

Karyotypic studies of the genus *Cuscuta* L. (Convolvulaceae) in Saudi Arabia and their taxonomic significance

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Sherif M. Sharawy, 2013. Karyotypic studies of the genus *Cuscuta* L. (Convolvulaceae) in Saudi Arabia and their taxonomic significance. *Taeckholmia* **33**: 65-82.

Chromosome numbers and karyotype criteria of nine species of the genus *Cuscuta* L. (Convolvulaceae) in Saudi Arabia were investigated. Chromosomes counts were recorded in four species for the first time in this study. Chromosome number, based on $x=7$ has been found in the majority of the studied *Cuscuta* species. A diploid number ($2n=14$) was recorded in five species, while tetraploid number ($2n=28$) was recorded in two species whereas $2n=56$ was recorded in *C. campestris*. However, $2n=30$ based on $x=6$ was recorded in *C. monogyna*. The chromosomes in the studied species of *Cuscuta* are generally small with a mean size ranging between 0.74 and 1.10 μm . Short chromosomes were particularly found in *C. planiflora* (MCL=0.74 μm) and *C. chinesis* (MCL=0.79), whereas longer chromosomes were scored in *C. campestris* (MCL=1.10). The karyotype in the studied species is mostly comprised of metacentric to submetacentric chromosomes as indicated by their mean arm ratio that ranges between 1.31 in *C. monogyna* and 1.83 in *C. planiflora*. The degree of karyotype asymmetry is indicated by high values of TF% ranges between 28.33 in *C. planiflora* and 40.54 in *C. campestris*. The A1 value ranged between 0.22 in *C. planiflora* and 0.43 in *C. palaestina*. Among the examined species, karyotype features were used to assess the

classification of the *Cuscuta* species in Saudi Arabia in the light of the current systems of classification.

Key words: Karyotype, *Cuscuta*, Chromosomes, Taxonomy, Saudi Arabia.

Introduction

The genus *Cuscuta* L. (dodders) is composed of approximately 200 species of tiny obligatory parasites that grow in a wide variety of climates and ecosystems (Yuncker, 1932; Stefanovic *et al.*, 2007; Welsh *et al.*, 2010 and Costea *et al.*, 2011). All members of this genus are vines with twining, slender, pale stems, with reduced, scale like leaves, and no roots. The parasitic stems are attached to the hosts by haustoria and depend entirely on their hosts to supply water and nutrients (Kujit, 1969; Dawson *et al.*, 1994). Most *Cuscuta* species are also characterized by reduced amounts or the complete absence of chlorophyll (van der Kooij *et al.*, 2000). *Cuscuta* is considered economically important because several species can cause significant losses to agricultural crops (Parker and Riches, 1993; Costea and Tardiff, 2006). However, many species are also ecologically important, acting as keystone species in their natural ecosystem (Press and Phoenix, 2005) and some dodders are in need of conservation (Costea and Stefanovic, 2009). *Cuscuta* is nearly cosmopolitan in distribution with its species found on every continent (except Antarctica), ranging from the 60th parallel north in Europe and Asia, to the Cape region of South Africa and as far as south as the 47th parallel in Argentina and Chile (Yuncker, 1932; Hunziker, 1950; Mabberley, 1987 and Stefanovic, *et al.*, 2007). In Saudi Arabia, the number of *Cuscuta* species increased with time since Migahid (1989) reported the presence of two species. Mandaville (1990) recorded three species while Collenette, (1999) confirmed the presence of nine species.

The genus was nested within Convolvulaceae under the subfamily Cuscutoidae (Engelmann, 1859; Bentham and Hooker, 1862; Bessey, 1915; Core, 1955; Engler, 1964; Hutchinson and Ashton, 1979). Other authors considered the species of the genus into a family of its own viz. Cuscutaceae depending on the mode of nutrition (Hutchinson, 1959; Cronquist, 1968; Hadac and Chrtek, 1970; Willis, 1973; Austin, 1975; Takhtajan, 1980). Engelmann (1859) divided the genus *Cuscuta* into three groups based primarily on stigma and style morphology. These groups were formally adopted by Peter (1897) and later by Yuncker (1932) as subgenera.

Subgenera *Monogyna* (*Monogynella*) has a single style with a variety of stigma shapes. Subgenera *Cuscuta* and *Grammica* are characterized by two distinct styles and can be distinguished by their stigma morphology (elongated and linear vs. short and capitate, respectively). Hadac and Chrtek (1970), on the bases of floral characters, accepted the Cuscutaceae but they added one more subgenus (*Kadurias*) to Engelmann's sub-genera. Recent molecular data (McNeal *et al.*, 2005; Stefanovic *et al.*, 2007 and Braukmann, *et al.*, 2013), also revealed a fourth major clade, consisting of *Cuscuta* species native to South Africa.

The importance of chromosomal information in plant systematics has attracted the attention of several authors. At the generic level and below chromosomes features provided a range of possibilities for understanding the affinities of taxa. The classification of a number of families has been either aided or substantiated by information from chromosome criteria or features as for example, chromosome size, the position of the centromere and special banding patterns (Badr and Elkington, 1978; Moore, 1978; Jackson, 1984; Badr and El-Kholy, 1986; Fernandez, *et al.*, 1993; El-Shazly and Abou El-Enain, 1999; Jianquan *et al.*, 2001; Badr and Sharawy, 2007; Sharawy, 2008). Karyotype studies were principally based on the bases that symmetrical karyotypes are more primitive than asymmetrical ones, longer chromosome than shorter ones, median centromeres with chromosome arms of equal length were more primitive than chromosome with arms of unequal length, low basic number has given rise to higher ones (Stebbins, 1956).

The aim of this study is to describe the karyotype criteria for nine species of *Cuscuta* in Saudi Arabia and discussing the impact of the variation in these criteria on the systematic treatments of the studied species.

Materials and Methods

The species used in this study are *Cuscuta brevistylosa*, *C. campestris*, *C. epilinum*, *C. chinesis*, *C. hyalina*, *C. monogyna*, *C. palaestina*, *C. pedicellata* and *C. planiflora*. These species represent each of the three subgenera in Engelman (1859) and Yuncker's (1932) taxonomic treatment of the genus *Cuscuta* (Table 1). Plant material was field collected from their natural habitats and also obtained from the living collection in different public botanical gardens in Saudi Arabia. Voucher specimens of field collected species are deposited in the herbaria of Biology Department, Faculty of Science, Hail University. The protocol used to obtain mitotic chromosomes from root tips of germinated seeds followed that of Cota and

Philbrick (1994). Permanent and semi permanent slides were made using Hoyer's fluid (Beek, 1955). The well-spread c-metaphase chromosomes were photographed from temporary preparation at magnifications of 2500 x (Fig. 1). Microscopic observations of chromosomes were made using Carl-Ziess photomicroscope III at x100.

Chromosome homology was assigned according to similarities in length, morphology and centromere position. Karyotype was constructed for each taxon by arranging the chromosomes in homologous pairs by order of their length and arm ratio as measured from the photographic prints. The chromosome asymmetry based on the position of the centromere was determined using the system developed by Levan *et al.* (1965). 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 was also deduced from the ratio between the short arms of the chromosomes and their total length as total form percent (TF %) as proposed by Huziwara (1962) as follows: [TF% = sum of short arm length / sum of total chromosome length \times 100]. In addition, karyotype asymmetry expressed by the ratio between chromosome arms has been estimated as the intrachromosomal asymmetry index (A_1) as suggested by Romero- Zarco (1986) as follows:

$$A_1 = 1 - \sum \frac{b_i}{B_i} / n_i$$

where (A_1) is the intrachromosomal asymmetry index and ranges from zero to one. The equation is formulated in order to obtain lower values when chromosomes tend to metacentric. (n_i) is the number of homologous chromosome pairs. (b_i) is the average length for short arms in every homologous chromosome pair and (B_i) is the average length for their long arms. Moreover, karyotype asymmetry due to ratio between the size of different chromosomes has been also estimated as the interchromosomal asymmetry index (A_2) using Pearson's dispersion coefficient (Romero-Zarco,1986).

$$A_2 = S / \bar{X}$$

where, A_2 is the interchromosomal asymmetry index, and (\bar{X}) is mean chromosome length (MCL) and (S) is its standard deviation.

To ascertain patterns of variation between indices and chromosomal variables, an analysis of variance was done using the mean value of five variables of the karyotypes of each taxon i.e. total chromosome length,

ploidy level, mean chromosome length, mean arm ratio, total form percent and intrachromosomal (A_1) index. The existence of previous chromosome counts for the studied taxa has been verified in the indexes of plant chromosome numbers by Index to Plant Chromosome Numbers (IPCN) (<http://www.tropicos.org/NameSearch.aspx>).

Results

The cytological data for the examined species is summarized in table 1 and their karyotypes are illustrated in Fig. 2. Chromosome number based on basic number of $x=7$ was found in the majority of the studied species, a diploid number of $2n=14$ is recorded in five species, whereas a tetraploid number ($2n=28$) is recorded in two species *C. chinensis* and *C. hyalina*. In addition, an octaploid number of $2n=56$ was recorded in *C. campestris*. Meanwhile, a number based on $x=6$ was scored *C. monogyna*, where a polyploidy number of $2n=30$ was scored. The chromosomes of the examined species of *Cuscuta* in Saudi Arabia are generally small (Table 1). Total chromosome length (TCL) varied between the studied species (Fig. 3). *Cuscuta planiflora* (TCL=4.98 μm) exhibit much shorter chromosomes compared to other species. Meanwhile, longest TCL (9.52 μm) have been found in *C. campestris*. In the remaining species, TCL ranged between 6.54 μm in the *C. chinensis* and 8.44 μm in the *C. epilinum* (Fig. 3). Similarly, shortest MCL was scored in *C. planiflora* ($0.74 \pm 0.009 \mu\text{m}$) and longest MCL in *C. campestris* ($1.10 \pm 0.18 \mu\text{m}$) (Fig. 4).

The karyotype in the studied species is mostly comprised of metacentric to submetacentric chromosomes as indicated by their mean arm ratio. This ratio ranged between 1.31 ± 0.19 in *C. monogyna* and 1.83 ± 0.22 in *C. epilinum* (Table 1). The low value of the standard error (SE) for mean arm ratio values indicated low degree of karyotype asymmetry in the studied species. The degree of karyotype asymmetry is also indicated by high values of TF% that ranged between 28.33% in *C. planiflora* and 29.74% in *C. monogyna* to 40.54% in *C. campestris*. Similarly, the A_1 value ranged between 0.22 in *C. planiflora* and 0.43 in *C. palaestina*. Also, A_2 ranged between 0.15 in *C. planiflora* and 0.2 in *C. palaestina* (Table 1). These values confirm the low karyotype asymmetry as indicated by the values of arm ratio and TF%.

The plotting of TCL against mean arm ratio (Fig. 5a) distinguish between most of diploid (*C. brevistylosa*, *C. epilinum*, *C. palaestina* and *C. pedicellate*), and the tetraploid species (*C. chinensis* and *C. hyalina*). In

particular, this figure clearly distinguished *C. monogyna* ($2n=30$, $x=6$) of subgenus Monogyna, and the diploid *C. planiflora* of subgenus Cuscuta as well as *C. campestris* ($2n=56$) of subgenus Grammica from the other species. The plotting of total chromosome length against the A1 values (Fig. 5b) also, clearly distinguished *C. planiflora*, *C. monogyna*, *C. palaestina* ($2n=14$, $x=7$) and *C. campestris*. The plotting diagram of total chromosome length against the total form percent (Fig. 5c) also, clearly distinguished *C. planiflora*, *C. chinesis* ($2n=28$, $x=7$) and *C. campestris* from the other species.

Discussion

The karyotype of five species in subgenus Cuscuta has been described in the present study viz: *C. brevistylosa*, *C. epilinum*, *C. palaestina*, *C. pedicelata* and *C. planiflora*. The karyotypes of three species viz: *C. campestris*, *C. chinesis* and *C. hyalina* of subgenus Grammica and of *C. monogyna* of subgenus Monogyna have been described in this study. The diploid number of $2n=14$ has been recorded only species belonging to subgenus Cuscuta. This agrees with the earlier records in *C. planiflora* by Pazy and Plitmann (1991). A tetraploid number of $2n=28$ was also recorded by Garcia and Castroviejo (2003) in this species. The diploid number $2n=14$ for *C. brevistylosa*, *C. epilinum*, *C. palaestina*, and *C. pedicelata* is recorded for first time in this study. While, the polyploid number ($2n=42$) of *C. epilinum* was recorded by Fogelberg (1938).

In subgenus Grammica, the studied species are uniform in chromosome number ($2n=28$), the only striking variation being found in *C. campestris*, which has $2n=56$ (Table 1). The tetraploid chromosome number ($2n=28$) in *C. hyalina* is recorded for first time in this study. The tetraploid ($2n=28$) and octaploid ($2n=56$) chromosome numbers were recorded in *C. chinesis* by Aryavand (1987), Sampathkumar (1979) and Mesicek and Sojak (1995) respectively. In addition, the chromosome number ($2n=56$) for *C. campestris* is similar to the previous counts reported by Aryavand (1987) and Garcia and Castroviejo (2003). Also, the tetraploid number of $2n=28$ was recorded by Ward (1984) and Khatoon and Ali (1993) for the same species from Mexico and Pakistan respectively. The karyological study of the three species of subgenus Grammica is in agreement with the previous classification of Engelmann (1859) and Yuncker (1932). They placed *C. chinesis* and *C. hyalina* ($2n=28$) in section *Eugrammica* and *C. campestris* in section *Cleistogrammica*. Also, the classification of the studied species of

subgenus *Grammica* is in agreement with previous results obtained from seed morphology by Abdel Khalik (2006), phylogeny by Costea *et al.* (2011), pollen and seed characters by Hamed (2005) and molecular characters by Stefanovic *et al.* (2007).

Striking variation being found in *C. monogyna* (Subgenera *Monogyna*), which has $2n=30$ based on $x=6$. This count is similar to the previous recorded by several authors: Pazy and Plitmann (1995, 2002) and Garcia and Castroviejo (2003). Yuncker (1932) split the subgenera *Monogyna* from the other two subgenera *Cuscuta* and *Grammica* on the basis of style characters. Also, the pollen and seed morphology confirms this division (Stefanovic *et al.*, 2002 and Welsh *et al.*, 2010); put the DNA sequence of multiple chloroplast loci does not support this separation (Stefanovic, *et al.* 2007).

Chromosome number and length variations have been shown in the genus *Cuscuta*, but closely related species have the same chromosome number and display variability in chromosome size (Fogelberg, 1938). These differences in chromosome size probably indicate genome restructuring and may be used for systematic purpose to distinguish closely related species (Johnson, 1980). In this study, the chromosome length in polyploid taxa was not found to increase or decrease significantly relative to the total chromosome length in diploids. However, the octaploid polyploid *C. campestris* ($2n=56$) clearly has the longest total chromosome length (9.52 μm) compared to diploid and other polyploid species of *Cuscuta* in this study. The MCL of the studied species of *Cuscuta* is small (Table 1), the shortest MCL was scored in *C. planiflora* (section *Cuscuta*) and the largest MCL in *C. campestris* (section *Grammica*). The karyotype in the studied species is comprised of metacentric to submetacentric chromosomes as indicated by their mean arm ratio. This ratio ranged between 1.58 ± 0.13 in *C. chinesis* to 1.83 ± 0.22 in *C. epilinum*. The degree of karyotype asymmetry in the studied species of *Cuscuta* is indicated by TF% that ranged between 32.33% in *C. planiflora* and 40.54% in *C. campestris* (Table 2).

The TF% in the studied species of subgenus *Cuscuta* ranged between 28.33 in *C. planiflora* to 38.92% in *C. pedicelata*. The delimitation of *C. pedicelata* from the other species of subgenus *Cuscuta* by the high value of TF% is in agreement with previous classification that placed *C. pedicelata* in section *Epistigma* and the remaining species in section *Cuscuta* based on variation in morphological traits, and seed morphology, as well as molecular

and micromorphological data (Yuncker, 1932; Sampathkumar and Ayyangar (1978); Stefanovic, *et al.*, 2002; Abdel Khalik, 2006; Costea, *et al.* 2006a, b, c, d, 2008 and Costea and Stefanovic, 2009b). Also, in subgenus *Grammica* the *C. campestris* (TF%=40.54%) was placed in section *Cleistogrammica* while the *C. chinensis* and *C. hyalina* (TF%=37.87 and 36.44) were placed in section *Eugrammica* based on exomorphological traits, seed morphology and phylogenetic (Yuncker, 1932; Abdel Khalik, 2006; Garcia and Martin, 2007; Stefanovic *et al.*, 2007).

The subgenus *Monogyna* was split from the subgenus *Cuscuta* on the basis of the single style (Yuncker, 1932). The chromosome number of *C. monogyna* ($2n=30$, $x=6$) was found to be also unique in the genus *Cuscuta* ($x=7$). In this study, most of the karyotype analysis confirmed this separation and are in congruent with the results of Stefanovic *et al.* (2002) and Abdel Khalik (2006) based on molecular and seed morphology characters.

Taxonomic significance of karyotype characters in *Cuscuta*

The division of the genus *Cuscuta* into the three subgenera *Cuscuta*, *Grammica* and *Monogyna* following Engelmann (1859) and Yuncker (1932). This arrangement has been largely confirmed by phylogenetic studies, the numerous sections and subsections created by Yuncker have been shown to be polyphyletic (Garcia and Martin 2007; Stefanovic *et al.*, 2007). At the species level, the systematic of *Cuscuta* is currently undergoing major taxonomic revisions through studies aimed at understanding the evolutionary relationships, specification and biogeography by using various molecular, morphological, and micromorphological data (Costea, *et al.* 2006a, b, c, d, 2008a; Costea and Stefanovic 2009b). It is clear that variation in chromosome number and karyotype criteria is important for future taxonomic revisions at the species level.

Acknowledgement

Thanks are due to Dr. Abdelfattah Badr, Professor of Genetics and Biosystematics, Botany Department, Faculty of Science, Helwan University for the final revision of the present article.

Table 1. A list of studied species, assigned to species subgenera and a summary of the karyotype features.

No.	Taxa	x	2n	TCL (μm)	MCL \pm SE (μm)	MAR \pm SE (r-value)	TF %	A ₁	A ₂	Chr. Type			Previous Chr. count
										m	sm	st	
I- Subgenus: Cuscuta (Eucuscuta)													
1	<i>C. brevistylosa</i> R.Br.	7	14*	8.30	0.93 \pm 0.13	1.63 \pm 0.12	33.67	0.36	0.16	3	4	-	-
2	<i>C. epilinum</i> Weihe	7	14	8.44	0.95 \pm 0.15	1.83 \pm 0.22	34.88	0.31	0.19	3	4	-	14,16,28, 30,32,42
3	<i>C. palaestina</i> Boiss.	7	14*	8.03	0.91 \pm 0.12	1.61 \pm 0.17	34.32	0.43	0.22	4	3	--	-
4	<i>C. pedicellata</i> Ledeb.	7	14*	7.99	0.87 \pm 0.10	1.81 \pm 0.20	38.92	0.37	0.19	2	4	1	-
5	<i>C. planiflora</i> Ten.	7	14	4.98	0.74 \pm 0.09	1.64 \pm 0.19	28.33	0.22	0.15	3	4	--	14,26,28
II- Subgenus: Grammica													
6	<i>C. campestris</i> Yuncker	7	56	9.52	1.10 \pm 0.18	1.71 \pm 0.24	40.54	0.41	0.21	1	5	1	28, 56
7	<i>C. chinensis</i> Lam.	7	28	6.54	0.79 \pm 0.09	1.58 \pm 0.19	37.87	0.33	0.16	3	3	1	28,32,56, 60
8	<i>C. hyalina</i> Roth.	7	28*	7.11	0.82 \pm 0.08	1.62 \pm 0.16	36.44	0.34	0.21	1	6	-	-
III- Subgenus: Monogyna (Monogynella)													
9	<i>C. monogyna</i> Vahl.	6	30	7.24	0.84 \pm 0.09	1.31 \pm 0.19	29.74	0.24	0.19	4	2	-	28,30
<p>TCL = Total Chromosome Length. MCL = Mean Chromosome Length. MAR = Mean Arm Ratio. SE = Standard Error. TF% = Total Form percent.</p>										<p>A₁ = Intrachromosomal asymmetry index. A₂ = Interchromosomal asymmetry index. Chr. = Chromosome. m = Metacentric region chromosome. sm = Submetacentric chromosome. st = Subtelocentric chromosome. * = New chromosome record.</p>			

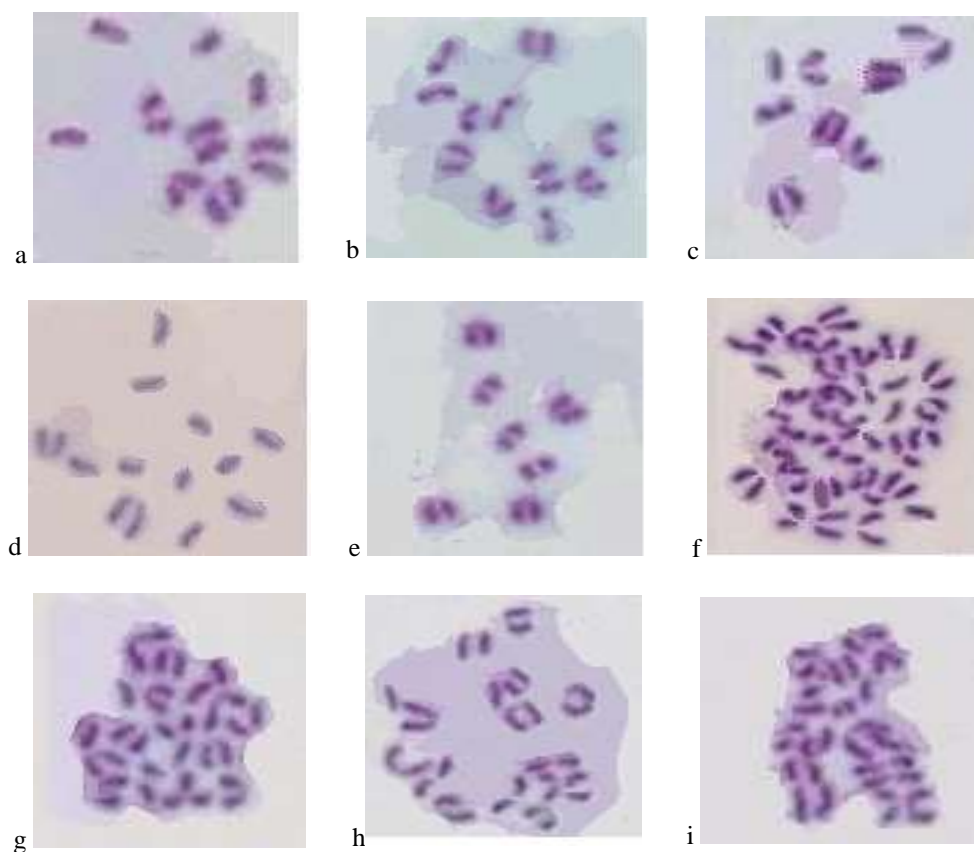


Fig. 1. Micrographs of the somatic metaphase chromosomes of nine Saudi Arabian species of the genus *Cuscuta*: a- *C. brevistylosa* ($2n = 2x = 14$), b- *C. epilinum* ($2n = 2x = 14$), c- *C. palaestina* ($2n = 2x = 14$), d- *C. pedicellata* ($2n = 2x = 14$), e- *C. planiflora* ($2n = 2x = 14$), f- *C. campestris* ($2n = 8x = 56$), g- *C. chinesis* ($2n = 4x = 28$), h- *C. hyalina* ($2n = 4x = 28$), i- *C. monogyna* ($2n = 4x = 30$). (Scale bars = $10 \mu\text{m}$).



Fig. 2. Karyotypes of 9 species of *Cuscuta* L. a: *C. brevistylosa*, b: *C. epilinum*, c: *C. palaestina*, d: *C. pedicellata*, e: *C. planiflora*, f: *C. campestris*, g: *C. chinesis*, h: *C. hyalina* and i: *C. monogyna*. Bar = 2 μ m.

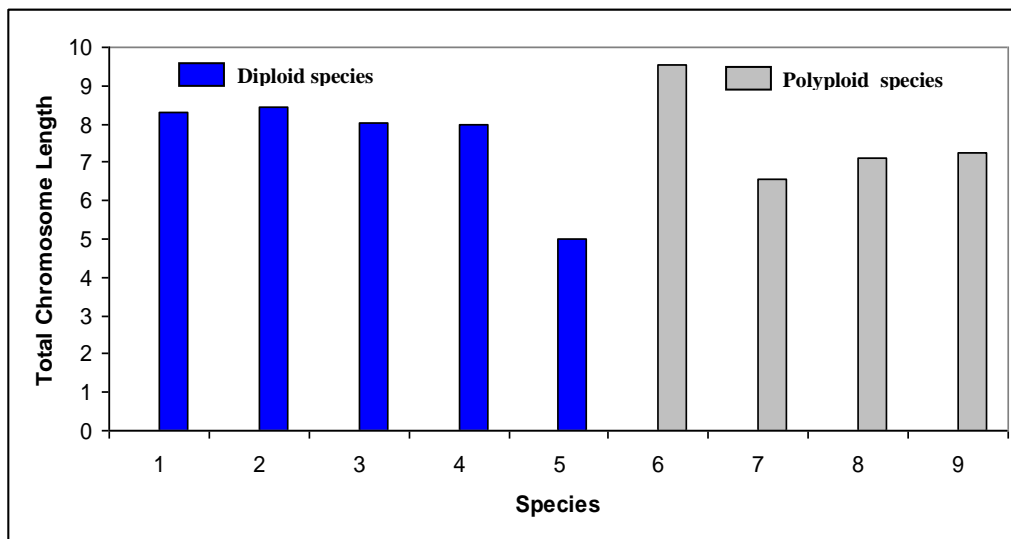


Fig. 3. Histogram showing the total chromosome length per species. (See Table 1 for species name).

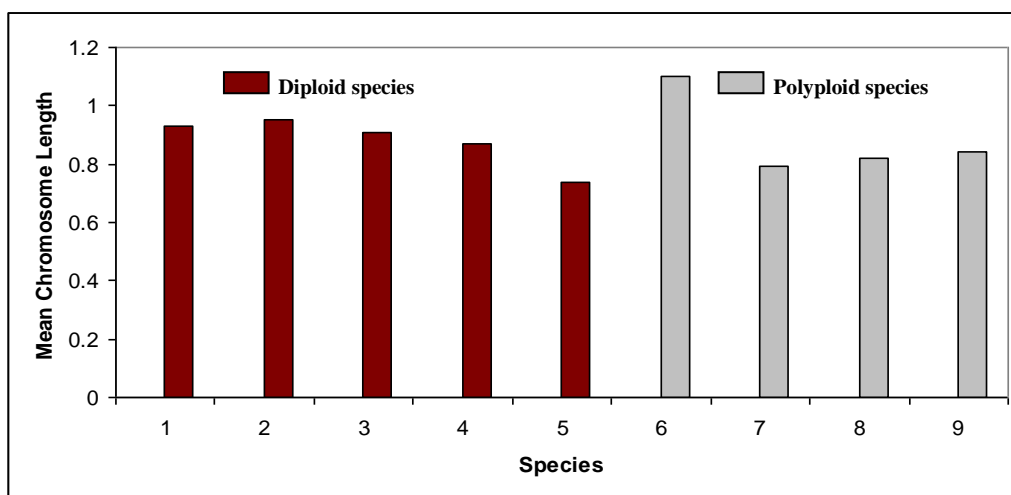


Fig. 4. Histogram showing the mean chromosome length per species. (See Table 1 for species name).

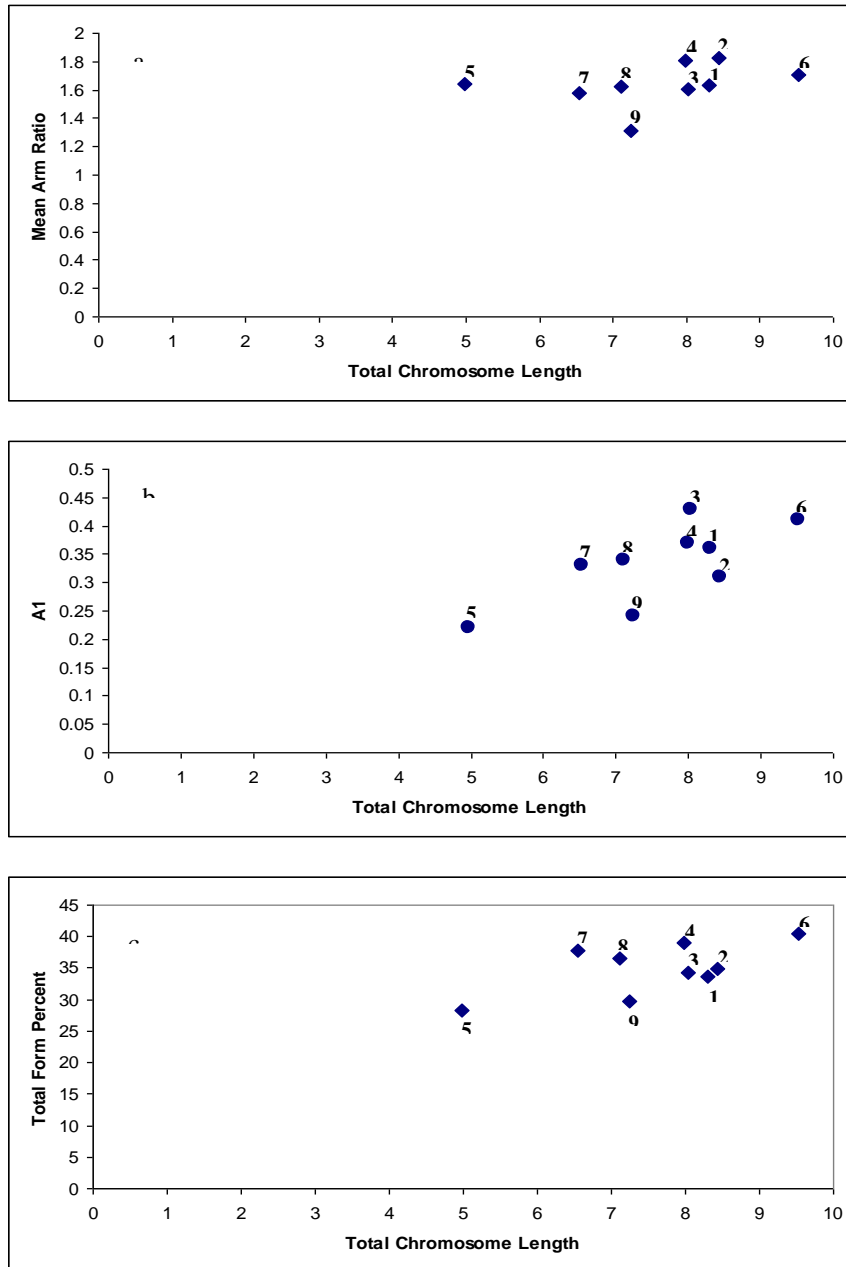


Fig. 5. The relationship between chromosome length and each of mean chromosome arm ratio (a), A1 values (b) and total form percent (c). (See Table 1 for species name).

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<http://www.tropicos.org/NameSearch.aspx>.

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