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Autor(en): Routsi, E. / Georgiadis, Th.

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## Cytogeographical study of *Centaurea* L. sect. *Acrocentron* (Cass.) DC. (Asteraceae) in Greece

## E. Routsi and Th. Georgiadis

Botanical Institute, Division of Plant Biology, Department of Biology, University of Patras, GR 265 00, Patras, Greece

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#### **Abstract**

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The cytology, cytogeography, morphology and systematics of *Centaurea* sect. *Acrocentron* in Greece are discussed. The species of this section are divided into groups of related taxa according to their morphology, cytology and geographical distribution. The chromosome numbers of 13 taxa are given for the first time and the numbers of 13 taxa are confirmed.

Key words: Asteraceae, Centaurea, Acrocentron - Cytogeography, Systematics.

#### Resumé

Etude cytologique, cytogéographique et systématique du genre *Centaurea* de la section *Acrocentron* en Grèce. Les espèces de cette section sont classifiées dans trois subsections en relation avec leur morphologie, cytologie et distribution géographique. Les nombres chromosomiques pour 13 taxa sont confirmés; les nombres d'autres 13 taxa sont nouveaux. La section *Acrocentron* possède deux nombres chromosomiques de base x=10 et x=11. Selon les données cytogéographiques et systématiques le nombre de base x=11 est probablement le plus ancien. L' etude cytogéographique a démontré une origine du Nord de la Gréce ou orientale de quelques espèces; mais la majorité des taxons sont endémiques de la Gréce.

#### Introduction

The genus *Centaurea* is represented in Greece by c.150 taxa (species and subspecies) which belong to 12 sections (Wagenitz 1986).

Centaurea sect. Acrocentron and C. sect. Acrolophus (Cass.) DC. are the two largest sections of the genus, and include a great number of Greek endemics. Together they represent more than 50% of the Greek Centaurea species. Following the biosystematic study of Centaurea sect. Acrolophus in Greece (Georgiadis 1980), the biosystematic study of C. sect. Acrocentron (in Greece) was undertaken, and the results of its cytogeographical and morphological study are presented in this paper.

The systematic problems of *C*. sect. *Acrocentron*, outlined by Gardou (1974), concern the taxonomic status of many taxa and the number of taxa belonging to the section. Twenty-six taxa (including varieties) were reported by Halácsy (1902) to be found in Greece, while Hayek (1931) reported 29 taxa (including varieties). In Flora Europaea (1976), Dostál classified these 29 taxa in five sections: *Acrocentron*, *Orientales*, *Carduiformes*, *Lopholoma*, *Rhizanthae*, and mentions only 6 taxa of *C*. sect. *Acrocentron* from the Greek region. According to Wagenitz & Gamal-Eldin (1985), *C*. sect. *Acrocentron* in Greece includes 31 taxa. Cytological studies by various authors – Guinochet & Foissac (1962); Gardou (1969, 1972, 1974); Damboldt & Matthäs (1975, 1979); Georgiadis (1980, 1983) – have shown that the genus *Centaurea* appears to have many basic chromosome numbers: x=8, 9, 10, 11, 12, 13, 14, 15, 16.

Centaurea sect. Acrocentron is characterized by 2n=2x=20, 2n=3x=30, 2n=4x=40, 2n=10x=100 and 2n=11x=110 (Runemark 1967, Gardou 1969, Phitos 1970, 1971, Damboldt & Matthäs 1975, Routsi & Georgiadis 1988), as well as 2n=2x=22 (Runemark 1967, Georgiadis & Christodoulakis 1984). Thus, C. sect. Acrocentron has two basic chromosome numbers: x=10 and x=11.

The appearance of two basic chromosome numbers and a great variety of morphological features show that *C.* sect. *Acrocentron* is neither cytologically nor morphologically homogeneous. A biosystematic study was therefore necessary to establish a comprehensible phylogenetic classification of this section.

## Cytology

Cytological investigations were carried out on root tips of plants raised from achenes collected in the wild. These were pretreated with 8-hydroxycinoline/colchine (3:1), fixed in Carnoy solution (3:1) and hydrolyzed with 1M HCl. They were later stained with Feulgen stain of the 31 taxa of the section, 26 taxa, belonging to 50 populations, were studied cytologically. The following five chromosome numbers were listed for the first time as new reports in Routsi & Georgiadis (1988) as follows:

1) C. redempta subsp. cytherea	2n = 20
2) C. ebenoides	2n = 20
3) C. macedonica	2n=20
4) C. lactucifolia	2n=20
5) C. psilacantha	2n=20

A further 8 chromosome numbers are also listed as new reports in this paper:

6)	C. rupestris subsp. athoa	2n=20
7)	C. rupestris subsp. parnonia	2n = 20
8)	C. rupestris subsp. kozanii	2n = 40
9)	C. grbavacensis	2n = 20
10)	C. laconica subsp. laconica	2n = 20
11)	C. rupestris subsp. finazzeri	2n = 20

This number has also been reported from the former Yugoslavia (Siljak-Yakovlev 1985) and Bulgaria (Kuzmanov et al. 1983).

12) C. salonitana 2n=20.

Diploid populations of *C. salonitana* have been found for the first time in Greece. Until now only tetraploid populations were known. For this taxon, the number 2n=20 has also been reported from Romania (Lungeanu 1975) and Bulgaria (Kuzmanov et al. 1979).

#### 13) C. immanuelis-loewii

2n=20.

The chromosome number of this species in Greece is given here for the first time. Prior to this study it was only known from Bulgaria (Kuzmanov et al. 1979, 1985).

The mitotic metaphases of the taxa mentioned above are represented in Figure 1. In addition, the chromosome numbers of 13 taxa (Fig. 2) of *C.* sect. *Acrocentron* have been confirmed after studying various populations as listed in Table 1.

The remaining 5 taxa have either not been studied cytologically or their chromosome numbers have been given by previous authors:

- The numbers of *C. raphanina* subsp. *raphanina*, *C. urvillei* subsp. *armata* and *C. laconica* subsp. *lineariloba* are known and are presented in Table 1.
- The population of *C. scabiosa* subsp. *fritschii* from Rhodopi the only location of *C. scabiosa* in Greece) has not yet been studied cytologically. The number 2n=20 has been reported from the former Yugoslavia (Lovka et al. 1971), Germany (Damboldt & Matthäs 1975) and Spain (Valdés-Bermejo 1983). The numbers 2n=20, 30, 40 have also been reported from France (Gardou 1969).
- The cytology of *C. tuntasia* has not been studied previously. This species was lost found in 1912 by Tuntas [W], and despite persistent attempts to relocate it, remains undiscovered. It seems possible that it was a rare, random and sterile hybrid between *C. salonitana* and *C. achaia*, as indicated by its intermediate morphological characters. It is possible that the taxon was found only in very limited areas and has become extinct due to because of urban and rural development and tourism activities in the region Attica where it was first found.

The cytological study of *Centaurea* sect. *Acrocentron* in Greece showed the existence of two basic chromosome numbers: x=10 and x=11. With x=10, diploids, tetraploids, decaploids and endecaploids are found, while with x=11 only diploids appear. All known chromosome numbers of the taxa belonging to C. sect. *Acrocentron* in Greece are presented in Table 1.

#### Morphology and systematics

Morphological studies were carried out on fresh material and herbarium specimens. All voucher specimens are deposited in UPA.

The morphological study of C. sect. Acrocentron, combined with the existence of the two basic chromosome numbers, led us to divide the section into three subsections (Routsi & Georgiadis 1994b): C. sect. Acrocentron subsect. Graecae Routsi & Georgiadis with x=10, C. sect. Acrocentron subsect. Atropurpureae Routsi and Georgiadis with x=11, and C. sect. Acrocentron subsect. Achaiae Routsi & Georgiadis with x=11. The two groups with x=11, although possessing the same basic chromosome number, are distinctly different as their different morphology shows.

Five groups of related taxa, with similar morphological features, were distinguished within *C.* sect. *Acrocentron* subsect. *Graecae*, taking into account their distinct morphological differences, as well as their different geographical distributions and origins (Table 1).



Fig. 1. Mitotic metaphases of: **a** Centaurea lactucifolia, **b** C. redempta subsp. cytherea, **c** C. ebenoides, **d** C. immanuelis-loewii, **e** C. grbavacensis, **f** C. psilacantha, **g** C. macedonica, **h** C. rupestris subsp. athoa, **i** C. rupestris subsp. finazzeri, **j** C. rupestris subsp. parnonia, **k** C. rupestris subsp. kozanii, **l** C. laconica, **m** C. salonitana



Fig. 2. Mitotic metaphases of: **a** Centaurea graeca, **b** C. atropurpurea, **c** C. redempta subsp. redempta, **d** C. raphanina subsp. mixta, **e** C. spruneri, **f** C. salonitana, **g** C. acicularis, **h** C. aetolica, **i** C. achaia, **j** C. euboica subsp. euboica, **k** C. korinthiaca, **l** C. rechingeri, **m** C. urvillei subsp. urvillei.

Table 1. Species, subspecies, varieties and chromosome numbers of Centaurea sect. Acrocentron in Greece.

N <sub>o</sub>	Taxon	Х	2n	Authors	Location
1.	C. laconica Boiss. subsp. laconica C. laconica Boiss. subsp. lineariloba	10	20 20	Routsi, 1993 Runemark, 1967	Prov. Cyclades: Andros, Sifnos
3	(Hal. & Doerfler) Gamal-Eldin & Wagenitz C. redempta Heldr. subsp. redempta	10	20	Phitos, 1970 Runemark, 1967 Phitos & Kamari, 1984 Tzanoudakis, 1986	Prov. Andros. Kreta: Prov. Chania: Kissamos Kreta: Prov. Rethymno Kreta: Prov. Chania: Topolia
4.		10	20	Routsi & Georg.*, 1988 Routsi & Georg., 1988	Kreta: Prov. Chania: Topolia Prov. Pireas Kythira: Kastro
5.	(Rech. I) Koutsi & Georgiadis C. spruneri Boiss. & Heldr.	10	100	Phitos & Kamari, 1973 Phitos, 1970	Prov. Attica: Sounio Prov. Kefallonia: Kefallonia
		10	110	Routsi, 1993 Phitos & Kamari, 1973	Prov. Kefallonia: Agon Prov. Attiki: Marathonas/ Prov. Attol/pia: Katonna & Kompoti
				Phitos, 1970 Phitos, 1971	Prov. Achaia: Mt. Chelmos Kreta: Prov. Heraklio: Siva, Moulia
.9	C. graeca Griseb.	10	20	Phitos, 1970 Strid & Franzen, 1981	Prov. Ioannina: Ioannina Prov. Pierria: Mt. Olympos (350 m)
7	C nsilacantha Boiss. & Heldr	10	20	Routsi & Georg., 1988 Routsi, 1993 Routsi & Georg. 1988	Prov. Larissa: Pinios Prov. Kozani: Siatista, Vourinos Prov. Viotia: Ås Lonkas/Prov. Fokida: Delnhi
∞ ∞		10	<u>5</u>	Routsi, 1993 Runemark, 1967	Prov. Fokida: Eleonas Creta: Prov. Lasithi: Sitia
9.	subsp. raphanina C. raphanina Sibth. & Sm.	10	20	Phitos, 1971 Runemark, 1967	Creta: Prov. Rethimno: Mt. Idi (1500 m) Creta: Prov. Attica: Porto Rafti/
	subsp. <i>mixta</i> (DC.) Kunemark			Phitos, 1971 Routsi & Georg., 1988 Routsi, 1993	Prov. Evia: Akr. Kafireus/Prov. Samos: Ikaria/ Prov. Cyclades: Naxos, Kufonisi Prov. Achaia: Mt. Panachaiko (1200m) Prov. Argolida: Nemea Prov. Cyclades: Serifos/Prov. Attica: Sounio/ Prov. Magnisia: Tsougria/Prov. Korinthia:
10.	C. urvillei DC. subsp. urvillei	10	40	Georg. & Christ.*, 1984	Prov. Samos: Samos
Ξ.	C. urvillei DC. subsp. armata Wagenitz Hal.	10	40	Koutst, 1993 Runemark, 1967 Gardon, 1975	Prov. Dodekanissa: Rodos Prov. Dodekanissa: Rodos
12.	C. acicularis Sibth. & Smith var. acicularis	10	20	Runemark, 1967 Georg. & Christ., 1984 Routsi & Georg., 1988	Prov. Dodekanissa: Nodos Prov. Samos: Samos: Kerkis, Ampelos Prov. Samos: Ikaria

\* Georg. = Georgiadis, Christ. = Christodoulakis, Mat. = Matthäs. The chromosome numbers given for a) C. psilacantha 2n=22, (Moore 1968, Taxon 17: 421) and b) C. grbavacensis 2n=22 (Strid 1980) appear to be incorrect.

## Key to the subsections and groups of Centaurea sect. Acrocentron

1 Appendages narrowly semi-lunate, very small (1/8–1/7 of the bract), dark brown. Florets brown-red.

**subsect.** *Atropurpureae* (basic chromosome number x=11)

- 1\* Appendages semi-lunate, broader, (1/3–3 times longer than the bract), triangular or convex.
- 2 Appendages convex, stramineous, covering the bracts (70%–100%), with stout terminal spines. Florets whitish to light purple. Leaves pinnatisect(-pinnate), with linear lanceolate segments.

**subsect.** *Achaiae* (basic chromosome number x=11)

2\* Appendages semi-lunate to triangular, usually not covering the bracts, straw-coloured to dark brown. Florets yellow, purple, browned. Leaves usually lyrate, pinnatilobed to pinnatisect(-pinnate), with ovate to linear segments.

**subsect.** *Graecae* (basic chromosome number x=10)

- 3 Florets pale yellow or yellow.
- 4 Appendages covering the bracts (90%–100%), slightly convex. Florets pale yellow. Leaves glabrous, pinnatilobed, with big, rounded segments.

C. lactucifolia group

- 4\* Appendages not covering the bracts. Florets yellow.
- 5 Short plants, almost without stems. Appendages triangular, usually with 3–5 spinules, straw-coloured. Leaves lyrate, pinnatisect, tomentose, with ovate segments.

C. acicularis group

- 5\* Tall plants, 30(-100) cm, rarely short. Appendages semi-lunate, with 6–12 cilia, straw coloured or dark brown. Leaves arachnoid, pinnatisect(-pinnate), with oblong segments.

  \*\*C. rupestris\* group\*\*
- 3\* Florets purple or brown-red.
- 6 Appendages not covering the bracts, semi-lunate, usually with a stout terminal spine, straw-coloured or sometimes dark brown. Florets usually purple, rarely dark red. Leaves lyrate, pinnatisect, with ovate segments.

C. laconica group

6\* Appendages usually covering the bracts (15%–50%), broadly triangular, decurrent, dark brown with 10–15 cilia. Florets brown-red, rarely red. Leaves pinnatisect(-pinnate), with lanceolate or linear segments, rarely lyrate.

C. scabiosa group

### Cytogeography

The cytology and morphology of the species of *C*. sect. *Acrocentron* seem to be closely linked to their geographical distribution. Thus, examining the groups and subgroups of this section, the following were observed:

- C. sect. Acrocentron subsect. Atropurpureae (x=11). This subsection includes C. rechingeri (2n=22) and C. atropurpurea (2n=22) which are endemic to the Aegean islands and are found on Samos the Sporades and the Cyclades. These isolated species are probably two of the most ancient species of C. sect. Acrocentron, as the absence of polyploidy,

higher basic chromosome number, morphological speciality, less developed floral characters and their geographical distribution suggest. This group was distributed in the region of Aegaeis and the dislocation of this land during the Miocene isolated these species into small populations on the Aegean islands.

- C. sect. Acrocentron subsect. Achaiae (x=11). This subsection, consisting of C. achaia, C. euboica, C. corinthiaca and C. aetolica, is distributed in Attica, Central and North Peloponnese, Sterea Hellas and Evia. It is a Greek endemic group, cytologically homogeneous (2n=22) and characterized by special morphological features, mainly concerning the appendages of the bracts. C. sibthorpii is another species of this section and is closely related to C. achaia. A detailed study of the populations of these two species using Correspondence Factor Analysis showed that C. achaia and C. sibthorpii are to the same species.
- C. sect. Acrocentron subsect. Graecae (x=10). This subsection includes the following groups and species:

## 1. C. lactucifolia group:

C. lactucifolia (2n=20) is a Greek endemic species, found in small populations on the Eastern Aegean Islands, Rhodos and Chalki. It is a taxonomically isolated species, without any close relatives, "possibly a relict from the Pliocene cliff flora" (Carlström 1986).

## 2. C. acicularis group:

C. acicularis (2n=20) is an Anatolian species, closely related to C. chrysantha (Wagenitz 1975b). It is found on the Eastern Aegean Islands and in Western Turkey.

3. C. laconica group: (C. redempta, C. laconica, C. raphanina, C. urvillei, C. spruneri, C. graeca, C. psilacantha)

The diploid species *C. redempta* and *C. laconica* (2n=20) are closely related and constitute an endemic Greek subgroup. They are located in Crete, Kythira, the Peloponnese and the West Cyclades. As shown by their close morphological similarities, these species must have been members of a subgroup distributed in the once-connected landmass of Crete-Kythira-Peloponnese-Cyclades, and were isolated after the dislocation of this land. This subgroup is related to *C. eryngiodes*, a species found in Lebanon, Syria and Egypt (Wagenitz 1975a). Similarities to Anatolian species and the distribution area of the subgroup indicate a possible Anatolian origin.

*C. raphanina*: is a Greek endemic and is found on Crete (subsp. *raphanina*), the Aegean Islands and in Eastern Greece (subsp. *mixta*). Both subspecies are diploid (2n=20).

On the Eastern Aegean Islands, *C. urvillei* is found. It is a very distinct taxon, which is closely related to the Anatolian species *C. lydia* (Wagenitz 1975b). Of the 5–6 subspecies described (Wagenitz 1975b), two are found on the Eastern Aegean Islands and they are both tetraploid (2n=40). Diploid populations (2n=20) of these subspecies have been reported from Turkey (Gardou & Tchehrehgocha 1975). The appearance of polyploidy at the western geographical limits of this species, as well as its similarities to Anatolian species confirm, its Asiatic origin.

*C. spruneri*, a species possessing the highest chromosome number within the section (2n=100, 110), has a relatively wide distribution area. It is found in Crete, Attica, North and Central Peloponnese, Western Greece, the Ionian Islands and Southern Albania.

C. graeca (2n=20), a species distributed in North, Central Greece and the former Yugoslavia, and along with the Greek endemic C. psilacantha (2n=20), found in Sterea Hellas, also belong to the C. laconica group.

## 4. C. rupestris group: (C. salonitana, C. macedonica, C. rupestris, C. tuntasia)

C. salonitana and C. macedonica, two closely related species, are distributed in Macedonia and Central Greece. C. macedonica (2n=20) is found in Central Macedonia and Central Albania. C. salonitana, a species with a wide distribution area (USSR, Turkey, Romania, Bulgaria, Hungary, the former Yugoslavia, Greece), appears in diploid and tetraploid populations. Diploid populations (2n=20) are found in East and Central Macedonia, while the tetraploid (2n=40) are distributed from Macedonia to Attica. The subgroup of C. rupestris (Routsi & Georgiadis 1994), which is represented in Greece by 4 subspecies, grows in NW Turkey, Greece (North Greece and Peloponnese), the former Yugoslavia, Bulgaria, Italy and France. The 3 subspecies, subsp. athoa, subsp. finazzeri and subsp. parnonia (a Greek endemic) are diploid (2n=20) and they are found in the mountains of Athos, Voras, Taygetos and Parnon. The fourth subspecies, subsp. kozanii, found in the area of Kozani, is also an endemic and is tetraploid (2n=40). The taxa of this subgroup are related to the C. scabiosa group, through C. ebenoides and C. immanuelis-loewii, with which they share many morphological similarities.

The *C. rupestris* subgroup seems to have a Northern origin, as indicated by its geographical distribution, its relationship with the *C. scabiosa* group, the lack of similarities with Anatolian species and the existence of polyploid populations in Greece (in their southern distribution area). The appearance of subsp. *athoa* (*C. athoa* DC.) in NW Turkey does not indicate about a possible Anatolian origin since no similarity with other Anatolian species has been observed (Wagenitz 1975b).

## 5. C. scabiosa group: (C. scabiosa, C. immanuelis-loewii, C. grbavacensis C. ebenoides)

C. scabiosa, C. immanuelis-loewii and C. grbavacensis are the southern members of a group originating from the North and are characterized by special morphological features. In Flora Europaea (1976) these species are classified in the sections Lopholoma and Orientales, which include Balkan and European species.

*C. grbavacensis* and *C. immanuelis-loewii*, both diploids (2n=20), are distributed mainly in North Greece and neighbouring former Yugoslavia and Bulgaria. The southern distribution area of this group is Mt. Olympus where *C. grbavacensis* is found. *C. scabiosa* is a species with a wide geographical distribution (USSR-Finland), one subspecies of which (subsp. *fritchii*) is found only in the area of Drama (Rhodopi) in Greece. Other reports from Greece (Pavlidis 1982) possibly concern *C. immanuelis-loewii*.

One species related to this group is *C. ebenoides* (2n=20), a Greek endemic from the Island of Evia. Its appendage morphology and head shape and size are similar to those of *C. immanuelis-loewii*.

#### **Discussion – Conclusions**

Studying the cytology of *Centaurea* sect. *Acrocentron* in Greece, the chromosome numbers of 26 taxa (13 taxa given for the first time) were determined by the study of 50 populations. Two basic chromosome numbers (x=10 and x=11), were found within C. sect. *Acrocentron* and diploid, tetraploid, decaploid and endecaploid species appeared. It is worth noting that the only species of C. sect. *Acrocentron* possessing the basic chromosome number of x=11 are Greek endemics. The fact that 31 taxa (the highest number in the countries of the Mediterranean area) of C. sect. *Acrocentron* are found in Greece, in addition to the existence of great morphological variety and the two basic chromosome numbers, suggest that the re-

gion of Greece played an important role in the differentiation of this section's species and emphasizes the interest of its biosystematic study (Routsi 1993).

Following the new cytological data the 8:12 ratio between the species with x=11 and x=10 as mentioned by Garcia Jacas and Susanna (1992) for the Eastern Mediterranean area, is modified and predominance of the species with x=10 becomes more evident. At the species level, this ratio reaches 6:16 and at the subspecies level reaches a striking 7:23.

In the Eastern Mediterranean, the taxa with x=11 have a distinct morphology, are taxonomically isolated and have restricted geographical distribution. In addition, the taxa with x=11 are diploid as opposed to the Western Mediterranean where only polyploid taxa (paleopolyploid) exist (Garcia Jacas & Susanna 1992). The evolutionary trend from larger to smaller chromosome numbers in the Composites is well known (Babcock 1947, Swanson 1957, Stebbins 1977, Solbrig 1977) and could support the assumption that the basic chromosome number of x=11 is the oldest one.

In the Eastern Mediterranean area, and Greece in particular, speciation in taxa with the basic chromosome number of x=10, seems to be as intensive as that observed in the Western Mediterranean. This could be explained by the existence of more x=10 taxa in the area (23) and also by their higher ploidy level in contrast to the fewer (7), always diploid, taxa with x=11.

The morphological study showed the existence of great variety within this section. Correlation of the cytological and morphological data resulted in *C*. sect. *Acrocentron* being divided into three subsections, one (subsect. *Graecae*) with x=10, and two (subsect. *Atropurpureae* and subsect. *Achaiae*) with x=11, each of them characterized by special morphological features. Within *C*. sect. *Acrocentron* subsect. *Graecae* there is great variety, and further

Tab. 2. Classification of section *Acrocentron* species in Greece according to their geographical distribution and origin.

Taxa with a northern distribution area	Taxa with their main distribution in Greece	Greek endemics	Anatolian species
		C. rechingeri C. atropurpurea	
		C. achaia C. euboica* C. corinthiaca C. aetolica	
C. scabiosa C. grbavacensis			
C. immanuelis-loewii		C. ebenoides	
C. rupestris subsp. finazzeri	C. rupestris subsp. athoa	C. rupestris subsp. kozanii	
C. salonitana	C. macedonica C. graeca	C. rupestris subsp. parnonia	
	C. spruneri	C. psilacantha C. raphanina* C. laconica	C. urvillei*
		C. redempta* C. lactucifolia	C. acicularis

<sup>\*</sup> The subspecies of these species are not mentioned because they belong to the same category

groups of morphologically related taxa can be distinguished. The groups and subgroups of *C*. sect. *Acrocentron* appear to have both different geographical distribution and origin. Thus, the following four categories can be distinguished: a) species originating from the North, b) species with their main distribution in Greece, c) species with exclusive distribution in Greece (Greek endemics), and d) Anatolian species (Table 2).

The division of *Centaurea* sect. *Acrocentron* into the sections proposed here, was based on a detailed cytological, cytogeographical and morphological study of the section *Acrocentron* in Greece. The above classification could be expanded for the total number of species of this section in the whole Mediterranean area by the creation of new subsections. This, however, demands the same detailed analysis for the remaining unknown species.

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