RESEARCH ARTICLE

Dated phylogeny, phylogeography, and classification of *Allium* subgenus *Amerallium* (Amaryllidaceae) from the Old World, based on six DNA fragments

Nikolai Friesen,[1] **Tobias Herden,**[1,2] **Mathias Leweke,**[1] **Lisa Grützmacher,**[1] **Ori Fragman-Sapir,**[3] **Herbert Hurka,**[1] **Frank R. Blattner**[4] **& Reinhard M. Fritsch**[4]

1 *Botanical Garden, University of Osnabrück, Albrechtstrasse 29, 49076 Osnabrück, Germany*

2 *Ingenieurbüro Prof. Dr. Jörg Oldenburg GmbH, Osterende 68, 21734 Oederquart, Germany*

3 *Jerusalem Botanical Gardens, the Hebrew University, Giv'at Ram, Jerusalem, 9021401, Israel*

4 *Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Corrensstrasse 3, 06466 Seeland OT Gatersleben, Germany*

Address for correspondence: *Nikolai Friesen, nfriesen@uni-osnabrueck.de*

Abstract The present study aims to reveal phylogenetic and phylogeographical patterns of the Eurasian species of *Allium* subg. *Amerallium*, covering 75% of the species in the Mediterranean region and East Asia, as well as 12 North American species representing most sections of the monophyletic American part of the subgenus. We sampled 38 species of *A.* subg. *Amerallium* out of a total of 52 species from the Old World. A molecular phylogeny of *A.* subg. *Amerallium* was constructed based on predominantly non-coding sequences from two nuclear ribosomal RNA regions (ITS, ETS), and four chloroplast (cp) regions (*trnL-trnF, atpB-rbcL, rpl32-trnL, rps16* intron). Most clades are well supported in the analyses of the combined nuclear and chloroplast data. However, analyses of single chloroplast fragments produced different topologies, which can be explained by differences in the variability of the chloroplast regions studied. Analysis of all cpDNA fragments combined produced a similar topology compared to the tree based on nuclear data. Most sections in the Eurasian part of *A.* subg. *Amerallium* are monophyletic. Only the position of *A. chamaemoly* (monotypic *A.* sect. *Chamaeprason*) nested in *A.* sect. *Molium* makes the latter paraphyletic. Different positions of *A. chamaemoly* within nuclear and plastid trees indicate an ancient hybridogenic origin. Monophyly of the American species of *A.* subg. *Amerallium* suggests an origin from extinct North Asian taxa. Currently, only one species from this ancient group, *A. kingdonii*, still exists in the eastern Himalayas.

Keywords biogeography; ETS; ITS; *rps16*; *atpB-rbcL*; *rpl32-trnL*; *trnL-trnF*; phylogeny

Supporting Information may be found online in the Supporting Information section at the end of the article.

■ INTRODUCTION

Allium L. is the largest genus of the petaloid monocotyledons, comprising more than 1018 species (Govaerts & al., 2005–2021). The main centre of diversity for the genus is in the Old World, where species richness is very high in Central Asia through SW Asia to the Mediterranean basin (Fritsch & Friesen, 2002; Khassanov, 2018; Friesen, 2022). The second centre of diversity, with about 100 species, is in the New World, mainly in western North America (Espejo Serna & Lopez-Ferrari, 1992; Nguyen & al., 2008; Wheeler & al., 2013; Mashayekhi & Columbus, 2014). At least 25 *Allium* species are cultivated as edible crops (Hanelt, 2001), including the economically important common onion (*A. cepa* L.), garlic (*A. sativum* L.), Japanese bunching onion (*A. fistulosum* L.), leek, kurrat, great-headed garlic (*A. ampeloprasum* L.) and rakkyo (*A. chinense* G.Don), as

well as species with medical properties and others of horticultural value (Fritsch & Friesen, 2002; Block, 2010; Fritsch, 2015; Friesen, 2022).

Allium is a member of the family Amaryllidaceae J.St.-Hil., subfamily Allioideae Herb., tribe Allieae Dumort. (Chase & al., 2009). Tribe Allieae comprises only *Allium* (including *Caloscordum* Herb., *Milula* Prain, and *Nectaroscordum* Lindl.).

Molecular phylogenies supported strongly the monophyly of *Allium* (Friesen & al., 2006; Nguyen & al., 2008; Li & al., 2010, 2012; Wheeler & al., 2013; Xie & al., 2019, 2020; Friesen, 2022). Within *Allium*, 15 subgenera and 74 sections were recognized by Friesen & al. (2006). In recent years some more *Allium* sections were newly combined and described: *A.* sect. *Longibidentata* (R.M.Fritsch) R.M.Fritsch (Fritsch, 2009: 465), sect. *Contortifolium* Yıld. (in Yıldırımlı & Kılıç, 2016: 20), sect. *Decipientia* (Omelczuk) R.M.Fritsch, sect. *Asteroprason* R.M.Fritsch, sect. *Stellata* (F.O.Khass.

& R.M.Fritsch) R.M.Fritsch, and sect. *Procerallium* R.M. Fritsch (in Fritsch & al., 2010: 168, 184, 187 and 199, respectively), sect. *Unicaulea* F.O.Khass. and sect. *Haneltia* F.O.Khass. (in Khassanov & al., 2011: 174), sect. *Rechingeria* F.O.Khass. & Tirkash. (in Khassanov & al., 2013: 214), sect. *Kingdonia* X.J.He & D.Q.Huang and sect. *Trifurcatum* X.J. He & D.Q.Huang (in Huang & al., 2014: 283 and 284, respectively), sect. *Pseudoscorodon* Brullo & al. (Brullo & al., 2019: 130), sect. *Tulipifolia* R.M.Fritsch & N.Friesen (in Friesen & al., 2021: 217), and sect. *Sulaimanicum* N.Friesen (in Khan & al., 2022: 05). There is also a good section, but unfortunately not properly described (nomen nudum): "*A.* sect. *Flavovirens*" Q.Q.Li & X.J.He (in Li & al., 2010).

About 145 species belong to the *Allium* subg. *Amerallium* Traub, which occur in North America, Europe, North Africa, the Middle East, and South-East Asia. The 45 Old World species and the 98 New World species of *A.* subg. *Amerallium* were divided into six (see Appendix 1) and four sections, respectively (Traub, 1968; Hanelt & al., 1992). The history of the classification of the Mediterranean *Allium* in the 20th century is complicated. Hermann (1939) described and typified 14 new *Allium* groups but without giving them a clear nomenclatural rank. However, Stearn (1946) accepted "*Chamaeprason* F.Herm.", and "*Haemoprason* F.Herm." at sectional rank, and later authors used Hermann's names for nomenclatural combinations, to give them clear and different ranks.

A new era of *Allium* nomenclature started when Traub (1968) introduced the subgeneric level in *Allium* classification by describing *A.* subg. *Amerallium* (in which he included *A.* sect. *Molium* G.Don, with subsect. *Xanthoprason* and subsect. *Chamaeprason*, and sect. *Ophioscorodon* (Wallr.) Endl.), and the rest of the Eurasian *Allium* sections placed in *A.* subg. *Allium* (including *A.* sect. *Microscordum* Maxim. and *A.* sect. *Nectaroscordum* (Lindl.) Gren. & Godr.). Shortly afterwards, Ekberg (1969) described *A.* subg. *Bromatorrhiza*, and Wendelbo (1969) raised *A.* sect. *Rhizirideum* G.Don, sect. *Molium*, and sect. *Melanocrommyum* Webb & Berthel. to subgeneric rank in the context of a regional revision. Kamelin (1973) was the first to apply the subgeneric rank to all the groups in the genus *Allium* in his classification, and since then the subgeneric rank has been widely used in *Allium* classifications.

Kamelin (1973) confirmed some groups of Hermann (1939) at sectional level but placed them in different subgenera: *A.* sect. *Narkissoprason* and sect. *Chamaeprason* in *A.* subg. *Rhizirideum*, and *A.* sect. *Xanthoprason* in *A.* subg. *Amerallium*. In contrast, De Wilde-Duyfjes (1976) simply included *A. chamaemoly* L., the only species in *A.* sect. *Chamaeprason*, in *A.* sect. *Molium* as one among many species. Hanelt & al. (1992) correctly assigned *A.* sect. *Narkissoprason* to *A.* subg. *Amerallium* for the first time and recognized two subsections, *A.* subsect. *Molium* and subsect. *Xanthoprason* within *A.* sect. *Molium*. The separation of *A. ursinum* L. as a monotypic section was never controversial, but there has been some dispute about the priority of the names "*Arctoprason*

Kirschl." or "*Ophioscorodon*" (Pastor & Valdes, 1983; Hanelt & al., 1992; Friesen & al., 2006; Friesen, 2022). There is another *Allium* species from North Africa that probably belongs to *A.* subg. *Amerallium* as well – *A. ruhmerianum* Asch. ex E.A.Durand & Barratte (Durand & Baratte, 1910: 234), an endemic *Allium* species from Cyrenaica. Morphologically *A. ruhmerianum* belongs to the *A.* subg. *Amerallium*, as De Wilde-Duyfjes (1976: 185) wrote: "*A. ruhmerianum* resembles certain small specimens of *A. roseum* L., and possibly its alliance is with this species. *A. roseum* differs essentially, even in small specimens, by a much stouter habit and consequently by, for instance, larger flowers and broader leaves. The spathe in *A. ruhmerianum* is 1- or 2-lobed, as against usually 3- or 4-lobed spathe in *A. roseum*." Brullo (in Bartolo & al., 1984) examined an accession of *A. ruhmerianum* from Cyrenaica in a karyological and anatomical study. He found a very unusual chromosome number of $2n = 33$, and after analysing the chromosomes he suggested that *A. ruhmerianum* is triploid with $n = 11$. The leaf cross section showed only one row of vascular bundles that fits very well within *A.* subg. *Amerallium* (Fritsch, 1988). These and other characters prompted *A. ruhmerianum* to be placed in the monotypic *A.* sect. *Rhynchocarpum* Brullo. In the Gatersleben classification (Hanelt & al., 1992) and in Friesen & al. (2006), *A. ruhmerianum* could not be examined and *A.* sect. *Rhynchocarpum* was placed with a question mark in *A.* subg. *Amerallium*. Unfortunately, we could not study this taxon for this work either, and its position in the classification remains questionable.

Current molecular data underlines the existence of two monophyletic sister alliances: a rather diverse Old World group and a derived New World group (Friesen & al., 2006; Li & al., 2010).

Allium subg. *Amerallium* with *A.* subg. *Nectaroscordum* and subg. *Microscordum* forms one phylogenetic clade and belongs to the first evolutionary lineage in the genus *Allium* (Fritsch & Friesen, 2002). This clade is sister to the rest of the genus (Dubouzet & Shinoda, 1999; Fritsch & Friesen, 2002; Friesen & al., 2006; Nguyen & al., 2008; Li & al., 2010; Wheeler & al., 2013; Friesen, 2022).

Allium subg. *Amerallium* is characterized by leaves with a single row of vascular bundles (Traub, 1968; Mashayekhi & Columbus, 2014) and subepidermal laticifers (Fritsch, 1988).

The Amerallium taxa are karyologically very diverse, with the dominating basic chromosome number of $x = 7$, which strongly supports their separate status in the genus *Allium*. This basic chromosome number is only predominant in the New World Amerallium taxa. In the Old World Amerallium taxa, besides $x = 7$, $x = 8$, 9, 10, and 11 also occur in several morphologically derived groups (Traub, 1968; Kollmann, 1969, 1970; Badr & Elkington, 1977; Pastor, 1982; Bartolo & al., 1984; Speta, 1989; Huang & al., 1995; Xu & al., 1998).

Li & al. (2012) gave a detailed review of the evolution of the base chromosome numbers in *Allium* and supposed that it was originally $x = 7$. Costa & al. (2020) came to a different result and regarded $n = 8$ as the archetype base chromosome number in *Allium*.

Molecular phylogenies clearly showed that the monophyletic New World Amerallium taxa evolved from a common Old-World ancestor (Hanelt & al., 1992; Dubouzet & Shinoda, 1999; Friesen & al., 2006; Nguyen & al., 2008; Li & al., 2010; Wheeler & al., 2013; Xie & al., 2020) and diversified on the way across the North American continent. Since all North American species of *Allium* subg. *Amerallium* have $x = 7$, we conclude that all New World species evolved from a common ancestor with a base chromosome number of $x = 7$ in the Mediterranean region or in eastern Asia. Hanelt & al. (1992) postulated an Asian origin and dispersal via the Bering land bridge. A recent biogeographic analysis also suggests that the common ancestor of the North American species originated from high latitudes in eastern Asia and dispersed to the New World via the Bering land bridge (Li & al., 2010, 2012). Wheeler & al. (2013), however, did not rule out a Mediterranean origin for this subgenus. Phylogenetic analyses based on ITS (Dubouzet & Shinoda, 1999; Friesen & al., 2006; Nguyen & al., 2008; Li & al., 2010) revealed no preferences for an East Asian or Mediterranean origin. Phylogenetic analyses based on chloroplast (cp) DNA sequences including representatives of Mediterranean, East Asian, and American Amerallium species have not yet been carried out.

Of today's East Asian representatives of *Allium* subg. *Amerallium*, only two species with $x = 7$ are possible common ancestors: *A. wallichii* Kunth (Kunth, 1843: 443) and *A. macranthum* Baker (Baker, 1874: 293). They belong to *A.* sect. *Bromatorrhiza*, but all species of this section have fleshy roots as a storage organ and do not develop bulbs (Li & al., 2012). This character is unique for Tibet, the Himalayas, and adjacent regions and is absent in the New World *Allium* species. Morphologically, the Mediterranean *Allium* species are more closely related to American species. To find the answer to this mystery, at least one species must be found among the Eurasian *Allium* taxa that could represent the common ancestor lineage of the Eurasian and American taxa. Huang & al. (2014) determined *A. kingdonii* Stearn (Stearn, 1960: 175) from *A.* sect. *Kingdonia* as such a species.

The present study aims to reveal phylogenetic and phylogeographical patterns within *Allium* subg. *Amerallium* species. A special focus is on the origin of the New World taxa.

■ MATERIALS AND METHODS

Taxon sampling. — We were able to study most of the species from the Mediterranean region and East Asia and to include 12 North American species covering most alliances of the monophyletic American part of *Allium* subg. *Amerallium*. Eurasian representatives of *A.* subg. *Amerallium* have very different shapes and colours of flowers (Figs. 1, 2). Plant material for DNA extraction was obtained from the *Allium* collections of the Botanical Garden of the University of Osnabrück, from Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, and different herbaria

(B, FR, GAT, HUJ, LE, MW, ORT, OSBU, TFC, W). Although some DNA samples from old or poorly dried herbarium specimens were highly degenerated, useable sequences of 36 species (from a total of 47, see suppl. Table S1), covering all taxonomic groups in the Old Word taxa, were obtained. Many species are represented by several accessions. Twelve New World species of *A.* subg. *Amerallium* were selected, representing all taxonomic groups following Nguyen & al. (2008) and Wheeler & al. (2013) (see Appendix 1).

Total genomic DNA was extracted using the "Innu-PREPP Plant DNA Kit" (Analytic Jena, Jena, Germany) according to the manufacturer's instructions and was used directly in PCR amplifications.

Karyological analysis. — Chromosomes were analysed in only two species that have strongly divergent chromosome numbers within *Allium* subg. *Amerallium*: *A. chamaemoly* ($2n = 22$) and *A. negevense* Kollmann ($2n = 20$), and for one species only the chromosome number was determined. Bulbs were planted in pots, and growing roots were used for the karyotype analysis. Root tips were excised from the bulbs and kept overnight in distilled water on ice. They were then transferred to room temperature for 20 minutes, pre-treated for three hours at room temperature in an aqueous solution of 0.1% colchicine, and were then fixed in a freshly prepared mixture of 96% ethanol and glacial acetic acid (3:1 v/v). Root tips were stained using hematoxylin according to the protocol reported by Smirnov (1968). Well-spread metaphase plates were digitally photographed, and finally, the chromosomes of the best plates were measured and pairwise arranged using the KaryoType software (Altinordu & al., 2016). For *A. chamaemoly* nine metaphase plates from one individual (Am543, see Appendix 1) and for *A. negevense* eight metaphase plates from two bulbs were analysed (Am942). Because the idiograms automatically assembled by the software were not satisfactory, we manually ordered the chromosome pairs according to their length and shape. The idiograms were designed using the bar graph function implemented in MS Excel. The terminology of Levan & al. (1964) was applied.

DNA sequencing. — We sequenced the external transcribed spacer (ETS) and internal transcribed spacer (ITS) from ribosomal nuclear DNA of most samples. Additionally, the plastid *trnL-trnF*, *atpB-rbcL*, *trnL-rpl32* intergenic spacer (IGS), and *rps16* intron regions were sequenced. GenBank accession numbers of the sequenced regions are shown in Appendix 1.

For most samples, the nrDNA ITS region (ITS1, 5.8S, ITS2) was amplified using primers ITS-A and ITS-B (Blattner, 1999). ITS1 and ITS2 were amplified separately when DNAs from old herbarium sheets were used. In these cases, the primers ITS-E and ITS-C (Blattner, 1999) together with primers ITS-A and ITS-B were used. The PCR conditions were identical to those described in Friesen & al. (2006).

The ETS region was amplified using the primers 18S-IGS (Baldwin & Markos, 1998) and ETS-all-f (Nguyen & al., 2008). The PCR conditions were identical to those described in Herden & al. (2012).

Fig. 1. Representative species of *Allium* subg. *Amerallium*: **A**, *Allium wallichii*; **B**, *A. hookeri* Am196; **C**, *A. fasciculatum* (Nepal); **D**, *A. narcissiflorum* Am79; **E**, *A. insubricum* Am289; **F**, *A. ursinum* Am322; **G**, *A. triquetrum* Am244; **H**, *A. pendulinum* (Corsica); **I**, *A. paradoxum* Am251; **J**, *A. scorzonerifolium* Am225; **K**, *A. moly* Am238; **L**, *A. chamaemoly* Am126. — Photos: A, G. Miehe; B, D–G & I–L, N. Friesen; C, Sherpaworld (*Allium fasciculatum*, image cropped, CC BY-SA 3.0); H, Tigerente (*Allium pendulinum*, image cropped, CC BY-SA 3.0). Origin of Am accessions, see Appendix 1.
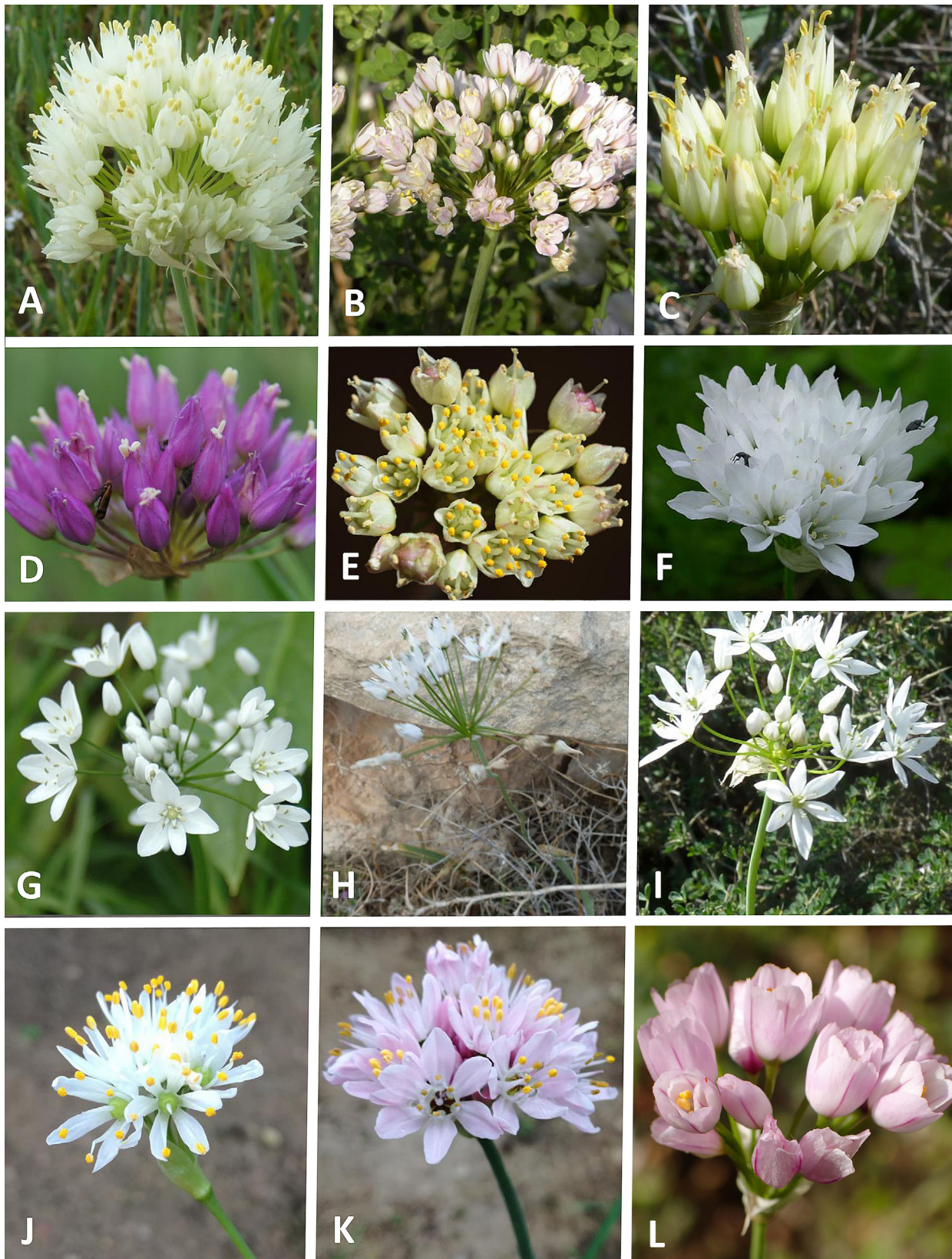
Fig. 2. Representative species of *Allium* sect. *Molium*: **A**, *A. qasyunense* Am334; **B**, *A. akirense* Am322; **C**, *A. erdelii* Am331; **D**, *A. longisepalum* (Iran); **E**, *A. papillare* (Negev, Halutsa); **F**, *A. candolleanum*; **G**, *A. neapolitanum* Am373; **H**, *A. palaestinum* Am337; **I**, *A. subhirsutum* Am283; **J**, *A. subvillosum* Am430; **K**, *A. canariense* (Lanzarote); **L**, *A. roseum*. — Photos: A–C & G–J, N. Friesen; D, H. Akhani; E, O. Fragman-Sapir; F, Pirogov (2009); K, K.G. Bernhardt; L, Pankova (2018). Origin of Am accessions, see Appendix 1.

Primers for the chloroplast regions were as follows: for the *rpl32-trnL* region as described in Shaw & al. (2007), for the *rps16* intron as described in Oxelman & al. (1997), for the *trnL-trnF* IGS as described in Taberlet & al. (1991), and for *atpB-rbcL* as described in Klaas & Friesen (2002). PCR products were sent to Microsynth SeqLab (https://srvweb.microsynth.ch/home/seqlab) for sequencing. Sequences from every individual were manually edited in CHROMAS Lite v.2.1 (Technesylum, https://chromas-lite.software.informer.com/2.1/). The sequences of all samples were aligned with CLUSTAL X2 (Thompson & al., 1997) and corrected manually in MEGA 7 (Kumar & al., 2016) where necessary.

Phylogenetic analyses. — *Nothoscordum bivalve* (L.) Britton and *Tulbaghia violacea* Harv. from tribes Leucocoryneae Ravenna emend. Sassone & al. and Tulbaghieae Endl. ex Meisn. (subfamily Allioideae) were chosen as the outgroup. *Allium siculum* L., *A. tripedale* Trautv. (*A.* subg. *Nectaroscordum*) and *A. monanthum* Maxim. (*A.* subg. *Microscordum*) were also included in the analysis to complete the first evolutionary lineage as recognized by Friesen & al. (2006). Parsimony analysis was performed with PAUP* v.4.0b10 (Swofford, 2002) using heuristic searches with tree bisection-reconnection (TBR) branch swapping and 100 random addition sequence replicates without tree limit. Bootstrap support (BS; Felsenstein, 1985) was estimated with 1000 bootstrap replicates, each with 100 random addition sequence searches. Bayesian analyses were conducted with MrBayes v.3.1.23 (Ronquist & Huelsenbeck, 2003). The sequence evolution model was chosen following the Akaike information criterion (AIC) obtained from jModelTest2 v.2.1.6 (Darriba & al., 2012). Two independent analyses with four Markov chains were run for 10 million generations, sampling trees every 100 generations. The first 25% of trees were discarded as burn-in. The remaining trees were combined into a single dataset, and a majority-rule consensus tree was obtained along with posterior probabilities (PP).

Divergence time estimation. — BEAST v.1.8 (Drummond & Rambaut, 2007) was used to estimate the divergence times in *Allium* subg. *Amerallium*. The BEAUti v.1.8 interface was used to create input files for BEAST and, where necessary, the XML files were manually adjusted.

For the estimation, a reduced subset of 55 accessions was selected from the ITS sequences. We used the uncorrelated lognormal relaxed clock (ucld) with the substitution model selected by the AIC (GTR + I + Γ). The Yule process was chosen as the speciation process. The ucld.mean for the ITS data was set to a normal distribution, with a mean of 4.13×10^{-9} substitutions per site per year (sub/site/yr) and a standard deviation (SD) of 1.0×10^{-9} according to Kay & al. (2006). Huang & al. (2012) stated a mean rate of 2.5×10^{-9} sub/site/yr (1.56×10^{-9} to 3.44×10^{-9} sub/site/yr). However, we decided against the usage of this rate for the same reasons as Friesen & al. (2015) did. The user-specified starting trees were inserted into the XML files in Newick format. Other parameters were set to default. First, several short BEAST runs were performed to examine the performance of the Markov chain Monte Carlo (MCMC). Additional runs were carried out with empty alignments to ensure that the priors alone did not determine the results. Finally, three independent BEAST runs were performed for every substitution rate setting with the MCMC chain length of 10^8 generations and a sampling frequency of every 100 generations.

The effective sample size (ESS) values were >200 with a 25% burn-in for all parameters as confirmed by analysing the output files with Tracer v.1.6. The tree output files from BEAST were summarised with LogCombiner v.1.8 and annotated with TreeAnnotator v.1.8. The mean node heights option was selected, and the posterior probability set to 0.5. The trees were visualised using FigTree v.1.4.0 with mean ages and 95% highest posterior density (HPD).

Ancestral range reconstruction. — The time divergence tree from BEAST analysis was used for ancestral range reconstruction. The analysis was carried out using the RASP4 v.4.4 ancestral state reconstruction tool (Yu & al., 2015, 2020). DEC, DIVALIKE and BAYAREALIKE biogeographic models were tested with and without corresponding jumping parameter (+J) using the BioGeoBEARS v.1.1.1 algorithm implemented in RASP through R (Matzke, 2013a,b, 2014). Finally, S-DIVA (Statistical Dispersal-Vicariance Analysis), BioGeoBEARS, and Bayesian Binary MCMC (BBM) were used for analysis.

Seven geographic entities were defined following the distribution patterns of the *Allium* species from the first evolutionary lineage: Eastern Mediterranean (A), western Mediterranean (B), Central, East and North Europe (C), South-East Asia (D), western North America (E), eastern North America (F), Far-East Asia (G). The maximum number of areas in which a species can coexist was set to three, following the widely distributed *A. ursinum* that can be found in three (A, B, C) out of the seven areas specified above.

■ RESULTS

In total, 759 new sequences (ITS, 136; ETS, 136; *rpl32-trnL*, 124; *rps16*, 124; *trnL-trnF*, 124; *atpB-rbcL*, 115) were generated and deposited in GenBank (accession numbers are given in Appendix 1).

nrETS. — Among the 127 accessions from *Allium* subg. *Amerallium* the length of the ETS region ranged from 453 base pairs (bp) in *A. scorzonerifolium* Desf. ex DC. and *A. subvillosum* Salzm. ex Schult. & Schult.f. to 470 bp in the American species *A. fimbriatum* S.Watson, *A. elmendorfii* M.E.Jones ex Ownbey and *A. validum* S.Watson. The alignment length was 498 bp (suppl. Appendix S1). Of these, 127 characters were constant, 38 variable characters were parsimony uninformative, and 333 were parsimony informative. The substitution model HKY + Γ was chosen by the AIC in jModelTest2 v.2.1.6 (Darriba & al., 2012) for the Bayesian analysis. Unweighted parsimony analysis resulted in 183,432 most parsimonious trees of 1112 steps (consistency index CI = 0.56;
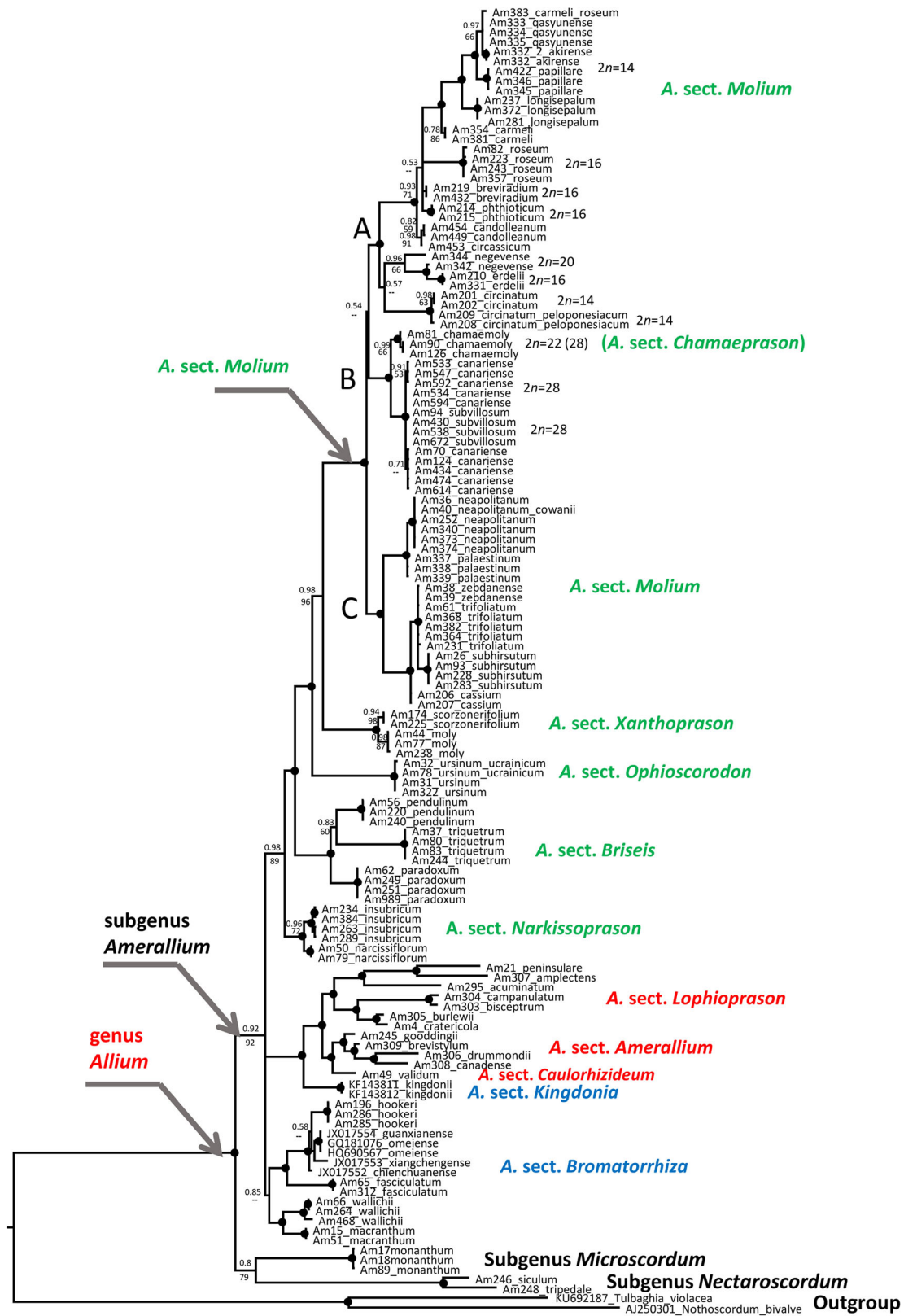
Fig. 3. Phylogenetic nrITS tree of the first evolutionary lineage of genus *Allium*. Numbers at nodes represent Bayesian posterior probabilities and bootstrap support (1000 replicates). The joint presence of Bayesian probabilities over 0.98 and bootstrap support over 95% is indicated with a black dot. For the origin of Am accessions, see Appendix 1. Sequences obtained from GenBank are given with GenBank accession number. Section names in green colour, Mediterranean distribution; blue, Asian distribution; red, North American distribution. A, B and C denote clades in *A.* sect. *Molium*.

retention index RI = 0.93). The topology of the nrETS parsimony tree (suppl. Fig. S1B) is similar to the nrITS tree (Fig. 3), but the Bayesian tree (suppl. Fig. S1A) shows a small difference in the basal part. American species of *A.* subg. *Amerallium*, although with very weak support (PP = 0.58), form the basal group, and the clade with representatives of *A.* subg. *Nectaroscordum* and subg. *Microscordum* are in a clade with *A.* sect. *Bromatorrhiza* and Mediterranean Amerallium taxa (suppl. Fig. S1A,B).

ITS. — Among the 132 *Allium* species from the first evolutionary lineage, the length of the ITS region ranged from 628 bp in *A. cassium* Boiss., *A. trifoliatum* Cirillo, *A. zebdanense* Boiss. & Noe und *A. subhirsutum* L. to 661 bp in *A. triquetrum* L., though most sequences were longer than 640 bp, which is slightly longer than the ITS sequences of *Allium* species in the second and third evolutionary lineages (Friesen & al., 2006). The alignment length was 729 bp (suppl. Appendix S2). Of these, 254 characters were constant, 55 variable characters were parsimony uninformative, and 420 were parsimony informative. The substitution model HKY + Γ was chosen by the AIC in jModelTest2 v.2.1.6 for the Bayesian analysis. Unweighted parsimony analysis resulted in 1,083,432 most parsimonious trees of 1656 steps (CI = 0.57; RI = 0.88).

Since we did not obtain the ETS sequences from all *Allium* species from which we have ITS sequences, especially not for the important species *A. kingdonii*, we analysed in detail the nrITS tree. The topology of the first evolutionary lineage of the genus *Allium* is similar to that published earlier in Friesen & al. (2006) with some differences in *A.* subg. *Amerallium*, as many more species were analysed (Fig. 3).

Members of *Allium* subg. *Microscordum* and subg. *Nectaroscordum* form a basally branching sister clade to *A.* subg. *Amerallium*. In *A.* subg. *Amerallium* we found three clades: (1) the Asian *A.* sect. *Bromatorrhiza* with weak support (PP = 0.85, BS –); (2) a very well-supported clade including all American *Allium* species of *A.* subg. *Amerallium* together with the Asian *A.* sect. *Kingdonia* (PP = 1.0, BS = 100); (3) all Eurasian-North African species from the Mediterranean area (PP = 0.98, BS = 89).

Most Mediterranean sections in *Allium* subg. *Amerallium* (i.e., sect. *Narkissoprason* (F.Herm.) Kamelin, sect. *Ophioscorodon*, sect. *Briseis* (Salisb.) Stearn, and sect. *Xanthoprason* (F.Herm.) Kamelin) form well-supported monophyletic clades. All species of *A.* sect. *Molium* and *A. chamaemoly* (sect. *Chamaeprason* (F.Herm.) Traub) form a strongly supported monophyletic clade, making *A.* sect. *Molium* paraphyletic. Within this clade are three distinct groups: Group A – *A. qasyunense* Mouterde, *A. akirense* N.Friesen & Fragman, *A. papillare* Boiss., *A. longisepalum* Bertol., *A. carmeli* Boiss., *A. roseum*, *A. breviradium* (Halácsy) Stearn, *A. phthioticum* Boiss. & Heldr., *A. candolleanum* Albov, *A. circassicum* Kolak., *A. negevense* Kollmann, *A. erdelii* Zucc. and *A. circinnatum* Sieber. Accessions from all these species form well-supported monophyletic clades, with the exception of accession Am383 of *A. carmeli* with pink

flowers, which is placed in a clade together with *A. qasyunense*. Group B – *A. chamaemoly* forms a strongly supported clade along with *A. subvillosum* and *A. canariense* (Regel) N.Friesen & P.Schönfelder. Group C consists of *A. neapolitanum* Cirillo, *A. palaestinum* Kollmann ex Fragm. & N.Friesen, *A. zebdanense*, *A. trifoliatum*, *A. subhirsutum*, and *A. cassium*, in which only *A. zebdanense* and *A. trifoliatum* are unresolved.

Plastid DNA sequence data. — The combined plastid data matrix of accessions of *Allium* subg. *Amerallium* and the outgroup included 3387 characters, divided into four partitions: 1–1180 *rpl32-trnL* IGS, 1181–1632 *trnL-trnF* IGS, 1633–2639 *rps16* intron, and 2640–3387 *atpB-rbcL* IGS (suppl. Appendix S3), of which 2360 were constant, 146 variable characters were parsimony uninformative, and 881 parsimony informative. The best-fit model for the combined cpDNA data selected by the AIC in jModelTest2 v.2.1.6 (Darriba & al., 2012) was GTR + Γ. The heuristic search found one most parsimonious tree that was 1818 steps long (CI = 0.69, RI = 0.67).

Individual trees of single plastid fragments show relatively strong differences in topology (suppl. Figs. S2–S5). Only the tree derived from the combined alignment of all four plastid fragments (Fig. 4) shows similar groupings of all taxa of *Allium* subg. *Amerallium* as in the ITS tree (Fig. 3).

Most Eurasian sections in *Allium* subg. *Amerallium* also form well-supported monophyletic clades in the plastid phylogeny, again with the exception of *A.* sect. *Molium*. All species of this section and *A. chamaemoly* (sect. *Chamaeprason*) form a very strongly supported monophyletic clade, which is divided into three clades (A, B and C). These groups are also present in the trees obtained from the separate analyses of the plastid regions, albeit in slightly different constellations. Within the relatively well-supported clade C (PP = 0.99, BS = 74), accessions of *A. chamaemoly* form a very strongly supported subclade (C2). The subclade is sister to the subclade C3 with *A. zebdanense*, *A. trifoliatum*, *A. subhirsutum* and *A. cassium*. Both subclades together are sister to subclade C1 with *A. circinnatum* and *A. erdelii*. The positions of *A. neapolitanum* and *A. palaestinum* also differ in the nuclear and plastid trees. In the ITS tree, these species are placed in clade C with *A. cassium*, *A. zebdanense*, *A. trifoliatum* and *A. subhirsutum*, and in the plastid tree they are a sister group to *A. canariense* and *A. subvillosum* in clade A.

Divergence time estimation. — Divergence time estimation analyses of the first evolutionary lineage in the genus *Allium* (Fig. 5) generated a highly congruent topology compared to the Bayesian inference analysis (Fig. 3). According to the analysed taxon sets, *A.* subg. *Amerallium* diversified into two sister groups (A, Eurasian taxa; B, North American taxa including *A. kingdonii*) around 39.54 million years ago (mya), in the middle Eocene. The separation of the East Asian *A.* sect. *Bromatorrhiza* from the Mediterranean species of *A.* subg. *Amerallium* is calculated at 36.49 mya. The separation of the American clade from East Asian *A. kingdonii* was
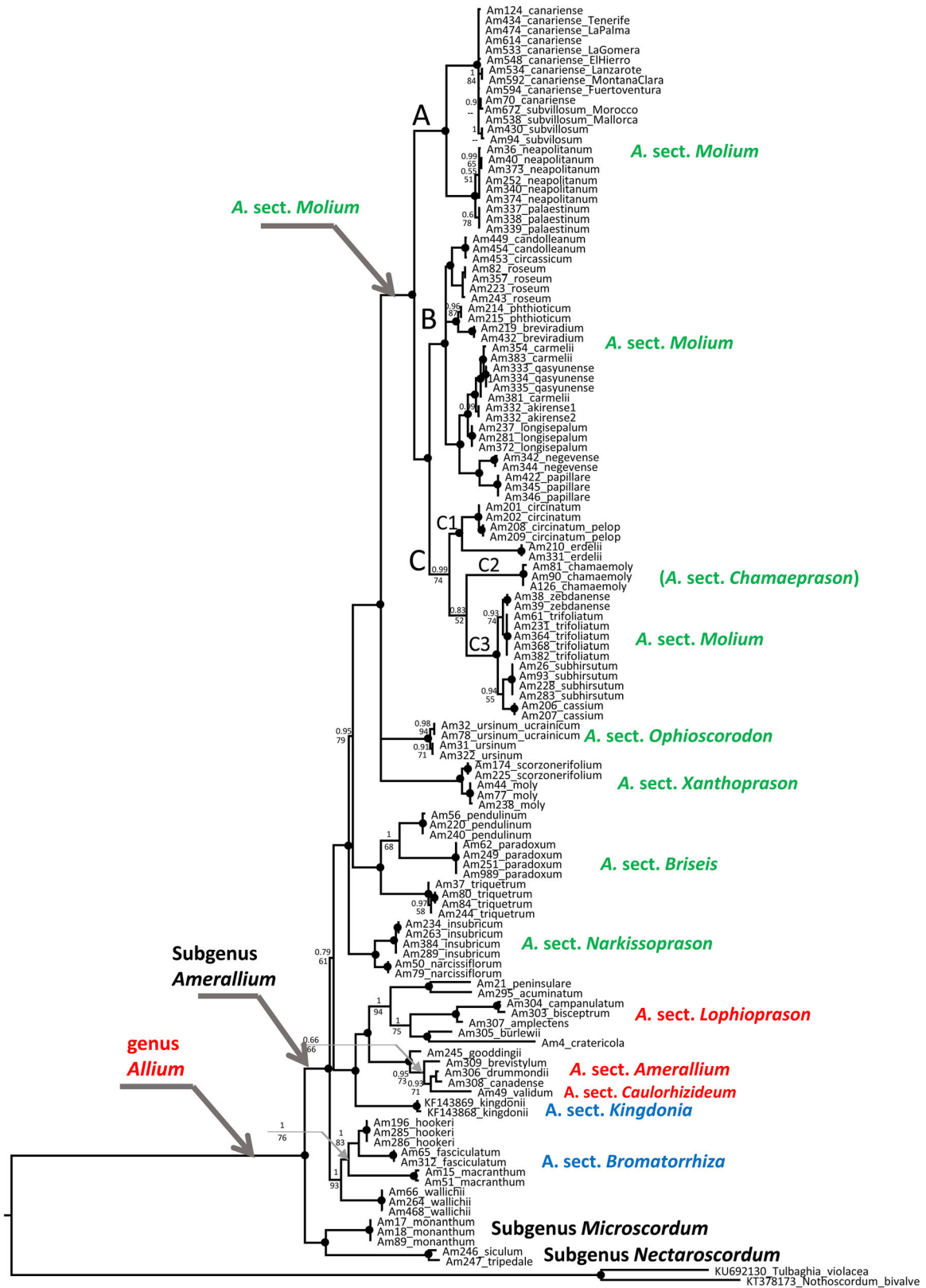
Fig. 4. Phylogenetic tree based on combined plastid fragments of the first evolutionary lineage of genus *Allium*. Numbers at nodes represent Bayesian posterior probabilities and bootstrap support (1000 replicates). The joint presence of Bayesian probabilities over 0.98 and bootstrap support over 95% is indicated with a black dot. For origin of Am accessions, see Appendix 1. Sequences from GenBank are given with GenBank accession number. Section names in green colour, Mediterranean distribution; blue, Asian distribution; red, North American distribution. A, B and C denote clades in *A.* sect. *Molium*.
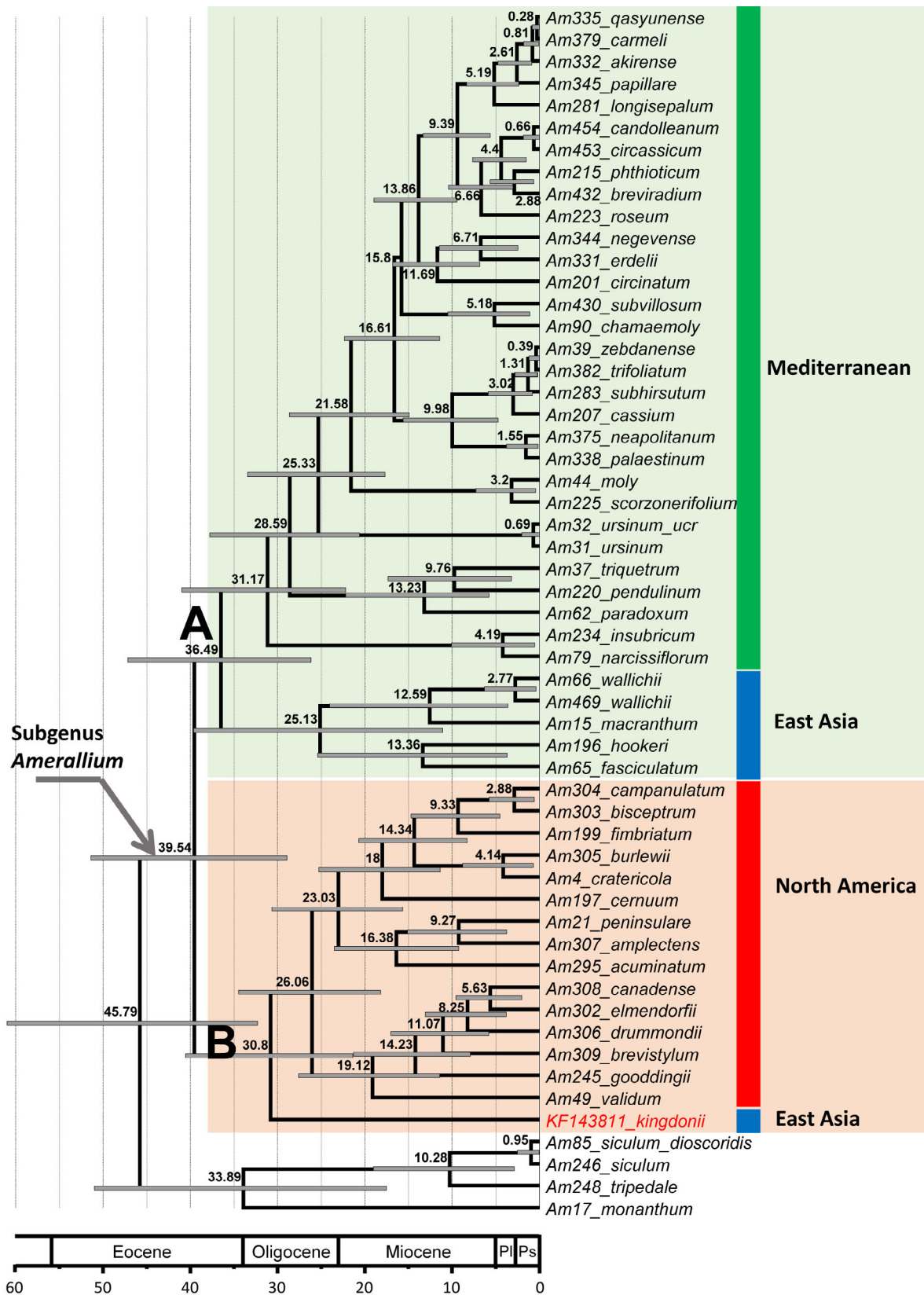
Fig. 5. Dated phylogeny based on ITS sequences of the first evolutionary lineage of the genus *Allium*. The median rate is given in units of substitutions per million years (including 95% confidence intervals) and the numbers at the nodes represent the absolute age in million years. A, Eurasian clade; B, North American clade, including *A. kingdonii* (highlighted). Pl, Pliocene; Ps, Pleistocene. The colour of the vertical bars shows the distribution pattern of clades.

calculated at 30.8 mya. Within the Mediterranean species, *A*. sect. *Narkissoprason* is basally branching (31.17 mya), followed by *A*. sect. *Briseis* (28.59 mya), sect. *Ophioscorodon* (25.33 mya), sect. *Xanthoprason* (21.58 mya), and lastly by sect. *Molium* including sect. *Chamaeprason* (16.61 mya).

Ancestral range reconstruction. — The result of the model test in BioGeoBEARS shows DIVALIKE and DIVA-LIKE+J as the most optimal model according to the AIC algorithm. The analysis using RASP4 v.4.4 ancestral state reconstruction tool with biogeographic models S-DIVA, DEC(+J), DIVALIKE(+J), BAYAREALIKE(+J) and Bayesian Binary MCMC have brought very similar results because the distribution patterns of the *Allium* species from the first evolutionary lineage are very clear and not overlapping (Fig. 6).

Karyology. — In this work we only examined the chromosomes of three species for which a deviating chromosome number was given in the literature or the chromosome number was unknown: *Allium chamaemoly* with $2n = 22$ and 28 for Spain (Pastor, 1982) and *A. negevense* with $2n = 20$. Our analysis confirms the chromosome numbers $2n = 22$ for *A. chamaemoly* (Fig. 7) and $2n = 20$ for *A. negevense* (Fig. 8). For *A. narcissiflorum* (accession Am79) only the number of chromosomes $2n = 14$ was counted.

■ DISCUSSION

With our work, we want to explain the phylogeny and biogeography of species of the three isolated distribution areas of *Allium* subg. *Amerallium*, which together with *A*. subg. *Nectaroscordum* (eastern Mediterranean, 2 species) and subg. *Microscordum* (Far-East Asia, 1 species) forms the first evolutionary lineage in *Allium* (Fritsch & Friesen, 2002).

The topologies of the ITS and ETS trees are similar, in contrast to the topologies of the individual trees based on the chloroplast fragments. Despite the differences in the individual trees of the chloroplast fragments (suppl. Figs. S2–S5), the tree based on the combined cpDNA fragments (Fig. 4.) shows a similar, albeit not identical, topology to the ITS tree (Fig. 3). This shows that phylogenetic studies in the genus *Allium* and in other taxa based on only one chloroplast fragment can lead to false/ambiguous phylogenetic conclusions.

Asiatic origin of the *Allium* subg. *Amerallium*. — The nuclear markers clearly show two monophyletic clades (North American and Eurasian) as sister groups. Within the Eurasian group, there are two monophyletic sister clades: Mediterranean and East Asian, with no inclination for relationships to the North American clade. The plastid markers show only one monophyletic clade, namely Mediterranean, whereas North American and East Asian taxa are left unresolved. One species of *Allium* subg. *Amerallium* from the Eastern Himalayas (*A. kingdonii*) is clearly placed in the North American clade in both analyses. This data supports the hypothesis that the New World species of *A*. subg. *Amerallium* have a common ancestor in East Asia. The separation of the

New World (including *A. kingdonii*) and Old World clades of *A*. subg. *Amerallium* occurred in the Late Eocene, and the separation of *A. kingdonii* ancestors and the American species of *A*. subg. *Amerallium* occurred in the Early Oligocene (Fig. 5). The Eurasian Late Eocene–Early Oligocene was characterized by significant tectonic activity, mainly linked to the collision of India and Asia, resulting in large-scale paleogeographic changes. The formation of an isolated Paratethys Sea started during the Eocene/Oligocene transition, and the closure of marine seaways culminated during the Early Oligocene. Continentalisation of Europe increased when the Turgai Strait closed and the Bering Bridge opened (Erdei & al., 2012; Hurka & al., 2019). At the same time the so-called "Eocene-Oligocene extinction event", also called the Eocene-Oligocene transition (EOT), an extinction event and faunal turnover, took place between 33.9 and 33.4 mya (Ivany & al., 2000; Lear & al., 2008).

A map of the Earth in the Eocene helps to understand the distribution and diversification of *Allium* species in the late Eocene (Fig. 9). We assume that the ancestor of *Allium* came to Eurasia via the Indian subcontinent, because the closest relatives of *Allium* are *Tulbaghia* from South Africa and the tribes Leucocoryneae and Gilliesieae from South America (Costa & al., 2020; Friesen, 2022). At that time, there were only two routes: a narrow land route to the west (into what is now the East Mediterranean) and a route to northeastern Asia.

All Mediterranean species are monophyletic and form the sister group to the Asian clade. Their separation occurred in the later Eocene or earlier Oligocene (Fig. 5). It is very interesting that the other two subgenera in the first evolutionary lineage have the same distribution pattern and a similar time of separation: *Allium monanthum* ($n = 8$, *A*. subg. *Microscordum*) is distributed only in East Asia (Korea, NE China, Far East Russia, Japan) and species from *A*. subg. *Nectaroscordum* ($n = 9$) are distributed only in the East Mediterranean (Friesen, 2022).

The two other American *Allium* species, *A. schoenoprasum* L. (*A*. subg. *Cepa* (Mill.) Radic, from the third evolutionary lineage) and *A. tricoccum* Aiton (*A*. subg. *Anguinum* (G.Don ex Koch) N.Friesen, from the second evolutionary lineage) also reached America via the Beringian Bridge (Friesen & Blattner, 2000; Herden & al., 2016).

The East Asian taxa of *Allium* subg. *Amerallium*. — The East Asian group of species from *Allium* subg. *Amerallium* consists of two sections: *A*. sect. *Bromatorrhiza* with eight species and two subspecies and *A*. sect. *Kingdonia* with only one species, *A. kingdonii*. One of the ancestors of *A. kingdonii* was distributed much further north in Siberia in the late Eocene/earlier Oligocene. With the opening of the Bering Bridge, at least one species reached North America, and over time almost a hundred species have evolved in the new area.

Allium sect. *Bromatorrhiza* is monophyletic and consists of two groups: *A. wallichii* and *A. macranthum* have the basic chromosome number $n = 7$, and the other group consists of
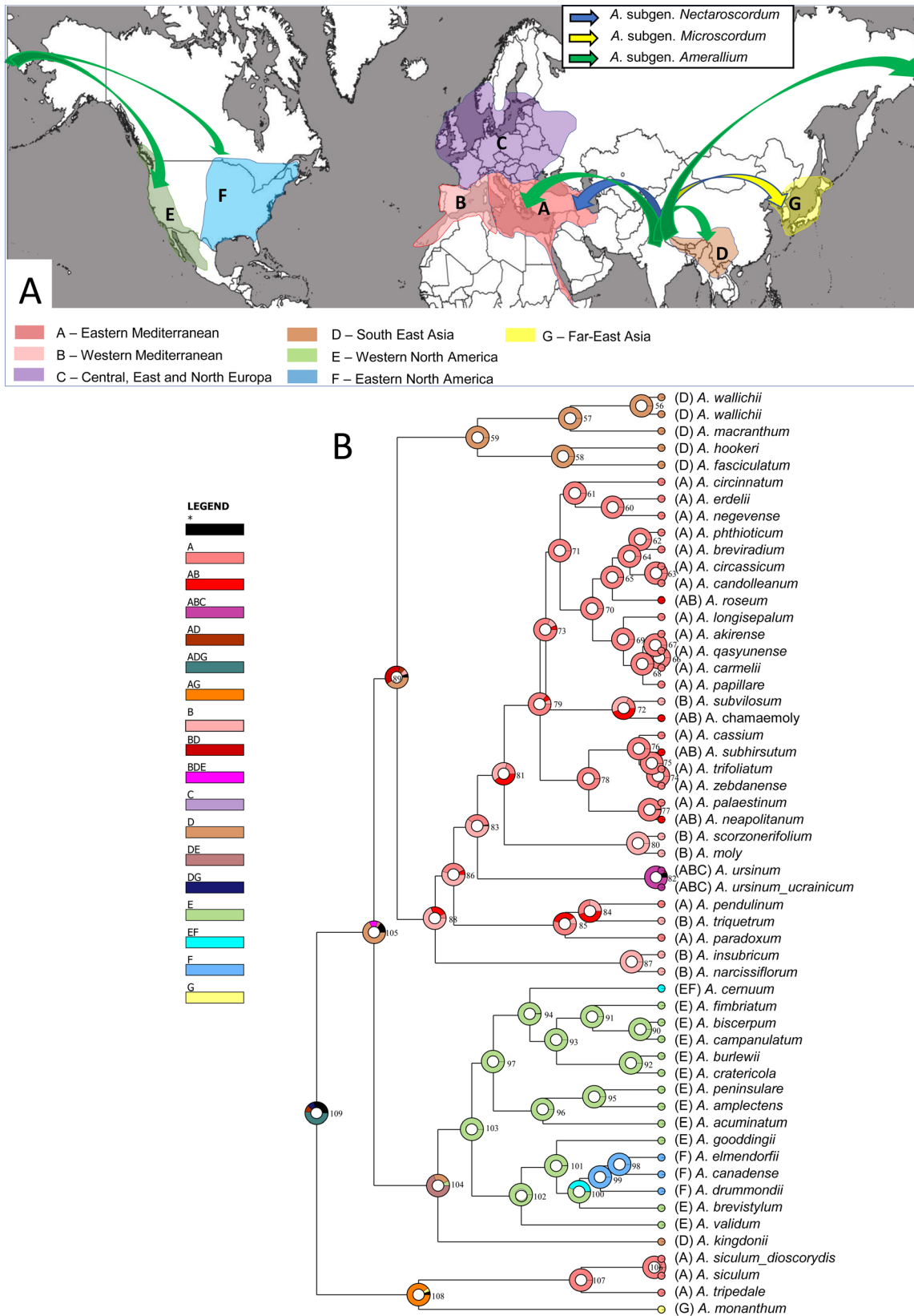
Fig. 6. Ancestral range reconstruction, based on a dated nuclear DNA phylogenetic tree of the first evolutionary lineage of *Allium* with DIVALIKE +J analysis: **A**, Geographic representation of biogeographic entities, where the arrows indicate the direction of the distribution shift. **B**, Tree representation of ancestral range reconstruction based on a divergence time tree retrieved from BEAST analysis under coalescent tree prior. Numbers next to pie charts are node numbering. See the explanation in suppl. Appendix S4.

A. hookeri Thwaites (*n* = 11) and *A. fasciculatum* Rendle (*n* = 10). These results are consistent with previous studies on *A.* sect. *Bromatorrhiza* (Li & al., 2010; Chen & al., 2022).

The Mediterranean taxa of *Allium* subg. *Amerallium*. — The phylogenetic trees (Figs. 3, 4) show that *Allium* sect. *Molium* (Mediterranean group) is paraphyletic and comprises at least three distinct groups. As part of the phylogenetic revision of the Eurasian representatives of *A.* subg. *Amerallium*, we discovered three new species of *A.* sect. *Molium*: *A. akirense* and *A. palaestinum* in Israel (Friesen & Fragman-Sapir, 2014; Fragman-Sapir & Friesen, 2017), and *A. canariense* in the Canary Islands. Although only *A. roseum* from sect. *Molium* was mentioned in floristic publications (Schönfelder & Schönfelder, 2008, 2012) on the flora of the Canary Islands, we were able to establish a correct taxonomic relationship of these plants in the Canary Islands – the endemic *A. canariense*, closely related to *A. subvillosum* (Friesen & al., 2015). Our results underline the affiliation of all 37 studied Mediterranean taxa to five sections (see also Appendix 1).

1. ***Allium*** sect. ***Narkissoprason*** (F.Herm.) Kamelin, Florogeneticheskij Analiz Estestvennoj Flory Gornoj Srednej Azii: 239. 1973 ≡ *Allium* [unranked] *Narkissoprason* F.Herm. in Repert. Spec. Nov. Regni Veg. 46: 58. 1939 – Type: *A. narcissiflorum* Vill.

Two rhizomatous endemics from high mountain pastures in the West and South-west Alps belong to this section: *Allium insubricum* Boiss. & Reut. (Fig. 1E) and *A. narcissiflorum* Vill. (Fig. 1D), both with 2*n* = 14 (Ohri & Pistrick, 2001; own count accession Am79). They form a strongly supported clade in the nuclear and plastid trees and are sister to all other Mediterranean species of *A.* subg. *Amerallium*. These species diverged from other members of Mediterranean Amerallium by about 31 mya (Fig. 5) and were probably able to survive the glacial period in the refugia at the foot of the South Alps.

2. ***Allium*** sect. ***Briseis*** (Salisb.) Stearn in Herbertia 11: 20. 1946 ≡ *Allium* [unranked] *Briseis* Salisb., Gen. Pl.: 92. 1866 – Type: *A. triquetrum* L.
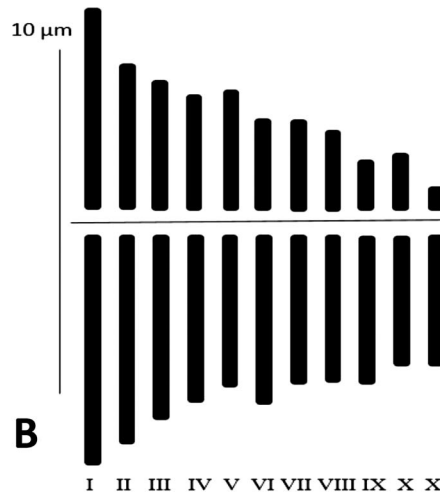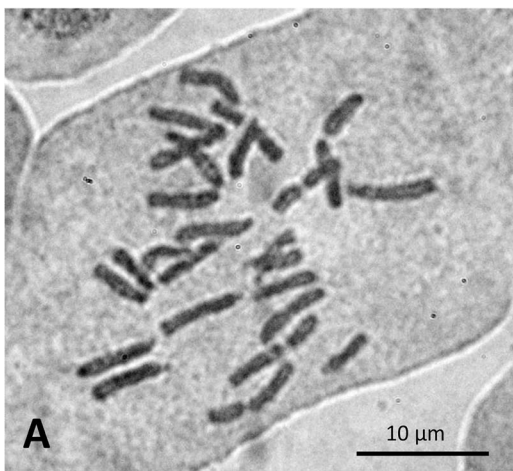

Fig. 7. Karyological analysis of *Allium chamaemoly* (Am126; origin of accession, see Appendix 1). **A**, Metaphase chromosomes 2*n* = 22; **B**, Idiogram.
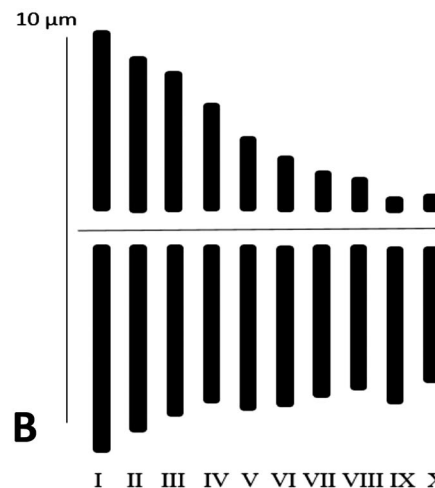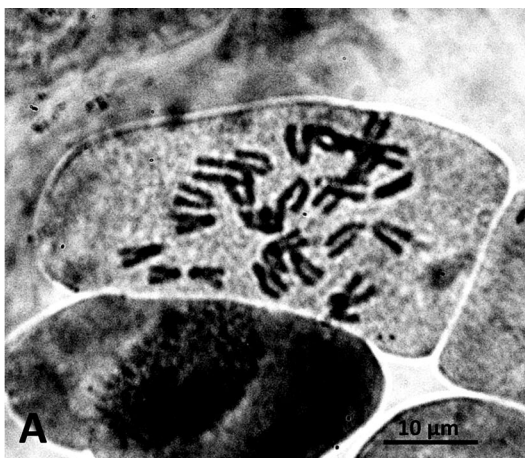

Fig. 8. Karyological analysis of *Allium negevense* (Am520; origin of accession, see Appendix 1). **A**, Metaphase chromosomes 2*n* = 20; **B**, Idiogram.

Allium sect. *Briseis* comprises three morphologically and karyologically very different species: (1) *A. paradoxum* (M.Bieb.) G.Don (Fig. 1I), with $2n = 16$ (Barling, 1954; Badr & Elkington, 1977; Vosa, 1977; Pogosian, 1983), naturally distributed in the East Caucasus and northern Iran and now widespread in Europe and North America as an invasive species; (2) *A. pendulinum* Ten. (Fig. 1H) with $2n = 14$ (Renzoni & Garbari, 1970), an endemic species from the Mediterranean islands of Corsica and Sardinia; (3) *A. triquetrum* (Fig. 1G) with $2n = 18$ (Renzoni & Garbari, 1970; Rickards, 1977; Badr & Elkington, 1977; Balog, 1979, 1982, 1984; Pastor, 1982; Ryan, 1983; Wittmann, 1984; Arends & Van der Laan, 1979; Leal Pérez-Chao & al., 1980; Ohri & Pistrick, 2001), originally from the western Mediterranean and now widespread in Europe, western North America, South Australia, and Argentina. All three species form a strongly supported clade in the nuclear and plastid trees and branch as second group after the Narkissoprason clade. The separation of the species in the section happened about 10 mya, before the Messinian salinity crisis (Krijgsman & al., 1999; Duggen & al., 2003; Agusti & al., 2006).

3. *Allium* sect. *Xanthoprason* (F.Herm.) Kamelin, Florogeneticheskij Analiz Estestvennoj Flory Gornoj Srednej Azii: 239. 1973 ≡ *Allium* [unranked] *Xanthoprason* F.Herm. in Repert. Spec. Nov. Regni Veg. 46: 58. 1939 – Type: *A. moly* L.

Allium sect. *Xanthoprason* comprises two West Mediterranean species with yellow flowers: *A. scorzonerifolium* (Fig. 1J) and *A. moly* (Fig. 1K), both with $2n = 14$ (Badr & Elkington, 1977, 1984; Pastor, 1982; Elena-Rosselló & al., 1987; Ohri & Pistrick, 2001), but triploids with $2n = 21$ were also found in *A. scorzonerifolium* (Pastor, 1982; Garcia Martinez, 2001). Both species form a strongly supported clade in the nuclear and plastid trees, which is accepted at sectional rank. The name of Hermann's Xanthoprason group was not validly published at section rank by Stearn (1946) (Art. 36.1 *ICN*), but this rank was validly accepted by Kamelin (1973).

4. *Allium* sect. *Ophioscorodon* (Wallr.) Endl., Gen. Pl.: 147. 1836 ≡ *Ophioscorodon* Wallr., Sched. Crit.: 129. 1822 – Type: *A. ursinum* L.

Only the taxon *Allium ursinum*, which is widely distributed in Europe and has two subspecies, belongs to this section. This species shows hypogeal seed germination, a unique seedling type, inverse leaf orientation, and large chromosomes ($2n = 14$) with an exceptionally high 2C DNA content of nearly 64 pg (Ohri & al., 1998; Ricroch & al., 2005), underlining the separate position of *A.* sect. *Ophioscorodon* in *A.* subg. *Amerallium* (Friesen & al., 2006; Herden & al., 2012). *Allium ursinum* subsp. *ucrainicum* Oksner & Kleopow is distributed in eastern and southern Europe, and *A. ursinum* subsp. *ursinum* (Fig. 1F) in western and central Europe. The fact that an intermediate zone exists (Rola, 2012) where both subspecies meet, suggests that two genotypes survived the last glacial maximum in different refugia, probably linked to beech refugia (Magri, 2008), and may have formed a hybrid zone during the interglacial period.

5. *Allium* sect. *Molium* G.Don in Mem. Wern. Nat. Hist. Soc. 6: 72. 1832 – Type: *A. roseum* L. [Fig. 2L]

This section is the most species-rich and diverse among the Mediterranean members of *Allium* subg. *Amerallium*. Analyses of nrITS and plastid markers resulted in three clades with very different compositions. This primarily concerns the position of *A. chamaemoly* (Fig. 1L).

Allium chamaemoly is karyologically ($2n = 22$) and morphologically distinct from most of the species in *A.* sect. *Molium*. Only *A. subvillosum* shares many characteristics (Speta, 1989). Hence, most authorities on *Allium* taxonomy put *A. chamaemoly* in the monotypic *A.* sect. *Chamaeprason* (Stearn, 1946, 1978; Kollmann, 1984; Hanelt & al., 1992). Traub (1972) assigned *A. chamaemoly* to *A.* subsect. *Chamaeprason* (F.Herm.) Traub of *A.* sect. *Molium* Endl. [sic]. Only De Wilde-Duyfjes (1976) simply included this species in *A.* sect. *Molium*. In both nuclear and plastid analyses, *A. chamaemoly* is positioned in *A.* sect. *Molium*, but in different groups. In the nuclear ITS tree, it stands together with *A. subvillosum* and *A. canariense* (Fig. 3). In the plastid tree
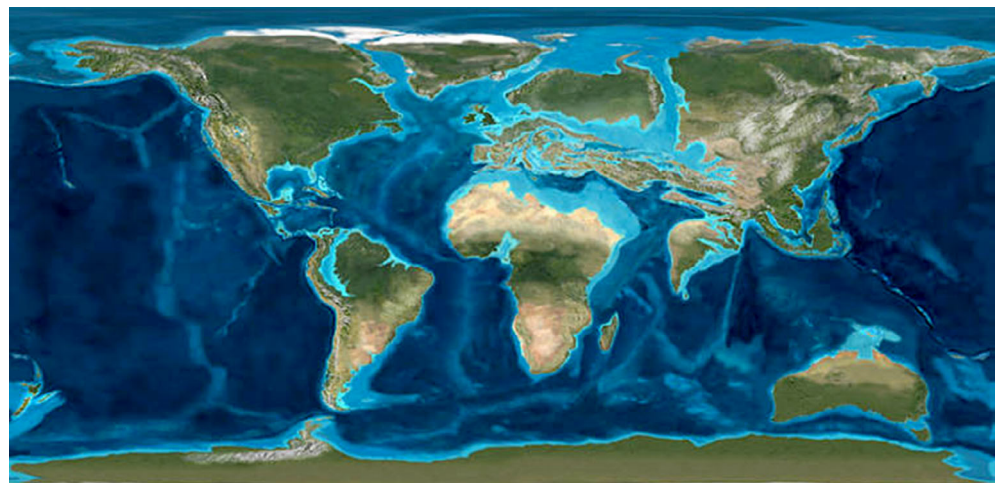

Fig. 9. Map of the Earth 50 mya in the Ypresian stage of the Eocene (Ron Blakey, Ypresian Earth 50 mya, CC BY-SA 4.0).

(Fig. 4), it appears somewhat isolated in clade C, closer to sub-clade C3 with *A. zebdanense*, *A. trifoliatum*, *A. subhirsutum* and *A. cassium*. These unclear positions most probably reflect an ancient hybrid origin of *A. chamaemoly* between *A. subvillosum* and the ancestor of subclade C2. That *A. subvillosum* was one of the parents of *A. chamaemoly* is shown not only by molecular data (nrITS tree), but also by some morphological characters: similar nectaries and structure of the bulbs. Speta (1989) already described this in his detailed morphological and karyological study of *A. chamaemoly*. The chromosome set of *A. chamaemoly* is very likely derived from an ancestral complement of $2n = 14$, because there are several telocentric chromosome pairs and one pair of satellite chromosomes. If we count all well-developed arms, except telocentric ones, we get 28 arms which corresponds to $2n = 14$. This explanation was already presented by Ved Brat (1965), Kollmann (1970), Pastor (1982), Pastor & Valdes (1983), Bartolo & al. (1984) and Tzanoudakis (1986, 1992). Pastor (1982) found $n = 14$ bivalents in the meiosis of *A. chamaemoly* from Spain, which disagrees with the results of Kollmann (1973), Marchi & al. (1974), Garbari (1975) and Speta (1989). Our mitotic count of the Spanish accession Am126 clearly shows 22 chromosomes (Fig. 7). With our chromosome count we do not want to question Pastor's (1982) chromosome count with $2n = 28$. It may well be that the only accession from Huelva that Pastor (1982) examined by chance had $2n = 28$. Additional cytological screening of the chromosome number in samples from Spain is needed to explain this phenomenon. Pastor & Valdes (1988) also examined the chromosomes in a plant from Italy and confirmed $2n = 22$. After morphological comparison of plants in several herbaria, they were able to determine small quantitative morphological differences between eastern and western plants and recorded $2n = 28$ chromosomes in all eastern plants of *A. chamaemoly*, which was the reason for describing *A. chamaemoly* subsp. *longicaule* Pastor & Valdes. This subspecies is, however, not recognized in *Flora Iberica* (Aedo, 2013).

Allium sect. *Molium* can be divided into at least three subsections (clades A, B and C) based on the nuclear tree and the plastid tree, but the groupings are different (Figs. 3, 4). Therefore, we renounce this division and recognize *A.* sect. *Molium* as a large polymorphic section including *A. chamaemoly*.

Key for Old World *Allium* sections from the first evolutionary lineage

1. Ovary semi-inferior; tepals 3- to 7-veined, leaves with wing-like median keel, scape terete .... *A.* subg./sect. *Nectaroscordum*
1. Ovary superior; tepals usually 1-veined, usually all alike; leaves with triangular keel or not keeled 2
2. Plants dioecious; umbel 1–2(3)-flowered ....*A.* subg. *Microscordum* (*A. monanthum*)
2. Plants monoecious (hermaphroditic); umbel 5- to many-flowered.....*A.* subg. *Amerallium* (and other alliums) 3
3. Roots thick, sometimes nearly tuberous; leaves linear, main nerve distinct .... *A.* sect. *Bromatorrhiza*

3. Roots thin, filiform; leaves without distinct nerve 4
4. Plant not rhizomatous; bulbs subcylindrical, ovoid, or subglobose; leaves almost basal, with short above-ground sheath, flat; scape terete or angled................................5
4. Bulbs narrowly conical to cylindrical, clustered on a short rhizome; scape 2-edged.............*A.* sect. *Narkissoprason*
5. Bulbs subcylindrical, outer tunics papery, with a few parallel fibres at base; leaves basal, petiolate, invertedly positioned ....*A.* sect. *Ophioscorodon* (*A. ursinum*)
5. Bulbs subglobose, tunics reticulate, papery, membranous, or coriaceous; leaves without petiole, usually linear or filiform to broadly lanceolate (*A. moly*), not inverted.......6
6. Perigone bright yellow ....*A.* sect. *Xanthoprason*
6. Perigone white, yellowish, crème, pink, greenish or purple 7
7. Leaves almost basal, with short above-ground sheath, so strongly keeled as to be triquetrous; scape triquetrous, flaccid after anthesis; tepals white............*A.* sect *Briseis*
7. Leaves linear, flat, or canaliculate, solid 8
8. Umbel lax; pedicels usually much longer than perigone; tepals never purple, after anthesis erect and enveloping the capsule; pedicels equal or subequal ....*A.* sect. *Molium*
8. Umbel few-flowered, pedicels very unequal, shorter or slightly longer than the purple tepals ....*A.* sect. *Kingdonia*

■ CONCLUSION

Most clades are well supported in the analyses of nuclear and combined chloroplast data. The single chloroplast fragment analyses produced different tree topologies, which can be explained by different phylogenetic variability of the chloroplast regions studied. Combined analysis of all cpDNA fragments produced a topology similar to that of the tree based on nuclear data. Most of the sections in the Eurasian part of *A.* subg. *Amerallium* are monophyletic. Only *A. chamaemoly* (monotypic *A.* sect. *Chamaeprason*) nested within the clade of *A.* sect. *Molium* makes this section paraphyletic. Different positions of *A. chamaemoly* in the nuclear and plastid trees indicate an ancient hybridogenic origin. The monophyletic American *Allium* species of *A.* subg. *Amerallium* originated from extinct North Asian taxa. Currently, only one species from this ancient group still exists in the Eastern Himalaya – *A. kingdonii*.

■ AUTHOR CONTRIBUTIONS

■ ACKNOWLEDGEMENTS

■ LITERATURE CITED

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Appendix 1. Voucher information for specimens used in this study.

Information is given here as follows: Taxon, Section, Accession, Locality, *Original collector and collection number*, Voucher (Herbarium code or living collection number in BG OSN), GenBank accession numbers (nrITS, nrETS, *atpB-rbcL*, *rps16* intron, *trnL-trnF*, *rpl32-trnL*). "–" indicates a missing sequence. All sequences were made as part of the Amerallium project, GenBank numbers beginning with NF were newly obtained for this study.

Allium subg. **Amerallium**: **Allium acuminatum** Hook., *Lophioprason*, Am295, U.S.A., Utah, Devonian BG, s.n. (OSN 2002-2344-W), HF934249, HF934478, HF934133, HF934362, HF934692, HF934590; **Allium akirense** N.Friesen & Fragman, *Molium*, Am382-1, Israel, Givat Brenner Kibbutz, *Friesen, N. s.n.* (OSBU 20932), HF934250, HF934479, HF934134, HF934363, HF934693, HF934591; Am332-2, Israel, Givat Brenner Kibbutz, *Friesen, N. s.n.* (OSBU 20932), HF934251, HF934480, HF934135, HF934364, HF934694, HF934592; Am332-3, Israel, Givat Brenner Kibbutz, *Friesen, N. s.n.* (OSBU 20932), HF934252, HF934481, HF934136, HF934365, HF934695, HF934593; **Allium amplectens** Torr., *Lophioprason*, Am307, U.S.A., California, Sierra Nevada, Stanislav River, *Friesen, N. s.n.* (OSN 2010-1173-W), HF934253, HF934482, HF934137, HF934366, HF934696, HF934594; **Allium bisceptrum** S.Watson, *Lophioprason*, Am303, U.S.A., California, San Bernadino, Big Bear, *Friesen, N. s.n.* (OSN 2010-1175-W), HF934254, HF934483, HF934138, HF934367, HF934697, HF934595. **Allium breviradium** (Halácsy) Stearn, *Molium*, Am219, Greece, Epirus, Grevenon, Vasilitsa, *Willing, R. & Willing, E. 21929* (B 100216574), HF934255, HF934484, HF934139, HF934368, HF934698, HF934596; Am432, Greece, Epirus, *Ioanninon, N. s.n.* (FR), HF934256, HF934485, HF934140, HF934369, HF934699, HF934597; **Allium brevistylum** S.Watson, *Caulorhizideum*, Am309, U.S.A., Colorado, La Plata River, *Friesen, N. s.n.* (OSN 2010-1179-W), HF934257, HF934486, HF934141, HF934370, HF934700, HF934598; **Allium burlewii** Davidson, *Lophioprason*, Am305, U.S.A., California, San Bernadino, Big Bear, *Friesen, N. s.n.* (OSN 2010-1176-W), HF934259, HF934487, HF934142, HF934371, HF934701, HF934599; **Allium campanulatum** S.Watson, *Lophioprason*, Am304, U.S.A., California, San Bernadino, Baldwin, *Friesen, N. s.n.* (OSN 2010-1178-W), HF934260, HF934488, HF934143, HF934372, HF934702, HF934600; **Allium canadense** L., *Amerallium*, Am308, U.S.A., Texas, *Friesen, N. s.n.* (OSN 2010-1191-W), HF934261, HF934489, HF934144, HF934373, HF934703, HF934601; **Allium canariense** (Regel) N.Friesen & P.Schönfelder, *Molium*, Am070, Spain, Teneriffa, Mirador de Baracan, *Blattner, F. s.n.* (OSN 2006-0728-W), HF934337, HF934565, HF934226, HF934455, HF934785, HF934672; Am123, Spain, Canary Islands, Teneriffa, *Friesen, N. s.n.* (OSN 2008-0568-W), HF934297, HF934528, HF934179, HF934408, HF934738, HF934632; Am124, Spain, Canary Islands, Teneriffa, *Blattner, F. s.n.* (OSN 08-02-0091-20), HF934298, HF934529, –, HF934180, HF934739, HF934633; Am434, Spain, Canary Islands, Teneriffa, *Blattner, R. s.n.* (OSN 2008-0569-W), HF934299, HF934530, –, LN823642, LN823700, LN823671; Am474 Spain, Canary Islands, La Palma, *Friesen, N. s.n.* (OSBU 22349), LN823590, LN823619, –, LN823649, LN823707, LN823678; Am533, Spain, Canary Islands, La Gomera, *Schönfelder, P. 10-15* (REG), LN823596, LN823625, –, LN823655, LN823713, LN823684; Am534, Spain, Canary Islands, Lanzarote, *Schönfelder, P. 09-21* (REG), LN823599, LN823628, –, LN823658, LN823716, LN823687; Am547, Spain, Canary Islands, El Hierro, *C. Stierstorfer 343* (B 100245045), LN823597, LN823626, –, LN823656, LN823714, LN823685; Am592, Spain, Canary Islands, Montana Clara, *Betancourt, A. s.n.* (ORT 39750), LN823602, LN823631, –, LN823661, LN823719, LN823690; Am594, Spain, Canary Islands, Fuerteventura, *Scholz, S. s.n.* (TFC 46482), LN823603, LN823632, –, LN823662, LN823720, LN823691; Am614, Spain, Canary Islands, Gran Canaria, *Schönfelder, P. 14-15* (REG), LN823594, LN823623, –, LN823653, LN823711, LN823682; **Allium candolleanum** Albov, *Molium*, Am449, Russia, Caucasus, Aigba Mts. *Shorina, N. s.n.* (MW0655279), HF934262, HF934490, HF934145, HF934374, HF934704, HF934602; Am454, Caucasus, Abkhazia, Gagra Massif, Berchil, *Kudrjashova, G. 3018* (LE), HF934263, HF934491, HF934146, HF934375, HF934705, HF934603; **Allium carmeli** Boiss., *Molium*, Am354, Israel, Judaean Mts., *Kollmann, F. 6628* (HUJ), HF934264, HF934492, HF934147, HF934376, HF934706, HF934604; Am379, Israel, Mt. Gilboa, *Kollmann, F. 5429* (HUJ), HF934265, HF934493, –, –, –, –; Am381, Israel, Mt Carmel, *Friesen, N. s.n.* (OSBU 20950), HF934266, HF934494, HF934148, HF934377, HF934707, HF934605; Am355, Israel, Judaean Mts., Yatir, *Bnur, M. 5456* (HUJ), HF934267, HF934495, –, –, –, –; Am383, Israel, Mt. Hermon, Majdal Shams, *Shmida, O. 5439* (HUJ), HF934268, HF934496, HF934149, HF934378, HF934708, HF934606; **Allium cassium** Boiss., *Molium*, Am206, Cyprus, entre Nata & Axylon, Paphos, *Alziar, G. 1080* (B 100216559), HF934269, HF934497, HF934150, HF934379, HF934709, HF934607; **Allium cassium** var. **hirtellum** Boiss., *Molium*, Am207, Cyprus, Stavros tis Psokkash, *Hand, R. 3215* (B 100192204), HF934270, HF934498, HF934151, HF934380, HF934710, HF934608; **Allium cernuum** Roth, *Lophioprason*, Am197, U.S.A., *Staudenfreunde 1984/755* (GAT 0682), AJ250289, HF934499, –, –, –, –; **Allium chamaemoly** L., *Chamaeprason*, Am081, Greece, Corinth Pr., Kalamata, *Guiol, F. 2353* (HUJ), HF934271, HF934500, HF934152, HF934381, HF934711, HF934609; Am090, Italy, Monte Testaccio, Roma, *Cacciato, A. s.n.* (W 1974/02005), –, HF934501, HF934153, HF934382, HF934712, HF934610; Am126, Spain, Malaga, Ardales, *Trissl, D. s.n.* (OSN 2007-0971-W), HE962504, HE859943, HF934154, HF934383, HF934713, HE859954; **Allium circassicum** Kolak., *Molium*, Am453, Russia, River Psou, Ajgba Mts., *Nikitin, F. s.n.* (LE), HF934272, HF934502, HF934155, HF934384, HF934714, HF934611; **Allium circinnatum** Sieber, *Molium*, Am201, Greece, Crete, prov. Iraklio,

Appendix 1. Continued.

Greuter, W. 19585 (B 100099078), HF934273, HF934503, HF934156, HF934385, HF934715, HF934612; Am202, Greece, Crete, Ep. Pedhiadhos, *Schwerdt-feger s.n.* (B 100673280), HF934274, HF934504, HF934157, HF934386, HF934716, HF934613; ***Allium circinnatum* subsp. *peloponnesiacum*** Tzanoud., *Molium*, Am208, Greece, Peloponnesus, Lakonia, *Jagel, A. s.n.* (B 100216563), HF934275, HF934505, HF934158, HF934387, HF934717, HF934614; Am209, Greece, Peloponnesus, Tenara, *Hellmann, V. s.n.* (B 100216564), HF934276, HF934506, HF934159, HF934388, HF934718, HF934615; ***Allium cratericola*** Eastw., *Lophioprason*, Am004, U.S.A., California, *Friesen, N. s.n.* (OSN 2003-1542-W), EU096146, EU162714, HF934160, HF934389, HF934719, HF934957; ***Allium drummondii*** Regel, *Caulorhizideum*, Am306, U.S.A., Texas, *Friesen, N. s.n.* (OSN 2010-1190-W), HF934277, HF934507, HF934161, HF934390, HF934720, HF934616; ***Allium elmendorfii*** M.E.Jones ex Ownbey, *Amerallium*, Am302, U.S.A., Texas, *Friesen, N. s.n.* (OSN 2010-1194-W), HF934278, HF934508, –, –, –, –; ***Allium erdelii*** Zucc., *Molium*, Am331, Israel, Ness Tsiyona, *Friesen, N. s.n.* (OSBU 20931), HF934279, HF934509, HF934162, HF934391, HF934721, HF934617; Am210, Israel, Philistean Plain, *Danin, A. 03.057* (B 100216565), HF934280, HF934510, HF934163, HF934392, HF934722, HF934618; Am349, Israel, *Kollmann, F. 5807* (HUJ), HF934281, HF934511, –, –, –, –; ***Allium fasciculatum*** Rendle, *Bromatorrhiza*, Am065, China, Xizang, Raka Tsangpo N of Sangsang, *Miehe, G. s.n.* (OSBU 21044), HF934282, HF934512, HF934164, HF934393, HF934723, HF934619; Am312, China, SE Tibet, W of Gongbogyamda, *Miehe, G. s.n.* (OSBU 21033), HF934284, HF934513, HF934165, HF934394, HF934724, HF934620; Am389, China, Tibet, *Miehe, G. s.n.* (OSBU 21037), HF934283, HF934514, –, –, –, –; ***Allium fimbriatum*** S.Watson, *Rhophetoprason*, Am199, U.S.A., California, Lake Co., *McNeal, D. s.n.* (GAT 3487), AJ411932, HF934515, AJ299125, –, –, –; ***Allium gooddingii*** Ownbey, *Caulorhizideum*, Am245, U.S.A., Arizona, *McDonough, M. s.n.* (GAT 3471), HF934285, HF934516, HF934166, HF934395, HF934725, HF934621; ***Allium hookeri*** Thwaites, *Bromatorrhiza*, Am196, China, prov. Yunnan, Kunming, *Hanelt, P. CHN-86/36* (GAT 2013), AK250297, HF934518, HF934167, HF934396, HF934726, HF934622; Am285, Thailand, Tak Province, village Mae Sot, *deVries, N. s.n.* (GAT 3081), HF934286, HF934517, HF934168, HF934397, HF934727, HF934623; Am286, Thailand, Mae Sot Province, *Buijsen, A. s.n.* (GAT 2926), HE962508, HE859944, HF934169, HF934398, HF934727, HE859963; ***Allium humile*** Kunth, *Bromatorrhiza*, Am205, India, Udai C. Pradeshan, Kalimpong, *Dickore, W.B. s.n.* (M 31627), HF934287, –, –, –, –, –; ***Allium insubricum*** Boiss. & Reuter, *Narkissoprason*, Am234, Italy, Insubria, Grigna Meridionale, *Cubr, M. 41856* (B 100126079), HF934288, HF934519, HF934170, HF934399, HF934729, HF934624; Am263, Italy, Insubria, Grigna, Barzio, *Neuffer, B. s.n.* (OSBU 367), HE962509, HE859946, HF934171, HF934400, HF934730, HE859959; Am289, Italy, Insubria, Lausanne BG, s.n. (GAT 3066), HF934289, HF934520, HF934172, HF934401, HF934731, HF934625; Am384, Italy, Insubria, Grigna, Piani Resenelli, *Friesen, N. s.n.* (OSBU 21207), HF934290, HF934521, HF934173, HF934402, HF934732, HF93462; ***Allium longisepalum*** Bertol., *Molium*, Am213, Iran, Kuh-Gerid, *Strauss, T. s.n.* (B 100216768), HF934291, HF934522, HF934174, HF934403, HF934733, HF934627; Am237, Iran, Kordestan, 80 km to Sanandaj, *MeZarre, S. 35233* (B 100216767), HF934292, HF934523, –, –, –, –; Am281, Iran, prov. Kohgiluye-va Buyerahmad, *Fritsch, R. IRN2010/1296* (GAT 6787), HF934293, HF934524, HF934175, HF934404, HF934734, HF934628; Am372, Iran, prov. W. Azerbaijan, valley Ghasemlu, *Fritsch, R. IRN2011/1353* (GAT 6896), HF934294, HF934525, HF934176, HF934405, HF934735, HF934629; ***Allium macranthum*** Baker, *Bromatorrhiza*, Am015, Bhutan, *Miehe, G. s.n.* (OSBU 21222), HF934295, HF934526, HF934177, HF934406, HF934736, HF934630; Am051, Bhutan, *Miehe, G. s.n.* (OSBU 21063), HF934296, HF934527, HF934178, HF934407, HF934737, HF934631; ***Allium moly*** L., *Xanthoprason*, Am044, Spain, Jaen, Sierra de Casorla, *Blattner, F. 501* (FR), HE962501, FN551226, HF934181, HF934410, HF934740, HE859953; Am077, Spain, Aragona, Puerto de Aldea, Chemnitz BG, s.n. (OSN 2005-1327-G), AJ412743, FN551227, HF934182, HF934411, HF934741, HE859952; Am291, Germany, Gatersleben, home garden, *Maass, H. s.n.* (GAT 1117), HF934300, HF934531, HF934183, HF934412, HF934742, HF934634; ***Allium narcissiflorum*** Vill., *Narkissoprason*, Am079, France, Villard de Lane, *Friesen, N. s.n.* (OSBU 19616), HE962503, HE859947, HF934187, HF934416, HF934746, HE859960; Am050, Italy, Cuneo, Vallone Grande E Trinita, *Hörandl, E., Hadachek, F. 6886* (W 2003/00024), F934304, HF934532, HF934188, HF934417, HF934747, HF934638; ***Allium neapolitanum*** Cirillo, *Molium*, Am36, Turkey, vilajet Antalya, Side, *Friesen, N. s.n.* (OSN-2012-0942-W), HF934305, HF934533, HF934189, HF934418, HF934748, HF934639; Am40, Turkey, *Friesen, N. s.n.* (OSN 2003-1645-G), HF693743, FN551228, HF934190, HF934419, HF934749, HE859951; Am252, Israel, Netanja, *Friesen, N. s.n.* (OSB 2010-1139-W), HF934306, –, HF934191, HF934420, HF934750, HF934640; Am340, Israel, Judaean Mts., *Friesen, N. s.n.* (OSBU 20937), HE962510, HE859941, HF934192, HF934421, HF934751, HF934641; Am341, Israel, Mt. Gilboa, *Friesen, N. s.n.* (OSBU 20938), HF953353, HF953352, HF934193, HF934422, HF934752, HF934642; Am373, Israel, Jerusalem, Mt. Scopus, *Friesen, N. s.n.* (OSN 2011-0928-W), HF934307, HF934535, –, –, –, –; Am374, Israel, Golan, *Friesen, N. s.n.* (OSBU 20948), HF934308, HF934536, HF934194, HF934423, HF934753, HF934643; Am375, Israel, Mt. Carmel, *Friesen, N. s.n.* (OSBU 20949), HF934309, HF934537, –, –, –, –; ***Allium palaestinum*** (Feinbrun) Kollmann ex Fragman & N.Friesen, *Molium*, Am337, Israel, Cefar Adumim, *Friesen, N. s.n.* (OSBU 20935), HF934310, HF934538, HF934195, HF934424, HF934754, HF934644; Am338, Israel, Road 90, East of Hemdat, *Friesen, N. s.n.* (OSBU 20936), HF934311, HF934539, HF934196, HF934425, HF934754, HF934645; Am339, Israel, Jerusalem, *Friesen, N. s.n.* (OSN 11-12-0010-20), HF934312, HF934540, HF934197, HF934426, HF934756, HF934646; ***Allium negevense*** Kollmann, *Molium*, Am342, Israel, between Feruham and Sede Rogenz, *Kollmann, F. 5972* (HUJ), HF934313, HF934541, HF934198, HF934427, HF934757, HF934647; Am343, Israel, Central Negev, Sede Zin, *Danin, A. 5956* (HUJ), HF934314, HF934542, –, –, –, –; Am344, Israel, Negev, Avdat, *Danin, A. 5987* (HUJ), HF934315, HF934543, HF934199, HF934428, HF934758, HF934648; ***Allium papillare*** Boiss., *Molium*, Am345, Egypt, N Sinai, 5 km SW of Rafah, *Shmida, A. s.n.* (HUJ), HF934316, HF934544, HF934200, HF934429, HF934759, HF934649; Am346, Israel, W Negev, Kerem Schalom, *Kollman, F. 6935* (HUJ), HF934317, HF934545, HF934201, HF934430, HF934760, HF934650; Am422, Israel, W Negev, Halutsa Sands, *Fragman-Sapir, O. s.n.* (HUJ), HF934318, HF934546, HF934202, HF934431, HF934761, HF934651; ***Allium paradoxum*** (M.Bieb.) G.Don var. **paradoxum**, *Briseis*, Am062, Germany, Leipzig BG, s.n. (GAT 1657), AJ412741, HF934547, HF934203, HF934432, HF934762, HF934652; ***Allium paradoxum*** var. **normale** Stearn, *Briseis*, Am249, Iran, prov. Golestan, Naitinga valley, *Fritsch, R. IRN 2004/1002* (GAT 6232), HE962505, HE859939, HF934204, HF934433, HF934763, HE859958; Am251, Iran, prov. Golestan, near pass Jakhtikalo, *Fritsch, R. IRN 2004/1017* (GAT 6247), HF934319, HF934548, HF934205, HF934434, HF934764, HF934653; ***Allium pendulinum*** Ten., *Briseis*, Am056, Italy, Sardinia, S. Girolamo, *Malicky, H. s.n.* (W 1982/09653), HF934320, HF934549, HF934206, HF934435, HF934765, HF934654; Am220, France, Corsica, *Cesca, G. & al. s.n.* (B 101252079), HE962506, HE859940, HF934207, HF934436, HF934766, HE859961; Am240, France, Corsica, Bastelica, *Lambinon, J. 84/Co/443* (B 100008135), HF934321, HF934550, HF934208, HF934437, HF934767, HF934655; ***Allium peninsulare*** Lemmon ex Greene, *Lophioprason*, Am021, U.S.A., California, *Friesen, N. s.n.* (OSN 2003-1546-W), HF934322, HF934551, HF934209, HF934438, HF934768, HF934656; ***Allium phthioticum*** Boiss. & Heldr., *Molium*, Am214, Greece, Nom Phthiotis, Iti Massif, *Willing, R. & Willing, E. 4640* (B 100216569), HF934323, HF934552, HF934210, HF934439, HF934769, HF934657; Am215, Greece, Nom Imathia, Vermion Massif, *Willing, R. & Willing, E. 9576* (B 100216570), HF934324, HF934553, HF934211, HF934440, HF934770, HF934658; ***Allium qasyunense*** Mouterde, *Molium*, Am333, Israel, Road 90, Um Zuqa, *Friesen, N. s.n.* (OSBU 20933), HF934325, HF934554, HF934212, HF934441, HF934771, HF934659; Am334, Israel, Mevo Hamma, *Friesen, N. s.n.* (OSBU 20039), HF934326, HF934555, HF934213, HF934442, HF934772, HF934660; Am335, Israel, east coast of Lake Galilee, *Friesen, N. s.n.* (OSBU 20934), HF934327, HF934556, HF934214, HF934443, HF934773, HF934661; ***Allium roseum*** L., *Molium*, Am082, Spain, Malaga, Ardales, Los Picachones, *Trissl, D. s.n.* (OSN 2006-1384-W), FR693745, FN551230, HF934215, HF934444, HF934774, HE859957; Am222, Greece, Peranthi, *Willing, R. & Willing, E. 136741* (B 100257074), HF934328, HF934557, HF934216, HF934445, HF934775, HF934662; Am243, Tunisia, 45 km west of Beja, *Hammer, K. TUN-92/95* (GAT 3266), HF934329, HF934558, HF934217, HF934446, HF934776, HF934663; Am357, Spain, Alicante, Denia, *Fort, J.M. s.n.* (HUJ), HF934330, HF934559, HF934218, HF934447, HF934777, HF934664; ***Allium scorzonerifolium*** Desf., *Xanthoprason*, Spain, Prov. Salamanca, Linares de Rio, *Ladero & al. 15747* (B 100216577), HF934331, HF934560, HF934219, HF934448, HF934778, HF934665; ***Allium scorzonerifolium*** var. **xericense** (Perez Lara) R.Fern., *Xanthoprason*, Am174, Spain, Madrid, La Pedriza, Stockholm BG, s.n. (GAT 3428), HE962500, FN551225, HF934220, HF934449, HF934779, HF934666; ***Allium subhirsutum*** L., *Molium*, Am026, Turkey, vilajet Aydin, Kusadasi, *Fritsch, R. & Friesen, N. TUR-1995-144* (GAT 5322), F934333, HF934561, HF934222, HF934451, HF934781, HF934668; Am093, Italy, Palermo, Adiacenze di Petralia, Palermo BG, s.n. (GAT 0023), HF934334, HF934562, HF934223, HF934452, HF934782, HF934669; Am228, Greece, Lakonia, Ep. Lakedhemonos, *Willing, R. & Willing, E. 91639*

Appendix 1. Continued.

(B 100073951), HF934335, HF934563, HF934224, HF934453, HF934783, HF934670; Am283, Italy, Monte Argentario Hal, Siena BG, s.n. (GAT 1461), HF934336, HF934564, HF934225, HF934454, HF934784, HF934671; ***Allium subvillosum*** Salzm. ex Schult. & Schult.f., *Molium*, Am094, Portugal, Algarve, *Trissl, D. s.n.* (OSBU 15067), HF934338, HF934566, HF934227, HF934456, HF934786, HF934673; Am430, Spain, Mallorca, Liuemajor, *Baumann, K. 6/94* (FR 0034934), HF934339, HF934567, HF934228, HF934457, HF934787, HF934674; Am538, Spain, Mallorca, Cabo Blanco, *Schönfelder, P. 99-3* (REG), LN823606, LN823635, –, –, –, –; Am670, Morocco, *Deil, U. s.n.* (FB 04746), LN823607, LN823636, –, LN823665, LN823723, LN823694; ***Allium trifoliatum*** Cirillo subsp. **hirsutum** (Regel) Kollmann, *Molium*, Am061, Cyprus, Paphos, Eledhion, Amargeti, *Vogt, R. s.n.* (OSN 2002-1284-Z), HF934347, HF934575, HF934229, HF934458, HF934788, HF934675; Am231, Greece, *Alziar, G. 0294* (B 100216579), HF934348, HF934576, HF934230, HF934459, HF934789, HF934676; Am359, Israel, Mt. Carmel, *Friesen, N. s.n.* (OSN 2011-0939-W), HF934349, HF934577, –, –, –, –; Am361, Israel, Mt. Carmel, *Friesen, N. s.n.* (OSBU 20940), HF934340, HF934568, –, –, –, –; Am362, Israel, Judaean Mts., *Friesen, N. s.n.* (OSBU 20941), HF934341, HF934569, –, –, –, –; Am363, Israel, Mt. Carmel, *Friesen, N. s.n.* (OSBU 20942), HF934342, HF934570, –, –, –, –; Am366, Israel, Judaean Mts., Ramat Raziel, *Friesen, N. s.n.* (OSBU 20944), HF934343, HF934572, –, –, –, –; Am364, Israel, Mt. Carmel, *Friesen, N. s.n.* (OSBU 20943), HF934344, HF934571, HF934231, HF934460, HF934790, HF934677; Am368, Israel. Judaean Mts, American Independence Park, *Friesen, N. s.n.* (OSN 2011-0942-W), HF934345, HF934573, HF934232, HF934461, HF934791, HF934678; Am382, Israel, Mt. Hermon, *Friesen, N. s.n.* (OSBU 20950), HF934346, HF934574, HF934233, HF934462, HF934792, HF934679; ***Allium triquetrum*** L. *Briseis*, Am037, Spain, *Trissl, D. s.n.* (OSN 2003-1630-G), HF934351, HF934578, HF934235, HF934464, HF934794, HF934681; Am080, Italy, Bonn BG, s.n. (IT-0-BONN-15215), HF934352, HF934579, HF934236, HF934465, HF934795, HF934682; Am084, Spain, Los Picachones, Ardales, *Trissl, D. s.n.* (OSN 2006-1386-W), HF934353, HF934580, HF934237, HF934466, HF934796, HF934683; Am244, Tunisia, 45 km west of Beja, *Hammer, K. TUN-92/93* (GAT 3269), HE962507, HE859938, HF934238, HF934467, HF934797, HE859962; Am292, Spain, Andalusia, Sotogrande, *Trissl, D. s.n.* (OSBU 20072), HF934354, HF934581, –, –, –, –; ***Allium ursinum*** L. subsp. **ursinum**, *Ophioscorodon*, Am031, Germany, Thuringia, Keula, *Fritsch, R. s.n.* (GAT 1350), HF934356, HF934583, HF934239, HF934468, HF934798, HF934684; Am200, Germany, Lower Saxony, Bad Iburg, *Friesen, N. s.n.* (OSBU 19824), FR682003, FN551205, –, –, FN550393, –; Am322, Northern Ireland, Belfast, near Castle, *Ahr, T. s.n.* (OSN 2010-1800-W), FR682002, HF934584, HF934242, HF934471, HF934799, HF934685; ***Allium ursinum*** subsp. **ucrainicum** Oksner & Kleopow, *Ophioscorodon*, Am032, Bulgaria, near Kjustendil, *Schulze-Motel, J. BGR-97/2* (GAT 2322), FR693742, FN551224, HF934241, HF934470, HF934800, HE859950; Am078, Poland, Carpathian, Prague BG, s.n. (OSN 2009-1629-Z), HF934355, HF934582, HF934240, HF934469, HF934801, HF934686; ***Allium validum*** S.Watson, *Caulorhizideum*, Am049, U.S.A., California, Silver Lake, *McNeil, D. s.n.* (GAT 1779), HF934357, HF934585, HF934243, HF934472, HF934802, HF934687; ***Allium wallichii*** Kunth, *Bromatorrhiza*, Am066, China, Xizang, *Miehe, G. s.n.* (OSBU 11560), HE962502, HE859945, HF934244, HF934473, HF934803, HE859964; Am264, Nepal, Himalaya, *Miehe, G. s.n.* (OSN 2010-1140-W), HF934358, HF934586, HF934245, HF934474, HF934804, HF934688; Am329, China, Xizang, upper Sun Kosi, S of Nyalam, *Miehe, G. s.n.* (OSBU 21258), HF934359, HF934587, HF934246, HF934475, HF934805, HF934689; ***Allium zebdanense*** Boiss. & Noe, *Molium*, Am038, Turkey, Gewiehs GmbH s.n. (OSN 2003-1632-G), HF934360, HF934588, HF934247, HF934476, HF934806, HF934690; Am039, Turkey, Gewiehs GmbH s.n. (OSN 2003-1633-G), HF934361, HF934589, HF934248, HF934477, HF934807, HF934691. — *Allium* subg. **Microscordum: Allium monanthum** Maxim., *Microscordum*, Am017, Russia, Chabarovskij Kraj, mouth of Ussuri, *Levichev, I. s.n.* (GAT 5617), HF934301, –, HF934184, HF934413, HF934743, HF934635; Am018, Russia, Vladivostok, *Levichev, I. s.n.* (GAT 5618), HF934302, –, HF934185, HF934414, HF934744, HF934636; Am089, Russia, north of Vladivostok, *Barkalov, V. s.n.* (OSN 2006-1644-W), HF934303, –, HF934186, HF934415, HF934745, HF934637; — ***Allium*** subg. **Nectaroscordum: Allium bulgaricum** (Janka) Prodan, *Nectaroscordum*, Am085, Bulgaria, dist. Burgas, Nos Emine, *Pistrik, K. BGR-87/6* (GAT 2192), HF934258, –, –, –, –, –; ***Allium siculum*** Ucria, *Nectaroscordum*, Am246, Turkey, vilajet Antalya, Termessos, *Friesen, N., Fritsch, R.M. TUR-95/1* (GAT 5354), HF934332, –, HF934221, HF934450, HF934780, HF934667; ***Allium tripedale*** Trautv., *Nectaroscordum*, Am247, Armenia, Aragats massif, Hamberd, *Fritsch, R. GEO/ARM-2002/98* (GAT 6082), HF934350, –, HF934234, HF934463, HF934793, HF934680.