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 because the method of control is species specific. 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 swarm. 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 sub-shrubs 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, intorse, 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, Rusciifoliae, Denudantes, Pallidae, Chilensis and Humiles. Species that are found in Botswana are from the series Pallidae and Chilensis as follows:


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 gum, 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 boresholes 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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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 Boks-pits, Rapplespan, Valhoek and Struizendam (BORA V AST), 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 BORA-V AST (Bokspits, Rapplespan, Vaalhoek and Struizendam) area form a hybrid swarm.

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 swarm 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 BORA V AST 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 BORA V AST area (Fig. 1), which includes villages of Boksplits (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,
The morphological characters were also subjected to canonical correlation analysis (Kaiser 1958).

Species composition in the BORAVAST area comprises several species, including Prosopis chilensis, P. juliflora, P. velutina, and P. glandulosa. These species are characterized by their specific leaflet, branch density, glabrous/pubescent, and pod thickness dimensions. The data for these characters are presented in Table 1.

### Quantitative morphological analyses (morphometrics)

Morphological characters were analyzed with STATISTICA (1998).

### Results

#### 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/pubescent, pod thickness, spine size, leaflet colour, pod width, and leaflet length comprised the left set, while the number of pinnae comprised the right set.

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Specimen and Voucher No</th>
<th>Leaflet width (mm)</th>
<th>Branch density (pairs/m)</th>
<th>Glabrous/Pubescent (%)</th>
<th>Pod thickness (mm)</th>
<th>Leaflet colour</th>
<th>Pod width (mm)</th>
<th>Leaflet length (cm)</th>
<th>Leaflet diameter (mm)</th>
<th>Number of pinnae</th>
<th>Leaflet pairs</th>
<th>Spine size</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. chilensis, 2007/03</td>
<td>1.79 ± 0.01</td>
<td>10 ± 0.02</td>
<td>10 ± 0.02</td>
<td>3.94 ± 0.02</td>
<td>10 ± 0.02</td>
<td>5.227 ± 0.28</td>
<td>11.25 ± 0.02</td>
<td>4.97 ± 0.28</td>
<td>2 ± 0.02</td>
<td>2 ± 0.02</td>
<td>4.70 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>

### 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, and P. chilensis x P. pallida. This group splits into two sub groups. One of the sub groups comprises P. chilensis x P. juliflora and P. chilensis x P. pallida as being closely related and the other sub groups comprise P. juliflora x P. glandulosa, P. chilensis x P. juliflora as being as closely related, and P. chilensis x P. pallida as being as 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 the 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 P. chilensis with three replicates each. Only mature and healthy specimens were picked.
Table 2 Grouping of various Prosopis species based on K – means clustering.

<table>
<thead>
<tr>
<th>Cluster 1</th>
<th>Cluster 2</th>
<th>Cluster 3</th>
<th>Cluster 4</th>
<th>Cluster 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. chilensis</td>
<td>P. glandulosa</td>
<td>P. juliflora x P. glandulosa</td>
<td>P. chilensis x P. juliflora</td>
<td>P. velutina</td>
</tr>
<tr>
<td>P. glandulosa</td>
<td>P. juliflora x P. pallida</td>
<td>P. juliflora</td>
<td>P. chilensis x P. juliflora</td>
<td>P. velutina</td>
</tr>
<tr>
<td>P. chilensis x P. glandulosa</td>
<td>P. chilensis x P. pallida</td>
<td>P. juliflora x P. glandulosa</td>
<td>P. chilensis x P. juliflora x P. glandulosa</td>
<td>P. velutina</td>
</tr>
<tr>
<td>P. glandulosa x P. chilensis</td>
<td>P. juliflora x P. pallida</td>
<td>P. juliflora x P. glandulosa</td>
<td>P. chilensis x P. juliflora x P. glandulosa</td>
<td>P. velutina</td>
</tr>
</tbody>
</table>

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 clas-
detected. For instance, because of the sympatric co-occurrence in the BORA VAST area forms a hybrid swam. And based on this, it can be concluded that species composition in BORA VAST revealed by canonical correlations (Table 4), which indicated that we were dealing with a hybrid swam. This according to Curtu et al. (2004) is evidence of a hybridization event.

Hybrid complexes

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. angustifolia and P. glandulosa x P. chilensis, which needs to be studied closely to determine the duration of the photosynthetic pathway, the C3 cycle (McPherson 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 BORA VAST area. 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 (Solitis and Solitis 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).

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