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

Abstract

Constantinidis, Th., Kamari, G. & Phitos, D.: A cytological study of 28 phanerogams from the mountains of SE Sterea Ellas, Greece. – Willdenowia 27: 121–142. 1997. – ISSN 0511–9618.

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

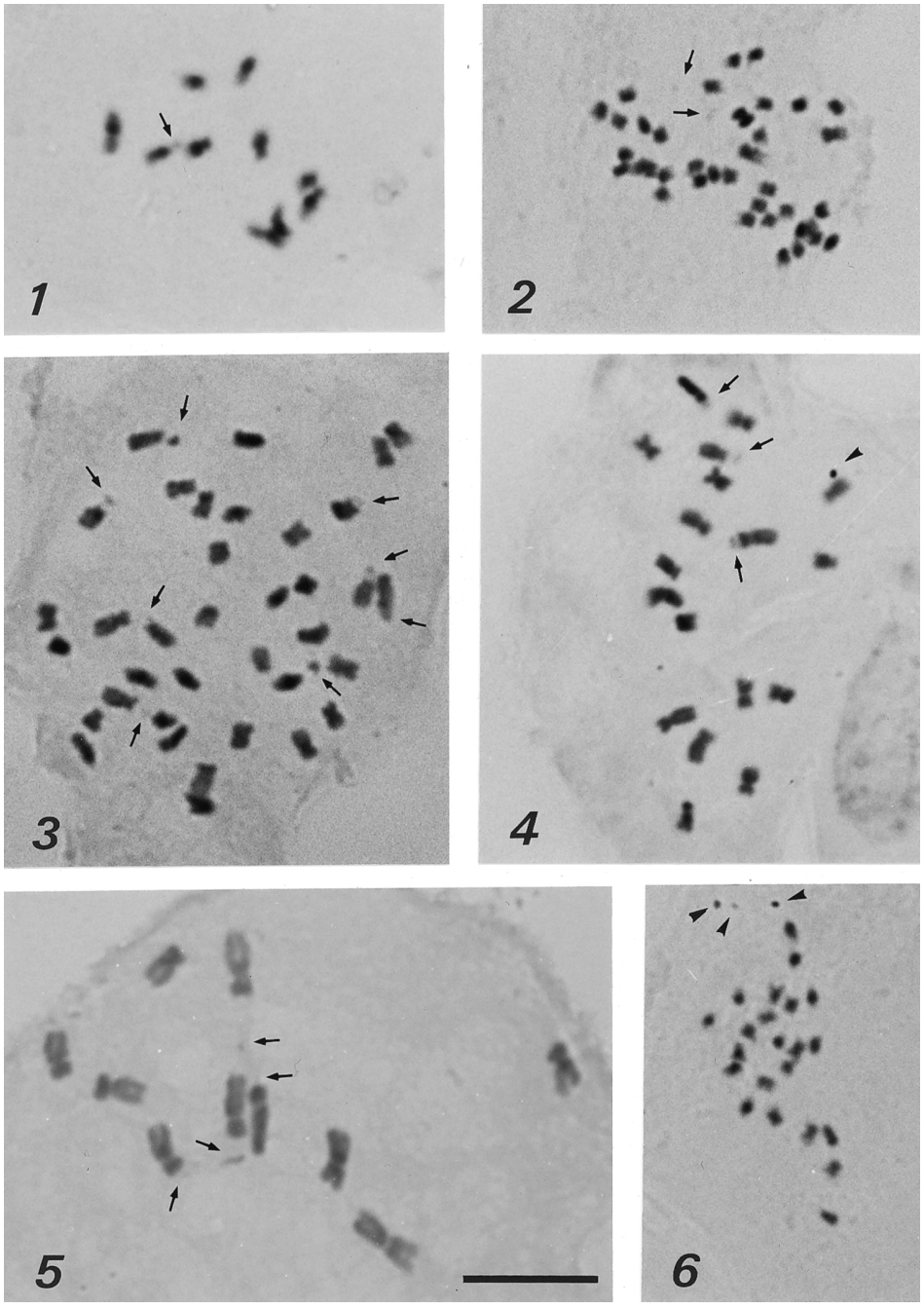
Caryophyllaceae

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. euboica* 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 µm, 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 µm.

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 ophiolithic 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 ophiolithic 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 µm 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 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 µm. 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 heterozygocity 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 ≡ *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 µm.

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).

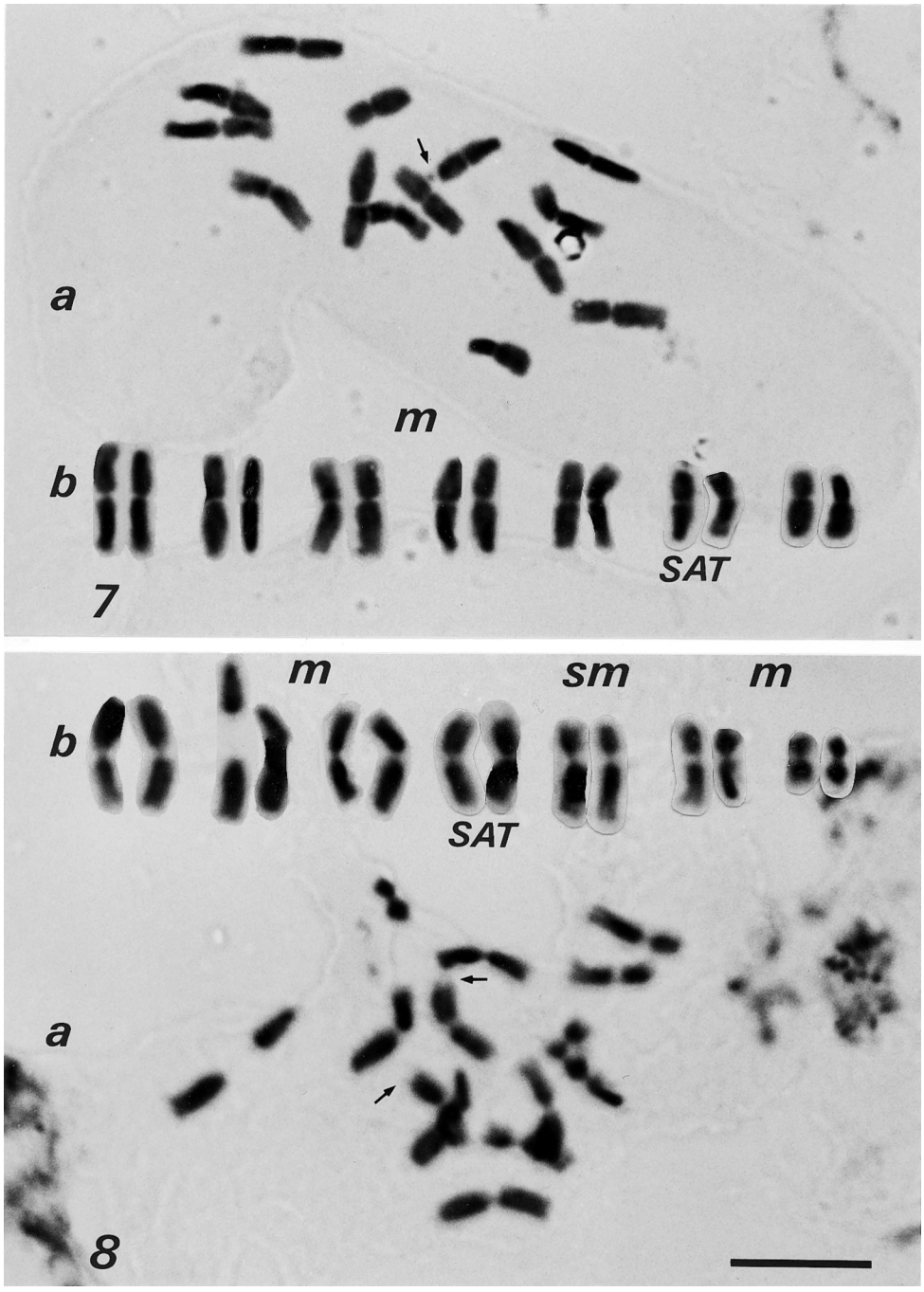
Scorzonera crocifolia is an endemic species of Central and S Greece, usually found at altitudes below 1000 m. A previous count in material from the island of Kithira, S Greece, revealed 2n = 14 (Damboldt 1968). This number is confirmed, and in addition the karyotype (Fig. 7a) and karyogram (Fig. 7b) 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 serpentinica 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).

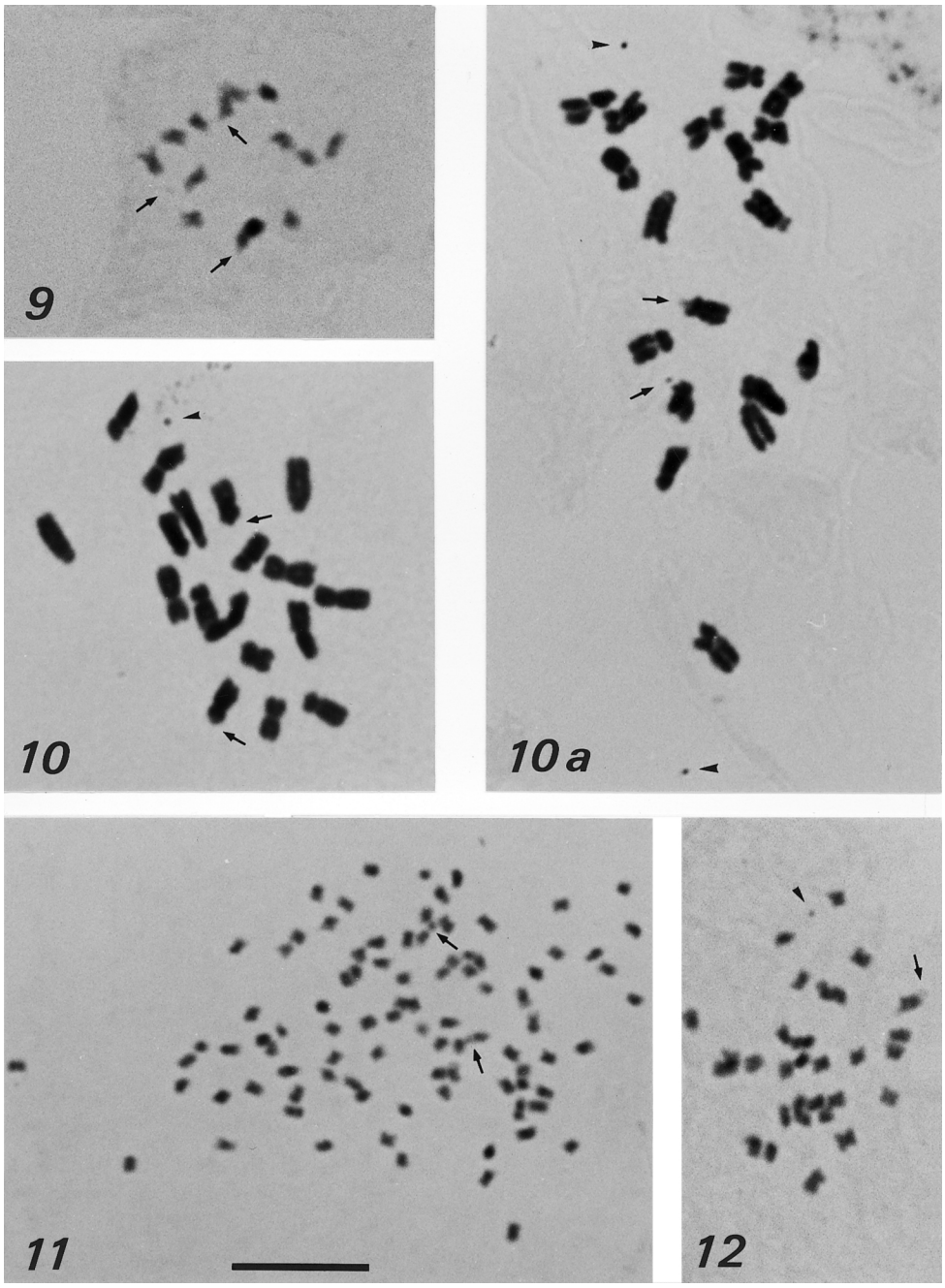
Scorzonera serpentinica is an endemic species, previously known only from Central and N Evvia where it grows on serpentine (Rechinger 1961). Material from the serpentine slopes of Mt Gerania approaches the description of *S. serpentinica* in many respects, but the taxonomic differences between the latter and *S. crocifolia* are somewhat vague. *S. serpentinica* 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. serpentinica*. 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. serpentinica* 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. serpentinica.* Also in contrast to *S. crocifolia*, the fourth largest chromosome of *S. serpentinica* is satellited; the satellits are faintly stained and not always visible. The karyotype formula of *S. serpentinica* 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. serpentinica*, 2n = 14. – Arrows indicate SAT-chromosomes. Scale bar: 10 µm.



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× = 90; 12: *Th. teucrioides* subsp. *candilicus*, 2n = 30 + 1B. – Arrows indicate SAT-chromosomes and arrowheads B-chromosomes. Scale bar: 10 µm.