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Karyotype characterization of Turkish taxa of the genus *Asperula* L. - section Thlipthisa (Rubiaceae)

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Abstract

Karyotype studies were performed in five taxas of *Asperula viz.*, *Asperula brevifolia, A. pseudochlorantha* var*. pseudochlorantha, A. pseudochlorantha* var*. antalyensis, A. serotina* and *A. purpurea* subsp. *apiculata* from the section Thlipthisa, and their diploid (2n), and the basic (x) chromosome numbers were determined as 2n=22 and x=11, respectively. Chromosome length varied from 0.80 µm (*A. serotina*) to 2.40µm (*A. brevifolia*). Chromosomes are principally metacentric except for *A serotina*, which has submetacentric chromosomes. *A. serotina* showed the highest intrachromosomal assymetry coefficient (A_1) index and *A. pseudochlorantha* var*. antalyensis* represented the highest interchromosomal asymmetry coefficient (A_2). The chromosome numbers of *A. brevifolia, A. serotina* and *A. purpurea* subsp. *apiculata* were reported for the first time.

Key words: *Asperula,* chromosome, karyology, rubiaceae

Introduction

The Rubiaceae (coffee family) is the fourth largest family of flowering plants after Asteraceae, Orchidaceae and Fabaceae. It contains approximately 13,200 species belonging to 620 genera, of which 26 genera have more than 100 species [1]. *Asperula* L., with a total of 183 species, is one of the most important genera in the family. The total number of *Asperula* taxa is 230 with subspecies and varieties [2].

The most comprehensive information on the distribution of *Asperula* in Turkey is in the Flora of Turkey and East Aegean Islands [3]. *Asperula* includes 51 taxa in six sections in the Turkish flora, 26 of which are endemic. *A. brevifolia* Vent., *A. pseudochlorantha* Ehrend. var. *pseudochlorantha*, *A. pseudochlorantha* var. *antalyensis* (Ehrend.) Minareci et Yildiz, *Asperula serotina* (Boiss. et Heldr.) Ehrend and *A. purpurea* (L.) Ehrend. subsp. *apiculata* (Sibth. et Sm.) Ehrend belongs to sect. *Thlipthisa* (Griseb.) Ehrend. The taxa in sect. *Thlipthisa* are all perennials. Their flowers are shortly pedicellate to sessile, subtended by short bracts and bracteoles; the corollas are mostly 4-merous, usually without filiform appendages.

In *Asperula*, multiple molecular genetic studies [4, 5], taxonomical studies [2, 3, 6] and studies on the chromosome number and morphology [2, 7-10] have been conducted. There are five *Asperula* taxa that takes place in *Thlipthisa* section in Turkey. Chromosome number of only two of them have been determined in the present study; the chromosome number 2n=22 have been found in *A. pseudochlorantha* var. *pseudochlorantha* and *A. pseudochlorantha* var. *antalyensis* [2]. The present investigation was underataken to study five realted texa with respect to karyology in order to better understand the taxonomy and evolution in the group concerned.

Materials and methods

Five taxa of *Asperula* section *Thlipthisa* which are endemic to Turkey were examined. Karyological observations were based on the material collected between 2005 and 2007 from natural populations (Table 1). The chromosome counts were obtained from root tip metaphases. The root tips were pretreated in an aqueous solution of 8-hydroxyquinoline (0.002% w/v) or a mixture of 1:1 (v/v) 8-hydroxyquinoline (0.002% w/v) : colchicine (0.3% w/v) for 3-24 h, depending on the material, and followed by fixation in 3:1 (v/v) absolute ethanol : glacial acetic acid for 24 h at 0-4 °C. Differentiation of the pretreatment was aiming at

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Table 1. The location and habitats of studied specimens of *Asperula*

Taxa	Specimen location and habitat
A. brevifolia	Mugla: Marmaris, Datça to Knidos, limestone rocks 480 m, 36°42'534"N, 27°34'223"E, 21.05.2007
A. pseudochlorantha var. *pseudochlorantha*	Antalya: Kemer, under *Pinus bruita* forest, 50–100 m, 36°35' 341"N, 30°30'311"E, 07.06.2007
A. pseudochlorantha var. *antalyensis*	Antalya: Göynük, west slope of town, rocky area, 60 m, 36°40' 307"N, 30°32'209"E, 11.06.2007
A. serotina	Karaman: Ermenek towards Mut 62. km, 885 m, 36°36'039"N, 33°03'195"E, 13.07.2006
A. purpurea subsp. *apiculata*	Tekirdað: Þarköy, 7 km from Uçmakdere to Yeniköy, 40°50' 175"N, 27°23'077"E, 14.06.2007

optimum spreading and optimum condensation of chromatin. Thus, 8-hydroxiquinoline was used for the taxa *A. pseudochlorantha* var. *pseudochlorantha*, *A. pseudochlorantha* var. *antalyensis* and *Asperula serotina*, a mixture of 8-hydroxyquinoline : colchicine for *A. brevifolia* and *A. purpurea* subsp. *apiculata*. Fixed root tips were stored at –20°C in 70% ethanol for one day or up to several weeks. After, they were hydrolysed in 1N HCl for 10-14 min at 60°C, and placed in Feulgen's stain for ca. 2-4 h. The stained root tips were macerated in 45% (v/v) acetic acid on a slide and counterstained in lacto-propionic orcein prior to squashing.

The preparations obtained were examined and photographed by using an Olympus trinocular microscope with D-plan 100-1.25 160/0.17 oil immersion objective and NFK x 3.3 LD 125 lens. A great number of seeds taken from at least 5 specimens belonging to each taxon were germinated for the study. At least ten well-spread metaphase plates of each taxon were analyzed karyologically measuring long arm (L), short arm (S) and total chromosome length (TCL); relative lengths (%); arm ratios (L/S); arm index and location of the centromere and were used to construct the ideograms. Relative chromosome length and arm ratios were calculated according to Krikorian *et al*. [11]. Absolute chromosome length and the centromeric index were used for comparison of the karyotypes. Chromosome morphology was determined according to Levan *et al*. [12]. Karyotype asymmetry was calculated according to the indexes suggested by Romero-Zarco [13].

Results and discussion

In this study, five taxa of *Asperula* were included of which four are endemic to Turkey. Chromosome numbers and the karyotype of these taxa were determined and presented in Tables 2 & 3. The karyomorphology of the taxa studied here have been described for the first time. The number of somatic chromosomes of the five taxa was 2n=22 (Fig. 1) with basic chromosome number x=11.

Karyotypes

A. brevifolia: Eleven pairs of metacentric chromosomes were observed at mitotic metaphase (Figs. 1A, 2A). The chromosome length ranged between 1.80µm and 2.40µm. The total haploid chromosome length was 22.50µm. It showed the lowest intrachromosomal and interchromosomal asymmetry coefficients (A_1, A_2) (Table 3, Fig. 3). *A. brevifolia* presents the most symmetric karyotype, as reflected by its highest mean centromeric index and lowest intrachromosomal asymmetry coefficient (A_1); both characters clearly differentiated this species from the remaining taxa.

Table 2. Karyological features of five *Asperula* taxa

Taxa	L	S	CL	TCL	Mean arm ratio L/S
A. brevifolia	1.08 (0.1)	0.97 (0.1)	2.05 (0.2)	22.50 (2.8)	1.12 (0.1)
A. pseudochlorantha var. *pseudochlorantha*	0.74 (0.2)	0.61 (0.1)	1.36 (0.2)	14.92 (1.9)	1.22 (0.2)
A. pseudochlorantha var. *antalyensis*	0.70 (0.2)	0.61 (0.1)	1.31 (0.3)	14.40 (1.6)	1.14 (0.1)
A. serotina	0.62 (0.1)	0.45 (0.1)	1.07 (0.2)	11.80 (1.4)	1.41 (0.3)
A. purpurea subsp. *apiculata*	1.00 (0.1)	0.82 (0.1)	1.82 (0.3)	19.97 (3.1)	1.24 (0.2)

L = Long arm, S = short arm, CL = mean chromosome length, TCL = total haploid chromosome length. Figure within parentheses indicates standard deviation (±)

Table 3. Comparison of chromosome properties of *Asperula* taxa

Taxa	Chrom. no. (2n)	Chromosome lenght (µm)			KF	CI	A1	A2
		Mean	Max	Min				
A. brevifolia	22	2.05	2.40	1.80	22m	47.3	0.099	0.114
A. pseudochlorantha var. *pseudochlorantha*	22	1.36	1.71	1.00	22m	45.5	0.153	0.171
A. pseudochlorantha var. *antalyensis*	22	1.31	1.85	0.82	22m	46.9	0.114	0.256
A. serotina	22	1.07	1.40	0.80	18m+4sm	42.2	0.254	0.172
A. purpurea subsp. *apiculata*	22	1.82	2.20	1.32	22m	44.9	0.179	0.139

m= metacentric, sm= submetacentric, KF= karyotype formula, CI= centromeric index, A_1= asyimetry coefficent intrachromosomic and A_2 = asyimetry coefficent interchromosomic.

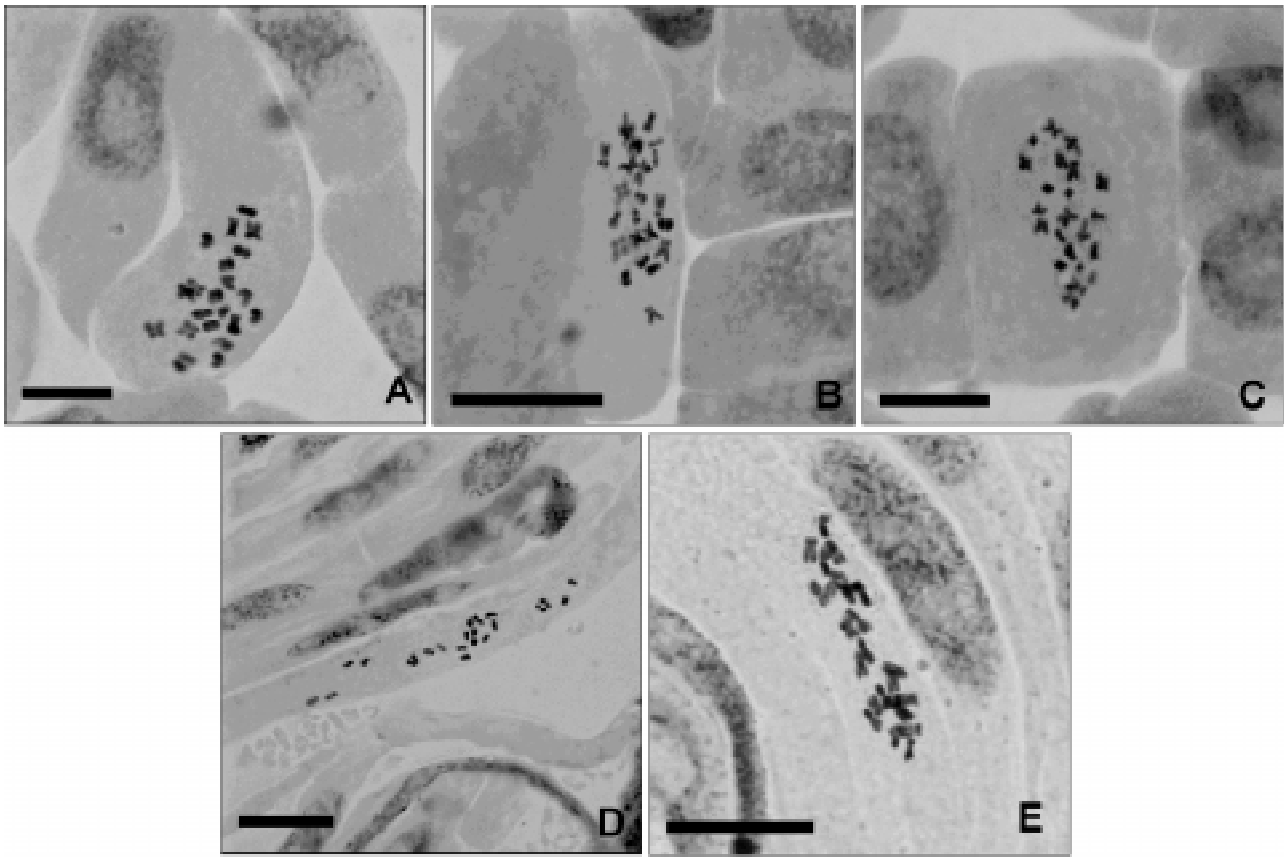

Fig. 1. Mitotic metaphase chromosomes of *Asperula* taxa, A = *A. brevifolia*; B = *A. pseudochlorantha* var. *pseudochlorantha*; C = *A. pseudochlorantha* var. *antalyensis*; D = *A. serotina* and E = *A. purpurea* subsp. *Apiculata*; Scale bar = 10 µm.

A. pseudochlorantha var. *pseudochlorantha*: Eleven pairs of metacentric chromosomes were observed at mitotic metaphase (Figs. 1B, 2B). The chromosome length varied between 1.00µm and 1.71 µm. The total haploid chromosome length was 14.92 µm. *A. pseudochlorantha* var. *pseudochlorantha* and *A. pseudochlorantha* var. *antalyensis* are the most similar taxa morphologically as well as in chromosome mean value. Similarly, the morphologically dissimilar taxa (*A. brevifolia* and *A. serotina*) were also found to be dissimilar in mean chromosome length. This result is in agreement with the findings of similar studies [2].

A. pseudochlorantha var. *antalyensis* : Eleven pairs of metacentric chromosomes were observed at mitotic metaphase (Figs. 1C, 2C). The chromosome length ranged between 0.82 µm and 1.85µm. The total haploid chromosome length was 14.40µm (Table 2). It showed the highest interchromosomal asymmetry coefficient (A_2) (Fig. 3) reflecting the amplitude of its chromosome size (Table 3, Fig. 3). The karyotype asymmetry index is a good expression of the general morphology of plant chromosomes and the magnitude of its difference is an indirect indication of the number of chromosome rearrangements that have occurred between species [14].

A. serotina. Nine pairs of metacentric and two pairs of submetacentric chromosomes were observed at mitotic metaphase (Figs. 1D, 2D). The chromosome length ranged between 0.80µm and 1.40µm. The total haploid chromosome length was 11.80µm. Among the taxa studied, it showed the highest intrachromosomal asymmetry coefficient (A_1) (Fig. 3).

A. purpurea subsp. *apiculata*. Eleven pairs of metacentric chromosomes were observed at mitotic metaphase (Figs. 1E, 2E). The chromosome length varied between 1.32µm and 2.20µm. The total haploid chromosome length was 19.97µm (Table 2).

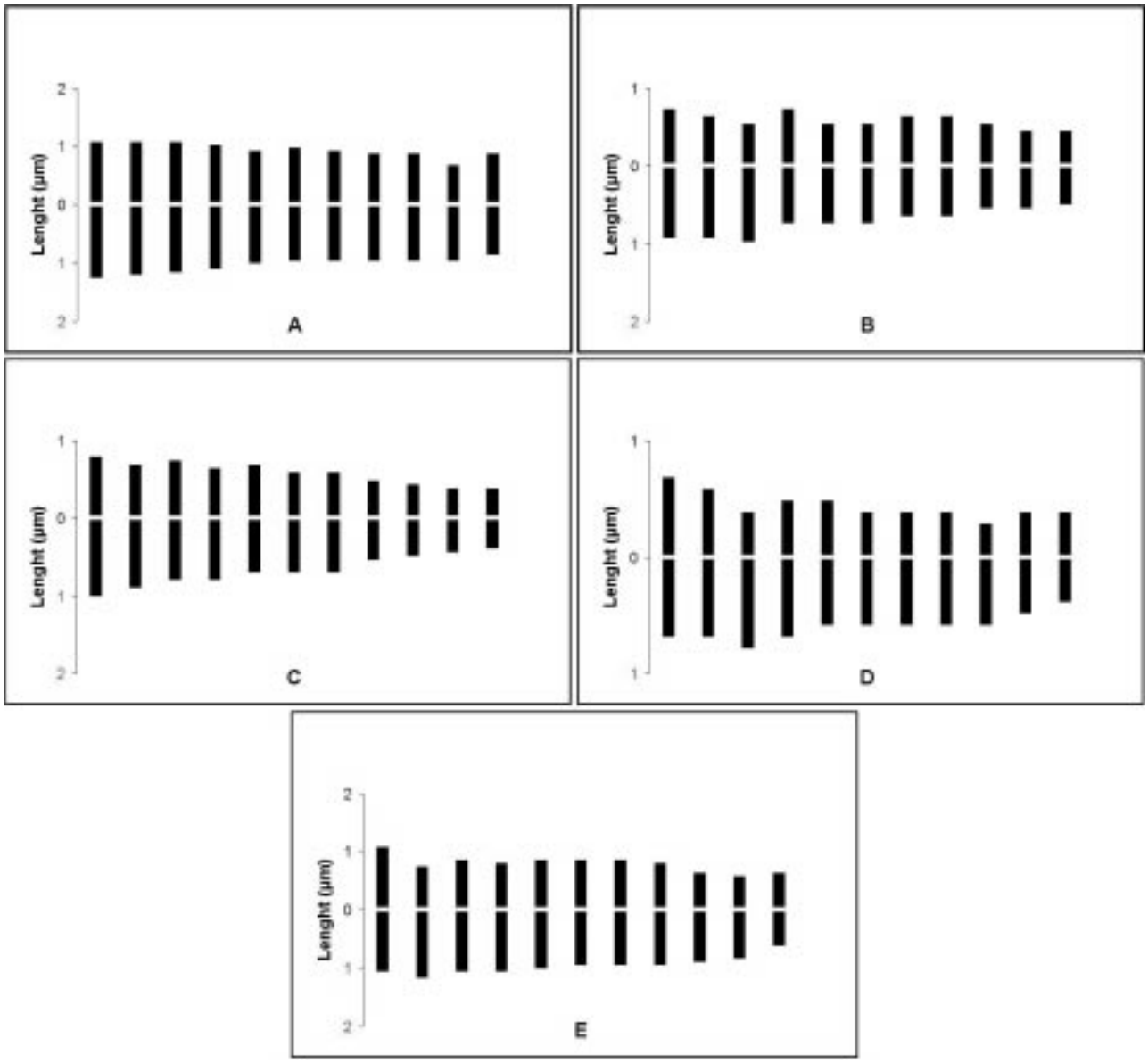

Fig. 2. Ideogram of *Asperula* taxa, A= *A. brevifolia;* B= *A. pseudochlorantha* var*. pseudochlorantha*; C= *A. pseudochlorantha* var*. antalyensis*; D= *A. Serotina* and E= *A. purpurea* subsp. *apiculata*.

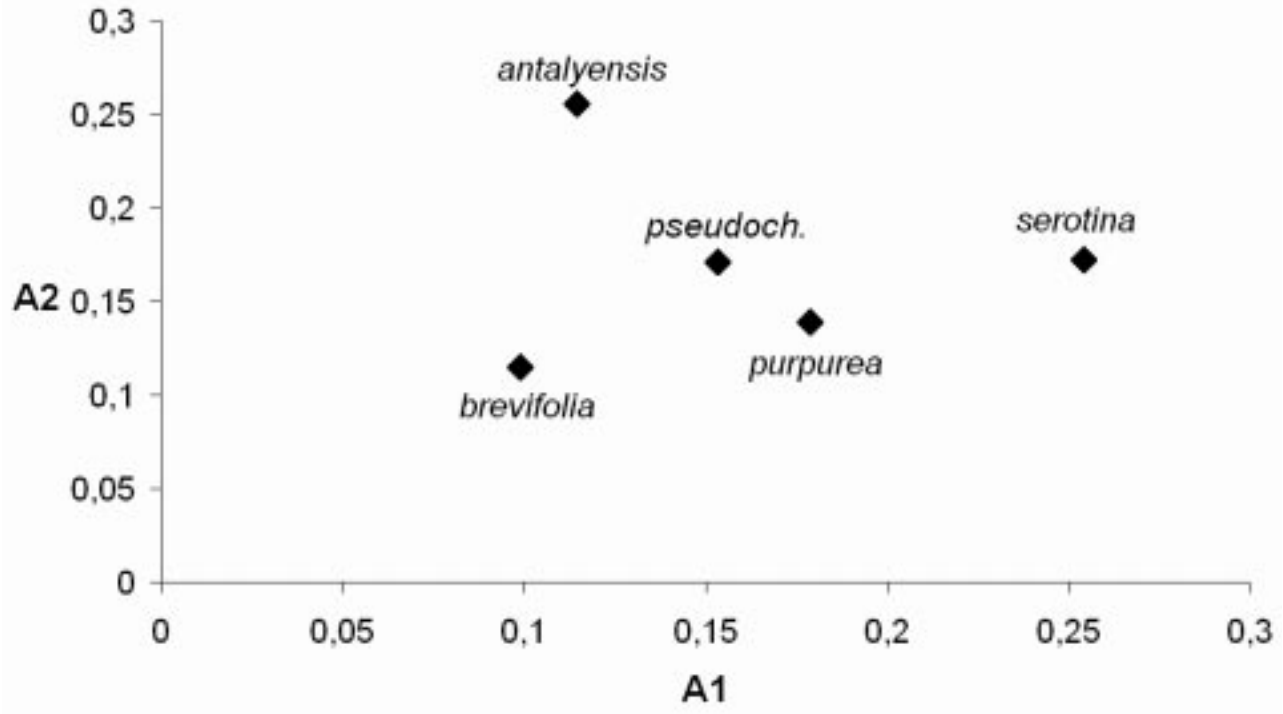

Fig. 3. Dispersion graphic of asymmetry coefficient, A_1 and A_2 of *Asperula* taxa

This study confirmed the diploid chromosome numbers of *A. pseudochlorantha* var. *pseudochlorantha* and *A. pseudochlorantha* var. *antalyensis* as 2n=22 with basic chromosome number as 11. Chromosomal counts of three new taxa namely, *A. brevifolia*, *A. serotina* and *A. purpurea* subsp. *apiculata* were also determined and it was found to be 2n=22.

Out of 230 taxa of *Asperula* worldwide, the chromosome number of about 32 *Asperula* taxa has thus far been determined. The chromosome numbers 2n=20, 22, 40, 44 have been reported. The previous studies [2, 7-10, 15] have reported that this genus has two different basic chromosome number i.e. x=10 and 11. The diploidy percentage is about 90% among the karyologically studied taxa of *Asperula*. Chromosome dimensions are comparable among the taxa which belong to the same section [9, 10]. Although, there are not big differences of chromosome dimensions among the taxa which have similar morphological features and belong to the same sections, but there are big differences of mean chromosome length between the taxa which have obvious morphological differences. It was observed that the differences of chromosome length are positively proportional to the morphological differences in *Asperula* taxa. In section *Thlipthisa*, *A. serotina* and *A. purpurea* subsp. *apiculata* are allied taxa morphologically [3], but differences in mean chromosome length were observed. Additional differences were recorded by means of other morphological and palynological studies as reported by Minareci *et al*. [6].

Chromosomal features of *A. pulvinaris* (Boiss.) Heldr. ex Boiss. were reported by to be 2n= 4x=44 [15], the population examined is tetraploid with x=11, chromosomes of small to medium size (between ca. 1.7 to 4.8µm), its karyotype is symmetrical with metacentric (m) and submetacentric (sm) chromosomes, at least one small pair of faintly stained satellites exists on a submetacentric chromosome pair (sm-SAT). This result thus contrasts with result of the current study in regard of the number and the mean length of chromosomes (Table 3). It can be explained as follows: *Cynanchicae* section that covers *A. pulvinaris* includes many polymorphic diploid and tetraploid races with basic chromosome number x=10. These races are connected with each other by transitional (perhaps hybridogenous) populations. These chromosome types are the most common in the family whereas subtelocentric are too rare [17-20]. The results of the present study will thus help in understanding the

differentiation of similar taxa and their taxonomic position, and may form the base for future biosystematic studies in *Asperula*.

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