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Allium makrianum (*Alliaceae*), a New Autumnal Species from Greece

By

Cristian BRULLO*), Salvatore BRULLO*), Gianpietro GIUSSO DEL GALDO*) and Cristina SALMERI**)

With 3 Figures

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Summary

BRULLO C., BRULLO S., GIUSSO DEL GALDO G. & SALMERI C. 2010. *Allium makrianum (Alliaceae)*, a new autumnal species from Greece. – Phyton (Horn, Austria) 49 (2): 267–278, with 3 figures.

Allium makrianum C. BRULLO, S. BRULLO, GUISSO & SALMERI is described as a new species growing on limestone slopes of Mt. Pilineon on Chios, a north-eastern Aegean island (Greece). *A. makrianum* is characterized by hairy leaves (blades and sheaths), glabrous and unilateral spathe, glabrous tepals, no interstaminal teeth and densely tuberculate ovary. The species is a very rare autumnal geophyte, showing some relationships to *A. archeotrichon* from Rhodos. Its morphology, chromosome number (2n=16), leaf anatomy, ecology and taxonomy are examined.

Zusammenfassung

BRULLO C., BRULLO S., GIUSSO DEL GALDO G. & SALMERI C. 2010. *Allium makrianum* (*Alliaceae*), a new autumnal species from Greece. [*Allium makrianum* (*Alliaceae*), eine neue, herbstblühende Art aus Griechenland] – Phyton (Horn, Austria) 49 (2): 267–278, mit 3 Abbildungen.

*) Dr. C. BRULLO, Prof. Dr. S. BRULLO and Dr. G. GIUSSO DEL GALDO, Dipartimento di Botanica, Università di Catania, via A. Longo, 19, 95125 Catania (Italy); e-mail: salvo.brullo@gmail.com

**) Prof. Assoc. Dr. C. SALMERI, Dipartimento di Scienze Botaniche, Università di Palermo, Via Archirafi, 38, 90123 Palermo (Italy); e-mail: cristinasalmeri@gmail.com

Allium makrianum C. BRULLO, S. BRULLO, GIUSSO & SALMERI, das auf Kalk-Abhängen des Mt. Pilineon auf Chios (NE ägäische Inseln, Griechenland) wächst, wird als neue Art beschrieben. *A. makrianum* ist durch behaarte Blätter (Spreiten und Scheiden), kahle, einseitige Spatha, kahle Tepalen, dicht warziges Ovar etc. ausgezeichnet. Es ist ein sehr seltener Geophyt, der gewisse Beziehungen zu *A. archeotrichon* von Rhodos zeigt. Morphologie, Chromosomenzahl (2n=16), Blattanatomie, Ökologie und systematische Stellung wurden untersucht.

1. Introduction

In the frame of taxonomical investigations on the genus *Allium* in Greece, a very peculiar population found on the island of Chios (NE Aegean area) is examined. This plant is localized in the northern part of the island near Vikion, where it grows within dwarf shrubby plant communities. It is likely that, due to its autumnal flowering (September-October), and its rarity, this geophyte characterized by hairy leaves, was never collected by earlier botanists. Nevertheless, in the recent flora of Chios (SNOGERUP & al. 2001) an *Allium* specimen is recorded for the same locality and identified by D. TZANOUDAKIS as *Allium paniculatum* L. subsp. *villosulum* (HALÁCSY) STEARN. Due to the rigid stem and hairy leaves our population can be easily confused with *A. paniculatum* subsp. *villosulum*, which is indeed differentiated by brown bulb tunics, hairy and opposite spathe valves, hairy tepals and interstaminal teeth (see BRULLO & al. 1998). On the contrary, the investigated plants show whitish bulb tunics, glabrous and unilateral spathe valves, glabrous tepals and no interstaminal teeth.

Morphological investigations on living wild material and cultivated specimens revealed that this population is closely related to some autumnal species of *Allium* sect. *Codonoprasum* RCHB. occurring in the Aegean area (TZANOUDAKIS & KYPRIOTAKIS 1993, 2008, BRULLO & al. 1997c, 1999, 2003a, 2003b, TZANOUDAKIS 2000, BIEL & al. 2006, BOGDANOVIĆ & al. 2009). This group is differentiated by some shared morphological characters, such as rigid and erect stems, leaf sheaths covering $^1/_2$ to entirely the stem, erect or slightly divaricate spathe valves, campanulate perigon, simple stamens normally included into the perigon. These Aegean autumnal species are: *Allium tardans* GREUTER & ZAHARIADI (Crete, Karpathos), *A. rausii* BRULLO & al. (Thessaly), *A. platakisii* TZANOUD. & KYPR. (Pondokonisi), *A. euboicum* RECH. f. (Evvoia), *A. aegilicum* TZANOUD. (Antikithira) and *A. archeotrichon* BRULLO, PAVONE & SALMERI (Rhodos). They all are endemic taxa, geographically strictly localized, and they are characterized by a diploid chromosome number (2n = 16). The plants belonging to the population found on Chios seem to be more closely related with *A. archeotrichon* mainly for the hairy leaves, but several remarkable features concerning the stem, leaves, spathe valves, flowers, and fruit allow to distinguish them from *A. archeotrichon*. Therefore, these plants are treated as a species new to science and named *A. makrianum*.

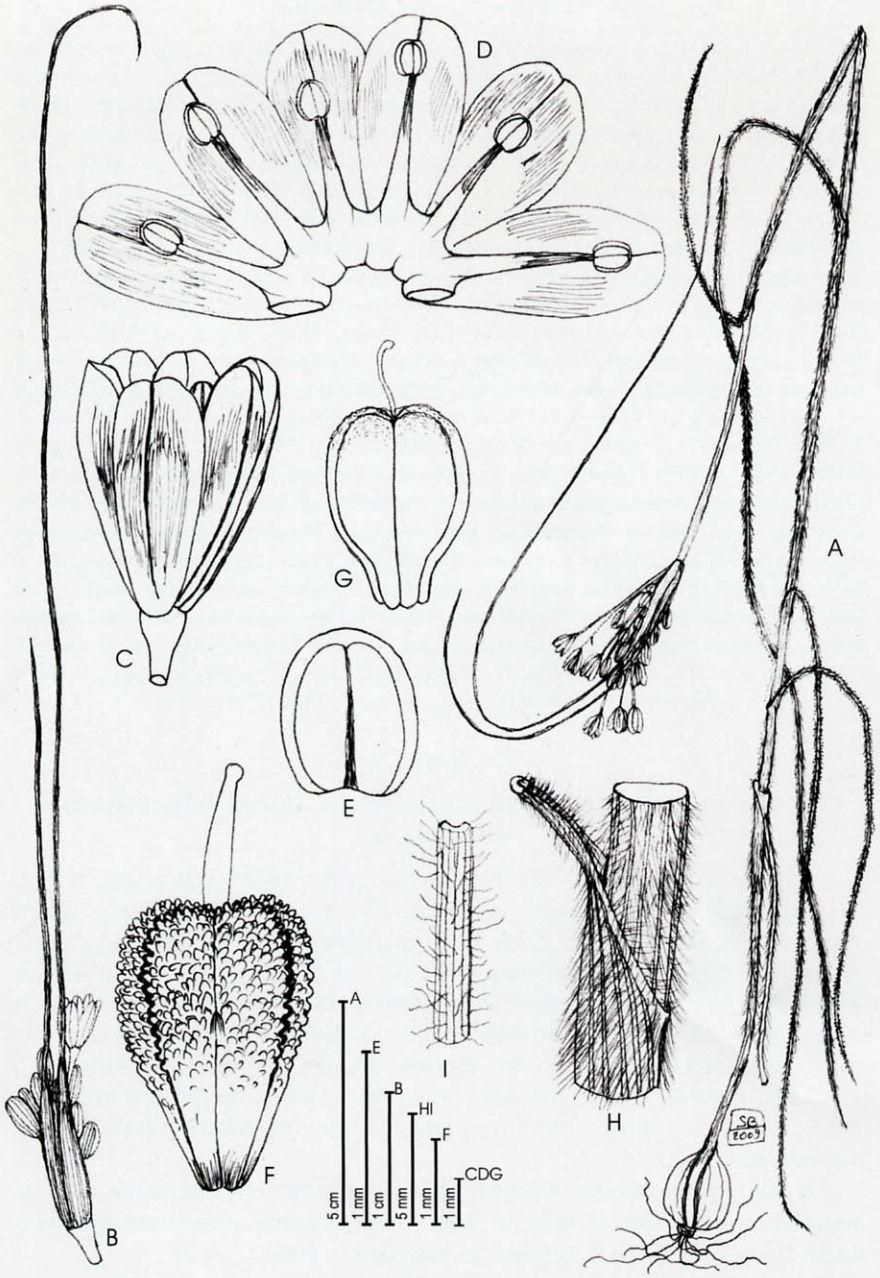

Fig. 1. *Allium makrianum*. A – habit, B – inflorescence, C – flower, D – perigon and stamens, E – anther, F – pistil, G – capsule, H – leaf indumentum (lower part), I – leaf blade indumentum.

2. Material and Methods

The morphological investigations were carried out both on living material from the type locality and specimens cultivated in the Botanical Garden of Catania (Italy). Qualitative and quantitative morphological features, which are diagnostic in the genus *Allium*, were measured and scored using fresh material. Leaf anatomy was studied on cultivated plants using leaf blades of maximum size in their optimal vegetative development, usually before the flowering stage. The leaf tissue was fixed in Karpechenko and embedded in paraffin; the transverse sections (10 μm thick) were stained with ruthenium red and light green. The drawing was created based on a photograph. Karyological analyses were performed on mitotic plates from root-tip cells of cultivated bulbs, pre-treated with 0.3% colchicine water solution for 3 hours, fixed in ethanol-acetic acid mixture (3:1) for 6 hours, then hydrolyzed in 1N HCl for 7' and stained according to the Feulgen method. Metaphase observations and chromosome measurements were conducted using the image analysis systems IKAROS 4.6 (Metasystem) and Zeiss AxioVision 5.1. Karyotyping was done with the specialized software Cromolab(c) 1.1 (BRULLO 2002–2003), which was used to recognize homologues, to order chromosomes by size and classify them according to their morphology and centromere position (LEVAN & al. 1964). Ten mitotic plates from five individuals were used for determining the karyotype. Measures of each chromosome were processed by an Excel worksheet to calculate mean and standard deviation of long and short arm lengths (satellites included), absolute and relative lengths, arm ratio and centromeric index. To estimate the karyotype asymmetry different numerical parameters were calculated, such as categories of STEBBINS 1971, REC and SYi (GREILHUBER & SPETA 1976), TF% (HUZIWARA 1962), A_1 and A_2 (ROMERO ZARCO 1986), DI (LAVANIA & SRIVASTAVA 1992), CV_{CI}, CV_{CL} and AI index (PASZKO 2006).

3. Results

3.1. *Allium makrianum* C. BRULLO, S. BRULLO, GIUSSO & SALMERI spec. nova (Fig. 1)

Diagnosis: Ab *Allio archeotrichone* bulbo 10–22 mm longo, tunicis externis albidis, scapo usque ad 40 cm alto, vaginis foliorum per 4/5 ad totaliter longitudinis tecto, foliis inconspicuis costatis, spathae valvis unilateralis, longissimis, valva majore usque ad 25 cm longa, valva minore usque ad 9 cm longa, tepalis 5–6 mm longis, albo-viridis, 2–2.7 mm latis, staminorum filamentis purpurascentibus superne et albidis inferne, exterioribus 1.5–2.5 mm longis, interioribus 3.2–3.5 mm longis, annulo 1–1.2 mm alto, antheris stramineis, subrotundatis, 0.9 mm longis, 0.8 mm latis, ovario 3–3.2 mm longo, 1.8–2 mm lato, capsula obovoidea, 4.5–4.8 × 3.3–3.5 mm, differt.

Holotypus: Grecia, Isola di Chios, versante settentrionale, nei cespuglieti spinosi presso Vikion a circa 700 m di quota, esemplare coltivato, 09.09.2008, BRULLO S. & GIUSSO DEL GALDO G., CAT.

Paratype: Grecia, Isola di Chios, versante settentrionale, nei cespuglieti spinosi a Sud di Vikion a circa 700 m di quota, 11.10.2007, BRULLO S. & GIUSSO DEL GALDO G., CAT.

Table 1. Measurements, classification and symmetry indices of the *Allium makrianum* chromosome complement

	Absolute length (μm)			Relative length %					
	Long arm	Short arm	Total length	Long arm	Short arm	Total length	Ratio		
Chrom.	*Mean ± S.D*	*Mean ± S.D.*	*Mean ± S.D.*	*Mean ± S.D.*	*Mean ± S.D.*	*Mean ± S.D.*	l/s	CI	Type
1	5.82 ± 0.88	5.18 ± 0.80	11.00 ± 1.54	4.31 ± 0.45	3.82 ± 0.16	8.13 ± 0.44	1.12	47.09	m
2	5.48 ± 0.72	4.88 ± 0.63	10.36 ± 1.20	4.07 ± 0.43	3.61 ± 0.12	7.68 ± 0.2	1.12	47.10	m
3	5.58 ± 0.99	4.44 ± 0.59	10.02 ± 1.45	4.12 ± 0.30	3.28 ± 0.07	7.41 ± 0.33	1.26	44.31	m
4	5.36 ± 0.58	4.12 ± 0.25	9.48 ± 0.80	3.97 ± 0.18	3.07 ± 0.18	7.04 ± 0.27	1.30	43.46	m
5	5.16 ± 0.88	4.66 ± 0.69	9.82 ± 1.54	3.80 ± 0.24	3.44 ± 0.23	7.25 ± 0.39	1.11	47.45	m
6	4.96 ± 0.82	4.40 ± 0.50	9.36 ± 1.30	3.66 ± 0.18	3.26 ± 0.15	6.92 ± 0.24	1.13	47.01	m
7	4.98 ± 0.69	4.02 ± 0.48	9.00 ± 1.14	3.68 ± 0.19	2.98 ± 0.08	6.66 ± 0.18	1.24	44.67	m
8	4.72 ± 0.68	3.88 ± 0.34	8.60 ± 0.84	3.49 ± 0.25	2.89 ± 0.25	6.38 0 0.21	1.22	45.12	m
9	4.28 ± 0.65	3.46 ± 0.37	7.80 ± 088	3.17 ± 0.28	2.57 ± 0.24	5.79 ± 0.32	1.24	44.36	m^{sat}
10	4.22 ± 0.58	3.54 ± 0.53	7.76 ± 0.92	3.13 ± 0.29	2.62 ± 0.10	5.75 ± 0.26	1.19	45.62	m^{sat}
11	4.08 ± 0.48	3.64 ± 044	7.72 ± 0.91	3.03 ± 0.20	2.70 ± 0.13	5.72 ± 0.32	1.12	47.15	m
12	3.72 ± 0.43	3.58 ± 0.51	7.30 ± 0.94	2.76 ± 0.18	2.65 ± 0.19	5.41 ± 0.37	1.04	40.04	m
13	4.08 ± 0.63	3.08 ± 0.50	7.16 ± 0.98	3.03 ± 0.34	2.28 ± 0.18	5.30 ± 0.30	1.32	43.02	m^{sat}
14	3.88 ± 0.54	3.08 ± 0.42	6.96 ± 0.87	2.88 ± 0.31	2.28 ± 0.17	5.16 ± 0.35	1.26	44.25	m^{sat}
15	3.42 ± 0.48	3.10 ± 0.58	6.52 ± 0.95	2.53 ± 0.14	2.29 ± 0.13	4.82 ± 0.17	1.10	47.55	m
16	3.18 ± 0.36	2.94 ± 0.40	6.22 ± 0.97	2.36 ± 0.12	2.17 ± 0.08	4.60 ± 0.26	1.08	47.27	m

Complement length (2*n*): 135.08 ± 16.14 μm

Symmetry indices: Stebbins' Cat.: 1A; AI: 0.46; CV_{CL}: 12.00; CV_{CI}: 3.87; A1: 0.15; A2: 0.12; REC: 75.20; SYi: 85.02; TF%: 45.90

Etymology: The specific epithet comes from „Makris", the ancient name of the island of Chios.

Description: Bulb ovoid, 10–22 × 7–16 mm, with outer tunics membranaceous, whitish. Stems 10–40 cm tall, cylindrical, glabrous, erect, covered by leaf sheaths for 4/5 to total length, inserted externally to the bulb. Leaves 5, green, entirely hairy, with hairs 1–2 mm long; blade 5–25 cm long, 1.8–2.3 mm wide, semicircular, more or less fistulous, with inconspicuous ribs. Spathe persistent, with two unequal valves, unilateral, much longer than the inflorescence, the larger 7-nerved and 5–25 cm long, the smaller 5-7-nerved and 2.5–9 cm long. Inflorescence lax and fastigiate, 10-25-flowered; pedicels erect, unequal, 7–40 mm long. Bostryces 8. Perigon campanulate, with tepals equal, greenish-white, tinged with purplish, elliptical, rounded at the apex, 5–6 mm long, the outer ones 2–2.5 mm wide, the inner ones 2.3–2.7 mm wide, midrib purplish-green. Stamens normally included, rarely slightly exserted from the perigon, with simple filaments, purplish above and white below, unequal, the outer ones 1.5–2.5 mm long, the inner ones 3.2–3.5 mm long, connate below into an annulus 1–1.2 mm

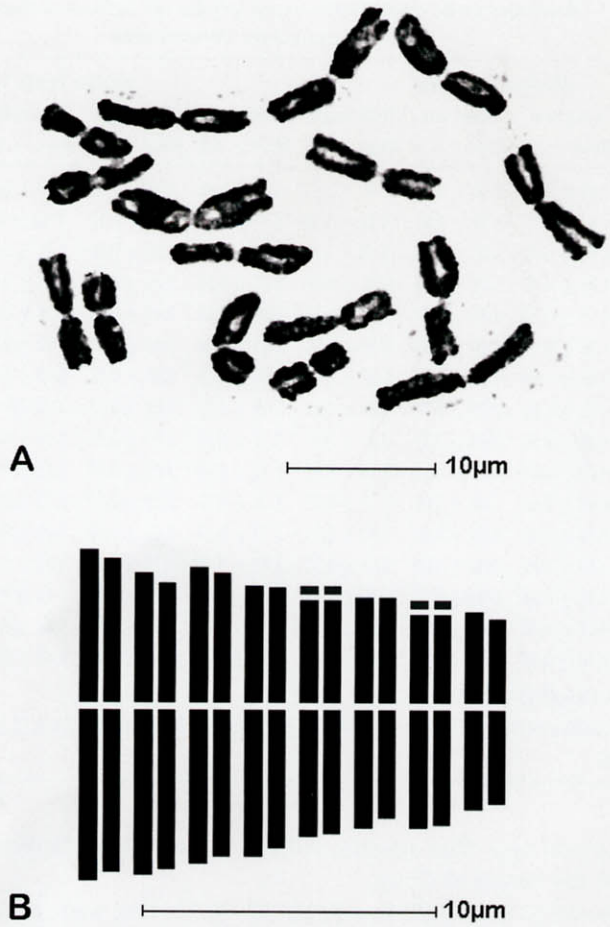

Fig. 2. *Allium makrianum*. A – metaphase plate of mitotic chromosomes (2n = 16), B – karyogram of the diploid set.

high; anthers straw-yellow, subrounded, 0.9 × 0.8 mm, rounded at the apex. Ovary obovoid, narrowed at the base, greenish-yellow, densely tuberculate above, 3–3.2 × 1.8–2 mm; style white, 0.5–1.5 mm long. Capsule trivalved, obovoid, 4.5–4.8 × 3.3–3.5 mm, green.

3.2. Chromosome Number

Allium makrianum is a diploid species with a somatic chromosome number of 2n = 16 (Fig. 2A). All examined specimens show a quite regular karyotype with metacentric chromosomes. Microsatellites are evident in some metaphase plates, occurring on the short arm of two chromosome

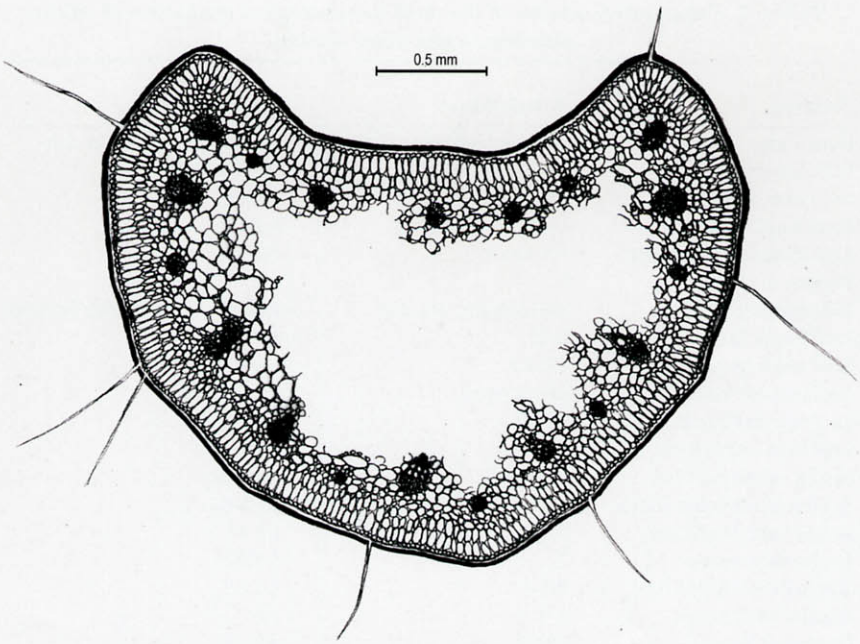

Fig. 3. Leaf blade cross section of *Allium makrianum*.

pairs (Fig. 2B). The karyotype formula can be resumed as 2n = 2x = 16: 12 m + 4 m^{sat}. The absolute chromosome length varies from 11 ± 1.54 μm for the longest chromosome to 6.22 ± 0.97 μm for the shortest one, while the relative length ranges from 8.13 ± 0.44 % to 4.60 ± 0.26 %. Many different indices were calculated to assess the degree of symmetry of the chromosome complement; all values remark a high homogeneity in the karyotype of *A. makrianum* (Table 1).

3.3. Leaf Anatomy

The leaf cross section of *A. makrianum* shows a semi-cylindrical outline, concave on the ventral face and provided with inconspicuous ribs on the dorsal one. The epidermis is covered by a welldeveloped cuticle. Stomata are distributed along the whole leaf perimeter while hairs are scattered distributed only in the abaxial surface. The palisade tissue is regular and compact, and arranged in two layers of cells; the outer layer shows large, elongated cells in transversal section, while the inner one shows cells which appear small and sub-orbicular. The spongy tissue is irregular and circumscribed to the leaf periphery since the leaf is widely fistulous. Many secretory canals occur under the palisade tissue. The vascular bundles are 18–20, of which 11–13 are abaxial and 7 are adaxial (Fig. 3).

Table 2. Comparative scheme of the main morphological characters of *Allium makrianum* and allied species

Character	***A. makrianum***	***A. archeotrichon***
Outer bulb tunics	whitish, membranaceous	brown-blackish, coriaceous
Bulb size (mm)	10–22 × 7–16	10–14 × 12–18
Bulb stem insertion	external	central
Stem length (cm)	10–40	5–25
Leaf sheath covering stem	4/5-totally	1/2-4/5
Number of leaves	5	4–5
Leaf shape in section	semicircular, fistulous	subcylindrical, slightly fistulous
Leaf length (cm)	5–25	8–32
Leaf width (mm)	1.8–2.3	1.5–2
Leaf palisade tissue	two-layered	one-layered
Leaf vascular bundless	18–20	9–20
Leaf hairs length (mm)	1–2	0.4–1
Spathe valves insertion	unilateral	opposite
Spathe valves indumentum	glabrous	glabrous
Larger valve length (cm)	5–25	1.5–4
Larger valve nerves	7	5–7(9)
Smaller valve length (cm)	2.5–9	1–2.5
Smaller valve nerves	5–7	3–5
Number of flowers (per inflorescence)	10–25	(4)6–20
Pedicel length (mm)	7–40	10–30
Tepals colour	white-greenish	white-pink
Tepal length (mm)	5–6	6
Tepal shape	elliptical	linear-elliptical
Outer tepal surface	glabrous	glabrous
Outer tepal width (mm)	2–2.5	1.5
Inner tepal width (mm)	2.3–2.7	1.5
Tepal apex	rounded	rounded
Stamen/perigon	included	included
Stamen filament (colour)	purplish above white below	white
Outer stamen length (mm)	1.5–2.5	2.5–3.2
Inner stamen length (mm)	3.2–3.5	4–4.5
Interstaminal teeth	absent	absent
Annulus height	1–1.2	1.2–1.4
Anther colour	straw-yellow	white-pink to pink-purplish
Anther shape	subrounded	elliptical
Anther apex	rounded	rounded
Anther size (mm)	0.9 x 0.8	0.8–1.1 × 0.5–0.6
Ovary size (mm)	3–3.2 × 1.8–2	2.8–3 × 1.5–1.6
Ovary shape	obovoid	obovoid
Ovary surface	densely tuberculate above	densely papillose above
Style length (mm)	0.5–1.5	1–1.2
Capsule shape	obovoid	subglobose
Capsule size (mm)	4.5–4.8 × 3.3–3.5	5 × 4.5
Flowering period	IX – X	IX – X

A. rhodopaeum subsp. *rhodopaeum*	*A. rhodopaeum* subsp. *turcicum*	*A. hirtovaginum*
pale brown, coriaceous	pale brown, coriaceous	dark brown, coriaceous
15–22 × 10–15	15–22 × 10–15	10–15 × 7–10
central	central	central
15–35(45)	15–35(45)	10–25
1/2–2/3	1/2–2/3	1/3–1/2
3–4	4–6	4
convolute, compact	flat, compact	–
10–30	10–30	8–18
4	2	1–1.5
two-layered	one-layered	–
25–27	18–20	–
0.3–0.8	0.3–0.8	0.1–0.2
opposite	opposite	opposite
hairy in the appendage	hairy in the appendage	hairy
4.5–10	4.5–10	6–12
5–7(8)	7–9	7
3.5–7.6	3.5–7.6	3.5–7
3.5	5	5
(10)15–35	(10)15–35	20–40
5–25	5–25	10–25
brownish-green	brownish-green	pink-purplish
5–6	5–6	4.5–5
oblong	oblong	elliptical
hairy above	hairy above	glabrous
2–2.7	2–2.7	2–2.5
2–2.8	2–2.8	2–2.5
obtuse	obtuse	apiculate
included	included	exserted
white above purplish below	white above purplish below	lilac above white below
1.8–2.3	1.8–2.3	25–30
2.3–3	2.3–3	4.5–4.7
present	present	absent
1–1.5	1–1.5	0.7–0.8
white-yellowish	white-yellowish	yellow
oblong	oblong	elliptical
rounded	apiculate	rounded
1–1.2 × 0.5–06	1–1.2 × 0.5–0.6	1.5–1.6 × 0.7–0.8
2.5–3 × 1.4–1.6	2.5–3 × 1.4–1.6	1.5–1.6 × 1.8–2
ellipsoid	submoniliform	subglobose
slightly papillose above	slightly papillose above	slightly papillose above
1–2	1–2	2
obovoid	obovoid-subglobose	obovoid
4.5–5 × 3.5–4	4.5–5 × 3.5–4	3.8–4 × 4–4.2
VII – VIII	VIII – IX	VI – VII

3.4. Distribution and Ecology

Allium makrianum occurs in the northern part of Chios (Greece), where it was found in a small area (about 200 sqm) near the village of Vikion. This species is represented by a few-numbered population of about 50 individuals, growing on the carbonatic substrates of the northern slope of Mt. Pilineon (about 700 m a.s.l.). It is a member of a thorny shrub plant community characterized by the dominance of *Sarcopoterium spinosum* (L.) SPACH and *Astragalus ptilodes* BOISS. This geophyte flowers in September-October after a long lasting vegetative phase with no dormancy.

3.5. Conservation Status

For its rarity and the low number of individuals in the only known population, which is particularly threatened by over-grazing, it is proposed to add *Allium makrianum* to the Regional Red List of IUCN as Critically Endangered (CR). In particular, based on the criteria adopted by IUCN 2001, 2003, 2006, the following category is proposed: CR A4cd, B2ab(iii, iv, v), D.

3.6. Taxonomic Remarks

The occurrence of a spathe with two persistent appendiculate valves, longer than the inflorescence, simple stamens and an ovary with inconspicuous nectaries allows to include *Allium makrianum* into the sect. *Codonoprasum* RCHB. Within this section, this species shows close relationships to *A. archeotrichon* described by BRULLO & al. 1999 from Rhodos (SE Aegean Island) for its habit, its hairy leaves and late flowering time. Several morphological features allow to distinguish quite well both these species (Tab. 2). In particular, *A. archeotrichon* is characterized by shorter, blackish-brown bulbs, with coriaceous outer tunics, and stems max. 25 cm tall, leaves subcylindrical, slightly fistulous, with palisade tissue one-layered and hairs 0.4–1 mm long, spathe valves opposite, equalling the inflorescence, tepals whitish-pink, linear-elliptical, 1.5 mm wide, stamen filaments white, longer (outer ones 2.5–3.2 mm long, inner ones 4–4.5 mm long), anthers white-pink to purplish-pink, elliptical (0.8–1.1 × 0.5–0.6 mm), ovary smaller (2.8–3 × 1.5–1.6 mm), papillose above, capsule subglobose, 5 × 4.5 mm.

Another species having some affinities with *A. makrianum* is *A. rhodopaeum* VELEN. [= *A. paniculatum* L. subsp. *villosulum* (HALÁCSY) STEARN], which is represented by the subsp. *rhodopaeum* and subsp. *turcicum* BRULLO, GUGLIELMO & TERRASI, both occurring on the Balkan peninsula (BRULLO & al. 1998). The type is known from Greece and Bulgaria and flowers from July to early August, while the other subspecies is localized in the European Turkey and flowers from late August to September. According to the literature, *A. rhodopaeum* is a diploid species sharing

with *A. makrianum* the same habit as well as leaf hairiness, but it differs in having outer bulb tunics coriaceous, pale brown, more robust stem, leaves with hairs 0.3–0.8 mm long, sheaths covering the stem for 1/2–2/3 of its length, blade convolute to flat, not fistulous, spathe valves opposite, max. 10 cm long, with hairy appendage, pedicels max. 25 mm long, tepals brownish-green, externally hairy, obtuse at apex, stamen filamens white above and purplish below, interstaminal teeth present, anthers oblong (1–1.2 × 0.5–0.6 mm), yellowish-white, ovary ellipsoid, smaller (2.5–3 × 1.4–1.6 mm), slightly papillose above, capsule 4.5–5 × 3.5–4 mm (see Tab. 2).

Due to the occurrence of hairy leaves and spathe valves with a long appendage, also *A. hirtovaginum* P. CANDARGY seems to have some affinities with *A. makrianum*. This species described from Lesbos (E Aegean island) was recently illustrated and cytotaxonomically examined, based on living material collected from the type locality (BRULLO & al. 2008). According to these data, *A. hirtovaginum* differs from *A. makrianum* in many morphological features as well as in the flowering period (see Tab. 2). In fact, it flowers in early summer (June-July) and it is characterized by smaller bulbs (10–15 × 7–10 mm), with dark brown, coriaceous tunics, stem max. 25 cm long, inserted in the centre of the bulb, leaves 4, with sheaths covering the stem for 1/3–1/2 and hairs 0.1–0.2 mm long, spathe valves hairy, opposite, patent, max. 12 mm long, inflorescence 20–40 flowered, tepals purplish-pink, 4.5–5 mm long, apiculate, stamen filaments exserted from the perigon, the outer ones 25-30 mm long, the inner ones 4.5–4.7mm long, anthers yellow, elliptical, 1.5–1.6 × 0.7–0.8 mm, ovary subglobose, smaller (1.5–1.6 × 1.8–2 mm), capsule 3.8–4 × 4–4.2 mm.

On the basis of its peculiar ontogenetic cycle, some morphological features as well as its punctiform distribution, *Allium makrianum* can be considered as a relict element of the old Tertiary flora, similarly to other autumn-flowering species of the genus *Allium* as already emphasized by KOLLMANN & al. 1991, TZANOUDAKIS & KYPRIOTAKIS 1993, 2008, BRULLO & al. 1997a, b, c, 1999, 2003a, and TZANOUDAKIS 2000.

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