Karyotype Analysis and Systematic Relationships in the Egyptian Astragalus L. (Fabaceae)

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Abstract: In this study the karyotype criteria of 35 taxa representing 24 species of Egyptian Astragalus have been analyzed and their impact on the systematic delimitation of the studied species is discussed. Chromosome numbers, based on x = 8 have been found in the majority of Astragalus species in Egypt. A diploid number (2n = 16) was recorded in 22 taxa representing 17 species and polyploid numbers are recorded in six taxa representing three species. Numbers based on x = 7 were recorded in four taxa of which three counts are tetraploid with 2n = 28 representing A. annulatis, A. marocottus and A. vogelii. In addition, numbers based on x = 6 were encountered in A. trimestris (2n = 12 and 2n = 24) and A. boetius (2n = 30). The chromosomes in the studied species of Astragalus are generally small with a mean size ranging between 0.82 and 1.59 μm. Short chromosomes were particularly found in A. vogelii (MCL = 0.82 μm) and A. boetius (MCL = 0.87 μm), whereas longer chromosomes were scored in A. sinuosus (MCL = 1.59 μm). The karyotype in the studied taxa is mostly comprised of metacentric to submetacentric chromosomes as indicated by their mean arm ratio that ranges between 1.35 in A. vogelii and 2.03 in A. asterias. The degree of karyotype asymmetry is indicated by high values of TP% that ranges between 36.11% in A. asterias and 47.48% in A. tribuloides. The A, value ranges between 0.40 in A. vogelii and 0.90-0.92 in samples of A. asterias. Distance trees illustrating the relationships of the studied taxa, based on the analyses of karyotype features, have been constructed using Dice and Jaccard similarity coefficients. The grouping of the examined species, in these trees, is discussed in the light of their previous systematic treatments.

Key words: Astragalus, Egyptian flora, chromosomes, karyotype, systematics

INTRODUCTION

The genus Astragalus L. of the family Fabaceae is the largest genus of flowering plants (Polhill, 1981) comprising over 2000 annual or perennial herbaceous or subshrub species grouped in 150 sections (Podleck, 1986). In Egypt, the genus is represented by 32-35 species, delimited in several sections and distributed in different phytogeographical regions of the country (El-Hadjidi and Fayed, 1995; Boulos, 1999). Many species of Astragalus exhibit valuable economic values. Astragalus hamosus L., A. multiceps Benth and A. tribuloides Del., are medically useful. Astragalus cicer L. is a promising legume species for forage production (Townsend, 1981). Some species yield gum tragacanth, which is used by printers and dyers (Ali, 1961). However, several species of Astragalus have poisonous effects on grazing animals (James, 1983; Daniel et al., 1984; Pantier and Hartley, 1989).

The first deliberate study on the chromosomes of Astragalus was made by Ledingham (1960) who reported chromosome numbers for 84 species giving counts for 53 species that were not previously made. He found that the species from the old world have a basic chromosome number of x = 8, while those from the new world have x = 11, 12 and 13. That report was substantiated by another report by Ledingham and Rever (1963) in which further counts for 83 species were given. The studies on the cytology of Astragalus have ever since been made on species in different geographic regions of the World (www.mobot.mobot.org).

Chromosome counts, based on x = 8 have been reported in the vast majority of Old World species. In addition, counts based on base numbers of x = 7 or x = 6 have been encountered in few species (Maassoumi, 1987; Badr et al., 1996; Malallah et al., 2001). Meanwhile, studies on the cytology of Astragalus in America (Ledingham and Pepper, 1973; Martinez, 1974; Liston, 1990; Dophiz et al., 1995) confirmed the existence of basic numbers ranging between 11 and 15. The preponderance of species with a basic number of x = 8 led Badr et al. (1996) to conclude that it is the primary basic number in Astragalus. They further assumed that the x = 7 and x = 6 numbers have been derived from x = 8 by

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aneuploid loss of chromosomes. However, comprehensive studies on the karyotype criteria of Astragalus species in relation to their systematic treatment are generally lacking.

The importance of chromosomal information in plant systematics and evolution has attracted the attention of several workers. At the generic level and below chromosome features have provided a range of possibilities for understanding the affinities of taxa. Examples illustrating the role of chromosomal data in solving systematic problems in plant genera are found in Allium (Badr and Elkington, 1978), Plantago (Badr and El-Kholy, 1986), Ulex (Fernandez et al., 1993), Sesbania (Abou El-Ein et al., 1998, El-Shazly and Abou El-Ein, 1999) and several others. As reported by several authors Ledingham (1960), Ledingham and Rever (1963) Ledingham and Pepper (1973), Martinez (1974), Dopchiz et al. (1995) and Badr et al. (1996), variation in chromosome number in Astragalus, differentiate old World species from those of America.

The cytology of the Egyptian Astragalus was first studied by Badr et al. (1996) who described the karyotype for 14 taxa representing eight species. Chromosome counts for species of Egyptian Astragalus were substantiated by Sharawy (2001) who reported counts for 22 species. However, detailed karyotype analysis has not been made. In this study, we describe the karyotype criteria for 35 taxa representing 24 species of the Egyptian Astragalus and discuss the impact of the analyses of variation in these criteria on the systematic treatment of species.

MATERIALS AND METHODS

Material of 35 taxa representing 24 species, two subspecies and five varieties of the Egyptian Astragalus L. were collected, through 2000 to 2005, as mature flowering plants, from different localities in Egypt (Table 1). Herbarial sheets for all taxa are deposited at the Herbarium of Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt. To obtain seeds for cytological preparations, pods of all taxa were collected from healthy plants; left to dry at room temperature, seeds were then obtained from dry pods and kept at 4°C until use. For cytological investigations, young root tips were obtained from seeds that had been germinated in Petri-dishes, pretreated with 0.05% colchicine solution for 3 h and fixed in 3:1 ethanol: glacial acetic acid for 24 h.

For cytological preparations, root tips were hydrolyzed for 6 min in 1 M HCl at 60°C, washed briefly in dd H2O and stained in Feulgen safranin solution for 1-2 h. Squashed preparation were made in 2 drops of 45% acetic acid and made permanent by rinsing in absolute alcohol and mounting in Euparal. Examination of chromosomes was made under the high power of light microscope using oil emersion lens. Photographs of well-spread chromosomes were made using Carl-Zeiss photomicroscope III, at a magnification of 2500 and chromosomal measurements were made from photographic prints. Somatic number of chromosomes (2n) as counted in cytological preparations.

Total chromosome length of the haploid genome and the mean chromosome length±standard error (MCL±SE). Mean arm ratio±standard error (arm ratio±SE) was calculated for each karyotype by dividing the sum of long arms length on the sum of short arms length. Based on the measurements of chromosome length and arm ratio, a karyotype has been constructed and a karyotype formula has been calculated, for each taxon, by arranging the chromosomes in homologous pairs or groups in order of decreasing length and arm ratio as proposed by Levan et al. (1965). The degree of karyotype asymmetry was also determined by calculating total form percent (TF%) using the equation of Huziwara (1962) as follows:-

\[
TF(\%) = \frac{\text{Sum of short arm length}}{\text{Sum of total chromosome length}} \times 100
\]

In addition, asymmetry based on the ratio between the chromosome arms ratio and length has been estimated for each taxon using the equation of Zarco (1986) as follows:-

\[
A_i = 1 - \frac{\sum b_i}{B_i} + n_i
\]

where:
- \(A_i\) = Intrachromosomal symmetry index that ranges from zero to one,
- \(n_i\) = Number of homologous chromosome pairs or groups,
- \(b_i\) = Average length for short arms in every homologous chromosome pair or group and
- \(B_i\) = Average length for their long arms. The equation is formulated in order to obtain lower values when chromosomes tend to be metacentric.

In order to find out relationships based on the karyotype features of the studied taxa, total chromosome length per genome of all taxa has been plotted against the measures of karyotype asymmetry values (mean arm ratio, TF% and \(A_i\) value). In addition, the recorded karyotype features have been coded and analyzed with the software NTSYS-pc 2.1 (Rohlf, 1993) using UPGMA (Sokal and Michener 1958) and Neighbor-joining (Saitou and Nei, 1987) methods in order to produce distance trees that illustrate the relationships among the studied taxa.
RESULTS AND DISCUSSION

The cytological data for the examined taxa is summarized in Table 1 and their karyotypes are illustrated in Fig. 1-3. Chromosome numbers based on the basic number of \(x = 8\) are found in the majority of the studied taxa, a diploid number of \(2n = 16\) is recorded in 22 taxa, whereas a triploid number (\(2n = 24\)) is recorded in one sample of \(A. asterias\) (section Sesamei). In addition, a tetraploid number (\(2n = 32\)) is counted in \(A. corrugatus\), of section Harpilobus and in three samples of \(A. homosus\) (section Buceras) and a hexaploid (\(2n = 64\)) is recorded in \(A. homosus\) var. brachyceras. Numbers based on \(x = 7\) are found in \(A. annularis\) (two samples) and \(A. mareoticus\) of section Harpilobus and in \(A. vogelii\) (section Herpocaulus); all these samples are tetraploid (\(2n = 28\)).
Fig. 1: Karyotypes of 12 taxa of Egyptian *Astragalus* L. a: *A. hispidus*, b: *A. hispanicus* spp. Kralikianus, c: *A. hamosus*, d: *A. hamosus* var. brachyceras, e: *A. hamosus* var. brachyceras, f: *A. hamosus* var. buceras, g: *A. carpinus*, h: *A. fruticosus*, i: *A. sieberi*, j: *A. trigonus*, k: *A. dactylocarpous* spp. acinactiflorus and l: *A. boeticus*
However, one additional sample of *A. annularis* is found diploid with 2n = 14. Meanwhile, numbers based on x = 6 are encountered in two samples of *A. trimestris* of section Harpilobus; one diploid with 2n = 12 and the other tetraploid with 2n = 24 and in *A. boeticus* (section Cyamodes) where a pentaploid number (2n = 30) is recorded.

The chromosomes of the examined taxa of *Astragalus* are generally small. Total chromosome length (TCL) varies about two folds between species (Table 1). *Astragalus boeticus* of section Cyamodes (TCL = 5.22) and *A. vogelii* of section Herpocaulus (TCL = 5.74 μm) exhibit much shorter chromosomes compared to other species. Meanwhile, longest TCL (12.72 μm) have been found in *A. sinaicus* (x = 8) of section Sesamei. In the remaining taxa, TCL ranges between 8.58-894 μm in the two samples of *A. trimestris* and 12.08 μm in *A. eremophilus*. Similarly, shortest MCL was scored in *A. boeticus* (0.82±0.05 μm) and longest MCL in *A. sinaicus* (1.59±0.08 μm).

The karyotype in the studied taxa is mostly comprised of metacentric to submetacentric chromosomes as indicated by their mean arm ratio. This ratio ranges between 1.35±0.16 in *A. vogelii* and 1.93±0.30 to 2.03±0.30 in *A. asterias* (Table 1). The low value of the Standard Error (SE) for mean arm ratio values indicates low degree of karyotype asymmetry in the studied taxa. The degree of karyotype asymmetry is also indicated by high values of TF% that ranges between 36.11% in *A. asterias* and 47.48% in *A. tribuloides*, the examined samples of the latter species and the two samples of *A. hispidulus* show higher TF% compared to other species. Similarly, the As ranges between 0.40 in *A. vogelii* and 0.90-0.92 in the two samples of *A. asterias*. These values confirm the low karyotype asymmetry as indicated by the values of arm ratio and TF%.

The plotting of TCL against arm ratio (Fig. 4a) distinguish most of the species that have base numbers of x = 6 and x = 7. In particular, this figure clearly distinguish *A. boeticus* (2n = 30, x = 6) of Section Cyamodes and *A. vogelii* (2n = 28, x = 7) of Section Herpocaulus, the two samples of *A. trimestris* (2n = 12, 24, x = 6) of section Harpilobus and the two samples of *A. asterias* (2n = 16, 24, x = 8) and *A. sinaicus* (2n = 16) of section Sesamei. The plotting diagram of total chromosome length against the A values (Fig. 4b) also clearly distinguished *A. boeticus*, *A. vogelii*, *A. trimestris*, *A. asterias* and *A. sinaicus*. The plotting diagram of total chromosome length against the values of total form percent (Fig. 4c) also clearly distinguished *A. boeticus*, *A. vogelii* and the two samples of *A. trimestris* and also the two samples of *A. hispidulus* (2n = 16, x = 8) of Section Ankylobus, the octaploid sample of *A. hamosus* (2n = 64, x = 8) of section Buceras and the four samples of *A. tribuloides* (2n = 16, 24, x = 8) of section Sesamei. The remaining taxa, that mostly have a numbers based x = 8 are not sufficiently differentiated by the plotting of chromosome length against the arm ratio or A values.

The neighbor joining tree illustrating the relationships between the studied samples of *Astragalus*.
is illustrated in Fig. 5. In this tree, *A. mareticus* (x = 7), *A. boeticus* (x = 6) and *A. vogelii* (x = 7) and the samples of *A. hamosus*, with polyploid numbers based on x = 8 are separated, as different clusters, from a large group that comprises other taxa. In the latter group, the two samples of *A. trimestris* (x = 6), *A. corrugatus* (x = 8) and the two samples of *A. annularis* (x = 7) are also distinguished separate clusters at high distance. The remaining taxa (all with x = 8) are divided in two major groups; the first comprises two subgroups, a large one that includes *A. sinaicus*, *A. stella*, *A. tribuloides*, *A. schimperi* and *A. asterias* and a smaller one comprised of *A. peregrinus* and *A. bombycinus*. In the other group, *A. eremophilus* and *A. kahiricus* as well as *A. spinosis* and *A. haurensii* are differentiated from a group comprising the remaining seven samples. This group is differentiated into two clusters; one including *A. trigonus*, *A. dactylocarpous* and *A. carpinus* and other *A. sieberi*, *A. fruticosus* and the two samples of *A. hispidulus*.

The UPGMA tree illustrating the relationships between the studied samples of *Astragalus* (Fig. 6) also separated *A. mareticus*, *A. boeticus* and *A. vogelii* and the samples of *A. hamosus*; as different clusters from other taxa. The two samples of *A. trimestris* are also clearly distinguished from other taxa. However, in this tree *A. corrugatus* and the two samples of *A. annularis* are not separated, as in the NJ tree whereas, the two samples of *A. hispidulus* are clearly delimited as a distinguished cluster. The remaining taxa are divided in two major groups; the first comprises two subgroups; a larger one that includes *A. stella*, *A. sinaicus*, *A. tribuloides*, *A. schimperi* and *A. asterias*; The other group is divided into three subgroups; the first comprises *A. corrugatus* and the two samples of *A. annularis*; the second of *A. peregrinus*, *A. bombycinus*, *A. eremophilus* and *A. kahiricus* and the third of *A. spinosis* and *A. haurensii* and a cluster comprised of *A. sieberi*, *A. fruticosus*, *A. trigonus*, *A. dactylocarpous* and *A. carpinus*.

![Diagram](image)

Fig. 5: A neighbor joining tree illustrating the relationships among the studied 35 taxa of *Astragalus*, based on the analysis of karyotype criteria.
The studied species of Egyptian Astragalus are delimited in 13 sections (Podlech, 1986; 1991, Table 1). Section Ankylopus is represented by two samples of *A. hispidulus*, one representing the type *A. hispidulus* and the other *A. hispidulus* ssp. *kraklrianus*. Both samples have 2n = 16 with metacentric and submetacentric chromosomes (Fig. 1a and b), the two samples also have closely similar chromosome length and mean arm ratio but slightly different A<sub>v</sub> value (Table 1). The subspecies *kraklrianus* differs from the type by shorter and broader pods and smaller number of seeds (Boulos, 1999). Podlech and Ayte (1998) favored the consideration of *A. hispidulus* ssp. *kraklrianus* as a separate species i.e., *A. krakli* Batt.; a view supported by the electrophoretic profile of storage seed protein (Al-Nowaihi et al., 2002). However, the karyotype features of the examined samples do not justify the proposed delimitation. The UPGMA tree appear to justify the delimitation of *A. hispidulus* in a separate section but in the NJ tree this species is associated with the species in section Carpinii (*A. carpinus*) and section Chronopinus (*A. dactylcarpus, A. fruticosus, A. sieberi* and *A. trigonus*). All these species have 2n = 16 and 9 similar TCL and mean arm ratio but slightly different A<sub>v</sub> value (Table 1).

Karyological similarities are evident among the examined four species of section Chronopinus i.e., *A. dactylcarpus, A. fruticosus, A. sieberi* and *A. trigonus*. All four species have 2n = 16 with closely similar chromosome length, arm ratio TP% and A<sub>v</sub> (Table 1). The resemblance in chromosome criteria among these species is correlated with similarities in their seed protein electrophoretic profiles (Al-Nowaihi et al., 2002). However, morphological and anatomical features distinguished *A. fruticosus* from the other three species (Sharawy, 2001). Podlech (Personal Communication, 2001) favored the delimitation of this species in subsection
Astragalus and the other three species in section Chronopus. However, this view is not substantiated by the karyotype features reported here. On the contrary, karyotype analysis and the analysis of chromosome data support close relationships between these species. Furthermore, the NJ tree, based on these data, indicates close relationships among the species of section Chronopus and A. carpinus of section Carpini and A. hispidulus (section Ankylopus).

Section Buceras is represented by four samples of A. hamosus; all have polyplioid chromosome numbers based on x = 8, three samples are tetraploid with 2n = 32 and one sample of A. hamosus spp. brachyceras has 2n = 64 (Table 1). The tetraploid number has been frequently reported for A. hamosus but pentaploid (2n = 40), hexaploid (2n = 48) and other aneuploid numbers were also recorded in this species (Looeve and Kjellquist, 1974; Horjales, 1976; Diaz Lifante et al., 1992). In Egyptian material, Badr et al. (1996) recorded 2n = 32 in A. hamosus and 2n = 40 in A. hamosus spp. buceras, however, 2n = 64 in A. hamosus spp. brachyceras is recorded here for the first time. The chromosomes of all samples of A. hamosus are metacentric and submetacentric (Fig. 1c-f) with similar TCL, MCL, arm ratio, TP% and A1 values (Table 1).

Podlech and Aytac (1998) regarded A. hamosus a polymorphic species in morphological characters, an observation supported by differences in chromosome number among different samples. However, the variation, in chromosome number among different samples of A. hamosus, is not associated with morphological and anatomical resemblances (Sharawy, 2001) and similarities in the electrophoretic profiles of seed protein (Al-Nowaili et al., 2002). The samples of A. hamosus are clearly delimited as a distinguished group in the NJ and UPGMA distance trees. The analysis of chromosome features thus support the position of A. hamosus in section Buceras as proposed by Podlech (1986 and 1991).

The chromosome number of 2n = 30 reported in A. boeticus of section Cyamodes is most likely pentaploid, based on x = 6 and is similar to previous counts reported for this species by several authors (Ledingham, 1960; Ledingham and Rever, 1963; Martinez, 1974; Ferrandes and Quiros, 1978; Badr et al., 1996). The karyotype is comprised of metacentric and submetacentric short chromosomes. The chromosomes of this species, like that of A. vogeli (2n = 28) of section Herpoeculas, are extremely short compared to other species. However, the karyotype of A. boeticus is more asymmetric as judged by higher mean arm ratio, TP% and A1 value compared to that of A. vogeli (Table 1).

The analyses of karyotype data separate A. vogeli and A. boeticus as well as A. mareoticus (2n = 28), of section Harpilobus, from other species, at high distance coefficients between them. In the UFGMA tree, these three species are clearly distinguished from all other taxa. The high distance coefficients among these species, in both the NJ and UFGMA trees, may be congruent with the delimitation of A. boeticus in section Cyamodes and A. vogeli in section Herpoeculas as proposed by Podlech (1986 and 1991) and agree with differences between them in morphological, anatomical and seed protein criteria as described by Sharawy (2001) and Al-Nowaili et al. (2002).

Both the NJ and UPGMA trees clearly indicated the separation of A. mareoticus from other species of section Harpilobus. This section is represented in this study by four species that have different chromosome numbers and exhibit variable arm ratio, TP% and A1 values. Numbers based on x = 8 are found in A. curvatus (2n = 32) and A. hauarensis (2n = 16) and numbers based on x = 6 in A. trimetrics (2n = 12, 24). Meanwhile, a tetraploid number, based on x = 7 (2n = 28) is found in A. mareoticus. The chromosome counts and karyotype features of these four species do not support their grouping in section Harpilobus (Podlech, 1991) and is contrary to similarities in their seed protein electrophoretic profile (Al-Nowaili et al., 2002). In the NJ tree, A. trimetrics and A. curvatus are associated together at high distance coefficients between them. In the UPGMA tree, A. curvatus appeared associated, at relatively high distance coefficient, with A. annularis (section Haematodes). In both trees A. hauarensis is grouped with A. spinosus of section Poteriun; both species have 2n = 16 and similar karyotype features (Table 1). The karyotype features and the analyses of chromosomal data thus do not support the grouping of the above four species in section Harpilobus as proposed by Podlech (1986 and 1991).

Two samples of A. annularis (section Haematodes) have been examined in this study, one has a diploid number of 2n = 14 and the second a tetraploid number of 2n = 28 (Fig. 2c and d). The diploid number was recorded in Egyptian material by Badr et al. (1996) and the tetraploid number is recorded here for the first time. The chromosomes of the two samples have similar length and arm ratio but the diploid sample shows slightly higher TP% and lower A1 value (Table 1). The karyotype criteria for A. annularis support the morphological and anatomical characters (Sharawy, 2001) and seed protein electrophoretic profile (Al-Nowaili et al., 2002) that justify the delimitation of this species in section Haematodes as proposed by Podlech (1986). This is confirmed by the position of the two samples of A. annularis in the NJ tree.
The association of this species with *A. corrugatus* in the UPGMA tree may be merely reflecting similarities in chromosome measurements.

A diploid number of 2n = 16 is reported for *A. kahircus* of section Eremophylla (Fig. 2a) and *A. eremophilus* of section Falcinellus (Fig. 2b). The same number was reported for material of *A. eremophilus* from Saudi Arabia (Badr and Gassim, 1992) and Egypt (Badr et al., 1996). The karyotype of both species is comprised of metacentric and submetacentric. The two species also have similar chromosome length, arm ratio, TF% and A₀ value (Table 1). These resemblances are reflected in the NJ tree; in which the two species are delimited together, at a relatively high distance coefficient. The grouping of these two species in the NJ tree is contrary to their separation in two sections by Podlech (1986 and 1991). In the UPGMA tree, *A. kahircus* and *A. eremophilus* are grouped at low distance coefficient but delimited with *A. peregrinus* and *A. bombycinus* of section Platygloittis; all four species have 2n = 16 but the former two species have longer chromosomes, lower arm ratio and higher TF% and A₀ value.

Section Platygloittis is represented in this study by two samples of *A. bombycinus* and one sample of *A. peregrinus*; all three samples are diploid with 2n = 16. The same number was reported in both species by Ledingham and Rever (1963) and in *A. peregrinus* by Brullo et al. (1991) and Badr et al. (1996). The karyotype of both samples of *A. bombycinus* is composed of metacentric and submetacentric chromosomes (Fig. 2k and 3a) and that of *A. peregrinus* have eight pairs of metacentric chromosomes (Fig. 3b). However, both species have similar arm ratio, TF% and A₀ value, but the latter species has shorter chromosomes (Table 1). The delimitation of *A. bombycinus* and *A. peregrinus*, as one group, in the NJ tree, agree with similarities among them in morphological and anatomical characteristics and pollen type (Saad and Taia, 1988). These data combined with similarities among these two species in sperm cell characteristics (Sharawy et al., 2003) and seed protein electrophoretic profiles (Al-Nowahi et al., 2002) support their delimitation together in section Platygloittis (Podlech, 1991).

Chromosome numbers based on n = 8 have been recorded in nine taxa representing five species of section Sesamei (Table 1). One sample of *A. asterias* has a triploid number of 2n = 24 and the other eight samples have a diploid number of 2n = 16. The same number was only reported, in Egyptian material, for *A. sinaicus* by Badr et al. (1996). The karyotype of the nine taxa is illustrated in Fig. 3d-l. They differ in chromosome length that ranges from 1.59±0.08 μm in *A. sinaicus* to 1.26±0.04 μm in *A. stella*. In the karyotype of the latter species (Fig. 3h), a satellite is observed on the short arms of the chromosome pair numbered 3. The two samples of *A. asterias* are distinguished by higher mean arm ratio and A₀ value that indicates asymmetric karyotype (Table 1).

In the NJ and UPGMA trees, the species of section Sesamei are clearly distinguished as one group; only *A. sinaicus* and *A. stella* are slightly distinct from the samples representing the other three species. This distinction is in agreement with the view of Gazer (1993), who proposed four groups for section Sesamei typified by *A. asterias*, *A. sinaicus*, *A. stella* and *A. schimperi* respectively. *Astragalus asterias* possesses sessile leaves and fruits with double incummentum (Sharawy, 2001). Saad and Taia (1988) also found that this species has *A. palaestinus* pollen type that differs from the pollen types in section Sesamei. Moreover, evidence from seed protein electrophoretic analysis indicated the grouping of *A. asterias* with *A. tribuloides* (Al-Nowahi et al., 2002) that is correlated with similarities between these two species in sperm cell characteristics (Sharawy et al., 2003). However, in this study samples representing both species are grouped with *A. schimperi* indicating close relationship between these three species. Chromosomal criteria and analysis of karyotype data, as presented here, support the grouping of the five species that has been delimited in section Sesamei by Podlech (1986 and 1991).

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