



A new *Allium* species from section *Molium* from Israel: *A. akirense* (Amaryllidaceae)

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Abstract

As part of the phylogenetic revision of the Eurasian representatives of the subgenus *Amerallium* we have discovered a new *Allium* species (section *Molium*) in Israel, related to *A. qasyunense*. It is described here as *Allium akirense*, based on living plants and recent herbarium specimens. Independence of the new species is confirmed by morphological and ecological features, and also by molecular ones. To learn more about the phylogenetic relationships within a group of closely related species of section *Molium*, we used maximum parsimony and Bayesian analyses of combined nuclear (ITS—internal transcribed and ETS—external transcribed spacers of rRNA genes) and chloroplast (*rpl32–trnL* intergenic spacer) dataset of 7 taxa. Discussion on geographic distribution, conservation status and habitat is provided, as well as an identification key including the closest related species.

Key words: *Allium*, *Allium akirense*, *Molium*, plant taxonomy, ITS, ETS, *rpl32–trnL*

Introduction

In the treatment of the genus *Allium* Linnaeus (1753: 143) in “Flora of Palaestina” (Kollmann 1986), eight species and subspecies from section *Molium* G. Don ex Koch (1837: 715) were listed: *A. trifoliatum* Cirillo (1788: 11) subsp. *hirsutum* (Regel 1875: 221) Kollmann in Kollmann & Steran (1975: 204), *A. erdelii* Zuccarini (1843: 236), *A. qasyunense* Mouterde (1953: 348), *A. carmeli* Boissier (1854: 28), *A. negevense* Kollmann (1969: 69), *A. roseum* Linnaeus (1753: 296) var. *tourneuxii* Boissier (1882: 274), *A. papillare* Boissier (1854: 27) and *A. neapolitanum* Cirillo (1788: 13). Kollmann (1971) affiliated all local species of section *Molium* into two (not validly published) series: “*Patentes*” (*A. neapolitanum* and *A. trifoliatum* subsp. *hirsutum*), and “*Campanulatae*” (*A. carmeli*, *A. erdelii*, *A. qasyunense*, *A. papillare*, *A. negevense* and *A. roseum* var. *tourneuxii*).

Later, some new *Allium* species were described (Brullo *et al.* 1991, Brullo *et al.* 2008, Fragman-Sapir & Fritsch 2011, Brullo *et al.* 2014).

A few years ago another *Allium* taxon was found in the southern Coastal Plain of Israel in the hills near Kibbutz Giv'at Brenner. These plants differ from the closely related *A. qasyunense* in many characters such as petal color, smaller flowers, and a stronger growth potential. In spring of 2011 and 2013, we collected some of these plants and other species from “series *Campanulata*” sensu Kollmann (1971) for a taxonomical study and analyzed molecular characters: DNA sequencing of non-coding sequences from two nuclear ribosomal RNA regions (ITS and ETS) as well as a chloroplast region (*rpl32–trnL*). DNA sequencing of non-coding fragments has been widely employed in *Allium* phylogenetic studies and has proven very useful in taxonomic investigations (Dubouzet & Shinoda 1999, Friesen *et al.* 2000, 2006, Klaas & Friesen 2002, Nguyen *et al.* 2008; Li *et al.* 2010; Wheeler *et al.* 2013, Mashayekhi & Columbus 2014).

Material and methods

Bulbs and leaf samples for DNA isolation were collected in spring 2011 in Israel and grown in the Botanical Gardens in Jerusalem and Osnabrück. Fourteen accessions of seven species (*A. akirense*, *A. qasyunense*, *A. papillare*, *A. erdelii*, *A. negevense*, *A. neapolitanum* and *A. longisepalum* Bertoloni (1842: 429) of section *Molium* were included in analysis (see Table 1). Bulbs were planted in pots and growing roots were used for the chromosome studies. The leaves for DNA isolation were dried with silica gel.

TABLE 1. Accessions of *Allium* species used in the study.

Accession	Species	2n	Origin	Voucher	nrITS	nrETS	<i>Rpl32-trnL</i>
Am-332-1	<i>A. akirense</i>	14	Israel, Kibbutz Givat Brenner	OSBU 20932	HF934250	HF934479	HF934591
Am-332-2	<i>A. akirense</i>	14	Israel, Kibbutz Givat Brenner	OSBU 20932	HF934251	HF934480	HF934592
Am-332-3	<i>A. akirense</i>	28	Israel, Kibbutz Givat Brenner	OSBU 20932	HF934252	HF934481	HF934593
Am-331	<i>A. erdelii</i>	16	Israel, Ness Tsiyona	OSBU 20931	HF934279	HF934509	HF934617
Am-281	<i>A. longisepalum</i>	?	Iran, prov. Kohgiluyeh - Boyer-Ahmad	GAT 6787	HF934293	HF934524	HF934628
Am-372	<i>A. longisepalum</i>	?	Iran, prov. W Azarb., valley Ghasemlu	GAT 6896	HF934294	HF934525	HF934629
Am-341	<i>A. neapolitanum</i>	28	Israel, Mt. Gilboa	OSBU 20938	HF953353	HF953352	HF934642
Am-342	<i>A. negevense</i>	20	Israel, Between Feruham and Sede Rogenz	HUJ 005972	HF934313	HF934541	HF934647
Am-344	<i>A. negevense</i>	20	Israel, Negev, Avdat	HUJ 005987	HF934315	HF934543	HF934648
Am-345	<i>A. papillare</i>	?	Egypt, N. Sinai, 5 km SW Rafah	HUJ, 17.02.1971	HF934316	HF934544	HF934649
Am-422	<i>A. papillare</i>	?	Israel, W Negev, Halutsa Sands	HUJ, 23.02.12	HF934318	HF934546	HF934651
Am-333	<i>A. qasyunense</i>	14	Israel, Road 90, Um Zuqa	OSBU 20933	HF934325	HF934554	HF934659
Am-334	<i>A. qasyunense</i>	14	Israel, Mevo Hamma	OSBU 20039	HF934326	HF934555	HF934660
Am-335	<i>A. qasyunense</i>	14	Israel, East coast of Lake Galilee at En Gev	OSBU 20934	HF934327	HF934556	HF934661

Root tips have been used for the study of chromosomes in mitosis. Excised roots were kept in distilled water on ice overnight. They were then transferred to room temperature for 20 min and pre-treated for 2 h at room temperature in an aqueous 0.1 % solution of colchicine. The tissue was fixed in a freshly prepared mixture of 96 % ethanol and glacial acetic acid (3:1). The haematoxylin staining according to Smirnov (1968) was used for imaging. For chromosome morphology, the classification of Levan *et al.* (1964) and Tzanoudakis (1983) has been followed. Karyotype symmetry was determined calculating the recent M_{CA} index (Peruzzi & Eroğlu 2013). The karyotype was studied on five metaphase plates.

Genomic DNA was sampled using the 'InnuPREPP Plant DNA kit' (Analytic Jena AG) according to the instructions of the manufacturer and used directly in PCR amplifications. All PCRs were carried out in a Biometra Professional Thermocycler gradient. For plastid DNA analyses we used the noncoding marker *trnL-rpl32*, which is according to Shaw *et al.* (2007) the most variable marker on the cpDNA. The same amplification and sequencing primers for ITS were used as given in Friesen *et al.* (2006). The ETS region was amplified using the primers 18S-IGS and ETS-all-f (Baldwin & Markos 1998, Nguyen *et al.* 2008). Primers for the chloroplast region *trnL-rpl32* were described in Shaw *et al.* (2007). Forward and reverse sequences from each individual were manually edited in CHROMAS Lite 2.1 (Technesium Pty Ltd.) and combined in single consensus sequences. The sequences of all samples were aligned with CLUSTAL X (Thompson *et al.* 1997), and the alignment was subsequently corrected manually in MEGA 5 (Tamura *et al.* 2011).

Phylogenetic analyses were carried out on both, individual and combined data sets (nuclear: ITS and ETS; and plastid DNA sequence) using parsimony and Bayesian methods. *Allium neapolitanum* has been chosen as the outgroup, based on the analyses of Friesen *et al.* (2006) and the taxonomical revision of Kollmann (1971). Parsimony analysis was performed with PAUP* 4.0b10 (Swofford 2002) using heuristic searches with TBR and 100 random addition sequence replicates. Bootstrap support (BS; Felsenstein 1985) was estimated with 100 bootstrap replicates, each with 100 random addition sequence searches. Bayesian analyses were implemented with MrBayes 3.1.23 (Ronquist & Huelsenbeck 2003). Sequence evolution models were evaluated using the Akaike Information Criterion (AIC) with the aid of Modeltest 3.7 (Posada & Crandall 1998). Two independent runs each of eight chains, 5 million generations, sampling every 1000 generations. 25% of initial trees were discarded as burn-in. The remaining 25 000 trees were combined into a 50% majority-rule consensus tree.

Results

Karyotype analysis:—The chromosome studies in the eight plants collected in the hills near the Kibbutz Giv'at Brenner revealed the chromosome number $2n = 2x = 14$ (Fig. 1) for seven plants, one plant was tetraploid with $2n = 4x = 28$. All chromosomes were metacentric and relatively large: the largest chromosome pair was 13.6 μm and the shortest 8.9 μm long (Fig. 2; Table 2).

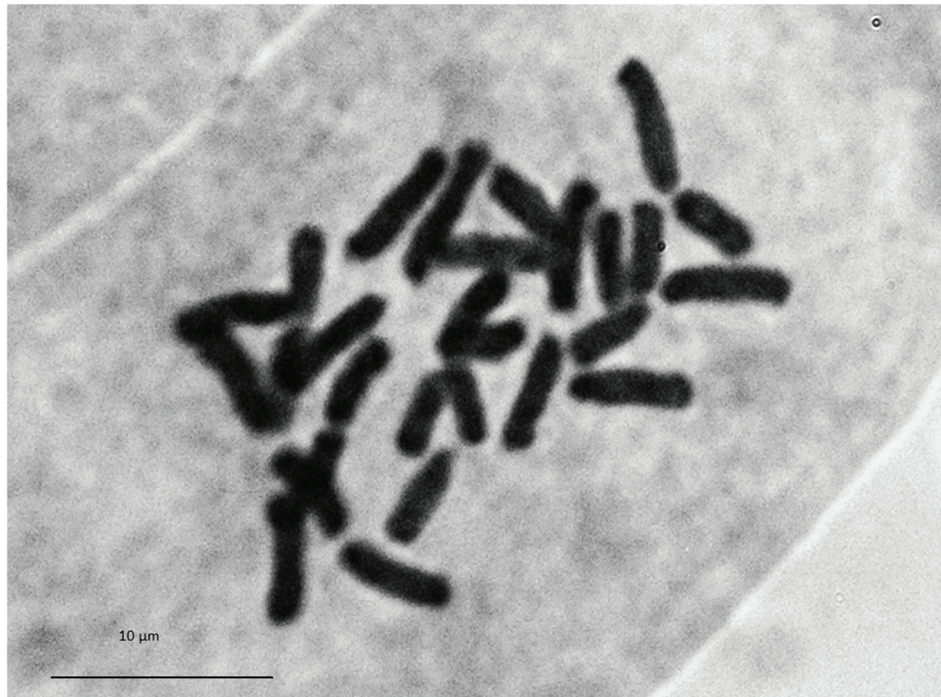


FIGURE 1. Metaphase chromosomes of *Allium akirense*, $2n = 14$.

Sequences Analysis:—Sequences of both diploid and the one tetraploid accessions of *A. akirense* did not show any intraspecific difference in all three DNA fragments studied, but each DNA fragment of *A. akirense* have some different mutations which distinguished it from other species of the section *Molium* (See Alignment in Supplement 1). Since there were no incongruent results in the separate analyses of ITS and ETS nrDNA and *rpl32-trnL* spacer of plastid DNA matrices (results not shown), we combined them in a joint analysis of 14 accessions, including *Allium neapolitanum* as outgroup. The combined data matrix included 1972 characters divided in three partitions (ITS nrDNA, 1–654; ETS nrDNA, 655–1121; *rpl32-trnL*, 1122–1972) of which 1608 were constant, 208 variable parsimony uninformative and 156 were parsimony informative characters. Parsimony and Bayesian analyses yielded the same topology but with lower bootstrap percentages (BP) than posterior probabilities (PP). The best fit model for combined ITS–ETS–*trnL*–*rpl32* data set was K80. The heuristic search found one most-parsimonious tree that was 427 steps long (CI = 0.899, RI = 0.858). The three accessions of *Allium akirense* build a strongly supported clade (100 % in BS and PP) which is clearly separated from *A. qasyunense*, which represents the sister clade (Fig. 3). Next related to *A. akirense* and *A. qasyunense* are *A. papillare* and *A. longisepalum* whereas *Allium negevense* and *A. erdelii* are outside the *A. akirense* clade within Kollmann’s “series *Campanulatae*”.

TABLE 2. Karyo-morphometric parameters for *A. akirense*. Mean values come from 5 good metaphase plates from different individuals of the type locality. Abbreviations: TAL = total absolute length; LA = long arm; SA = short arm; CI = centromeric index; Type = chromosome nomenclature according to Levan *et al.* (1964) and Tsounodakis (1983); TKL = total karyotype length. M_{CA} = mean centromeric asymmetry.

Pair n.	TAL	TRL	LA	SA	CI	Type
I	13.6 ± 0.3	8.5 ± 0.2	7.1 ± 0.4	6.5 ± 0.1	47.8	m
II	12.9 ± 0.4	8.1 ± 0.4	6.9 ± 0.3	6.0 ± 0.3	46.5	m
III	12.1 ± 0.6	7.6 ± 0.3	6.8 ± 0.6	5.3 ± 0.5	43.8	m
IV	11.6 ± 0.5	7.3 ± 0.4	6.2 ± 0.4	5.3 ± 0.3	46.1	m
V	10.8 ± 0.4	6.8 ± 0.2	6.0 ± 0.5	4.8 ± 0.4	44.4	m
VI	9.7 ± 0.6	6.1 ± 0.4	5.3 ± 0.5	4.4 ± 0.5	45.3	m
VII	8.9 ± 1.0	5.6 ± 0.5	4.7 ± 0.8	4.2 ± 0.3	47.2	m
TKL = 159 ± 1.4; M_{CA} : 8.18						

Discussion

The karyotype of diploid plants of *A. akirense* (Fig. 2) is very similar to the karyotypes of other diploid species with $2n = 14$ of section *Molium* and especially to *Allium qasyunense* which was studied by Kollmann (1969, 1970, 1973). One tetraploid plant with $2n = 28$ we found within the Giv'at Brenner population shows no differences in molecular data. It is probably an autotetraploid, although further studies are needed to confirm this hypothesis.

Molecular results clearly point out towards an independent evolution of *A. akirense* for a relatively long period. *Allium akirense* shows also morphological, geographical and ecological differences to all related species. These results indicate certainly a very well established taxon, which we describe here as a new species.

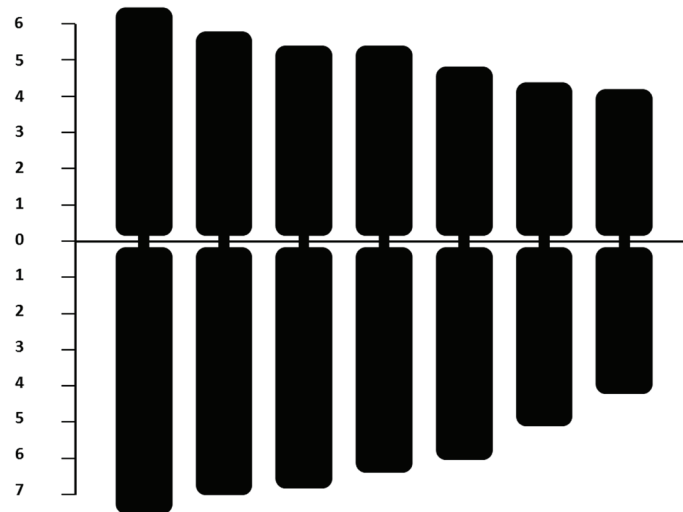


FIGURE 2. Idiogram of *Allium akirense*, $n = 7$.

Description of the new species

Allium akirense N.Friesen & Fragman, *sp. nov.* (Figs 2A–E)

From the closely related *Allium qasyunense* it differs in white-pinkish perigone, smaller only 3–5 mm long flowers, smaller capsules, included (to equal) stamens, and in a completely different habitat.

Type:—ISRAEL. Hill near kibbutz Giv'at-Brenner, N31867130, E34807520, $h = 70$ m, Batha and garigue on calcified sandstone, 2 April 2013. *O. Fragman-Sapir* (holotype, HUJ123409!, isotypes HUI!, OSBU!).

Bulb subglobose, $15\text{--}25 \times 7\text{--}10$ mm, with dusty brown, coriaceous outer and white papery inner tunics. Roots 10–15. Stem 15–40(–50) cm tall, cylindrical, glabrous, erect, sometimes bent on lower part, covered by leaf sheaths for 1/5–1/3 of total length. Leaves (2–)3–4(–5), green (usually dry or almost dry in bloom), linear, flat; leaf blade 12–40 cm long (the lower leaf is the longest), 1–3 mm wide in the lower part, gradually narrowing to the tip; sheath densely-minutely velutinous, blade sparsely-minutely velutinous. Spathe persistent, much shorter than pedicels, with 1 valve and 2–3 acute lobes. Inflorescence lax, many flowered, 40–65 mm across, fastigiate to hemispherical, pedicels subequal, 18–24 mm long. Perigone campanulate, segments white to pinkish, elliptical, rounded at the apex, 3–5 mm long, midrib white or pinkish-green. Stamens included in the perigone or equal to it, with simple filaments, white; anthers yellow, oblong. Style included or slightly exerted. Ovary sub-cylindrical, and slightly narrowed in the middle and at the apex, greenish-yellow, densely roughish above, $4\text{--}4.2 \times 2\text{--}2.2$ mm; style white, 0.4–0.6 mm long. Capsule 2–2.5 mm across, depressed globose, enclosed by the dry perigone.

Additional specimens seen (paratypes):—ISRAEL, hill near kibbutz Giv'at-Brenner, N31°51'56", E034°48'24.7", $h = 69$ m, 21 March 2011. *N. Friesen* (OSBU20932!).

Etymology:—The plant is named *akirense* after the Hebrew Biblical name “Ekron” and the Arabic name “Akir”, both relate to the hills and villages where the species is found.

Distribution:—*Allium akirense* was found in 8 neighbouring sites in the southern Coastal Plain of Israel around Kibbutz Giv'at Brenner (Fig. 2). Since the coastal plain of Israel is densely settled, we cannot know the historical full range of the species. We believe it could have been growing in more sites that are now urban.

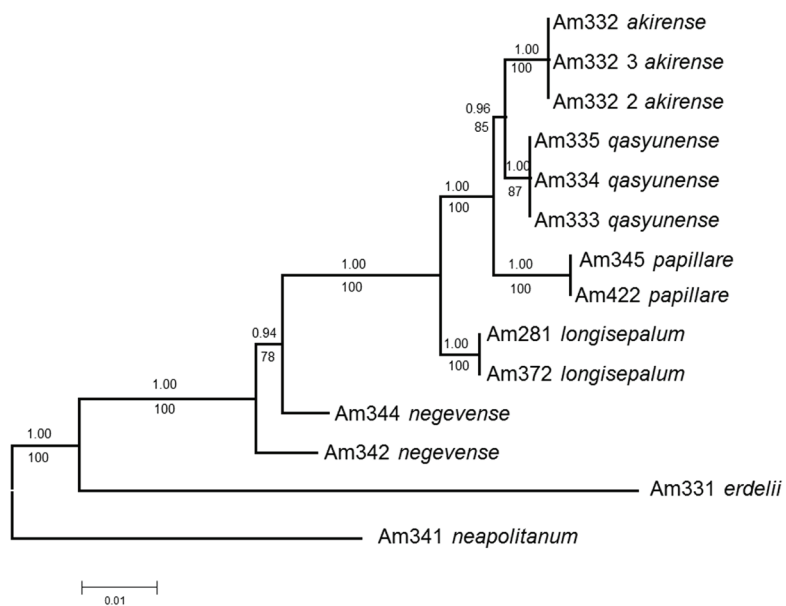


FIGURE 3. Phylogenetic tree based on a combined ITS, ETS and *rpl32-trnL* spacer data set. Bayesian posterior probabilities (PP) are given above branches, bootstrap support (BS) values over 50% from maximum parsimony analysis below branches.



FIGURE 4. Distribution map of *Allium akirense* in Israel (black dots).

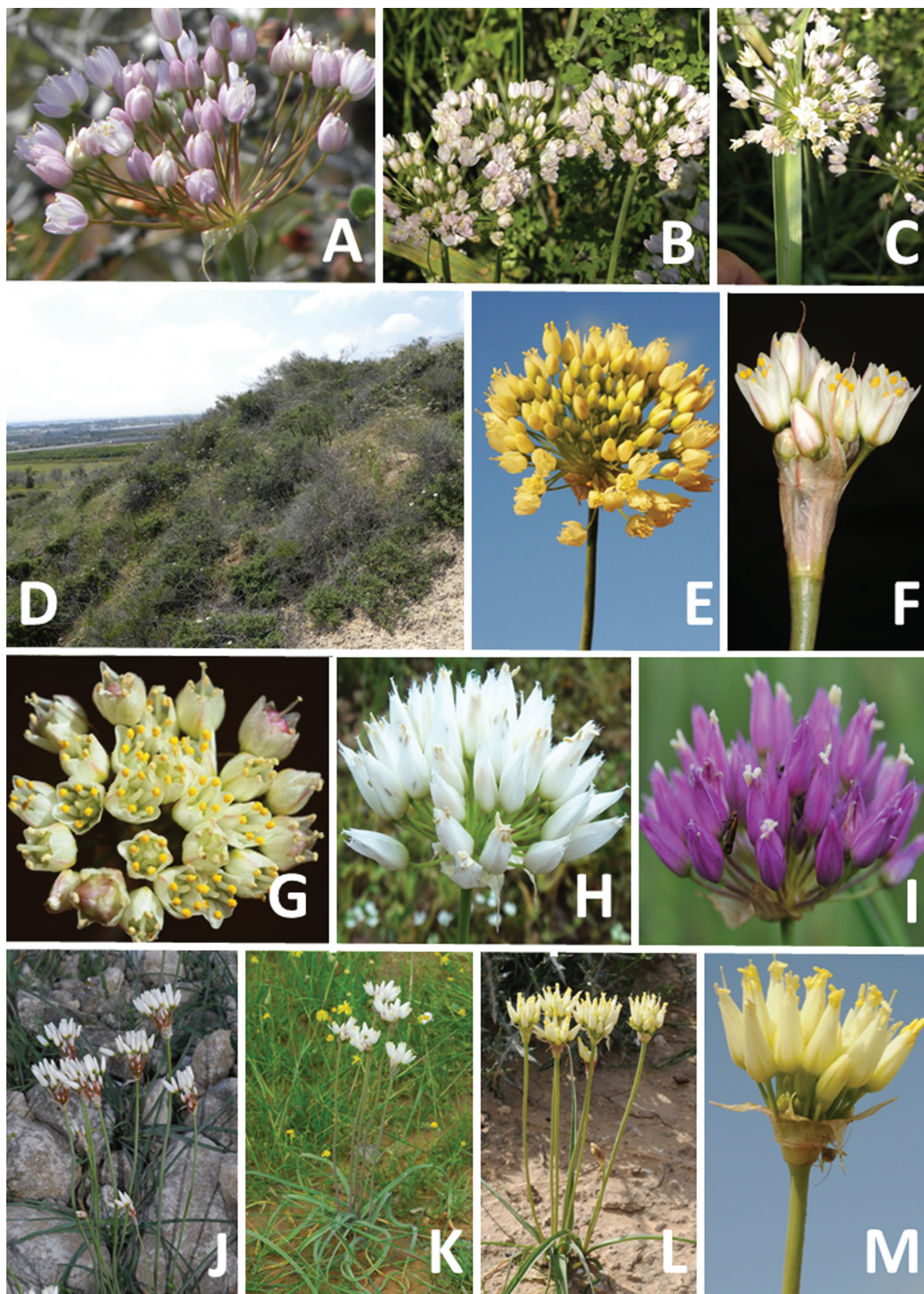


FIGURE 5. Umbels and habitats of the species from the sect. *Molium*. A–C. *Allium akirense* (photos O. Hochberg). D. Sandstone hill near Kibbutz Giv’at-Brenner with *A. akirense* (photo N. Friesen). E. *A. qasyunense* (Western Golan Heights above Ein Gev). F–G. *A. papillare* (Western Negev, F—Sede Yitzhak, G—Halutza, photos O. Fragman-Sapir). H–I. *A. longisepalum* (Iran, H—photo Akhani; I—photo H. Razifard). J–K. *A. negevense* (Negev, K-Rachme Ridge). L–M. *A. erdelii* (North Negev – Goral Hills, photos O. Fragman-Sapir).

Ecology:—*Allium akirense* grows on coastal calcified sandstone, locally known as “kurkar”. The vegetation of the sites is primarily Mediterranean batha and garique (phrygana) dominated by *Thymbra capitata* (Linnaeus 1753: 568) Cavanilles (1803: 37), *Cistus salviifolius* Linnaeus (1753: 524), and *Hyparrhenia hirta* (Linnaeus 1753: 1046) Stapf (1918: 315). *A. akirense* is a good example of an arid taxon that penetrated the Mediterranean Coastal Plain on sandy soils and evolved here into a new narrow endemic. Other desert species or those of arid origin in the coast are *Retama raetam* (Forsskal 1775: 214) Webb & Berthelot (1842: 56), *Salvia lanigera* Poir. in Lamarck (1817: 49), *Asparagus horridus* Linnaeus (1774: 274), and *Allium tel-avivense* Eig in Eig *et al.* (1931: 75).

Conservation:—The rich habitat of *Allium akirense* together with other coastal sandy habitats in the coastal plain were assigned to the list of the most vulnerable areas in Israel, suffering from heavy urban, industrial and agricultural developments (Shmida *et al.* 2011). Specifically, all *A. akirense* sites are under immediate danger, as not even one of these sites is to be found within a nature reserve. Thus, there is a real threat to the survival of the species in nature. In three sites there are hundreds of plants, but in the other five just a few. It is estimated that there is a total of around 3000 plants all together. The species range sums up to less than 3 sq. km. Thus, based on the IUCN criteria (2014), it is proposed to include it in the following category: critically endangered CR B2 b(II, III). Due to the vulnerability of the plant, an ex situ conservation program has begun in the Jerusalem Botanical Gardens, where reproduction under cultivation is being tested. Reproduced plants will be dispersed in several botanical gardens and plant shelter gardens in order to back up the few wild populations.

Identification key to *Allium akirense* and its close relatives

1. Flowers narrow campanulate, sometimes constricted in the upper part.....2
- Flowers broad campanulate, not constricted in the upper part.....4
2. Leaves with long soft hairs, inflorescences narrowly fastigiate; lower arid parts of Mediterranean zone to semi-desert.....
-*A. erdelii*
- Leaves short-haired, inflorescences not narrow3
3. Flowers 10–15 mm long.....4
- Flowers shorter than 7 mm.....5
4. Pedicels 15–25(–30) mm long, flowers rose to pink or pure white..... *A. longisepalum*
- Pedicel 10–15 mm long, flowers white-yellowish; rocky semi-desert highlands.....*A. negevense*
5. Flowers 3–5 mm long, white-pinkish; coastal hills.....*A. akirense*
- Flowers 5–7 mm long cream coloured6
6. Leaf sheaths and part of blades covered by thick edged, backwards pointed tiny hairs, perigone segments blunt with a prominent purplish midvein; desert sands*A. papillare*
- Leaf sheaths and blades covered by erect very short hairs, perigone segments pointed, without a prominent midvein; Med.-desert transition zone to lowland semidesert*A. qasyunense*

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