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Karyomorphological study of some Cretan archeophytes

Abstract

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A karyological study of six characteristic archeophytes from the island of Crete is presented, along with comments concerning their taxonomy and IUCN conservation status, whenever appropriate. For *Leontice leontopetalum* subsp. *leontopetalum* the chromosome number 2*n* = 16 is given for the first time in material from Greece. Additionally, new chromosome numbers for *Geranium tuberosum* and *Ranunculus asiaticus* var. *sanguineus* are reported. Karyotype microphotographs and karyological features for all taxa examined are provided and their karyotype morphology is also discussed.

Key words: chromosome numbers, conservation, distribution, karyotype morphology, taxonomy.

Introduction

Changes from traditional to modern agricultural practices on the island of Crete, during the 20th century, have adversely affected the non-invasive Mediterranean weed flora. Many of these species were presumably originally introduced by humans (such plants are termed archeophytes), since the island was cultivated more than 9000 years ago (Phitos & Kamari 2009), and they often exhibit peculiar restricted distributions, reflecting the isolation under which the traditional agriculture developed (Turland & al. 2004). The declining populations of archeophytes are an important but overlooked issue in the conservation of Mediterranean biodiversity. Since insufficient comparative historic data exist for the last century, it is currently difficult or sometimes impossible to measure population decline or threat levels of Cretan weeds. This is at least partly because weeds tend to be undercollected during botanical excursions.

In spring of 2003, a research project took place entitled “Threatened weeds in the traditional agriculture of Crete” and financially supported by the National Geographic Society. The project was a collaboration between the Missouri Botanical Garden, St. Louis U.S.A. (MO) and the Botanical Institute of the University of Patras, Greece (UPA). The goal of this project was to carry out a thorough floristic survey of weeds in sites of traditional agriculture of Crete, Greece (Turland & al. 2004).

During this project, living material was collected for cytological investigation. As a result of this study, the chromosome number, karyotype morphology and geographical distribution of some endemic, rare and interesting archeophytes of Crete are presented along with issues of taxonomy, phytogeography and conservation.

We are pleased to have the opportunity to publish this work as a tribute to our distinguished friend Prof. Francesco M. Raimondo, as recognition of his efforts and achievements.

Materials and methods

During the fieldwork in Crete, fifty cultivated localities (fields, vineyards and olive groves) were surveyed in detail for vascular plant taxa. The presence of 'traditional agriculture' was inferred from (1) a rich weed flora, (2) the absence or non-dominance of the highly invasive S African geophyte, *Oxalis pes-caprae* L., (3) the absence of herbicidal spraying, (4) the absence of irrigation pipe systems (Turland & al. 2004).

Living plants for karyological study were collected from several regions and cultivated in the experimental botanical garden of the Botanical Institute of Patras University. Chromosome counts were obtained from root tip metaphases, using the squash technique (Östergren & Heneen 1962) with some small modifications. Root tips of *Leontice leontopetalum* L. subsp. *leontopetalum, Tulipa doerfleri* Gand., *T. saxatilis* Sieber ex Spreng., *Anemone hortensis* subsp. *heldreichii* (Boiss.) Rech. f. and *Ranunculus asiaticus* var. *sanguineus* (Mill.) DC., were pretreated for 6 h in an 1:1 mixture of 8-hydroxyquinoline (0.002% w/v):colchicine 0.2% w/v. For *Geranium tuberosum* L. an aqueous solution of 8-hydroxyquinoline (0,002% w/v) was used. Fixation in Carnoy (3:1 (v/v) absolute ethanol:glacial acetic acid) was implemented for 24 h at 0-4 ^{o}C. Afterwards, the root tips were hydrolyzed in 1N HCl for 12 min at 60 ^{o}C and placed in Feulgen's stain (Darlington & La Cour 1969) for ca. 4 h.

At least five photomicrographs of each population were examined, taken with a Zeiss Axiophot photomicroscope. Chromosome terminology follows Levan & al. (1965), Stebbins (1971) and Kamari (1976), taking into consideration comments and suggestions by Sybenga (1959), Bentzer & al. (1971) and Favarger (1978). For the characterization of the karyotypes the following chromosome features were calculated: (1) total chromosome length (TLC); (2) mean arm ratio (MAR); (3) centromeric index range (CIR); and (4) arm difference ratio range (ADR); (5) relative length range (RLR). Moreover, following Paszko's (2006) method the additional parameters are given: (1) shortest (SC) and longest (LC) chromosome length; (2) longest to shortest chromosome ratio (LC/SC); (3) mean long-arm length (p); (4) mean short-arm length (q); (5) mean chromosome length; (6) mean centromeric index (CI) (Table 1).

Table 1. Chromosome characteristics of the studied taxa.

Taxon	Ploidy level	Range SC-LC (µm)	Ratio LC/SC	p (µm) Mean (±SD)	q (µm) Mean (±SD)	CL (µm) Mean(±SD)	TLC (µm)	ARR (µm)	MAR (µm) (±SD)	CIR (µm)	CI (µm) Mean (±SD)	ADR (µm)	RLR (µm)	Karyotype morphology
Leontice leontopetalum subsp. *leontopetalum*	2x	5.06-14.95	2.95	6,44 (±1.45)	3.93 (±1.67)	10.37 (±2.65)	165.99	1.00-4.00	1.89 (±0.81)	0.50-0.80	0.63 (±0.09)	0.00-0.60	3.25-9.01	8m + 6sm + 2st-SAT
Geranium tuberosum	2x	1.45-5.07	3.50	1.75 (±0.60)	0.81 (±0.24)	2.55(±0.55)	51.09	1.01-4.00	2.27 (±0.76)	0.50-0.80	0.68 (±0.07)	0.00-0.60	3.10-8.38	4m + 14sm + 2st-SAT
Tulipa doerfleri	3x	3.66-7.98	2.18	4,27 (±0.94)	1.37 (±0.46)	5.63(±1.04)	202.82	1.60-8.00	3.51 (±1.54)	0.62-0.89	0.76 (±0.07)	0.23-0.78	1.81-3.92	21sm + 15st
Tulipa saxatilis	2x	4.86-10.23	2.11	5.49 (±1.34)	1.71 (±0.67)	7.19(±1.48)	172.69	1.50-8.67	3.65 (±1.69)	0.60-0.90	0.76 (±0.08)	0.20-0.79	2.76-6.02	12sm + 10st + 2t
Anemone hortensis subsp. *heldreichii*	2x	6.02-11.54	1.92	5.69 (±0.44)	3.17 (±2.01)	8.86(±2.06)	141.75	1.05-12.00	3.92 (±3.82)	0.51-0.92	0.68 (±0.17)	0.02-0.85	4.25-8.14	10m + 2st + 2st-SAT + 2t
Ranunculus asiaticus var. *sanguineus*	2x	4.26-8.55	2.01	4.15 (±0.66)	2.26 (±0.95)	6.71 (±1.31)	102.59	1.01-3.50	2.14 (±0.85)	0.50-0.83	0.66 (±0.12)	0.00-0.56	4.15-8.33	6m + 6sm + 4st
Ranunculus asiaticus var. *sanguineus*	3x	4.53-9.62	2.12	4.46 (±0.57)	2.45 (±1.27)	6.41 (±1.15)	165.82	1.00-4.75	2.42 (±1.30)	0.50-0.78	0.65 (±0.10)	0.00-0.65	3.10-10.62	12m + 3sm + 9st

Abbreviations: (SC) shortest and (LC) longest chromosome length; (p) mean long-arm length; (q) mean short-arml ength; (CL) mean chromosome length; (TLC) total chromosome length; (ARR) arm ratio range; (MAR) mean arm ratio; (CIR) centromeric index range; (CI) mean centromeric index; (ADR) arm difference ratio range; (RLR) relative length range; (SD) standard deviation.

Results and discussion

Berberidaceae

Leontice leontopetalum L. subsp. ***leontopetalum*** (Fig. 1). — 2*n* = 2x = 16 (Fig. 7).

Gr: Nomos Irakliou, Prov. Pirgiotissis: 1 km N of Kamilari, 35° 02’ 45.8” N, 24° 47’ 37.6” E, alt. 55 m, level open olive groves, area 2100 m^2, surrounded by young and older olive groves and a few small fields with cereal crops, 16 Apr 2003, *Kyriakopoulos & Turland* sub *Turland 1215* (MO, UPA).

Leontice leontopetalum is a conspicuous geophytic, obligate weed of traditional agriculture with a tough, deeply rooted tuber. According to Coode (1965) the taxon is divided into three subspecies: *L. leontopetalum* subsp. *leontopetalum* is found from the E Mediterranean area to N Iraq and N Iran; *L. leontopetalum* subsp. *ewersmannii* (Bunge) Coode is distributed in Turkestan, Iran and W Pakistan; and *L. leontopetalum* subsp. *armeniacum* (Boivin) Coode grows in Jordan, Syria and Armenia. The two subspecies found in the E parts of this large distribution area grow in semi-natural, steppe-like vegetation, while the typical subspecies has probably evolved as an ecotype with the development of agriculture (Phitos & Strid 2002). *L. leontopetalum* subsp. *leontopetalum* grows in clayey soils sticky in spring and compacted in summer at attitudes up to 1100 m, with other characteristic taxa of similar habitats such as *Ornithogalum nutans* L., *Geranium tuberosum* L., *Aegilops triuncialis* L., *Consolida regalis* Gray, *Cerastium semidecandrum* L., *Brassica geniculata* (Desf.) Ball, *Papaver rhoeas* L., *Delphinium peregrinum* L., *Ranunculus muricatus* L., *Centaurea calcitrapa* L., *C. solstitialis* L. subsp. *solstitialis*, *Mantisalca salmantica* (L.) Briq. & Cavill., *Scolymus hispanicus* L., *Reseda lutea* L., *Eryngium campestre* L., *Hypericum triquetrifolium* Turra.

Due to the relatively limited distribution range of the subspecies, the scattered and small size of the populations, the specificity of the habitat, the gradual decline of the older populations and changes associated with the use or intensive cultivation of agricultural land the taxon has been characterized as Vulnerable (VU) according to the IUCN (2001, 2003, 2006) criteria A4(c); B1b(iii)c(iii); C2a(i) (Krigas & al. 2009).

In Crete it is very rare and has been decreasing over the last century. During our fieldwork it was found at only three localities. The chromosome number 2*n* = 16 is given by several authors from elsewhere (Toren 1962, 1965; Markova 1969; Kosenko 1977). Moreover, Toren (1962) reported the gametophytic count *n* = 9, which, however is considered doubtful (Phitos & Strid 2002). The chromosome number 2*n* = 16 given in the present study is, to the best of our knowledge, counted for the first time from Greece. The karyotype, which has the following formula 2*n* = 8m + 6sm + 2st-SAT = 16, consists of mostly long chromosomes, varying in size from 5.06-14.95 μm. The total chromosome length is 165.99 μm, while the mean arm ratio is given as 1.89 (Table 1). The satellites of the acrocentric chromosomes are small, spherical and not always visible.

Geraniaceae

Geranium tuberosum L. (Fig. 2). — 2*n* = 2x = 20 (Fig. 8).

Gr: Crete, Nomos Lasithiou, Prov. Lasithiou: Lasithi plain, 500 m S of Agios Konstantinos, 35° 10' 14.8" N, 25° 30' 03.5" E, alt. 850 m, level field with a few fruit trees, 3/4 fallow, 1/4 with cereal crop, area 1200 m^2, many similar fields in area, 7 Apr 2003, *Bareka & Turland* sub *Turland 1080* (MO, UPA).

— Crete, Nom. Irakliou, Prov. Monofatsiou: Ahendrias, within village, 34° 59' 27" N, 25° 13' 25" E, alt. 685 m, field with cereal crop and many weeds, 15 Apr 2003, *Kyriakopoulos & Turland* sub *Turland 1199* (B, BM, MO, UPA).

Geranium tuberosum is one of the most reliable indicators of traditional agriculture, in that it is restricted (obligate) to such habitats. The taxon is distributed in S Europe, from SE France to the Aegean region, extending E to Crimea, Caucasia, the Syrian desert, N Iraq and N Iran (Webb & Ferguson 1968). It is a rare archeophytic weed of cultivated and fallow fields (Lasithi plain and Ahendrias in the eastern Asterousia mountains) (Fielding & Turland 2005).

The chromosome number 2*n* = 28 for *Geranium tuberosum* has been previously reported for this taxon in material from Sterea Hellas (Van Loon & Oudemans 1982). However, our counts from both studied populations in Crete revealed the chromosome number 2*n* = 20. The karyotype consists of small mostly submetacentric (sm) chromosomes varying in size from 1.45-5.07 µm (Fig. 8). The arm ratio varies from 1.01 to 4.00 and the centromeric index from 0.50 to 0.80. The mean chromosome length is given as 3.50 µm, while the total chromosome length is 51.09 µm. Only one chromosome pair is acrocentric and bears big spherical satellites (st-SAT) longer than the short arms. The karyotype formula is given as: 2*n* = 4m + 14sm + 2st-SAT = 20 chromosomes.

Liliaceae

Tulipa doerfleri Gand. (Fig. 3). — 2*n* = 3x = 36 (Fig. 9).

Gr: Nom. Rethimnis, Prov. Amariou: Gious Kambos plain ESE of Spili, beside stream on W part of plain, 35° 12' 50.4" N, 24° 33' 47.3" E, alt. 755 m, fallow field, partly with deep soil over limestone, partly with very stony soil on schist, area 1600 m^2, many similar fields in area, some fallow, some with cereal crops, 21 Apr 2003, *Phitos, Kamari & Turland* sub *Turland 1306* (B, BM, MO, UPA).

Tulipa doerfleri is an endemic taxon to WC Crete, from Angouseliana (Prov. Agiou Vasiliou) to Moni Asomaton (Prov. Amariou). It is an obligate weed of traditionally cultivated land (the only endemic weed in the Cretan flora) occurring in cultivated and fallow fields, and persisting in abandoned fields, at altitudes of 330-750 m (Turland 2009). It grows with other weeds of cultivation, including the geophytes *Allium nigrum* L., *Gladiolus italicus* Mill., *Muscari comosum* (L.) Mill., *Ranunculus ficaria* subsp.

chrysocephalus P. D. Sell and *Smyrnium perfoliatum* subsp. *rotundifolium* (Mill.) Hartvig and a wide variety of annuals including *Anagallis arvensis* L., *Eruca vesicaria* (L.) Cav., *Glebionis segetum* (L.) Fourr., *Papaver rhoeas* L. and *Scandix pecten-veneris* L.

Tulipa doerfleri has been treated as *T. orphanidea* Boiss. & Heldr. (e.g. by Grey-Wilson & Matthews 1980), which occurs in S Greece (Peloponnisos and Attiki) and W Turkey (Persson 1991). According to Zonneveld (2009) in his revision of the genus, based on nuclear genome size measurements, *T. doerfleri* has been included in *T. orphanidea* as a subspecies [*T. orphanidea* subsp. *doerfleri* (Gand.) Zonn.].

Whereas *Tulipa orphanidea* has an orange to brick-red or rarely yellow perianth, *T. doerfleri* has a deep red perianth. Moreover, *T. orphanidea* is characterized by several ploidy levels from diploid with 2*n* = 2x = 24 chromosomes (Southern 1967; Fedorov 1969; Blakey & Vosa 1981; Zonneveld 2009) to tetraploid with 2*n* = 4x = 48 chromosomes (Athanasiou 1988; Persson 1991), with triploid karyotypes being the most common (Athanasiou 1988; Tzanoudakis & al. 1991; Başak & Özhatay 1997; Zonneveld 2009). *Tulipa doerfleri*, on the other hand, is always triploid with 2*n* = 3x = 36 chromosomes (Sonderhousen 1977; Tzanoudakis & al. 1991 as *"T. orphanidea"*). Based on the present authors' observations, *T. doerfleri* appears to be completely sterile, spreading vegetatively by subterranean stolons but never forming viable seeds. This biology is compatible with a sterile triploid.

Changes to traditional agricultural practices, such as abandonment of cultivated fields or deep-ploughing with modern machinery, threaten the survival of *Tulipa doerfleri*. For that reason the category Vulnerable (VU) was proposed in the Red Data Book of Rare and Threatened Plants of Greece (Turland 2009) based on criteria B1ab(iii,v)c(iv)+2ab(iii,v)c(iv); D2 (IUCN 2001).

The karyological features of the taxon are given in Table 1. The karyotype comprises of 2*n* = 3x = 21sm + 15st = 36 chromosomes, ranging in size from 3.66 to 7.98 µm.

Tulipa saxatilis Sieber ex Spreng. (Fig. 4). — 2*n* = 2x = 24 (Fig. 10).

Gr: Nom. Lasithiou, Prov. Lasithiou: Lasithi plain, between Agios Konstandinos and Koudoumalia, N side of road, 35° 10’ 08.5” N, 25° 29’ 24.4” E, alt. 835 m, ploughed field without crop, area 600 m^2, surrounded on 3 sides (N, W, S) by similar fields with some fruit trees, 7 Apr 2003, *Bareka & Turland* sub *Turland 1085* (B, BM, MO, UPA).

— Nom. Hanion, Prov. Selinou: N part of Omalos plain, near Omalos village, 35° 20’ 26.8” N, 23° 54’ 06.4” E, alt.1055 m, more or less level fallow field, area 3500 m^2, several similar fields on plain, 1 May 2003, *Karakitsos & Turland* sub *Turland 1380* (B, BM, MO, UPA).

Tulipa saxatilis grows on ledges and in crevices of calcareous cliffs, rocky slopes and screes, stream banks in scrub, flat clayey and cultivated, fallow or abandoned fields, often on mountain plains such as those of Lasithi and Omalos. The species is scattered around Crete and also occurs in Karpathos, the E Aegean island of Rodos and SW Turkey (Fielding & Turland 2005).

Figs 1-4. Photos of: **1,** *Leontice leontopetalum* subsp. *leontopetalum*; **2,** *Geranium tuberosum*; **3,** *Tulipa doerfleri*; **4,** *Tulipa saxatilis*.

This taxon was considered as “normally triploid” since most of the chromosome counts reported revealed a triploid chromosome number (Southern 1967; Yoshida 1980; Blakey & Vosa 1981; Tzanoudakis & al. 1991; Raamsdonk & Vries 1992). However, most of the Cretan populations examined previously by Tzanoudakis & al. (1991) were diploid with the exception of one triploid population.

Both populations studied during this project are diploid and their karyotype consists $2n = 2x = 12sm + 10st + 2t = 24$ chromosomes, varying in size between 4.86-10.23µm. The total length of the chromosome complement is 172.69 µm and the mean chromosome size is 7.19 µm. The mean arm ratio is given as 3.65 while the relative length of the chromosomes ranges from 2.76 to 6.02 (Table 1).

Ranunculaceae

Anemone hortensis subsp. ***heldreichii*** (Boiss.) Rech. f. (Fig. 5). — $2n = 2x = 16$ (Fig. 11).

Gr: Nom. Lasithiou, Prov. Mirambellou: S of Katharo plain, on road to Giannitsi, 35° 07’ 48.6” N, 25° 33’ 56.0” E, alt.1140 m, small field inaccessible to tractors, schist substrate, without obvious crop, inorganic fertilizer applied, area 700 m^2, surrounded by phrygana on schist, 6 Apr 2003, *Bareka & Turland* sub *Turland 1068* (B, BM, MO, UPA).

Anemone hortensis L. s. l. is a tuberous C Mediterranean species, extending E to the S Aegean region, belonging to *A.* subg. *Anemone* and to the “*Coronaria* group” together with all tuberous anemones from the Mediterranean region and SC USA with one disjunct species in South America (Mlinarec & al. 2006). It is divided into two subspecies: *A. hortensis* subsp. *hortensis* is distributed throughout most of the range of the species, while *A. hortensis* subsp. *heldreichii* is an endemic taxon of the islands of the Crete-Karpathos area, where it grows in phrygana, open calcareous woods, olive groves garigue and rocky places at an altitude from 0 to 1850 m (Fielding & Turland 2005).

The somatic number $2n = 16$ counted here for *A. hortensis* subsp. *heldreichii* has been previously given for *A. hortensis* s.l. by several authors (Tzanoudakis 1986 in material from Crete probably referring to *A. hortensis* subsp. *heldreichii*, Druskovic & Lovka 1995; Lovka 1995; Mlinarec & al. 2006 in material from elsewhere). It is the most common somatic number in all representatives of *A.* subg. *Anemone*. The karyotype is symmetrical, consisting of $2n = 2x = 10m + 2st + 2st\text{-}SAT + 2t = 16$ chromosomes, ranging in size from 6.02 to 11.54 µm. The morphometric analysis of the taxon (Table 1) gave similar results to those given by Mlinarec & al. (2006) in material from Croatia. It is also noteworthy that the satellites observed in an acrocentric chromosome pair are longer than the short arm of the homologues.

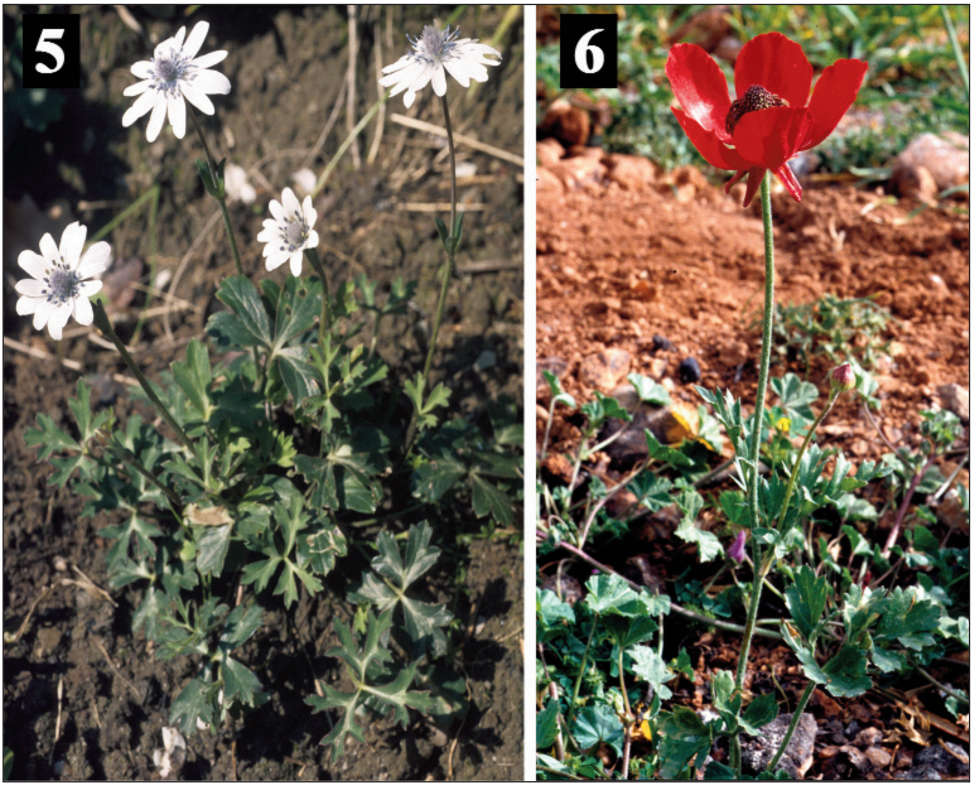
Ranunculus asiaticus var. ***sanguineus*** (Mill.) DC. (Fig. 6). — $2n = 2x = 16$ (Fig. 12a) & $2n = 3x = 24$ (Fig. 12b).

Gr: Nom. Hanion, Prov. Apokoronou: S of Vrises on road to Hora Sfakion, 4.4 km by road from river in Vrises, 35° 20’ 59.2” N, 24° 12’ 08.8” E), alt. 280 m, terraced olive grove with various fruit and nut trees, area 650 m^2, surrounded by similar olive groves, 19 Apr 2003, *Phitos*, *Kamari & Turland* sub *Turland 1272* (B, BM, MO, UPA).

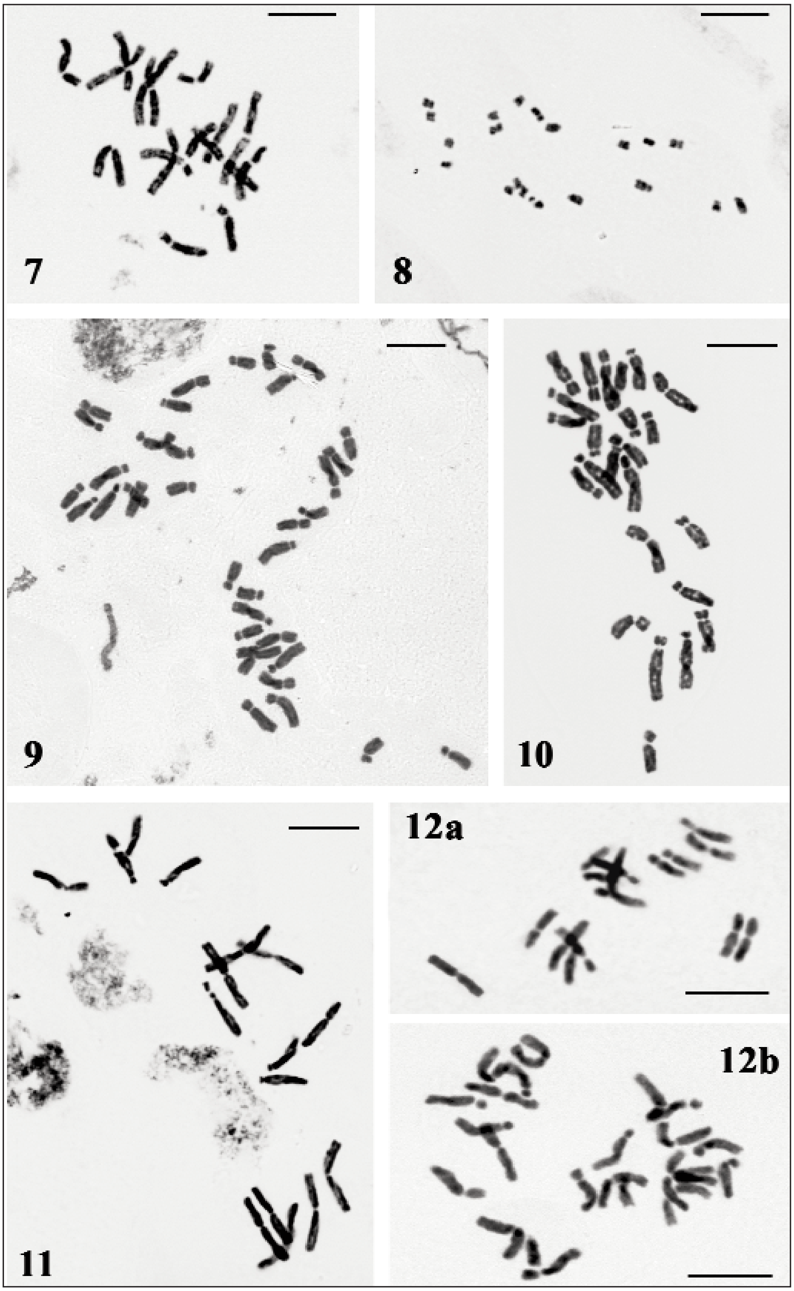
— Nom. Rethimnis, Prov. Amariou: N part of Fourfouras village, SW of road junction to Platania, 35° 12' 53.0" N, 24° 42' 40.1" E, alt. 430 m, open olive grove, area 1000 m^2, surrounded by similar olive groves and small fields with cereal crops, 20 Apr 2003, *Kamari & Turland* sub *Turland 1280* (B, BM, MO, UPA).

— Nom. Hanion, Prov. Sfakion: W edge of Anopoli village, S side of road to Aradena, 35° 13' 10.0" N, 24° 04' 53.9" E, alt. 585 m, small level stony fallow field, area 300 m^2, surrounded by fallow and cultivated (with cereals) fields, vineyards and olive groves, 22 Apr 2003, *Kamari & Turland* sub *Turland 1375* (MO, UPA).

Ranunculus asiaticus L. s. l. is a very variable species occurring mainly in the E Mediterranean area, e.g. Libya, Egypt, the Aegean area, Cyprus and through Anatolia to Syria, W Iraq &W Iran (Strid 2002). The red-flowered variety of this tuberous geophyte in Crete, var. *sanguineus*, has a completely different ecological niche to the plants with yellow flowers (var. *flavus* Dörfl.) and white or pink flowers (var. *albus* Hayek) (Turland & al. 2004). The red-flowered variety occurs exclusively in cultivated ground, while the other two grow in



Figs 5-6. Photos of: **5**, *Anemone hortensis* subsp. *heldreichii*; **6,** *Ranunculus asiaticus* subsp. *sanguineus*.



Figs 7-12. Microphotographs of mitotic metaphase plates of: **7**, *Leontice leontopetalum* subsp. *leontopetalum*, 2*n* = 2x = 16; **8**, *Geranium tuberosum*, 2*n* = 2x = 20; **9**, *Tulipa doerfleri*, 2*n* = 3x = 36; **10**, *Tulipa saxatilis*, 2*n* = 2x = 24; **11**, *Anemone hortensis* subsp. *heldreichii*, 2*n* = 2x = 16 and **12**, *Ranunculus asiaticus* subsp. *sanguineus*, **12a**, 2*n* = 2x = 16; **12b**, 2*n* = 3x = 24. – Scale bars = 10 µm.

semi-natural phrygana and garigue habitats. According to Turland & al. (2004) the collected plants have underground stolons, by which they were spreading vegetatively.

The chromosome number 2*n* = 2x = 16 found here in var. *sanguineus* has also been reported for *Ranunculus asiaticus* s. l. and *R. asiaticus* var. *albus* (Montmollin 1984; Tzanoudakis 1986; Tak & Wafai 1996; Chen & al. 2003; Baltisberger & Widmer 2004, 2005). However, within the studied populations of *R. asiaticus* var. *sanguineus* we also found triploid individuals with 2*n* = 3x = 24 reported here for the first time. The karyotype features for both diploid and triploid individuals are given in Table 1. The main difference between diploid and triploid karyotypes is the number of submetacentric and acrocetric chromosomes. The diploid karyotype consists of 2*n* = 2x = 6m + 6sm + 4st = 16 chromosomes, while the morphology of the triploid karyotype is given as 2*n* = 3x = 12m + 3sm + 9st = 24 chromosomes. The karyotype morphology reported by Tzanoudakis (1986) for *R. asiaticus* s.l. is 2*n* = 2x = 6m + 10st = 16 chromosomes. Baltisberger & Widmer (2005) reported the karyotype formula 2*n* = 2x = 6m + 6sm/st + 4st = 16 chromosomes for *R. asiaticus* var. *albus*, which is quite similar with the karyotype morphology reported here for *R. asiaticus* var. *sanguineus.*

Concluding remarks

In the present study a detailed karyotype analysis of six characteristic representatives of archeophytes in the island of Crete (Greece) was implemented, focusing specifically on the determination of the chromosome number and the morphology of the karyotype with the use of classical karyological methodology (squash technique, followed by Feulgen staining). A detailed karyotype analysis with twelve different karyological features is given for the studied taxa. Additionally, data on the distribution, systematics and ecology of the taxa are given.

The chromosome number (2*n* = 16) of *Leontice leontopetalum* subsp. *leontopetalum* is given for the first time from Greek material.

For *Geranium tuberosum* a new chromosome number (2*n* = 20) is reported for the first time.

For *Ranunculus asiaticus* var. *sanguineus* triploid (2*n* = 3x = 24) individuals have been found for the first time; this is together with diploid (2*n* = 2x = 16) individuals as previously reported.

The majority of the investigated taxa are diploid: *Leontice leontopetalum* subsp. *leontopetalum* (2*n* = 2x = 16), *Tulipa saxatilis* (2*n* = 2x = 24), *Anemone hortensis* subsp. *heldreichii* (2*n* = 2x = 16) and *Ranunculus asiaticus* var. *sanguineus* (2*n* = 2x = 16).

For the genus *Geranium* several different basic chromosome numbers have been reported: x = 10, 12, 13, 14, 16 (Petrova & Stanimirova 2001, 2003) The most common basic chromosome number is x = 14 (Albers 1990). All individuals and both populations of *G. tuberosum* studied revealed the new chromosome number 2*n* = 20, instead of 2*n* = 28 which has been previously reported. This is not a very common phenomenon for the genus. On the contrary, several species of the genus have a diploid together with a tetraploid chromosome number in the same individual (Petrova & Stanimirova 2001, 2003). The phenomenon is noted as endomitosis (Geitler 1939) conducting to endopolyploidy (Löve & Löve 1975).

Polyploidy in the genus *Tulipa* is very common and it seems to play a significant role in the speciation of the genus (Zonneveld 2009), where diploid species are the most common (2*n* = 2x = 24). However, triploid (2*n* = 3x = 36) and tetraploid forms (2*n* = 4x = 48) have been reported (Southern 1967; Zonneveld 2009). Pentaploid forms (2*n* = 5x = 60) are recorded only in *T. clusiana* Redouté, and Kroon & Jongerius (1986) found a hexaploid karyotype (2*n* = 6x = 72) in *T. polychroma* Stapf.

Both *Tulipa doerfleri* (2*n* = 3x = 36) and *T. saxatilis* (2*n* = 2x = 24), examined here, belong to *T.* subg. *Eriostemones*. As already mentioned here, Zonneveld (2009) included *T. doerfleri* sub *T. orphanidea*, as *T. orphanidea* subsp. *doerfleri*. However, the morphological and karyological differences between these two taxa, as well as the polymorphism in the *T. orphanidea* group make urgent the need for further study in order to resolve any taxonomic question. For all the above reasons we prefer now to maintain *T. doerfleri* as a separate species.

Fluorescence in situ hybridization (FISH) is an excellent tool for chromosome identification and studies of chromosome evolution and genome organization (Bareka & al. 2012). Many studies, have been published on the distribution, organization, and activity of ribosomal RNA (rRNA) genes for studying the evolutionary relationship within many genera and elucidating many taxonomical issues, e.g., in the genera *Artemisia* L. (Garcia & al. 2007), *Bellevalia* Lapeyr. (Bareka & al. 2012), *Cedrus* Trew (Bou Dagher-Kharrat & al. 2001), *Hypochaeris* L. (Cerbach & al. 1998; Weiss-Schneeweiss & al. 2003), *Lilium* L. (Siljak-Yakovlev & al. 2003; Muratovič & al. 2005), *Quercus* L. (Zoldoš & al. 1999) and *Reichardia* Roth (Siljak-Yakovlev & al. 1998). We believe that a molecular cytogenetic study on the representatives of this group could answer questions concerning the taxonomic relationships between Greek representatives of *Tulipa*.

In the Mediterranean *Anemone* species, two distinct karyotypes have been found so far: one with three acrocentric and five metacentric to submetacentric chromosomes (*A. coronaria* L., *A. pavonina* Lam.) and another with four acrocentric and four metacentric chromosomes (*A. blanda S*chott & Kotschy, *A. palmata* L.) (Marks & Schweizer 1971; Medail & al. 2002). The karyotype morphology of *A. hortensis* studied from Croatia (Mlinarec & al. 2006) is in accordance with the karyotypes of *A. coronaria* – *A. pavonina* group. Those three species have also the same heterochromatin banding pattern (Marks & Schweizer 1971) and they differ only by a variable telomeric DAPI banding pattern of the fourth-in-size chromosome pair (Mlinarec & al. 2006). The karyotype morphology found here for *A. hortensis* subsp. *heldreichii* is also in accordance with the results given by Mlinarec & al. (2006).

The present study of these six characteristic archeophytes from Crete can be regarded as a small but significant contribution in the effort to further advance the biosystematic study of the non-invasive Mediterranean weed flora.

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