

BASED ON CYTOTAXONOMICAL AND MORPHOLOGICAL EVIDENCE ASTRAGALUS REMOTIFLORUS SUBSP. NIGROHIRSUTUS IS RAISED TO SPECIES RANK

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Astragalus remotiflorus Boiss. from section *Anthylloidei* DC. has three subspecies, that are endemic to Iran. *A. remotiflorus* subsp. *nigrohirsutus* is an endemic species in Fars province and is found on the mountain of Kharman kuh, 1760–3122 m. In this study cytological and morphological study were performed for this subspecies and *A. remotiflorus* subsp. *remotiflorus*. Also, the morphological characteristics of *A. remotiflorus* subsp. *melanogramma* were examined. The results indicate that *A. remotiflorus* subsp. *nigrohirsutus* is a hexaploid ($2n=6x=48$) with the karyotype formula of $15m+9sm$. Symmetrical karyotype of this subspecies is in the class 2A. Morphological characteristics indicate that this subspecies with a small amount of mixed black hairs ca. 1 – 2.5 mm long on the peduncle and calyx distinguishes it from the other two subspecies. Also, it differs from subsp. *melanogramma* in having longer peduncle and lax inflorescence. *A. remotiflorus* subsp. *nigrohirsutus* is raised to the rank of species as *A. nigrohirsutus*.

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Key words. Karyotype, *Astragalus remotiflorus*, *Astragalus nigrohirsutus*, endemic, Fars, Iran.

تغییر جایگاه رده بندی *Astragalus remotiflorus* subsp. *nigrohirsutus* به سطح گونه بر اساس مطالعات ریخت شناسی و گزارش جدید کروموزومی

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گونه *Astragalus remotiflorus* با سه زیر گونه معرفی شده که همگی انحصاری ایران می باشند. زیرگونه *remotiflorus* subsp. *Astragalus nigrohirsutus* انحصاری استان فارس است و در ارتفاعات خرمن کوه فسا یافت می گردد. در این تحقیق این زیرگونه با زیرگونه *Astragalus remotiflorus* subsp. *remotifluors* مورد مطالعه سیتولوژیکی و مورفولوژیک قرار گرفت. همچنین خصوصیات مورفولوژیکی آن با زیرگونه *Astragalus remotiflors* subsp. *melanogramma* نیز بررسی گردید. نتایج نشان می دهد که این زیر گونه، هگزاپلوئید ($2n=6x=48$) بوده و فرمول کاریوتیپی آن $15m+9sm$ می باشد و از این نظر بر اساس تقارن کاریوتیپی استیبینز در کلاس 2A قرار می گیرد. زیرگونه *Astragalus remotiflorus* subsp. *remotiflorus* دیپلوئید ($2n=2x=16$) و فرمول کاریوتیپی آن $3m+5sm$ بوده و از نظر تقارن کاریوتیپی در کلاس 2B قرار می گیرد. از نظر خصوصیات مورفولوژیکی، این زیرگونه با داشتن مقدار کمی از مخلوط کرک های سیاه به طول ۱ تا ۲/۵ میلی متر روی دم گل آذین و کاسه گل با دو زیرگونه دیگر متفاوت است. و اختلاف اساسی آن با زیرگونه

subsp. *melanogramma* در داشتن دم‌گل‌آذین بلندتر با گل‌های پراکنده و تنک می‌باشد. با توجه به وجود تفاوت‌های اساسی در خصوصیات مورفولوژیکی و کروموزومی این زیرگونه با دو زیرگونه دیگر، *Astragalus remotiflorus* subsp. *nigrohirsutus* به سطح گونه ارتقا، می‌یابد.

INTRODUCTION

Astragalus is the most diverse genus of all Angiosperms, with more than 2,500 species (Maassoumi 1998). This genus is mainly distributed through arid and temperate regions of the Northern Hemisphere and South America. It is especially abundant in SW Asia, where 1500 species are found (Sanderson & Wojciechowski 2000). *Astragalus remotiflorus* from sect. *Anthylloidei* DC. has three subspecies. *Astragalus remotiflorus* Boiss. subsp. *remotiflorus*, *Astragalus remotiflorus* Boiss. subsp. *melanogramma* (Boiss.) Tietz & Zarre and *Astragalus remotiflorus* subsp. *nigrohirsutus* Tietz & Zarre (Maassoumi 2003). In the whole world chromosomal studies done on *Astragalus* including Iran (Aryavand 1977, Cartier 1979, Magulaev 1980, Löve & Löve 1982, Parfitt et al. 1985, Maassoumi 1986, Maassoumi 1987, Badr et al. 1996, Javadi 2006, Sheidai & Ghahremani-nejad 2009, Hesamzadeh Hejazi & Ziaei Nasab 2009), and according to that IPCN, reports the basic chromosome numbers of $x = 7, 8, 11-15$ and diploid, tetraploid, hexaploid and octaploid levels for the genus *Astragalus*. The aim of this paper is to present result of cytological studies on *Astragalus remotiflorus* and to introduce a new combination.

MATERIAL AND METHODS

Materials

1. *Astragalus remotiflorus* subsp. *nigrohirsutus* (Fars: Fasa, Kharman kuh, 3122 m, N: 29, 12; E: 53, 34, Borjjan 1366).
2. *Astragalus remotiflorus* subsp. *remotiflorus* (Fars: Fasa, Kharman kuh, 1980 m, N: 29, 12; E: 53, 33, Borjjan 1375).
3. *Astragalus remotiflorus* subsp. *melanogramma* (Hamedan: Avaj, Sultan-Bulagh mt., 2000–2400 m, Maassoumi & Mirhosseini 59404).

The voucher specimens are deposited in TARI and herbarium of Science and Research Branch, Islamic Azad University, Tehran (IAUH).

Cytological Studies

Selected healthy seeds of collected samples were germinated on moisturized filter paper in room temperature (22°C–25°C). The healthy roots which were brittle, translucent and white were collected at 9:30–10:45 am (Dayer 1979). 2–3 mm of root tips were cut and treated with 0.002 M 8-hydroxyquinoline for 5

h at 18°C. Then fixed in 3:1 ethanol: glacial acetic acid for at least 48 h, and stored at 4°C. They were washed in distilled water to remove the fixative, hydrolysed in 1N HCl for 5–7 min. at 60°C, and stained with 2% aceto-orcein for 1 h. The shoot tips were squashed in 45% acetic acid solution (Sharma & Sharma, 1980). For their chromosome observation, at least 5 metaphase plates were scanned and drawn for different chromosome measurements. Microphotographs were taken with Labomed microscope with photographic attachment ($\times 1000$). Using Micro-measure software developed by the Department of Biology, Colorado State University, USA (<http://www.colostate.edu/Depts/Biology/Micrommeasure>) in order to prepare the karyotype. Measurements of long arm, short arm, total chromosome length and centromeric index were used. Total form percentage (TF %) of chromosomal complements was also calculated (Levan et al. 1964). Karyotype of the subspecies was constructed from the microphotographs of respective metaphase plates.

RESULTS

Somatic chromosomes and karyotype of the *Astragalus remotiflorus* Boiss. subsp. *remotiflorus* are illustrated in Fig. 1 A, B. The karyotypic characters are shown in Table 1. Cytological studies in this subspecies shown that it is diploid and $2n=16$ ($2n=2X=16$). Karyotypic formula is $3m+5sm$, and karyotypic symmetry is in class 2B. The average length of a set of haploid chromosome ($n=8$) is $21.91\mu\text{m}$ and the average percentage relative length is %12.50. Average absolute length of chromosomes is $2.74\mu\text{m}$, and the largest chromosome of submetacentric, with absolute length $3.46\mu\text{m}$ and the relative length %15.80. Smallest chromosome of metacentric, with absolute length $1.71\mu\text{m}$ and the relative length equal to 7.78%. The range of the long arm length varies from $0.96\mu\text{m}$ on chromosome no.8 to $2.00\mu\text{m}$ in chromosome no.4. The range of the short arm length varies from $0.75\mu\text{m}$ in chromosome no.8 to $1.30\mu\text{m}$ in chromosome no.1. The average ratio of long arm to short arm (AR) in this karyotype is 1.75. The relative amount of chromatin in this subspecies was 2.73. Differences in the relative length (DRL) of chromosomes are $4.01\mu\text{m}$ and total form percentage (TF%) is 37.33%.

Somatic chromosomes and karyotype of the *Astragalus remotiflorus* Boiss. subsp. *nigrohirsutus* are

Table 1. Average characteristics of karyotype in haploid genome, *Astragalus remotiflorus* subsp. *remotiflorus*.

No.	TL	RL%	L	L%	S	S%	AR	CI
1	3.46±0.17	7.90	2.16±0.16	4.93	1.30±0.30	2.97	1.97	0.37
2	3.26±0.20	7.44	2.16±0.19	4.94	1.10±0.10	2.50	2.09	0.34
3	3.18±0.10	7.26	1.90±0.11	4.33	1.28±0.28	2.93	1.54	0.40
4	2.99±0.06	6.81	2.00±0.08	4.57	0.98±0.98	2.24	2.13	0.33
5	2.79±0.06	6.36	1.80±0.06	4.12	0.98±0.98	2.24	1.86	0.35
6	1.60±0.06	5.92	1.67±0.03	3.81	0.93±0.93	2.12	1.81	0.36
7	1.93±0.08	4.41	1.07±0.02	2.45	0.86±0.86	1.96	1.27	0.44
8	1.71±0.06	3.89	0.96±0.01	2.19	0.75±0.75	1.70	1.30	0.44

Total chromosome length (TL). Relative percentage length of chromosome (RL%). Long arm length (L). The relative percentage length of the long arm (L%). Short arm length (S). Relative percentage length of the short arm (S%). Arm ratio (AR). Centromeric index (CI).

Table 2. Average characteristics of karyotype in haploid genome, *Astragalus remotiflorus* subsp. *nigrohirsutus*.

No.	TL	RL (%)	L	L (%)	S	S (%)	AR	CI
1	3.44±0.09	5.18	0.97	3.48	0.47±0.08	1.70	2.18	0.33
2	3.39±0.10	5.01	0.84	3.03	0.55±0.07	1.99	1.58	0.40
3	3.36±0.10	4.89	0.81	2.92	0.55±0.02	1.97	1.51	0.41
4	2.33±0.09	4.80	0.81	2.90	0.50±0.09	1.81	1.86	0.37
5	2.29±0.08	4.66	0.79	2.83	0.51±0.15	1.82	1.63	0.39
6	1.27±0.09	4.56	0.76	2.75	0.50±0.05	1.81	1.55	0.40
7	1.24±0.09	4.47	0.74	2.66	0.50±0.07	1.81	1.53	0.40
8	1.23±0.08	4.42	0.77	2.77	0.46±0.03	1.64	1.74	0.37
9	1.20±0.09	4.34	0.73	2.65	0.47±0.03	1.69	1.59	0.39
10	1.19±0.09	4.28	0.75	2.69	0.44±0.02	1.59	1.74	0.37
11	1.17±0.08	4.20	0.68	2.45	0.49±0.07	1.75	1.47	0.41
12	1.15±0.08	4.15	0.73	2.62	0.43±0.02	1.53	1.73	0.37
13	1.14±0.08	4.10	0.69	2.48	0.45±0.06	1.61	1.62	0.39
14	1.13±0.08	4.05	0.68	2.46	0.44±0.07	1.60	1.67	0.39
15	1.11±0.07	3.99	0.63	2.28	0.48±0.04	1.72	1.34	0.43
16	1.09±0.07	3.91	0.67	2.42	0.41±0.04	1.49	1.68	0.38
17	1.08±0.07	3.88	0.61	2.19	0.47±0.05	1.69	1.34	0.43
18	1.07±0.07	3.86	0.66	2.39	0.41±0.04	1.47	1.73	0.39
19	1.06±0.08	3.80	0.68	2.46	0.37±0.06	1.34	2.00	0.35
20	1.03±0.08	3.72	0.60	2.16	0.43±0.07	1.56	1.47	0.41
21	1.01±0.08	3.65	0.55	2.98	0.46±0.03	1.67	1.19	0.46
22	0.98±0.08	3.52	0.59	2.14	0.38±0.02	1.38	1.61	0.40
23	0.95±0.06	3.41	0.55	1.97	0.40±0.50	1.44	1.42	0.42
24	0.88±0.06	3.19	0.52	1.87	0.37±0.03	1.32	1.43	0.41

Total chromosome length (TL). Relative percentage length of chromosome (RL%). Long arm length (L). The relative percentage length of the long arm (L%). Short arm length(S). Relative percentage length of the short arm (S%). Arm ratio (AR). Centromeric index (CI).

illustrated in Fig. 2 A, B. The karyotypic characters are shown in Table 2. Cytological studies in this subspecies shown that it is hexaploid and have 48 chromosomes (2n=6X=48). Karyotypic formula is 15m+9sm, and karyotypic symmetry is in class 2A. The average length of a set of haploid chromosome (n=24), is 27.77µm and the average percentage relative length is 4.16%. Average absolute length of chromosomes is 1.15 µm,

and the largest chromosome of submetacentric, with absolute length 1.43 µm and the relative length 5.17%. Smallest chromosome of metacentric, with absolute length 0.88 µm and the relative length equal to 3.18%. The range of the long arm length varies from 0.52 µm for chromosome no.24 to 0.97 µm for chromosome no.1. The range of the short arm length varies from 0.36 µm in chromosome no.24 to 0.55 µm in

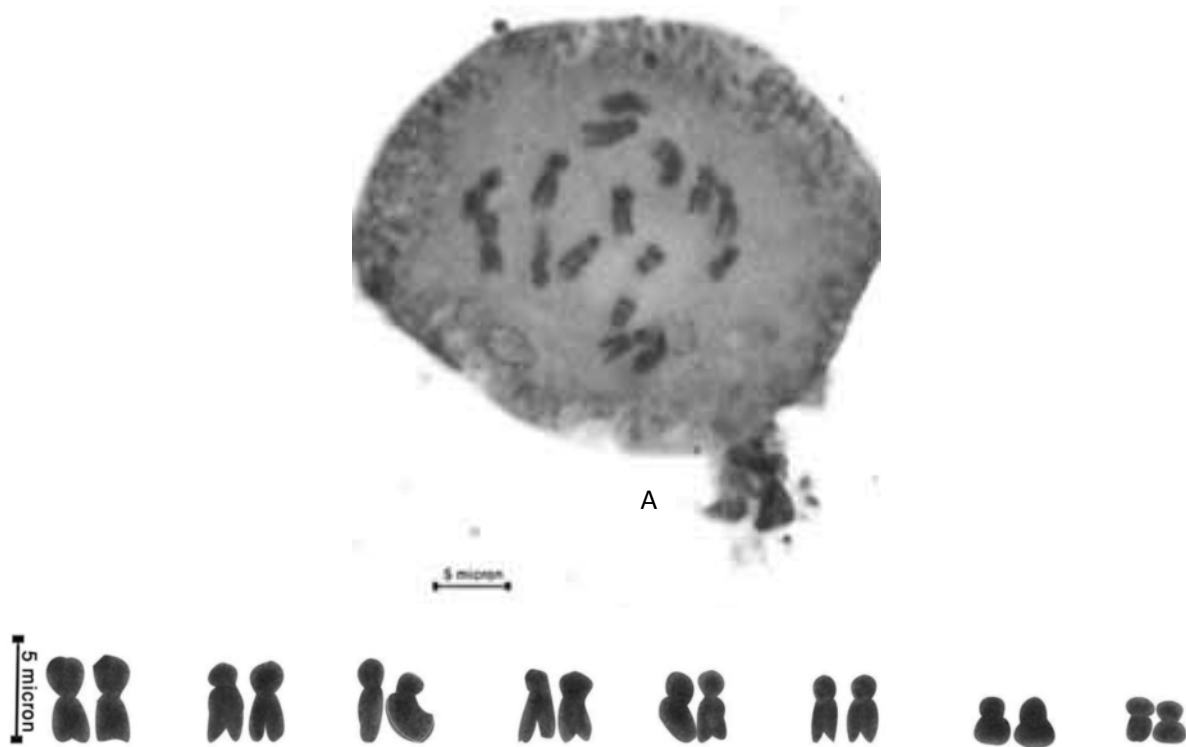


Fig. 1. Somatic metaphase chromosomes (A) and karyotype of *Astragalus remotiflorus* subsp. *remotiflorus* (B).

chromosome no. 2. The average ratio of long arm to short arm (AR) in this karyotype is 1.60. The relative amount of chromatin in this subspecies was 1.15. Differences in the relative length (DRL) of chromosomes are 1.99 μm and total form percentage (TF%) is 39.39%.

DISCUSSION

Cytological studies indicate that the two subspecies *Astragalus remotiflorus* subsp. *remotiflorus* and *A. remotiflorus* subsp. *nigrohirsutus* have different levels of ploidy, subsp. *nigrohirsutus* is probably an allohexaploid which represents a hybrid origin where there are two or more different genomes and subsp. *remotiflorus* is diploid. In most cases, the number of chromosomes is correlated with specialization of the species. So that, the species with higher degree of specificity, has the lower number of chromosomes (Levitsky 1980). As shown in Table 3, according to total form percentage (TF%) (Huziwara 1962) in these two examples, subsp. *nigrohirsutus* with (TF%=39/39), had a more symmetrical karyotype. Maximum difference between relative length of the longest and

the shortest chromosomes (DRL) was present in the subspecies *remotiflorus* (8.02), indicates that this subspecies has a lower symmetry. The highest relative amount of chromatin (VRC) was observed in subspecies *remotiflorus* (2.73). This shows that, this subspecies have a higher degree of specificity. Karyotype status of subspecies in evolutionary classification method based on karyotype asymmetry (Stebbins 1971) revealed two different classes 2A and 2B in this configuration. Accordingly, the subspecies *remotiflorus* is more asymmetrical and has a higher degree of evolution, although it has declined in the ploidy level. It should be noted that in none of these subspecies, the satellites and nucleolar organizer regions were observed. Morphological studies indicate that subspecies *nigrohirsutus* with a small amount of mixed black hairs ca. 1 – 2.5 mm long on the peduncle and calyx distinguish it from the other two subspecies. Also, it differs from subsp. *melanogramma* in having longer peduncle and lax inflorescence. Based on the morphological and chromosomal studies in this research, *Astragalus remotiflorus* subsp. *nigrohirsutus* Tietz & Zarre. raised to species rank.

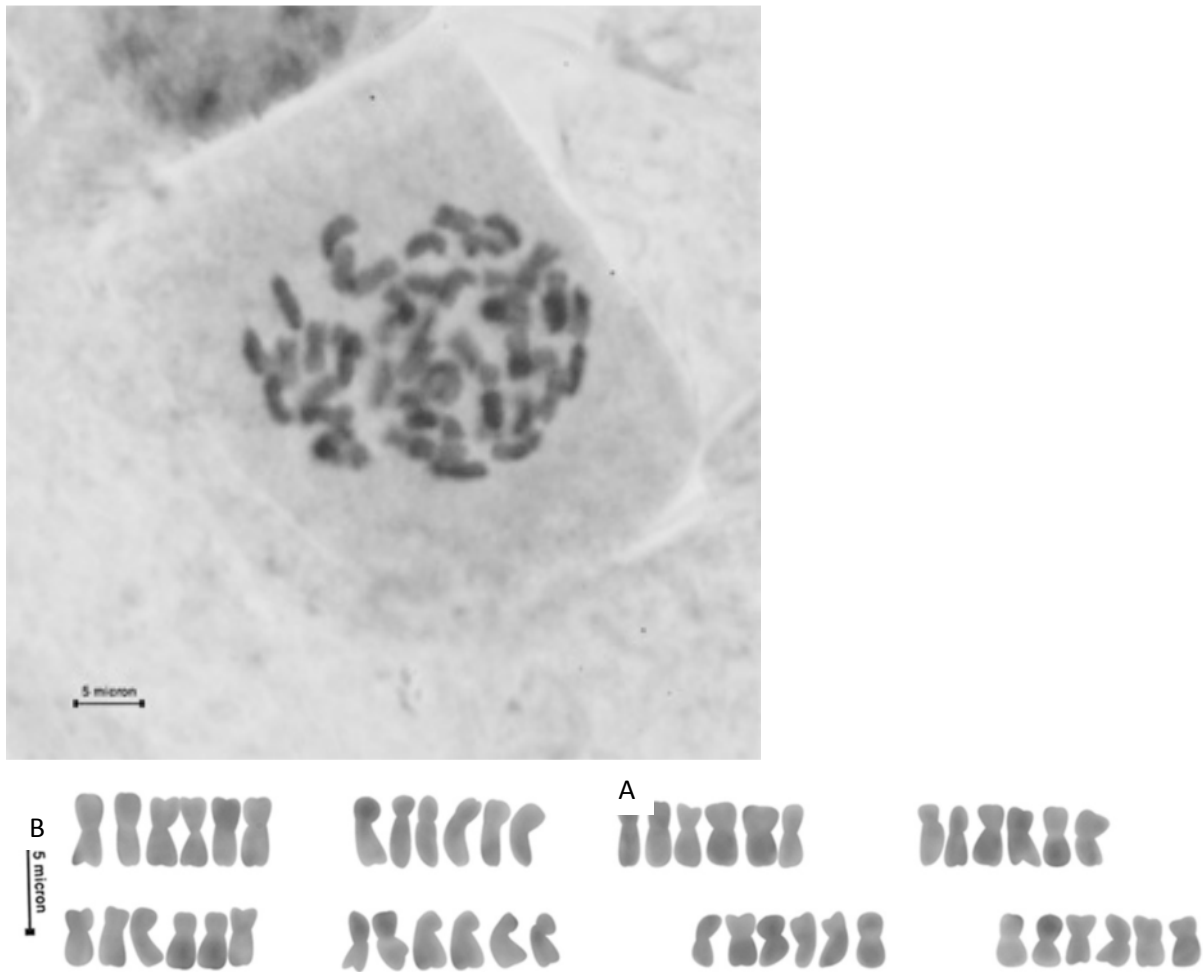


Fig. 2. Somatic metaphase chromosomes (A) and karyotype of *Astragalus remotiflorus* subsp. *nigrohirsutus* (B).

Taxonomic Treatment

Astragalus nigrohirsutus (Tiets & Zarre) Borjian, Maassoumi & Assadi. **comb. et stat. nov.**

Syn. *Astragalus remotiflorus* Boiss. subsp. *nigrohirsutus* Tiets & Zarre, Sendtnera 2: 331 (1994).

Plant 20 - 54 cm tall, densely cushion from, ca. 30 - 60 cm in diameter, densely divided from the base. Stem of the current year ca. 5 cm long densely covered with stipules. Stipules membranous, 7 - 13 cm long, acuminate, ciliate, yellowish white, densely covered with short spreading hairs at the margin, ca. 13 mm long, at the base ca. 7 mm adnate to the petiole, along the stem ca. 3 - 4 mm jointed to each other. Leaves 15 - 20 cm long; petiole ca. 3 - 5 cm long; both petiole and rachis densely covered with short spreading hairs. Leaflets 12 - 24 pairs, remote, oblong-elliptic or in upper part obovate, 5 - 10 mm long and 3 - 5 mm wide; both sides densely covered with spreading to

subappressed white hairs; upper side slightly rugose. Raceme 8 - 15 cm long with remote flowers; peduncle ca. 9 - 15 cm long, at the base covered with short spreading white hairs, but below the raceme mixed with black hairs. Bracts falling soon, 5 - 8 mm long, 1.5 - 3.5 mm wide, hairy. Calyx tubular in flowering time, but later inflated, ca. 10 - 22 mm long and 12 - 20 mm wide, with dark purple parallel nerve, densely covered with short black and white hairs; teeth 2.5 - 5 mm long. Corolla yellow to yellowish white; standard 16 - 22 mm long; limb ca. 8 - 10 mm long and 7 - 10 mm wide, ovate to oblong-elliptic, slightly retuse at the apex; wings 16 - 21 mm long; the limb 5 - 9 mm long and 2 - 3 mm wide; keel 14 - 19 mm long; limb 4 - 6 mm long and 2 - 3 wide, triangular - ovate to oblong. Ovary hairy; style at base pilose. Pods elliptic, 5.5 - 9 mm long and 4 - 8 mm wide, and 2 - 3 mm in diameter, dorsiventrally compressed, densely covered

Table 3. Karyotypic features and karyotype symmetry measurement parameters.

Taxon	KF	2n	SC	AR	CI	VRC	DRL	TF%
<i>Astragalus remotiflorus</i> subsp. <i>remotiflorus</i>	5sm + 3m	2m = 2x = 16	2B	1.75	0.379	2.73	8.02	33.37
<i>Astragalus remotiflorus</i> subsp. <i>nigrohirsutus</i>	15m+9sm	2n = 6x= 48	2A	1.60	0.396	1.15	1.99	39.39

Karyotype formula (KF). Chromosome number (2n). Stebbin's asymmetry category (SC). Arm ratio (AR). Centromeric index (CI). Relative value of chromatine (VRC). difference between relative length of the longest and the shortest chromosomes (DRL). Total form percentage (TF%).

with spreading to subappressed hairs. Seed 4 – 6 mm long, 3 – 3.5 mm wide, reniform, brownish – green.

Specimens seen: Fars: Fasa, Kharman kuh, 3122 m, N: 29, 12 ; E: 53, 34, Borjian s. n.; Fasa, Kharman kuh, 3122 m, N: 29, 12 ; E: 53, 34, Borjian s. n.; Fasa, Kharman kuh, 3122 m, N: 29, 12 ; E: 53, 34, Borjian 1366.

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