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An Assessment of *Prosopis* L. in the Bokspits Area, South-Western Botswana, Based on Morphology

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ABSTRACT

The genus *Prosopis* comprises 44 species. Extensive introgression and hybridization is suspected in the genus, which makes identification of the species very difficult. Accurate identification of the species is crucial in controlling invasive species, within this genus. That is so because the method of control is species specific. The objective of the study was to determine the taxonomic structure of *Prosopis* species in Southern Botswana. A systematic qualitative approach was used to sample the species such that specimen selection was based on observable morphological discontinuities. The morphological characters were subjected to multivariate analysis since the analysis has been reported to be good at identifying hybrids. The multivariate analysis included anova, cluster analysis, factor analysis and canonical correlation. The study revealed four pure lines of *Prosopis* and nine hybrid species. The pure lines are *Prosopis chilensis*, *P. juliflora*, *P. velutina* and *P. glandulosa* and they all belong to one section, ALGAROBIA. The observed hybrids were *P. chilensis x P. glandulosa*, *P. glandulosa x P. chilensis*, *P. juliflora x P. glandulosa*, *P. chilensis x P. juliflora*, *Acacia karroo x P. juliflora*, *P. glandulosa x P. pallida*, *P. juliflora x P. pallida*, *P. chilensis x P. juliflora x P. glandulosa* and *P. chilensis x P. pallida*. It was therefore concluded that the *Prosopis* species in Southern Botswana have formed a hybrid swam. And for the hybrids *P. chilensis x P. glandulosa* and *P. glandulosa x P. chilensis* it was concluded that gene flow between *P. chilensis* and *P. glandulosa* is bidirectional. For the hybrid *P. juliflora x P. glandulosa* it was concluded that gene flow was from *P. juliflora* to *P. glandulosa*.

Keywords: ALGAROBIA, hybrid – swam, multivariate analysis, PAUP

INTRODUCTION

The genus *Prosopis* L. comprises of trees, shrubs or subshrubs that are spiny or rarely unarmed. The leaves are bipinnate, with few pairs of opposite pinnae; petiole with circular, sessile, apical gland and sometimes smaller, similar ones on rachis of pinnae. Leaflets are small, many, mostly opposite, linear, oblong and fusiform. Inflorescence is spike-like, axillary and sometimes with globose heads. Flowers are bisexual and small with campanulate 5-merous calyx. The corolla has 5 linear petals that can be fused or free, glabrous or pubescent and frequently villous or pilose inside towards tip. The stamens are 5 + 5 and free. Anthers are elliptic, dorsifixed, introrse, with an apical, pedicellate, globose or ovoid connectival gland. Pods are linear, straight, falcate and annular to spiral coils. Seeds are ovoid, compressed, hard and brown. The genus is reported to exhibit high levels of polyploidy with $x = 13, 14$ (Linnaeus 1767; Johnston 1962; Hutchinson 1964; Burkart 1976 and Germishuizen 2000).

Prosopis is classified into 44 species with five sections as follows: Section PROSOPIS, Section ANONYCHIUM, Section STROMBOCARPA, Section MONILICARPA and Section ALGAROBIA. Section ALGAROBIA is the biggest of the five sections and is further divided into seven series which are Sericanthae, Ruscifoliae, Denudantes, Pallidae, Chilensis and Humiles. Species that are found in Botswana are from the series Pallidae and Chilensis as follows:

1) Series Pallidae; *Prosopis. pallida* (Humboldt & Bonpland ex Willd.) H.B.K, 2) Series Chilensis; *P. chilensis* (Molina) Stuntz emend Burkart, *P. glandulosa* Torrey, *P. juliflora* Swartz DC, and *P. velutina* Wooton.

These species are indigenous to South America and were purposely introduced into Botswana by the Ministry of Agriculture with objectives of controlling desertification and the spread of sand dunes (BCAPR 2004). Members of this genus are generally known as mesquites. In general, the introduced species can have both positive and adverse effects.

The positive effects include economic benefits as outlined below. These include *Prosopis juliflora* pods, which can be used as a coffee substitute, bread flour and medicine for treating skin lesions and digestive disturbances. Flour from such pods can also be used as a lactation enhancer and an aphrodisiac (Rocha 1986). *Prosopis chilensis* on the other hand stabilizes sand dunes and makes good feed for sheep and goats (Mustafa 1986), while *Prosopis glandulosa* is a medicine for gout, dropsy and oedema (Najila *et al.* 2002). And wood from most species of *Prosopis* is a good fuel and can improve fertility of the soil through nitrogen fixation (Pasiecznik *et al.* 2004).

Negative impacts as reported by dwellers of BORAVAST include the following; blockage of boreholes due to the dense root system, depletion of water tables and nutrients at deep soil profiles and allelopathic effects that enable the *Prosopis* to invade the area. And, according to Oba *et al.* (2000), bush cover that exceeds 30% degrades rangeland condition.

The bush thickets caused by the *Prosopis* also act as hiding places for criminals as reported by the villagers. For instance, police officers in Bokspits allege that criminals easily smuggle livestock to South Africa by taking advantage of the dense bush thickets formed by the *Prosopis*. And the bush thickets along road sides contribute to an increase in road accidents that involve livestock and vehicles (BCAPR 2004).

There also are complaints by the villagers that the *Prosopis* plants activate allergic conditions them. And pricks by *Prosopis* thorns cause wounds that are painful and difficult to heal (BCAPR 2004).

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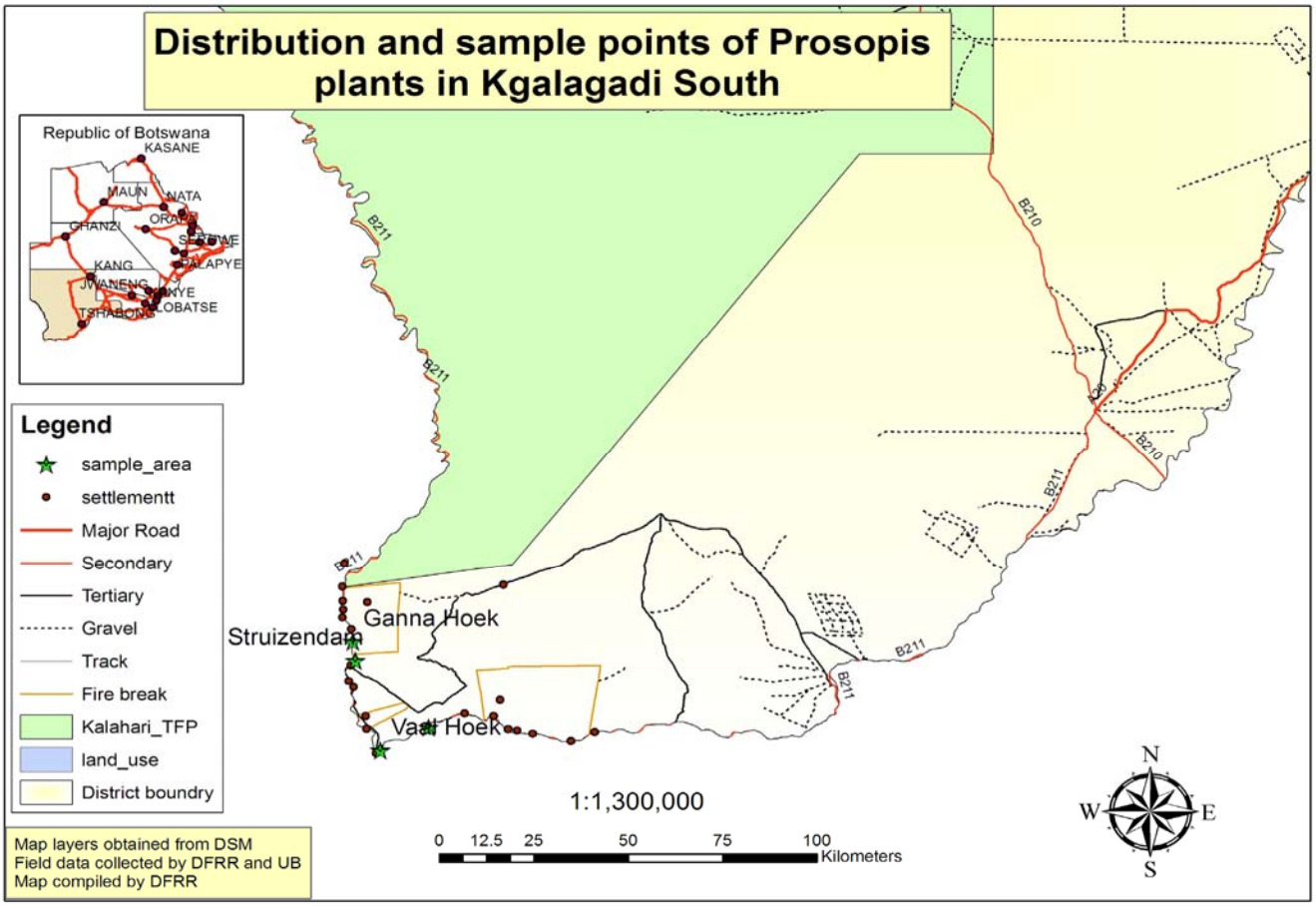

Fig. 1 ***Prosopis*** **sampling points in BORAVAST, Kgalagadi.**

Although, the negative effects of *Prosopis* far outweigh the positive ones, there is a realisation that complete eradication may bring some unwanted environmental impacts (BCAPR 2004).

Therefore, in trying to assist the communities of Bokspits, Rapplespan, Valhoek and Struizendam (BORAVAST), a study was designed that would assist in generating data to be used in designing a management and control strategy for the spread of *Prosopis* in the area. But then the challenge was that there are different methods of controlling the spread and invasion of *Prosopis*, depending on the species in question. As such, a baseline taxonomic study to determine *Prosopis* species in the area was carried out. From this, it was hypothesized that *Prosopis* species in the BORAVAST (Bokspits, Rapplespan, Vaalhoek and Struizendam) area form a hybrid swam.

Hybrid swams occur as a result of gene exchange between nuclear DNA or cytoplasmic DNA (i.e., cpDNA or mtDNA) of species. This is known as introgression. Introgression can be infraspecific, interspecific or intergeneric (Soltis and Soltis 1998). Infraspecific introgression involves formation of morphotypes within the same species. And interspecific introgression forms hybrids between different species of the same genus, while intergeneric introgression is between different genera (Soltis and Soltis 1998). Interspecific and intergeneric introgression can also lead to speciation, which is the stabilization of a particular hybrid to become a recognized species (Grant 1971).

The exchange of genetic material as described under introgression above can be very complex. This can lead to a situation where introgression is detected in the morphology of the species but not in the nuclear DNA of the species (Soltis and Soltis 1998). In other situations genetic exchange can be detected in the nuclear DNA and not in the cytoplasmic DNA and vice-versa. Therefore, all studies of introgression (i.e., using morphology or DNA) are important because through these, the direction of gene transfer and the age of the hybrid swam can be determined.

The impact of introgression on plant diversity has been a subject of much debate. It can be an evolutionary dead end (Grant 1971), or it can reinforce the survival of the species (Soltis and Soltis 1998). The evolutionary dead ends are faced with extinction as they will find it difficult to adapt to changing environmental conditions. On the other hand, introgression can strengthen the genetic diversity of the species such that they proliferate and lead to speciation through reticulate evolution (Grant 1971). An analysis of gene flow is crucial in understanding speciation events and maintenance of species integrity (Curtu *et al* 2007).

Therefore, it was necessary to design a study whose objectives were to determine the various *Prosopis* taxonomic lineages and hybrid swams in the BORAVAST area. The study utilized morphology to assess the hybrid swams. The morphological characters were also subjected to multivariate analysis since the analysis has been reported to be good at identifying intermediate morphological forms in plants (Kremer *et al.* 2002).

MATERIALS AND METHODS

Sampling

Systematic qualitative sampling of *Prosopis* species was done in the BORAVAST area (**Fig. 1**), which includes villages of Bokspits (26° 53′ 16.88″ S, 20° 41′ 30.63″E), Rapplespan (26° 49′50.20″S, 20° 48′ 52.89″E), Vaalhoek (26° 53′19.96″S, 20° 42′ 01.95″E) and Struizendam (26° 39′28.23″S, 20° 38′ 27.38″E). Sampling was qualitative in the sense that the trees/shrubs were selected based on diagnostic characters.

Data collection

Data was collected from both vegetative and reproductive characters. Thirteen different types of *Prosopis* forms were sampled,

Table 1 Character dimensions of the different species of *Prosopis* species in BORAVAST.

Species Specimen and Voucher No	Leaflet width	Branch density	Glabrous/ Pubescent	Pod thickness	Leaflet colour	Pod width	Leaflet length	Leaflet diameter	Number of pinnae	Leaflet pairs	Spine size
P. chilensis, 2007/03	1.79 ± 0.01	10 ± 10.09	10 ± 24.96	3.94 ± 0.25	10 ± 10.38	5.227 ± 0.28	11.25 ± 1.02	4.97 ± 0.06	2 ± 0.38	2 ± 5.85	4.70 ± 0.02
P. glandulosa, 2007/11	2.33 ± 0.02	10 ± 10.09	10 ± 24.96	4.39 ± 0.27	10 ± 10.38	7.38 ± 0.39	13.5 ± 1.22	4.55 ± 0.05	2 ± 0.38	26.5 ± 5.85	3.04 ± 0.01
P. chilensis x P. glandulosa, PB2/2008	1.26 ± 0.01	10 ± 10.09	10 ± 24.96	5.45 ± 0.34	10 ± 10.38	13.41 ± 0.71	19.73 ± 1.79	6.07 ± 0.07	2 ± 0.38	12.5 ± 5.85	0.00 ± 0.01
P. glandulosa x P. chilensis, PV1/2008	2.34 ± 0.02	10 ± 10.09	10 ± 24.96	6.95 ± 0.43	10 ± 10.38	11.75 ± 0.62	31.65 ± 2.87	7.05 ± 0.08	2 ± 0.38	21 ± 5.85	2.38 ± 0.01
P. juliflora x P. glandulosa, PS1/2008	2.64 ± 0.02	20 ± 10.09	10 ± 24.96	3.71 ± 0.23	30 ± 10.38	9.05 ± 0.48	22.54 ± 2.05	6.76 ± 0.08	2 ± 0.38	10 ± 5.85	1.10 ± 0.01
P. velutina, PS5/2008	3.46 ± 0.03	20 ± 10.09	10 ± 24.96	4.21 ± 0.26	30 ± 10.38	11.86 ± 0.63	16 ± 1.45	5.28 ± 0.06	2 ± 0.38	13.6 ± 5.85	0.00 ± 0.01
P. juliflora, 2007/08	0.74 ± 0.01	30 ± 10.09	10 ± 24.96	1.32 ± 0.08	30 ± 10.38	6.2 ± 0.33	13.87 ± 1.26	4.06 ± 0.05	2 ± 0.38	18.5 ± 5.85	0.00 ± 0.01
P. chilensis x P. juliflora, PS3/2008	1.11 ± 0.01	10 ± 10.09	10 ± 24.96	6.13 ± 0.38	30 ± 10.38	7.27 ± 0.39	15.41 ± 1.4	4.37 ± 0.05	2 ± 0.38	16.2 ± 5.85	2.16 ± 0.01
A. karroo x P. juliflora, PB4/2008	1.84 ± 0.01	10 ± 10.09	10 ± 24.96	3.17 ± 0.2	30 ± 10.38	6.82 ± 0.36	6.95 ± 0.63	2.52 ± 0.03	2 ± 0.38	10.75 ± 5.85	1.38 ± 0.01
P.glandulosa x P. pallida, 2007/06	1.83 ± 0.01	35 ± 10.09	10 ± 24.96	2.37 ± 0.15	10 ± 10.38	7.96 ± 0.42	29.94 ± 2.72	5.43 ± 0.07	2 ± 0.38	17.5 ± 5.85	0.01 ± 0.01
P. juliflora x P. pallida, PB3/2008	1.33 ± 0.01	30 ± 10.09	10 ± 24.96	5.01 ± 0.31	10 ± 10.38	8.23 ± 0.44	15.86 ± 1.44	5.34 ± 0.06	2 ± 0.38	16.67 ± 5.85	0.00 ± 0.01
*P. chilensis x P. juliflora x P. glandulosa**, PR1/2008	2.55 ± 0.02	30 ± 10.09	10 ± 24.96	3.95 ± 0.25	30 ± 10.38	5.227 ± 0.28	25.7 ± 2.33	5.48 ± 0.07	2 ± 0.38	15 ± 5.85	2.28 ± 0.01
P.chilensis x P. pallida, PR2/2008	2.52 ± 0.02	30 ± 10.09	10 ± 24.96	4.01 ± 0.25	10 ± 10.38	10.95 ± 0.58	17.65 ± 1.6	4.95 ± 0.06	2 ± 0.38	15 ± 5.85	2.38 ± 0.01

with three replicates each. Only mature and healthy specimens were picked.

Quantitative morphological analyses (morphometrics)

Morphological characters were analyzed with STATISTICA (1998).

1. Cluster analysis

Tree joining and K – means analysis were employed, with Euclidian distances and single linkage amalgamation rule enforced. Analysis of variance (ANOVA) under cluster analysis was also performed.

2. Factor analysis

Variable correlations were checked and factor loadings run. Ten factors were initially selected. The Eigen values recommend four factors i.e., those greater than one (Kaiser 1960) for initial analysis and the factor loadings indicated the contribution of variables to be concentrated on the four factors. All the four factors were retained because Thurstone (1947) recommends the use of at least two factors in factor analysis. Factors were also rotated using varimax (Kaiser 1958).

3. Canonical correlation

The morphological characters were also subjected to canonical correlation analysis, with the review descriptive statistics and correlation matrix activated. Ten variables which are, the distance between leaflets, leaflet diameter, leaflet width, branch density, glabrous/pubescence, pod thickness, spine size, leaflet colour, pod width, and leaflet length comprised the left set, while the number of pinnae comprised the right set.

RESULTS

Species composition

Species composition in the BORAVAST area comprises *Prosopis chilensis*, *P. juliflora*, *P. velutina* and *P. glandulosa* which are the pure lines. And the hybrids are *P. chilensis x P. glandulosa*, *P. glandulosa x P. chilensis*, *P. juliflora x P. glandulosa*, *P. chilensis x P. juliflora*, *Acacia karroo x P. juliflora*, *P. glandulosa x P. pallida*, *P. juliflora x P. pallida*, *P. chilensis x P. juliflora x P. glandulosa* and *P. chilensis x P. pallida*. Dimensions for characters of the various species in the study are recorded in **Table 1**.

Cluster analysis

1. Tree joining

Tree joining (**Fig. 2**) classified the 13 *Prosopis* species into four main groups. Group one comprised *P. chilensis*, *P. glandulosa*, *P. chilensis x P. glandulosa* and *P. glandulosa x P. chilensis*. In this group *P. chilensis x P. glandulosa* and *P. glandulosa x P. chilensis* are more closely related to one another, with *P. chilensis* being their closest sister taxa and *P. glandulosa* being sister taxa to the three taxa. Group two comprises *P. glandulosa x P. pallida*, *P. juliflora x P. pallida*, and *P. chilensis x P. pallida*, which is also the closest sister group to Group one. Group three comprises *P. juliflora x P. glandulosa*, *P. juliflora*, *P. chilensis x P. juliflora x P. glandulosa*, *P. chilensis x P. juliflora* and *Acacia karroo x P. juliflora*. This group splits into two sub groups. One of the subgroups comprises *P. chilensis x P. juliflora* and *Acacia karroo x P. juliflora* as being closely related and the other sub group comprises *P. juliflora x P. glandulosa* and *P. chilensis x P. juliflora x P. glandulosa* as being more closely related, with *P. juliflora* acting as their closest sister. These two sub groups ultimately pair as sister taxa of one another. Group four comprises *P. velutina*, and resolves as a standalone taxon that is a sister taxon of all species in the analysis.

2. K – means clustering

There was perfect congruence between K – means clustering and tree joining as the results were identical. K – means clustering (**Table 2**) identifies five clusters of *Prosopis*, of which one of the clusters splits into two to represent the subgroups of group three in tree joining (**Fig. 2**). Of these clusters, cluster one comprises *P. chilensis*, *P. glandulosa*, *P. chilensis x P. glandulosa* and *P. glandulosa x*

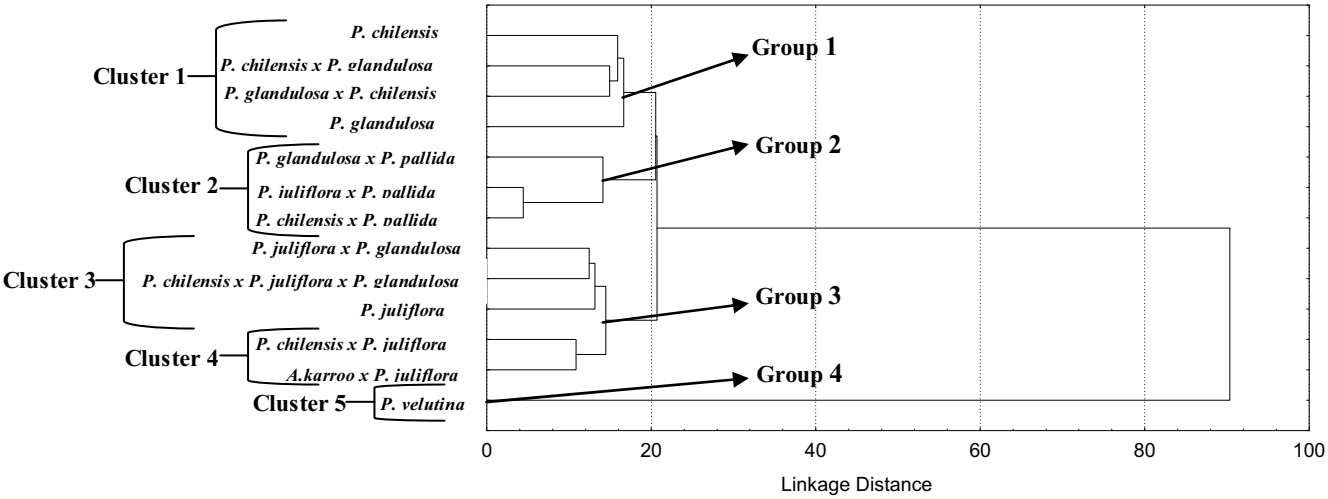

Fig. 2 Hierarchical tree for *Prosopis* species using tree joining with single linkage and Euclidian distances enforced.

Table 2 Grouping of various *Prosopis* species based on K – means clustering.

Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
P. chilensis	*P. glandulosa x P. pallida*	*P. juliflora x P. glandulosa*	*P. chilensis x P. juliflora*	*P. velutina*
P. glandulosa	*P. juliflora x P. pallida*	*P. juliflora*	*Acacia karroo x P. juliflora*	
P. chilensis x P. glandulosa	*P. chilensis x P. pallida*	*P. chilensis x P. juliflora x P. glandulosa*		
P. glandulosa x P. chilensis				

P. chilensis. Cluster two comprises *P. glandulosa x P. pallida*, *P. juliflora x P. pallida*, and *P. chilensis x P. pallida* while cluster three comprises *P. juliflora x P. glandulosa*, *P. juliflora*, *P. chilensis x P. juliflora*, *Acacia karroo x P. juliflora* and *P. chilensis x P. juliflora x P. glandulosa*.

Cluster four comprises *P. chilensis x P. juliflora* and *Acacia karroo* x *P. juliflora*. Cluster 5 is *P. velutina*.

Analysis of variance (ANOVA)

The tree joining (**Fig. 2**) and K – means clustering results (**Table 2**) were confirmed via the ANOVA (**Table 3**). The ANOVA indicated high significance ($P < 0.03$) for all the *Prosopis* species except for *P. velutina*.

Canonical correlation

The canonical analysis (**Table 4**) sort to analyze the characters that were used to come up with the hierarchical tree from tree joining, the K – means clustering and conclusions of the ANOVA. According to canonical correlation ten characters, which are leaflet width, branch density, glabrous/ pubescent (i.e., surface texture), pod thickness, spine size, leaflet colour, pod width, leaflet length and the distance between leaflets were highly correlated (R = 0. 9809) with high significance ($P = 0.03$) in determining the above referred clusters (**Table 2**) and groups of *Prosopis* (**Fig. 2**). But, there was very little variance (11. 69%) among the analyzed taxa.

On the other hand when a chi square test was performed, with successive roots removed (**Table 5**), it was revealed that the chi-square was significant ($P = 0.03$), but only when all the ten characters that comprised the left set (**Table 4**) were present.

DISCUSSION

Morphological and statistical analysis

Both the tree joining analysis (**Fig. 2**) and K – means clustering (**Table 2**) sort the studied taxa into four parental (pure) species and nine hybrids. And it was noted that taxonomic delimitations established by the two analysis (tree joining and K – means clustering) were identical, implying congruence between the two analysis. This provided confidence in the data and supports Kremer *et al.* (2002) and Curtu *et al.* (2007) who reported that morphological characters if subjected to multivariate analysis can detect hybridization in plants.

Also, according to canonical correlations (**Table 4**), all the 10 characters which are leaflet width, branch density, glabrous/pubescent (i.e., surface texture), pod thickness, spine size, leaflet colour, pod width, leaflet length and the distance between leaflets contributed in determining clus-

Table 3 Analysis of variance (ANOVA) of the different *Prosopis* species in BORAVAST.

	Between SS	df	Within SS	df	F	Signif. p
P. chilensis	81.97	1	72.16	9	10.22	0.01
P. glandulosa	302.65	1	236.32	9	11.53	0.01
P. chilensis x P. glandulosa	156.19	1	181.35	9	7.75	0.02
P. glandulosa x P. chilensis	349.75	1	464.49	9	6.78	0.03
P. juliflora x P. glandulosa	582.41	1	357.27	9	14.67	0.00
P. velutina	2720.53	1	5379.77	9	4.55	0.06
P. juliflora	920.87	1	376.48	9	22.01	0.00
P. chilensis x P. juliflora	437.25	1	303.42	9	12.97	0.01
A. karroo x P. juliflora	326.83	1	377.79	9	7.79	0.02
P. glandulosa x P. pallida	805.74	1	574.98	9	12.61	0.01
P. juliflora x P. pallida	459.94	1	320.60	9	12.91	0.01
P. chilensis x P. juliflora x P. glandulosa	960.12	1	350.57	9	24.65	0.00
P. chilensis x P. pallida	411.16	1	333.86	9	11.08	0.01

Table 4 Canonical summary of the *Prosopis* species in BORAVAST.

N =13	Canonical R = 0. 9809; Chi^2 (10) = 19. 63; P = 0. 03297	
	Left set	**Right set**
No. of variables	10	1
Variance extracted	11. 69%	100.00%
Total redundancy	11. 25%	96. 21%
Variables 1	Leaflet width	No. of pinnae
Variables 2	Branch density	
Variables 3	Glabrous /pubescent	
Variables 4	Pod thickness	
Variables 5	Spine size	
Variables 6	Leaflet colour	
Variables 7	Pod width	
Variables 8	Leaflet length	
Variables 9	Distance between leaflets	
Variables 10	Leaflet pairs	

Table 5 Chi-square tests with successive roots removed for BORAVAST *Prosopis* species.

Canonical R	Canonical R-square	Chi -square	d. f	p
0.980853	0.962072	19. 63241	10	0.032965

ters and groups as according to the tree joining analysis and K - means clustering. The reason could be that these characters were highly correlated (R = 0. 98085) and highly significant (P = 0. 03). This was also supported by the ANOVA analysis (**Table 3**), which based on the reliability of these characters indicated significant differences between the different species and hybrids in question ($P < 0.03$). An interpretation of this implies that the ANOVA analysis (**Table 3**), recognized species boundaries due to the reliable 10 characters revealed by canonical correlations (**Table 4**). And the fact that canonical correlations (**Table 4**) extracted very little variance (11. 6882%) among the characters in question, also indicated that we were dealing with a hybrid swam.

Species composition

All in all, four pure parental species and nine hybrids are recognized in the BORAVAST area. The four pure species have distinct morphological boundaries, while the nine hybrids have morphological characters that are intermediates of the pure species. This according to Curtu *et al.* (2007) and Archibald *et al.* (2004) is evidence of a hybridization event. And based on this, it can be concluded that species composition in the BORAVAST area forms a hybrid swam.

However, it is possible that some hybrids might have been missed out, especially during the sampling process. That is so because some hybrids that we thought to be obvious to be growing in the BORAVAST area could not be detected. For instance, because of the sympatric co-occurrence of *Prosopis velutina* and *Prosopis glandulosa* in BORAVAST, we expected the hybrid *Prosopis velutina x Prosopis glandulosa* to be present in the area since there are no reproductive isolation mechanisms between two species as this hybrid has been recorded in Australia (CRC 2003).

Hybrid complexities

In most situations, gene flow was observed to be in one direction, except between *Prosopis glandulosa* and *P. chilensis* in which the gene flow was seen to be bidirectional. Hence, the existence of the two hybrids *P. chilensis x P. glandulosa* and *P. glandulosa x P. chilensis*. The conclusion that gene flow between these two species is in both directions was derived from characteristics of pod width and leaflet length.

Pod width in the hybrids *P. chilensis x P. glandulosa* (13.41 ± 2.66 mm) and *P. glandulosa x P. chilensis* (11.75 ± 2.66 mm) is almost double the size that of *P. glandulosa* (7.38 ± 2.66 mm) and *P. chilensis* (5. 23 ± 2.66 mm). This doubling of pod width is an indication of the presence of polyploidy (Garcia-Jacas *et al.* 2009) in *P. chilensis x P. glandulosa and P. glandulosa x P. chilensis*, which is also regarded as direct evidence of introgression in plants (Garcia-Jacas *et al.* 2009). And as for gene flow, with respect to pod width, it is probable that pod width in the hybrid *P. chilensis x P. glandulosa* (13.41 ± 2.66 mm) was inherited from *P. glandulosa* (7.38 ± 2.66 mm). This could have happened because of chromosomal aberrations, leading to size doubling of structural features as in the genus *Lavandula angustifolia* (Urwin *et al.* 2007). If this has happened to the *Prosopis* species in question, then it would mean that gene flow was from *P. glandulosa* to *P. chilensis*. And pod width for the hybrid *P. glandulosa x P. chilensis* (11.75 ± 2.66 mm) would have been inherited from *P. chilensis* (5.23 ± 2.66 mm) in the same manner described above, implying that gene flow was from *P. chilensis* to *P. glandulosa*. Hence, bidirectional.

On the other hand, leaflet length was probably inherited in the direction that is opposite to pod width. That is so because, in the hybrid *P. chilensis x P. glandulosa* leaflet length (19.73 ± 7.24 mm) is seen to be almost double that of *P. chilensis* (11.25 ± 7.24 mm), while the leaflet length (31.65 ± 7.24 mm) in the hybrid *P. glandulosa x P. chilensis* is seen to be almost triple the size that of *P. glandulosa* (13.5 ± 7.24 mm). In this case, gene flow for the hybrid *P. chilensis x P. glandulosa* was probably from *P. chilensis* to *P. glandulosa*, while for the hybrid *P. glandulosa x P. chilensis* it was from *P. glandulosa* to *P. chilensis*.

Spine size, on the other hand, was inherited in the normal diploid manner as there were no observable spine size changes. But gene flow was also bidirectional. That is so because, it is probable that the hybrid *P. chilensis x P. glandulosa* (1.08 ± 0.99 mm) inherited spine size from *P. chilensis* (1.08 ± 0.99 mm), while the hybrid *P. glandulosa x P. chilensis* (0.144 ± 0.99 mm) inherited spine size from *P. glandulosa* (0.144 ± 0.99 mm).

Another hybridization event worth mentioning was that between *Acacia karroo* and *Prosopis juliflora*. This is a hybridization event between two different genera, which is quite a rare phenomenon. And it marks a remarkable speciation event, speciated introgression (Soltis and Soltis 1998), which needs to be studied closely to determine the duration it will take for the hybrids (from this combination) to stabilize, indicating the occurrence of a new species.

But even if this combination is rare (i.e., between two genera) it is not a surprising thing since the two species *Acacia karroo* and *Prosopis juliflora* share a common photosynthetic pathway, the C3 cycle (McPherson *et al.* 1993).

CONCLUSION

There seems to be no reproductive isolation mechanisms between *P. chilensis*, *P. glandulosa* and *P. juliflora* as evidenced by the presence of hybrid swam in the BORAVAST area. And gene flow between *P. chilensis* and *P. glandulosa* is bidirectional. This is most probable because even on site hybrids of these species are the most abundant.

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