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Cytological investigations on some Ranunculus-species from Crete

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ABSTRACT

BALTISBERGER, M. & A. WIDMER (2005). Cytological investigations on some Ranunculus-species from Crete. *Candollea* 60: 335-344. In English, English and French abstracts.

The chromosome numbers are presented for seven species (20 sites) of *Ranunculus* L. from Crete, and taxonomic and phytogeographic aspects are discussed. The number of chromosomes for *Ranunculus cupreus* Boiss. & Heldr. ($2n = 16$) is recorded for the first time. A new ploidy level was found in *Ranunculus paludosus* Poir. (pentaploid with $2n = 5x = 40$), and karyotypes are given for *Ranunculus asiaticus* L., *Ranunculus creticus* L. and *Ranunculus cupreus*.

RÉSUMÉ

BALTISBERGER, M. & A. WIDMER (2005). Données cytologiques de quelques espèces du genre Ranunculus de Crète. *Candollea* 60: 335-344. En anglais, résumés anglais et français.

Les nombres chromosomiques de sept espèces de *Ranunculus* (20 localités) de Crète sont présentés, et des aspects taxonomiques et phytogéographiques sont commentés. Le nombre de chromosomes pour *Ranunculus cupreus* Boiss. & Heldr. ($2n = 16$) est compté pour la première fois. Un nouveau degré de ploïdie a été trouvé pour *Ranunculus paludosus* Poir. (pentaploïde avec $2n = 5x = 40$), et les caryotypes de *Ranunculus asiaticus* L., *Ranunculus creticus* L. et de *Ranunculus cupreus* sont discutés.

KEY-WORDS: Ranunculus – Crete – Chromosome numbers – Karyotypes

Introduction

During an excursion to Israel and Crete in March 2000, plants were collected for biosystematic and cytological analyses and were transferred alive to the greenhouse of our institute. On these plants cytological investigations have been carried out. The results of the Israelian plants have been published recently (BALTISBERGER & WIDMER, 2004).

The cytological variability (chromosome numbers, karyotypes, ploidy levels) within the genus *Ranunculus* is rather high and not fully known, and karyological features are important for the understanding of natural groups within the genus (HÖRANDL & al., 2005). Additionally, as the genus *Ranunculus* is of special interest for us (see e.g. BALTISBERGER, 1980, 1992, 1994; MÜLLER & BALTISBERGER, 1984), we collected the *Ranunculus*-species met in Crete. Where possible, plants were collected from several sites. Cytological investigations have been carried out and the results are presented here. The seven investigated species are arranged in alphabetical order. Nomenclature follows *Flora Hellenica* (STRID, 2002).

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The chromosome numbers are presented and discussed, including interesting aspects of systematics and/or geographic distribution. Literature concerning chromosome numbers was checked using FEDEROV (1974), VAN LOON (1987), as well as the series "Index to plant chromosome numbers" (MOORE, 1970, 1971, 1972, 1973, 1974, 1977; GOLDBLATT, 1981, 1984, 1985, 1988; GOLDBLATT & JOHNSON, 1990, 1991, 1994, 1996, 1998, 2000, 2003). In *Ranunculus asiaticus* L., *R. creticus* L. and *R. cupreus* Boiss. & Heldr., karyotypes are discussed.

Materials and methods

The cytological investigations were carried out on root tips. These were pretreated with colchicine (0.05%) for 2 hours, then fixed in ethanol/acetic acid (3:1), and stained and squashed in lacto-propionic orcein (DYER, 1963). Five to ten metaphases were counted out of each individual to determine the chromosome number, and if possible several individuals were investigated of each species (or each site, respectively). The numbers of investigated individuals are indicated in Table 1.

Karyotypes are discussed using the terminology for chromosome morphology proposed by LEVAN & al. (1964). The chromosomes are named according to the position of the centromers, which is expressed with the arm ratio, "long arm to short arm". The following terms are used:

– metacentric	arm ratio	1.0-1.7;
– submetacentric	arm ratio	1.7-3.0;
– subtelocentric	arm ratio	3.0-7.0;
– acrocentric	arm ratio	more than 7.0;
– telocentric	only one arm.	

Plant material

After the indications of the site, date of sampling, number of herbarium specimen (in parenthesis specimen number of cultivated plants) are listed. Specimens are deposited in Z + ZT.

1. *Ranunculus asiaticus* L.

GREECE. Crete: Meadow, Moni Kardiotissis, near Krasion, N. Iraklion, 18.IV.1996, leg. F. Klötzli *(M. Baltisberger 13230)*; Open slope, W of Mires, N. Iraklion, 18.III.2000, *M. Baltisberger & A. Widmer 13545 (M. Baltisberger 13731)*; Rocky slope, NNW of Ag. Galini, on the road from Ag. Galini to Spili, N. Rethimno, 19.III.2000, *(M. Baltisberger 13740)*; Bushland near Lampini, WNW of Spili, N. Rethimno, 20.III.2000, *(M. Baltisberger 13744)*; Rocky slope between Selia and Rodakino, W of Spili, N. Rethimno, 20.III.2000, *(M. Baltisberger 13741)*.

2. *Ranunculus creticus* L.

GREECE. Crete: Rocks in the gorge of Therisso, near Omalos, SSW of Chania, N. Chania, 18.IV.1991, *M. Eggenberger & B. Merz (M. Baltisberger 12793)*; N exposed, rocky slope, NNW of Ag. Galini, on the road from Ag. Galini to Spili, N. Rethimno, 19.III.2000, *M. Baltisberger & A. Widmer 13555 (M. Baltisberger 13716)*; Gorge S of Ag. Ioanis, on the road from Rethimno to Selia, SSW of Rethimno, N. Rethimno, 20.III.2000, *M. Baltisberger & A. Widmer 13559 (M. Baltisberger 13722)*; N exposed rocks near the branches to Roustika off the road from Atsipopoulo to Episkopi, WSW of Rethimno, N. Rethimno, 21.III.2000, *M. Baltisberger & A. Widmer 13561 (M. Baltisberger 13726)*.

3. *Ranunculus cupreus* Boiss. & Heldr.

GREECE. Crete: Rocky slope between Selia and Rodakino, W of Spili, N. Rethimno, 20.III.2000, *M. Baltisberger & A. Widmer 13558 (M. Baltisberger 13721, M. Baltisberger 13811, M. Baltisberger 13892*; duplicates in herb. Kit Tan, Copenhagen).

4. *Ranunculus ficaria* L.

GREECE. Crete: Wet place in culture of *Olea europaea* L., near Lampini, WNW of Spili, N. Rethimno, 20.III.2000, *M. Baltisberger & A. Widmer 13557 (M. Baltisberger 13719)*; Wet place in culture of Olea europaea L., between Episkopi and Kournas, WSW of Rethimno, N. Chania, 21.III.2000, *M. Baltisberger & A. Widmer 13560 (M. Baltisberger 13724).*

5. *Ranunculus marginatus* d'Urv.

GREECE. Crete: Wet ruderal place, NW of Ag. Galini, on the road from Ag. Galini to Spili, N. Rethimno, 19.III.2000, (*M. Baltisberger 13717*).

6. *Ranunculus muricatus* L.

GREECE. Crete: Wet ruderal place, NW of Ag. Galini, on the road from Ag. Galini to Spili, N. Rethimno, 19.III.2000, *(M. Baltisberger 13718)*; Open wet place in culture of Olea europaea L., near Lampini, WNW of Spili, N. Rethimno, 20.III.2000, *(M. Baltisberger 13720)*; Wet ruderal place in the gorge S of Ag. Ioanis, on the road from Rethimno to Selia, SSW of Rethimno, N. Rethimno, 20.III.2000, *(M. Baltisberger 13723)*; Open wet place in culture of Olea europaea L., between Episkopi and Kournas, WSW of Rethimno, N. Chania, 21.III.2000, *(M. Baltisberger 13725).*

7. *Ranunculus paludosus* Poir.

GREECE. Crete: Slope near Krasion, N. Iraklion, 17.IV.1996, leg. F. Klötzli *(M. Baltisberger 13231)*; Culture of Olea europaea L., NW of Ag. Galini, on the road from Ag. Galini to Spili, N. Rethimno, 19.III.2000, *(M. Baltisberger 13814)*; Bushland near Lampini, WNW of Spili, N. Rethimno, 20.III.2000, *(M. Baltisberger 13815).*

Results and discussion

1. *Ranunculus asiaticus* ($2n = 16$; Fig. 1A)

Ranunculus asiaticus is a rather variable species growing in open and waste places and shrubby vegetations in the Mediterranean and W Irano-Turanian area. It varies in the shape of the leaves and the colour of the flowers (red, white, and yellow), and several variants have been named. All plants from all five sites have undivided or 3-fid lower leaves with broad lobes as well as white flowers, only few plants from Lampini *(M. Baltisberger 13744)* showed flowers tinged pale rose-pink. As different morphological characters may occur in the same population, all varieties seem to fall within one range of variation of *R. asiaticus* and have therefore no systematic value (Strid, 2002). But there seems to be a tendency for the geographical distribution of the flower colours, plants from Greece showing mostly white flowers and plants from more eastern parts as Turkey (Davis, 1965) or Israel (Baltisberger & Widmer, 2004) are red flowered.

All plants investigated from all five sites were diploid with $2n = 16$ chromosomes. The chromosome number corresponds with the indications in literature. The karyotype consists of 6 metacentric, 8 submeta- to subtelocentric and 2 acrocentric chromosomes (6m 8sm/st 2a), similar karyotypes are given by GOEPFERT (1974), TZANOUDAKIS (1986), TAK & WAFAI (1996), and BALTISBERGER & WIDMER (2004). As similar karyotypes have been found in plants from both colour types no cytological difference seems to exist between white or red coloured plants from the respective areas.

2. *Ranunculus creticus* ($2n = 16$; Fig. 1B)

Ranunculus creticus occurs in the S Aegean area and grows in shaddy places on rocky ledges and in gorges. It is a distinct, beautiful and often rather tall species. As it grows in similar habitats and looks like the even larger *R. cortusifolius* Willd. from the Macaronesian islands it was suggested that these two species are closely related (GOEPFERT, 1976). But molecular data suggest (PAUN & al., 2005) that the two species are not as closely related. They join the same clade but they are each nested within a group of more or less the same phytogeographical region (W- respectively E-Mediterranean). The similar habit is probably an independent adaptation to the shaded habitat. Nevertheless, based on data of chloroplast DNA, JOHANNSON (1998) found a strongly supported monophyletic group with both *R. cortusifolius* and *R. creticus* as well as some other species (e.g. *R. asiaticus*).

All plants investigated from all four sites had 16 chromosomes and were thus diploid. This corresponds with GOEPFERT (1974), MONTMOLLIN (1986), and TZANOUDAKIS (1986). The karyotype consists of 6 metacentric, 8 submeta- to subtelocentric and 2 acrocentric chromosomes, the latter with satellites (6m 8sm/st $2a_{SAT}$). Similar karyotypes are given by GOEPFERT (1974) and TZANOUDAKIS (1986). These findings for *R. creticus* correspond with the karyotype data for other *Ranunculus*-species, e.g. *R. asiaticus* (see above) and *R. cortusifolius* (GOEPFERT, 1974; DALGAARD, 1985; BALTISBERGER & al., 1990). These three species are not closely related (PAUN & al., 2005) but belong to a monophyletic group (JOHANNSON, 1998).

3. *Ranunculus cupreus* ($2n = 16$; Fig. 1C)

Ranunculus cupreus is an endemic species of Crete growing in crevices and ledges on limestone, often with phrygana. It is a small species (up to 15 cm) characterized by its sparsely pubescent leaves 3-partite to base with rather deeply 3-partite lobes and the honey-leaves yellow above but copper-coloured beneath. However, the plants on which the cytological investigations were based have basal leaves which are completely glabrous and more than normally lobed (see Fig. 2), and they lack the reddish tinge of the outside of the honey-leaves (fide Kit Tan). *Ranunculus cupreus* looks rather similar to *R. millefoliatus* Vahl differing in size, less divided leaves, and the copper-coloured honey-leaves. The close relationship of these two species is supported by molecular data (PAUN & al., 2005).

The chromosome number of *R. cupreus* was not known up to now. With 16 chromosomes the plants proved to be diploid. The same number is given for the related *R. millefoliatus* (GARBARI & al., 1973; POPOVA, 1973; GOEPFERT, 1974; MARCHI & VISONA, 1982; SHOPOVA & SEKOVSKI, 1982). The karyotype of *R. cupreus* consists of 3 pairs of metacentric, 3 pairs of meta- to submetacentric, 1 pair of subtelocentric and 1 pair of acrocentric chromosomes, the subtelo- and acrocentric chromosomes bearing satellites (6m 6m/sm $2st_{SAT}$ $2a_{SAT}$). Similar karyotypes are given for *R. millefoliatus* by GOEPFERT (1974), MARCHI & VISONA (1982) and SHOPOVA & SEKOVSKI (1982).

4. *Ranunculus ficaria* ($2n = 32$)

Ranunculus ficaria is widespread on wet and shady places in Europe, Asia, and N Africa and introduced in northern America. It is very variable and taxonomically difficult, and several ploidy levels (viz. $2x$, $3x$, $4x$) as well as aneuploid individuals (HESS & al., 1977) are known. Based on morphological characters some previous authors (e.g. JANCHEN, 1949) suggested to treat *R. ficaria* and its allied species as a seperate genus *Ficaria* Schaeff. but in most floras it remained within *Ranunculus*. Nevertheless molecular data support the separation of *Ficaria* as a distinct genus (HÖRANDL & al., 2005; PAUN & al., 2005).

Plants from the Mediterranean region have been referred to *Ranunculus ficaria* subsp. *ficariiformis* (F. W. Schultz) Rouy & Foucaud. Greek plants normally lack bulbils in the leaf axils which is typical for this subspecies; they therefore have been recently described as a distinct tetraploid subspecies, *R. ficaria* subsp. *chrysocephalus* P. D. Sell (SELL, 1991), and this seems to be the only taxon of the group occuring in S Greece and the Aegean region (STRID, 2002). Plants of both sites from Crete proved to be tetraploid with $2n = 4x = 32$ chromosomes confirming the indication by SELL (1991).

5. *Ranunculus marginatus* ($2n = 16$)

Ranunculus marginatus grows in open vegetations on wet places, in abandoned fields and on roadsides. Its distribution is said to cover SE Europe. Two varieties are distinguished (TUTIN & AKEROYD, 1993). Typical *R. marginatus* with smooth achenes is diploid with $2n = 2x = 16$ chromosomes (STRID & FRANZEN, 1981; LENTINI & al., 1988; BALTISBERGER & al., 1993) whereas *R. marginatus* var. *trachycarpus* (Fisch. & C. A. Mey.) Azn. with tuberculate achenes is tetraploid (LANGLET, 1927; GREGSON, 1965; GOEPFERT, 1974; SLAVIK & al., 1993; BALTISBERGER & BALTISBERGER, 1995). As many annuals, *R. marginatus* varies greatly in size, branching mode, and number of flowers. But it also varies in taxonomically relevant morphological characters of the achenes such as size, broadness of the margin, length of the beak, and surface (smooth, variously tuberculate till even muricate). This is also the case with the plants from Crete showing achenes with the surface from completely smooth to densely tuberculate (Fig. 3). All plants investigated were diploid with $2n = 2x = 16$, even these plants with tuberculate achenes. So the correlation between ploidy level and variety seems to be unexistent (at least for the material from Crete).

Ranunculus marginatus often looks similar to the annual, widespread and also very variable *R. sardous* Crantz, and the two taxa are therefore often difficult to distinguish. *R. sardous* is diploid or tetraploid, and the surface of the achenes is also variable as in *R. marginatus*. At least at present no geographical pattern based on morphology and/or cytology can be detected within this complex, and it therefore was proposed to treat the respective taxa as a variable complex under the name of *R. sardous* (STRID, 2002). But molecular data indicate that the two species are not (HÖRANDL & al., 2005) or at least not closely related (PAUN & al., 2005).

6. *Ranunculus muricatus* ($2n = 48$)

Ranunculus muricatus is a widespread annual in the Mediterranean area and introduced in other regions with similar climate. It grows in open vegetation on wet places, in abandoned fields and disturbed grounds. The species is polyploid, and three levels are known viz. tetraploid with 32 (VAN LOON & DE JONG, 1978), hexaploid with 48 (e.g. DIOSDADO & PASTOR, 1996), and octoploid with 64 chromosomes (FUJISHIMA, 1986). Based on ploidy levels no geographical pattern seems to exist. All investigated plants from all four sites from Crete proved to be hexaploid with $2n = 6x = 48$ chromosomes which is the most common ploidy level within *R. muricatus*.

7. *Ranunculus paludosus* ($2n = 32, 40$; Fig. 1D)

Ranunculus paludosus grows in dry or seasonally wet meadows, in abandoned fields and open places in bushland. It is a variable and widespread species in the Mediterranean area. Two ploidy levels are known viz. diploid with $2n = 2x = 16$ and tetraploid with $2n = 4x = 32$. The investigated plants from Krasion proved to be tetraploid, confirming the ploidy level more frequently indicated in literature. But all plants from the other two sites were pentaploid with $2n = 5x = 40$ which was unknown within this species up to now (Fig. 1D).

The pentaploid plants could originate by combining a reduced gamete of a diploid with a unreduced gamete of a tetraploid plant. But as diploid *R. paludosus* is not known from Crete the origin of the pentaploid plants remains unclear. Pentaploid plants show an uneven ploidy level. Complete pairing of chromosomes in meiosis is therefore not possible. The result is an unregular meiosis, and those plants are probably not fertile. But as all plants produce subterranean stolons (up to ten stolons per plant!) which grow very fast and build remarcable rosettes of leaves within some weeks (at least in the greenhouse) the propagation of these pentaploid plants seems to be no problem. This was probably also the case in nature because both sites were rather densely covered with numerous rosettes of this species. A similar strategy was described for plants of *R. dissectus* subsp. *sibthorpii* Davis with an uneven ploidy level (here triploid) from Ulu Dag in Turkey (BALTISBERGER & TAN, 1991). This taxon usually is diploid and without stolons but a population of triploid plants was found producing subterranean stolons.

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Table 1. – Alphabetical list of investigated species (* first record, + new ploidy level).

Species	Voucher number	Number of individuals investigated	2*n*
Ranunculus asiaticus	13230	1	16
R. asiaticus	13545	9	16
R asiaticus	13740	5	16
R. asiaticus	13744	9	16
R. asiaticus	13741	9	16
R. creticus	12793	9	16
R. creticus	13555	12	16
R. creticus	13559	8	16
R. creticus	13561	10	16
R. cupreus	13558	10	16*
R. ficaria	13557	13	32
R. ficaria	13560	6	32
R. marginatus	13717	6	16
R. muricatus	13718	7	48
R. muricatus	13720	15	48
R. muricatus	13723	4	48
R. muricatus	13725	9	48
R. paludosus	13231	3	32
R. paludosus	13814	14	40+
R. paludosus	13815	13	40

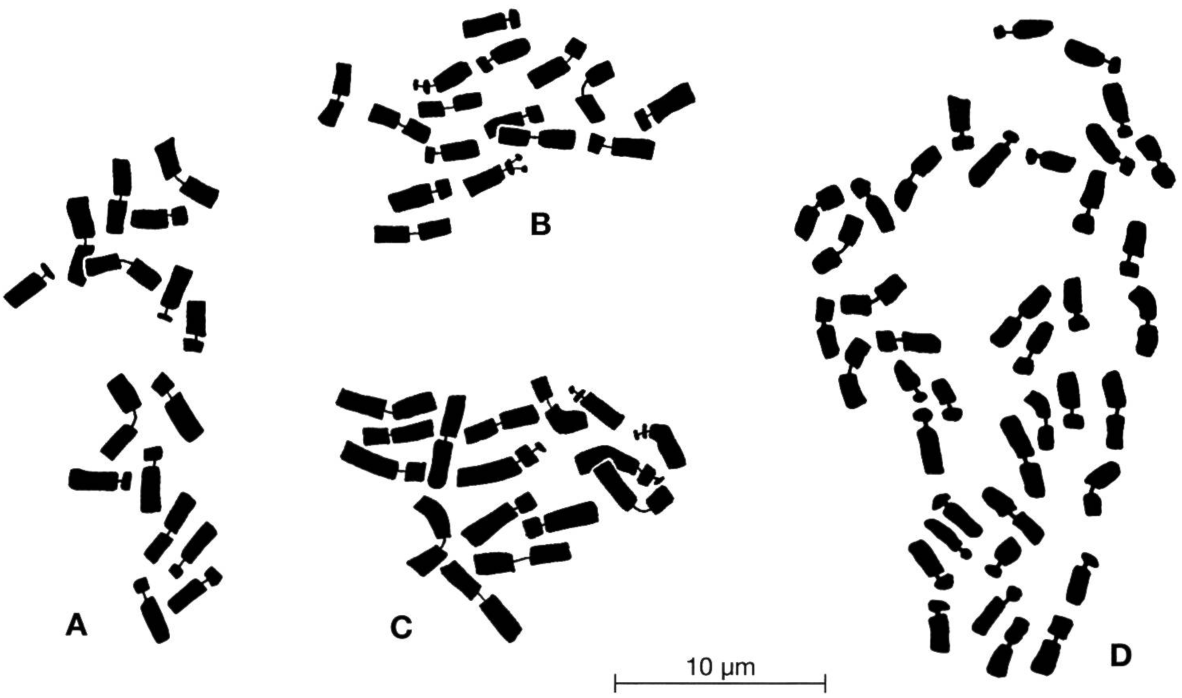

Fig. 1. – Somatic metaphases. **A.** *Ranunculus asiaticus* L. *(M. Baltisberger & A. Widmer 13545;* 2*n* = 2*x* = 16); **B.** *Ranunculus creticus* L. *(M. Baltisberger & A. Widmer 13561;* 2*n* = 2*x* = 16); **C.** *Ranunculus cupreus* Boiss. & Heldr. *(M. Baltisberger & A. Widmer 13558;* 2*n* = 2*x* = 16); **D.** *Ranunculus paludosus* Poir. *(M. Baltisberger 13815;* 2*n* = 5*x* = 40).

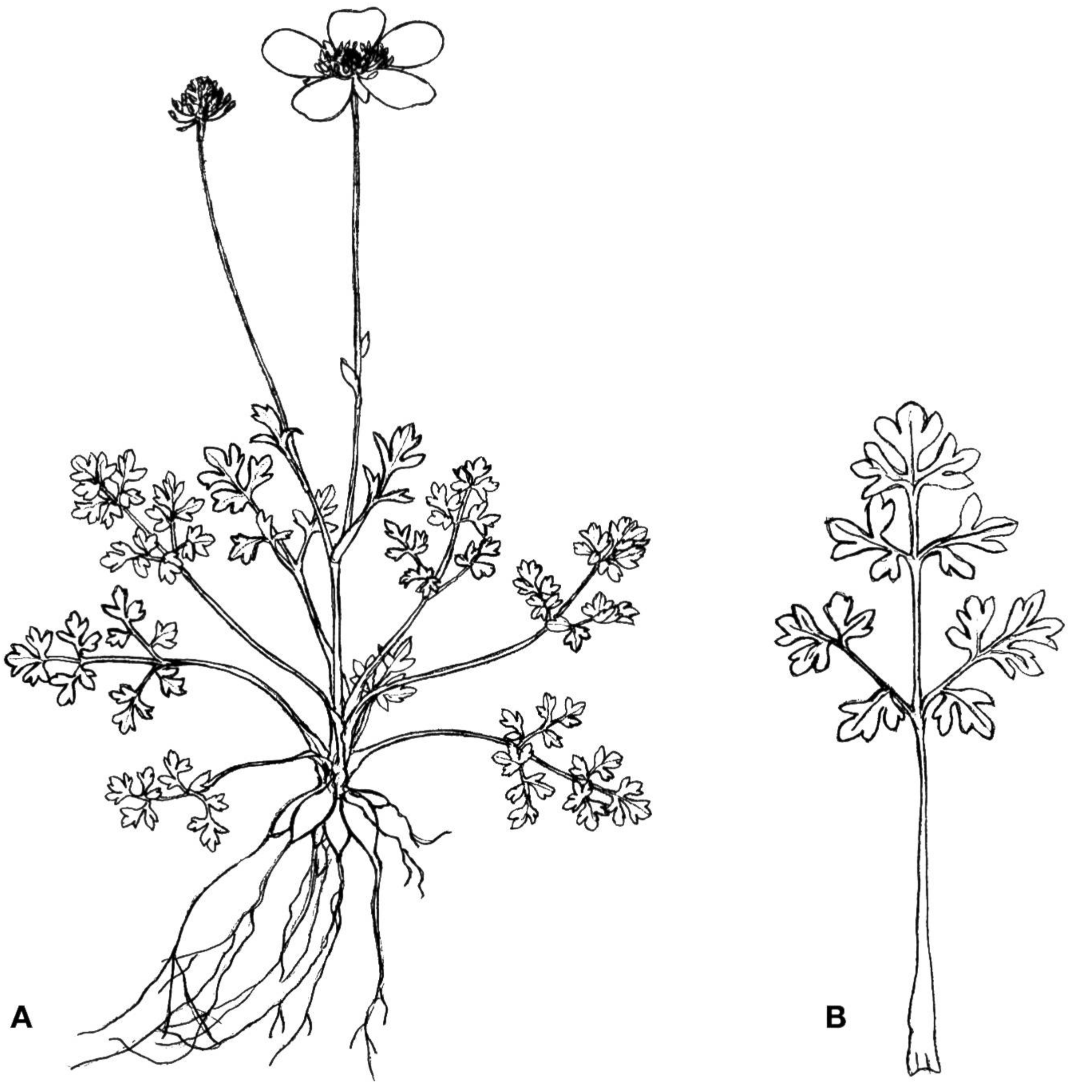

Fig. 2. – *Ranunculus cupreus* Boiss & Heldr. *(M. Baltisberger & A. Widmer 13558)*. **A.** Habit; **B.** Basal leaf.

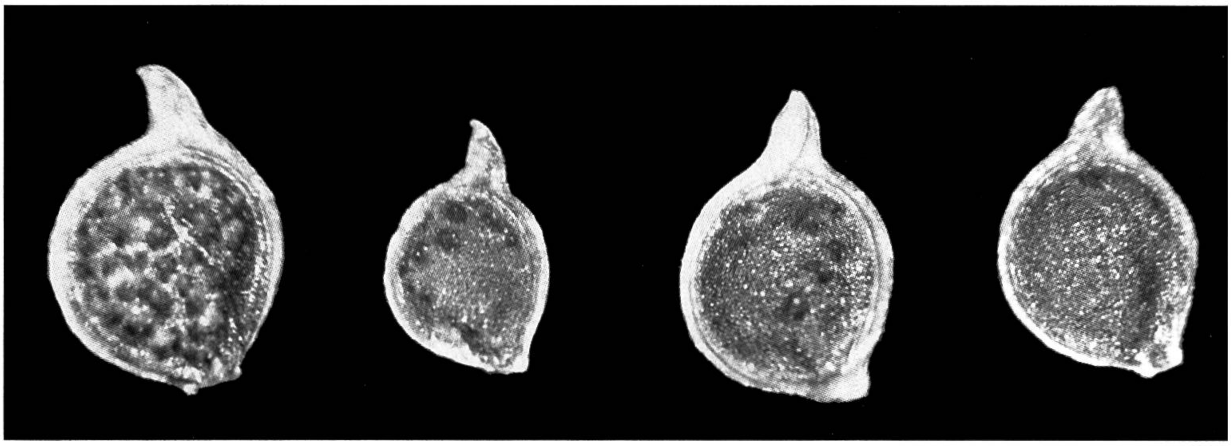

Fig. 3. – Achenes of *Ranunculus marginatus* d'Urv. *(M. Baltisberger 13717)* showing the surface variability from tuberculate (left) to smooth (right).