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## Karyological studies on some endemic and rare species of Kefalonia, Ionian Islands, Greece

### Abstract

Samaropoulou, S., Bareka, P., Artelari, R. & Kamari, G.: Karyological studies on some endemic and rare species of Kefalonia, Ionian Islands, Greece. — Fl. Medit. 23: 215-221. 2013. — ISSN: 1120-4052 printed, 2240-4538 online.

The chromosome numbers and the karyotype morphology of five taxa originating from Kefalonia, Ionian Islands, are given. Regarding *Saponaria aenesia*, a karyotype analysis is reported here for the first time, while for *Fritillaria mutabilis* it is presented for the first time from Kefalonia island. The remaining data are in accordance with previous references for the respective taxa. Additionally, data for all studied taxa concerning their habitat and potential threats are discussed. Finally, an attempt is done to assess the conservation status of their populations and to suggest measures for their protection and sustainable management.

*Key words:* Karyotype analysis, *Centaurea subciliaris*, *Cymbalaria microcalyx*, *Fritillaria mutabilis*, *Saponaria aenesia*, *Stachys parolinii*, conservation status.

### Introduction

Kefalonia is the largest island of the Ionian Islands (West Greece) and has a very rich flora. However, only 6 taxa have been found to be stenoendemic of the island [i.e. *Ajuga orientalis* subsp. *aenesia* (Heldr.) Phitos & Damboldt, *Limonium cephalonicum* Artelari, *Saponaria aenesia* Heldr., *Scutellaria rupestris* subsp. *cephalonica* (Bornm.) Greuter & Burdet, *Silene cephalenia* Heldr. subsp. *cephallenia*, *Viola cephalonica* Bornm.], while 61 are Greek endemic species (Katsouni & al. 2009; Karagianni 2010; Katsouni 2012). Among other things, this implies that Kefalonia is an island, derived from the mainland rather than the sea. It is very significant that Kefalonia is the only island of Greece characterized by an *Abies cephalonica* fir forest, with Mt. Ainos being its 'locus classicus'. According to its floristic elements and their distribution, Kefalonia belongs to the Adriatic-Ionian phytogeographical region (Phitos & Damboldt 1985).

So far, not many of the 1088 taxa, occurring on Kefalonia island have been investigated karyologically (Phitos & Damboldt 1971, 1985; Kapasa & al. 2001). This paper constitutes part of the first author's MSc thesis and it presents a karyological study on some endemic and rare species of this Greek island. The main aim of this thesis is to combine

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\* Extended and enriched version of the poster presented at the XIV Optima meeting in Palermo, 9-15 Sept. 2013.

information and comments about the karyology of some very important species with data about their habitat and potential threats. Finally, an attempt is being made to assess the conservation status of their populations and to suggest measures for their protection and sustainable management.

## Material and method

Living plants or seeds of the investigated taxa (Table 1) were cultivated in the Experimental Botanic Garden of the University of Patras. The cytological study is based on the squash technique and the chromosome counts were made from root tip metaphases (Östergren & Heneen 1962; Kamari 1976).

The root tips were pretreated in an aqueous solution of 8-hydroxyquinoline (0,002% w/v) for 5-6 hours – in the case of *Fritillaria mutabilis* in a mixture of 1:1 8-hydroxyquinoline (0,002% w/v): colchicine (0.3 w/v) for 3 hrs (Kamari 1984) – and fixed in 3:1 (v/v) absolute ethanol:glacial acetic acid for 24 hours at 4 °C. Fixed root tips were stored at -20 °C at 75% ethanol for some days to months.

Before their use, the root tips were hydrolyzed in 1N HCl 60 °C for 10-12 min and stained in Feulgen for 3 hrs (Darlington & La Cour 1969). Prior to squashing, the stained root tips were put on a slide with a drop of 45% (v/v) acetic acid. The slides were observed and photos were taken with AXIOPHOT Zeiss microscope, equipped with a Leica digital camera.

Table 1: Alphabetical list of the investigated taxa with location and chromosome number – \*: first report; †: first report of karyotype morphology.

<b>Taxon</b>	<b>Location</b>	<b>2n</b>
<i>Centaurea subciliaris</i>	Mt. Ainos	36 +1B
<i>Cymbalaria microcalyx</i> subsp. <i>minor</i>	Mt. Ainos	28
<i>Fritillaria mutabilis</i>	Mt. Roudi	24 †
<i>Saponaria aenesia</i>	Mt. Ainos	28 *, †
<i>Stachys parolinii</i>	Mt. Roudi	34

## Results

### *Caryophyllaceae*

*Saponaria aenesia* Heldr. — 2n = 28 (Fig. 1A).

**Gr:** Ionian Islands, Isl. Kephallinia, Mt. Ainos, 38° 07' 45'' N, 20° 41' 12'' E, alt. 1367 m, 05 Jul 2009, *Karagianni s.n.* (seeds), *Samaropoulou* cult. no *SK15* (UPA).

This is a stenoendemic species of Kephallonia island, located mainly on calcareous ground at 400-1500 m alt. A typical characteristic of its subpopulations' status is their

sparse and interspersed distribution, thus never forming a large and united population (Katsouni & al. 2009).

*Saponaria aenesia* has been characterized, according to the IUCN 2001 criteria, as an Endangered (EN) species, due to the above mentioned restricted and disjunct distribution, as well as the detection of very few mature individuals. The species is mainly subjected to fire threats and intense grazing (Katsouni & al. 2009).

The chromosome number,  $2n = 28$  and the karyotype morphology of *Saponaria aenesia* are recorded for the first time. The karyotype is diploid, symmetrical, consisting of mostly metacentric (m) chromosomes, ranging in size between 1.47 and 2.19  $\mu\text{m}$  (Fig. 1A).

### Compositae

*Centaurea subciliaris* Boiss. & Heldr. —  $2n = 36 + 1B$  (Fig. 1B).

**Gr:** Ionian Islands, Isl. Kephallinia, Mt. Ainos,  $38^{\circ} 08' 24''$  N,  $20^{\circ} 39' 33''$  E, alt. 1596 m, 16 Aug 2008, *Karagianni s.n.* (seeds), *Samaropoulou* cult. no *SK12* (UPA).

Karaplianis & Constantinidis (2009), in accordance to Matthäs (1976) suggested that the plants from the Ionian Islands (Kefalonia and Lefkada) belong to the typical subspecies of *Centaurea subciliaris*, while those from Akarnania (Sterea Ellas) to the subspecies *acarnanica* Matthäs, both taxa being endemic to their distributional areas. However, Greuter & Raab-Straube (2008) raised subspecies *acarnanica* to species level and reported that *C. subciliaris* is also distributed in Bulgaria. More detailed study, using material from more localities and applying new karyological techniques will help elucidate the story behind this very interesting *Centaurea* group.

*C. subciliaris* occurs in calcareous places, uncultivated fields and at the edges of the roads. Most of Kefalonia's subpopulations are found at 400-600 m alt., however this species also sporadically appears on Mt. Ainos up to an altitude of 1600 m.

Road widening and intense grazing are the main threats for the subpopulations of the taxon.

Previous chromosome reports refer to diploid ( $2n = 18 + 2B$ ) and tetraploid ( $2n = 4x = 36 + 0-4B$ ) chromosome numbers (Phitos & Damboldt 1971, 1985). Our results from a sub-

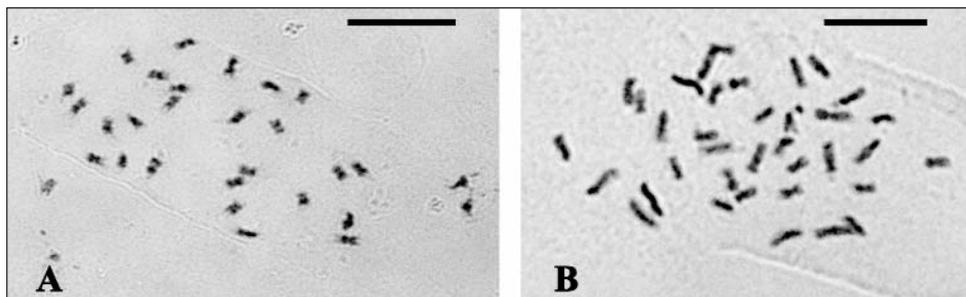


Fig. 1. Microphotographs of mitotic metaphase plates of: **A.** *Saponaria aenesia*,  $2n = 28$ ; **B.** *Centaurea subciliaris*,  $2n = 4x = 36 + 1B$ . – Scale bars = 10  $\mu\text{m}$ .

population of Mt. Ainos are in agreement with the latter reports ( $2n = 4x = 36 + 1B$ -chromosomes). The karyotype is found to be symmetrical with metacentric (m) and submetacentric (sm) chromosomes, ranging in size between 2.13-3.63  $\mu\text{m}$ . Moreover, the presence of one B-chromosome is observed, as well as a pair of SAT-chromosomes (Fig. 1B).

### *Labiatae*

*Stachys parolinii* Vis. —  $2n = 34$  (Fig. 2A).

**Gr:** Ionian Islands, Isl. Kephallinia, Mt. Roudi, 38° 11' 40'' N, 20° 37' 28'' E, alt. 820 m, 01 Jul 2009, *Karagianni s.n.* (seeds), *Samaropoulou* cult. no SK17 (UPA).

*Stachys parolinii* is a Greek endemic species, which occurs in N Peloponnisos, Sterea Hellas and on Ionian Islands (Kephallonia and Lefkada). It is localized in calcareous grounds and open, rocky places with macchie and phrygana, at 10-900 m alt. (Phitos & Damboldt 1985, Tan & Iatrou 2001).

Intense grazing, especially outside the protected area of the Aenos National Park, is the main activity threatening the subpopulations of the taxon.

The chromosome number  $2n = 34$  agrees with the previous records for the species (Baltisberger & Lenherr 1984). The karyotype is diploid and symmetrical, as far as morphology is concerned. The chromosomes range between 1.11 and 2.22  $\mu\text{m}$  (Fig. 2A).

### *Liliaceae*

*Fritillaria mutabilis* Kamari —  $2n = 24$  (Fig. 3).

**Gr:** Ionian Islands, Isl. Kephallinia, Mt. Roudi, at the summit area Gioupari, 38° 11' 08'' N, 20° 36' 56'' E, alt. 1050 m, 01 May 2013, *Phitos & Kamari 21775*, *Samaropoulou* cult. no SK83 (UPA).

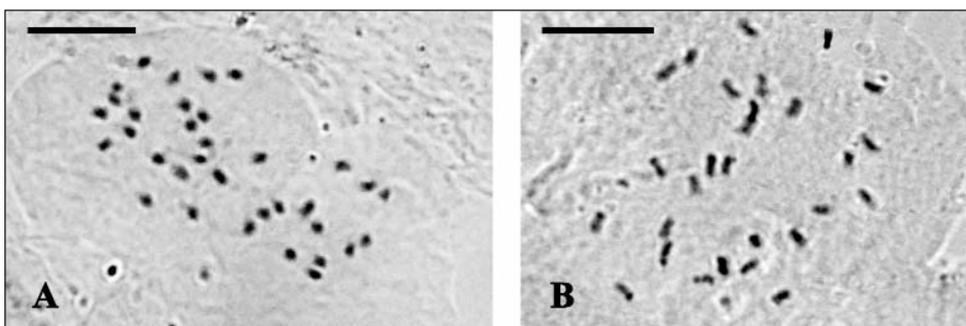


Fig. 2. Microphotographs of mitotic metaphase plates of: **A.** *Stachys parolinii*,  $2n = 34$ ; **B.** *Cymbalaria microcalyx* subsp. *minor*,  $2n = 28$ . – Scale bars = 10  $\mu\text{m}$ .

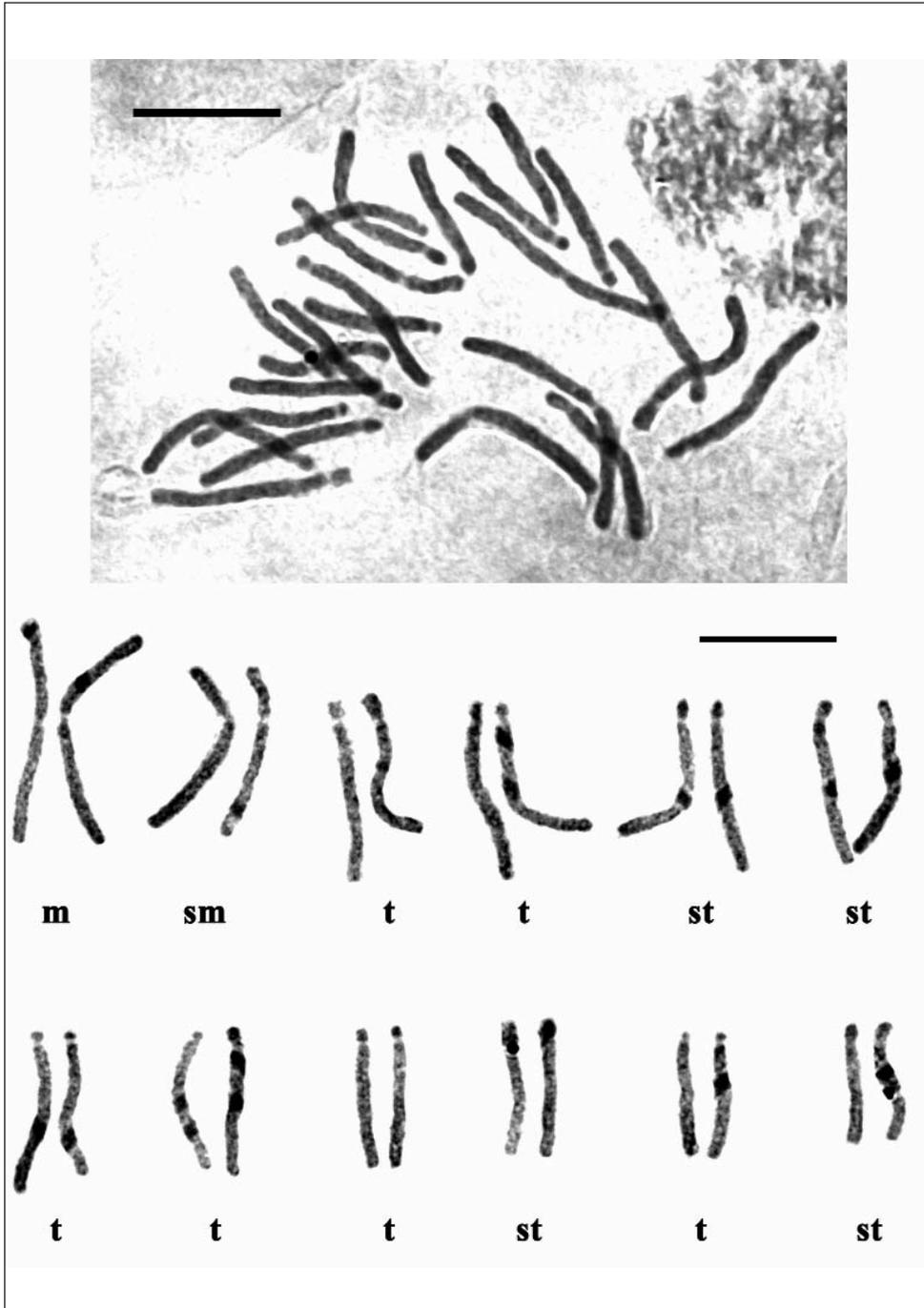


Fig. 3. Microphotograph of mitotic metaphase plates and karyogram of *Fritillaria mutabilis*,  $2n = 24$ .  
– Scale bars = 10  $\mu$ m.

This is a Greek endemic *Fritillaria* species, appearing on the mountains of N Peloponissos, Sterea Hellas, in S Pindos and on Ionian Islands (Kefalonia and Lefkada). It is found on rocky slopes with macchie or in montane to subalpine meadows and openings of *Abies cephalonica* forest. It prefers limestone, schist or rarely ophiolitic substrates, at (400-) 600-2100 m alt. (Kamari 1991a).

*Fritillaria mutabilis* is mainly threatened in Kefalonia due to the very intense grazing practices.

The karyotype of a subpopulation of Mt. Roudi consists of  $2n = 2m + 2sm + 6st + 2st-SAT + 12t = 24$  chromosomes and it is diploid, asymmetrical, as far as its morphology is concerned. Chromosome size ranges between 10.8-19.5  $\mu\text{m}$  (Fig. 3). Previous reports for the species demonstrate  $2n = 24 + 0-2B$ , as well as an acrocentric pair of SAT-chromosomes, bearing very big and long satellites (Kamari 1991b).

### Scrophulariaceae

*Cymbalaria microcalyx* (Boiss.) Wettst. subsp. *minor* (Cufod.) Greuter —  $2n = 28$  (Fig. 2B).

**Gr:** Ionian Islands, Isl. Kephallinia, Mt. Ainos, 38° 09' 34'' N, 20° 37' 24'' E, alt. 1073 m, 27 Jul 2008, *Karagianni s.n.* (seeds), *Samaropoulou* cult. no SK25 (UPA).

*Cymbalaria microcalyx* subsp. *minor* is an endemic taxon of Greece and its distribution includes Ionian Islands (Kefallinia), Peloponissos and S Pindos. The species is localized in rock crevices or stony places, up to 1950 m alt. (Raus 1991).

The chromosome number  $2n = 4x = 28$  agrees with previous reports for the subspecies by Damboldt & Phitos (1971) and Speta (1986). Its karyotype is tetraploid and symmetrical, consisting of metacentric (m) and submetacentric (sm) chromosomes, varying in size between 1.33-2.67  $\mu\text{m}$  (Fig. 2B).

### Acknowledgements

We would like to thank the employees of the Management Body of the Aenos National Park for their important collaboration particularly in the form of support during plant collection and the provision of bulbs or seeds that were used in the karyological study.

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