

Taxonomy, chorology and evolution of *Allium lusitanicum* - the European "*A. senescens*"

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Abstract: The karyology and phylogeny of *Allium lusitanicum* LAMARCK [*A. senescens* subsp. *montanum* (FRIES) HOLUB] and its relation to other related species of sect. *Rhizirideum* was studied. A random amplified polymorphic DNA (RAPD) analysis revealed a position of *A. lusitanicum* apart from the Asian species *A. senescens*. A distribution map and a complete synonymy of *A. lusitanicum* is given, together with a phylogenetic hypothesis of the red flowering species of sect. *Rhizirideum*.

Introduction

Allium lusitanicum LAM. is commonly given the rank of a subspecies by the name of *A. senescens* L. subsp. *montanum* (F.W. SCHMIDT) HOLUB, and it has a very complicated taxonomic and nomenclatural history. A discussion of some aspects of its nomenclature is found in the works of HYLANDER (1945) and HOLUB (1970). STEARN (1978) has conclusively distinguished correct names at all ranks for this taxon: if given the rank of an independent species, it should accordingly be named *A. lusitanicum* LAM., as subspecies *A. senescens* subsp. *montanum* (FRIES) HOLUB; and finally, as a variety, the name *A. senescens* var. *calcareum* (WALLROTH) HYLANDER would be appropriate. *Allium senescens* L. was described by LINNAEUS (1753), completely based on GMELIN's taxon *Allium scapo ancipiti teretiusculo, foliis ensiformibus, hinc paulo convexioribus* from Siberia (GMELIN 1747, tab. 11, fig.2). VVEDENSKY (1935) in the "Flora of SSSR" splitted *A. senescens* s. l. into four races: a European race (*A. montanum* SCHMIDT or *A. fallax* ROEM. et SCHULT.), an Altai-Sayanian race, a Transbaikalian race (*A. senescens* s. str. = *A. baicalense* WILLD.), and a race from the Russian Far East (*A. spirale* WILLD.). Because of the weak differences in morphology and the complicated nomenclature, VVEDENSKY did not consider these races as separate species. He also did not decide ranking of the taxa and did not propose valid names for them. FRIESEN (1987, 1988) on the basis of morphological, chorological, and karyological characters distinguished four species within the Siberian *A. senescens* s. l., which more or less correspond to the races of VVEDENSKY: *A. austrosibiricum* FRIESEN (2n=16 Altai-Sayanian race); *A. senescens* L. s. str. (2n=32, 48 Transbaikalian race); *A. spirale* WILLD. (2n=32 Far East race), and in addition a Daurian race *A. dauricum* FRIESEN (2n=32). He distinguished the European race of the *A. senescens* complex (2n=32) as an independent species. Morphologically (Table 1 and Fig. 1, 2) and chorologically (Fig. 3), this European race of *A. senescens* s. l.



A. angulosum L.



A. lusitanicum Lam.



A. lusitanicum Lam.



A. senescens L.



A. austrosibiricum Friesen



A. incensiodorum Radic



A. senescens L.



A. nutans L.

Fig. 1. *Allium lusitanicum* and its related species from section *Rhizirideum*.

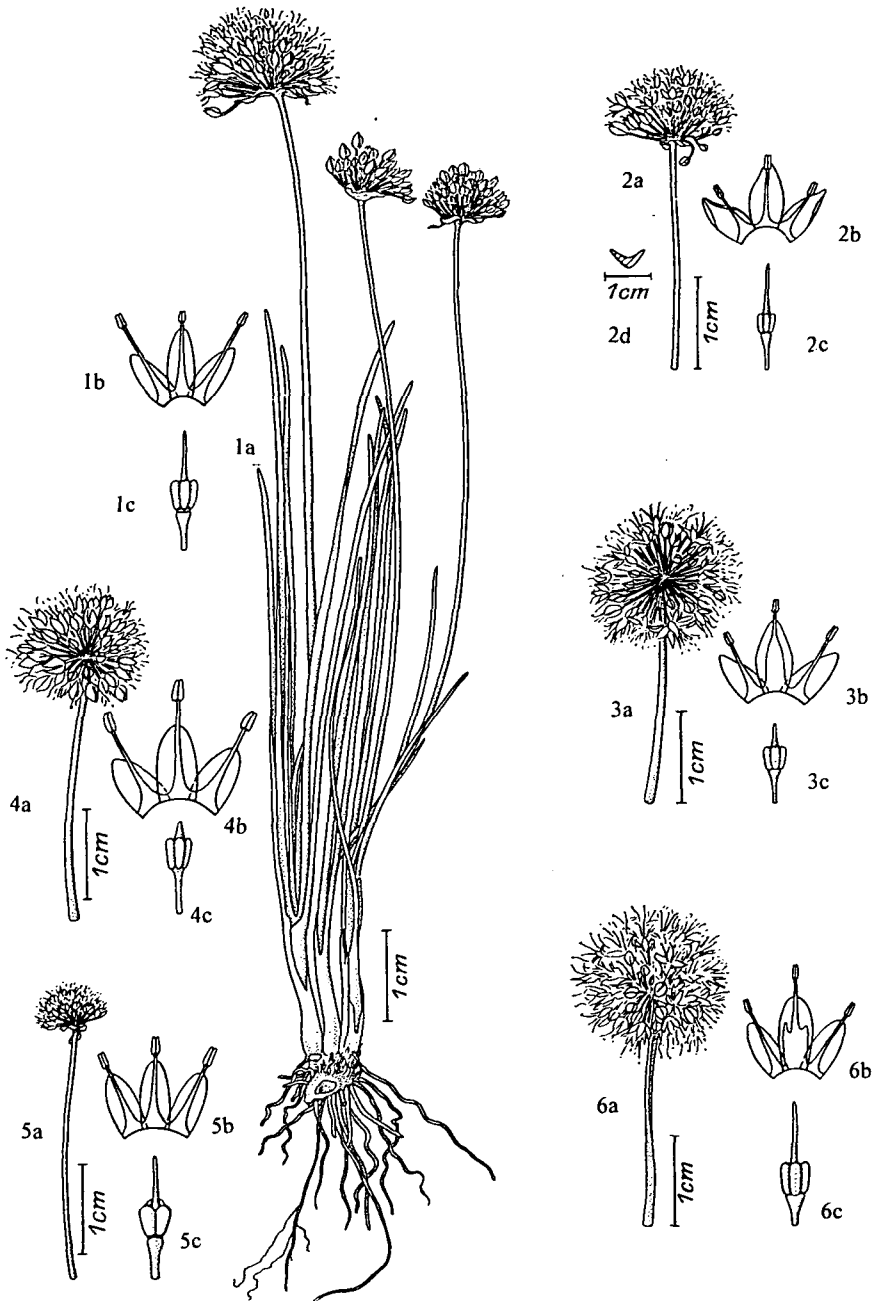


Fig. 2. 1 - *A. lusitanicum* (TAX 1355), 2 - *A. angulosum* (TAX 1746), 3 - *A. senescens* s. str. (TAX 2352), 4 - *A. austrosibiricum* (TAX 2747), 5 - *A. incisioidorum* (TAX 3764), 6 - *A. nutans* (TAX 3194). a - habitus or umbel; b - filaments and perigon; c - ovary and style; d - cross-section of leaf blade

is separated from the Siberian races. The disjunction between the European and the Siberian habitats of *A. senescens* s. l. is more than three thousand kilometres.

The area of *A. lusitanicum* (Fig. 3) can be described using the fine-scaled map of oceanity by JAEGER (1968): sm-temp c2-5EUR. The formula reveals, that the species is restricted to Europe and has a rather closed distribution in the submeridional and south-temperate zones in suboceanic areas. Only in southern Italy the species invades the meridional zone, but avoids the extreme summer-dry areas of Apulia and Sicily. It occurs there in slightly more humid mountain areas in altitudes up to 1500 (2200) m (PIGNATTI 1982). In northern Italy *A. lusitanicum* also appears in lowland areas (POLDINI 1991). The western limit of the species coherent distribution area is in northern Spain, only one record is known from Portugal. In the Pyrenees *A. lusitanicum* occurs between 100 and 2200 m (BOLOS 1990). The course of the north-western boundary enclosing the Massif Central in Southern France and Central Germany points to the above-mentioned avoidance of areas with euoceanic climate. Single records within the north temperate zone are known from southern Scandinavia and Lithuania. On the basis of the available floristic data from Poland the distribution limit can only be estimated within this area. Eastern-most occurrences were found in the Ukraine, Kiev area. Records from the Saratov area in Russia (FEDTSCHENKO 1939) are (probably) doubtful. In Romania *A. lusitanicum* seems to be restricted to the Carpathian mountains. In Bulgaria the species has been recorded only at one locality in the Stara Planina (STEFANOV 1943). The distribution area of *A. lusitanicum* in the countries of the former Yugoslavia is only insufficiently known and has to be regarded as preliminary.

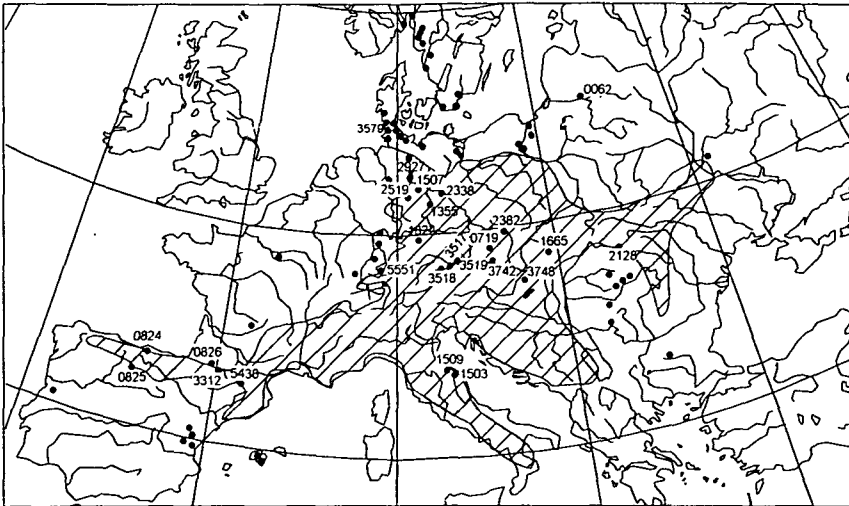


Fig. 3. Distribution map of *A. lusitanicum* with TAX numbers of studied accession.

In this paper we will dealing with the question of the taxonomical rank and we will try to elucidate the evolution of the European *A. lusitanicum* (*A. senescens* subsp. *montanum*). To resolve this problem we used karyological analysis and a Random Amplified Polymorphic DNA analysis (RAPD) (WILLIAMS et al. 1990, WELSH & MCCLELLAND 1990)

for a more detailed investigation of *A. senescens* ssp. *montanum* and its systematic position within the related species of sect. *Rhizirideum*.

RAPD analyses reveal even small genetic differences, since a large part of the nuclear genome will be scanned, as can be seen by mapping studies of segregating markers in a wide variety of plant families and is successful used for the clarification of the phylogeographical questions (e.g., BACHMANN & HOMBERGEN 1996, GABRIELSON et al. 1997, PURPS & KADEREIT 1998, TOLLEFSRUD et al. 1998, FRIESEN et al. in press).

Material and methods

Plant materials - A total of 35 accessions of 9 species from *Allium* section *Rhizirideum* from the living collection of the Department of Taxonomy and the Genebank of the IPK Gatersleben were investigated: *A. lusitanicum* (24 accessions), *A. angulosum* (3 accessions), *A. senescens* (2 accessions) and one accession each of *A. incesciodorum*, *A. spirale*, *A. austrosibiricum*, *A. albidum*, *A. nutans* and *A. stellerianum* as related species. Accession numbers and the origins are listed in table 2. The material was carefully selected because *A. lusitanicum* and *A. senescens* easily hybridise when grown together. The investigated accessions of *A. lusitanicum* represent the entire geographical range of the species (Fig. 3).

Chromosome preparation - Excised roots were kept in distilled water on ice overnight. They were then transferred to room temperature for 20 min and pre-treated for 1.5 h at room temperature in an aqueous 0.05% solution of colchicine. The tissue was fixed in a freshly prepared solution of 96% ethanol/glacial acetic acid (3:1). Meristems were hydrolysed in 0.1 N HCl for 8 min. at 60° C, dissected on a slide in 45% carmin acetic acid and squashed under a coverslip. Chromosome nomenclature followed LEVAN et al. (1964).

Isolation of DNA - DNA was isolated with the NucleoSpin Plant kit (Macherey-Nagel, Düren, Germany) according to the instructions of the manufacturer. 10 µl of the isolated DNA were dissolved in 150 µl of water and 4 µl (approximately 50 ng) of this DNA solution were used for each PCR amplification.

RAPD analysis - Amplification was carried out using up to 11 arbitrary 10 bp primers (A04, A16, A19, AA17, AA18, AB04, AB18, AC19, C05, C07, G13, D01, and D03) obtained from Operon Technologies, Alameda, CA. The amplification conditions were optimised according to FRIESEN et al. (1997). One third of the reaction mixtures was separated on 1.5% agarose gels in 0.5xTBE, followed by staining with ethidium bromide (SAMBROOK, FRITSCH & MANIATIS 1989). The DNA profiles were scored manually for presence (1) or absence (0) of bands from enlarged photographs of the gels. From the resulting 1/0 data matrix distances and a character based analysis were conducted. Wagner parsimony trees were calculated with PAUP 3.1.1, using the heuristic search algorithm with MULPARS, ACCTRAN, and TBR branch swapping. Pairwise genetic distances were calculated using the JACCARD coefficient. Finally, a phenogram was prepared based on UPGMA (unweighted pair-group method, arithmetic average) cluster analysis of the genetic distance matrix. The genetic distance matrix was also subjected to a principal co-ordinate analysis (PCA). From the distances, new independent axial co-ordinates are calculated which represent most of the variability of the original data. The

taxa are then plotted as points in a three dimensional continuous space defined by the first three co-ordinates. These calculations were done in the NTSYS-PC program (Applied Biostatistic Inc. New York, 1993, version 1.8).

Results and discussion

Karyology

Different chromosome numbers have been published for the European *A. senescens* subsp. *montanum*: $2n = 16, 24,$ and 32 . BAKSAY (1956) reported $2n = 24$ from Hungary; SHOPOVA (1966) $2n=32$ from central and east Europe; GADELLA and KLIPHUIS (1970) $2n = 32$ from the French Alps; HOLUB et al. (1970) $2n = 32+0-4B$ from Czechoslovakia; FERNANDES-CASAS et al. (1978) $2n = 16+0-5B$ from Hueska (Spain); ZABORSKY (1978) $2n = 32$ from Czechoslovakia; PASTOR (1982) $2n = 16$ and 32 from the Pyrenees (Spain) and SPETA (1984) $2n = 32$ and 24 from Austria. Diploid plants of *A. senescens* subsp. *montanum* ($2n = 16$) were reported only from Spain, and triploids ones ($2n = 24$) from Hungary and Austria only. Most populations of the European *A. senescens* are tetraploid which corresponded well with our own results. All investigated accessions of *A. lusitanicum* were tetraploids ($2n = 32$), *A. angulosum* and *A. incensiodorum* were diploids ($2n = 16$) (Tab. 3 and Fig. 4). In Europe we have two diploid species (*A. angulosum* and *A. incensiodorum*) and one tetraploid species (*A. lusitanicum*). The diploid number of chromosomes (together with the tetraploid number) for *A. lusitanicum* was found only in Spain. Morphologically, the $2x$ and $4x$ plants do not show any difference (PASTOR 1982).

The karyotype was studied in one accession of *A. lusitanicum*, *A. angulosum* and *A. incensiodorum*, respectively.

A. lusitanicum LAMARCK

TAX 2385 (Czech Republic). $2n = 32$

$K2n = 22m + 6sm + 2sm(sat) + 2sa(sat)$. $CI = 40.8\%$, $L2n = 294.4 \mu m$

A. angulosum L.

TAX 2806 (Germany, Mansfelder Land). $2n = 16$.

$K2n = 10m + 4sm + 2sa(sat)$. $CI = 39.0\%$. $L2n = 133.2 \mu m$

A. incensiodorum RADIC

TAX 3764 (Croatia). $2n=16$.

$K2n = 14m + 2sm(sat)$. $CI = 41.4\%$. $L2n = 171.9 \mu m$

The karyotype of *A. angulosum* is more asymmetrical and shorter in comparison with karyotypes of *A. lusitanicum* and *A. incensiodorum*. Morphologically, the karyotypes are very similar to those of the other species of the section *Rhizirideum* s. str. (FRIESEN 1988).

RAPD analysis

Two groups of accessions were studied using RAPDs. In the first group most species of the section *Rhizirideum* were included in order to define the phylogenetic relations of *A. lusitanicum* within the section, and its relationship to *A. senescens* s. str. In the second group all available accessions of *A. lusitanicum* (24 accessions), one accession of *A.*

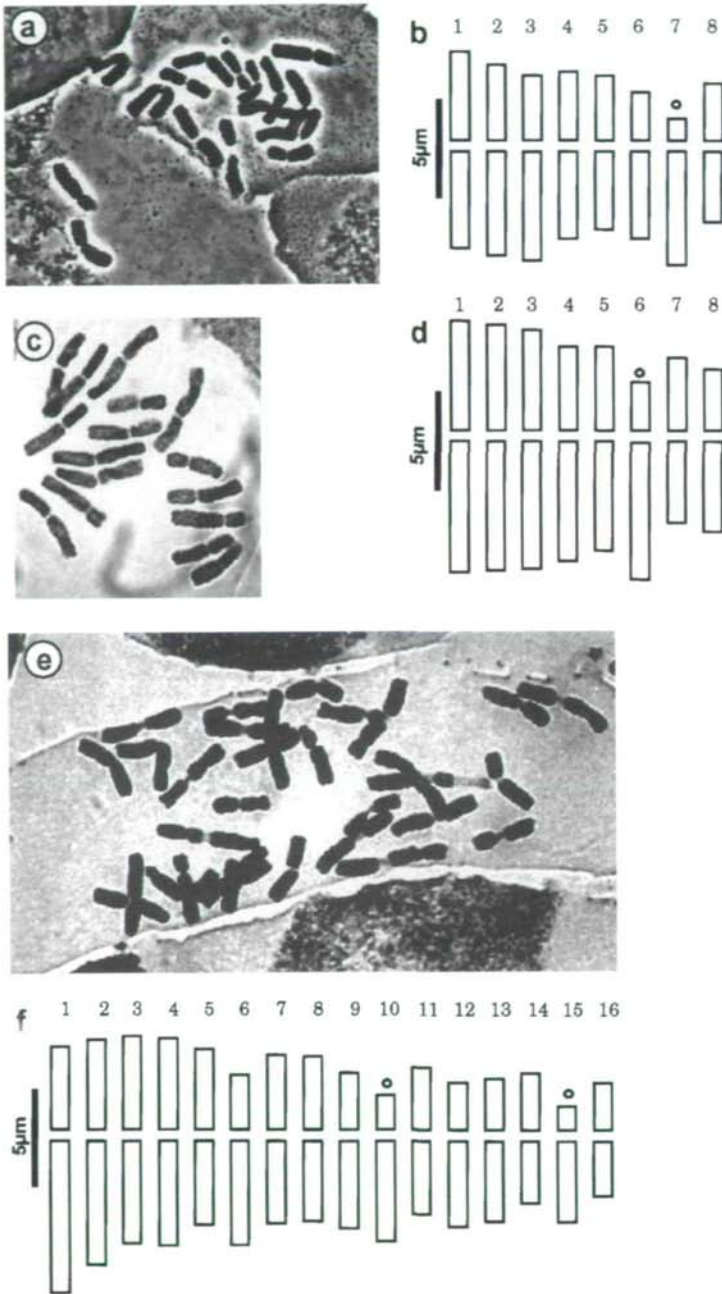


Fig. 4. Somatic cells of *A. angulosum* (a), *A. incisiodorum* (c), *A. lusitanicum* (e) and their idiograms (b, d, f respectively).

senescens, one accession of *A. austrosibiricum*, one accession of *A. incensiodorum*, and two accession of *A. angulosum* were included to determine the intraspecific variation of *A. lusitanicum*. From the raw data, distance matrices were calculated with the JACCARD coefficient (the use of other coefficients yielded similar groupings, results not shown). From the distance matrix a dendrogram was constructed by UPGMA clustering. In the first analysis a total of 111 polymorphic DNA fragments were scored with nine Operon primers (A04, A16, A19, AA17, AB04, AC19, C07, G13, D01, and D03). Three distinct groups are evident in the dendrogram (Fig. 5): 1 - *A. albidum* and *A. stellerianum*; 2 - *A. angulosum*, *A. incensiodorum* and *A. lusitanicum*; 3 - *A. austrosibiricum*, *A. nutans*; *A. senescens* and *A. spirale*. *A. lusitanicum* is clearly distinguished from *A. senescens* and placed together with *A. angulosum* and the other recently newly described European diploid *A. incensiodorum*. It is interesting that in the dendrogram the yellow flowered species (*A. albidum* and *A. stellerianum*) are distinguished from other, red or pinkish flowered species. On the other hand, the European species (also including accession of *A. angulosum* from West Siberia, TAX 2778) with red flowers are clearly distinguished from the Asiatic species.

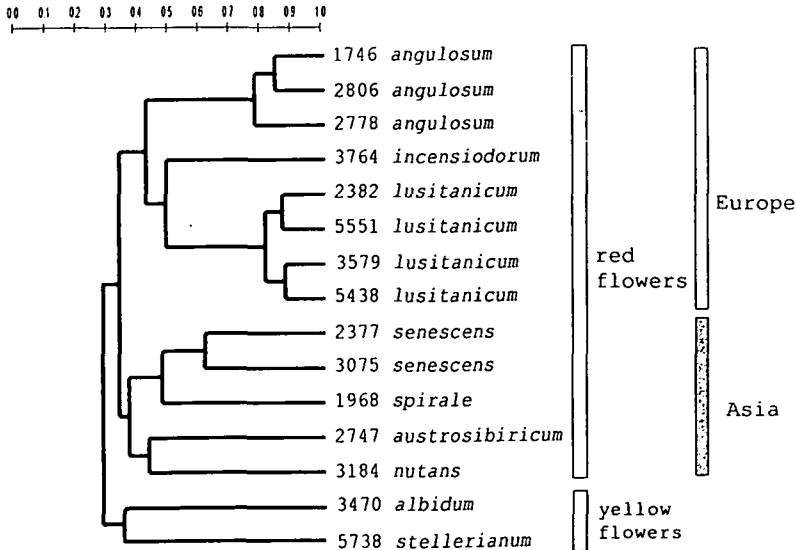


Fig. 5. UPGMA dendrogram of 15 accessions of nine species of sect. *Rhizirideum*, based on 111 RAPD markers.

In the second analysis a total of 166 polymorphic DNA fragments were scored with eleven Operon Primers (A04, A16, A19, AA17, AA18, AB04, AB18, AC19, C07, G13, D01, and D03). The arrangement of the species in this group (Fig. 5) is similar to the groups in the first investigation (Fig. 6). The Siberian species *A. senescens* and *A. austrosibiricum* form a sister group to the European species *A. angulosum*, *A. incensiodorum*, and *A. lusitanicum*. All *A. lusitanicum* accessions form a monophyletic unit which is subdivided in three subgroups. These subgroups correspond very well with

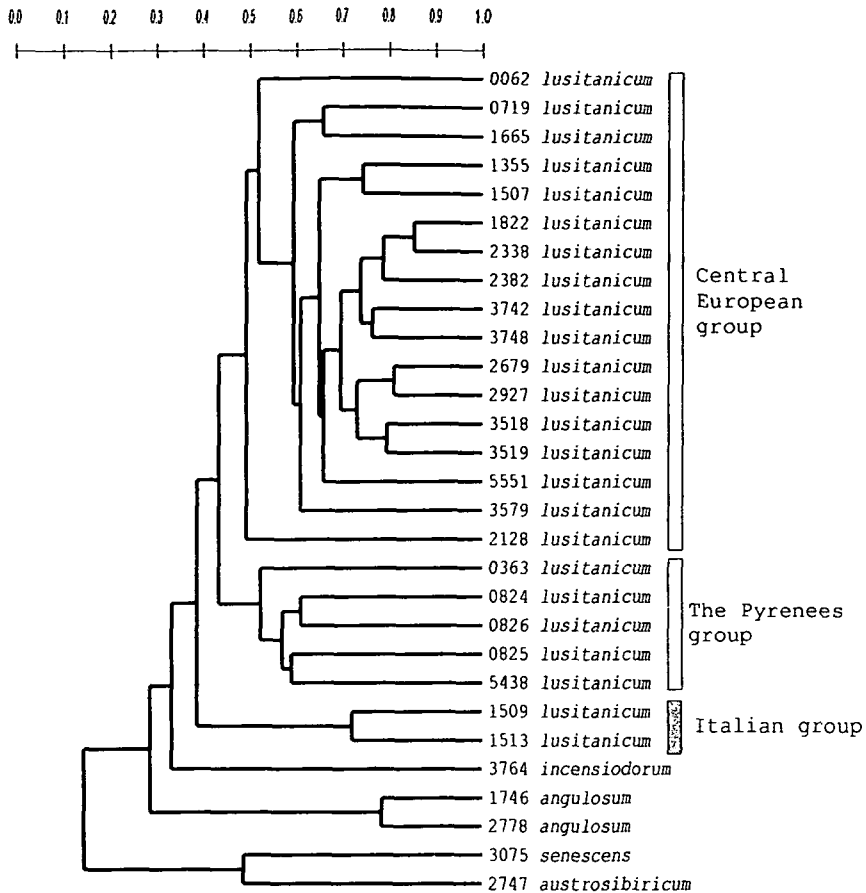


Fig. 6. UPGMA dendrogram of 24 accessions of *A. lusitanicum* and five accessions of related species, based on 166 RAPD markers.

the geographical distribution of the investigated accessions: they are the Italian group, the Pyrenean group, and the Central European group (Fig. 7, see also Fig. 3). Certainly, the subdivision in such geographical groups inside *A. lusitanicum* has some phylogenetical significance, but the inclusion of farther geographically intermediate accessions of *A. lusitanicum* from northern Italy and southern France possibly would weaken this clear geographical distinction.

The division of the species of section *Rhizirideum* into two groups in the dendrogram (Fig. 5), which corresponded to the colour of the petals, as well as the subdivision of the monophyletic red flowered species into a European and an Asiatic subgroup, showed that this character might be phylogenetically old in this section, very likely might be present from the Palaeocene. The reconstruction of the evolution among red flowered species in the section *Rhizirideum* can be approached in the following way: In the Palaeocene on

the Eurasian Continent one paleotaxon existed. In the Eocene, the Euroasiatic continent was divided by the opening of the Turgai Strait (VINOGRADOV 1967-68, TIFFNEY 1985)) and the joint area of this species was separated. These separated species were the ancestors for the European species on one side and the Asiatic species on the other side: an European with - *A. angulosum*, *A. incensiodorum* and *A. lusitanicum*; an Asiatic subgroup with: *A. senescens*, *A. austrosibiricum*, *A. nutans*, *A. dauricum*, *A. spirale* and others. After retreat of Turgai Straits in the Oligocene one European species (*A. angulosum*) spread to West Siberia. In the „yellow petals“ group we can be predict a similar scenario.

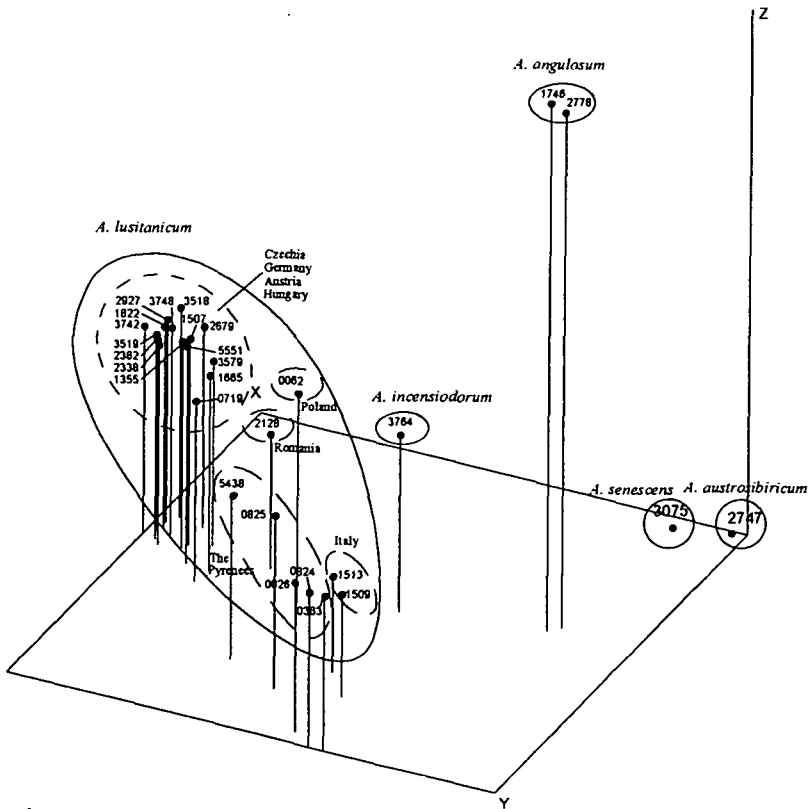


Fig. 7. Three-dimensional plot of the first three principal co-ordinates, calculated from the same RAPD distance matrix used for the UPGMA analysis in Fig. 6.

The distribution of the second diploid European species with reddish flowers, *A. incensiodorum*, is insufficiently known. It was only reported from several places along the Adriatic Sea coast (RADIC 1989).

Nomenclature and synonymy of the European species of sect. *Rhizirideum* with red petals.

Allium lusitanicum LAMARCK 1783

Encycl. Meth. Bot. 1: 70 excl. syn Tournefortii. Typus: In Lusitania. (P!). (Fig. 8).

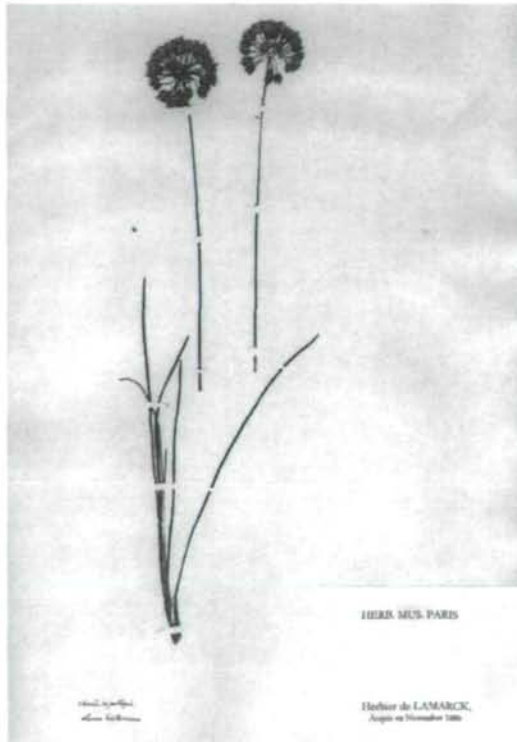


Fig. 8. Typus of *A. lusitanicum* LAMARCK.

- *A. senescens* L. 1753 p. p., quoad pl. Europaeam BAUHINII et HALLERI.
- *A. montanum* F.W. SCHMIDT 1794 Fl. Boem. 4: 28, non *A. montanum* SCHRANK in SCHRANK & MOLL 1785 Naturh. Briefe über Oesterr. 2: 220.
- *A. serotinum* LAPEYR. 1813 Hist. Abr. Pl. Pyr.: 179.
- *A. angulosum* var. *calcareum* WALLROTH 1822 Sched. Crit. 1: 134.
- *A. calcareum* (WALLROTH) WALLROTH 1840 Linnaea 14: 113.
- *A. fallax* SCHULTES & SCHULTES fil. 1830 Syst. Veg. 7: 1072, nomen illegit.
- *A. fallax* subsp. *montanum* FRIES 1839 Novit. Fl. Suec. , Mant. 2: 18.
- *A. senescens* var. *montanum* G. BECK 1890 Fl. Nieder-Österr. 1: 168.
- *A. senescens* var. *calcareum* (WALLROTH) HYLANDER 1945 Nomenkl. Syst. Studien: 113 vel Uppsala Univ Arsskr. 1945 no 7: 113.
- *A. leptophyllum* SCHUR 1866 Enum. Pl. Transs.: 674, non *A. leptophyllum* WALL.
- *A. montanum* subsp. *glaucum* (SCHRADER) HOLUB 1950 in Dostal Květena CSR: 1763.
- *A. senescens* subsp. *montanum* (FRIES) HOLUB 1970 in Folia Geobot. Phytotax. (Praha) 5: 341.
- *A. montanum* F.W. SCHMIDT f. *subbulbilliferum* PRISZTER 1972 in Bot. Közlem. 59. 1: 46.

- *A. montanum* F.W. SCHMIDT subsp. *leptophyllum* (SCHUR) SOÓ 1977, publ. 1978 in Acta Bot. Acad. Sci. Hung. 23: 387.
- *A. senescens* subsp. *glaucum* (SCHRADER ex POIR) J. DOSTAL 1984 in Folia Mus. Rer. Nat. Bohem. Occid. Bot. 21: 16.
- *A. senescens* subsp. *lusitanicum* (LINK. et REGEL) J. DOSTAL 1984 in Folia Mus. Rer. Nat. Bohem. Occid. Bot. 21: 15, nom nudum.

***A. incensiodorum* RADIC 1989**

Acta Biokovica (Makarska) 5: 29, 33, fig. p. 36. Typus: rocky and sandy soil of maritime side from Biokovo mountain. (Herbarium IPM Makarska)

- *A. polycormium* LOVRIC 1993 in Optima Abstr. VII Meeting, Bulgaria: 123, nomen invalidum. Typus: North Adriatic islands, dunes, Zarok near port Baska. 7.10.67. Leg. A.Z.Lovric. (ADRZ).

***A. angulosum* L. 1753**

Sp. Pl. : 300. Typus: In Sibiria, Germaniae humidiusculis. (LINN!)

- *A. acutangulum* SCHRADER ex SCHLECHTENDAL 1814 in Enum. Pl. Horti Berol. Suppl. : 16.
- *A. laxum* G. DON 1827 Monogr. Genus *Allium* : 63.
- *A. andersonii* G. DON 1827 Monogr. Genus *Allium*: 59.

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Table 1. Morphological differences of *A. lusitanicum* and its related species.

	<i>Allium lusitanicum</i>	<i>Allium incensiodorum</i>	<i>Allium angulosum</i>	<i>Allium senescens</i>	<i>Allium austrosibiricum</i>	<i>Allium nutans</i>
Bulb	0.8-1 cm, narrowly conical	0.5-1 cm, narrowly conical	0.5-1 cm, cylindrical or narrowly conical	1.5-1.8 (2) cm, conical	0.6-1 cm, narrowly conical	1.5-2 cm, conical
Leaves	4-6 mm, green, canaliculate, not keeled	4-8 mm, green, slightly ribbed, canaliculate, sometimes basal slightly keeled	4-8 mm, green, canaliculate, sharply keeled, equaling or broader than scape	5-11 mm, graysh, flat, always broader than scape	1.5-4 mm, graysh, canaliculate, narrow or equaling to the width of the scape	6-15 mm, green, flat, always broader than scape
Umbel	2-3 cm, hemispherical or nearly spherical	1.5-3 cm, hemispherical	2.5-4 cm, hemispherical or fasciculate	3-5 cm, spherical or nearly hemispherical	2.5-4 cm, hemispherical	4-6 cm, spherical
Filaments	1.2x longer than tepals	1.5x longer than tepals	equaling tepals	inner filaments at base broadened, 1.2x longer than tepals	1.5x longer than tepals	inner filaments with one acute tooth at each side
Ovary with stylus	longer than tepals	longer than tepals	longer than tepals	mouth longer than tepals	equal or longer than tepals	longer than tepals
Time of flowering	IX-X	VIII-IX	VII (VIII)	VII-VIII	VI-VII	VII-VIII

Table 2. The origin of the accessions of *Allium lusitanicum* and some other related species from sect. *Rhizirideum* investigated with RAPD. (TAX - accession number of the Department of Taxonomy, Institute for Plant Genetics and Crop Plant Research)

TAXon	Accession number and origin of plants
<i>A. lusitanicum</i> LAM.	TAX 0062 (Poland, Czerwony Chodel); TAX 0363 (Spain, Vaqueira Val d'Arab); TAX 0719 (Austria, Zöbing); TAX 0824 (Spain, Prov. Santander); TAX 0825 (Spain, Prov. Palencia); TAX 0826 (France, the Pyrenees); TAX 1355 (Germany, Jena); TAX 1507 (Germany, Sangerhausen); TAX 1509 (Italy, Monte Sibillini); TAX 1513 (Italy, Monte Acuto); TAX 1665 (Hungary, Zemplen mt.); TAX 1822 (Germany, Fränkische Schweiz); TAX 2128 (Rumania, Cluj-Napoca); TAX 2338 (Germany, Machedern), TAX 2385 (Czech Republic, Moravia); TAX 2679 (Germany, Reinsburg); TAX 2927 (Germany, Benzingerode); TAX 3518 (Germany, Berchtesgadener Land); TAX 3519 (Austria, Schafberg); TAX 3579 (Germany, Borsbüll); TAX 3742 (Austria, Körmelgrus); TAX 3748 (Hungary, Balaton); TAX 5438 (Andorra), TAX 5551 (Germany, Schwäbische Alb).
<i>A. incensiodorum</i> RADIC	TAX 3764 (Croatia)
<i>A. angulosum</i> L.	TAX 1746 (Hungary); TAX 2778 (North Kazakhstan, Kievskoe); TAX 2806 (Germany, Mansfelder Hügelland)
<i>A. senescens</i> L.	TAX 2377 (Mongolia); TAX 3075 (Russia, Jakutia)
<i>A. spirale</i> WILLD.	TAX 1968 (North Korea, Kanwon)
<i>A. albidum</i> FISCH. ex BIEB.	TAX 3470 (Georgia)
<i>A. nutans</i> L.	TAX 3184 (Russia, Altai)
<i>A. stellerianum</i> WILLD.	TAX 5738 (Russia, Baikal)

Table 3. Chromosome number of investigated accessions of *A. angulosum*, *A. incensiodorum* and *A. lusitanicum* from the living collection of the Department of Taxonomy, Institute for Plant Genetics and Crop Plant Research

Taxon	TAX No	Chromosome number 2n	Origin
<i>A. angulosum</i>	0007	16	Italy, Puglia
	0534	16	Russia, Voronezh
	0720	16	Germany, Bodensee
	1746	16	Hungary
	1833	16	Germany, Baden-Württemberg
	1853	16	Germany, Dessau
	2773	16	North Kazakhstan, Kievskoe
	2778	16	Russia, West Siberia, Poltavka
	2806	16	Germany, Mansfelder Hügelland
	3314	16	Hungary, Gödöllö
<i>A. incensiodorum</i>	3764	16	Croatia
<i>A. lusitanicum</i>	0062	32	Poland, Czerwony Chodel
	0363	32	Spain, Vaqueira Val d'Arab
	0719	32	Austria, Zöbing
	0824	32	Spain, Prov. Santander
	0825	32	Spain, Prov. Palencia
	0826	32	France, the Pyrenees
	1355	32	Germany, Jena
	1507	32	Germany, Sangerhausen
	1509	32	Italy, Monte Sibillini
	1513	32	Italy, Monte Acuto
	1665	32	Hungary, Zemplen Mt.
	1822	32	Germany, Fränkische Schweiz
	2128	32	Romania, Cluj-Napoca
	2338	32	Germany, Machern
	2385	32	Czech Republic, Moravia
	2679	32	Germany, Reinsburg
	2927	32	Germany, Benzingerode
	3312	32	France, The Pyrenees
	3518	32	Germany, Berchtesgadener Land
	3519	32	Austria, Schafberg
	3579	32	Germany, Borsbüll
	3742	32	Austria, Körmelgrus
	3748	32	Hungary, Balaton
	5438	32	Andorra, the Pyrenees
	5551	32	Germany, Schwäbisch Alb
	5817	32	Germany, Kyffhäuser