with reduced paucifoliate leaves, but normally the specimens show bipinnate leaves with a few pairs of opposite pinnae bearing numerous, usually small, leaflets. Most of the species have a modified indehiscent legume called ‘drupaceous loment’ (Burkart 1952).

The genus *Prosopis* includes ~45 species, distributed in south-western Asia, Africa and predominantly in America (Burkart 1976a, 1976b; Hunziker *et al.* 1986; Palacios and Brizuela 2005; Palacios 2006), where they mainly grow in two important areas: the Mexican–Texan centre and the Argentine–Paraguayan–Chilean one, where most species occur (Burkart 1976a).

At present, Argentina is the major centre of diversity for the genus with ~27 species (Hunziker *et al.* 1986; Palacios *et al.* 1988). Therefore, nearly 60% of the species of the genus are found throughout the continental Argentinean territory, lacking only on the southern part of the Patagonian Region and the Misiones province (Burkart 1976a; Hunziker *et al.* 1986; Palacios *et al.* 1988). Thirteen species are endemic to Argentina, some of them with very restricted distributions,

such as *P. argentina*, *P. calingastana*, *P. castellanosi* and *P. ruizleali* (Hunziker *et al.* 1975; Correa 1984). There are several reports of interspecific hybridisation between some species of this genus in the Argentinean Chaco region (Hunziker *et al.* 1986; Ramirez *et al.* 1999; Burghardt *et al.* 2004; Vega and Hernandez 2005).

The tree species of *Prosopis* are an important component of many agroforestry systems in arid zones worldwide (Vega and Hernandez 2005). *Prosopis alba*, *P. nigra*, *P. juliflora*, *P. tamarugo* and *P. pallida* are suitable species for this purpose. Several species of the genus are promissory as multipurpose crop plants. They could colonise arid and semiarid regions providing shade, food and derivatives, fodder, firewood, timber for building furniture and wooden floors, gums and possibly tannins (Hunziker *et al.* 1986; Felker and Moss 1996).

Burkart’s monograph (1976a, 1976b) is the most extensive taxonomic study of the genus. In that work, the genus was divided into five sections on the basis of morphological traits, particularly using the vegetative diversification of the spines. Three of these sections, *Algarobia*, *Monilicarpa* and *Strombocarpa*, are distributed in America.

The section *Algarobia* has the largest number of species with 31 taxa, including spiny or rarely unarmed trees, shrubs or subshrubs with axillary caulinar spines. Its representatives are distributed in warmer and drier parts of America. This section was divided by Burkart (1976a, 1976b) into the following six series: *Sericanthae*, *Ruscifoliae*, *Humiles*, *Denudantes*, *Pallidae* and *Chilenses*.

The section *Strombocarpa* includes two series (*Strombocarpae* and *Cavenicarpae*), with species usually called ‘screwbeans’ or ‘tornillos’ because of the shape of their fruits, commonly coiled into dense spirals. Members of this section are shrubs or trees with stipular spines, distributed in the south-western United States, Mexico and South America.

The monotypic section, *Monilicarpa*, is represented by *Prosopis argentina*, an endemic shrub with caulinar and frequently apical spines. It is restricted to western Argentina where it inhabits sandy soils.

Although Burkart’s monograph is the only exhaustive morphological treatment of the genus found in the literature, several authors have published valuable contributions to corroborate the infra-generic divisions within *Prosopis*. For example, Caccavari de Filice (1972) in her study of pollen morphology, indicated differences between species of sections *Strombocarpa* and *Algarobia*. Furthermore, the foliar architecture distinguishes species belonging to different sections (Martinez 1984). On the other hand, Castro (1989), in analysing structural and ultra structural characters of the secondary xylem, found a clear separation between species of sections *Algarobia* and *Strombocarpa*, pointing out that *P. argentina* (within *Monilicarpa*) shares characters with almost all the species of *Algarobia*.

Biochemical and molecular analyses have greatly improved the knowledge of the relationships among species of Leguminosae (Burghardt 1996b; Bessega *et al.* 2006; Landeras *et al.* 2006; Espert *et al.* 2007). These tools also show their utility in *Prosopis*. Specifically, the biochemical data seems to be congruent with morphological observations. Chromatographic analyses of amino acids (Carman *et al.* 1974) and flavonoids (Carman *et al.* 1974; Gitelli *et al.* 1984) showed qualitative and quantitative dissimilarities among species from different sections. Moreover, immunological assays confirmed Burkart’s classification into sections (Cohen *et al.* 1967), and Saidman (1985), in her electrophoretic studies of enzymes, pointed out that the genetic differences between the sections *Algarobia* and *Strombocarpa* are great enough to prevent the correct inference of the homologies between isozymic bands. Finally, polypeptidic patterns have provided enough evidence to distinguish the three American sections of the genus (Burghardt and Palacios 1997). Seed-storage proteins are a valuable source of information, especially in legumes, where they have been used to clarify species boundaries and to study supra- and intra-specific relationships (Sammour 1994; Maquet *et al.* 1999; Burghardt 2000a; Espert and Burghardt 2003).

The major aim of the present study is to generate a morphological and biochemical phylogeny, in order to elucidate systematic relationships among the American sections of *Prosopis*. Phylogenetic relationships within *Prosopis* have remained largely unexplored, since all previous studies focused on genetic variability or phenetic analysis (Saidman and Vilardi 1987; Bessega *et al.* 2005; Vega and Hernandez 2005). Therefore, this work constitutes the first integral systematic approach to the study of the genus, in which *P. argentina* from the section *Monilicarpa*, representatives of five of the six series of the section *Algarobia* and of the two series of the section *Strombocarpa* were analysed, by using two different sources of evidence.

Materials and methods

Taxon sampling

The list of the taxa examined, their taxonomic positions in Burkart’s system (1976a), their origin and voucher information are provided in Table 1.

Morphological data

Twenty-two morphological characters were scored for the species of *Prosopis* and the outgroup, *Prosopidastrum angusticarpum*. The list of all included morphological characters and character states is shown in Table 2. All characters analysed were obtained from the herbarium specimens listed in Table 1, except for the *Prosopis palmeri* data that were obtained from Burkart (1976a, 1976b). *In situ* field observations of the South American species made at the moment of collection, allowed us to accurately verify the plant habit.

Biochemical data

Storage-seed proteins were extracted from single mature seeds in the case of the species of sections *Algarobia* and *Monilicarpa*. Because of the small size of the seeds in some species of the section *Strombocarpa*, three to four seeds were used. Seeds were extracted by grinding with mortar and pestle; the resultant powder was mixed with 0.5 mL of aluminium lactate buffer at pH 3.6 plus 3 M urea. After 30 min, the samples were centrifuged at 10 000 rpm for 30 min at 4°C, and the supernatant was submitted to electrophoresis.

Electrophoresis was performed by using 7% polyacrylamide gels made in water. Prior to electrophoresis, the gels were equilibrated in the extraction buffer at 4°C for at least 48 h. Electrophoresis runs were conducted for ~6 h at 400–600 V, with aluminium lactate buffer (pH 3.6) in both reservoirs. Gels were stained with 0.005 % w/v Coomassie brilliant blue (Sigma) in a solution of MeOH–HOAc–H₂O (4 : 1 : 10) for 18 h.

The data matrix was constructed by scoring the resultant protein bands as double state absence/presence characters.

Phylogenetic analysis

Prior to the analysis, congruence among datasets was measured by the incongruence length difference test (Farris *et al.* 1995) with Winclada (Nixon 2002). The datasets were analysed with T.N.T. ver 1.0 (Goloboff *et al.* 2003), with different costs for the biochemical characters. Differential costs can be applied to events; however, typically insufficient information exists to justify this and so events are treated equally (Jackson 2004). Despite this observation, we applied a weighting scheme, on the basis of the assumption that the acquisition of the same amino acid chain is more unlikely than the loss of the protein. Therefore, different set of costs (1/1, 2/1 and 10/1) for the transition between absence to presence of the protein were tried.

The analyses were conducted by heuristic search methods, with 20 random addition sequences plus TBR, retaining 10 cladograms per replicate, keeping up to 10 000 trees.

Bremer supports (Bremer 1994) were calculated, finding up to 10 extra steps suboptimal trees and retaining 10 000 trees in the memory buffer. Jackknife values (Farris *et al.* 1996) were found by resampling the matrix 1000 times, with a 36 removal probability.

Table 1. List of the taxa and accessions analysed

Abbreviations for collectors: N Bacigalupo (NB), R Braun Wilke (RBW), L Bravo (LB), A Burghardt (ADB), A Carter (AC), M Correa (MC), O Di Iorio (ODI), P Hoc (PSH), J Hunziker (JHH), C Muñoz (CM), C Naranjo (CAN), R Palacios (RAP), S Enus Zeiger (SEZ), O Solbrig (OTS) and B Simpson (BS)

Taxon	Geographical origin (country and provinces), voucher information (collector and accession number) and depository
<i>Prosopidastrum</i> Burkart	
<i>P. angusticarpum</i> R.A.Palacios & P.S.Hoc	Argentina: Rio Negro (RAP & ADB 1803, 1804) BAFC
<i>Prosopis</i> L.	
Section <i>Strombocarpa</i>	
Series <i>Strombocarpae</i>	
<i>P. burkartii</i> Muñoz	Chile: Iquique (RAP 1574, 1575, 1625) BAFC
<i>P. palmeri</i> Watson	Mexico: Baja California (AC 4275, 4034) UT
<i>P. reptans</i> Benth.	Argentina: Córdoba (RAP, ADB <i>et al.</i> 1635) BAFC
<i>P. strombulifera</i> (Lam.) Benth.	Argentina: Catamarca (JHH 9563); San Juan (RAP 1630); Mendoza (RAP 1603); San Luis (RAP & ADB 1732, 1733; ADB <i>et al.</i> 1637) BAFC CHILE: Iquique (RAP 1590) BAFC
<i>P. torquata</i> (Cav. ex Lag.) DC.	Argentina: Catamarca (JHH 9571; JHH, CAN & RAP 9191); La Rioja (JHH w/n°); San Luis (RAP & ADB 1730, 1731) BAFC
Series <i>Cavenicarpae</i>	
<i>P. ferox</i> Griseb.	Argentina: Jujuy (MC and NB w/n°) SI, (RBW 119, 120, 121, 141, 142) BAFC
<i>P. tamarugo</i> Phil.	Chile: Tarapacá (CM w/n°) SI, (JHH 9839) BAFC; Iquique (RAP 1576 to 1579); Arica (RAP 1585) BAFC
Section <i>Monilicarpa</i>	
<i>P. argentina</i> Burkart	Argentina: Catamarca (JHH 9564) BAFC
Section <i>Algarobia</i>	
Series <i>Chilenses</i>	
<i>P. alba</i> Griseb.	Argentina: Formosa (RAP 314, 323, 329, 330, 464, 548; RAP, ADB & PSH 797) BAFC, (OTS 4238, 4247) GH; Santiago del Estero (RAP 512, 522; RAP, ADB & PSH 818) BAFC, (OTS-BS 4273) GH; Chaco (RAP 496, 523, 529, 580, 920, 922, 923) BAFC, (OTS-BS 4281) GH; Santa Fe (RAP 677) BAFC
<i>P. alpataco</i> Phil.	Argentina: San Luis (JHH, CAN & RAP 9053); La Pampa (SEZ 108); Mendoza (RAP 1651 to 1659); Neuquén (RAP 1665, 1666); Río Negro (JHH, CAN & RAP 8663) BAFC
<i>P. caldenia</i> Burkart	Argentina: La Pampa (SEZ 115; JHH 9758) BAFC
<i>P. chilensis</i> (Molina) Stuntz emend. Burkart	Argentina: La Rioja (JHH 9737) BAFC
<i>P. flexuosa</i> DC.	Argentina: Catamarca (JHH, CAN & RAP 9199); San Juan (JHH, CAN & RAP 9054, 9808); Mendoza (RAP & ADB 1747); San Luis (JHH, CAN & RAP 9054; RAP & ADB 1734) BAFC
<i>P. glandulosa</i> Torrey	U.S.A.: Nuevo México (BS 2215–1, 2216–1); Texas (BS 2218–1, 2219–1) GH
<i>P. juliflora</i> (Swartz) DC.	COLOMBIA: Magdalena (JHH 10046, 10048) BAFC
<i>P. pugionata</i> Burkart	Argentina: San Luis (RAP & ADB 1736, 1737); Mendoza (RAP & ADB 1739) BAFC
<i>P. nigra</i> (Griseb.) Hieron.	Argentina: Formosa (RAP 465, 473, 487) BAFC, (OTS 4255, 4267) GH; Santiago del Estero (RAP 578; RAP, ADB & PSH 830); Chaco (RAP 499, 524, 525, 528, 535); Salta (RAP 927 to 931) BAFC
<i>P. velutina</i> Wooton	U.S.A.: Arizona (BS 2227–1, 2229–1) GH. MEXICO: Sonora (BS 2211/1) GH
Series <i>Sericanthae</i>	
<i>P. kuntzei</i> Harms	Argentina: Chaco (ODI w/n°) BAFC
Series <i>Denudantes</i>	
<i>P. castellanosi</i> Burkart	Argentina: Neuquén (RAP & ADB 1774 to 1776) BAFC
<i>P. denudans</i> Benth.	Argentina: Río Negro (RAP & ADB 1784 to 1790) BAFC
<i>P. ruizleali</i> Burkart	Argentina: Mendoza (RAP 1643, 1645, 1648 to 1650; RAP & ADB 1755 to 1764); Neuquén (RAP 1662 to 1664; RAP & ADB 1769, 1772) BAFC
Series <i>Pallidae</i>	
<i>P. affinis</i> Spreng	Argentina: Entre Ríos (CAN 288, 364, 365, 272) BAFC
<i>P. pallida</i> (H & B ex Willdenow) HBK	Peru: Majoro (JHH 10008) BAFC
<i>P. rubriflora</i> Hassler	Paraguay: Concepción (RAP 1681) BAFC
Series <i>Ruscifoliae</i>	
<i>P. hassleri</i> Harms	Argentina: Formosa (RAP 311, 316, 320, 322, 462, 480, 556 to 558, 560, 561, 563; RAP, ADB & LB 683, 684, 708; RAP, ADB & PSH 796, 802, 805, 807) BAFC
<i>P. ruscifolia</i> Griseb.	Argentina: Formosa (RAP 307, 321, 332, 334, 478, 539, 540, 550, 554; RAP, ADB & PSH 811, 813 to 816, 835 to 837) BAFC, (OTS 4266) GH; Santiago del Estero (BS 1037–1, 1037–4, 1037–5) GH, (RAP 511, 513, 516, 518 to 521, 571 to 573, 576, 577, 667 to 669, 671, 829, 946, 947; RAP, ADB & PSH 838 to 842); Chaco (RAP 489, 498, 526, 527, 530, 533, 534, 536, 538); Tucumán (RAP 661 to 664); Salta (RAP 926, 937, 938) BAFC

Table 2. List of morphological characters analysed
Codification of each character state is indicated in parentheses

1. Habit:	Shrub (0), tree (1)
2. Striated stems:	Absent (0), present (1)
3. Gemiferous stems:	Absent (0), present (1)
4. Lignified stipules:	Absent (0), present (1)
5. Caulinar spines:	Absent (0), present (1)
6. Glands at the base of the stipule:	Absent (0), present (1)
7. Leaves:	Unijugate (0), multijugate (1)
8. Leaf epidermis:	Glabrous (0), pubescent (1)
9. Leaflet apex:	Obtuse (0), acute (1)
10. Acumen:	Absent (0), present (1)
11. Inflorescences:	Spike like raceme (0), heads (1), aments (2)
12. Flower color:	Yellow green (0), red (1)
13. Corolla:	Free petals (0), gamopetalous (1)
14. Petals:	Glabrous (0), pilose (1)
15. Mesocarp:	Dry (0), fleshy (1)
16. Legume:	Cylindrical (0), compressed (1)
17. Fruit shape:	Coiled (0), not coiled (1)
18. Sutures:	Moniliform (0), parallel or slightly coiled (1)
19. Fruit colour:	Yellow (0), purple to brown (1), red (2), black (3), dark grey to brown (4), brown (5)
20. Endocarp segments:	Coriaceous (0), bony (1), membranous (2)
21. Number of seed rows per fruit:	One (0), two (1)
22. Seed disposition:	Longitudinal (0), transversal (1)

Results

Morphological data

From the observations of the plant material (Table 1) and data obtained from the literature, a matrix with 22 vegetative and reproductive characters was constructed, in which 18 were parsimony informative (Table 3).

Biochemical data

In total, 149 protein bands were detected in the electrophoregrams of all the species analysed (not shown, but see Burghardt and Palacios 1997 for more details). Most were observed for the genus *Prosopis* (137 bands), whereas for the outgroup species, *Prosopidastrum angusticarpum*, 12 unique bands were found from a total of 19 fractions.

Twenty bands were exclusive to the section *Strombocarpa*; these fractions were not present in any of the species of the other sections studied. Nevertheless, only one of these bands was shared by almost all species of *Strombocarpa*, *P. palmeri* being the only taxon where this fraction was not observed. Moreover, this latter species displayed only 2 of the 20 exclusive bands mentioned above. All species of the section *Strombocarpa* shared with the taxa of the section *Algarobia* one polypeptidic fraction, which was not present in *P. argentina*. The latter, the only species of the monotypic section *Monilicarpa*, had four exclusive protein bands. In the protein pattern of this taxon were observed four bands that also appear in the species of the section *Strombocarpa*, whereas seven bands are present in the species of both section *Monilicarpa* and section *Algarobia*.

Seventy-five of the 137 bands observed in *Prosopis* are present exclusively in the species of the section

Table 3. Character state matrix

Characters are listed in the same order as Table 2. ?, missing data; *polymorphism

Species	1	2
<i>Prosopidastrum angusticarpum</i>	1234567890123456789012	00?1100110100001114010
<i>Prosopis chilensis</i>	1000101011200111110101	1000101011200111110101
<i>P. glandulosa</i>	10?0100000200111100100	1000101000200111010101
<i>P. caldenia</i>	1000101000200111010101	1010101000200111101100
<i>P. flexuosa</i>	1010101000200111101100	100010010020011111110?
<i>P. velutina</i>	10001000?0200111101101	100010001?200111110111
<i>P. pugionata</i>	100010001?200111110111	1000100001200111110101
<i>P. alba</i>	1000100001200111110101	100010100?200111101121
<i>P. juliflora</i>	100010100?200111101121	0010100001200111110101
<i>P. nigra</i>	0010100001200111110101	1000101101200111110101
<i>P. alpataco</i>	10001011?210111110101	100010*1?0200111101101
<i>P. pallida</i>	100010*1?0200111101101	01001001??000101112001
<i>P. rubriflora</i>	01001000??200111113101	01001000?1200111113101
<i>P. affinis</i>	01001000?1200111113101	1000101101200111113111
<i>P. castellanosi</i>	1010100010200111101100	1000100001200111110111
<i>P. denudans</i>	0010100100200110102100	1001000001001101115210
<i>P. ruizleali</i>	1001010000001100005100	0011000100101100010000
<i>P. kuntzei</i>	0011000100101100010000	0011000100101100010000
<i>P. ruscifolia</i>	0001000100001100005110	1001000100001100115011
<i>P. hassleri</i>	10010001?1001100110011	
<i>P. argentina</i>		
<i>P. palmeri</i>		
<i>P. burkartii</i>		
<i>P. strombulifera</i>		
<i>P. reptans</i>		
<i>P. torquata</i>		
<i>P. tamarugo</i>		
<i>P. ferox</i>		

Algarobia, but only three of them are marker bands of the section.

Phylogenetic analysis

The ILD test showed that the datasets are congruent ($P=0.005$); therefore, a simultaneous analysis was performed. The morphological and biochemical datasets were combined into a single matrix of 28 taxa and 170 characters (112 parsimony informative) and submitted to a heuristic search. Under equal weights, 31 trees of length 338 were obtained ($CI=0.352$, $RI=0.644$). The consensus with the node support is shown (Fig. 1). In this tree, species of the section *Strombocarpa* are grouped in a single clade, whereas species of the section *Algarobia*, along with *P. argentina*, comprise a second monophyletic group, although both clades have low support values.

When a set of costs 2/1 was applied (i.e. transition between absence of protein band to its presence has cost 2, and presence to absence has cost 1), two trees 481 steps long were obtained ($CI=0.247$, $RI=0.625$); we present here the consensus of these two optimal trees (Fig. 2). This cladogram shows little resolution, since two polytomies are observed near the base of the *Prosopis* clade. Most of the species of the section *Strombocarpa* are grouped in a single clade as in Fig. 1, the only exception being *P. palmeri*, which appears in the basal polytomy; however, in one of the two most parsimonious

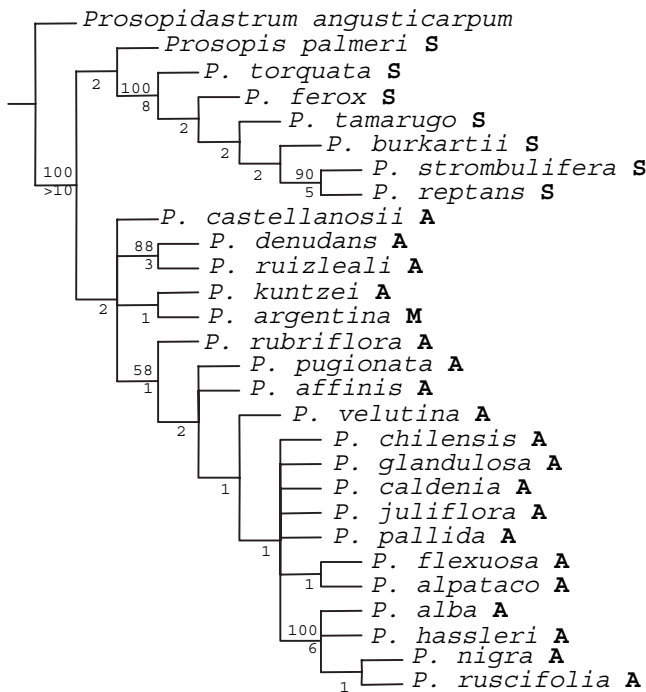


Fig. 1. Strict consensus tree of 31 optimal cladograms, obtained under an equal weight cladistic analysis of morphological and biochemical data. Jackknife values over 50% and Bremer support values are shown above and below branches, respectively. Sections of *Prosopis* are indicated with capital letters (A, *Algarobia*; M, *Monilicarpa* and S, *Strombocarpa*).

trees, all species of the section comprise a monophyletic clade. *Prosopis argentina* (section *Monilicarpa*) is found basal to the clade where most of the species of the section *Algarobia* are grouped, although with low support values. This clade comprises species of series *Ruscifoliae*, *Pallidae* and *Chilenses*, whereas relationships between species of series *Denudantes* and *Sericanthae* remain unresolved. However, when a new analysis constraining monophyly of section *Algarobia* was performed, one most parsimonious tree was found only three steps longer (length 484).

Finally, under transformation costs 10/1 the searches resulted in two trees of 609 steps ($CI=0.195$, $RI=0.737$), and the consensus tree was obtained (Fig. 3). Once again, species of the section *Strombocarpa* comprise a monophyletic clade, and the two series defined by the current *Prosopis* classification system are separated into two monophyletic groups. Members of section *Algarobia* are spread in different clades of this consensus tree. A monophyletic group contains species of series *Pallidae*, *Chilenses* and *Ruscifoliae*, whereas a second clade, more related to section *Strombocarpa*, is comprised of members of *Denudantes*, *P. kuntzei* and the only species of section *Monilicarpa*, *P. argentina*. When the monophyly of section *Algarobia* is constrained, two optimal trees of length 625 are obtained. This is 16 steps longer than the original search.

Discussion

Several hypotheses can be inferred following the cladistic analyses of the morphological and biochemical character states

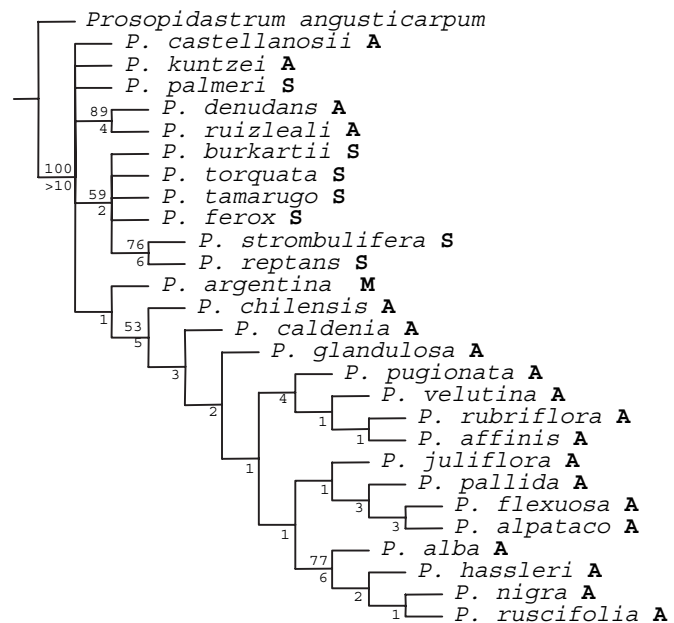


Fig. 2. Strict consensus tree recovered when a set of costs 2/1 was applied to the combined dataset.

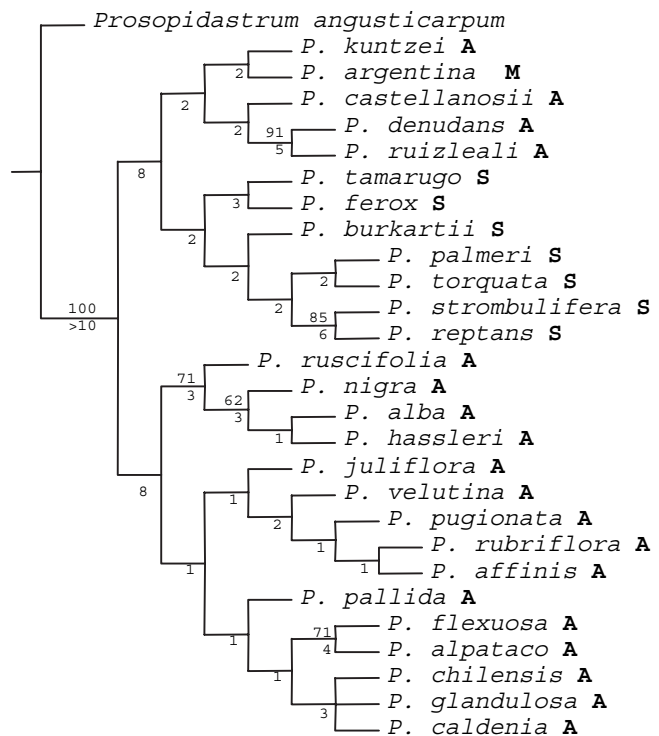


Fig. 3. Strict consensus tree obtained under transformation costs 10/1.

of 27 species of the genus *Prosopis*. These hypotheses represent interesting contributions to the classification structure of this important genus.

Section *Strombocarpa*

The results strongly suggest that section *Strombocarpa* constitutes a natural taxon, since all its species belong to a monophyletic clade in almost all the shortest trees of weighted and unweighted analyses. This hypothesis is contrary to that of Bessega *et al.* (2006), who concluded that the section is not monophyletic. Nevertheless, these authors only included three species, whereas our study included seven of the nine species of the section.

The gamopetalous corolla and the presence of foliar spines are synapomorphies for this section. In addition, species of the series *Cavenicarpae* (*P. tamarugo* and *P. ferox*) are always associated in a clade, although with low support values. When transition costs of 10/1 are applied, these species are separated from those of series *Strombocarpae*, the other group of the section *Strombocarpa*. The two series constitute clades with low support values; this feature is in agreement with the fact that there are no protein bands that are present exclusively in all the species of series *Strombocarpae* or *Cavenicarpae*. The pattern of presence/absence of the remaining polypeptidic fractions do not concord with the taxonomic division of the section proposed by Burkart (1976b). Also, only one morphological character (seed disposition) constitutes a synapomorphy of each series: *Cavenicarpae* has seeds transversally placed in the pod, whereas the seeds of *Strombocarpae* are oriented longitudinally.

Moreover, the lack of support for the series found in the present study is in agreement with the possible existence of hybridisation between the species of *Cavenicarpae* and *Strombocarpae* (Palacios *et al.* 1991).

Prosopis strombulifera and *P. reptans*, of series *Strombocarpae*, are joined in highly supported monophyletic clades in all the analyses performed, which are congruent with Burkart's observation (1976a, 1976b) about the possible subspecies status of these two taxa. This hypothesis was also proposed by Saidman (1985) on the basis of isoenzymatic studies, and Burghardt (2000b) in her phenetic study of the section.

Prosopis palmeri was initially considered as a member of the section *Algarobia* (Watson 1889), later it was treated by Britton and Rose (1928) as a new genus (*Sopropis*). Burkart (1940) considered this species to be in the section *Anonychium*, but a few years later placed *P. palmeri* in the section *Strombocarpa* (Burkart 1976a, 1976b). In our study, although *P. palmeri* has alternative placements in the weighted and unweighted optimal trees obtained, this species has a clear affinity with the members of the section *Strombocarpa*, supporting its inclusion within this group in agreement with Burkart (1976a).

Section *Algarobia*

This section, as defined by Burkart (1976a), seems to be an artificial grouping of species, according to our results and those of Bessega *et al.* (2006). Members of the series *Denudantes* (three of its four species were studied here) appear isolated from the remaining species of *Algarobia*. *Prosopis denudans* and *P. ruizleali* always conform to a highly supported clade (support values 88–91%); the suggestion that *P. ruizleali* is a subspecies of *P. denudans* (Burkart 1976b) is in agreement with these results. The three species of *Denudantes* do not compose a monophyletic

clade on the consensus of the analysis performed with equal transition costs (Fig. 1); however, when the monophyly of this section is constrained, the most parsimonious trees (MPT) recovered are only three steps longer than the cladograms obtained with no constraints applied. When transition costs 2/1 are applied before the cladistic searches, the species of *Denudantes* are associated into a monophyletic clade in some of the most parsimonious trees recovered; and with 10/1 costs, this same clade appears in the consensus of the most parsimonious trees (Fig. 3). We propose that this series should be treated as a new section of the genus *Prosopis*, since its members are clearly isolated from the remaining species of the section *Algarobia*. This separation is supported by the presence of striated stems and of several unique protein bands. This suggestion agrees with previous phenetic studies (Burghardt 1992).

According to our results, *P. kuntzei* (section *Algarobia*, series *Sericanthae*) has an uncertain placement within the genus *Prosopis*. The addition of more *Sericanthae* species and new characters from different sources, would contribute towards the resolution of the taxonomic status of this series.

Members of the series *Chilenses*, *Ruscifoliae* and *Pallidae* are always united in a monophyletic clade, which means that they form a natural group and deserve a taxonomic rank as a whole, perhaps as a section *Algarobia sensu stricto*. However, the boundaries of the three groups inside this clade are unclear. Burghardt (2000a) made a phenetic analyses of this section, and pointed out that the relationships observed among the three series mentioned above could not be completely correlated with the system proposed by Burkart (1976a, 1976b).

Prosopis nigra (series *Chilenses*) seems to be more closely related to *P. ruscifolia* of the series *Ruscifoliae*, and not to *P. alba*, which belongs to the same series as *P. nigra*, following Burkart's system. In addition, *P. alba* appears closely related to another member of *Ruscifoliae*, *P. hassleri*. These four species form a monophyletic clade in the consensus obtained, with good support values (71 to 100%). Saidman (1985) and Burghardt and Palacios (1991) suggested that all these taxa should be considered as semi-species, although Burghardt (1996a) found evidence to consider them valid biological species. We prefer the hypothesis of the existence of a common ancestor for these four taxa, rather than them being the product of successive hybridisation events, a scenario that actually occurs in the genus but does not seem to be responsible for the variability found in the area where this group of species resides (Burghardt 1996b). On the other hand, the variability found for the species of the series *Sericanthae* and *Denudantes*, along with their geographic distribution (especially of the latter one) and morphological specialisation, seem to indicate that the allopatric genetic divergence played a major role in the speciation of this group.

Section *Monilicarpa*

There is no evidence to confirm the existence of this section. *Prosopis argentina* has an uncertain position in all the cladograms obtained, and its phylogenetic affinities still remain ambiguous. This species appeared inside the *Algarobia* clade (see Fig. 1), or in a basal position, next to the species of the series *Chilenses*, *Ruscifoliae* and *Pallidae* (Fig. 2), or finally, it was on a totally different position when transformation costs 10/1 are applied. If, before the analyses with costs 10/1, the monophyly of

the section *Algarobia* is constrained, *P. argentina* is placed inside the *Strombocarpa* clade, next to *P. ferox*. Clearly, additional studies should be conducted in order to resolve the position of this species in a *Prosopis* natural classification system.

Biogeographical considerations

An interesting conclusion could be obtained from the results of the present work regarding the geographic distribution of the genus and the diversification of its species. Given that the sections *Strombocarpa* and *Algarobia* have species that grow on North and South America, and that these species are found merged in single clades (see Figs 1 and 3), it could be inferred that both centres of distribution of the genus comprised an ancestral palaeoflora. In some cases, diversification might have taken place before the separation of these areas.

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