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## Chromosome counts and karyotype analysis of species of family Apocynaceae from Egypt

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**Abstract.** The chromosome counts of 13 species of family Apocynaceae in the flora of Egypt have been reported; one species from subfamily Periplocoideae and the other 12 species from subfamily Asclepiadoideae. The chromosome numbers are  $2n = 22$  for *Periploca angustifolia*, *Glossonema boveanum*, *Pentatropis nivalis*, *Cynanchum acutum*, *Calotropis procera*, *Gomphocarpus sinaicus*, *Pergularia daemia* and *Pergularia tomentosa*;  $2n = 24$  for *Leptadenia arborea* and *Solenostemma argel*;  $2n = 22, 44$  for *Caudanthera edulis*, *Caudanthera sinaica* and *Desmidorchis acutangulus*. The chromosome numbers and karyotype analyses were firstly reported in *Leptadenia arborea* ( $2n = 24$ ). The polyploid nature was demonstrated by the prevalence of cells with  $2n = 4x = 44$  chromosomes in *Caudanthera edulis*, *Caudanthera sinaica* and *Desmidorchis acutangulus*. The chromosomes are median and submedian as most species in the Apocynaceae. The intrachromosomal asymmetry and interchromosomal asymmetry were estimated with  $M_{CA}$  and  $CV_{CL}$  values. In intrachromosomal asymmetry, *Desmidorchis acutangulus* is the most symmetrical karyotype, while *Pergularia tomentosa* is the most asymmetrical karyotype. In interchromosomal asymmetry, *Glossonema boveanum* is the most symmetrical karyotype, while *Cynanchum acutum* is the most asymmetrical karyotype.

**Keywords.** Apocynaceae, chromosome number, Egyptian flora, karyotype asymmetry.

### INTRODUCTION

The family Apocynaceae comprises 366 genera and *ca.* 5100 species (Meve, 2002; Endress et al., 2014). This family is currently divided into five subfamilies; Periplocoideae, Asclepiadoideae, Apocynoideae, Rauvolfioideae, Secamonoideae (Endress and Bruyns, 2000; Endress et al., 2014). The majority of species represented in the Egyptian flora are classified in the two subfamilies Periplocoideae and Asclepiadoideae. The subfamily Periplocoideae is a small group of species comprising only *ca.* 195 species in 33 genera (Heneidak and Naidoo, 2015). On the other hand, Asclepiadoideae is the largest

subfamily of the Apocynaceae and contains about 3000 species in 164 genera of five tribes. The tribes are divided into 15 subtribes (Meve, 2002; Endress et al., 2014).

Chromosome data have been constantly used for systematic purposes but chromosome number alone is not sufficient to exactly trace the evolutionary history of taxonomic groups. However, comparative karyotype analysis of related species has traditionally been used to describe patterns and directions of chromosomal evolution within plant groups and to infer the evolutionary role of chromosomal changes in plant evolution (Stebbins, 1971; Badr et al, 1997; 2009; Eroğlu et al., 2013; Kamel et al. 2014). More detailed information about the karyotype has been found necessary in order to provide diagnostics criteria for the systematics and phylogeny of plants (Altay et al., 2017). In fact, karyological features are evaluated as important taxonomic characters only when provide additional information and allow conclusions about evolutionary events in the group of interest (Badr and Elkington, 1977; Peruzzi and Eroğlu, 2013).

Survey of chromosome counts in the Apocynaceae in chromosome count reports, particularly the *Index to Plant Chromosome Numbers of the Missouri Botanical Garden* (<http://www.tropicos.org/Project/IPCN>) and the Chromosome Counts Database (CCDB) (<http://ccdb.tau.ac.il>) which is a community resource of plant chromosome numbers (New Phytol. 206(1): 19-26) as well as the old counts reported in Federov (1969) as well as the chromosome count reports that was frequently published in the Journal Taxon indicated that several authors have reported chromosome numbers of many species of the Apocynaceae. Several authors have reported chromosome numbers of many species of the Apocynaceae (Francini, 1927; Mitra and Datta, 1967; Federov, 1969; Arrigoni and Mori, 1976; Albers and Delfs, 1983; Albers and Austmann, 1987; Khatoon and Ali 1993; Liede 1996; Albers et al., 1993; Albers and Meve, 2001; Kamel et al., 2014). These studies showed that the family is karyologically almost entirely homogenous, especially subfamilies Asclepiadoideae and Periplocoideae, with nearly 96% of the taxa investigated so far having chromosome complements in multiples of a basic number of  $x = 11$ , with a few deviating numbers.

Deviating chromosome numbers were reported with  $2n = 18, 24$  in *Funastrum clausum* (Jacq.) Schulr. and *Funastrum cynanchoides* (Decne.) Schulr. (tribe Asclepiadeae) (Albers et al., 1993),  $2n = 20$  in *Microloma incanum* Decne. *Microloma calycinum* E. Mey., *Microloma sagittatum* (L.) R. Br. and *Microloma tenuifolium* (L.) K. Schum. (tribe Asclepiadeae) (Albers et al., 1993) and  $2n = 24$  in *Periploca graeca* L. (subfamily Periplocoideae) (Pesci, 1971). In literature,  $x = 9$  was only reported

in *Cynanchum acutum* L. and *Pergularia tomentosa* L. (Federov, 1969). The deviating chromosome numbers, i.e.  $2n = 24$  and  $x = 9$  that were found previously and in the present work were reported a deviating base chromosome numbers in the genera *Cynanchum*, *Microloma*, and *Sarcostemma* (Albers et al. 1993). These authors gave an account of previously published deviating chromosome numbers in the Asclepiadaceae.

In subfamily Asclepiadoideae, the polyploidy rate is approximately 6%. The polyploid species are mostly tetraploid (85%) with  $2n = 44$  and only a few are hexaploid with  $2n = 66$  (Albers and Meve, 2001). Albers (1983) reported the polyploid taxa in most of the genera of tribe Ceropegieae. Albers and Meve (1991) observed that the proportion of polyploid cells in the meristems of adventives roots is significantly higher than in the meristems of primary and secondary roots in genera *Duvalia* Haw., *Hoodia* Sweet ex Decne., *Orbea* Haw., *Pectinaria* Haw., *Stapelia*, *Trichocaulon* N.E.Br. and *Tridentea* Haw. High ploidy levels were recorded in *Tylophora anomala* N. E. Br.; for example the decatetraploid ( $2n = 132-154$ ) and the hexaploid ( $2n = 66$ ) (Meve, 1999).

In Apocynaceae, the counting and measuring of small size of the chromosomes is difficult. The chromosomes form a graded series with only very slight differences in morphology (Albers, 1983). Within a single karyotype the chromosomes are comparatively similar in size. The heterogeneous karyotypes were only found where chromosome sizes varied considerably in the subfamilies Periplocoideae, Asclepiadoideae and Secamoideae (Albers and Meve, 2001).

In the present study, 13 species of the family Apocynaceae were investigated karyologically to determine the chromosome numbers and to compare with earlier results. In addition, the karyotype of the examined species growing in Egypt has been analysed using a number of chromosome characterizing parameters such as variations in length, arm ration and centromeric asymmetry indices in order to gather more information that might help a better understanding of the taxonomic treatment of the species of Apocynaceae in the Egyptian flora.

## MATERIALS AND METHODS

### *Plant materials*

Seeds of 13 species of Apocynaceae were collected from mature flowers from sites in their natural habitats as given in Table 1 and mapped as in Figure 1. Voucher specimens of the examined species are kept at Suez University Herbarium. In the two succulent species (*Caudanthera edulis* and *Desmidorchis acutangulus*),

**Table 1.** List of species examined and the localities from which plants used for chromosome counts were collected and date of collection.

Taxa	Date	Locality
1. <i>Periploca angustifolia</i> Labillardiere	12.06.2009	El-Salûm: Wadi Salufa, 31°37'24"N–25°09'00" E, Morsy et al. s.n.
2. <i>Caudanthera edulis</i> (Edgew) Meve & Liede	27.01.2009	Gebel Elba: Wadi Yahameib, 22°25'18"N–36°18'33"E, Morsy et al. s.n.
3. <i>Caudanthera sinaica</i> (Decne.) Plowes	10.05.2009	North Sinai: Gidda Pass, 30°13'06"N–33°03'04"E, Heneidak s.n.
4. <i>Desmidorchis acutangulus</i> Decne.	23.08.2009	Gebel Elba: Wadi Aideib, 22°15'00"N–36°26'12"E, Morsy et al. s.n.
5. <i>Leptadenia arborea</i> (ForssK.) Schweinf.	17.02.2009	Aswan: 24°05'00"N–32°54'18"E, Heneidak s.n.
6. <i>Glossonema boveanum</i> (Decne.) Decne.	09.04. 2009	Sharm El Sheikh: Nabq protectorate, South Sinai, 28°07'00"N–34°25'00"E, Heneidak s.n.
7. <i>Solenostemma arghel</i> (Delile) Hayne	25.11. 2009	Dahab: South Sinai, 28°29'05"N–34°31'18"E, Heneidak s.n.
8. <i>Pentatropis nivalis</i> (J. F. Gmel.) D. V. Field & J. R. I. Wood	30.05.2009	Gebel Elba: Abu Ramad, 22°20'00"N–36°34'00"E, Morsy et al. s.n.
9. <i>Cynanchum acutum</i> L.	07.10. 2009	Suez: Shalufa, 30°07'03"N–32°32'27"E, Heneidak s.n.
10. <i>Calotropis procera</i> (Willd.) R. Br.	06.10. 2009	Ismailia: 30°64'00"N–32°27'00"E, 06.10.2009, Heneidak s.n.
11. <i>Gomphocarpus sinaicus</i> Boiss.	15.04. 2009	Saint Catherine: Wadi El Arbeen, South Sinai, 28°32'12"N–33°95'00"E, Heneidak s.n.
12. <i>Pergularia daemia</i> (Forssk.) Chiov.	25.10. 2009	Gebel Elba: Wadi Acaw, 22°15'31"N–36°21'00"E, Morsy et al. s.n.
13. <i>Pergularia tomentosa</i> L.	13.12. 2009	Ismailia: Suez desert road, 29°38'33"N–32°16'32"E, Heneidak s.n.

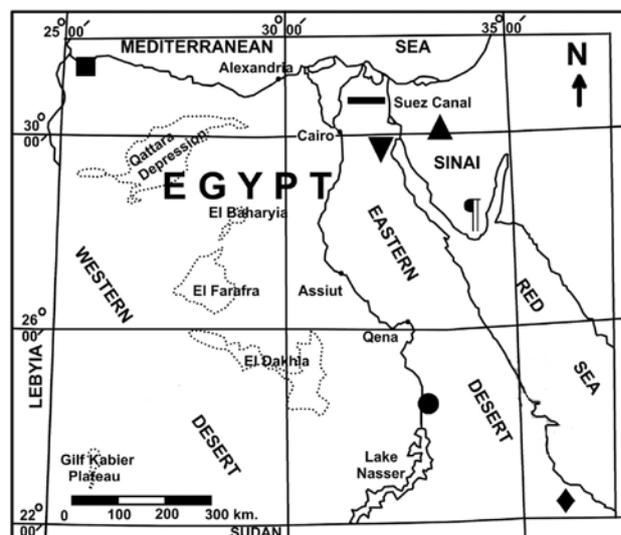
the root tips of adventitious roots were collected from plants, except *Caudanthera sinaica* from seedlings.

#### Cytogenetic procedure

For cytological preparations, seeds were germinated on moist Whatman paper and actively-growing root tips were pre-treated in saturated aqueous  $\alpha$ -bromonaphthalene at 4°C for 24 hours, or in a solution of 0.002 M 8-hydroxyquinoline at 18°C for 5-6 hours. They were fixed in absolute ethanol:acetic acid (3:1) for at least one hour, hydrolysed in 1N HCl at 60°C for 8 minutes and stained in Feulgen staining solution. The slides were mounted in Euparal for long-term storage (Martin et al., 2011). Photographs of chromosome spreads were taken using a Carl Zeiss Axiostar Plus microscope fitted with a Canon (Pc 1200 Power shoot A641) digital camera.

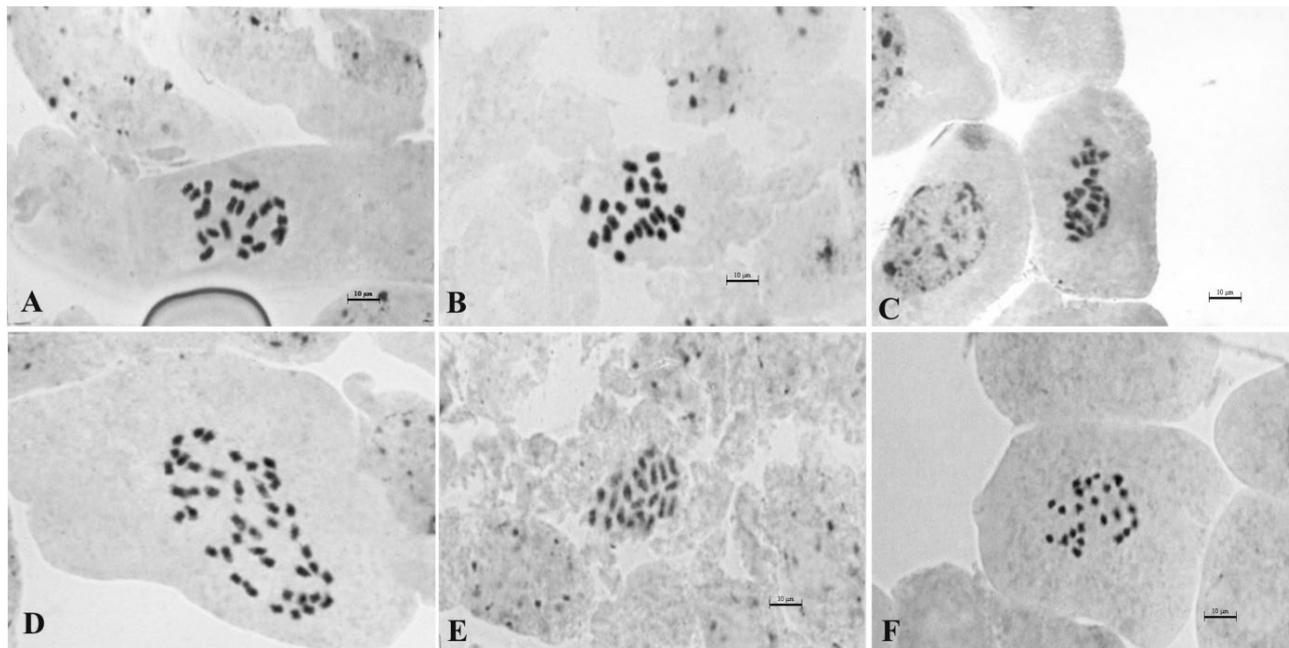
The number of somatic chromosomes was carefully counted in five slides for each species. Karyotype analyses were made by using Bs200Pro Image Analysis Software. Homologous pairs of somatic chromosomes were determined according to their total and relative lengths for each species.

The following parameters were used to characterize the chromosomes: long arm (LA), short arm (SA), total length (TL = LA + SA) and arm ratio (LA / SA). Total haploid lengths and mean haploid lengths were calculated. For the karyotype formula, chromosomes were classified using the nomenclature of Levan et al. (1964).



**Figure 1.** The distribution map of the studied species in Egypt. *Periploca angustifolia* (■); *Caudanthera edulis*, *Desmidorchis acutangulus*, *Pentatropis nivalis*, *Pergularia daemia* (◆); *Caudanthera sinaica* (▲); *Leptadenia arborea* (●); *Glossonema boveanum*, *Solenostemma arghel*, *Gomphocarpus sinaicus* (♣); *Cynanchum acutum* (▼); *Calotropis procera*, *Pergularia tomentosa* (■).

Several karyotype symmetry indices have been applied to express the asymmetry of the karyotype. Karyotype asymmetries were estimated by mean centromeric asymmetry ( $M_{CA}$ ) (Peruzzi and Eroğlu, 2013) and coefficient of variation of chromosome length ( $CV_{CL}$ ) (Paszko, 2006). The intrachromosomal asymmetry was



**Figure 2.** Photomicrographs of somatic metaphase chromosomes in root tip cells: *Periploca angustifolia* (A), *Caudanthera sinaica* (B), diploid *Caudanthera edulis* (C), tetraploid *Caudanthera edulis* (D), *Desmidorchis acutangulus* (E), *Leptadenia arborea* (F). Scale bar = 10 µm.

calculated with  $M_{CA} = [\text{mean } (L - S) / (L + S)] \times 100$ . The formula contains the length of long arm (L) and short arm (S) of each chromosome. The interchromosomal asymmetry was calculated with  $CV_{CL} = [\text{standard deviation} / \text{mean chromosome length}] \times 100$ . Finally, a scatter diagram between intrachromosomal asymmetry ( $M_{CA}$ ) and interchromosomal asymmetry ( $CV_{CL}$ ) was drawn.

## RESULTS AND DISCUSSION

The subfamily Periplocoideae is represented with one species in tribe Periploceae. The subfamily Asclepiadoideae is represented with 12 species in tribe Ceropogoneae and Asclepiadeae. The photographs illustrating the chromosomes of the studied species are shown in Figures 2 and 3. The ideograms are given in Figure 4.

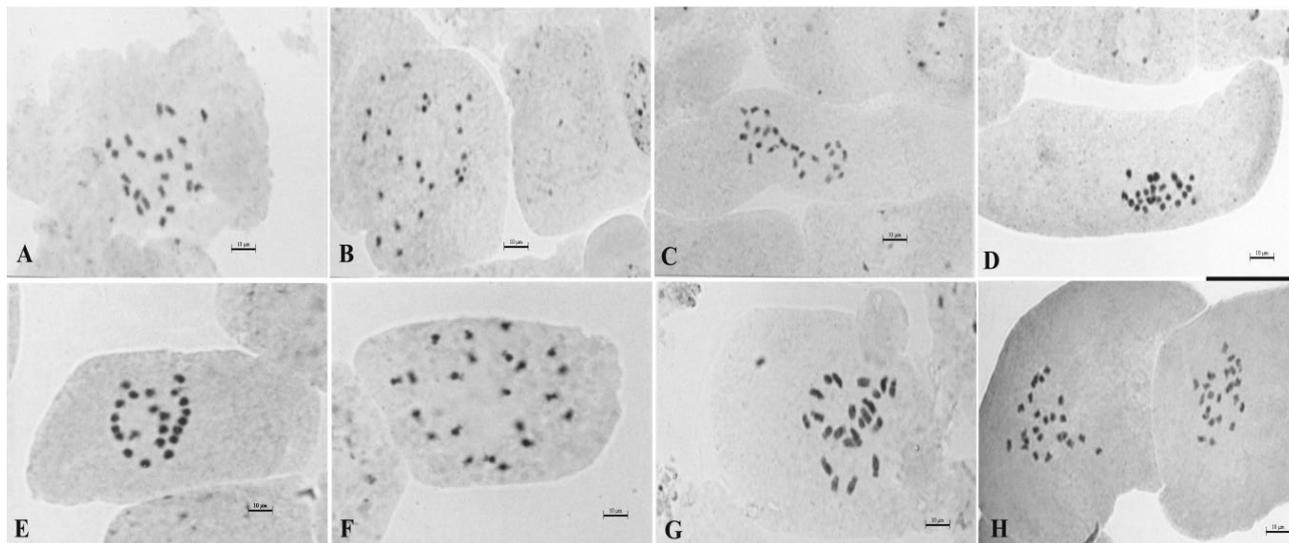
The gametic and somatic chromosome counts of the investigated species in present and previous studies are given in Table 2. Detailed chromosomal data are given in Table 3.

### Chromosome numbers

Table 2 summarizes the chromosome number and the previous counts for the studied species of Apocyn-

aceae. Eleven of the 13 species examined here have  $2n = 22$ , based on a basic number of  $x = 11$ . These results confirmed previous records for other species, and therefore, it is clear that the dominance of a basic number of  $x = 11$  and a majority of  $2n = 22$  is the base in the subfamilies Periplocoideae and Asclepiadoideae. It is the first time to count the chromosomes of *Leptadenia arborea* ( $2n = 24$ ); (Figure 2F).

Both diploid chromosome number ( $2n = 22$ ) and tetraploid chromosome number ( $2n = 44$ ) cells were scored in the three succulent species, which belong to tribe Ceropogoneae; i.e. *Caudanthera sinaica*, *C. edulis* (Figures 2C, 2D) and *Desmidorchis acutangulus*. Diploid number ( $2n = 22$ ) is reported also in *Caudanthera edulis* by Albers and Meve (2001), in *Caudanthera sinaica* by Albers and Meve (2001), Kamel et al. (2014), and in *Desmidorchis acutangulus* by Albers and Delfs (1983), Albers and Meve (2001). However, tetraploid number ( $2n = 44$ ) is recorded also in *Caudanthera edulis* by Albers and Austmann (1987), in *Desmidorchis acutangulus* by Kamel et al. (2014), while in *Caudanthera sinaica* recorded in the present study only). Polyploidy is known to occur in 11 genera of subfamily Asclepiadoideae with eight genera belonging to tribe Ceropogoneae (Albers and Meve, 2001). There are different patterns (mixoploidy) in terms of the number of chromosomes. This is probably the state of the endopolyploidy that is the result of enderoduplication. No odd-number polyploidy was



**Figure 3.** Photomicrographs of somatic metaphase chromosomes in root tip cells: *Pentatropis nivalis* (A), *Glossonema boveanum* (B), *Solenostemma arghel* (C), *Cynanchum acutum* (D), *Pergularia tomentosa* (E), *Pergularia daemia* (F), *Gomphocarpus sinaicus* (G), *Calotropis procera* (H). Scale bar = 10  $\mu$ m.

found. The diploid count supports the findings of Albers and Meve (2001); while the tetraploid count in this study supports the findings of Albers and Austmann (1987). Albers and Meve (1991) reported that the frequency of tetraploid cells in the adventitious roots is higher than in the primary and the secondary roots. This phenomenon may lead to a complete polyploidization of adventitious roots, and can be ascribed to ecological rather than morphological or genetic factors (Albers and Meve, 1991).

Diploid chromosome number ( $2n = 22$ ) is recorded in *Periploca angustifolia* in this study and by Arri-goni and Mori (1976). Deviations from this number are absent in this species as reported before in subfamily Periplocoideae by Albers and Meve (2001). In the current study, three species; *Glossonema boveanum*, *Pentatropis nivalis* and *Gomphocarpus sinaicus* was also found to have a diploid chromosome number of  $2n = 22$  as scored by Albers and Meve (2001), Kamel et al. (2014) and other four *Gomphocarpus* species examined by Albers and Meve (2001). The same for *Cynanchum acutum* was also found to have a diploid chromosome number of  $2x = 22$  as recorded by Kamel et al. (2014) and in other 25 *Cynanchum* species examined by Albers and Meve (2001). The old records of earlier numbers of  $n = 9$  and  $2n = 18$  in *Cynanchum acutum* quoted in Francini (1927) and Federov (1969) as well as the count of  $2n = 24$  in *Cynanchum virens* (Albers et al., 1993) may be regarded as deviating numbers as argued by Albers et al. (1993).

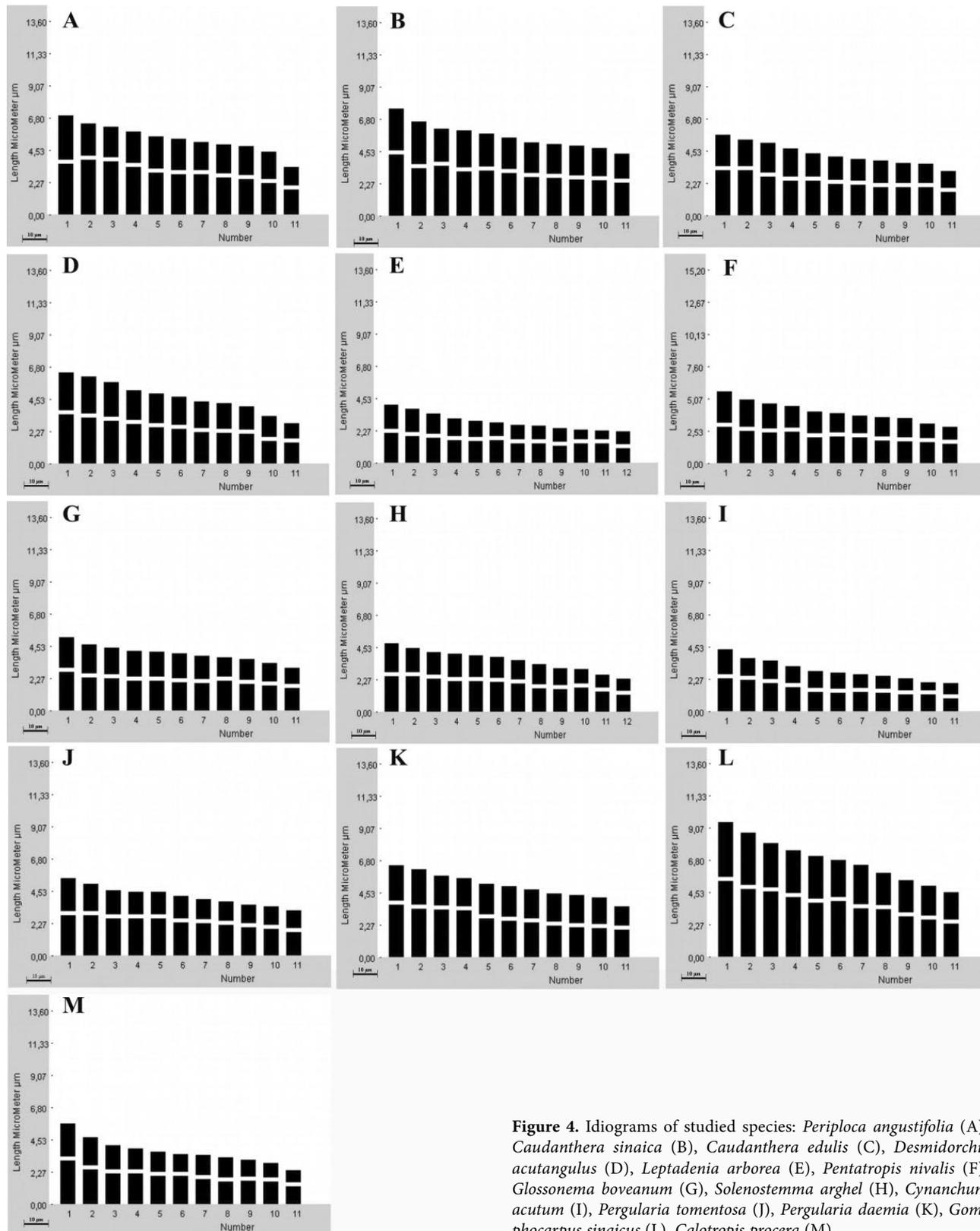
*Calotropis procera* was also found to have a diploid chromosome number of  $2n = 22$  as recorded by Fedorov

(1969), Albers and Meve (2001) and Kamel et al. (2014). The other number of  $2n = 26$  recorded for this species by Bramwell et al. (1972) may be regarded as deviating number as argued by Albers et al. (1993). The two *Pergularia* species were also found to have a diploid chromosome number of  $2n = 22$  as recorded by Albers and Meve (2001) and Kamel et al. (2014) in *Pergularia daemia* or by Albers and Meve (2001) in *Pergularia tomentosa*. The old records of earlier numbers of  $n = 9$  in *Pergularia tomentosa* quoted in Federov (1969) as well as the count of  $2n = 24$  in *Pergularia daemia* (Mitra and Datta, 1967) may be regarded as deviating numbers as argued by Albers et al. (1993).

Chromosome number of *Leptadenia arborea* was  $2n = 24$  in this report, while Albers and Meve (2001) found  $2n = 22$  in two *Leptadenia* species (*L. pyrotechnica* Decne. and *L. hastata* (Pers.) Decne.). This may be regarded as deviating number as argued by Albers et al. (1993). The same for *Solenostemma arghel* was also found to have a diploid chromosome number of  $2n = 24$  in this study, while Kamel et al. (2014) found  $2n = 22$  in this species.

#### Karyotype analyses

The chromosomes of the examined species are all small with slight morphological differences among the complements of the studied samples. When compared the chromosome morphology among the species, the smallest mean chromosome length (2.60  $\mu$ m)



**Figure 4.** Idiograms of studied species: *Periploca angustifolia* (A), *Caudanthera sinaica* (B), *Caudanthera edulis* (C), *Desmidorchis acutangulus* (D), *Leptadenia arborea* (E), *Pentatropis nivalis* (F), *Glossonema boveanum* (G), *Solenostemma arghel* (H), *Cynanchum acutum* (I), *Pergularia tomentosa* (J), *Pergularia daemia* (K), *Gomphocarpus sinaicus* (L), *Calotropis procera* (M).

**Table 2.** The gametic and somatic chromosome counts of the investigated species in present and previous studies.

Subfamily, Tribe, Subtribe	Species	Previous results		Reference	Present counts <i>2n</i>	Explanation
		<i>n</i>	<i>2n</i>			
Subfamily Periplocoideae						
Tribe Periploceae	<i>Periploca angustifolia</i>	—	22	Arrigoni and Mori (1976)	22	Detailed measurements
Subfamily Asclepiadoideae						
Tribe Ceropegieae						
Subtribe Stapeliinae	<i>Caudanthera edulis</i>	—	22	Albers and Meve (2001)	22	Detailed measurements
		—	44	Albers and Austmann (1987)	44	
	<i>Caudanthera sinaica</i>	—	22	Albers and Meve (2001), Kamel et al. (2014)	22 & 44	New count & detailed measurements
		—	22	Albers and Delfs (1983), Albers and Meve (2001)	22	
<i>Desmidorchis acutangulus</i>	—	22	Kamel et al. (2014)	44	Detailed measurements	
	—	44				
Subtribe Leptadeniinae	<i>Leptadenia arborea</i>	—	—	—	24	First report
Tribe Asclepiadeae						
Subtribe Asclepiadinae	<i>Calotropis procera</i>	11	—	Fedorov (1969)	22	Detailed measurements
		—	22	Albers and Meve (2001), Kamel et al. (2014)		
		—	26	Bramwell et al. (1972)		
		—	44	Kamel et al. (2014)		
	<i>Gomphocarpus sinaicus</i>	—	22	Kamel et al. (2014)	22	Detailed measurements
		—	22	Albers and Meve (2001), Kamel et al. (2014)	22	
	<i>Pergularia daemia</i>	—	24	Mitra and Datta (1967)	22	Detailed measurements
		9	—	Fedorov (1969)		
<i>Pergularia tomentosa</i>	—	22	Albers and Meve (2001)	22	Detailed measurements	
	—	44	Kamel et al. (2014)			
<i>Solenostemma arghel</i>	—	22	Kamel et al. (2014)	24	New count	
Subtribe Cynanchinae						
Subtribe Cynanchinae	<i>Cynanchum acutum</i>	9	—	Fedorov (1969)	22	Detailed measurements
		—	18	Francini (1927)		
		—	22	Kamel et al. (2014)		
<i>Glossonema boveanum</i>	—	22	Albers and Meve (2001), Kamel et al. (2014)	22	Detailed measurements	
Subtribe Tylophorinae	<i>Pentatropis nivalis</i>	—	22	Albers and Meve (2001), Kamel et al. (2014)	22	Detailed measurements

was observed in *Cynanchum acutum* of tribe Asclepiadeae. In contrast the largest mean length (6.51µm) was observed in *Gomphocarpus sinaicus* of tribe Asclepiadeae. Albers and Meve (2001) concluded that the average karyotype size diminished from rather large chromosomes in the Periplocoideae to the smallest karyotype length in the presumed most advanced tribe of the Asclepiadoideae, the Asclepiadeae.

The mean chromosomes length in *Leptadenia arborea* is 2.61µm, whereas Albers and Meve (2001) noticed an average length of 0.72 µm in two *Leptadenia* species (*L. pyrotechnica* and *L. hastate*). In this study, mean chromosome lengths in *Caudanthera sinaica*, *Desmidor-*

*chis acutangulus* and *Caudanthera edulis* were relatively larger (7.25, 6.14 and 5.38 µm, respectively). These three species also express evolutionarily basic morphological characters (Albers and Meve, 2001). Meve and Heneidak (2005) reported that the average mean chromosome length is (1.06-1.38 µm) in *Apteranthes europaea* of tribe Ceropegieae. The chromosomes of the three polyploid species studied here are usually smaller than those of diploid ones as reported before in polyploidy taxa by Albers and Meve (2001). A general tendency of size reduction can be seen starting with the presumably most primitive subfamily Periplocoideae to the more evolved Asclepiadoideae, and within the latter subfamily starting

**Table 3.** The measurement data of the studied Apocynaceae species.

Species	KF	SC ( $\mu\text{m}$ )	LC ( $\mu\text{m}$ )	RL (%) SC-LC	THL ( $\mu\text{m}$ )	MCL ( $\mu\text{m}$ )	CV <sub>CL</sub>	M <sub>CA</sub>
<i>Periploca angustifolia</i>	20m + 2sm	3.08	6.68	5.52-11.99	55.73	5.06	19.87	15.37
<i>Caudanthera edulis</i>	20m + 2sm	2.84	5.38	6.37-12.07	44.55	4.05	19.34	16.45
<i>Caudanthera sinaica</i>	22m	4.08	7.25	6.95-12.37	58.61	5.33	17.35	13.01
<i>Desmidorchis acutangulus</i>	22m	2.59	6.14	5.31-12.59	48.74	4.43	25.05	10.81
<i>Leptadenia arborea</i>	20m + 4sm	1.88	3.80	5.99-12.11	31.35	2.61	23.20	16.29
<i>Glossonema boveanum</i>	22m	2.70	4.88	6.59-11.92	40.97	3.72	16.63	11.20
<i>Solenostemma arghel</i>	24m	2.04	4.54	5.11-11.34	40.00	3.33	22.57	17.71
<i>Pentatropis nivalis</i>	22m	2.52	5.32	6.13-12.93	41.17	3.74	21.85	12.07
<i>Cynanchum acutum</i>	18m + 4sm	1.67	4.10	5.82-14.31	28.63	2.60	29.16	16.98
<i>Calotropis procera</i>	22m	2.08	5.37	5.53-14.26	37.67	3.42	26.27	15.19
<i>Gomphocarpus sinaicus</i>	22m	4.20	9.20	5.86-12.83	71.71	6.51	23.94	14.42
<i>Pergularia daemia</i>	22m	3.30	6.19	6.31-11.83	52.36	4.76	18.65	12.82
<i>Pergularia tomentosa</i>	22m	2.88	5.12	6.71-11.92	42.94	3.90	17.72	19.94

Abbreviations: karyotype formula (KF), shortest chromosome length (SC), longest chromosome length (LC), relative length (RL), total haploid chromosome length (THL), mean chromosome length (MCL).

with the most primitive Fockeeae to the most advanced Asclepiadeae, a decrease in chromosome size has taken place (Albers and Meve, 2001).

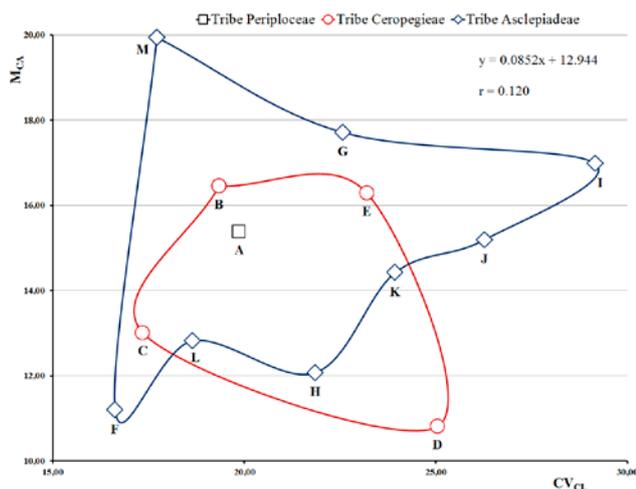
The chromosomes of most karyotypes are comparatively similar in size. Only rarely were heterogeneous karyotypes found where chromosome size varied considerably (Albers and Meve, 2001). The smallest arm ratio was observed in *Desmidorchis acutangulus* (1.06) and the highest one was observed in *Leptadenia arborea* (2.12). *Cynanchum acutum* has the smallest chromosome length as 1.67  $\mu\text{m}$  and the biggest chromosome length is measured in *Gomphocarpus sinaicus* as 9.20  $\mu\text{m}$ . Liede et al. (2002) also found that the chromosomes are generally short and varying in length, one pair of the large sized chromosomes in *Glossonema boveanum*. In Apocynaceae, chromosomes are typically submetacentric, rarely acrocentric with one pair of chromosomes possessing secondary constrictions with satellites (Albers, 1983; Albers and Meve, 2001). Albers and Meve (2001) found the smaller chromosomes in tribe Asclepiadeae, in particular the subtribes Asclepiadinae, Astephaninae and Metastelminae where mean length ranges from 0.70 to 1.15  $\mu\text{m}$ .

In tribe Ceropegieae, the M<sub>CA</sub> values indicated that *Desmidorchis acutangulus* is the most symmetrical karyotype, while *Caudanthera edulis* is the most asymmetrical karyotype. Whereas, the CV<sub>CL</sub> values indicated that the most homogeneous centromere position is observed in *Caudanthera sinaica*. On the other hand the most heterogeneous centromere position is observed in *Desmidorchis acutangulus*.

In tribe Asclepiadeae, the M<sub>CA</sub> values indicated that *Glossonema boveanum* is the most symmetrical karyotype, while *Pergularia tomentosa* is the most asymmetrical karyotype. The CV<sub>CL</sub> values indicated that the most homogeneous centromere position is observed in *Glossonema boveanum*. On the other hand the most heterogeneous centromere position is observed in *Cynanchum acutum*.

In all tribe, the symmetrical and asymmetrical karyotypes are quite different. In parallel, a weak positive correlation is determined between M<sub>CA</sub> and CV<sub>CL</sub> ( $r = 0.120$ ) (Figure 5). In Figure 5, three tribes of family Apocynaceae have different karyotypes in terms of asymmetry degrees: tribe Asclepiadeae with higher intrachromosomal asymmetry and interchromosomal asymmetry, tribe Ceropegieae with lower intrachromosomal asymmetry and interchromosomal asymmetry, one species of tribe Periploceae with relatively average intrachromosomal and interchromosomal asymmetry. On the other hand the results need to be supported by data from more species, because the species number investigated (per tribe) is much too low.

The possible origin of deviating chromosome numbers called numerical aneuploidy are defects in cell division as anaphase lagging, nondisjunction or presence of B-chromosomes. B-chromosomes, which are also known as supernumerary chromosomes, are a major source of intraspecific variation in nuclear DNA (Jones et al., 2008). The general consideration is that B-chromosomes are derived from the A-chromosomes. Probably, a B-chromosome may have originated from paracentro-



**Figure 5.** Scatter diagram between  $M_{CA}$  and  $CV_{CL}$ : *Periploca angustifolia* (A), *Caudanthera edulis* (B), *Caudanthera sinaica* (C), *Desmidorchis acutangulus* (D), *Leptadenia arborea* (E), *Glossonema boveanum* (F), *Solenostemma arghel* (G), *Pentatropis nivalis* (H), *Cynanchum acutum* (I), *Calotropis procera* (J), *Gomphocarpus sinaicus* (K), *Pergularia daemia* (L), *Pergularia tomentosa* (M).

meric region amplification of a fragmented A chromosome or from A chromosome fusions.

## CONCLUSION

With this study, new chromosome data were given for 13 taxa of family Apocynaceae. More karyological data are needed to understand the phylogeny of Apocynaceae. In conclusion, some intrageneric relationships within Apocynaceae will clarify with comparative chromosomal analysis. Also, additional comparative high-resolution molecular cytogenetic studies will be necessary to clarify phylogenetic relationships between genera or species.

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