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CYTOTAXONOMICAL INVESTIGATIONS OF THE TRIBES ASCLEPIADEAE AND CEROPEGIEAE OF THE SUBFAMILY ASCLEPIADOIDEAE-APOCYNACEAE

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Abstract

In this study, karyotype criteria of 27 accessions (17 taxa) of the two tribes Asclepiadeae (20 accessions) and Ceropegieae (7 accessions) from Egypt and Saudi Arabia belonging to subfamily Asclepiadoideae are described and polyploid variations are also discussed. Detailed karyotype features, *i.e.* total chromosome length (TCL), mean chromosome length (MCL) and karyotype asymmetry expressed as arm ratio (MAR), total form percent (TF %), intrachromosomal asymmetry (A_1) and interchromosomal asymmetry (A_2), are also described. Karyotype features of the studied accessions were used to assess the tribal relationships within the subfamily Asclepiadoideae to differentiate between taxa that belonging to the tribes Asclepiadeae and Ceropegieae in the light of the current systems of classification. In addition, new chromosome counts of 16 taxa or accessions are reported here for the first time.

Introduction

The family Apocynaceae includes trees, shrubs, herbs, and lianas, commonly called the dogbane family. Members of the family are native to European, Asian, African, Australian and American tropics or subtropics, with some temperate members (Endress & Bruyns, 2000). The only genera found in temperate Europe away from the Mediterranean are *Vinca* (Apocynoideae) and *Vincetoxicum* (Asclepiadoideae). The family, as currently recognized, includes some 1500 species divided in about 424 genera. (Endress & Bruyns, 2000).

Many species are tall trees found in tropical rainforests, but some grow in tropical dry (xeric) environments. There are also perennial herbs from temperate zones. Many of these plants have milky sap, and many species are poisonous if ingested.

There are 5 subfamilies: Apocynoideae, Asclepiadoideae, Periplocoideae, Rauvolfioideae and Secamonoideae (Endress & Bruyns, 2000). The former two subfamilies were part of the Apocynaceae *sensu stricto*, whilst the latter three subfamilies used to belong to the Asclepiadaceae. The expended family Apocynaceae is the result of a conflation of the two families.

The Asclepiadaceae is a former plant family now treated as a subfamily (subfamily Asclepiadoideae) in the Apocynaceae. In 2000, Endress & Bruyns concluded that the family 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. This view was also up held by the Angiosperm Phylogeny Group (APG, 2003).
They form a group of perennial herbs, twining shrubs, lianas or rarely trees but notably also contain a significant number of leafless stem succulents, all belonging to the order Gentianales. The name comes from the type genus *Asclepias* (milkweeds). There are 348 genera, with about 2,900 species. They are mainly located in the tropics to subtropics, especially in Africa and South America.

The florally advanced tribe Stapeliae within this family contains the relatively familiar stem succulent genera such as *Huernia, Stapelia* and *Hoodia*. They are remarkable for the complex mechanisms they have developed for pollination, which independently parallel the unrelated Orchidaceae, especially in the grouping of their pollen into pollinia. The fragrance from the flowers, often called "carrion", attracts flies that pollinate the flowers.

The tribal subdivision of subfamily Asclepiadoideae is based largely on the organization of the androecium (Huber, 1983, Endress & 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 & 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).

Chromosomes have been considered as sources of valid taxonomic criteria. The classification of a number of families has been aided or substantiated by information on chromosome criteria or features (Moore, 1978 and Jackson, 1984). The chromosome size, the position of the centromere and special banding patterns may also be systematically informative (Judd *et al*., 1999).

Though focus on phylogenetic studies have been shifted towards DNA based studies (Shinwari *et al.*, 2011), yet chromosomes can still provide supplementary data to clarify ambaguities. The Gramineae provide many classical examples of the value of the chromosome data in tribal delimitation. *Spartina*, for example, was for long placed in tribe Chlorideae (x = 10), although its chromosomes (x = 7) were at variance. Marchant (1968) showed the genus to have in fact x = 10 small chromosomes and dilemma was solved (Moore, 1978). Other examples demonstrating the role of chromosomal criteria in the taxonomy at the family level include the work of Behera & Patnaik (1974) on Amaranthaceae, Badr *et al*. (1997a) on the Solanaceae, Kamel (1996) on the Asteraceae and Aboel Atta *et al*., (1999) on the Ranunculaceae. The karyotype data also appear to be of taxonomic value in providing a logical basis for the redistribution of genera in tribes. For example of the usefulness of karyotype in clarifying taxonomic relationships is the family Ranunculaceae (Gregory, 1941).

Karyotype studies were principally based on the bases that symmetrical karyotypes are more primitive than asymmetrical ones, longer chromosomes than shorter ones, median centromeres with chromosome arms of equal length were more primitive than chromosomes with arms of unequal length, low basic numbers had given rise to higher ones. These features are based on the comparison between karyotypes of known relative antiquity, as determined through classical taxonomy (Sharma, 1990).

At the generic level and below, the chromosomes provided a range of cytological possibilities for understanding the limitations, affinities and evolution of taxa (Badr *et al*., 1997b, Abou El-Enain, 1999, Kamel, 1999a, 1999b, 2000, 2001, Vallés *et al*., 2001, De Loewnstern & Garbari, 2004, Kamel, 2004, Sevimay, 2005, Kamel, 2006, Khatoon & Ali, 2006, Badr, 2007, Badr & Sharawy, 2007, Sharawy, 2008, Badr *et al*., 2009, Kamel *et al*., 2009, Melahat *et al*., 2011, Tabur *et al*., 2012).

In this work, chromosome numbers and detailed karyotype features of 27 accessions of the two tribes, Asclepiadeae (20 accessions) and Ceropegieae (7 accessions) were studied, karyotype features were used to assess the tribal relationships within the subfamily Asclepiadoideae and to differentiate between the taxa that belonging to the tribes Asclepiadeae and Ceropegieae in the light of the current systems of classification.

Materials and Methods

Plant Materials: Materials of the 27 accessions were collected from various habitats in Egypt and Saudi Arabia. Seeds of 27 accessions, representing 13 genera and 17 species of subfamily Asclepiadoideae (Apocynaceae), 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 Täckholm (1974), Mandaville (1990), Migahid (1996), Migahid & Hammouda (1978), Collenette (1985 & 1999), and Boulos (1999). Herbarium specimens of the examined accessions 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

Table 1. A list of the examined taxa of the subfamily Asclepiadoideae, their tribe delimitation and their sources & localities.

No.*	Taxa	Sources & Localites
	Tribe: Asclepiadeae	
1.	*Calotropis procera* (Ait.) Ait.f.	Hail - Al Madinah road, Saudi Arabia.
2.	*Calotropis procera* (Ait.) Ait.f.	Hail - Al Jouf road, Saudi Arabia.
3.	*Calotropis procera* (Ait.) Ait.f.	Cairo - Suez road, Egypt.
4.	*Calotropis procera* (Ait.) Ait.f.	Orman Botanical Garden, Egypt.
9.	*Cynanchum acutum* L.	Hail Botanical Garden, Hail, Saudi Arabia.
10.	*Cynanchum acutum* L.	Orman Botanical Garden, Egypt.
11.	*Glossonema boveanum* (Decne.) Decne.	Makkah - Jiddah road, Saudi Arabia.
12.	*Gomphocarpus fruticosus* (L.) Ait.	Aja Mountain, Hail, Saudi Arabia.
13.	*Gomphocarpus fruticosus* (L.) Ait.	Saint Catherine, Sinai, Egypt.
14.	*Gomphocarpus sinaicus* Boiss.	Hema Faid region, Hail, Saudi Arabia.
15.	*Gomphocarpus sinaicus* Boiss.	Wadi El Arish, Sinai, Egypt.
17.	*Kanahia laniflora* (Forssk.) R.Br.	Al Madinah - Makkah road, Saudi Arabia.
20.	*Odontanthera radians* (Forssk.) D.V.Field.	Makkah - Jiddah road, Saudi Arabia.
21.	*Pentatropis nivalis*	Al Madinah - Makkah road, Saudi Arabia.
22.	*Pergularia daemia* Forssk.	Al Madinah - Makkah road, Saudi Arabia.
23.	*Pergularia tomentosa* L.	Aja Mountain, Hail, Saudi Arabia.
24.	*Pergularia tomentosa* L.	Saint Catherine, Sinai, Egypt.
25.	*Solenostemma argel* (Del.) Hayne.	Aja Mountain, Hail, Saudi Arabia.
26.	*Solenostemma argel* (Del.) Hayne.	Saint Catherine, Sinai, Egypt.
27.	*Solenostemma argel* (Del.) Hayne.	Hail - Al Jouf road, Saudi Arabia.
	Tribe: Ceropegieae	
5.	*Caralluma penicillata* White et Sloane	Al Madinah - Makkah road, Saudi Arabia.
6.	*Caralluma retrospiciens* (Ehrenb.) N.E.Br.	Hail - Al Madinah road, Saudi Arabia.
7.	*Caralluma sinaica* (Decne.) A.Berger	Saint Catherine, Sinai, Egypt.
8.	*Ceropegia arabica* H.Huber.	Fayfa Mountain, Jizan, Saudi Arabia.
16.	*Huernia lodarensis* Lavranos.	Al Taif - Abha road, Saudi Arabia.
18.	*Leptadenia pyrotechnica* (Forssk.) Decne.	Aja Mountain, Hail, Saudi Arabia.
19.	*Leptadenia pyrotechnica* (Forssk.) Decne.	Cairo - Suez road, Egypt.

No.* = Numbers of taxa as listed in Figs. 1-6.

Cytological studies: Cytological preparations were carried out on root tips obtained from seeds germinated on sterile moist filter paper in Petri dishes at 25°C. Roots were pretreated with 0.05% colchicine solution for 2-3 hrs. and fixed in Carnoy for 24 hr. and then stored in 70 % ethanol at 4°C. Cytological preparations were made using the Feulgen squash method. The well-spread c-metaphase chromosomes were photographed from temporary preparations at magnifications of 2500 *x*.

A karyogram for each taxon was constructed by arranging the chromosomes in homologous pairs by order of their length and arm ratio as measured from the photographic prints. The number of chromosome types was determined as described by Levan *et al*. (1965). Measurements of chromosome length were taken on the same photographs of the karyogram.

The variation in chromosome length (TCL & MCL) and chromosome arm ratio (MAR) within the karyotype has been estimated by calculating the standard error (SE) of these parameters. Karyotype asymmetry deduced from the ratio between the short arms of the chromosomes and their total length was expressed as total form percent (TF%) as proposed by Huziwara (1962). Karyotype asymmetry expressed by the ratio between chromosome arms has been also estimated as the intrachromosomal asymmetry index (A_1) as suggested by Romero-Zarco (1986).

The value of A_1 is considered to be close to zero if all chromosomes are metacentric and near to one if all chromosomes are telocentric. Karyotype asymmetry due to the ratio between size of different chromosomes has been also estimated as the interchromosomal asymmetry index (A_2) using Pearson's dispersion coefficient, that is the ratio between the standard deviation and the mean chromosome length (Romero-Zarco, 1986).
The existence of previous chromosome counts for the studied taxa has been verified in the index of plant chromosome numbers by IPCN Chromosome Reports (http://www.tropicos.org/NameSearch.aspx).

Results

Cytological observations of tribe Asclepiadeae: The tribe Asclepiadeae is represented in this study by 20 accessions (11 taxa) (Table 2 and Figs. 1, 3, 4, 5 & 6). Chromosome counts and karyotype description of 13 accessions are reported here for the first time.

All accessions were found to have a basic chromosome number of *x* = 11, on the other hand, somatic chromosome numbers varied between 2*n* = 22 in 15 accessions (4, 9, 10, 11, 12, 13, 14, 15, 17, 20, 21, 22, 25, 26 & 27) to 2*n* = 44 in 5 accessions (1, 2, 3, 23 & 24). Among the 20 accessions studied of Asclepiadeae somatic chromosome number of 2*n* = 22 (a diploid of *x* = 11) has been found in 15 accessions and 2*n* = 44 (a tetraploid of *x* = 11) in the remaining accessions.

Total chromosome length (TCL) values varied between accessions studied (Table 2). The highest value (76.31 µm) is recorded in *Pergularia tomentosa* L. (23) (Aja Mountain, Hail, Saudi Arabia) with mean chromosome length (MCL) of 6.94±0.41 µm, while the lowest value (37.90 µm) was found in *Pentatropis nivalis* (21) (Al Madinah - Makkah road, Saudi Arabia) with mean chromosome length (MCL) of 3.45±0.19 µm.

The most variable chromosomes in length were found in *Calotropis procera* (Ait.) Ait. F. (2) (Hail - Al Jouf road, Saudi Arabia) (SE of MCL ±0.57 µm), whereas the most similar chromosomes were scored in *Glossonema boveanum* (Decne.) Decne. (11) (Makkah - Jiddah road, Saudi Arabia) (SE of MCL ±0.07 µm).

The highest MAR value (3.88±1.21) was recorded in *Cynanchum acutum* L. (10) (Orman Botanical Garden, Egypt), whereas the lowest (1.15±0.04) was found in *Kanahia lanifolia* (Forssk.) R. Br. (17) (Al Madinah - Makkah road, Saudi Arabia). Of the studied accessions, the highest TF% (46.04%) was recorded in *Kanahia lanifolia* (Forssk.) R. Br. (17) (Al Madinah - Makkah road, Saudi Arabia), whereas, the lowest TF% (28.97%) was recorded in *Cynanchum acutum* L. (10) (Orman Botanical Garden, Egypt).

Most of the accessions studied have karyotypes of metacentric to submetacentric chromosomes. The degree of karyotype asymmetry as indicated by TF% values are correlated with A_1 and A_2 values show the high degree of karyotype symmetry in the majority of the accessions (Table 2). Eleven of the studied accessions (1, 2, 3, 9, 12, 15, 17, 20, 24, 25, & 26) are characterized by the presence of A_1 values less than 0.39.

Cytological observations of tribe Ceropegieae: The chromosome number and karyotype description is shown for 7 accessions (6 taxa) of the tribe Ceropegieae (Table 2 and Figs. 2 & 4). Chromosome count of three accessions from this tribe are presented here for the first time, these accessions are, *Caralluma retrospiciens* (Ehrenb.) N. E. Br. (6), *Ceropegia arabica* H. Huber. (8), *Huernia lodarensis* Lavranos. (16) and *Leptadenia pyrotechnica* (Forssk.) Decne. (18). All accessions were found to have a basic chromosome number of *x* = 11, on the other hand, somatic chromosome numbers were varied between 2*n* = 22 in *Caralluma penicillata* (5) (Al Madinah - Makkah road, Saudi Arabia), *Caralluma sinaica* (7) (Saint Catherine, Sinai, Egypt), *Huernia lodarensis* (16) (Al Taif - Abha road, Saudi Arabia), *Leptadenia pyrotechnica* (18) (Aja Mountain, Hail, Saudi Arabia) and *Leptadenia pyrotechnica* (19) (Cairo - Suez road, Egypt) to 2*n* = 44 in *Caralluma retrospiciens* (6) (Hail - Al Madinah road, Saudi Arabia) and *Ceropegia arabica* (8) (Fayfa Mountain, Jizan, Saudi Arabia). Among the 7 studied accessions a somatic chromosome number of 2*n* = 22 (a diploid of *x* = 11) has been found in five accessions (5, 7, 16, 18 & 19) and 2*n* = 44 (a tetraploid of *x* = 11) in the rest accessions (6 & 8).

Total chromosome length (TCL) values varied between studied accessions (Table 2). The highest value (71.75 µm) was recorded in *Leptadenia pyrotechnica* (18) (Aja Mountain, Hail, Saudi Arabia) with mean chromosome length (MCL) of 6.52±0.61 µm, while the lowest value (37.14 µm) was found in *Ceropegia arabica* (8) (Fayfa Mountain, Jizan, Saudi Arabia) with mean chromosome length (MCL) of 3.38±0.23 µm.

Table 2. The cytological features of the studied taxa of the tribes Asclepiadeae and Ceropegieae.

No.	Tribe	Taxa	Subtribe	*x*	2*n*	TCL (µm)	MCL ± SE (µm)	Long. Chr. (µm)	Short Chr. (µm)	Diff. (µm)	MAR ± SE (*r*-value)	TF %
1.	Asclepiadeae	*Calotropis procera* (Ait.) Ait.F.	Asclepiadinae	11	44 *	73.16	6.65±0.54	9.19	4.00	5.19	1.49±0.16	40.54
2.	,,	*Calotropis procera* (Ait.) Ait.F.	,,	11	44 *	58.67	5.33±0.57	8.50	2.59	5.91	1.41±0.09	41.86
3.	,,	*Calotropis procera* (Ait.) Ait.F.	,,	11	44 *	69.31	6.31±0.53	9.25	3.81	5.44	1.49±0.06	40.35
4.	,,	*Calotropis procera* (Ait.) Ait.F.	,,	11	22	71.50	6.50±0.26	7.75	5.25	2.50	1.85±0.16	36.01
5.	Ceropegieae	*Caralluma penicillata* White et Sloane.	Stapeliinae	11	22	54.78	4.98±0.19	6.13	4.10	2.03	1.82±0.16	36.24
6.	,,	*Caralluma retrospiciens* (Ehrenb.) N.E.Br.	,,	11	44 *	60.63	5.51±0.54	8.19	3.06	5.13	1.66±0.10	37.57
7.	,,	*Caralluma sinaica* (Decne) A.Berger.	,,	11	22	53.75	4.89±0.53	8.15	2.00	6.15	1.65±0.12	37.95
8.	,,	*Ceropegia arabica* H.Huber.	,,	11	44 *	37.14	3.38±0.23	4.44	1.93	2.51	1.20±0.05	45.93
9.	Asclepiadeae	*Cynanchum acutum* L	Cynanchinae	11	22	48.85	4.44±0.29	6.00	2.80	3.20	1.64±0.07	41.04
10.	,,	*Cynanchum acutum* L.	,,	11	22	41.60	3.78±0.32	5.15	2.00	3.15	3.88±1.21	28.97
11.	,,	*Glossonema boveanum* (Decne.) Decne.	Glossonematinae	11	22	49.25	4.48±0.07	5.00	4.25	0.75	1.48±0.06	40.61
12.	,,	*Gomphocarpus fruticosus* (L.) Ait.	Asclepiadinae	11	22	58.50	5.32±0.27	6.50	3.00	3.50	1.76±0.19	37.61
13.	,,	*Gomphocarpus fruticosus* (L.) Ait.	,,	11	22	56.25	5.11±0.23	6.50	4.00	2.50	1.86±0.09	35.11
14.	,,	*Gomphocarpus sinaicus* Boiss.	,,	11	22 *	60.25	5.48±0.29	6.75	3.75	3.00	1.92±0.18	35.27
15.	,,	*Gomphocarpus sinaicus* Boiss.	,,	11	22 *	56.75	5.16±0.29	7.00	4.00	3.00	1.46±0.07	40.53
16.	Ceropegieae	*Huernia lodarensis* Lavranos.	Stapeliinae	11	22 *	41.80	3.80±0.27	5.00	2.25	2.75	1.51±0.11	40.67
17.	Asclepiadeae	*Kanahia lanifolia* (Forssk.) R.Br.	Asclepiadinae	11	22 *	42.90	3.90±0.21	5.00	3.00	2.00	1.15±0.04	46.04
18.	Ceropegieae	*Leptadenia pyrotechnica* (Forssk.) Decne.	Leptadeniinae	11	22	71.75	6.52±0.61	9.25	3.75	5.50	1.87±0.12	34.84
19.	,,	*Leptadenia pyrotechnica* (Forssk.) Decne.	,,	11	22	71.00	6.45±0.73	9.25	3.25	6.00	2.29±0.28	30.63
20.	Asclepiadeae	*Odontanthera radians* (Forssk.) D.V.Field.	Glossonematinae	11	22 *	49.50	4.50±0.24	6.00	3.50	2.50	1.50±0.11	40.91
21.	,,	*Pentatropis spiralis* (Forssk.) Decne.	Astephaninae	11	22 *	37.90	3.45±0.19	4.50	2.50	2.00	2.73±0.83	32.98
22.	,,	*Pergularia daemia* Forssk.	,,	11	22	51.85	4.71±0.33	6.75	3.00	3.75	1.87±0.19	36.64
23.	,,	*Pergularia tomentosa* L.	,,	11	44 *	76.31	6.94±0.41	9.25	4.50	4.75	1.99±0.10	33.74
24.	,,	*Pergularia tomentosa* L.	,,	11	44 *	51.63	4.69±0.54	7.25	2.25	5.00	1.85±0.21	35.35
25.	,,	*Solenostemma argel* (Del.) Hayne.	Glossonematinae	11	22 *	58.85	5.35±0.52	8.50	2.80	5.70	1.45±0.11	42.01
26.	,,	*Solenostemma argel* (Del.) Hayne.	,,	11	22 *	41.83	3.80±0.29	5.38	2.10	3.28	1.69±0.12	37.89
27.	,,	*Solenostemma argel* (Del.) Hayne.	,,	11	22 *	59.96	5.45±0.34	7.50	4.05	3.45	1.81±0.16	35.86

Table 2. (Cont'd.).

No.	Tribe	Taxa	Subtribe	A_1	A_2	SAT	Chr. Type					Previous chr. count
							M	m	sm	st	t	
1.	Asclepiadeae	*Calotropis procera* (Ait.) Ait.F.	Asclepiadinae	0.28	0.27	--	--	10	--	1	--	22
2.	,,	*Calotropis procera* (Ait.) Ait.F.	,,	0.26	0.35	--	--	9	2	--	--	22
3.	,,	*Calotropis procera* (Ait.) Ait.F.	,,	0.33	0.28	--	--	10	1	--	--	22
4.	,,	*Calotropis procera* (Ait.) Ait.F.	,,	0.41	0.13	--	--	5	6	--	--	22
5.	Ceropegieae	*Caralluma penicillata* White et Sloane.	Stapeliinae	0.42	0.12	--	--	4	6	1	--	22
6.	,,	*Caralluma retrospiciens* (Ehrenb.) N.E.Br.	,,	0.37	0.32	--	--	6	5	--	--	--
7.	,,	*Caralluma sinaica* (Decne) A.Berger.	,,	0.35	0.36	--	2	3	6	--	--	22
8.	,,	*Ceropegia arabica* H.Huber.	,,	0.15	0.23	--	--	11	--	--	--	--
9.	Asclepiadeae	*Cynanchum acutum* L	Cynanchinae	0.30	0.21	--	1	8	2	--	--	22
10.	,,	*Cynanchum acutum* L.	,,	0.54	0.28	--	1	2	5	1	2	22
11.	,,	*Glossonema boveanum* (Decne.) Decne.	Glossonematinae	0.45	0.05	--	--	10	1	--	--	22
12.	,,	*Gomphocarpus fruticosus* (L.) Ait.	Asclepiadinae	0.36	0.17	--	2	4	5	--	--	22
13.	,,	*Gomphocarpus fruticosus* (L.) Ait.	,,	0.45	0.15	--	3	8	--	--	--	22
14.	,,	*Gomphocarpus sinaicus* Boiss.	,,	0.44	0.19	--	--	3	7	1	--	--
15.	,,	*Gomphocarpus sinaicus* Boiss.	,,	0.30	0.19	--	1	9	1	--	--	--
16.	Ceropegieae	*Huernia lodarensis* Lavranos.	Stapeliinae	0.30	0.24	--	1	6	4	--	--	--
17.	Asclepiadeae	*Kanahia lanifolia* (Forssk.) R.Br.	Asclepiadinae	0.14	0.18	--	3	8	--	--	--	--
18.	Ceropegieae	*Leptadenia pyrotechnica* (Forssk.) Decne.	Leptadeniinae	0.44	0.31	--	--	5	6	--	--	22
19.	,,	*Leptadenia pyrotechnica* (Forssk.) Decne.	,,	0.51	0.38	--	--	3	7	1	--	22
20.	Asclepiadeae	*Odontanthera radians* (Forssk.) D.V.Field.	Glossonematinae	0.30	0.17	--	2	4	5	--	--	--
21.	,,	*Pentatropis spiralis* (Forssk.) Decne.	Astephaninae	0.50	0.18	--	--	4	6	--	1	--
22.	,,	*Pergularia daemia* Forssk.	,,	0.42	0.23	--	--	5	5	1	--	22
23.	,,	*Pergularia tomentosa* L.	,,	0.48	0.20	--	--	2	9	--	--	--
24.	,,	*Pergularia tomentosa* L.	,,	0.39	0.31	--	1	5	4	1	--	--
25.	,,	*Solenostemma argel* (Del.) Hayne.	Glossonematinae	0.28	0.32	--	1	7	3	--	--	--
26.	,,	*Solenostemma argel* (Del.) Hayne.	,,	0.37	0.25	--	1	6	4	--	--	--
27.	,,	*Solenostemma argel* (Del.) Hayne.	,,	0.40	0.21	--	--	5	6	--	--	--

MCL = Mean chromosome length, MAR = Mean arm ratio, SE = Standard error, TF % = Total form percent,
A_1 = Intrachromosomal asymmetry index, A_2 = Interchromosomal asymmetry index
SAT = A satellite, Chr. = Chromosome, M = Metacentric chromosome, m = Metacentric region chromosome, sm = Submetacentric chromosome, st = Subtelocentric chromosome
t = Acrocentric chromosome, * = New chromosome record, Long. = Longes, Short. = Shortest, Diff. = Difference

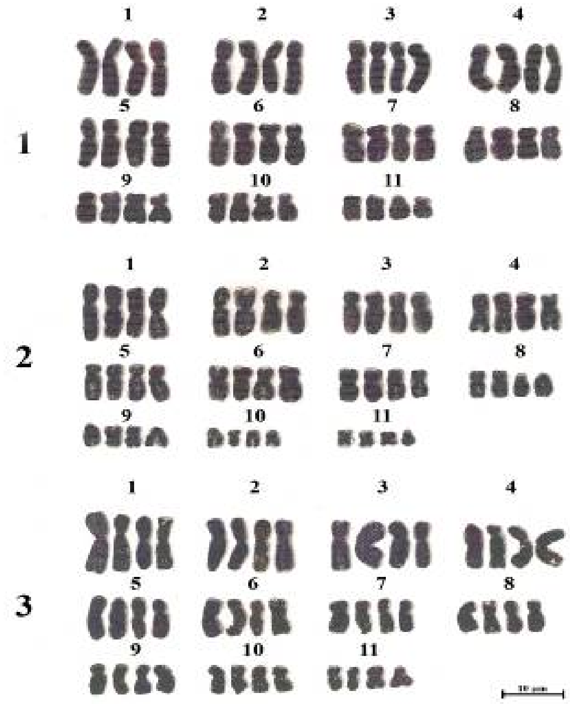

Fig. 1. Karyotype of tribe Asclepiadeae; (1) *Calotropis procera* (Ait.) Ait. F., (2) *Calotropis procera* (Ait.) Ait. F. and (3) *Calotropis procera* (Ait.) Ait. F.

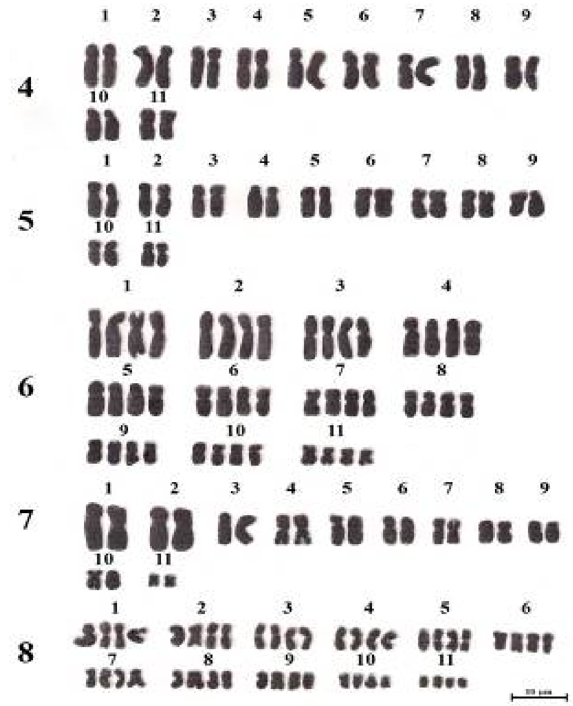

Fig. 2. Karyotype of tribes Ceropegieae and Asclepiadeae; (4) *Calotropis procera* (Ait.) Ait. F., (5) *Caralluma penicillata* White et Sloane., (6) *Caralluma retrospiciens* (Ehrenb.) N. E. Br., (7) *Caralluma sinaica* (Decne) A. Berger. and (8) *Ceropegia arabica* H. Huber.

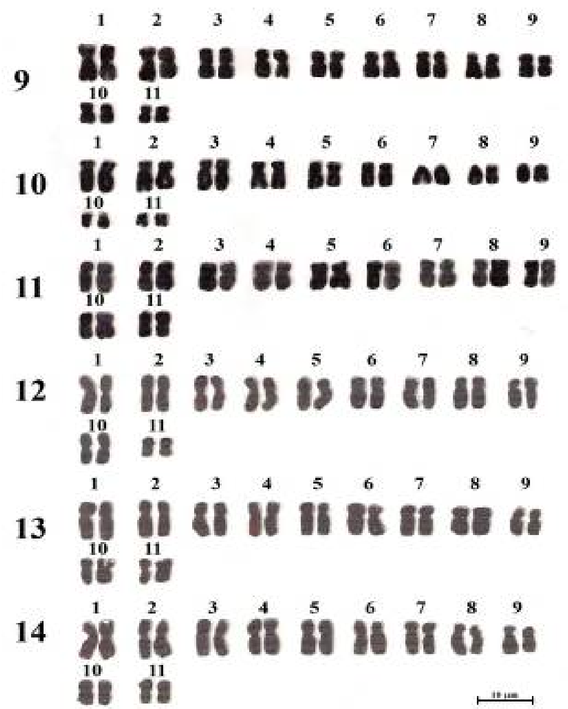

Fig. 3. Karyotype of tribe Asclepiadeae; (9) *Cynanchum acutum* L., (10) *Cynanchum acutum* L., (11) *Glossonema boveanum* (Decne.) Decne., (12) *Gomphocarpus fruticosus* (L.) Ait., (13) *Gomphocarpus fruticosus* (L.) Ait. and (14) *Gomphocarpus sinaicus* Boiss.

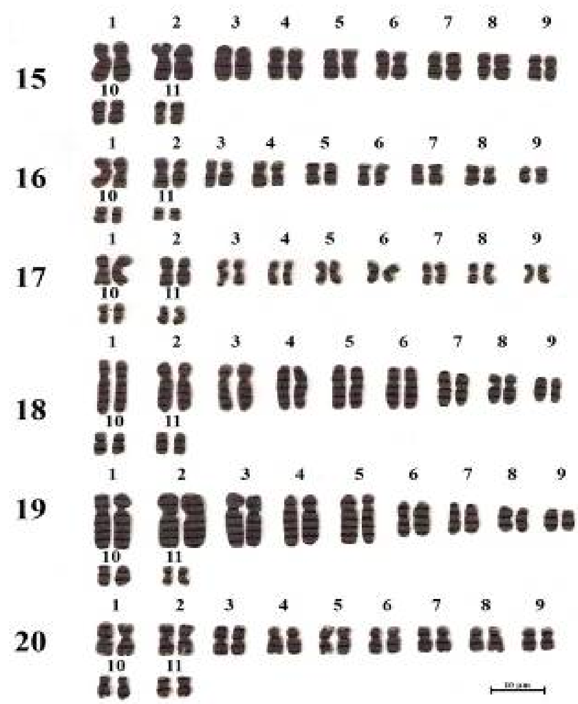

Fig. 4. Karyotype of tribes Asclepiadeae and Ceropegieae; (15) *Gomphocarpus sinaicus* Boiss., (16) *Huernia lodarensis* Lavranos., (17) *Kanahia lanifolia* (Forssk.) R. Br., (18) *Leptadenia pyrotechnica* (Forssk.) Decne., (19) *Leptadenia pyrotechnica* (Forssk.) Decne. and (20) *Odontanthera radians* (Forssk.) D. V. Field.

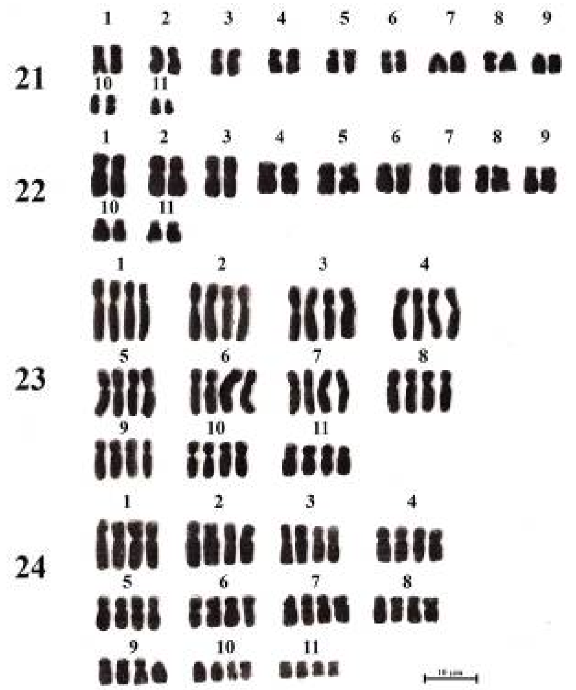

Fig. 5. Karyotype of tribe Asclepiadeae; (21) *Pentatropis nivalis* (22) *Pergularia daemia* Forssk., (23) *Pergularia tomentosa* L., and (24) *Pergularia tomentosa* L.

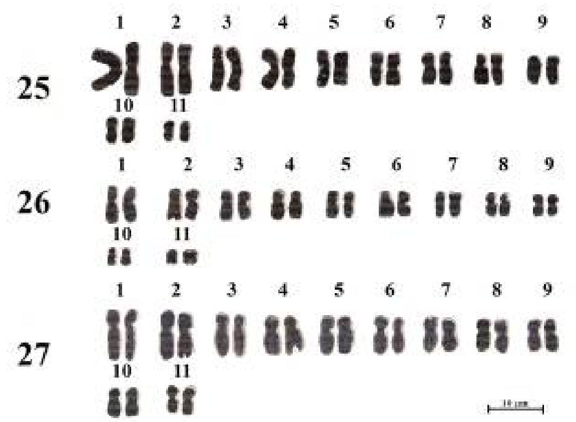

Fig. 6. Karyotype of tribe Asclepiadeae; (25) *Solenostemma argel* (Del.) Hayne., (26) *Solenostemma argel* (Del.) Hayne. and (27) *Solenostemma argel* (Del.) Hayne.

The most variable chromosomes in length were found in *Leptadenia pyrotechnica* (19) (Cairo - Suez road, Egypt) (SE of MCL ±0.73 µm), whereas the most similar chromosomes were scored in *Caralluma penicillata* (5) (Al Madinah - Makkah road, Saudi Arabia) (SE of MCL ±0.19 µm).

The highest MAR value (2.29±0.28) was recorded in *Leptadenia pyrotechnica* (19) (Cairo - Suez road, Egypt), whereas the lowest (1.20±0.05) was found in *Ceropegia arabica* (8) (Fayfa Mountain, Jizan, Saudi Arabia). The highest TF% (45.93%) was recorded in *Ceropegia arabica* (8) (Fayfa Mountain, Jizan, Saudi Arabia), whereas, the lowest TF% (30.63%) was recorded in *Leptadenia pyrotechnica* (19) (Cairo - Suez road, Egypt). Five of the studied accessions within the tribe were found to have TF% less than 38% indicating a low degree of karyotype symmetry. These five accessions (no, 5, 6, 7, 18 & 19) were found to have asymmetry karyotypes as indicated by the values of A_1 and A_2.

Discussion

Based on morphological characters, the two tribes, Asclepiadeae and Ceropegieae of subfamily Asclepiadoideae (Apocynaceae) are separated (Endlicher, 1938, Cronquist, 1968, Markgraf, 1972, Spellman, 1977, Takhtajan, 1980 and Rosatti, 1989). In addition, Sundell (1980), Kunz (1995), Swarupanandan *et al.* (1996) and Endress & 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 & 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 and Mahmood *et al.*, 2010).

There are few reports on the chromosome count, karyotyping or molecular studies of some members of Asclepiadeae especially the endemic taxa of Egypt or Saudi Arabia. Al-Turki *et al.,* (2000) carried out karyological studies of *Leptadenia pyrotechnica* (Forssk.) Decne. (Asclepiadaceae) from Saudi Arabia, Albers & Meve (2001) on the Asclepiadoideae (Periplocoideae and Secamonoideae) and De Loewnstern & Garbari (2004) on the Stapelieae (Asclepiadaceae) of Jordan.

On the other hand, the molecular and phylogenetic studies included, Meve *et al.,* (2001) on the *Ceropegia aristolochioides* complex (Apocynaceae-Ceropegieae), Liede & Kunze (2002) on *Cynanchum* and the Cynanchinae (Apocynaceae-Asclepiadoideae), Nair & Keshavachandran (2006) on *Gymnema sylvestre* R.Br., Jürgens *et al.,* (2008) on the Asclepiadoideae-Asclepiadeae, Mahmood *et al.,* (2010) on *Caralluma* species, Vajha *et al.,* (2011) on *Caralluma* spp. to understand species homology, Viviana *et al*., (2011) on *Diplolepis*, and Liede *et al.,* (2013) on the *Sarcostemma* group of *Cynanchum*.

In addition, taxonomical and floristic studies which had carried on the member of the Asclepiadoideae included for example, Sennblad & Bremer (2002) on the classification of Apocynaceae, Malpure *et al.* (2006) on *Ceropegia* L. (Asclepiadaceae) from the Western Ghats of India, Bensusan (2009) on the taxonomy and conservation status of Moroccan stapeliads (Apocynaceae-Asclepiadoideae-Ceropegieae-Stapeliinae), Bruyns (2011) on *Sarcostemma*, Ramachandran *et al.,* (2011a) on *Caralluma bicolor*, Ramachandran *et al.,* (2011b) on *Caralluma diffusa* (Wight) N.E. Br. (Asclepiadaceae) and Kullayiswamy *et al.,* (2012) on *Ceropegia pullaiahii*.

The karyotype features of species help us as a taxonomic value in providing a logical basis for the redistribution of genera in tribes. In karyotype, symmetrical karyotypes are more primitive than asymmetrical ones, longer chromosomes are more primitive than shorter ones, metacentric chromosomes are more primitive than sub-metacentric to acrocentric chromosomes, low basic numbers are primitive than high ones (Sharma, 1990). In several instances, studies of karyotype morphology have led the way to a new and fuller understanding of the systematic relationships within a major group of plants and to a complete reorganization of the taxonomic system of the group (Stebbins, 1956).

The karyological features observed of the studied taxa of tribes Asclepiadeae and Ceropegieae showed some relationships within these members, all members of both tribes are characterized by the same basic number (x = 11). Chromosomes base-numbers are usually considered as having a great evolutionary significance and taxonomic value. In the Poaceae, for example, the subfamilies are characterized by different base-numbers: Bambuusoideae have mostly x = 12, Arundinoideae mostly x = 9 or 12, Chloridoideae mostly x = 9 or 10, Panicoideae mostly x = 5, 9 or 10 and Pooideae mostly x = 7. However, within Pooideae some tribes, e. g., Glycerieae with x = 9 or 10, or genera, e. g., *Anthoxanthum* (tribe Phalarideae) with x = 5, deviate, the base-numbers in such cases providing important diagnostic characters (Stebbins, 1971).

Polyploidy numbers of chromosomes were recorded within the studied taxa of both tribes as a tetraploid of 2n = 44 (five accessions in tribe Asclepiadeae, 25% and two in Ceropegieae, 28.6%). In tribe Asclepiadeae, the three accessions of *Calotropis orocera* (1, 2 & 3) and the two accessions of *Pergularia tomentosa* (23 & 24) are characterized by polyploidy numbers of chromosomes as a tetraploid of 2n = 44. While, in tribe Ceropegieae polyploidy numbers observed in the two accessions, *Caralluma retrospiciens* (Ehrenb.) N.E.Br. (6) and *Ceropegia arabica* H. Huber. (8).

Chromosome size may vary as much as 20-fold between genera of the same family having the same or similar basic chromosome number. Plant chromosomes can be categorized into two types based on size, the large type (8-10 µm or less) and the small type (2-3 µm or less). All the studied taxa of both tribes have chromosomes larger than 3 µm specially the members of tribe Asclepiadeae.

In addition, the karyological features observed of the studied taxa of tribes Asclepiadeae and Ceropegieae showed that, mean chromosome length (MCL) of tribe Asclepiadeae was 6.94±0.41 µm and the same value of tribe Ceropegieae was 6.52±0.61 µm, from these results it is clear that both tribes have more or less similar lengths of chromosomes.

In addition, the chromosomal complements or karyotypes of most species of plants consist of chromosomes, which are comparable to each other in size. There are, however, many complements, which contain chromosomes of two contrasting sizes, large and small. In the present study, the longest pair of chromosomes was 9.25 µm and recorded in four accessions: *Calotropis procera* (Ait.) Ait. F. (3) and *Pergularia tomentosa* L. (23) from the tribe Asclepiadeae and the two accessions of *Leptadenia pyrotechnica* (Forssk.) Decne. (18 & 19) from the tribe Ceropegieae. On the other hand, the smallest pair of chromosomes was 1.93 µm and recorded in *Ceropegia arabica* H. Huber. (8) from the tribe Ceropegieae.

The tribe Asclepiadeae is characterized by long chromosomes, most of the studied taxa were found to have long chromosomes as indicated by mean chromosome length (MCL) values, 14 accessions were found to have MCL above 4.50 µm. *Cynanchum acutum* L. (10) (Orman Botanical Garden, Egypt) and ***P. nivalis*** (21) (Al Madinah - Makkah road, Saudi Arabia) are characterized from the remaining accessions by short chromosomes with mean chromosome length (MCL) of 3.78±0.32 & 3.45±0.19 µm and the presence of two & one pair of t type chromosome (Acrocentric), respectively. These findings are also confirmed by the highest values of MAR (3.88 & 2.73, respectively).

On the other hand, within the seven accessions of tribe Ceropegieae, the two accessions of *Leptadenia pyrotechnica* are characterized by having the longest chromosomes, 6.52±0.61 and 6.45±0.73 µm, this is congruent with their delimitation in subtribe Leptadeniinae by Endlicher (1938) and Meve & Liede (2002).

Variations in mean chromosome length (MCL) between the three species of *Caralluma* (with longer chromosomes having MCL values from 4.89 to 5.51 µm), *Ceropegia arabica* (with shorter chromosomes having MCL value 3.38 µm) and *Huernia* (with shorter chromosomes having MCL value 3.80 µm) do not supports the grouping of *Huernia* in subtribe Stapeliinae section *Caralluma* as previously proposed by Audissou (2005) and Fennane ***et al.*** (2007).

The most important morphological variable in chromosomes is the variation in size between the chromosomes of one genome. This aspect is more significant than overall measurements because artificial variation in size is likely to apply equally to all the chromosomes in a cell. The chromosomes in a genome can vary from being virtually all identical in size to exhibit a size difference ratio of 5 more (Stace, 2000).

In this work, the most unequal chromosomes were found in the tribe Ceropegieae, the highest difference between the chromosome sizes was 6.15 µm in *Caralluma sinaica* (Decne) A. Berger (7) and the the lowest difference between the chromosome sizes was 2.51 µm in *Ceropegia arabica* H. Huber (8). In the tribe Asclepiadeae, the highest difference between the chromosome sizes was 5.91 µm in *Calotropis procera* (Ait.) Ait. F. (2) and the the lowest difference between the chromosome sizes was 0.75 µm in *Glossonema boveanum* (Decne.) Decne. (11).

According to Sharma (1990), the members of tribe Ceropegieae are characterized by unequal chromosomes or asymmetric karyotypes than members of the other tribe Asclepiadeae which are characterized by equal chromosomes or symmetric karyotypes. These results are in agreement with the separation of both tribes within subfamily Asclepiadoideae (Apocynaceae) in the previous taxonomic classification by Endlicher (1938), Cronquist (1968), Markgraf (1972), Spellman (1977), Takhtajan (1980), Rosatti (1989) based on the morphological characters.

The mean arm ratio varied within the studied tribes, the highest MAR value recorded in tribe Asclepiadeae was (3.88±1.21), while in tribe Ceropegieae it was (2.29±0.28). On the other hand, the lowest MAR observed was (1.15±0.04) in tribe Asclepiadeae and (1.20±0.05) in tribe Ceropegieae.

The range of mean arm ratio observed within the studied taxa in tribe Asclepiadeae was (1.15 - 3.88), while in tribe Ceropegieae it was (1.20 - 2.29). Based on this finding, most karyotypes of the studied taxa of the tribe Asclepiadeae have metacentric (m), submetacentric (sm) and subtelocentric (st) types of chromosomes, while the studied taxa of the tribe Ceropegieae have metacentric (m) and submetacentric (sm) types of chromosomes.

In addition, five of the studied taxa of tribe Asclepiadeae are characterized by the presence of one pair of st type of chromosome (subtelocentric) in their karyotype formula: *Calotropis procera* (Ait.) Ait. F. (1) (from Hail - Al Madinah road, Saudi Arabia.), *Cynanchum acutum* L. (10) (from Orman Botanical Garden, Egypt.), *Gomphocarpus sinaicus* Boiss. (14) (from Hema Faid region, Hail, Saudi Arabia.), *Pergularia daemia* Forssk. (22) (from Al Madinah - Makkah road, Saudi Arabia.) and *Pergularia tomentosa* L. (24) (from Saint Catherine, Sinai, Egypt.).

The range of total form (TF%) observed within the studied taxa in tribe Asclepiadeae was (28.97 - 46.04%), while in tribe Ceropegieae it was (30.63 - 45.93%). It is clear from these results that member of both tribes have a low degree of karyotype symmetry.

The *Ceropegia arabica* H. Huber. of tribe Ceropegieae is characterized from the other species of the same tribe in having short chromosomes with one type (m) and the highest symmetric karyotype (TF = 45.93%, A_1 = 0.15 and A_2 = 0.23) in this study. The phylogenetic studies carried by Fishbein (2001), Bensusan (2009) and Bartosz *et al*., (2010**)** confirmed the separation of *Ceropegia arabica* of tribe Ceropegieae from the other species of the same tribe, the present findings support this separation.

In the present work, new chromosome counts were scored for the first time for 16 accessions of tribes Asclepiadeae and Ceropegieae and karyotype analysis was made on those accessions. In conclusion, the karyotype features were useful in establishing differences between taxa and both tribes of subfamily Asclepiadoideae. Most of data observed from karyotypes analysis confirm the systematic relationships within tribes of subfamily Asclepiadoideae.

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