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THEOPHANIS CONSTANTINIDIS, GEORGIA KAMARI & DIMITRIOS PHITOS

## A cytological study of 28 phanerogams from the mountains of SE Sterea Ellas, Greece

### Abstract

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28 phanerogams of various families, from the mountains of Gerania, Pateras, Kitheron, Pastra and Elikon (SE Sterea Ellas, Greece) are cytologically studied. The chromosome numbers of 13 taxa (*Aristolochia microstoma*, *Asperula baenitzii*, *A. pulvinaris*, *A. rigidula*, *Centaurea subsericans*, *Conium divaricatum*, *Johrenia distans*, *Peucedanum vittijugum* subsp. *vittijugum*, *Scorzonera serpentina*, *Thlaspi pindicum*, *Thymus parnassicus*, *Th. teucrioides* subsp. *candilicus*, and *Verbascum boissieri*) are presented for the first time. In addition, Greek populations of 11 taxa are cytologically examined for the first time. Mitotic metaphase photomicrographs and/or karyograms are presented for the majority of taxa studied. Brief comments are given on the karyotype morphology, cytogeography and relationships of selected taxa.

### Introduction

Extensive cytological studies have been programmed as part of the “Flora Hellenica” project, mainly concentrated on endemic and phytogeographically interesting taxa of the Greek flora. The cytological investigation presented here deals with taxa from the mountains of Gerania, Pateras, Kitheron, Pastra and Elikon (SE Sterea Ellas, Greece). Where available, additional material of the same taxa but of different provenance has been included. In most cases, the karyotype morphology of the taxa studied is illustrated (photomicrographs and/or karyograms are presented) and comments on their karyotype, cytogeography and other aspects of their biology are given. Our results are summarized in Tab. 1. This paper forms part of the first author’s thesis, currently in progress, which deals mainly with the flora and vegetation of the mountains Gerania, Pateras and Kitheron.

### Material and methods

Living plants or seeds of all taxa investigated were collected by the first author (abbreviated as *Const.* in the text) during several field trips, and cultivated in the experimental botanical garden of the University of Patras. Vouchers are deposited in the herbarium of the University of Patras (UPA).

The nomenclature follows Greuter & al. (1984–89), Strid (1986), Strid & Tan (1991) and Tutin & al. (1968–80, 1993).

Tab. 1. Alphabetical list of the taxa investigated with provenance and chromosome number; ● = first report; \* = first report for a Greek population; ⊕ = new ploidy level.

Taxon	Provenance	2n
<i>Aceras anthropophorum</i>	Mt Pateras	42 *
<i>Aristolochia microstoma</i>	Mt Kitheron	10 ●
<i>Asperula baenitzii</i>	Mt Pateras	22 ●
<i>A. pulvinaris</i>	Mt Pateras	44 ●
<i>A. rigidula</i>	Mt Pateras	44 ●
<i>Bellevalia ciliata</i>	Mt Kitheron, Viotia area	8 *
<i>Bufonia stricta</i> subsp. <i>stricta</i>	Mt Gerania	18
<i>Centaurea attica</i> subsp. <i>megarensis</i>	Mt Gerania	36 ⊕
<i>C. subsericans</i>	Mt Elikon	18 ●
<i>Cephalaria setulifera</i>	Mt Pateras	18 + 0–1B *
<i>Cerastium dichotomum</i>	Mt Pateras	38 + 0–2B *
<i>Clematis cirrhosa</i>	Mt Gerania	16 + 0–1B *
<i>Conium divaricatum</i>	Mt Pastra	22 + 0– B ●
<i>Coris monspeliensis</i>	Mt Gerania	18 *
<i>Delphinium fissum</i> subsp. <i>fissum</i>	Mt Kitheron	16 *
<i>D. peregrinum</i>	Mt Pateras	16 *
<i>Helminthotheca echioides</i>	Mt Pastra	10 *
<i>Johrenia distans</i>	Mts Pateras, Ipaton, Parnassos & Athos	22 + 0–1 B ●
<i>Malabaila aurea</i>	Mt Pateras	20
<i>Mantisalca salmantica</i>	Mt Pateras	22 + 0–3 B *
<i>Peucedanum vittijugum</i> subsp. <i>vittijugum</i>	Mt Pateras	22 + 0–1 B ●
<i>Scorzonera crocifolia</i>	Mt Pateras	14
<i>S. serpentinica</i>	Mt Gerania	14 ●
<i>Senecio macedonicus</i>	Mt Kitheron	40 *
<i>Thlaspi pindicum</i>	Mt Gerania	14 ●
<i>Thymus parnassicus</i>	Mt Kitheron	90 ●
<i>Th. teucrioides</i> subsp. <i>candilicus</i>	Mt Gerania	30 + 0–1 B ●
<i>Verbascum boissieri</i>	Mt Gerania	36 ●

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), a mixture of 1 : 1 (v/v) 8-hydroxyquinoline (0.002% w/v) : colchicine (0.3% w/v), or a saturated aqueous solution of  $\alpha$ -bromonaphthalene 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 optimum spreading and optimum condensation of chromatin. Thus, 8-hydroxyquinoline was used for the genera *Aceras*, *Bufonia*, *Centaurea*, *Cerastium*, *Conium*, *Coris*, *Helminthotheca*, *Johrenia*, *Malabaila*, *Mantisalca*, *Peucedanum*, *Thlaspi*, and *Thymus*, a mixture of 8-hydroxyquinoline : colchicine for *Bellevalia*, *Cephalaria*, *Clematis*, *Delphinium*, *Scorzonera*, and *Senecio*, and  $\alpha$ -bromonaphthalene for *Aristolochia*, *Asperula*, and *Verbascum*. 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 c. 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.

At least five photomicrographs of each taxon were examined, taken with a Zeiss Axiophot photomicroscope. Permanent preparations of all taxa examined were made, following, with

minor modifications, the method described by Östergren & Heneen (1962), and are kept in the Botanical Institute of the University of Patras.

Chromosome terminology follows principally Levan & al. (1964) and Stebbins (1971). Comments and suggestions given by Sybenga (1959), Bentzer & al. (1971) and Favarger (1978) were also taken into consideration.

All taxa investigated are listed by their families, in alphabetical order.

## Results

### *Aristolochiaceae*

*Aristolochia microstoma* Boiss. & Spruner – Fig. 1.

$2n = 10$

GREECE: Sterea Ellas, Mt Kitheron, the summit Korifi and its southern and western slopes, open, stony places, c. 1000 m, 38°11'N, 23°18'E, 16.4.1994, *Const. 4435* (UPA).

*Aristolochia microstoma* is a distinct species endemic to Greece, distributed in southern parts of Sterea Ellas and the northern Peloponnisos (Nardi 1991). Its chromosome number,  $2n = 10$ , and karyotype are presented here for the first time. The chromosomes are small, c. 1.2 to 3.1  $\mu\text{m}$ , metacentric (m) or submetacentric (sm), with one pair of satellites clearly evident on a metacentric chromosome pair (m-SAT) (Fig. 1). Nardi (1991) suggested that this species shows affinities to the *A. pallida* group, which includes *A. pallida* Willd., *A. lutea* Desf., *A. tyrrhena* Nardi & Arrigoni, and *A. elongata* (Duchartre) Nardi, as well as to *A. clusii* Lojak. Three of these five species have the same chromosome number  $2n = 10$ , while *A. lutea* and *A. clusii* have  $2n = 8$  and  $2n = 12$ , respectively (Nardi 1984, 1989, 1991).

### *Caryophyllaceae*

*Bufonia stricta* (Sm.) Gürke subsp. *stricta*

$2n = 18$

GREECE: Sterea Ellas, Mt Gerania, c. 2.5 km east of the summit Makriplagi, limestone rocks, c. 1000 m, 38°01'N, 23°09'E, 20.6.1993, *Const. 3901* (UPA).

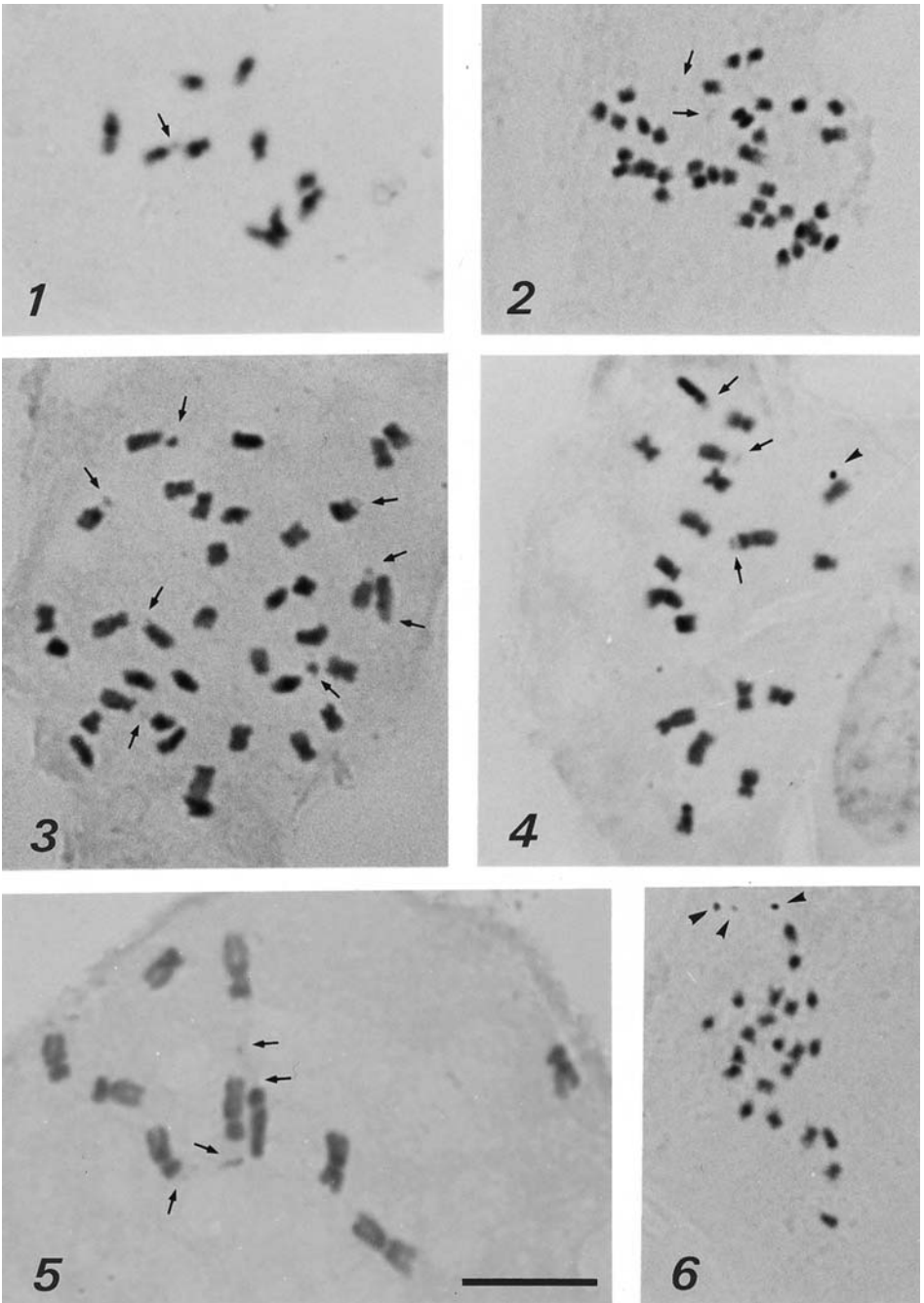
*Bufonia stricta* subsp. *stricta*, a Greek endemic, is known from several localities in Crete, but is rare on the mainland, where it is only known from Mt Parnon (Peloponnisos) and a few mountains in Attiki. Montmollin (1984, 1986) studied two Cretan populations and counted  $2n = 18$  and  $n = 9$  respectively. Our results, the first from the Greek mainland, confirm the previous counts. The related, recently described *B. euvoica* Phitos & Kamari also has  $2n = 18$  (Phitos & Kamari 1992).

*Cerastium dichotomum* L. – Fig. 2.

$2n = 38$

GREECE: Sterea Ellas, Mt Pateras, north-west of the village of Veniza, edges of cultivated fields, c. 380 m, 38°05'N, 23°16'E, 21.4.1991, *Const. 1428* (UPA).

*Cerastium dichotomum* is known from a few localities on the Greek mainland and Crete where it is mostly associated with cultivated land. The chromosome number  $2n = 38$  and, in some cases, karyotype drawings were previously given by Brett (1952, 1955, origin of material not indicated), Söllner (1952, 1954, material from Algeria and Iran), Aryavand & Favarger (1980, material from Iran), Galland (1988, material from Morocco), Celebioglu & Favarger (1993, material from Anatolia), and Lara Ruiz (1993, material from Spain). Our chromosome counts seem to be the first from Greece and confirm the previous counts; in addition, a karyotype photomicrograph of *C. dichotomum* is presented here (Fig. 2). The chromosomes are small, c. 1.0 to 2.0  $\mu\text{m}$ , therefore detailed observation of their morphology is difficult. The majority, however, appear to be metacentric (m), and two of them are satellited (m-SAT).



Figs 1–6. Mitotic metaphase plates – 1: *Aristolochia microstoma*,  $2n = 10$ ; 2: *Cerastium dichotomum*,  $2n = 38$ ; 3: *Centaurea attica* subsp. *megarensis*,  $2n = 36$ ; 4: *Centaurea subsericans*,  $2n = 18 + 1B$ ; 5: *Helminthotheca echioides*,  $2n = 10$ ; 6: *Mantisalca salmantica*,  $2n = 22 + 3B$ . – Arrows indicate SAT-chromosomes and arrowheads B-chromosomes. Scale bar: 10  $\mu\text{m}$ .

**Compositae*****Centaurea attica* subsp. *megarensis* (Halácsy) Dostál – Fig. 3.** $2n = 4x = 36$ 

GREECE: Sterea Ellas, Mt Gerania, the western and south-western slopes of the summit Korifi, sparse bushes on ophiolitic substrate, c. 900 m, 38°02'N, 23°04'E, 20.6.1994, *Const.* 4829 (UPA).

*Centaurea attica* is a polymorphic species endemic to E Central Greece, which, according to Georgiadis (1980), comprises four subspecies. Subsp. *megarensis* is restricted to ophiolitic substrates on Mt Gerania above 500 m. Previous examination of this subspecies by Georgiadis & Phitos (1976) revealed a chromosome number of  $2n = 18$ . Our count of  $2n = 36$  indicates the additional existence of a tetraploid cytotype of this subspecies. The chromosomes (Fig. 3) are of about 1.5 to 3.1  $\mu\text{m}$  in size, the majority is submetacentric (sm), the rest metacentric (m). Eight satellites were found in the complement, four of them being quite large and situated on the short arm of submetacentric chromosomes (sm-SAT), the remaining four are smaller and not always visible.

***Centaurea subsericans* Halácsy – Fig. 4.** $2n = 18 + 0-1 \text{ B}$ 

GREECE: Sterea Ellas, Mt Elikon, on the southern and south-western slopes of the summit Paliovouna, calcareous rocks above the timberline, c. 1500–1600 m, 38°17'N, 22°52'E, 16.7.1994, *Const.* 5013 (UPA).

*Centaurea subsericans* is a critical endemic species of *C.* sect. *Acrolophus*, described from Mt Pateras and known only from its classical locality and Mt Elikon. This is the first report of its chromosome number and karyotype. The chromosomes are small, c. 1.6 to 3.0  $\mu\text{m}$ . Four pairs of metacentric (m), two pairs of submetacentric (sm) and four pairs of submetacentric to acrocentric (sm/st) chromosomes were observed. The largest chromosome pair shows structural heterozygosity with respect to the centromeric position. The two longest chromosomes and two of the submetacentric ones bear clearly visible satellites on their short arms (sm-SAT). A small B-chromosome is sometimes present in the complement (Fig. 4).

Wagenitz (1989) and Gamal-Eldin & Wagenitz (1991) reported the close affinity of *C. subsericans* to *C. pseudocadmea* Wagenitz, questioning whether the latter can be maintained as a distinct species despite its placement in a different section, *C.* sect. *Phalolepis*. Recent collections confirm the pronounced variability of some *Centaurea* populations on Mts Pateras, Kitheron and Elikon, with *C. subsericans*, *C. pseudocadmea* and *C. attica* Nym. subsp. *pateraea* (Halácsy) Georg. exhibiting a pattern of variation that could be due to hybridization. According to our present knowledge, *C. pseudocadmea* has a tetraploid karyotype with  $2n = 4x = 36$  (Constantinidis & Kamari 1994), while both *C. attica* subsp. *pateraea* (Georgiadis 1980) and *C. subsericans* are diploids ( $2n = 18$ ). The two latter taxa grow on the same mountain (Mt Pateras) but in different parts and are distinct, although clearly related.

***Helminthotheca echioides* (L.) Holub  $\equiv$  *Picris echioides* L. – Fig. 5.** $2n = 10$ 

GREECE: Sterea Ellas, Mt Pastra, the area between the summits Panorama and Korifoula, calcareous slopes and dolines, c. 850 m, 38°12'N, 23°27'E, 7.7.1995, *Const.* 5687 (UPA).

*Helminthotheca echioides*, being widespread in the Mediterranean area and introduced elsewhere (e.g. Holzapfel 1994), has been cytologically examined on material from several countries (see Pastor & al. 1990, Luque & Díaz Lifante 1991, Kuzmanov 1993, Oberprieler & Vogt 1993, Holzapfel 1994) but no chromosome count in Greek plants has been reported so far. Our count of  $2n = 10$  agrees with earlier reports. The two longest chromosomes in the complement are metacentric (m), appearing unequal in size, while the rest are submetacentric (sm). Four of the submetacentric chromosomes bear satellites on their short arms (sm-SAT) (Fig. 5). The chromosome size ranges between 3.4 to 5.5  $\mu\text{m}$ .

***Mantisalca salmantica* (L.) Briq. & Cavill. – Fig. 6.**

2n = 22 + 0–3B

GREECE: Sterea Ellas, Mt Pateras, close to the village of Ano Alepochori, abandoned land, c. 320 m, 38°05'N, 23°12'E, 16.6.1991, *Const. 2003* (UPA).

*Mantisalca salmantica* has a predominantly W Mediterranean distribution, with few occurrences in Greece. Earlier counts of 2n = 18 (Chiappini 1954, material from Sardinia as *Centaurea salmantica* L.) and n = 10 (Guinochet 1957, material from France as *C. salmantica*) have not been confirmed recently and are probably erroneous. The chromosome number of 2n = 22 was counted in material from Portugal (Guinochet & Foissac 1962, Fernandes & Queirós 1971, Queirós 1973), France (Rashid 1974), Italy (Raimondo & Garbari 1975), Spain (Horjales 1976 as *Microlonchus salmanticus* DC., Hellwig 1994), Libya (Brullo & al. 1990), and Morocco (Oberprieler & Vogt 1993). No previous count is known from Greece. Our count of 2n = 22 is in agreement with the above reports. Furthermore, up to 3 small and unequal B-chromosomes were observed in some metaphase plates. The chromosomes are very small, c. 0.9 to 1.5 µm, and usually metacentric (m). A pair of small satellites exist in the complement but is not always visible.

***Scorzonera crocifolia* Sm. – Figs 7a-b.**

2n = 14

GREECE: Sterea Ellas, Mt Pateras, east of the village of Psatha, phrygana on limestone substrate, c. 480 m, 38°06'N, 23°14'E, 12.5.1991, *Const. 1742* (UPA).

*Scorzonera crocifolia* is an endemic species of Central and S Greece, usually found at altitudes below 1000 m. A previous count in material from the island of Kithira, S Greece, revealed 2n = 14 (Damboldt 1968). This number is confirmed, and in addition the karyotype (Fig. 7a) and karyogram (Fig. 7b) are presented for the first time. The karyotype appears symmetrical, with all chromosomes being metacentric, gradually decreasing in size and ranging from 4.6 to 7.8 µm. One of the two small chromosome pairs bears a small but conspicuous satellite on its short arm. Thus, the karyotype formula of the species is 2n = 12m + 2m-SAT = 14 (Fig. 7b).

***Scorzonera serpentonica* Rech. f. – Figs 8a-b.**

2n = 14

GREECE: Sterea Ellas, Mt Gerania, between the villages of Mazi and Schinos, open *Pinus* forest, ophiolithic substrate, c. 520 m, 38°02'N, 23°07'E, 23.5.1992, *Const. 2436* (UPA).

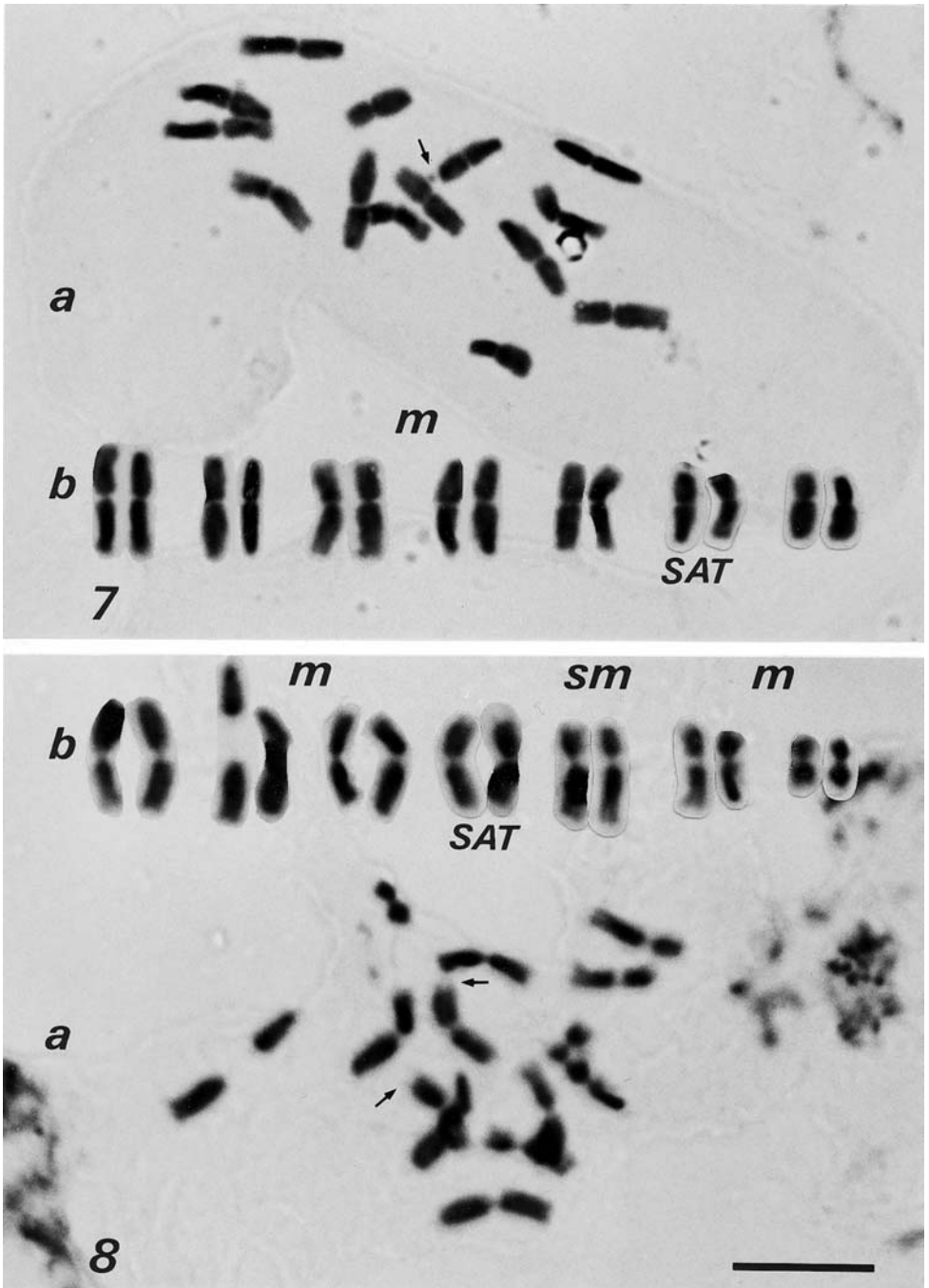
*Scorzonera serpentonica* is an endemic species, previously known only from Central and N Evvia where it grows on serpentine (Rechinger 1961). Material from the serpentine slopes of Mt Gerania approaches the description of *S. serpentonica* in many respects, but the taxonomic differences between the latter and *S. crocifolia* are somewhat vague. *S. serpentonica* probably represents a serpentine adaptation of *S. crocifolia*, with minor modifications especially in the vegetative parts.

Compared to the karyotype of *S. crocifolia*, however, some significant differences were observed in the karyotype of *S. serpentonica*. The latter consists of twelve metacentric and two submetacentric chromosomes (Figs 8a-b) while in *S. crocifolia* all chromosomes are metacentric (Figs 7a-b). The smallest chromosome pair of *S. serpentonica* differs considerably in size from the rest of the complement as well as from that of *S. crocifolia*, resulting in a somewhat more asymmetrical karyotype in *S. serpentonica*. Also in contrast to *S. crocifolia*, the fourth largest chromosome of *S. serpentonica* is satellited; the satellites are faintly stained and not always visible. The karyotype formula of *S. serpentonica* is 2n = 10m + 2m-SAT + 2sm = 14. The chromosome size ranges from c. 3.4 to 7.9 µm.

***Senecio macedonicus* Griseb.**

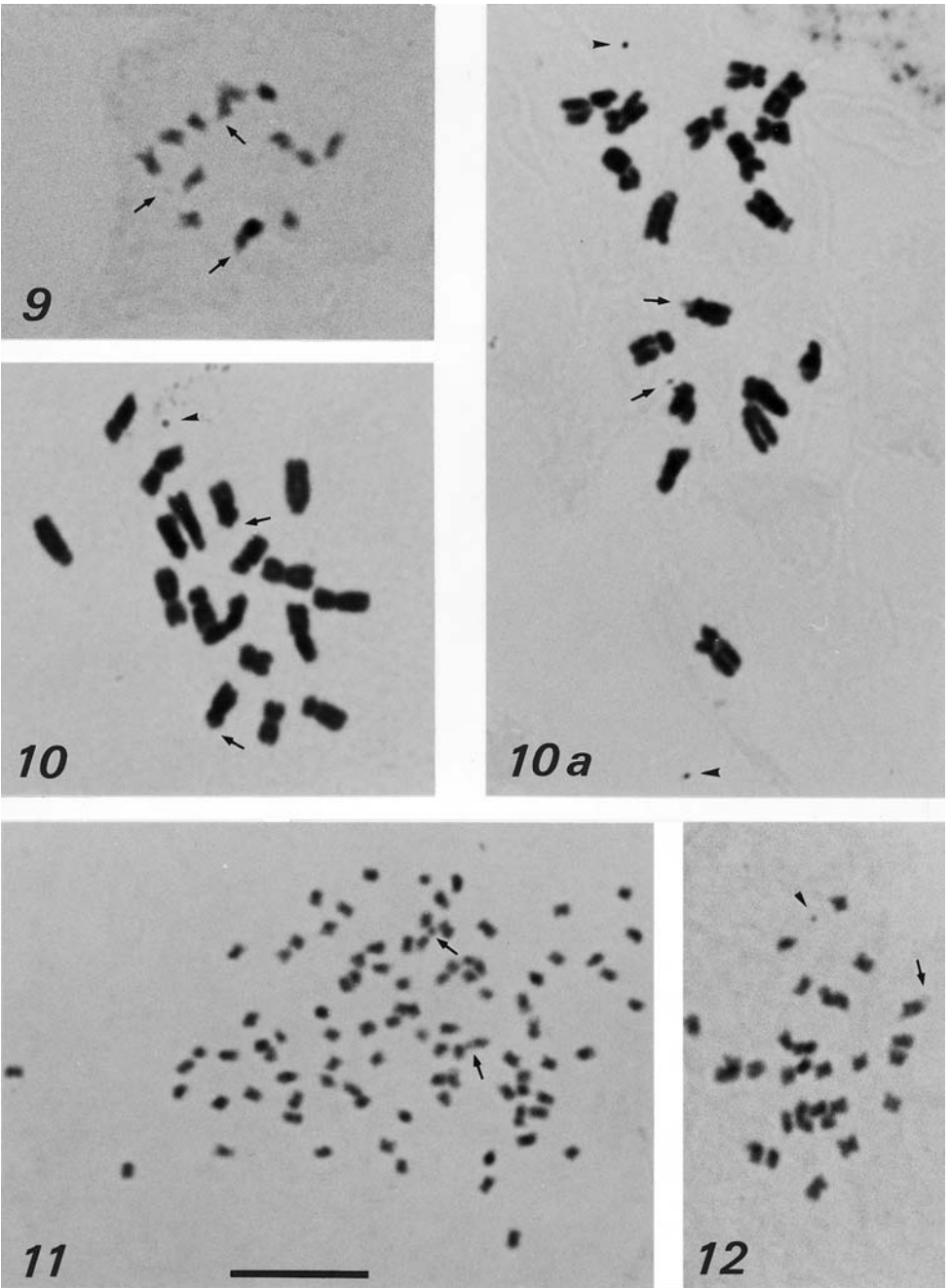
2n = 40

GREECE: Sterea Ellas, Mt Kitheron, the south-western slopes of the summit Gennimata, open stony area, c. 900–960 m, 38°11'N, 23°19'E, 23.4.1995, *Const. 5361* (UPA).



Figs. 7–8. Mitotic metaphase plates (a) and karyograms (b) – 7: *Scorzonera crocifolia*,  $2n = 14$ ; 8: *S. serpentina*,  $2n = 14$ . – Arrows indicate SAT-chromosomes. Scale bar: 10  $\mu\text{m}$ .





Figs 9–12. Mitotic metaphase plates – 9: *Thlaspi pindicum*,  $2n = 14$ ; 10,10a: *Cephalaria setulifera*,  $2n = 18 + 1B, 18 + 2B$ ; 11: *Thymus parnassicus*,  $2n = 6 \times = 90$ ; 12: *Th. teucrioides* subsp. *candilicus*,  $2n = 30 + 1B$ . – Arrows indicate SAT-chromosomes and arrowheads B-chromosomes. Scale bar: 10  $\mu\text{m}$ .

This perennial of the S Balkans is usually found at altitudes above 1000 m. Our chromosome count of  $2n = 40$ , being the first report from Greece, agrees with a previous report from Bulgaria (Kuzmanov & Georgieva 1983).

### Cruciferae

*Thlaspi pindicum* Hausskn. = *Th. tymphaeum* Hausskn. – Fig. 9.

$2n = 14$

GREECE: Sterea Ellas, Mt Gerania, between the summits Makriplagi and Tris Portes, ophiolitic slopes, c. 600–850 m,  $38^{\circ}01'N$ ,  $23^{\circ}06'E$ , 8.4.1995, *Const.* 5242 (UPA).

*Thlaspi pindicum* is endemic to Albania and Greece (Franzén 1986). It is found mainly on serpentine, apparently depicting a disjunct distribution pattern with Mt Gerania as its southernmost limit. To our knowledge, no previous chromosome count of this species has ever been reported. The chromosome number of  $2n = 14$  and a photomicrograph of a metaphase plate (Fig. 9) are presented here. The population examined is diploid, with mostly metacentric (m) small (c. 1.0 to 1.9  $\mu\text{m}$ ) chromosomes. Three to four small, poorly stained satellites were observed in some preparations.

### Dipsacaceae

*Cephalaria setulifera* Boiss. & Heldr. – Figs 10, 10a.

$2n = 18 + 0-2B$

GREECE: Sterea Ellas, Mt Pateras, the summit of Mikri Kolosoura, stony calcareous slopes, c. 850 m,  $38^{\circ}06'N$ ,  $23^{\circ}17'E$ , 29.9.1991, *Const.* 2280 (UPA).

The rare *Cephalaria setulifera* has been reported from Montenegro (Verlaque 1985: 211) and a few localities in Central Greece. Kokkini (1991), however, questions Verlaque's record and considers the taxon (sub *Cephalaria flava* subsp. *setulifera* (Boiss. & Heldr.) Kokkini) as endemic to Greece. Our count of  $2n = 18$  (Figs 10, 10a), to our knowledge the first one from Greece, is in agreement with previous reports by Verlaque (1975, 1977, 1985) on material from Montenegro. The chromosome complement consists of eight metacentric, six submetacentric and four acrocentric chromosomes, ranging in size between 2.5 and 4.4  $\mu\text{m}$ . One submetacentric chromosome pair bears small satellites on its short arms. One to two small, spherical B-chromosomes were detected in some metaphase plates. The karyotype formula is  $2n = 2x = 8m + 4sm + 2sm\text{-SAT} + 4st = 18 + 0-2B$  (Figs 10, 10a). The karyotype of the material examined here shows considerable differences compared to the drawing and idiogram given by Verlaque (1985). Apparently, the taxonomic relationship between the Greek and Yugoslavian populations needs further investigations, but no herbarium material from Montenegro was available to the authors.

### Labiatae

*Thymus parnassicus* Halácsy – Fig. 11.

$2n = 6x = 90$

GREECE: Sterea Ellas, Mt Kitheron, the summit Profitis Ilias, close to an abandoned military camp, calcareous substrate, c. 1380 m,  $38^{\circ}11'N$ ,  $23^{\circ}14'E$ , 12.7.1992, *Const.* 2710 (UPA).

*Thymus parnassicus*, known from a few mountains of Central Greece, is also reported from the south of the former Yugoslavia and appears again disjunct in Central Anatolia (Baden 1991). Its chromosome number of  $2n = 90$  and a photomicrograph of a somatic metaphase (Fig. 11) are given here for the first time. A fairly wide range of chromosome numbers ( $n = 6, 7, 8, 9, 10, 13$  and  $15$ ) have been reported for *Thymus* (Darlington & Wylie 1961, Löve & Löve 1961, 1974, Fedorov 1969, and van Loon 1987). *Th. parnassicus* has a hexaploid cytotype ( $x = 15$ ) with very small chromosomes of c. 0.7 to 1.6  $\mu\text{m}$ , not suitable for detailed morphological studies. Three to four satellites, two of which are considerably larger, can be observed in most metaphase plates. Such a high chromosome number in the genus *Thymus* is quite rare, and to

our knowledge other examples of a hexaploid cytotype with  $x = 15$  have been reported only for *Th. zygioides* var. *lycaonicus* (Čelak.) Ronniger by Jalas & Uotila (1976) in material from Turkey sub *Th. longedentatus* (Degen & Urum.) Ronniger, as well as by Markova (1989) and Markova & Goranova (1994) in material from Bulgaria.

***Thymus teucrioides* subsp. *candilicus* (Beauv.) Hartvig – Fig. 12.**

$2n = 30 + 0-1B$ .

GREECE: Sterea Ellas, Mt Gerania, the western and south-western slopes of the summit Korifi, bushes and shrubs on ophiolithic substrate, c. 900 m,  $38^{\circ}02'N$ ,  $23^{\circ}04'E$ , 20.6.1994, *Const.* 4825 (UPA).

*Thymus teucrioides* subsp. *candilicus* is endemic to and scatteredly distributed in Greece, known mainly from localities in Central Greece (Hartvig 1987). It shows a particular preference for serpentine substrates, and its occurrence on Mt Gerania is the southernmost known limit of its distribution area. Its chromosome number as well as a photomicrograph of a somatic metaphase (Fig. 11) are presented here for the first time. Our investigated population is possibly diploid, with  $x = 15$ . The chromosomes appear to be metacentric (m) and submetacentric (sm), small in size (c. 0.8 to 1.4  $\mu\text{m}$ ), with one submetacentric chromosome pair bearing small satellites (sm-SAT).

**Liliaceae**

***Bellevalia ciliata* (Cyr.) Nees – Figs 13a-d.**

$2n = 8$

GREECE: Sterea Ellas, Mt Kitheron, c. 1.5 km south-west of the village of Kaparelli, edges of cultivated fields, c. 450 m,  $38^{\circ}13'N$ ,  $23^{\circ}12'E$ , 17.4.1993, *Const.* 2984 (UPA); Sterea Ellas, c. 5 km north of Ritsona area, cultivated land, c. 240–270 m,  $38^{\circ}25'N$ ,  $23^{\circ}31'E$ , 20.3.1994, *Const.* 4322 (UPA); Sterea Ellas, c. 2.5 km from the village of Dafni on the way to the village of Dafnoula, cultivated fields, c. 400 m,  $38^{\circ}14'N$ ,  $23^{\circ}26'E$ , 16.4.1994, *Const.* 4436 (UPA).

The combination of perigon colours during flower maturation in plants of the above cited populations of *Bellevalia ciliata* differs from those reported by Feinbrun (1938–40), and a more detailed study of the morphology of the species based on living material would be desirable (see also Berg & al. 1989). Earlier reports for *B. ciliata* (Feinbrun 1938–40, Chiarugi 1949, Fedorov 1969) all give the diploid chromosome number of  $2n = 8$ . This number is confirmed, and the karyotype and karyogram (Figs 13a-d) are presented here for the first time from Greece.

The largest pair of chromosomes (c. 14.5 to 18.9  $\mu\text{m}$  in size) is metacentric in all populations examined and bears two small but conspicuous double satellites. It is followed by a pair of acrocentric chromosomes (c. 11.9 to 16.9  $\mu\text{m}$ ) which usually has a small satellite on its short arm (Figs 13a-b) and sometimes on its long arm too (Figs 13c-d). The last two pairs are metacentric to submetacentric, c. 7.8 to 12.5  $\mu\text{m}$  in size, and bear small satellites on their short arms. The shorter pair of the metacentric to submetacentric chromosomes bears additional satellites on its long arms. Noteworthy is the presence of secondary constrictions in most chromosomes of the complement. Such constrictions are found in both arms close to the telomere in the longest metacentric chromosomes (Figs 13a-b), and in both arms but close to the centromere in the acrocentric chromosomes (Figs 13a,c). Constrictions occasionally occur also in around the middle of the short arm of the metacentric to submetacentric chromosome pair (Fig. 13a). A considerable variation was observed among the populations examined, particularly concerning the presence or absence of satellites and secondary constrictions. As a result, two karyotype formulas are given here:  $2n = 4m\text{-SAT} + 2m/sm + 2st\text{-SAT} = 8$  (*Const.* 2984) and  $2n = 2m\text{-SAT} + 4m/sm\text{-SAT} + 2st\text{-SAT} = 8$  (*Const.* 4322).

**Orchidaceae**

***Aceras anthropophorum* (L.) W.T. Aiton – Fig. 14.**

$2n = 42$

GREECE: Sterea Ellas, Mt Pateras, the area between the summits Liondari and Agios Ilias, stony

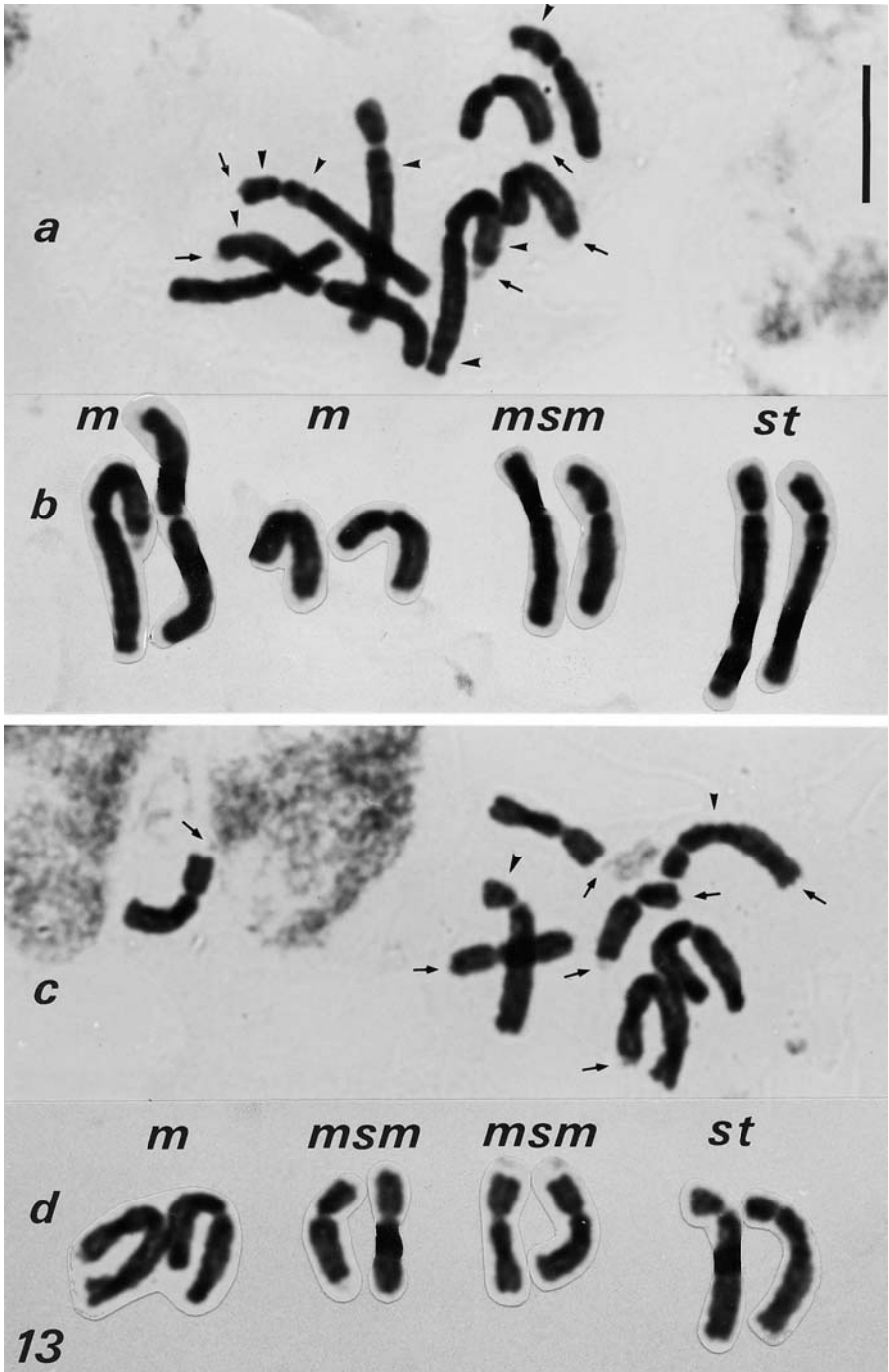


Fig. 13. Mitotic metaphase plates (a,c) and karyograms (b,d) of *Bellevalia ciliata*,  $2n = 8$ ; 13a-b: material from Mt Kitheron (*Const.* 2984); 13c-d: material from the area of Ritsona (*Const.* 4322). – Arrows indicate SAT-chromosomes and arrowheads secondary constrictions. Scale bar: 10  $\mu$ m.

places with *Arbutus andrachne* and other bushes, c. 850–900 m, 38°06'N, 23°20'E, 29.4.1994, *Const.* 4475 (UPA).

The only member of the monotypic genus *Aceras* is widely distributed in the Mediterranean area and also in W and Central Europe. A chromosome number of  $2n = 42$  was first counted in Swiss material (Heusser 1938), and has been reported later from several other countries, namely Britain (Fedorov 1969), the Netherlands (Kliphuis 1963) Spain (Löve & Kjellqvist 1973, Ruiz de Clavio Jiménez 1988), Italy (Scrugli 1977, Del Prete 1978, D' Emerico & al. 1993), and France (Balayer 1986, Cauwet-Marc & Balayer 1986). This is the first chromosome report based on Greek material. In our preparations, the chromosomes were strongly stained and not clear enough for detailed morphological analysis. However, most of them seem to be metacentric (m) (Fig. 14). The chromosome size ranges between c. 1.9 to 2.9  $\mu\text{m}$ .

### *Primulaceae*

*Coris monspeliensis* L. – Fig. 15.

$2n = 18$

GREECE: Sterea Ellas, Mt Gerania, between the village of Pissia and the nunnery of Osios Patapios, calcareous slopes, c. 650 m, 38°00'N, 22°57'E, 5.6.1993, *Const.* 3737 (UPA).

*Coris monspeliensis* is rare in Greece, known only from a few localities, which apparently form the easternmost limit of its European distribution. Previous cytological reports, all giving  $2n = 18$ , include those by Puech (1963, 1968, material from France), Kress (1963, material from Italy), Delay (1971, material from France), Löve & Kjellqvist (1974, material from Spain), and Baldini (1988, material from Italy). A different chromosome number of  $2n = 56$  reported from Europe (see Ferguson 1972) is unusual and needs confirmation. No chromosome count based on Greek material is known to the authors. Our count of  $2n = 18$  confirms earlier records. The chromosomes are predominately metacentric (Fig. 15), varying in size from 1.6 to 3.1  $\mu\text{m}$ . One of the metacentric pairs bears small satellites. The karyotype formula of the examined material can be given as  $2n = 2x = 10m + 2m\text{-SAT} + 4sm + 2sm/st = 18$ .

### *Ranunculaceae*

*Clematis cirrhosa* L. – Fig. 16.

$2n = 16 + 0\text{-}1\text{B}$

GREECE: Sterea Ellas, Mt Gerania, place known as Selki, between the villages of Schinos and Pissia, clearings of *Pinus* forest, c. 500 m, 38°02'N, 22°59'E, 6.2.1993, *Const.* 2853 (UPA).

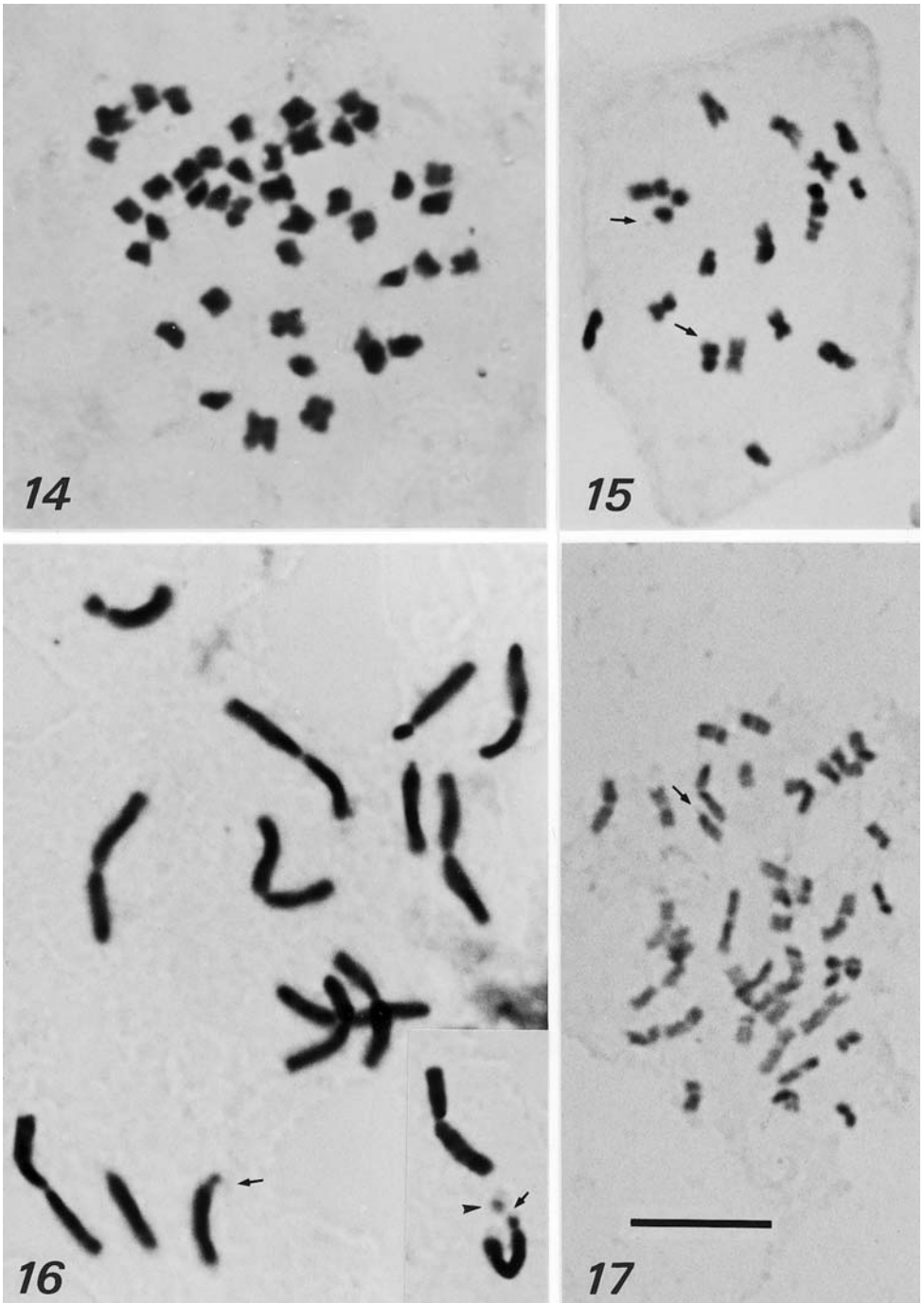
*Clematis cirrhosa* is a Mediterranean element with an early flowering period (winter to early spring). The species has been previously cytologically examined by Dahlgren & al. (1971) and Cardona & Contandriopoulos (1980) in material from the Balearic islands, as well as by Romano & al. (1987) in material from Sicily. They all give  $2n = 16$ . Our results, the first based on Greek material, are in agreement with these earlier records. The karyotype of the species is given in Fig. 16. Five chromosome pairs are metacentric, one is acrocentric and two are subtelocentric, one of which bears unequal satellites on its short arms. A small, spherical B-chromosome is usually present in the complement. The karyotype formula is  $2n = 2x = 10m + 2st + 2t + 2t\text{-SAT} = 16 + 0\text{-}1\text{B}$ . The chromosome size ranges between 7.0 and 12.5  $\mu\text{m}$ .

*Delphinium fissum* Waldst. & Kit. subsp. *fissum* – Figs 18a-b.

$2n = 16 + 0\text{-}1\text{B}$

GREECE: Sterea Ellas, Mt Kitheron, between the summits Profitis Ilias and Rachi Frasouri, edges of *Abies* forest, c. 1200 m, 38°11' N, 23°14' E, 28.5.1994, *Const.* 4695 (UPA).

To our knowledge, this is the first report of a chromosome count for *Delphinium fissum* subsp. *fissum* based on Greek material.  $2n = 16$  is the most common number found in this species, and has been reported from several other countries (see Simon & al. 1995 for references). The largest chromosome pair is metacentric, bearing clearly visible satellites, unequal in



Figs. 14–17. Mitotic metaphase plates – 14: *Aceras anthropophorum*,  $2n = 42$ ; 15: *Coris monspeliensis*,  $2n = 18$ ; 16: *Clematis cirrhosa*,  $2n = 16 + 1B$ ; 17: *Asperula pulvinaris*,  $2n = 44$ . – Arrows indicate SAT-chromosomes and the arrowhead a B-chromosome. Scale bar: 10  $\mu\text{m}$ .

size (Figs 18a-b). The second largest pair is submetacentric, followed by six pairs of acrocentric to subtelocentric chromosomes, one pair of which has satellites on its short arms. Moreover, a small submetacentric B-chromosome is sometimes observed in metaphase plates. Thus, the karyotype formula is  $2n = 2x = 2m\text{-SAT} + 2sm + 8st + 2st\text{-SAT} + 2st/t = 16 + 0\text{-1B}$ . The chromosome size varies between 4.2 and 13.0  $\mu\text{m}$ .

***Delphinium peregrinum* L. – Fig. 19.**

$2n = 16$

GREECE: Sterea Ellas, Mt Pateras, western slopes close to Petra Korakou summit, rocky calcareous substrate, c. 500–700 m, 38°07'N, 23°14'E, 16.6.1991, *Const. 2046* (UPA).

*Delphinium peregrinum* is an annual species with a predominantly E Mediterranean distribution. Previous chromosome counts are reported from plants of unknown origin (Gregory 1941), from Turkey (Demiriz & Misirdali 1980, Baltisberger 1991a) and Bulgaria (see Kuzmanov 1993), but not from Greece. Our count of  $2n = 16$ , apparently the most common chromosome number in the genus *Delphinium*, is in agreement with all previous reports for this species. The chromosomes (Fig. 19) vary in size from about 5.6 to 10.6  $\mu\text{m}$ , depending on the degree of chromosome contraction. The largest chromosome pair in the complement is metacentric to submetacentric, the remaining seven pairs are acrocentric to subtelocentric and gradually decrease in size. One of the acrocentric pairs has large satellites on the short arms and a clear secondary constriction on its long arm close to the centromere. The karyotype formula is  $2n = 2x = 2m/sm + 4 st + 2st\text{-SAT} + 2st/t + 6t = 16$ .

**Rubiaceae**

***Asperula baenitzii* Heldr. ex Boiss.**

$2n = 22$

GREECE: Sterea Ellas, Mt Pateras, small ravine north-east of the summit Liondari, gravel, c. 860 m, 38°06'N, 23°21'E, 22.6.1991, *Const. 2126* (UPA).

This endemic species, belonging to *A. sect. Thlipthisa*, is only known from a few localities in Attiki. Its chromosome number is given here for the first time. According to Schönbeck-Temesy & Ehrendorfer (1985) the members of this section are all “palaeo-Mediterranean” endemics, centred in the E Mediterranean area and showing the same diploid chromosome number of  $2n = 22$ .

***Asperula pulvinaris* (Boiss.) Heldr. ex Boiss. – Fig. 17.**

$2n = 4x = 44$

GREECE: Sterea Ellas, Mt Pateras, the summit Megali Kolosoura, open calcareous slopes, c. 1000–1100 m, 38°06'N, 23°17'E, 28.5.1994, *Const. 4714* (UPA).

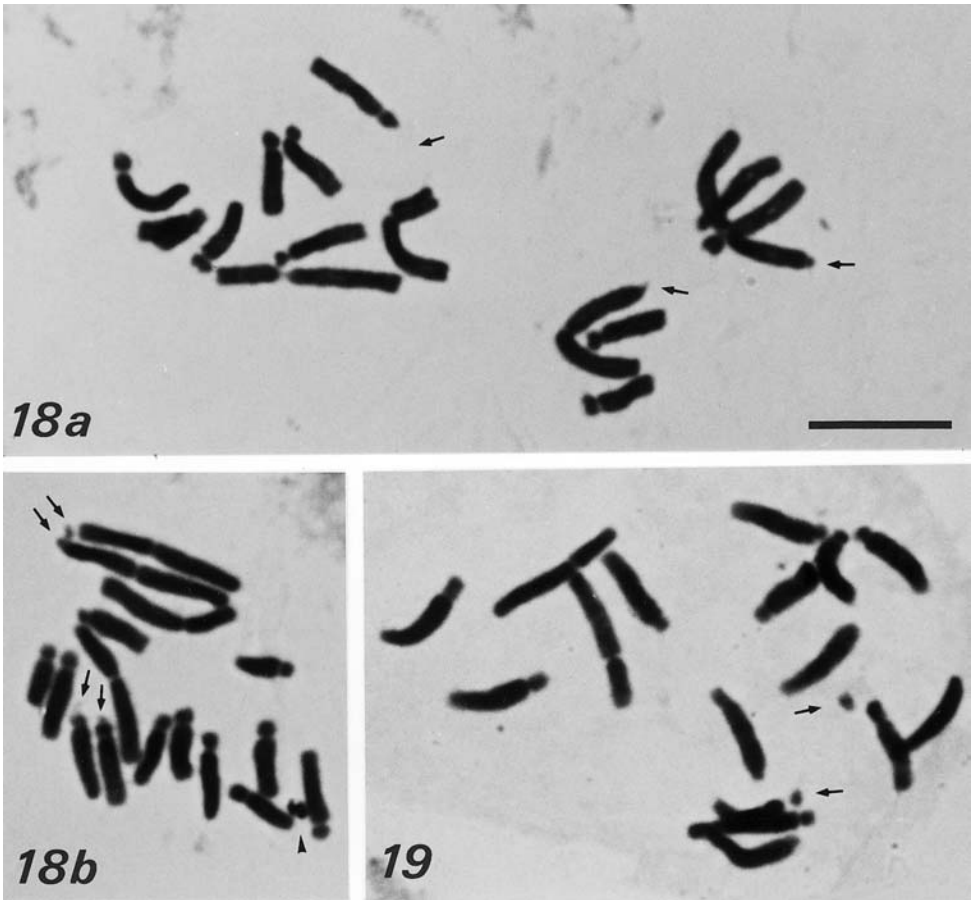
*Asperula pulvinaris* is restricted to the mountains of Attiki, where it forms densely pulvinate cushions at usually above 1000 m altitude (Schönbeck-Temesy & Ehrendorfer 1991). No previous chromosome number reports of this species were found in the literature; thus, the number  $2n = 4x = 44$  and a metaphase photomicrograph are reported here for the first time. The population examined is tetraploid with  $x = 11$  and chromosomes of small to medium size (between c. 1.7 to 4.8  $\mu\text{m}$ ). Its karyotype (Fig. 17) 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).

***Asperula rigidula* Halácsy**

$2n = 44$

GREECE: Sterea Ellas, Mt Pateras, south-east of the village of Psatha, rocky places along a little frequented road, c. 300 m, 38°05'N, 23°13'E, 17.5.1994, *Const. 5895* (UPA).

*Asperula rigidula* is an endemic species of E Sterea Ellas, Evvia and Peloponnisos, closely related to *A. lutea* Sm. Its chromosome number, being  $2n = 4x = 44$ , is reported here for the first time.



Figs 18–19. Mitotic metaphase plates – 18: *Delphinium fissum* subsp. *fissum*,  $2n = 16$  (a) and  $2n = 16 + 1B$  (b); 19: *D. peregrinum*,  $2n = 16$ . – Arrows indicate SAT-chromosomes and arrowhead B-chromosome. Scale bar:  $10\ \mu\text{m}$ .

### Scrophulariaceae

*Verbascum boissieri* (Heldr. & Sart. ex Boiss.) Kuntze – Fig. 20.

$2n = 36$

GREECE: Sterea Ellas, Mt Gerania, foothills between the settlements of Pefkogiali and Mavrolimni, ophiolitic substrate, c. 10–20 m,  $38^{\circ}03'N$ ,  $23^{\circ}07'E$ , 30.4.1993, *Const.* 3237 (UPA).

*Verbascum boissieri* is an endemic species of *V.* subg. *Celsia*, distributed in Central Greece. Its chromosome number of  $2n = 36$  and a metaphase photomicrograph (Fig. 20) are presented here for the first time. The chromosomes are small, c. 0.8 to 1.6  $\mu\text{m}$ , and because of their size no detailed karyological analysis was carried out. In some cells up to four distinct satellites are visible, two of them quite large and always evident.

### Umbelliferae

*Conium divaricatum* Boiss. & Orph. – Fig. 21.

$2n = 22 + 0-1B$



GREECE: Sterea Ellas, Mt Pastra, c. 8 km east-northeast of the village of Erithres, limestone rocks, c. 450 m, 38°14'N, 23°23'E, 16.4.1994, *Const. 4409* (UPA).

*Conium divaricatum* is retained as a separate species here, following Leute (1971). The species seems to differ from *C. maculatum* L. in both its morphology and ecological requirements, as it is found in drier and less disturbed habitats, such as at the base of limestone rocks, on screes, and sometimes also as a true chasmophyte. Its chromosome number and karyotype are given here for the first time. The species is diploid with  $2n = 22$  (Fig. 21), thus not differing from the more common *C. maculatum* in this respect (see Fedorov 1969, Goldblatt 1981, 1984 for references). The karyotype is somewhat asymmetrical with respect to relative chromosome size, consisting of predominantly metacentric chromosomes ranging from 1.7 to 5.0  $\mu\text{m}$ . A large, usually double satellite is apparent on the short arm of a submetacentric to acrocentric chromosome, but is not always visible on its homologue. In addition, two metacentric chromosomes seem to be satellited on their long arm and a small B-chromosome is sometimes observed in the complement. The karyotype formula is  $2n = 16m + 2m\text{-SAT} + 2sm + 2sm\text{/st-SAT} = 22 + 0\text{-}1\text{B}$ .

***Johrenia distans*** (Griseb.) Halácsy – Figs 22a-b.

$2n = 22$

GREECE: Sterea Ellas, Mt Pateras, western slopes close to Petra Korakou summit, limestone, c. 520 m, 38°07'N, 23°14'E, 16.6.1991, *Const. 2060* (UPA); Sterea Ellas, Mt Ipaton, close to the summit, remnants of deciduous *Quercus* forest, c. 730–765 m, 38°24'N, 23°24'E, 4.7.1993, *Const. 3981* (UPA); Sterea Ellas, Mt Parnassos, along the road leading to Mana Nerou spring, gravelly slopes on bauxite, c. 1100 m, 38°29'N, 22°35'E, 19.7.1994, *Const. 5048* (UPA); Macedonia, Mt Athos (Agion Oros), between the small monastic communities (= Skites) of Agia Anna and Mikra Agia Anna, calcareous rocky slopes and gravel, c. 300 m, 40°08'N, 24°18'E, 21.8.1995, *Const. 5896* (UPA).

*Johrenia distans* is a Greek endemic of this mainly Asiatic genus. This first report of its chromosome number is based on the examination of four populations. The species is diploid with  $2n = 22$ , and the chromosomes are of medium size (c. 2.8 to 4.8  $\mu\text{m}$ ). In the karyotypes (Figs 22a from Mt Parnassos and 22b from Mt Ipaton), 14 chromosomes appear to be metacentric, six submetacentric and two acrocentric; thus, the karyotype formula is  $2n = 2x = 14m + 6sm + 2st = 22$ . Within the tribe *Peucedaneae*, to which the genus *Johrenia* belongs, the basic number  $x = 11$  is found in the vast majority of the species examined (Moore 1971).

***Malabaila aurea*** (Sm.) Boiss. – Fig. 23.

$2n = 20$

GREECE: Sterea Ellas, Mt Pateras, the low summit Kandili, calcareous rocks and gravel, c. 350–500 m, 38°03'N, 23°24'E, 1.5.1994, *Const. 4507* (UPA).

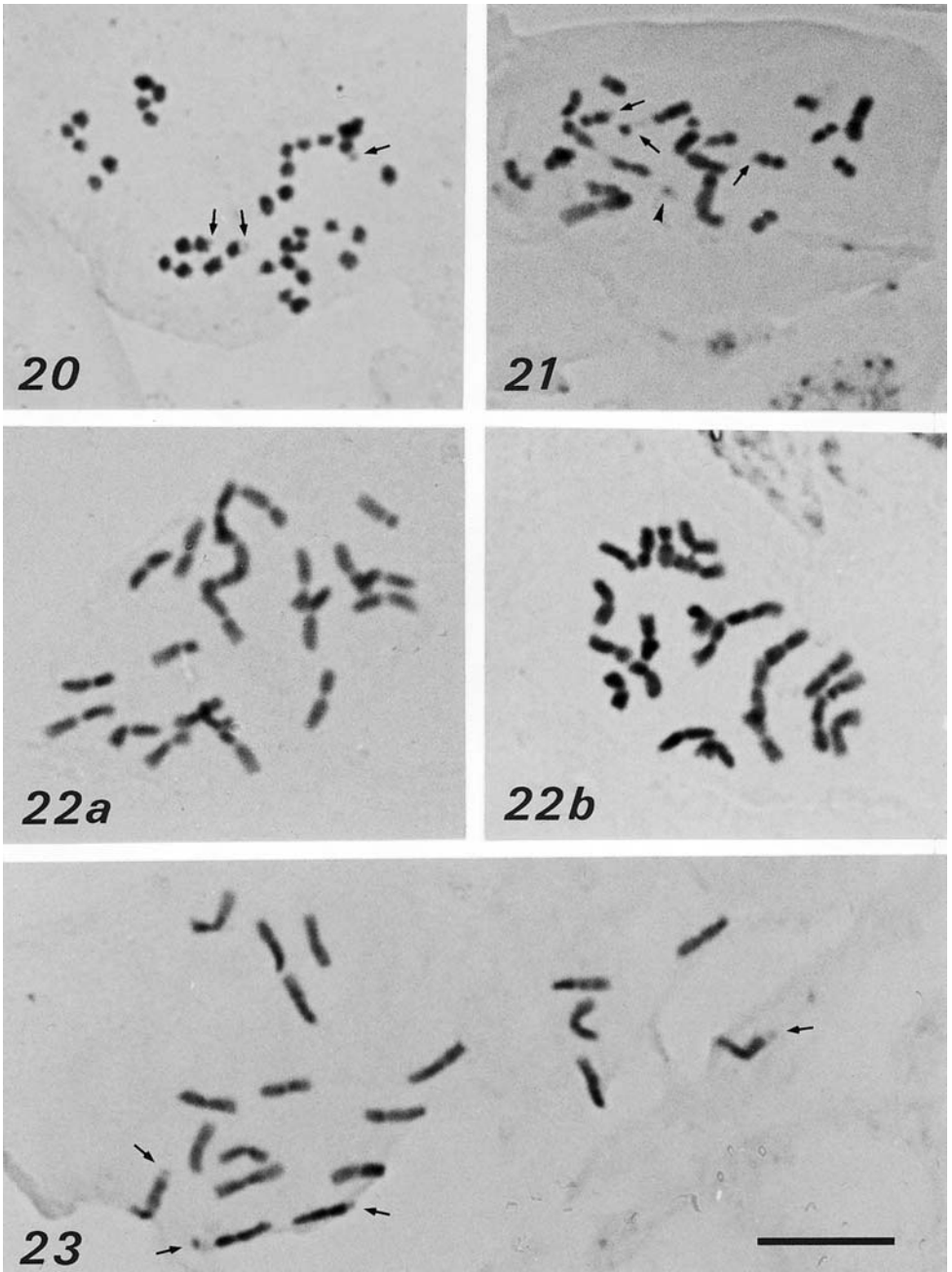
Two different chromosome numbers have been reported for *Malabaila aurea*; van Loon & Snelders (1979) and Moore (1982) reported  $2n = 22$ , the former in material from Macedonia, Greece, while the latter does not mention a provenance; Baltisberger (1991b), however, gives  $2n = 20$ , based on material from the Peloponnisos. Our results are in agreement with this last report. A metaphase photomicrograph is shown in Fig. 23. The karyotype is symmetrical with 12 submetacentric and 8 metacentric chromosomes, ranging in size from 3.6 to 5.1  $\mu\text{m}$ . Two pairs of submetacentric chromosomes are clearly satellited; thus, the karyotype formula is  $2n = 8m + 8sm + 4sm\text{-SAT} = 20$ .

***Peucedanum vittijugum*** Boiss. subsp. *vittijugum* – Figs 24a-b.

$2n = 22 + 0\text{-}1\text{B}$

GREECE: Sterea Ellas, Mt Pateras, northern slopes of the summit Mikri Kolosoura, clearings of *Pinus* forest, c. 700–800 m, 38°07'N, 23°17'E, 1.7.1994, *Const. 4905* (UPA).

Two subspecies have been recognized in the Balkan endemic *Peucedanum vittijugum*. To our knowledge, no previous chromosome count seems to exist for subsp. *vittijugum*, whereas



Figs. 20–23. Mitotic metaphase plates – 20: *Verbascum boissieri*,  $2n = 36$ ; 21: *Conium divaricatum*,  $2n = 22 + 1B$ ; 22: *Johrenia distans*, material from Mt Parnassos (a) and Mt Ipaton (b),  $2n = 22$ ; 23: *Malabaila aurea*,  $2n = 20$ . – Arrows indicate SAT-chromosomes and arrowhead B-chromosome. Scale bar: 10  $\mu$ m.

Kuzmanov & al. (1977, 1987) counted  $2n = 22$  for subsp. *minutifolium* (Janka) Kuzm. & Andreev in Bulgarian material. Our results show that subsp. *vittijugum* is also diploid with  $2n =$

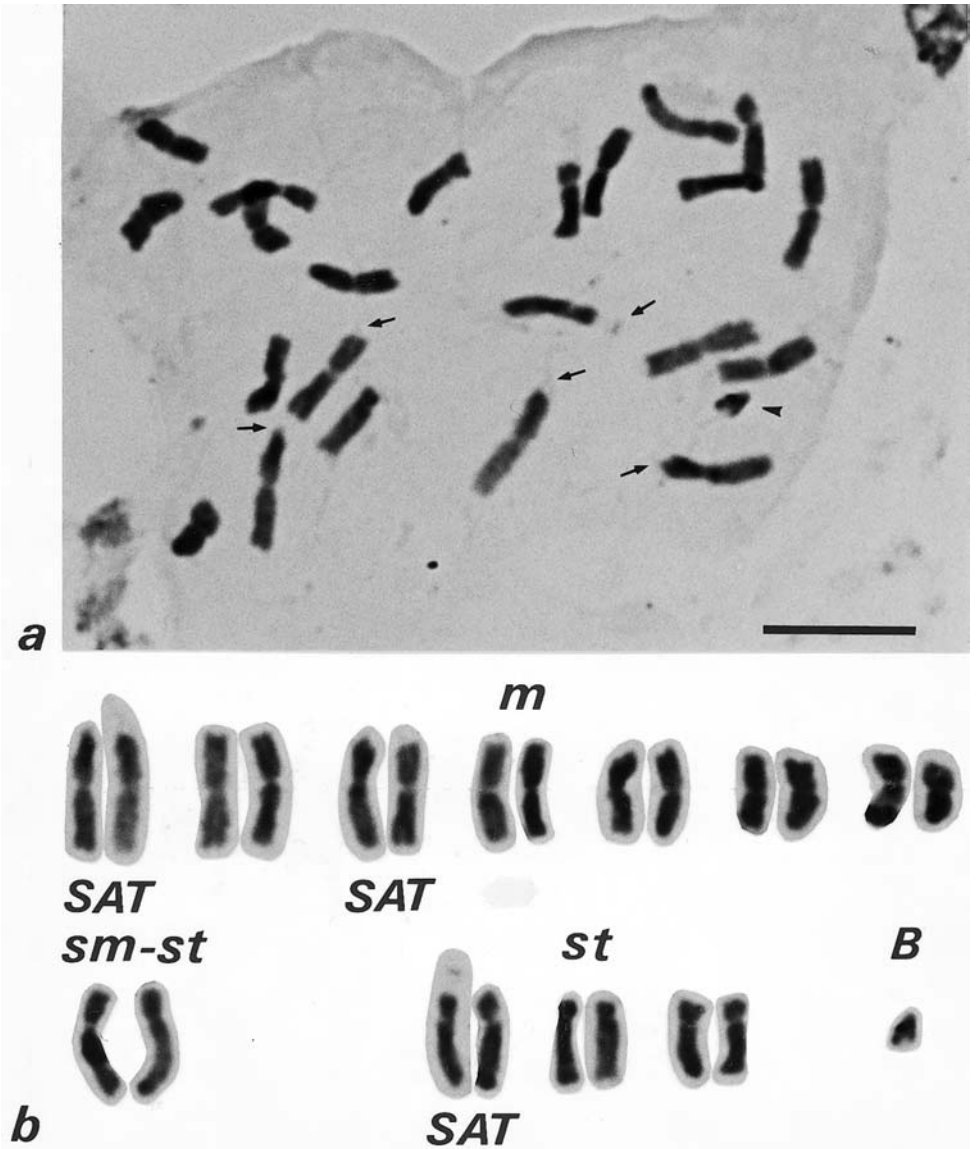


Fig. 24. Mitotic metaphase plate (a) and karyogram (b) of *Peucedanum vittijugum* subsp. *vittijugum*,  $2n = 22 + 1B$ . – Arrows indicate SAT-chromosomes and arrowhead B-chromosome. Scale bar: 10  $\mu$ m.

22. Most of the chromosomes are metacentric, four of them bearing small satellites. Satellites are also visible on a pair of acrocentric chromosomes. In addition, a B-chromosome was usually found in the complement (Fig. 24b). The karyotype formula is  $2n = 2x = 10m + 4m - SAT + 2sm/st + 4st + 2st - SAT = 22 + 0 - 1B$ . In the karyotype (Fig. 24a) and karyogram (Fig. 24b) presented here, the chromosomes range in size from 4.2 to 7.9  $\mu$ m.

There are significant differences between the karyotypes of the two subspecies of *P. vittijugum*. Greek material of subsp. *vittijugum* differs from the Bulgarian subsp. *minutifolium* in the

presence of six arcocentric and one B-chromosome. In subsp. *minutifolium* only metacentric and submetacentric chromosomes were observed (Kuzmanov & al. 1987).

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