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## Karyosystematic study of some taxa from *Campanula* section *Quinqueloculares* (*Campanulaceae*). I.

### Abstract

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Chromosome numbers and karyotypes are given for 12 taxa of *Campanula* section *Quinqueloculares*. All the examined taxa are distributed in Greece including the phytogeographical regions of Crete and Karpathos, Cyclades and East Aegean Islands. The chromosome number  $2n = 2x = 34$  is found in all examined taxa with the exception of *C. laciniata* ( $2n = 4x = 68$ ), which is a new chromosome number for the taxon from Crete. Their karyotypes are symmetrical comprising of mostly metacentric and submetacentric chromosomes, small in size, but they differ in the presence and the size of satellites. The chromosome count ( $2n = 34$ ) and karyotype morphology of *C. topaliana* subsp. *delphica* is given for first time. New populations of *C. cymaea*, *C. kamariana*, *C. pelviformis*, and *C. topaliana* subsp. *cordifolia* were karyologically investigated confirming the previous references. The karyotype morphology of *C. anchusiflora*, *C. andrewsii* subsp. *hirsutula*, *C. kamariana*, *C. lavrensis*, *C. merxmulleri*, *C. nisyria*, and *C. rupestris* is given for first time. Additionally, microphotographs are firstly provided hereby for all investigated taxa.

Keywords: Chromosome number, distribution, endemism, Greece, karyomorphology.

### Introduction

*Campanula* L. section *Quinqueloculares* (Boiss.) Phitos encompasses ca. 39 species mostly distributed in Greece and/or W Turkey (Liveri & al. 2019). Only two of the species (*C. crispa* Lam., *C. lyrata* Lam.) extend their distribution to Transcaucasia, Armenia, Iran and Jordan, whereas one species (*C. medium* L.) is restricted to Italy and France (Castroviejo & al. 2010). Greece and W Turkey harbor 24 and 12 species, respectively, in addition to 2 species (*C. hagielia* Boiss. and *C. lyrata*) which occur both in East Aegean Islands and the adjacent Turkish coastline.

The species of *Campanula* sect. *Quinqueloculares* are characterized by an extreme morphological polymorphism, which renders their identification hard, or even dubious in some cases (Phitos 1965, 1969, 2016; Eddie & Ingrouille 1999). In general, they are biennial (monocarpic) or, more rarely, perennial plants, mostly chasmophytic, featured by a 5-locular ovary, style with 5 stigmas, capsule opening with 5 pores, and calyx

appendages more or less covering the ovary (Phitos 1965, 1969; Liveri & al. 2019, 2020). However, in some species, the number of the ovary locules varies from 3 to 5 (Liveri & al. 2019, 2020). A small group of six species with a predominantly trilocular ovary has been recognized by Phitos (1963a) and referred to as “*Campanula rupestris* group” or “trilocular *Campanula* species” (Phitos 1964a) or “*Triloculares*” (Phitos 2016). Recently, the first phylogenetic study of *C.* sect. *Quinqueloculares* based on molecular data was performed revealing the polyphyletic origin of the traditionally circumscribed section (Liveri & al. 2020).

Karyological studies of 28 species belonging to *Campanula* sect. *Quinqueloculares* resulted in a consistent chromosome count ( $2n = 2x = 34$ ) for all of them (Marchal 1920; Phitos 1963a, b, 1964a, b, 1965, 1969; Contandriopoulos 1964, 1970; Gadella 1964; Zhukova 1967; Tornadore & al. 1974; Papatsou & Phitos 1975; Phitos & Kamari 1984; Montmollin 1986; Runemark & Phitos 1996; Strid 2015; Kyriakopoulos & al. 2017). The chromosome number  $2n = 2x = 32$  given in *C. medium* (Chen & al. 2003), is questionable.

Polyploidy is not common in *C.* sect. *Quinqueloculares*. So far, the only known polyploidy number is  $2n = 6x = 102$  in *C. laciniata* L. (Sugiura 1942).

Although the chromosome number for the majority of the species has been given in the past, the karyotype morphology of most species is still unknown.

The current karyological study provides chromosome number, ploidy level, type of symmetry and average maximum and minimum length of chromosomes. For each taxon, karyotype microphotograph is also provided and karyotype morphology is discussed.

**1982. *Campanula anchusiflora* Sm. —  $2n = 2x = 34$  (Figs 1a, 2a & 2b).**

**Gr:** Isl. Hydra: W side of the port, on the coastal road from the port towards Kamini village, alt. 6-10 m., 37° 21' 5" N, 23° 27' 51" E, 27 Apr 2016, leg. E. Liveri & V. Ketsilis-Rinis 188 (Herb. Phitos & Kamari in UPA).

*Campanula anchusiflora* (Fig. 1a) is a typical quinquelocular species restricted in Hydra island (Argosaronikos Gulf). However, morphologically deviating forms can be found on the adjacent mainland in Peloponnisos (Phitos 1965, 1969).

One population of *Campanula anchusiflora* in material from Hydra has been studied karyologically resulted to the chromosome number  $2n = 34$  (Phitos 1963a, 1969).

The chromosome number  $2n = 34$  for *Campanula anchusiflora* in material from Hydra is confirmed in our study. The karyotype is diploid and symmetrical, consisting of mostly metacentric (m) and submetacentric (sm) chromosomes which vary in size between 1.00-1.55  $\mu\text{m}$  (Fig. 2a; Table 1). In several karyotypes of the examined material, two submetacentric (sm) chromosomes are observed, bearing large satellites, similar in size to the short (s) arm (Fig. 2b). Due to the heterochromatin structure and the long distance from the short arm caused by the squashing technique, these satellites can be considered as B-chromosomes. The given chromosome number is confirmed by previous studies (Phitos 1963a, 1969); microphotographs of the karyotype are presented here for the first time (Figs 2a, b).

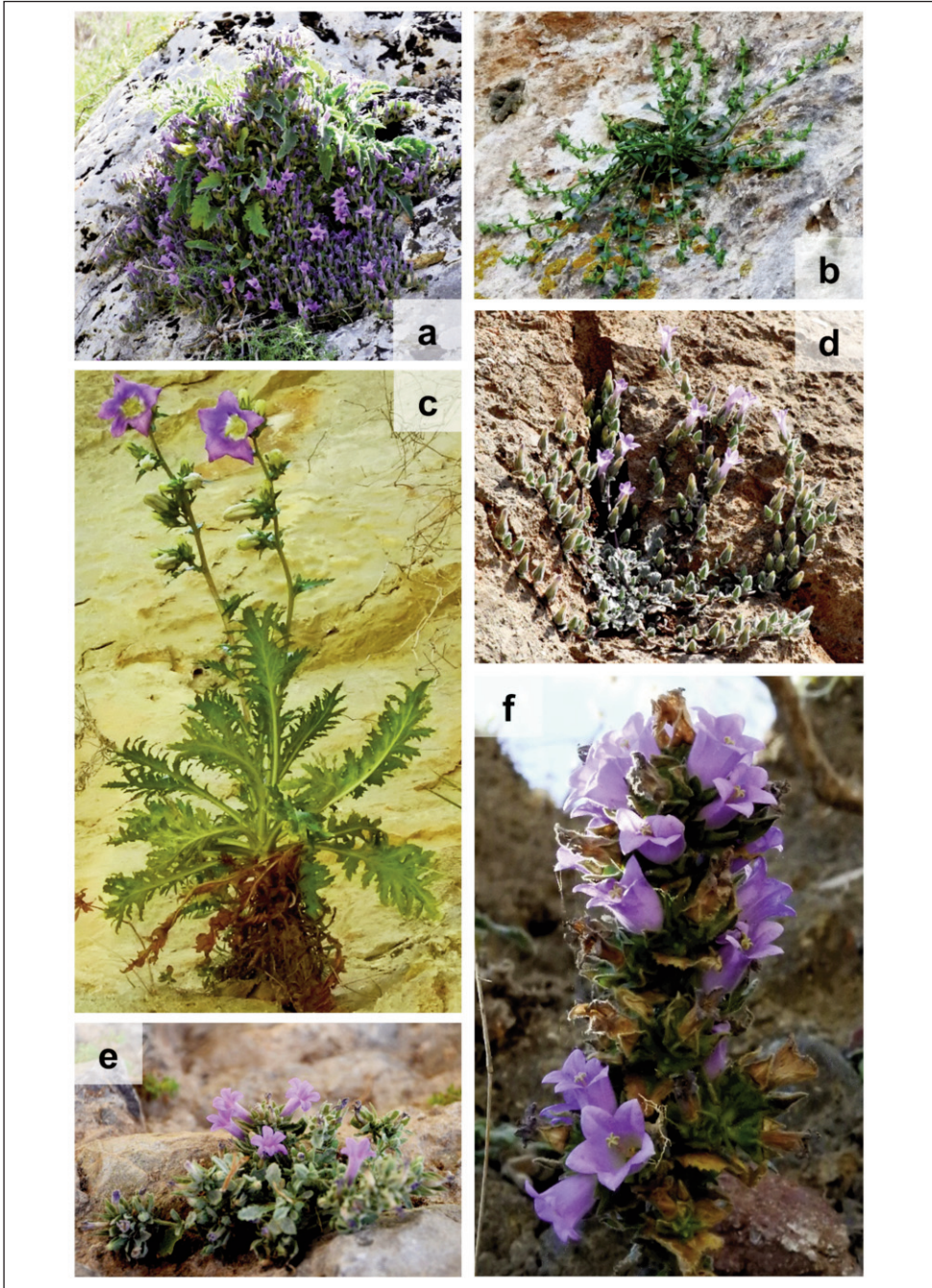


Fig. 1. Some taxa of *Campanula* section *Quinqueloculares*: **a**, *C. anchusiflora* (Hydra); **b**, *C. merxmulleri* (Skyros); **c**, *C. laciniata* (Crete); **d**, *C. topaliana* subsp. *delphica* (Delphi); **e**, *C. andrewsii* subsp. *hirsutula* (Monemvasia); **f**, *C. nisyria* (Nisyros). – Photos by E. Liveri.

**1983.** *Campanula andrewsii* subsp. *hirsutula* Phitos —  $2n = 2x = 34$  (Figs 1e & 2c).

**Gr:** Prov. Lakonia: Castle of Monemvasia, alt. 0-5 m., 36° 41' N, 23° 03' E, 21 May 2016, leg. E. Liveri & V. Ketsilis-Rinis 225 (Herb. Phitos & Kamari in UPA).

*Campanula andrewsii* is distributed in eastern Peloponnisos. It comprises of two subspecies: 1) the typical subsp. *andrewsii*, mostly growing in northeastern Peloponnisos (Argolis peninsula, Korinthos); and 2) subsp. *hirsutula* (Fig. 1e), which was originally described from the castle of Monemvasia (*locus classicus*) and it is also found in south-eastern Peloponnisos (Phitos 1965, 1969; Fedorov & Kovanda 1976) and in a single locality of northeastern Peloponnisos, in Methana peninsula (Kougioumoutzis & al. 2012a). *C. andrewsii* subsp. *hirsutula* differs morphologically from the typical subspecies mainly in its indumentum (hirsute calyx), corolla (shorter tube) and calyx appendages (very short and acuminate) (Phitos 1965, 1969; Fedorov & Kovanda 1976).

The chromosome count  $2n = 34$  has been recorded in material from the *locus classicus* (Phitos 1965, 1969). Additionally, Contandriopoulos (1964) reports the same chromosome number and provides a karyotype drawing for *C. andrewsii* s.l. in material from Mt. Parnassos, but most probably refers to a different taxon.

The examined material of *C. andrewsii* subsp. *hirsutula* shows a diploid, symmetrical karyotype comprised of  $2n = 2x = 34$  chromosomes, mainly metacentric (m) and submetacentric (sm), varying in size between 1.02 and 1.45  $\mu\text{m}$ ; one submetacentric (sm) chromosome pair bears large satellites (Fig. 2c; Table 1). Previous karyological references confirm the same chromosome number (Phitos 1965, 1969); although, the karyotype morphology and microphotograph are presented here for first time.

**1984.** *Campanula cymaea* Phitos —  $2n = 2x = 34$  (Fig. 2d).

**Gr:** Isl. Evia: village Vrysi, 15 km SW of the town Kymi, alt. 115 m., 38° 33' 06.56" N, 24° 02' 38.57" E, 6 May 2017, leg. E. Liveri, V. Ketsilis-Rinis & N. Cellinese 294 (Herb. Phitos & Kamari in UPA).

*Campanula cymaea* is a representative of “*Triloculares*”, endemic to eastern part of central Evia, especially around the town of Kymi (Phitos 1969; Phitos & Kamari 1995). It is included in the first *Red Data Book of Rare and Threatened Plants of Greece* as Rare (R), according to IUCN (1994) criteria (Phitos & Kamari 1995) and in the Greek Presidential Decree 67/1981.

The chromosome count  $2n = 2x = 34$  has been reported in material from two localities (Kymi and Potamia) accompanied with a karyotype drawing indicating one pair of large SAT-chromosomes (Phitos 1964a, 1969). Phitos (1964a) notices structural heterozygosity in the satellited pair in most individuals of the second population.

The examined material of *Campanula cymaea* is also diploid ( $2n = 2x = 34$ ) and has a symmetrical karyotype which includes mostly metacentric (m) and submetacentric (sm) chromosomes; one submetacentric (sm) chromosome pair bears satellites (Fig. 2d). The average maximum and minimum chromosome length equals to 0.86  $\mu\text{m}$

and 1.25  $\mu\text{m}$ , respectively (Table 1). The chromosome count and karyotype morphology of the newly examined subpopulation of *C. cymaea* is in accordance with previous studies (Phitos 1964a, 1969), but a microphotograph of the karyotype is provided for the first time (Fig. 2d).

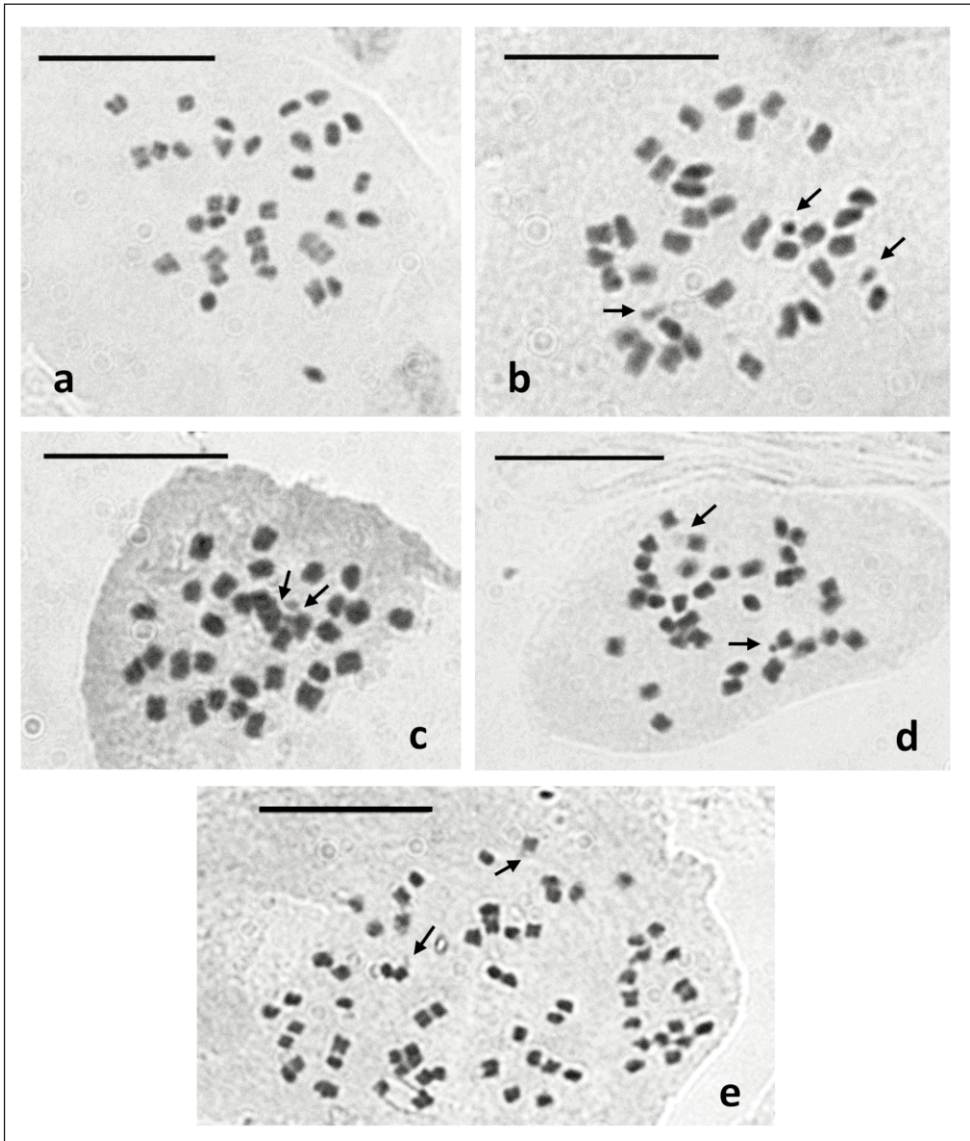


Fig. 2. Microphotographs of mitotic metaphase plates of *Campanula* section *Quinqueloculares*: **a**, and **b**, *C. anchusiflora*,  $2n = 34$ ; **c**, *C. andrewsii* subsp. *hirsutula*,  $2n = 34$ ; **d**, *C. cymaea*,  $2n = 34$ ; **e**, *C. laciniata*,  $2n = 68$ . – Arrows indicate satellites. Scale bars = 10  $\mu\text{m}$ .

**1985. *Campanula kamariana*** Kyriak., Liveri & Phitos —  $2n = 2x = 34$  (Fig. 3a).

**Gr:** Prov. Lakonia: south of village Itilo, castle of Kelefa, alt. 230 m.,  $36^{\circ} 42' 4.385''$  N,  $22^{\circ} 23' 47.327''$  E, 17 Jun 2018, leg. *E. Liveri & V. Ketsilis-Rinis 237* (Herb. Phitos & Kamari in UPA).

*Campanula kamariana* is a recently described species of *C.* sect. *Quinqueloculares*, which is easily distinguished from the other species of the section mostly by its strongly lacerate, sparsely pubescent to glabrescent rosette leaves with long, sparsely lobed petioles (Kyriakopoulos & al. 2017). Up to now, it is found in the description locality, i.e. Limeni, a small village closes to the town Areopolis (Messinian Bay - southern Peloponnisos) and a second locality firstly mentioned in the current study, i.e. the castle of Kelefa.

Concerning karyology of *Campanula kamariana* from the *locus classicus*, Kyriakopoulos & al. (2017) mention the chromosome number  $2n = 34$  and a symmetrical karyotype including mostly metacentric (m) chromosomes.

The examined material of *Campanula kamariana* shows also a diploid and symmetrical karyotype ( $2n = 2x = 34$ ) comprised of mostly metacentric (m) and submetacentric (sm) chromosomes, varying in size from 0.73 to 2.05  $\mu\text{m}$  (Fig. 3a, Table 1). One submetacentric (sm) chromosome pair is predominantly observed bearing large satellites. The chromosome number of *C. kamariana* is confirmed from a new subpopulation with the addition of a microphotograph. The presence of satellited chromosomes is firstly mentioned hereby (Fig. 3a).

**1986. *Campanula laciniata*** Lam. —  $2n = 4x = 68$  (Figs 1c & 2e).

**Cr:** Prov. Chania: Aradaina gorge, alt. 70 m.,  $35^{\circ} 12' 22.410''$  N,  $24^{\circ} 3' 12.084''$  E, 10 May 2017, leg. *E. Liveri, V. Ketsilis-Rinis & N. Cellinese 289* (Herb. Phitos & Kamari in UPA).

The large, broadly campanulate flowers and the laciniate, rather thick rosette leaves render *C. laciniata* (Fig. 1c) among the most showy and distinct members of *Campanula* sect. *Quinqueloculares* (Phitos 1965, 1969, 1995a; Cellinese & al. 2009; Strid 2016a, 2016b; Liveri & al. 2020). *Campanula laciniata* is distributed in the extended Cretan area (Crete and Karpathos), the southern Cyclades (Amorgos, Anafi, Folegandros, Kardiotisa, Sifnos, Sikinos), and Dodekanissos islands (Astypalea) (Phitos 1964b, 1965, 1969; Greuter 1973; Fedorov & Kovanda 1976; Phitos & Kamari 1984; Fielding & Turland 2005; Cellinese & al. 2009; Kougioumoutzis & al. 2012b; Strid 2016a, 2016b; Liveri & al. 2020). The populations of *C. laciniata* are scattered, consisting of a few individuals. This species is included in the first *Red Data Book of Rare and Threatened Plants of Greece* as Rare (R), according to IUCN (1994) criteria (Phitos 1995a), as well as in the Greek Presidential Decree 67/1981.

Diploid and hexaploid populations have been reported for *Campanula laciniata*:  $2n = 2x = 34$  chromosomes are reported in material from Folegandros (Phitos 1964b) and Karpathos (Phitos & Kamari 1984); while Sugiura (1942) reports an hexaploid karyotype with  $2n = 6x = 102$  chromosomes, from Greek material of an unknown locality.

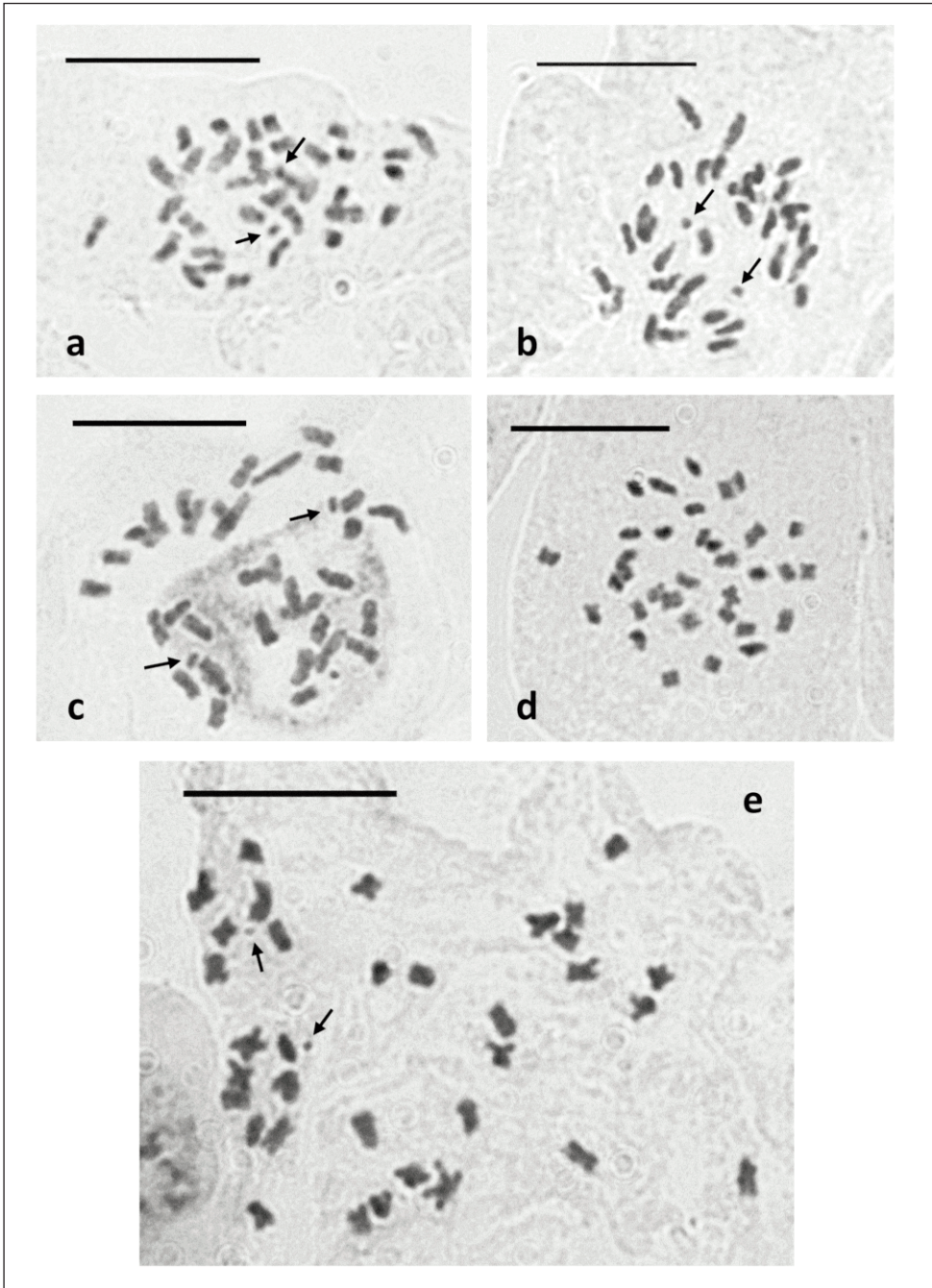


Fig. 3. Microphotographs of mitotic metaphase plates of *Campanula* section *Quinqueloculares*: **a**, *C. kamariana*,  $2n = 34$ ; **b**, *C. lavrensis*,  $2n = 34$ ; **c**, *C. merxmulleri*,  $2n = 34$ ; **d**, *C. pelviformis*,  $2n = 34$ ; **e**, *C. nisyria*,  $2n = 34$ . – Arrows indicate satellites. Scale bars = 10  $\mu\text{m}$ .

Our results reveal a new ploidy level for *Campanula laciniata* in material from Crete, which is studied karyologically for the first time here. The tetraploid chromosome count for the species is  $2n = 4x = 68$  chromosomes. The karyotype is symmetrical including mainly metacentric (m) and submetacentric (sm) chromosomes; four chromosomes have small spherical satellites, not all of them always visible (Fig. 2e). The chromosome size ranges from 0.78 to 1.29  $\mu\text{m}$  (Table 1). The chromosome morphology of the Cretan population is similar to that given for diploid population in material from Folegandros (Phitos 1964b). According to the karyotype drawing for the latter material, four SAT-chromosomes are observed (Phitos 1964b), in accordance with our results. The karyotype of *C. laciniata* is depicted for the first time with a microphotograph here (Fig. 2e).

**1987. *Campanula lavrensis*** (Toel & Rohlena) Phitos —  $2n = 2x = 34$  (Fig. 3b).

**Gr:** Prov. Chalkidiki: Athos peninsula, Moni Megistis Lavras, alt. 154 m., 40° 10' 14.23" N, 24° 23' 2.88" E, 5 Jun 2018, leg. *A. Karydas s.n.* (UPA 24662).

The northernmost distribution limit of *Campanula* sect. *Quinqueloculares* is Athos peninsula, or commonly known in Greece as the Agion Oros, where *Campanula lavrensis* occurs (Phitos 1969; Fedorov & Kovanda 1976). This species grows on the cliffs and old walls of the numerous monasteries located in this area. Due to its morphological variability, two varieties have been recognized: 1) var. *lavrensis*; and 2) var. *breueri* Phitos (Phitos 1965). According to Strid (2016a, b), material from Samothraki island also belongs to *C. lavrensis*, which previously was referred to as *C. rupestris* (Ade & Rechinger 1938; Stojanov & Kitanov 1944).

The only chromosome count given for *Campanula lavrensis* is  $2n = 34$  in material from the *locus classicus*, i.e. Moni Megistis Lavras, which is the largest and oldest monastery in Athos peninsula (Phitos 1965, 1969).

The material of *Campanula lavrensis* examined here has a diploid ( $2n = 2x = 34$ ) and symmetrical karyotype with metacentric (m) and submetacentric (sm) chromosomes, ranging from 1.06 to 1.99  $\mu\text{m}$  (Fig. 3b; Table 1). One pair of submetacentric (sm) chromosomes bears large satellites. The chromosome count confirms previous studies (Phitos 1965, 1969), but the karyotype morphology and a microphotograph are provided for first time.

**1988. *Campanula merxmuellerei*** Phitos —  $2n = 2x = 34$  (Figs 1b & 3c).

**Gr:** Isl. Skyros: Mt. Kochilas, Monastery Agios Artemios, alt. 430 m., 38° 51' 04.76" N, 24° 35' 39.72" E, 7 May 2017, leg. *E. Liveri, V. Ketsilis-Rinis & N. Cellinese 291* (UPA 24649).

One of the most distinct members of *Campanula* sect. *Quinqueloculares* is *Campanula merxmuellerei* (Fig. 1b), characterized by its glabrous and fleshy leaves combined with the entire petiole and elliptic-spathulate lamina (Phitos 1963b, 1965, 1995b; Phitos & Kamari 2009; Liveri & al. 2020). *C. merxmuellerei* is distributed in



Skyros and adjacent small islands, as well as in Psara island (eastern Aegean) (Phitos & Kamari 2009; Baliouis 2016). It is noteworthy that the first specimen from Psara island was collected by P. Saliaris in 2007 (kept in Herb. Phitos & Kamari). Due to its peculiar distribution, it is considered as a relict endemic of the Aegean region (Phitos 2016). It is included in the *Red Data Books of Rare and Threatened Plants of Greece* as Vulnerable (VU), according to IUCN (1994, 2003) criteria (Phitos 1995b; Phitos & Kamari 2009) and in the Greek Presidential Decree 67/1981.

The only chromosome count given for *C. merxmuelleri* is  $2n = 34$  in material from the *locus classicus*, ie. Monastery Agios Artemios in Mt. Kochilas (Phitos 1963b, 1969). In the karyotype drawing of the species two SAT-chromosomes are indicated (Phitos 1969).

According to our data, *Campanula merxmuelleri* has  $2n = 2x = 34$  chromosomes, metacentric (m) and submetacentric (sm) in morphology, ranging in size between 1.18

Table 1. Chromosome data of the examined taxa of *Campanula* section *Quinqueloculares*. MAX and MIN correspond to average maximum and minimum length of chromosomes, respectively. SAT corresponds to the maximum number of observed SAT-chromosomes. All measurements are given in  $\mu\text{m}$ . Standard Deviation (SD) is given in parenthesis.

| Taxon   | Chromosome number | SAT | MIN            | MAX            |
|---|-------------------|-----|----------------|----------------|
| <i>C. anchusiflora</i>                          | $2n = 2x = 34$    | 2   | 1.00<br>(0.08) | 1.55<br>(0.13) |
| <i>C. andrewsii</i><br>subsp. <i>hirsutula</i>  | $2n = 2x = 34$    | 2   | 1.02<br>(0.12) | 1.45<br>(0.12) |
| <i>C. cymaea</i>                                | $2n = 2x = 34$    | 2   | 0.86<br>(0.05) | 1.25<br>(0.08) |
| <i>C. kamariana</i>                             | $2n = 2x = 34$    | 2   | 0.73<br>(0.16) | 2.05<br>(0.38) |
| <i>C. laciniata</i>                             | $2n = 4x = 68$    | 4   | 0.78<br>(0.05) | 1.29<br>(0.24) |
| <i>C. lavrensis</i>                             | $2n = 2x = 34$    | 2   | 1.06<br>(0.34) | 1.99<br>(0.50) |
| <i>C. merxmuelleri</i>                          | $2n = 2x = 34$    | 2   | 1.18<br>(0.31) | 2.49<br>(0.72) |
| <i>C. nisyria</i>                               | $2n = 2x = 34$    | 2   | 0.91<br>(0.08) | 1.95<br>(0.19) |
| <i>C. pelviformis</i>                           | $2n = 2x = 34$    | 0   | 0.88<br>(0.11) | 1.61<br>(0.30) |
| <i>C. rupestris</i>                             | $2n = 2x = 34$    | 2   | 1.02<br>(0.20) | 1.95<br>(0.16) |
| <i>C. topaliana</i><br>subsp. <i>cordifolia</i> | $2n = 2x = 34$    | 2   | 0.85<br>(0.17) | 1.73<br>(0.39) |
| <i>C. topaliana</i><br>subsp. <i>delphica</i>   | $2n = 2x = 34$    | 2   | 0.83<br>(0.10) | 1.17<br>(0.33) |

and 2.49  $\mu\text{m}$  (Fig. 3c, Table 1). The karyotype is symmetrical and diploid whereas two large satellites are observed in most karyotypes. Our findings are in accordance with previous studies (Phitos 1963b, 1969). Additionally, a microphotograph is provided here for first time (Fig. 3c).

**1989. *Campanula nisyria*** Papatsou & Phitos —  $2n = 2x = 34$  (Figs 1f & 3e).

**AE:** Isl. Nisyros: Evangelistria, alt. 170 m., 36° 36' 22.331'' N, 27° 8' 41.394'' E, 8 May 2018, leg. E. Liveri, V. Ketsilis-Rinis & N. Cellinese 303 (UPA 24650).

*Campanula nisyria* (Fig. 1f) is endemic to the volcanic island Nisyros (Papatsou & Phitos 1975). Nisyros is the easternmost active volcano of the South Aegean Volcanic Arc (Sachpazi & al. 2002; Higgins 2009; Kougioumoutzis & Tiniakou 2014). Additionally, it is the only oceanic island in the Aegean; i.e. it has never been connected to a continental land-mass (Tibaldi & al. 2008). After the first description of *C. nisyria*, Burton (1991) referred that it is an extreme isolated form of *C. lyrata*, while Strid (2016a) considered it as a synonym of *C. hagielia*. However, recent molecular data (Liveri & al. 2020) reveal that *C. nisyria* belongs to a well-supported clade together with five Turkish endemics, whereas *C. lyrata* and *C. hagielia* form a different clade; both clades though are included in a larger clade comprised of *Campanula* taxa distributed in southeastern Aegean and Anatolia.

The only chromosome record ( $2n = 34$ ) for *Campanula nisyria* originates from the *locus classicus* (Papatsou & Phitos 1975).

The karyotype of *Campanula nisyria* examined here is diploid ( $2n = 2x = 34$ ), symmetrical, consisting of mostly metacentric (m) and submetacentric (sm) chromosomes; two of them bearing small spherical satellites which are rarely found in this size in the section (Fig. 3e). The chromosome length varies from 0.91  $\mu\text{m}$  to 1.95  $\mu\text{m}$  (Table 1). The chromosome count is in accordance with the previous study by Papatsou & Phitos (1975). However, the karyotype morphology and a microphotograph are provided here for first time (Fig. 3e).

**1990. *Campanula pelviformis*** Lam. —  $2n = 2x = 34$  (Fig. 3d).

**Cr:** Prov. Heraklio: New national road Iraklio-Agios Nikolaos, 42° km, close to Malia, alt. 100-120 m., 35° 18' 24.563'' N, 25° 22' 43.745'' E, 11 May 2017, leg. E. Liveri, V. Ketsilis-Rinis & N. Cellinese 295 (Herb. Phitos & Kamari in UPA).

*Campanula pelviformis* is endemic to Crete, growing mainly in the eastern part of the island (Phitos 1965; Fedorov & Kovanda 1976; Fielding & Turland 2005; Strid 2016a, b). Except from inhabiting calcareous cliffs and rocky slopes like the most of *Campanula* sect. *Quinqueloculares* species, *C. pelviformis* is also found growing along steep, rocky, roadside banks (Fielding & Turland 2005; Cellinese & al. 2009).

Phitos (1964b) reports the chromosome number  $2n = 34$  for *C. pelviformis* in material from Crete of unknown locality and provides a karyotype drawing indicating two satellites.

The same chromosome count has been also mentioned for the species in material of unknown origin by Sugiura (1938, 1942).

The examined material of *Campanula pelviformis* has a diploid ( $2n = 2x = 34$ ) and symmetrical karyotype consisting of mostly metacentric (m) and submetacentric (sm) chromosomes (Fig. 3d). The chromosome length varies from 0.88  $\mu\text{m}$  to 1.61  $\mu\text{m}$  (Table 1). Our findings confirm previous studies (Sugiura 1938, 1942; Phitos 1964b), however, no SAT-chromosomes are observed in the material examined here. The karyotype is firstly presented with a microphotograph hereby (Fig. 3d).

**1991.** *Campanula rupestris* Sm. —  $2n = 2x = 34$  (Fig. 4a).

**Gr:** Prov. Viotia: Springs Krya, alt. 300-400 m., 38° 25.908' N, 22° 52.540' E, 5 May 2016, leg. E. Liveri, A. A. Crowl & N. Cellinese 209 (UPA 24653).

*Campanula rupestris* is characterized by a consistently trilocular ovary with three stigmas, which is also found in other five species of the section; in total six species comprising the “*C. rupestris* group” (Phitos 1963a, 1964a). The species was discovered by Sibthorp, based on material from “Livadia” (Sterea Hellas) and was consequently described by Smith (Sibthorp & Smith 1819). The exact *locus classicus* of *C. rupestris* was enigmatic since no more details of the locality were known, until Phitos re-discovered it in 1962 (see Phitos 1964a, 1995c, 2016). Before 1964, various *Campanula* taxa were erroneously referred to *C. rupestris* in literature causing much confusion due to the lack of herbarium material and the morphological variability of “*C. rupestris* group”. Most likely, the species is also extended to Mt. Elikon (Phitos 1964a, 1995c). Eddie & Ingrouille (1999) studied four populations of *C. rupestris*; two of them should be re-examined because of possible confusion with *C. topaliana* Beauverd subsp. *delphica* Phitos, while for one population the mentioned locality is rather unclear (Phitos 2016).

*C. rupestris* is recognized according to IUCN (1994) criteria as Vulnerable (VU) in the first *Red Data Book of Rare and Threatened Plants of Greece* (Phitos 1995c) and it is also included in the Greek Presidential Decree 67/1981.

The karyological study of *Campanula rupestris* from its *locus classicus* revealed  $2n = 34$  chromosomes, accompanied with a karyotype drawing indicating one pair of SAT-chromosomes (Phitos 1964a). There are also two more references reporting the same chromosome number for *C. rupestris* in material from Peloponnisos (Phitos 1963a; Contandriopoulos 1964); most probably these references are related to *C. topaliana* subsp. *cordifolia* Phitos. Gadella (1964) also gives the same chromosome count for cultivated material of *C. rupestris* originated from England and the former Soviet Union.

The examined material of *Campanula rupestris* was also collected from the *locus classicus* and shows the same chromosome number and morphology. *C. rupestris* has diploid ( $2n = 2x = 34$ ) and symmetrical karyotype, consisting of mostly metacentric (m) and submetacentric (sm) chromosomes with one pair of SAT-chromosomes (Fig. 4a). The chromosome length ranges from 1.02  $\mu\text{m}$  to 1.95  $\mu\text{m}$  (Table 1). Microphotograph is firstly provided hereby (Fig. 4a).

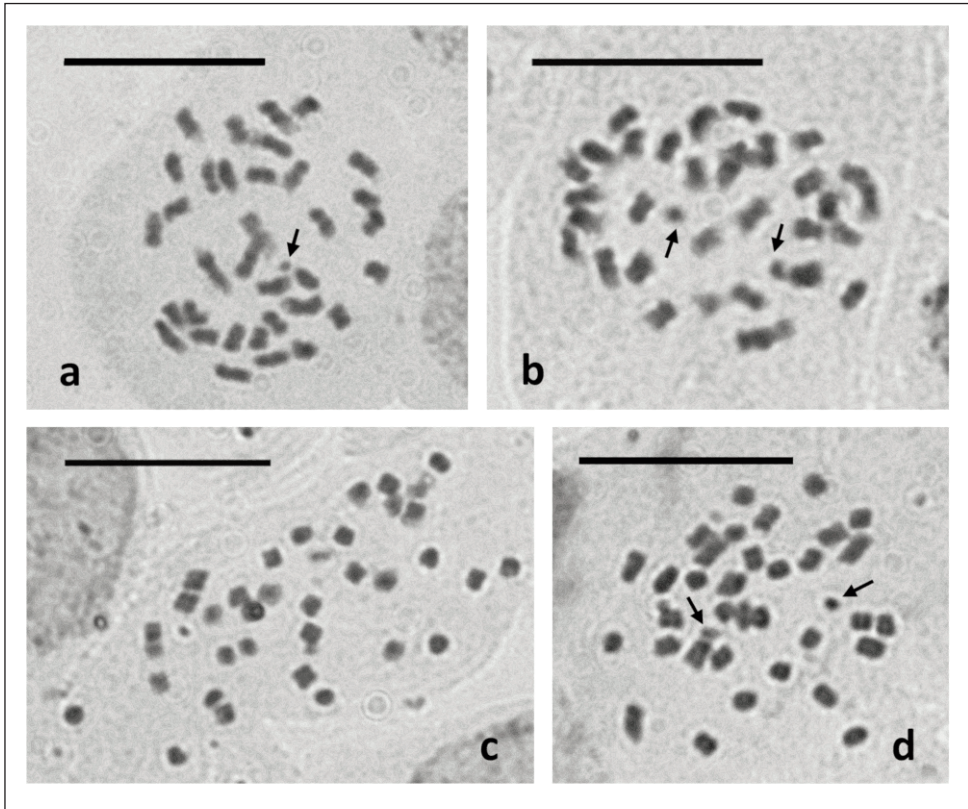


Fig. 4. Microphotographs of mitotic metaphase plates of *Campanula* section *Quinqueloculares*: **a**, *C. rupestris*,  $2n = 34$ ; **b**, *C. topaliana* subsp. *cordifolia*,  $2n = 34$ ; **c**, and **d**, *C. topaliana* subsp. *delphica*,  $2n = 34$ . – Arrows indicate satellites. Scale bars = 10  $\mu$ m.

**1992.** *Campanula topaliana* subsp. *cordifolia* Phitos —  $2n = 2x = 34$  (Fig. 4b).

**Gr:** Prov. Iliia: Thermal springs of Kaiafas, alt. 0-5 m., 37° 30' 52.49" N, 21° 36' 54.94" E, 8 Apr 2017, leg. *E. Liveri* & *V. Ketsilis-Rinis* 266 (Herb. Phitos & Kamari in UPA).

*Campanula topaliana* is a variable species which includes three subspecies *sensu* Phitos (1965): 1) subsp. *topaliana*, distributed in a very restricted region between Kalavryta and Monastery Agia Lavra (Prov. Achaia); 2) subsp. *cordifolia*, which occurs in the most part of Peloponnisos (Prov. Achaia, Argolida, Arkadia, Korinthia, Lakonia, Messinia); and 3) subsp. *delphica*, growing in Delphi (Prov. Fokida) and the nearby area. The three subspecies mainly differ in the shape of basal leaves and the size of corolla (Phitos 1965, 1969; Fedorov & Kovanda 1976).

The chromosome count from a new population studied here is in accordance with previous reports (Phitos 1965, 1969) in material from *locus classicus* (Zachlorou near

Kalavrita). The examined material shows a diploid ( $2n = 2x = 34$ ), symmetrical karyotype, consisting of mostly metacentric (m) and submetacentric (sm) chromosomes; two large satellites are observed in most karyotypes (Fig. 4b). The chromosome length ranges from 0.85  $\mu\text{m}$  to 1.73  $\mu\text{m}$  (Table 1). Karyotype morphology and a microphotograph are given for first time here (Fig. 4b).

**1993. *Campanula topaliana* subsp. *delphica*** Phitos —  $2n = 2x = 34$  (Figs 1d, 4c & 4d).

**Gr:** Prov. Fokida: close to Delphi, alt. 450 m., 38° 29' 15.21'' N, 22° 28' 11.37'' E, 9 Apr 2017, leg. E. Liveri & V. Ketsilis-Rinis 258 (Herb. Phitos & Kamari in UPA).

*Campanula topaliana* subsp. *delphica* (Fig. 1d) differs from the other subspecies in its grayish-sericeous basal leaves, long racemose to paniculate stems and their long, thin branches (Phitos 1965; Fedorov & Kovanda 1976).

Our results show that *C. topaliana* subsp. *delphica* has also a diploid ( $2n = 2x = 34$ ), symmetrical karyotype comprised of mostly metacentric (m) and submetacentric (sm) chromosomes (Fig. 4c); one pair of submetacentric (sm) SAT-chromosomes is observed in most metaphase plates (Fig. 4d). The chromosome size varies between 0.83 and 1.17  $\mu\text{m}$  (Table 1). To our knowledge, these are the first chromosome data for the subspecies.

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