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Taxonomic Relationships of Some Taxa of Subfamily Asclepiadoideae (Apocynaceae) as Reflected by Morphological Variations and Polymorphism in Seed Protein and RAPD Electrophoretic Profile

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Abstract: Taxonomic status of some members of the subfamily Asclepiadoideae (Apocynaceae) was analysed using variation of morphological features, seed protein electrophoretic pattern and molecular RAPD-PCR markers. The relationships between the examined taxa have been expressed as UPGMA trees, based on the coefficient of similarity using the NTSYS-pc software program. In all trees, the species of tribe Ceropegieae have been delimited together as one group from another major group that comprises species of tribe Asclepiadeae. The present work supports the earlier classification that transferred the genus *Ceropegia* from subtribe Stapeliinae to subtribe Ceropegiinae. The delimitation of species of tribe Asclepiadeae in four groups supports their previous subtribal delimitation.

Key words: Apocynaceae, Asclepiadaceae, Asclepiadoideae, RAPD-PCR, Seed protein electrophoresis, Taxonomy

INTRODUCTION

Robert Brown (Brown, 1810, 1811) separated the Asclepiadeae (Asclepiadaceae) from the Apocineae (Apocynaceae) of Antoine-Laurent de (1789). That separation was based on the morphological characters of androecium, pollen transfer system, gynoecium and the more or less presence of extra whorl of corona attached to petal which is present in Asclepiadaceae and lacking in Apocynaceae (Al Nawaihi *et al*., 2006). On the other hand, new evidence from more detailed and extensive morphological and palynological evidences as well as the rapidly growing body of molecular information (Nilsson *et al*., 1993; Struwe *et al*., 1994; Endress and Albert, 1995; Sennblad and Bremer, 1996; Civeyrel *et al*., 1998; Sennblad *et al*., 1998; Potgieter, 1999; Livshultz, 2010), suggests that Brown's delimitation does not reflect natural relationships and support the recognition of a single entity. Endress and Bruyns (2000) concluded that the Asclepiadaceae is an apomorphic derivative of the Apocynaceae; thus making the latter family monophyletic and are better considered Asclepiadaceae as a subfamily of the latter; a view which was also held by Angiosperm Phylogeny Group (2003).

In the subfamily Asclepiadoideae Liede and Albers (1994) recognized five tribes: Fockeeae H. Kunze, Meve and Liede, Marsdenieae Benth., Stapelieae Decne., Gonolobeae Reichb. ex Don and Asclepiadeae (R. Br.) Duby. Six years later, Endress and Bruyns (2000) reduced the tribes to three by abandoning Fockeeae and placing it into the tribe Marsdenieae and abandoning Gonolobeae and placing it within tribe Asclepiadeae. Further, the name Ceropegieae was adopted for the tribe formerly known as Stapelieae. The current three tribes in the subfamily Asclepiadoideae, in their treatment are thus: Marsdenieae, Ceropegieae and Asclepiadeae.

The tribal subdivision of subfamily Asclepiadoideae is based largely on the organization of the androecium (Huber, 1983; Endress and Bruyns, 2000); the pollinaria are directly attached to the corpusculum as in Marsdenieae or attached to the corpusculum via caudicles, additional arm-like appendages of the translator that are synapomorphic for the other two tribes of the subfamily (Asclepiadeae and Ceropegieae). The pollinia in the pollen sacs are oriented upwardly in tribes Ceropegieae and Marsdenieae or horizontally to pendulous in tribe Asclepiadeae in relation to translator (Endress and Bruyns, 2000). In addition to the orientation of the pollen sacs, the morphology of the anther (whether or not embedded in the tissue of the anther wings) and the position of anther wings with respect to the anther sacs were suggested as supplementary characters for tribal classification of the family. The characters of the gynoecium, particularly the presence or absence of true styles and the sharp constriction between stigma-head and ovaries have also been suggested as useful in differentiating Asclepiadeae and Ceropegieae (Swarupanandan *et al*., 1996).

The variations in the electrophoretic profile of storage seed protein SDS-PAGE have been found useful

in the study of systematics and evolution of plant species (Ladizinsky and Hymowitz, 1979; Vaughan, 1983) and may provide an understanding of the phylogenetic relationships of some taxa (Bergner and Jensen, 1989). However, valid assessment of taxonomic relationships among species and higher taxonomic ranks should necessarily be obtained when these data are considered with other lines of evidence particularly from morphology and cytology (Badr, 1995; Badr *et al*., 1998; Khalifa *et al*., 1998; Albers and Meve, 2002; Sharawy, 2008; Sharawy and Badr, 2008).

In recent years molecular data have been increasingly applied to resolve taxonomic and phylogenetic problems in plant systematics. In Asclepiadoideae, Meve and Liede (2004) analyzed the relationships of Ceropegieae and Marsdenieae, the two Asclepiadoideae tribes possessing erect pollinia by molecular investigation of non-coding cpDNA markers (*trnT-L* and *trnL-F* spacers), and the *trnL* intron. More recently, the PCR based approach Randomly Amplified Polymorphic DNA (RAPD) was widely used to address taxonomic issues in the subfamily. Goyder *et al*. (2007) studied relationships in subtribe Asclepiadinae and Jiu-Xiang *et al*. (2007) studied the affinities of 12 species of Apocynaceae. Mahmood *et al*. (2010) employed RAPD technique using 10 primers to assess genetic diversity and inter-specific relationships between two species of genus *Caralluma* that belong to tribe Ceropegieae.

In the present research work variation in morphological characters, polymorphism in seed protein electrophoretic profile as revealed by SDS-PAGE and the DNA molecular characters as generated by RAPD markers are applied to reassess the taxonomic relationships of 27 samples representing 13 genera and 17 species of subfamily Asclepiadoideae (Apocynaceae), in the light of their previous taxonomic treatments.

MATERIALS AND METHODS

The taxa used in this study, were collected from their natural habitats in Egypt and Saudi Arabia and from public gardens in Egypt and Saudi Arabia during the period 2006-2009. The tribe delimitation and localities of the examined materials are given in Table 1. The studied species were identified according to Tackholm and Drar (1974), Mandaville (1990), Moslem (1999), Collenette (1999) and Boulos (1999). Herbarium specimens of the examined taxa are deposited at the Herbarium of Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt and at the Museum of Biology Department, Faculty of Science, Hail University, Hail, Saudi Arabia. A total of 44 morphological characters were considered, the examined characters including 24 two-state characters and 20 multi-state characters. A list of these characters and their states are given in Table 2.

Table 1: A list of the examined taxa of the subfamily asclepiadoideae, their tribe delimitation and their localities

TAXA	Locality
Tribe: Asclepiadeae	
Calotropis procera (Ait.) Ait. F.	Hail-Al Madinah road, Saudi Arabia
Calotropis procera (Ait.) Ait. F.	Hail-Al Jouf road, Saudi Arabia
Calotropis procera (Ait.) Ait. F.	Cairo-Suez road, Egypt
Calotropis procera (Ait.) Ait. F.	Orman Botanical Garden, Egypt
Cynanchum acutum L.	Hail Botanical Garden, Hail, Saudi Arabia
Cynanchum acutum L.	Orman Botanical Garden, Egypt
Glossonema boveanum Decne.) Decne.	Makkah-Jiddah road, Saudi Arabia
Gomphocarpus fruticosus (L.) Ait.	Aja Mountain, Hail, Saudi Arabia
Gomphocarpus fruticosus (L.) Ait.	Saint Catherine, Sinai, Egypt
Gomphocarpus sinaicus Boiss.	Hema Faid region, Hail, Saudi Arabia
Gomphocarpus sinaicus Boiss.	Wadi El Arish, Sinai, Egypt
Kanahia laniflora (Forssk.) R. Br.	Al Madinah-Makkah road, Saudi Arabia
Odontanthera radians (Forssk.) D.V.Field	Makkah-Jiddah road, Saudi Arabia
Pentatropis spiralis (Forssk.) Decne.	Al Madinah-Makkah road, Saudi Arabia
Pergularia daemia Forsk	Al Madinah-Makkah road, Saudi Arabia
Pergularia tomentosa L.	Aja Mountain, Hail, Saudi Arabia
Pergularia tomentosa L.	Saint Catherine, Sinai, Egypt
Solenostemma argel (Del.) Hayne	Aja Mountain, Hail, Saudi Arabia
Solenostemma argel (Del.) Hayne	Saint Catherine, Sinai, Egypt
Solenostemma argel (Del.) Hayne	Hail-Al Jouf road, Saudi Arabia
Tribe: Ceropegieae	
Caralluma penicillata White et Sloane	Al Madinah-Makkah road, Saudi Arabia
Caralluma retrospiciens (Ehrenb.) N.E.Br.	Hail-Al Madinah road, Saudi Arabia
Caralluma sinaica (Decne) A. Berger	Saint Catherine, Sinai, Egypt
Ceropegia arabica H.Huber	Fayfa Mountain, Jizan, Saudi Arabia
Huernia lodarensis Lavranos	Al Taif-Abha road, Saudi Arabia
Leptadenia pyrotechnica (Forssk.) Decne.	Aja Mountain, Hail, Saudi Arabia
Leptadenia pyrotechnica (Forssk.) Decne.	Cairo-Suez road, Egypt

For protein extraction, 0.2 g seeds were powdered and mixed with 2 ml Tris-HCl buffer (Tris-SDS, β-mercaptoethanol and PMSF at pH = 8) for 1 h at room temperature. The mixture was centrifuged for 10 min at 12,000 rpm and 50 μL of supernatant (protein extract) was electrophoresed in 12.5% acrylamide that was prepared as described by Laemmli (1970) in Consort vertical slab gel apparatus. Gels were then stained for 16 h in sufficient amount of Comassie blue and distained for 12 h. the banding profile of the examined species was photographed while the gels were wet. The number of bands was scored by direct observation of gels and photograph. Each band was considered as a character and its presence or absence was coded for analysis.

RAPD-PCR reactions were conducted using 10 random primers from OPC series were tested for the

Table 2: A list of examined morphological characters and their codes for numerical analysis

Character	State	Code	Character	State	Code
Habit	Annual	0	Corolla colour	White	0
	Perennial	1		White with brown apex	1
Gross morphology	Tree or shrub	0		Yellow	2
	Herb	1		Pink	3
	Succulent	2		Purple	4
Root	Tuberous	0	Corolla lobe length	Shorter than tube	0
	Tuberless	1		Longer than tube	1
Stem	Erect	0	Corolla lobe	Flesh	0
	Climbing	1		Not fleshy	1
	Prostrate1	2	Corolla lobe apex	Acute	0
Stem Texture	Glabrous	0		Acuminate	1
	Hairy	1		Obtuse	2
Stem Cross section	Cylindrical	0	Corolla in the flower buds	Inflexed	0
	Angular	1		Not inflexed	1
	Irregular	2	Corona	Absent	0
Leaf duration	Leafless	0		Present	1
	Soon leafless	1	Corona insertion	On corolla	0
	Leafy	2		On stamina tube	1
Leaf arrangement	Opposite	0	Corona lobe	Free	0
	Alternate	1		United	1
Leaf shape	Ovate	0	Corona whorl	Single	0
	Lanceolate	1		Double	1
	Cordate	2	Corona colour	White	0
	Linear	3		Pink	1
Leaf texture	Coriaceous	0		Purple	2
	Succulent	1		Red	3
Leaf base	Exstipulate	0	Corona lobe shape	Ovate	0
	Stipulate	1		Elliptic	1
Leaf apex	Acute	0	Corona spreading	Erect	0
	Obtuse	1		Slightly erect	1
	Notched	2		Flat	2
Leaf blade base	Obtuse	0	Corpusculum shape	Oblong	0
	Cuneate	1		Triangular	1
	Rounded	2	Pollinia	Erect	0
	cordate	3		Pendulous	1
Leaf blade surface	Glabrous	0	Anther	Without apical appendages	0
	Hairy	1		With apical appendages	1
	Spiny	2	Style	Absent	0
Leaf venation	Brochidodromous	0		Present	1
	Acrodromous	1	Clavuncular morphology	Absent	0
Vein prominence	Obscure	0	(constriction between	Present	1
	Conspicuous	1	stigma-head and ovaries)		
The lowest pair of lateral veins	About half way to apex	0	Fruit shape	Ovate	0
	To apex	1		Linear	1
Inflorescence	Cyme	0		Inflated	2
	Umbel	1	Fruit surface	Glabrous	0
	Linear	2		Hairy	1
Pedicel hairs	Absent	0		Spiny	2
	Scattered	1	Seed colour	Brown	0
	Dense	2		Black	1
Indumentum	Absent	0	Seed shape	Ovate	0
	Scattered	1		Compressed	1
	Dense	2		Spathulate	2
Sepal lobe shape	Ovate	0	Seed surface	Glabrous	0
	Oblong ovate	1		Hairy	1
	Lanceolate	2	Seed coma	Short	0
				Long	1

amplification of DNA. The conditions reported by Khanuja *et al*. (1999) and Michiels *et al*. (2003) were used for RAPD-PCR. Amplification was achieved in a Techne (UK) Progene thermocycler programmed as follows: 94°C/4 min (1 cycle); 94°C/1 min, 37°C/1 min, 72°C/2 min (44 cycles); 72°C/7 min (1 cycle) and 4°C (infinitive). After the cycling was completed, 15 μL of the reaction products were analysed alongside small molecular weight markers on a 2% agarose gel in the presence of ethidium bromide and photographed under UV light.

For data analysis, the NTSYS-pc version 2.2 programs (Rohlf, 2000) was used. The most common use

of this program is for performing various types of agglomerative cluster analyses of some type of similarity or dissimilarity matrix. The relationships between the examined taxa have been expressed using the coefficient of similarity proposed by Dice (1945) as calculated by the program. Construction of trees illustrating the relationships between the studied taxa was constructed using the Unweighted Pair Group Method using Arithmetic Average (UPGMA) proposed by Sokal and Michener (1958).

RESULTS

Relationships based on morphological attributes: The UPGMA cluster analysis illustrating the relationships based on morphological attributes is shown in Fig. 1. In this tree, seven taxa representing the six species of tribe Ceropegieae are delimited together as a separate group from another major group that comprises the 20 taxa representing 11 species of tribe Asclepiadeae at a UPGMA distance coefficient of about 1.40. In the former group, the *Ceropegia arabica* is clearly distinguished from the other three species of the same tribe (Ceropegieae) at distance coefficient about 1.04. At distance coefficient of 0.90 the two samples of *Leptadenia pyrotechnica* are separated from the other species of the same tribe. Also, at distance coefficient of about 0.8 the *Huernia lodarensis* is separated from the three species of *Caralluma*. The second group comprising the species of tribe Asclepiadeae is divided into two subgroups; the first includes the eight taxa representing five species and the second comprises 12 taxa representing six species. The former subgroup is delimited at distance coefficient of about 1.15 into two clusters; one comprising *Pentatropis spiralis*, *Solenostemma argel*, (three samples), *Odonthanthera radians* and *Glossonema boveanum* and the second comprising the two samples of *Cynanchum acutum*. In first cluster, *Pentatropis spiralis* is clearly differentiated from the other species at taxonomic distance coefficient of about 1.10. The second subgroup includes 12 taxa representing six species and is divided into two clusters at taxonomic distance coefficient of about 0.75, the first comprising *Prgularia daemia*, *P. tomentosa* (2 samples), *Kanahia laniflora*, *Gomphocarpus sinaicus* (two samples) and *G. fruticosus* (two samples) while the second cluster comprising the four samples of *Calatropis procera*.

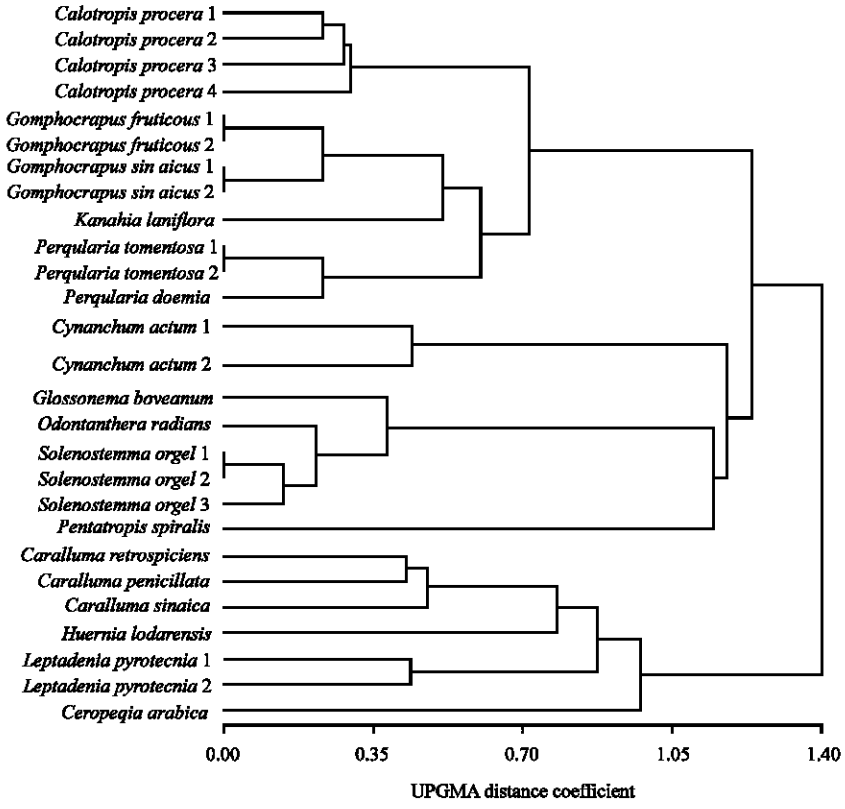

Fig. 1: UGMA tree illustrating the relationship between the studied samples of subfamily Asclepiadoideae, based on variation in morphological characters

Relationships based on attributes of seed protein electrophoresis: A total of 52 protein bands have been revealed in the electrophoretic profiles of the examined taxa of subfamily Asclepiadoideae (Plate 1). The molecular size of the bands scored ranges between over 100 KDa to about 10 KDa; the majority of the bands have been found polymorphic across the examined taxa. A UPGMA tree illustrating the relationships between the examined species based on the polymorphism in the seed protein electrophoretic profile is shown in Fig. 2. The topology of this tree generally resembles that of the tree based on morphological criteria. The examined species are delimited in two groups, one comprising seven taxa representing six species (*Leptadenia pyrotechnica*, *Ceropegia arabica*, *Huernia lodarensis*, *Caralluma sinaica*, *C. retrospiciens* and *C. penicillata*) and the other group includes 20 taxa representing the remaining 11 species. In the former cluster the two samples of *Leptadenia pyrotechnica* are clearly distinguished from the other species at a UPGMA distance coefficient of about 1.20. *Ceropegia arabica* and *Hurenia lodarensis* are separated from the three species of *Caralluma* at taxonomic distance coefficient of about 1.00 and 0.95 spontaneously. The second group is divided into two subgroups; the first includes eight taxa representing five species delimited in one small cluster comprised of the samples of *Cynanchum acutum* groups and another cluster comprising *Pentatropis spiralis*, *Solenostemma argel* (three samples), *Odontanthera radians* and *Glossonema boveanum*. The second subgroup cluster comprises 12 taxa representing six species and is divided into two clusters, a small one comprising the 4 samples of *Calotropis procera*. The second cluster comprises *Pergularia daemia*, *P. tomentosa* (two samples), *Kanahia laniflora*, *Gomphocarpus sinaicus* (two samples) and *G. fruticosus* (two samples).

Relationships based on attributes of RAPD fingerprinting: The bands produced by 10 of the 20 used random primers ranges in size from 500-2000 bp. Some of the bands were monomorphic but the majority of bands are polymorphic (Plate 2). The dendogram based on

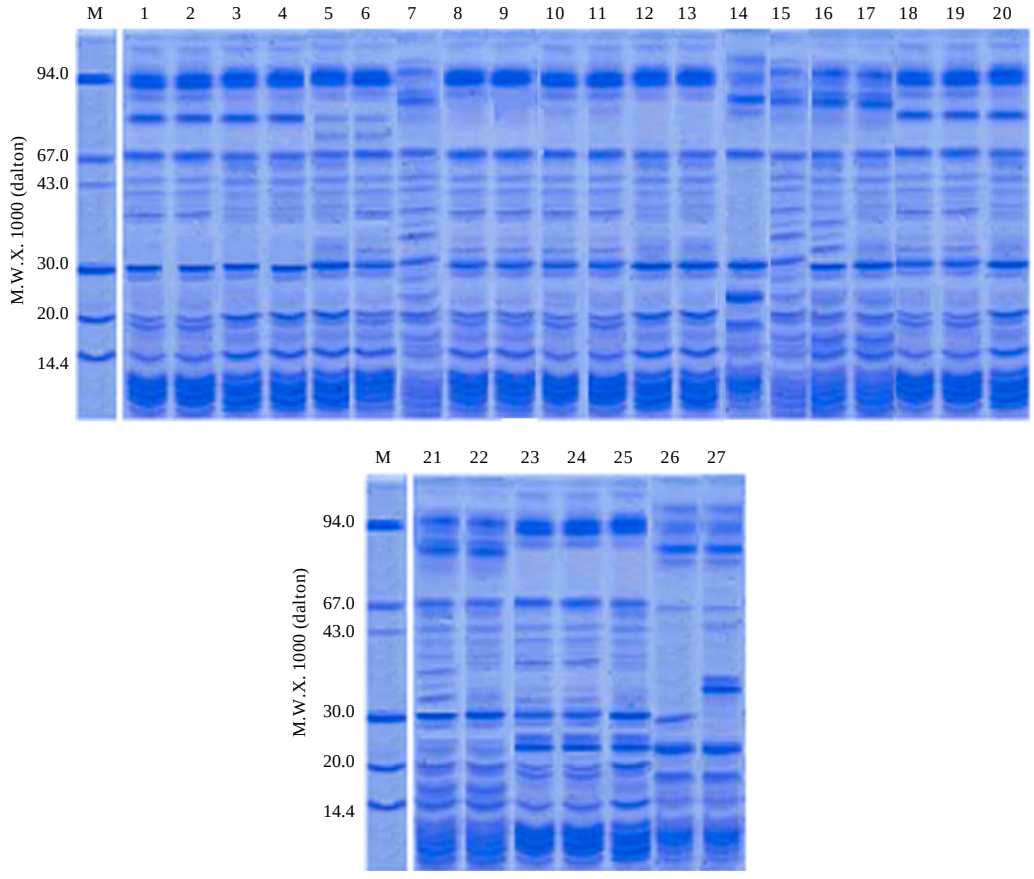

Plate 1: Photographs of polyacrylamide gel illustrating electrophoretic band profiles of seed proteins of the studied taxa extracted in Tris-HCl buffer (Numbers of taxa are as in (Table 1)

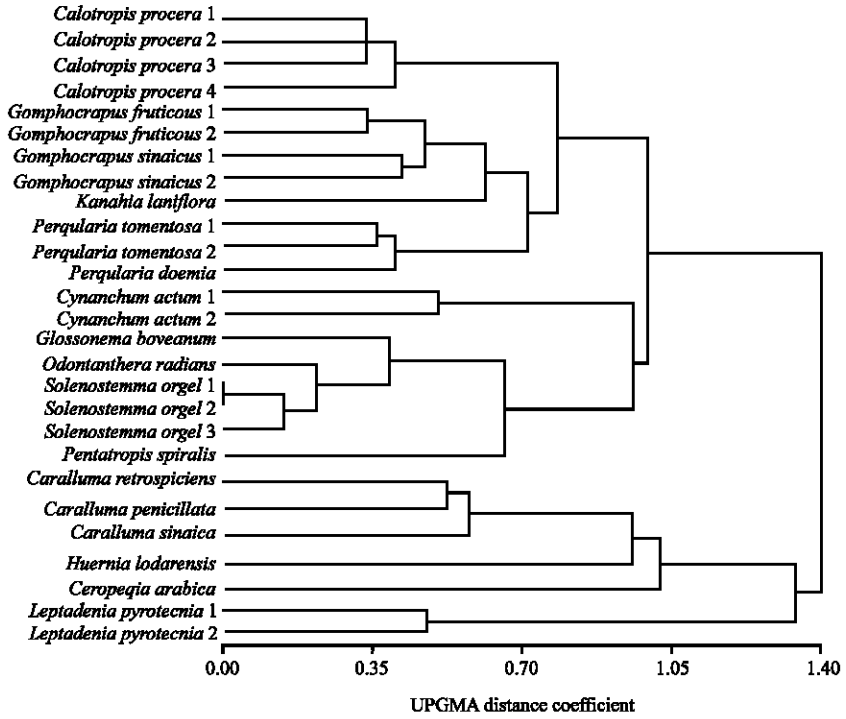

Fig. 2: UGMA tree illustrating the relationship between the studied samples of subfamily Asclepiadoideae, based on polymorphism in seed protein electrophoretic profiles

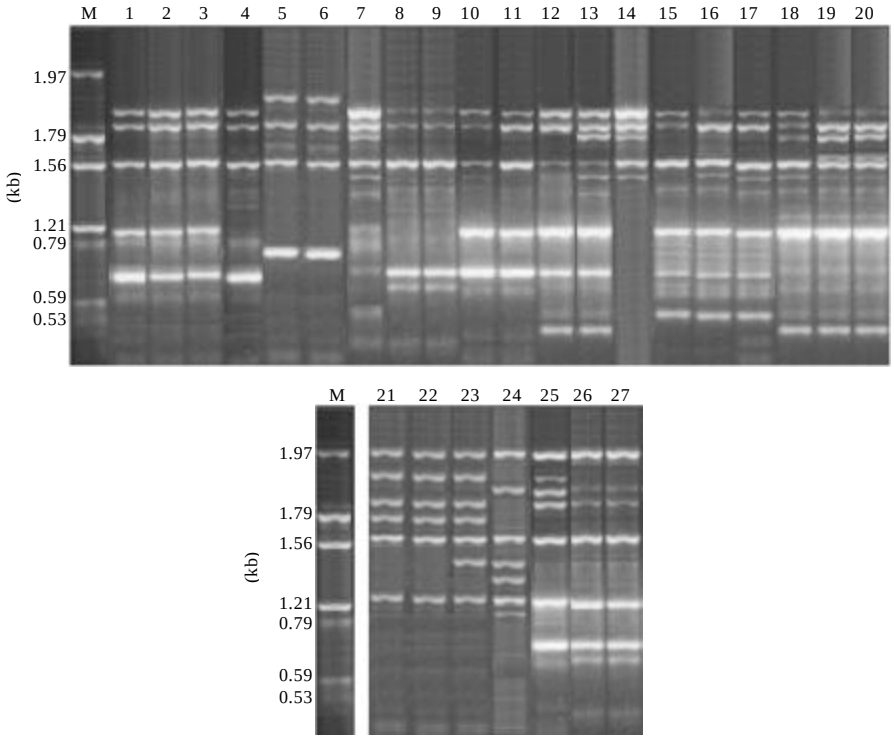

Plate 2: PCR-RAPD pattern of the studied taxa using different primers, (No. of taxa are as in Table 1)

RAPD-PCR divided the studied taxa into two main groups; the first includes seven taxa representing the six species of tribe Ceropegieae and the second comprises the 20 taxa representing 11 species of tribe Asclepiadeae (Fig. 3). In the first group *Ceropegia arabica* and *Huernia lodarensis* are separated from the remaining species at high distance coefficient of 1.40 and 1.00, respectively. The remaining species in this group are divided into two subgroups the first comprising the two samples of *Leptadenia pyrotechnica* and the second group including the three species of *Caralluma*. In the second group, the two samples of *Cynanchum acutum* are separated from the remaining species at a distance of about 1.10. The remaining species are then separated into two subgroups at a distance coefficient of about 1.00. In the first subgroups *Pentatropis spiralis* is separated from the other species in this cluster at a distance of 0.85. The species in the first subgroups is divided into clusters; one is comprised of three samples of *Solenostemma argel* and the second includes the two species *Odontanthera radians* and *Glossonema boveanum.* The second subgroups comprises 12 taxa representing six species and is divided into two clusters; the first comprises *Pergularia daemia*, *P. tomentosa* (2 samples), *Kanahia laniflora*, *Gomphocarpus sinaicus* (two samples) and *G. fruticosus* (two samples) and comprises the four samples of *Calotropis procera.*

Relationships based on morphological variations and polymorphism in seed protein electrophoretic profile and RAPD fingerprinting: The overall relationship between the examined taxa based on variation in morphological criteria and polymorphism in seed protein electrophoretic profile and RAPD fingerprinting is illustrated by the UPGMA tree shown in Fig. 4. In this tree, the studied taxa are also divided in two groups; one comprising the seven taxa representing the six species of tribe Ceropegieae and the second comprises the 20 taxa representing 11 species of tribe Asclepiadeae at UPGMA distance coefficient of about 1.37. In the first group *Ceropegia arabica* is separated from the remaining species at high distance coefficient of about 1.20. The remaining species in this group are divided into two subgroups; the first comprising the two samples of *Leptadenia pyrotechnica* and the second includes the three species of *Caralluma* and *Huernia lodarensis*. In the second group, the two samples of *Cynanchum acutum* are separated from the remaining species at a distance of about 1.06. The

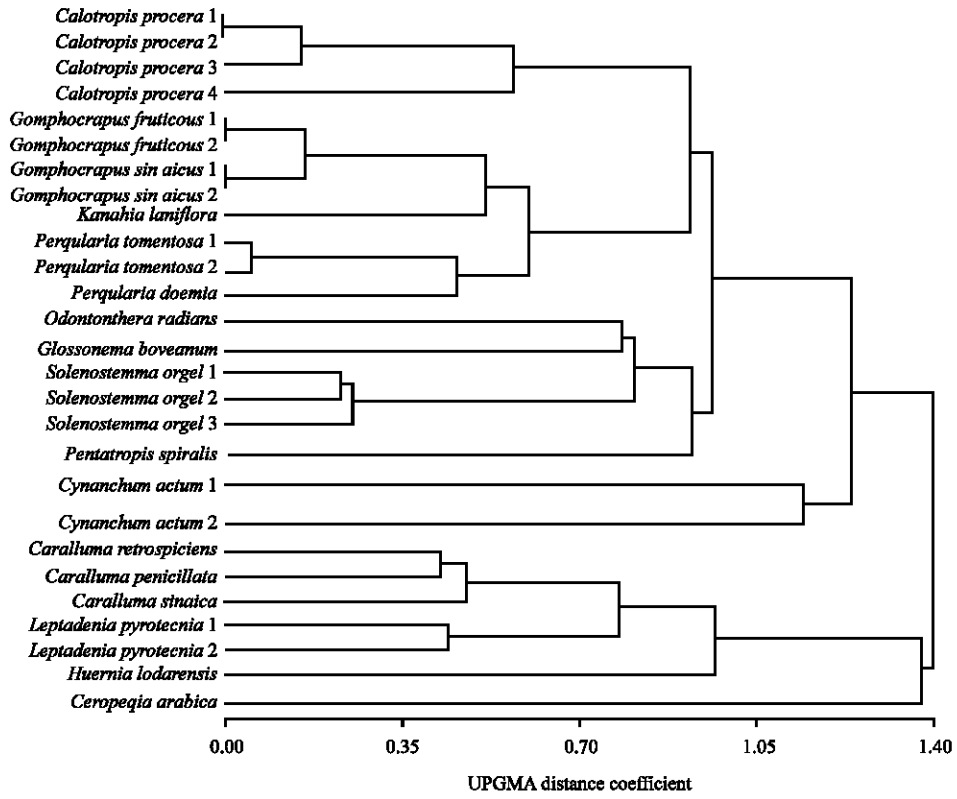

Fig. 3: UPGMA tree illustrating the relationships between the studied species based on analysis of RAPD fingerprinting

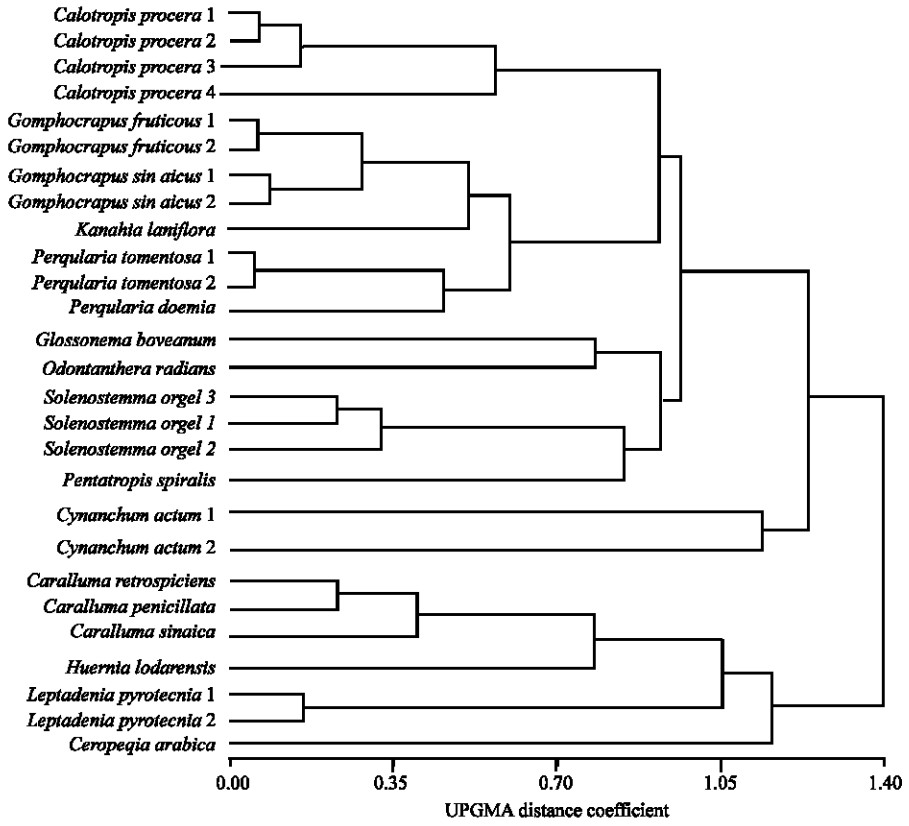

Fig. 4: UPGMA tree illustrating the relationships between the studied species based on variation in morphological characters, polymorphism in seed protein electrophoretic profiles and RAPD analysis

remaining species are separated into one small subgroups comprised of six taxa representing four species and a major subgroups comprised of 12 taxa representing six species. The former subgroup comprises *Pentatropis spiralis*, *Solenostemma argel* (three samples), *Odontanthera radians* and *Glossonema boveanum*. The latter larger subgroup includes the remaining species; *Pergularia daemia*, *P. tomentosa* (two samples), *Kanahia lanifolia*, *Gomphocarpus sinaicus* (two samples), *G. fruticosus* and the four samples of *Calotropis procera*.

DISCUSSION

The relationship between the examined species of the subfamily Asclepiadoideae (Apocynaceae) are discussed in the present work through the numerical analysis of all attributes viz. morphological characters, seed protein electrophoresis and RAPD-PCR analysis (Fig. 4). The six species of tribe Ceropegieae are delimited together as a separate group from another major group that comprises 11 species of tribe Asclepiadeae. The separation of species in the two tribes is in agreement with previous taxonomic classification by Endlicher (1938), Cronquist (1968), Markgraf (1972), Spellman (1977), Takhtajan (1980) and Rosatti (1989) based on the morphological characters. In addition Sundell (1980), Kunz (1995), Swarupanandan *et al.* (1996) and Endress and Bruyns (2000) recognized these two tribes based on the organization of the androecium, where in tribe Asclepiadeae the pollinia in pollen sacs are horizontally or pendulous while in tribe Ceropegieae are oriented upwardly. Liede and Albers (1994) and Goyder (2006) supported the separation between the two tribes (Asclepiadeae and Ceropegieae) by the characters of gynoecium, particularly the presence or absence of true styles and the sharp constriction between stigma-head and ovaries. Phylogenetic studies based genetic variations have also confirmed the delimitation between the two tribes as observed in this study (Liede, 1996, Fishbein, 2001; Goyder *et al*., 2007; Mahmood *et al*., 2010).

The six species of tribe Ceropegieae in all analyses used in this study are delimited into 3 groups.

The three species of *Caralluma* (*C. penicillata*, *C. retrospiciens* and *C. sinaica*) and *Huernia lodarensis* of subtribe Stapeliinae are grouped together in the most analyses in this study. The relatively high distance between *H. lodarensis* and the three species of *Caralluma* as indicated in Fig. 4 do not supports the grouping of *H. lodarensis* in section *Caralluma* as was proposed by Audissou (2005), and Ramachandran *et al*., (2011). Moreover, the three species of genus *Caralluma* are clustered at a low distance coefficient (about 0.25) indicating close morphological resemblance between the genotype of *Caralluma* species. The two samples of *Leptadenia pyrotechnica* were also separated from the other studied species of tribe Ceropegieae in the all analyses. This is congruent with their delimitation in subtribe Leptadeniinae by Endlicher (1938) and Meve and Liede (2002).

The *Ceropegia arabica* of tribe Ceropegieae is separated from the other species of same tribe in all analyses used in this study. Forster and Bruyns (1992), Kunz (1995) and Swarupanandan *et al*. (1996) included the genus *Ceropegia* in the subtribe Stapeliinae of tribe Ceropegieae referring to common characters of latex, lack of anther appendage and mode of attachment of the pollinia to the translator in addition to the stylar characters. Also, by using molecular evidence Liede (1997) and Meve and Liede (2004) pointed to the same direction. As early as, Bentham and Hooker (1876) separated the genus *Ceropegia* in subtribe Ceropegiinae according to the stem morphological characters. Recently the phylogenetic studies carried by Fishbein (2001), Bensusan (2009), Bartosz *et al*. (2010) and Kullayiswamy *et al*. (2012) confirmed the separation of *Ceropegia arabica* of tribe Ceropegieae from the other species of the same tribe; the present results support this separation.

In tribe Asclepiadeae; the separation of *Calotropis procera* (4 samples) *Gomphocarpus fruticosus* (2 samples), *G. sinaicus* (2 samples), *Kanahia laniflora*, *Pergularia daemia*, and *P. tomentosa* (2 samples) in a separate group is in agreement with their previous delimitation in subtribe Asclepiadinae by Sundell (1980), Li *et al*. (1995), Swarupanandan *et al*. (1996) Liede (1997, 1999) and Fishbein (2001) based on the vegetative and corona characters as well as style structure and mode of pollinia attachment to the translator. Otherwise, the grouping of the genus *Pergularia* in subtribe Asclepiadinae in the present work is congruent with its transfer from the subtribe Cynanchinae to subtribe Asclepiadinae by Liede and Meve (1996) based on the analysis of ITS region of the nuclear genome.

Except for *Pentatropis spiralis* of subtribe Astephaninae the grouping of *Glossonema boveanum*, *Odonthera radians*, and *Solenostema argel* together is in agreement with their previous delimitation in subtribe Glossonematinae by Liede *et al*. (2002). The grouping of *Pentatropis spiralis* (Subtribe Astephaninae) with the species of subtribe Glossonematinae is ascertained with the analyses based on seed protein characters (Fig. 2). However, it contradicts the analyses based on morphological criteria and RAPD-PCR (Fig. 1, 3). Meanwhile only a few morphological similarities exist between *P. spiralis* and the other four species of the same tribe. All of them are characterized by brochidodromous leaf venation, white colour of both corolla and corona and brown ovate seeds.

Liede *et al*. (2002) showed the subtribe Glossonematinae of the tribe Asclepiadeae, hitherto composed of Arabian and North African genera *Glossonema*, *Odonthanthera* and *Solenostemma*, not to be monophyletic. Also, he suggested that *Glossonema* and *Odontanthera* are closely allied to *Pentarrhinum*, an African genus of five species belonging to subtribe Cynanchinae based on molecular, karyological and morphological evidences. This is supported by the relationships as expressed in the tree based on RAPD analysis. In the meantime *Cynanchum acutum* of subtribe Cynanchinae is delimited from all taxa of tribe Asclepiadeae in all analyses. This species is morphologically characterized by often growing from rhizomes, opposite petiolated leaves, gynostegial origin of corona and the fusion of staminal and intrastaminal parts. This delimitation is congregant with previous results based on molecular evidences carried by Sennblad and Bremer (1996) and Liede *et al*. (2002).

CONCLUSION

Relationships between the studied species of subfamily Asclepiadoideae (Apocynaceae), based on morphological variations and polymorphism in seed protein electrophoretic profile and RAPD fingerprinting support the delimitation of the examined species in the two tribes Asclepiadeae and Ceropegieae. Moreover, the present study supports the transfer of the genus *Ceropegia* from subtribe Stapeliinae to subtribe Ceropegiinae and does not support the grouping of *Huernia lodarensis* in section *Caralluma*. Although, the present study separates the two samples of *Cynanchum acutum* from all taxa of tribe Asclepiadeae, the results are in agreement with the delimitation of the remaining studied species of tribe Ceropegieae in two subtribes Stapeliinae and Leptadeniinae.

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