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Full Length Research Paper

Multivariate analysis of *Harpagophytum* DC. ex Meisn (Pedaliaceae) based on fruit characters

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Harpagophytum **is bitypic and native to Southern Africa. Its two species are** ***Harpagophytum procumbens*** **and** ***H. zeyheri*****.** ***H. procumbens*** **is medicinal. A reliable method of identifying the species is through its fruit. However, distinguishing between** ***H. procumbens*** **and** ***H. zeyheri*** **can be difficult because of the various morphotypes. Hence, possibilities of introgression are hypothesized. The objective of this study was to test for interspecific introgression between the two species. Diagnostic characters of the fruit were subjected to multivariate analysis. Discriminant function analysis was used to identify and classify the 21 specimen types. Cluster analysis was used to test for possible appurtenance of individual fruit specimens to either the parental species (*** ***H. procumbens*** **or** ***H. zeyheri*****) or to the hybrid (*** ***H. procumbens*** **X** ***H. zeyheri*****). The study inferred the existence of hybridisation (introgression) between the two species. The hybrids can be characterised by fruit length, fruit width, arm width, arm length, and the number of seed rows. In the hybrids, the number of seed rows per loculus comes in various combinations (for example 3,3 and 3,2). And this was found to be quite important in identifying the hybrids. However, it was difficult to determine the direction of gene flow, thus, we recommend molecular analysis of the hybrids.**

Key words: Harpagophytum, fruit, introgression, multivariate.

INTRODUCTION

Harpagophytum DC. ex Meisn (Pedaliaceae) is a bitypic genus, with both species being native to Southern Africa. The two species are *Harpagophytum procumbens* and *H. zeyheri*. *H. procumbens*. The two species are perennial herbs with several prostrate annual stems that emanate from a succulent tuberous taproot. For an elaborate taxonomy of the genus see Ihlenfeldt (1988). This study focused on the biology of the fruit as it is perceived to play a major role in the taxonomy of the genus. The fruit of *Harpagophytum* is a woody capsule that is imperfectly dehiscent along its longitudinal length (Figure 1). The fruit is laterally compressed, with two obtuse protuberances on each face, armed with two rows of curved arms along the edges. Each arm bears re-curved spines. Inside the fruit are numerous seeds that are stacked in rows of 2's or 4's in each loculus, depending on the species. The two (2) rows are a diagnostic character for *H. zeyheri,* while the four (4) rows are a diagnostic character for *H. procumbens* (Ihlenfeldt, 1988).

Harpagophytum fruit capsules are morphologically variable and therefore present several taxonomic problems. It is not clear whether the various morphotypes exhibited by the species are an indication of hybridisation within the genus or they are just ecotypes of the same species. Hybridization between species often forms hybrid swams (Grant, 1971). Hybrid swams occur as a result of gene exchange between nuclear DNA or cyto-plasmic DNA (that is cpDNA or mtDNA) between species and is known as introgression. Introgression can be infraspecific, interspecific or intergeneric (Reiseberg and Brunsfeld, 1998). Infraspecific introgression involves the formation of morphotypes within the same species, interspecific introgression forms hybrids between different species of the same genus, while intergeneric

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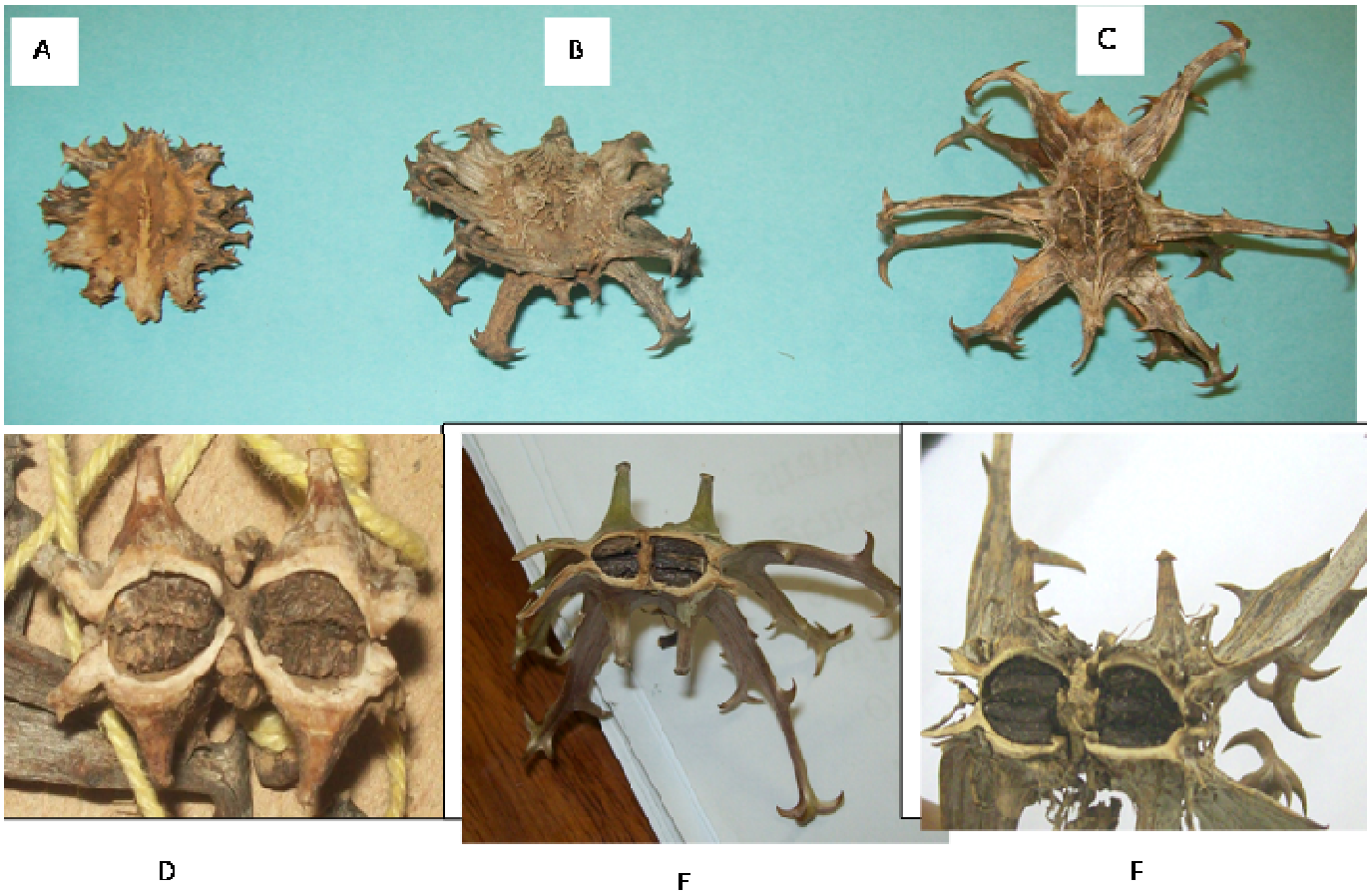

Figure 1. *Harpagophytum* fruits showing the three different forms: A) H. *zeyheri*; B) Hybrid; C) *H. procumbens*; D) two seed rows in *H. zeyheri*; E) three seed rows in one of the hybrids; F) four seed rows in *H. procumbens*.

introgression is between species of different genera (Reiseberg and Brunsfeld, 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 in introgression can be very complex and can lead to a situation where introgression is detected in the morphology of the species but not in the nuclear DNA (Reiseberg and Brunsfeld, 1998). In other situations genetic exchange can be detected in the nuclear DNA and not in the cytoplasmic DNA or vice-versa. Therefore, all studies of introgression (that is 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 (Reiseberg and Brunsfeld, 1998; Arnold and Martin, 2009). Introgression can be an evolutionary dead end (Grant, 1971), thereby reducing possibilities of genetic gain in plant breeding programs by scientists (Rangel et al., 2008) or it can reinforce the survival of the species (Reiseberg and Brunsfeld, 1998, Lanta et al., 2003, Arnold and Martin, 2009). The evolutionary dead ends are faced with extinction (Ellstrand, 1999) as they may find it difficult to adapt to changing environmental conditions. On the other hand, introgression can strengthen the genetic diversity of the species as in, for example, adaptive radiation (Grant, 1971). An analysis of gene flow is crucial in understanding speciation events and maintenance of species integrity (Curtu et al., 2007).

The main objective of the study, therefore, was to determine whether the various morphological patterns exhibited by *Harpagophytum* fruits are an indication of hybridization (introgression) between the two species or just ecotypes (Jordan, 2010) of the same species.

MATERIALS AND METHODS

Nomenclature

Diagnostic keys of Ihlenfeldt (1988) were used to distinguish between the types of *Harpagophytum* and their hybrids.

Sampling sites

Specimens were collected from six different areas of southern Botswana and deposited at UCBG herbarium in Gaborone, Botswana. The sites included the following (Table 4):

Table 1. Factor Loadings (Varimax normalized) to indicate principal components that contributed to taxa delimitation. Only factor loadings greater than 0.70 are indicated.

Characters	Factor 1	Factor 2	Factor 3
Arm length	0.74		
Arm width		0.85	
Column height	0.82		
Seed rows		0.85	
Fruit length	0.95		
Fruit width	0.77		
Fruit circumference			0.86

Sympatric zones

1) Kumakwane
2) Malotwana
3) Mmamashia
4) Oodi

Pure type zones

1) Bella - Bella)
2) Sekoma

Sampling technique

The sampling technique was systematic qualitative. Some 105 fruit specimens were collected, which comprised five (5) fruit specimens collected from 21 plant types (with the five fruits from each plant treated as duplicates of the voucher specimen). Fruits were chosen for further analysis because they play a great role in the taxonomy of the genus, and they are relatively uniform when compared to plastic traits like vegetative features.

Multivariate analysis

Morphometric analyses were conducted on each of the five replicate fruit specimens and the mean value recorded. The plant parts analyzed were as follows:

1) Length of the longest arm
2) The widest part of the longest arm (that is arm width)
3) Fruit specimen width
4) Fruit specimen length
5) The number of seed rows
6) Height of column that enclosed the seed rows
7) Fruit circumference.

Firstly, a one - way ANOVA model in STATISTICA (Anonym, 1995) was used to test for significant differences among the collected fruit specimens. Then a discriminant function analysis was used to test the hypothesis that there are three groups of *Harpagophytum*, namely *H. procumbens*, *H. zeyheri* and their hybrids. Under discriminant function analysis the scatter plot, classification matrix, box and whisker plot analysis were employed. The classification matrix was used to check for reliability of the scatterplot groupings (that is testing for confidence in the results), while the box and whisker tested for details of how the three groups differed. And finally, a cluster analysis was carried out using un-weighted pair – group averaging (UPGMA) in order to check for possible appurtenance of individual fruit specimen to either the parental/type species (*H. procumbens* or *H. zeyheri*) or to the expected hybrid (*H. procumbens* X *H. zeyheri*).

RESULTS

General morphology and number of seed rows

Based on fruit type, various morphotypes of *Harpagophytum* were observed. Features that characterize the morphotypes include arm length, arm width, fruit width, fruit length and the number of seed rows (Table 1). The number of seed rows - as a character - plays a significant role in the taxonomy of *Harpagophytum* as it immediately identifies the hybrids since the number of seed rows for *Harpagophytum* are four (4) and two (2) on each seed column for *H. procumbens* and *H. zeyheri* respectively (Ihlenfeldt, 1988). However, for the hybrids, the numbers of seed rows come in various combinations (Figure 1 and Table 2). For instance three hybrid specimens showed three (3) seed rows in one of the seed loculus and two (2) seed rows on the other (that is 3.2), while the other two hybrid specimens showed three (3) seed rows on both seed loculus (that is 3.3). Hence, upon noticing the importance of seed rows in the taxonomy of the genus, we placed a higher weight on the character prior to multivariate analysis (apriori weighting). However, the character was standardized, prior to analysis, and therefore excluded from the box and whisker analysis.

Discriminant function analysis

Scatter plot

According to the ordination scatterplot, three groups of *Harpagophytum* exist in southern Botswana (Figure 2). The groups are *H. procumbens*, *H. zeyheri* and hybrid(s) of the two species (*H. zeyheri* X *H. procumbens*). Root 1 separated the hybrids from the pure *H. procumbens* and the pure *H. zeyheri* and by so doing recognising them as a group. Root 2 allocated the hybrids to two groups as follows:

1) Those biased towards the *H. zeyheri* pure line
2) Those biased towards the *H. procumbens* pure line.

Classification matrix

Reliability on the classification of specimens into three groups was tested using the classification matrix. According to the classification matrix (Table 3), classification of the specimens into *H. procumbens, H. zeyheri* and their hybrids was perfectly precise (that is 100%

Table 2. Character parameters for the various voucher specimens used in the study.

Voucher number	Species[Ψ] identity	Arm length (mm)	Arm width (mm)	Seed rows* P < 0.00		Seed column height (mm)	Fruit length (mm)	Fruit width (mm)	Fruit circumference (mm)	Signif. p
				Seed column1	Seed column2					
OODI L3	Hybrid	7.8 ± 2.32	6.7 ± 0.76	2	2	3.40 ± 0.50	33.90 ± 3.44	16.60 ± 4.28	130.21 ± 3.17	0.00
LSHBL1	Hybrid	18.44 ± 4.32	9.6 ± 0.83	3	3	5.90 ± 0.50	36.20 ± 3.44	19.40 ± 4.03	82.50 ± 3.17	0.00
SKL1	*H. procumbens*	30.5 ± 6.58	9.1 ± 0.82	4	4	5.20 ± 1.12	47.80 ± 6.76	20.30 ± 4.28	90.01 ± 24.28	0.00
KKMWL5	Hybrid	21.6 ± 4.91	12.6 ± 0.9	3	3	4.30 ± 0.50	36.00 ± 3.44	21.10 ± 4.28	148.30 ± 3.17	0.00
KKWNL1	Hybrid	64.6 ± 12.98	3.2 ± 0.68	2	2	6.38 ± 0.50	58.74 ± 3.44	37.78 ± 4.28	122.34 ± 3.17	0.00
BBL5	Hybrid	7.52 ± 2.27	2.66 ± 0.67	2	2	2.28 ± 0.50	21.50 ± 3.44	16.38 ± 4.28	51.10 ± 3.17	0.00
KKMWL2	*H. procumbens*	43.3 ± 8.98	7.57 ± 0.78	2	2	6.89 ± 1.12	40.81 ± 6.76	24.03 ± 4.03	87.53 ± 24.28	0.00
LSBL3	Hybrid	21.11 ± 4.82	11.87 ± 0.88	2	2	5.35 ± 0.50	43.35 ± 3.44	32.21 ± 4.28	93.29 ± 3.17	0.00
BBL1	*H. zeyheri*	7.21 ± 2.21	4.99 ± 0.72	2	2	4.05 ± 0.77	32.90 ± 1.35	15.86 ± 2.99	108.10 ± 16.93	0.00
OODIL4	Hybrid	5.9 ± 1.96	3.53 ± 0.69	3	2	3.13 ± 0.50	30.02 ± 3.44	16.24 ± 4.28	69.54 ± 3.17	0.00
SKL2	*H. procumbens*	79.62 ± 15.8	9.9 ± 0.84	4	4	5.90 ± 1.12	57.01 ± 6.76	22.66 ± 4.03	11.30 ± 24.28	0.00
LSBL2	Hybrid	17.3 ± 4.1	6.48 ± 0.76	3	2	5.10 ± 0.50	51.38 ± 3.44	31.00 ± 4.28	97.52 ± 3.17	0.00
MMSL2	Hybrid	40.78 ± 8.51	13.1 ± 0.91	3	2	5.78 ± 0.50	51.58 ± 3.44	22.62 ± 4.28	113.76 ± 3.17	0.00
BBL2	Hybrid	4.2 ± 1.64	6.59 ± 0.76	2	2	4.92 ± 0.50	42.46 ± 3.44	25.54 ± 4.28	95.60 ± 3.17	0.00
OODI L2	Hybrid	12.1 ± 3.13	15.6 ± 0.97	2	2	4.90 ± 0.50	29.30 ± 3.44	19.40 ± 4.28	107.90 ± 3.17	0.00
MMSL1	Hybrid	44.28 ± 9.17	11 ± 0.86	2	2	6.49 ± 0.50	57.01 ± 3.44	22.28 ± 4.28	113.76 ± 3.17	0.00
SKL3	*H. procumbens*	64.4 ± 12.94	9.38 ± 0.82	4	4	10.30 ± 1.12	72.12 ± 6.76	27.50 ± 4.03	167. 52 ± 24.28	0.00
KKMWL4	Hybrid	3.5 ± 1.51	7 ± 0.77	2	2	9.32 ± 0.50	58.74 ± 3.44	37.78 ± 4.28	122.34 ± 3.17	0.00
BBL4	*H. zeyheri*	0.6 ± 0.97	4.74 ± 0.72	2	2	7.90 ± 0.77	38.50 ± 1.35	25.50 ± 2.99	90.30 ± 16.93	0.00
BBL3	*H. zeyheri*	5.68 ± 1.92	3.2 ± 0.68	2	2	6.00 ± 0.77	34.94 ± 1.35	19.90 ± 2.99	95.10 ± 16.93	0.00
BBL6	*H. zeyheri*	4.21 ± 1.65	2.2 ± 0.66	2	2	5.92 ± 0.77	32.56 ± 1.35	18.24 ± 2.99	83.14 ± 16.93	0.33

[Ψ] Hybrid refers to *H. zeyheri* X *H. procumbens*.
± represents standard error.
* Standard error not calculated since the character is qualitative as opposed to quantitative.

correct).

Box and whisker

The three groups revealed in the scatter plot graph (Figure 2) were further analyzed using box and whisker plots (Figure 3). Box and whisker plots provided morphometric details of how fruit length (Figure 3A), fruit width (Figure 3B), arm width (Figure 3C) and arm length (Figure 3D) were distinguished between the three groups. According to the box and whisker plots, *H. procumbens* generally posseses elongated fruits, while *H. zeyheri* has shorter fruits and the hybrids are intermediates of the two species (Figure 3A). The same pattern is repeated for arm width (Figure 3C) and arm length (Figure 3D). For fruit width, however, the hybrids posses the widest fruits followed by *H. procumbens* (Figures 1B and 3C).

Cluster analysis

Hierarchical tree

The hierarchical tree also recognized the presence

Table 3. Classification Matrix to indicate precision of classification

Rows: Observed classifications				
Columns: Predicted classifications				
Taxa	**Percent correct**	***H. zeyheri***	***H. procumbens***	**Hybrid**
H. zeyheri	100	4	0	0
H .procumbens	100	0	4	0
Hybrid	100	0	0	13
Total	100	4	4	13

Table 4. Collection sites of vouchers specimens used in the study.

Voucher Number	**Species**	**Herbarium***	**Collection site**
OODI L3	Hybrid	UCBG	**Oodi** – 24° 28' 25.98" S 26° 02' 30.22" E
LSHBL1	Hybrid	UCBG	**Malotwana -** 24° 17' 31.93" S 26° 08' 15.64" E
SKL1	*H. procumbens*	UCBG	**Sekoma** -24° 30' 15.82" S 23° 02' 30.22" E
KKMWL5	Hybrid	UCBG	**Kumakwane** -24° 41' 42.13 S" 26° 02' 16.99" E.
KKWNL1	Hybrid	UCBG	**Kumakwane** -24° 41' 42.13" S 26° 02 16.99" E.
BBL5	Hybrid	UCBG	**Bella - Bella** -24° 30' 00.95" S 26° 02 16.99 E"
KKMWL2	*H. procumbens*	UCBG	**Kumakwane** -24° 41' 42.13" S 26° 02' 16.99" E.
LSBL3	Hybrid	UCBG	**Malotwana -** 24° 17' 31.93" S 26° 08' 15.64" E
BBL1	*H. zeyheri*	UCBG	**Bella - Bella** -24° 30' 00.95" S 26° 02 16.99 E"
OODIL4	Hybrid	UCBG	**Oodi** – 24° 28' 25.98" S 26° 02' 30.22" E
SKL2	*H. procumbens*	UCBG	**Sekoma** -24° 30' 15.82" S 23° 02' 30.22" E
LSBL2	Hybrid	UCBG	**Malotwana -** 24° 17' 31.93" S 26° 08' 15.64" E
MMSL2	Hybrid	UCBG	**Mmamashia -** 24° 30' 59.80" S 25° 58' 51.91" E

Table 4. Cont.

BBL2	Hybrid	UCBG	**Bella - Bella** -24° 30' 00.95 S" 26° 02' 16.99 E"
OODI L2	Hybrid	UCBG	**Oodi** – 24° 28' 25.98" S 26° 02 30.22" E
MMSL1	Hybrid	UCBG	**Mmamashia -** 24° 30' 59.80" S 25° 58' 51.91" E
SKL3	*H. procumbens*	UCBG	**Sekoma** -24° 30' 15.82" S 23° 02' 30.22" E
KKMWL4	Hybrid	UCBG	**Kumakwane** -24° 41' 42.13" S 26° 02' 16.99" E.
BBL4	*H. zeyheri*	UCBG	**Bella – Bella** -24° 30' 00.95" S 26° 02' 16.99" E
BBL3	*H. zeyheri*	UCBG	**Bella - Bella** -24° 30' 00.95" S 26° 02' 16.99" E
BBL6	*H. zeyheri*	UCBG	**Bella - Bella** -24° 30' 00.95" S 26° 02' 16.99 "E

UCBG* - University College of Botswana Gaborone

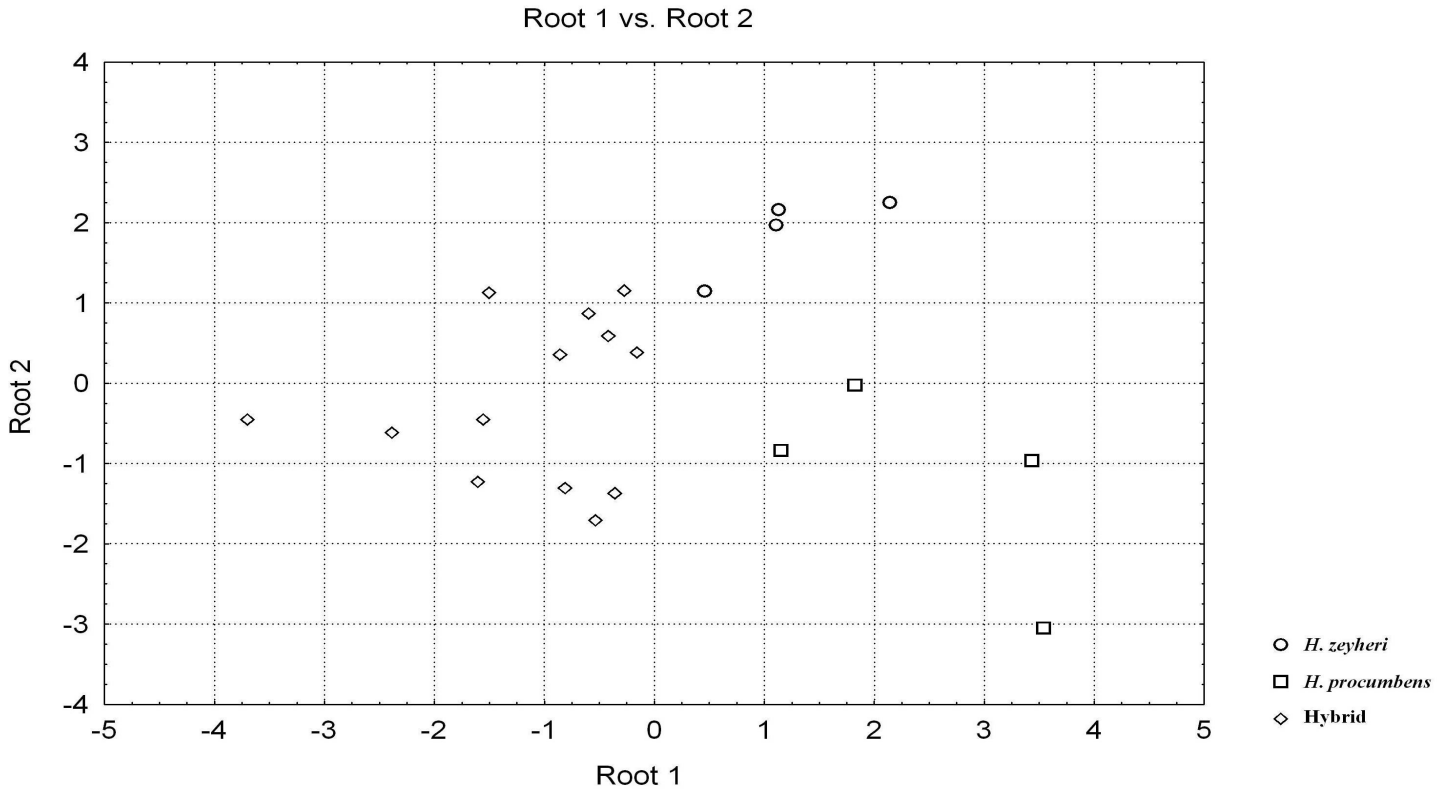

Figure 2. Scatter plot to indicate the three groups of *Harpagophytum.*

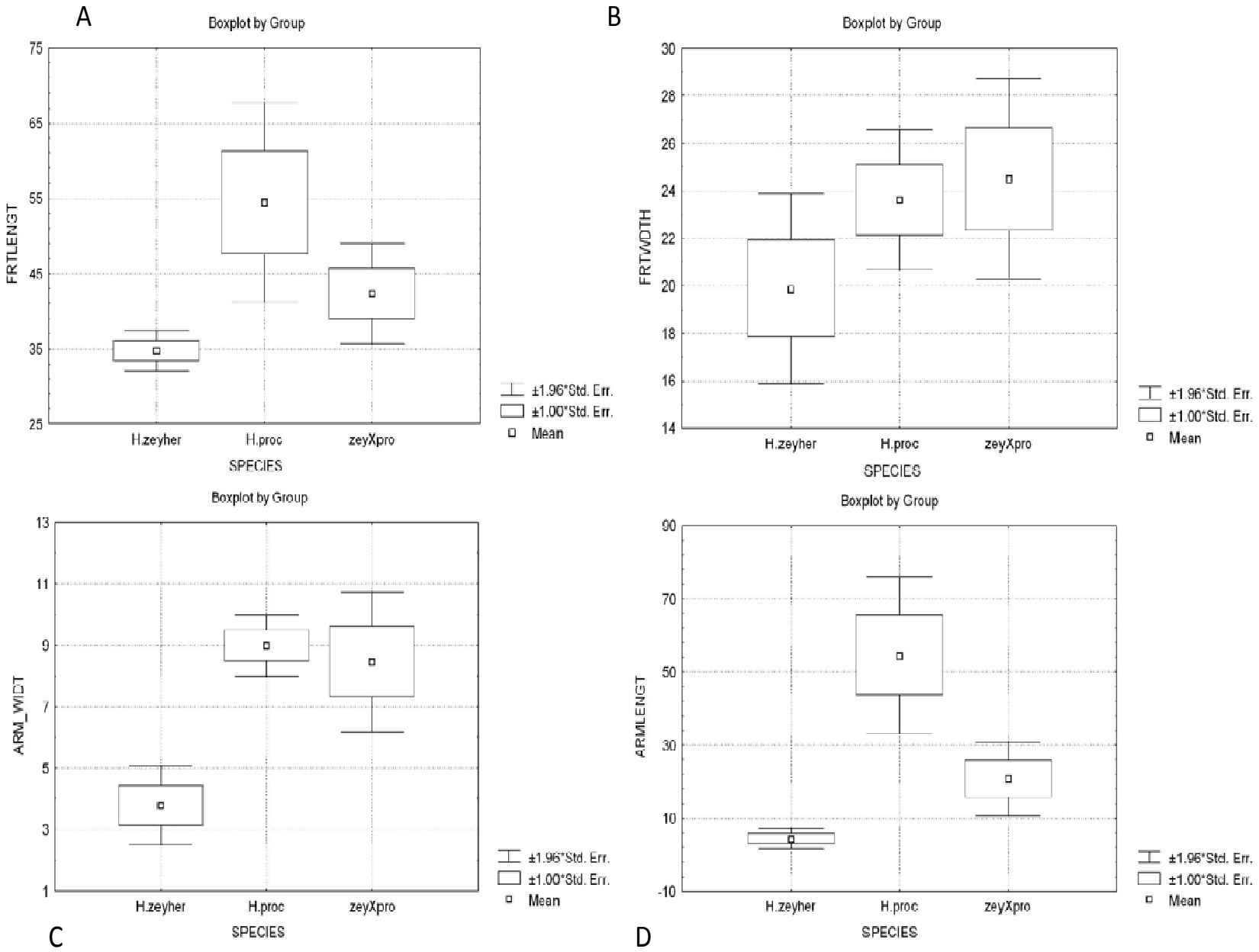

Figure 3. Box and whisker plots for the three *Harpagophytum* species: A) Fruit length; B) Fruit width; C) Arm width; D) Arm length.

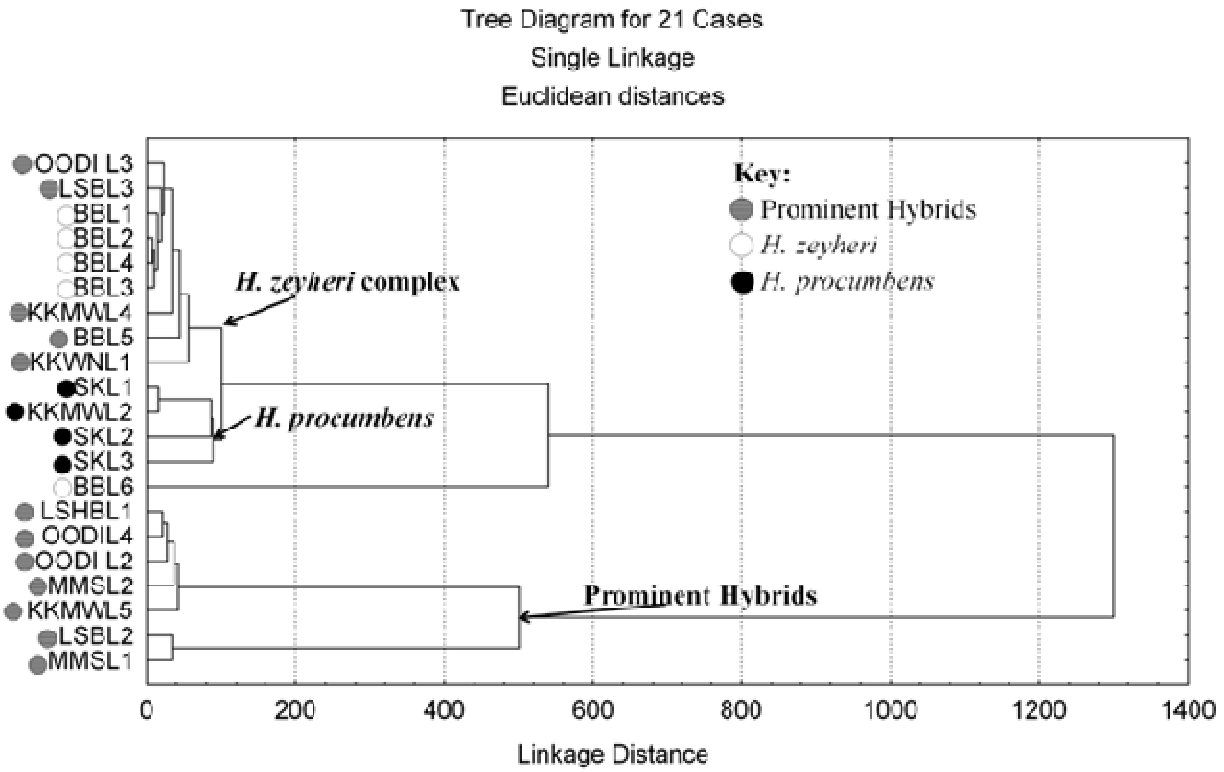

Figure 4. Tree diagram to show how the different *Harpagophytum* voucher specimens clustered.

of three groups in the study namely the *H. zeyheri* group, the *H. procumbens* group and the hybrids (Figure 1).

DISCUSSION

General morphology and number of seed rows

Number of seed rows

The three (3) seed rows in *Harpagophytum* have never been recorded in literature and are associated with speceis that we identified as hybrids. Therefore, we concluded that the three (3) seed rows are a direct evidence of introgression in *Harpagphytum*. From the literature (for example Hartmann and Ihlenfeldt, 1967; 1988) it is known that *H. zeyheri* is characterized by two seed rows on both of its seed loculus, while *H. procumbens* is characterized by four seed rows on both of its seed loculus. Hence, any deviation from that could be an indication of a new undescribed species or a hybridization event. The possibility of having a new species was ruled out because combinations in the number of seed rows per loculus have not yet stabilized (Table 2). A stabilized character is constant within different specimens of the same species and if the character was produced because of hybridization, its stabilization marks a speciation event (Walter et al., 1984).

Scatter plot and the hierachical tree

The hybrid complex (Figure 4), as identified by the hierachical tree, represents those hybrids with intermediate features (that is mid way blends). Such specimens are very difficult to classify and as such one can never tell whether the specimen is a *H. zeyheri* or a *H. procumbens*. All specimens with three seed loculus in at least one of the seed loculus fall into this category. The *H. zeyheri* complex contains those hybrids whose characters are biased towards the *H. zeyheri* species. On the other hand, Root 2 (Figure 2) in the scatter plot was well represented by specimen MMSL2, which looks like *H. procumbens* on the outside, but with the 3.2 seed row combination.

Box and whisker

The box and whisker analysis provided a quick guide to the identification of *Harpagophytum* and their hybrids. The pure lines fall within quantitative boundaries of their taxonomic descriptions as described by Ihlenfeldt and Hartmann (1970) and Ihlenfeldt (1988). And, from the results, we concluded the following to be some of the diagnostic features for the hybrids:

1) Fruits that are shorter (that is length) than those of *H. procumbens* but longer than those of *H. zeyheri*

2) Fruits that are generally wider (that is fruit width) than those of *H. procumbens* and *H. zeyheri*
3) Fruit arms are thinner (that is arm width) and shorter (that is arm length) than those of *H. procumbens* but wider and longer than those of *H. zeyheri*.

Conclusions

Morphometric analysis of fruits of *Harpagophytum* species revealed the possibility of gene flow between *H. procumbens* and *H. zeyheri*. But at this point it is difficult to determine the direction of gene flow and the frequency of hybridization. Diagnostic characters that distinguish pure lines from hybrids are the number of seed rows, fruit size (that is fruit length and fruit width) and fruit arms (that is arm width and arm length).

RECOMMENDATIONS

There is a need to do chromosomal and molecular work to get a full understanding of the *Harpagophytum* hybrid swam. The 5SDNA gene family, because it has multiple tandem repeats, should be used to determine the percentage of parental contribution by either species. Random Fragment length Polymorphism (RFLP) markers should be used to detail the frequency of occurrence and extent of introgression. In addition, cladistic analysis should be used to test for congruence since incongruence between the morphological and cpDNA data indicates the existence of localized introgression (Ferguson and Jansen, 2002).

REFERENCES

Anonym (1995). Statistica for windows volumes I and II, 2nd edition. Statsoft, U.S.A.

Arnold ML, Martin NH (2009). Adaptation by introgression. J. Biol., 8: 82.

Curtu AL, Gailing O, Finkeldey R (2007). Evidence for hybridization and introgression within a species – rich oak (Quercus spp.) community. *BMC.* Evol. Biol., 7: 1 -15.

Ellastrand CN, Prentice, HC, Hancock FJ (1999). Gene flow and introgression from domesticated plants into their wild relatives. Ann. Rev. Ecol. Syst., 30: 539 – 563.

Ferguson C, Jansen RK (2002). A Chloroplast DNA Phylogeny of Eastern *Phlox* (Polemoniaceae): Implications of Congruence and Incongruence with the *ITS* Phylogeny. Am. J. Bot., 89(8): 1324 – 1335.

Grant V (1971). Plant speciation. New York, NY, USA: Columbia University Press. *Harpagophytum procumbens* (Devil's Claw), Pedaliaceae. S. Afr. J. Bot. (In Press).

Ihlenfeldt HD (1988) Pedaliaceae. *F.Z* .8(3): 86 – 113.

Ihlenfeldt HD, Hartmann HEK (1970). *Harpagophytum. Mitt. Staatsinst. Allg.* Bot. Hamburg., 13: 15 – 69.

Jordaan A (2010). Seed coat development, anatomy and scanning electron microscopy of

Lanta V, Havrenek P, Ondrej V (2003). Morphometry Analysis and Seed Germination of *Amaranthus cruentus, A. retroflexux* and their Hybrid (*A.* X *turicensis*). Plant Soil Environ., 49 (8): 364–369.

Rangel PN, Brondani, RPV, Rangeland, PHN, Brondani C (2008). Agronomic and molecular characterization of introgression lines from the interspecific cross *Oryza sativa* (BG90-2) x *Oryza glumaepatula* (RS-16). Genet. Mol. Res., 7(1): 184-195.

Reiseberg LH, Brunsfeld SJ (1998). *Molecular Evidence and Plant Introgression*. In: Soltis DE, Solits PS Doyle JJ (eds). Molecular Systematics of Plants II: DNA Sequencing. Boston Kluwer, New York.

Walter GH, Hulley PE, Craig JFK (1984). Speciation, adaptation and interspecific competition. Oikos, 43: 246 -248.