

Phylogeny and biogeography of *Allium* (Amaryllidaceae: Allieae) based on nuclear ribosomal internal transcribed spacer and chloroplast *rps16* sequences, focusing on the inclusion of species endemic to China

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- **Background and Aims** The genus *Allium* comprises more than 800 species, placing it among the largest monocotyledonous genera. It is a variable group that is spread widely across the Holarctic region. Previous studies of *Allium* have been useful in identifying and assessing its evolutionary lineages. However, there are still many gaps in our knowledge of infrageneric taxonomy and evolution of *Allium*. Further understanding of its phylogeny and biogeography will be achieved only through continued phylogenetic studies, especially of those species endemic to China that have often been excluded from previous analyses. Earlier molecular studies have shown that Chinese *Allium* is not monophyletic, so the goal of the present study was to infer the phylogeny and biogeography of *Allium* and to provide a classification of Chinese *Allium* by placement of Chinese species in the context of the entire phylogeny.
- **Methods** Phylogenetic studies were based on sequence data of the nuclear ribosomal internal transcribed spacer (ITS) and chloroplast *rps16* intron, analysed using parsimony and Bayesian approaches. Biogeographical patterns were conducted using statistical dispersal–vicariance analysis (S-DIVA).
- **Key Results** Phylogenetic analyses indicate that *Allium* is monophyletic and consists of three major clades. Optimal reconstructions have favoured the ancestors of *Amerallium*, *Anguinum*, *Vvedenskya*, *Porphyroprason* and *Melanocrommyum* as originating in eastern Asia.
- **Conclusions** Phylogenetic analyses reveal that *Allium* is monophyletic but that some subgenera are not. The large genetic distances imply that *Allium* is of ancient origin. Molecular data suggest that its evolution proceeded along three separate evolutionary lines. S-DIVA indicates that the ancestor of *Amerallium*, *Anguinum*, *Vvedenskya*, *Porphyroprason* and *Melanocrommyum* originated from eastern Asia and underwent different biogeographical pathways. A taxonomic synopsis of Chinese *Allium* at sectional level is given, which divides Chinese *Allium* into 13 subgenera and 34 sections.

Key words: *Allium*, biogeography, classification, ITS, molecular phylogeny, *rps16*.

INTRODUCTION

The genus *Allium* L. comprises more than 800 species (Fritsch *et al.*, 2010), making it one of the largest monocotyledonous genera; it is a variable group that is spread widely across the Holarctic region from the dry subtropics to the boreal zone. *Allium dregeanum* is the only exception; it is native to South Africa (De Wilde-Duyfjes, 1976). This genus has a major centre of diversity stretching from the Mediterranean Basin to Central Asia and Pakistan and a second less pronounced one located in western North America. It consists of perennial herbs mostly characterized by tunicated bulbs, narrow basal leaves, umbellate or head-like inflorescences, flowers with six free or almost free tepals, superior ovaries with one to several ovules per locule, septa often containing nectaries opening by pores at the base of the ovary, entire or three-cleft stigma, loculicidal capsule, rhomboidal or spheroidal black seeds, and an onion-like odour and taste due to the presence of cystine sulphoxides. The genus is diverse in cytology. The most common basic chromosome number is $x = 8$, but other numbers ($x = 7, 9, 10, 11$) and variation in ploidy also occurs (Traub, 1968; Friesen, 1992; Huang *et al.*, 1995;

Xu *et al.*, 1998; Zhou *et al.*, 2007). *Allium* contains many economically important species, including garlic, leek, onion, shallot, bunching onion, chives and Chinese chives cultivated as vegetables or spices, and species used as herbal crops, as traditional medicines and as ornamental plants (Fritsch and Friesen, 2002). *Allium* is a member of the family Amaryllidaceae J.St.-Hil., subfamily Allioideae Herb., tribe Allieae Dumort. (Fay and Chase, 1996; APG III, 2009; Chase *et al.*, 2009). After Fay and Chase (1996), Friesen *et al.* (2000) and Chase *et al.* (2009), *Allium* (including *Caloscordum* Herb., *Milula* Prain and *Nectaroscordum* Lindl.) is the only genus in tribe Allieae.

The history of infrageneric classification in *Allium* dates back to Linnaeus (1753) who accepted 30 species in three alliances. Later authors recognized an increasing number of infrageneric groups: six sections and 285 species (Regel, 1875, 1887); nine sections and 228 species for the former USSR (Vvedensky, 1935) alone; three subgenera, 36 sections and subsections, and about 600 species (Traub, 1968); six subgenera, and 44 sections and subsections (Kamelin, 1973); three subgenera and 12 sections (Stearn, 1980); five subgenera and 16 sections (Hanelt, 1990). A recent classification was

proposed by Hanelt *et al.* (1992), including six subgenera, 50 sections and subsections for 600–700 species based on a multidisciplinary approach including morphological, anatomical, karyological, serological and numerical investigations as well as studies of life cycles, distribution, ecology and isozyme data. Friesen *et al.* (2006) presented a new classification of the genus consisting of 15 subgenera and 72 sections for about 780 species based on their phylogenetic study. Many morphological and anatomical studies on *Allium* have also been performed, and numerous data have been published dealing with newly described taxa and regional revisions (e.g. Brullo *et al.*, 1991; Cheremushkina, 1992; Kamenetsky, 1992; Hanelt and Fritsch, 1994; Friesen, 1995; Mathew, 1996; Khassanov, 1997; Xu and Kamelin, 2000; Dale *et al.*, 2002; Fritsch, 2009; Fritsch and Friesen, 2009; Kovtonyuk *et al.*, 2009). All of the above-mentioned works have been helpful in establishing and assessing the evolutionary lineages in the genus. However, due to their close morphological similarities, over-reliance on dried specimens, remarkable degrees of polymorphism and disagreements regarding the taxonomic importance of specific morphological traits (Hanelt *et al.*, 1992; Khassanov and Fritsch, 1994; Khassanov, 1997; Mes *et al.*, 1997; Gregory *et al.*, 1998), there are still many gaps in our knowledge of infrageneric taxonomy and differentiation and evolution in the genus.

Recently, molecular approaches using plastid DNA and nuclear ribosomal DNA (nrDNA) sequences have been applied to understand the evolutionary processes and taxonomic relations within the genus. A first approach to structuring the genus *Allium* by molecular markers was published by Linne von Berg *et al.* (1996). The resulting phenogram largely confirmed the subgeneric classification based on an integration of morphological and other methods, but found that subgenera *Amerallium* Traub and *Bromatorrhiza* Ekberg could not be clearly distinguished. Later molecular studies focused on the classification and phylogeny of the entire genus *Allium* (Mes *et al.*, 1997; Dubouzet and Shinoda, 1999; He *et al.*, 2000; Fritsch and Friesen, 2002; Friesen *et al.*, 2006) or specific subgenera such as *Amerallium* (Samoylov *et al.*, 1995, 1999), *Melanocrommyum* (Webb & Berth.) Rouy (Dubouzet and Shinoda, 1998; Mes *et al.*, 1999; Gurushidze *et al.*, 2008, 2010; Fritsch *et al.*, 2010) and *Rhizirideum* (G.Don ex Koch) Wendelbo (Dubouzet *et al.*, 1997). Other researchers focused on the origins of major *Allium* crops (e.g. Friesen and Klaas, 1998; Friesen *et al.*, 1999; Blattner and Friesen, 2006), phylogenetic relationships between *Allium* and the monotypic Himalayan genus *Milula* Prain (Friesen *et al.*, 2000), the phylogeny of section *Cepa* (Mill.) Prokh (Gurushidze *et al.*, 2007), the phylogenetic position of western North American species and their adaptation to serpentine soils (Nguyen *et al.*, 2008), and the origins of *A. ampeloprasum* horticultural groups and a molecular phylogenetic analysis of the section *Allium* (Hirschegger *et al.*, 2010). However, relatively few species endemic to China were included in these investigations.

One hundred and thirty-eight species of *Allium* (50 endemic, five introduced) occur in China (Xu and Kamelin, 2000), accounting for about one-sixth of recognized species. These represent five subgenera, namely *Allium*, *Rhizirideum*, *Melanocrommyum*, *Bromatorrhiza* and *Caloscordum* (Herb.)

R.M.Fritsch (not the subgenus *Amerallium*) according to the treatment of the Gatersleben group (Hanelt *et al.*, 1992), and four basic chromosome numbers, $x = 7, 8, 10, 11$ (not $x = 9$). These characteristics indicate that Chinese *Allium* is of particular interest for students of speciation, classification and phylogeny. Further understanding of *Allium* phylogeny and biogeography will be achieved only through continued phylogenetic studies, especially of those species endemic to China that have often been excluded from previous analyses.

The internal transcribed spacer (ITS) region of nrDNA is a valuable source of phylogenetic information at the generic and subgeneric level (Baldwin, 1992; Baldwin *et al.*, 1995; Blattner, 2004; Hörandl *et al.*, 2005) and has also proved to be informative in *Allium* (Dubouzet and Shinoda, 1999; Friesen *et al.*, 2000, 2006; Gurushidze *et al.*, 2007, 2008; Nguyen *et al.*, 2008). The intron of the *rps16* gene was first used for phylogenetic studies by Oxelman *et al.* (1997), and it has been shown to provide good resolution at the generic levels (e.g. Ingram and Doyle 2003; Marazzi *et al.*, 2006).

In this study, we present the results of a molecular systematic study of *Allium* based on ITS and *rps16* intron sequences. Previous molecular studies have shown that the Chinese *Allium* is not monophyletic, so the goals of the present study were to (1) clarify the phylogenetic relationships between subgenera within the genus, (2) infer the biogeography of *Allium* and (3) provide an intrageneric classification of Chinese *Allium* based on the placement of these Chinese species in the context of the entire genus.

MATERIALS AND METHODS

Taxon sampling

In total, 341 taxa (60 ITS sequences were generated in this study) were included in the present study for the single-marker (ITS) *Allium* phylogenetic analysis. The ingroup comprised 331 taxa covering all subgenera and almost all sections of the genus (see Friesen *et al.*, 2006). *Ipheion uniflorum*, two *Tulbaghia* species, two *Nothoscordum* species and five *Dichelostemma* species were designated as outgroups according to previous phylogenetic analyses (Fay and Chase, 1996; Mes *et al.*, 1997; Fay *et al.*, 2000; Friesen *et al.*, 2000, 2006; Nguyen *et al.*, 2008). Fifty-five *rps16* sequences generated in this study were combined with five *rps16* sequences obtained from the GenBank database with a focus on Chinese *Allium*, with 58 ingroup taxa and two outgroup species, namely *Nothoscordum gracile* and *Tulbaghia violacea*.

All accessions in the collection stem from populations collected during field trips or from botanical gardens (Chengdu Botanical Garden and Kunming Botanical Garden for cultivated species and outgroups). Voucher specimens were deposited in the herbarium of the Sichuan University (SZ). Voucher information and GenBank accession numbers (GQ181063–GQ181108 and GU566611–GU566624 for ITS; GU566625–GU566679 for *rps16*) are listed in Appendix 1. ITS and *rps16* accessions obtained from GenBank are presented in Appendix 2.

DNA extraction, amplification and sequencing

Genomic DNA was extracted from silica gel-dried or fresh leaves using the method of Doyle and Doyle (1987). Primers ITS4 and ITS5 (White *et al.*, 1990) were used to amplify the ITS region. The PCR programme was as follows: 94 °C for 5 min; 30 cycles of 94 °C for 45 s, 55 °C for 45 s and 72 °C for 1 min; and 72 °C for 7 min. The *rps16* intron was amplified with primers rpsF and rpsR2 (Oxelman *et al.*, 1997) in accordance with the protocol of Marazzi *et al.* (2006). PCR products were separated using 1.5 % (w/v) agarose TAE gel and purified using Wizard PCR preps DNA Purification System (Promega, Madison, WI, USA) following the manufacturer's instructions. The purified PCR products were sequenced in an ABI 310 Genetic Analyzer (Applied Biosystems Inc.) in both directions using the PCR primers.

Sequence comparisons and phylogenetic analyses

DNA sequences were initially aligned using the default pairwise and multiple alignment parameters in Clustal X (Jeanmougin *et al.*, 1998) and then rechecked and adjusted manually as necessary using MEGA4 (Tamura *et al.*, 2007). Gaps were positioned to minimize nucleotide mismatches and treated as missing data in phylogenetic analyses.

Exploratory phylogenetic analyses were conducted separately with each dataset (ITS matrix with a global sampling of *Allium* species, a subset of ITS matrix including only 60 taxa focusing on Chinese *Allium*, *rps16* matrix including the same 60 taxa, and the combined data matrix for the 60 taxa common to both ITS and *rps16*). Results from the ITS (a subset of ITS matrix) and *rps16* analyses (Supplementary Data Figs 1 and 2, available online) did not show any major topological conflict and provided higher resolution when analysed together than separately. Consequently, molecular datasets were combined (ITS + *rps16*) in a single analysis for Chinese *Allium*. Finally, phylogenetic analyses for the individual data matrix (ITS matrix with a global sampling of *Allium* species) and combined datasets (ITS and *rps16*) were conducted by employing maximum-parsimony (MP) criteria and Bayesian inference (BI), using the programs PAUP* version 4.0b10 (Swofford, 2003) and MrBayes version 3.1.2 (Ronquist and Huerstenbeck, 2003), respectively. For MP, heuristic searches were carried with 1000 random addition sequence replicates. One tree was saved at each step during stepwise addition, tree-bisection-reconnection (TBR) was used to swap branches, and the maximum number of trees was set to 10 000. All characters were unordered and equally weighted. Gaps were treated as missing data. Bootstrap values were calculated from 1000 000 replicate analyses using 'fast' stepwise-addition of taxa and only those values compatible with the majority-rule consensus tree were recorded. Prior to a Bayesian analysis, MrModeltest version 2.2 (Nylander, 2004) was used to select a best-fit model of nucleotide substitution, and the GTR + I + G model under the Akaike information criterion was selected. The Bayesian Markov chain Monte Carlo (MCMC) algorithm was run for 2000 000 generations with one cold chain and three heated chains, starting from random trees and sampling trees every 100 generations. The first 5000 trees were considered as the

burn-in and were discarded. A 50 % majority-rule consensus tree of the remaining trees was produced.

The incongruence length difference (ILD) test of ITS and *rps16* intron datasets for the same 60 taxa was carried out in PAUP* (Farris *et al.*, 1994) to assess potential conflicts between different DNA fragments. This test was implemented with 100 partition-homogeneity test replicates, using a heuristic search option with simple addition of taxa, TBR branch swapping and MaxTrees set to 1000.

Biogeographical analysis

Dispersal–vicariance analysis (DIVA) (Ronquist, 1996, 1997, 2001) is one of the most widely used methods for inferring biogeographical histories. Although model-based methods for inferring biogeography are available (e.g. Ree *et al.*, 2005; Ree and Smith, 2008; Sanmartín *et al.*, 2008), DIVA remains popular because it provides rapid results, requires little prior information, and gives results comparable with the model-based likelihood method Lagrange of Ree *et al.* (2005) and Ree and Smith (2008) (e.g. Ree *et al.*, 2005; Burbrink and Lawson, 2007; Velazco and Patterson, 2008; Xiang and Thomas, 2008). Statistical DIVA (S-DIVA) (Yu *et al.*, 2010) is a program which complements DIVA, implements the methods of Nylander *et al.* (2008) and Harris and Xiang (2009), and determines statistical support for ancestral range reconstructions using a novel method. Both the previous studies (Fritsch, 2001; Fritsch and Friesen, 2002; Friesen *et al.*, 2006) and our molecular analyses indicate that evolution in *Allium* proceeded along three separate evolutionary lines, so we attempted to carry out a geographical analysis for each separate evolutionary line. Three separate taxonomic subsets with a focus on each evolutionary line with the ITS data realigned where necessary were obtained and then phylogenetic analyses for each evolutionary line were conducted by employing BI with the methods described above. Because of the lack of resolution within the third evolutionary line, however, we could not analyse its biogeography using S-DIVA. So finally, potential biogeographical scenarios of several specific subgenera [including *Amerallium*, *Anguinum*, *Vvedenskya* (Kamelin) R.M.Fritsch, *Porphyroprason* (Ekberg) R.M.Fritsch and *Melanocrommyum*] in the first and second evolutionary line were tested using statistical dispersal–vicariance analysis implemented in S-DIVA. Distribution areas of these subgenera and their close allies were defined according to the World Checklist of Selected Plant Families maintained by the Royal Botanic Gardens, Kew, UK (<http://apps.kew.org/wcsp/home.do>) and taxonomic and geographical studies of these *Allium* species (e.g. Xu and Kamelin, 2000; Dale *et al.*, 2002). S-DIVA requires a fully resolved tree, and therefore polytomies were arbitrarily resolved. Allowing reconstruction, two optimizations were performed: first, with an unconstrained number of unit areas for each ancestral node; and second, with the number of ancestral areas restricted to two. The rationale for such a constraint is that vicariance is a proximate consequence of dispersal. Moreover, extant taxa used in the analyses rarely occur in more than two individual areas. Extinction events in DIVA are usually inferred *ad hoc* after the analysis in order to explain widespread ancestral distributions among areas that are not geographically adjacent (Sanmartín, 2003)

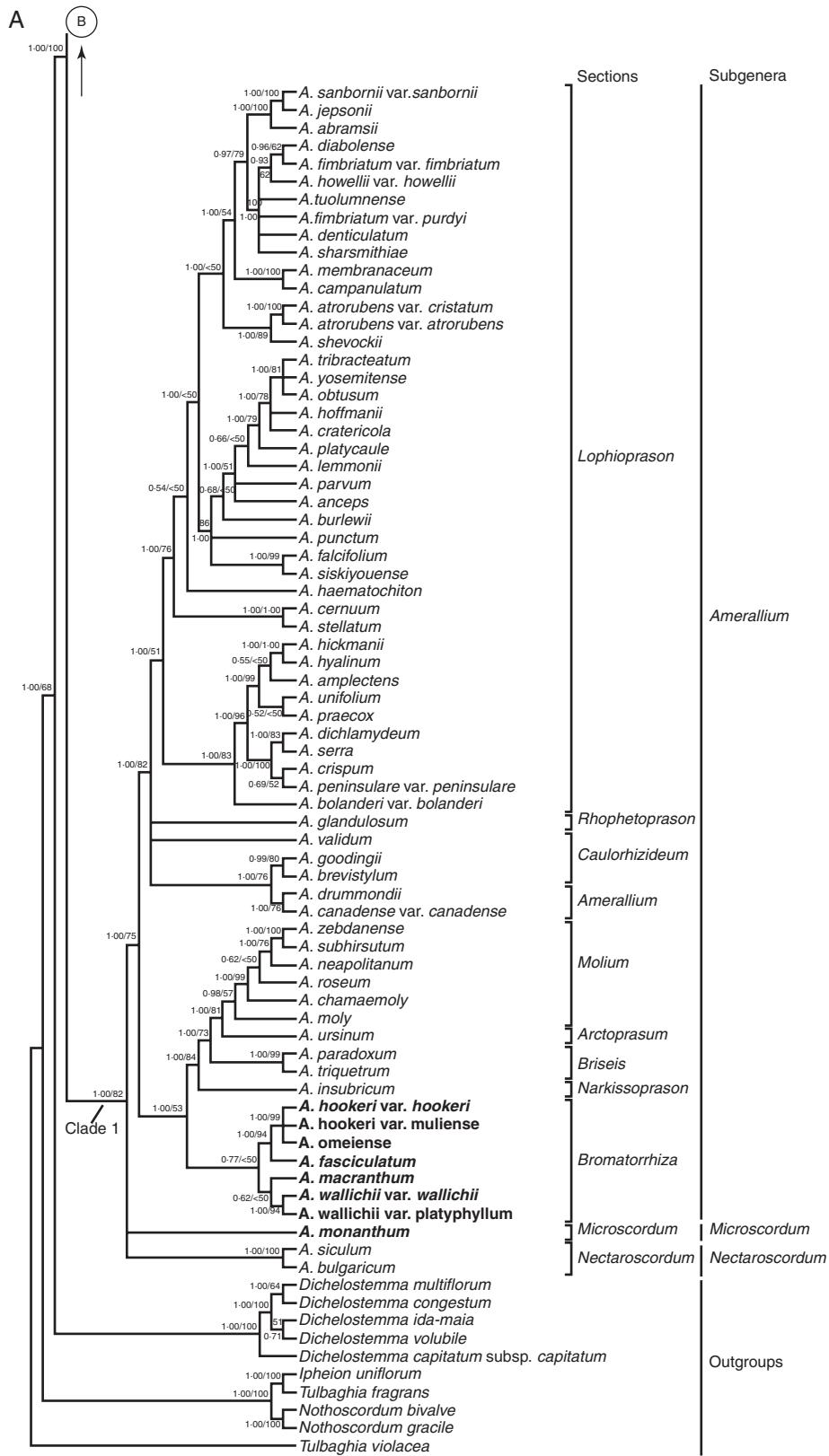


FIG. 1. Phylogenetic tree resulting from a Bayesian analysis of the ITS sequences from species of *Allium* and ten outgroup species. The subgeneric and sectional classification according to Hanelt *et al.* (1992), Dubouzet and Shinoda (1999), Friesen *et al.* (2006), Gurushidze *et al.* (2008), Nguyen *et al.* (2008), Kovtonyuk *et al.* (2009), Fritsch *et al.* (2010) and our own results is indicated on the right. Values along branches represent Bayesian posterior probabilities (PP) and parsimony bootstrap (BS), respectively. Scientific names given in bold are those endemic to China, in bold italics those distributed in China and other areas, and in italics species distributed in other areas of the world.

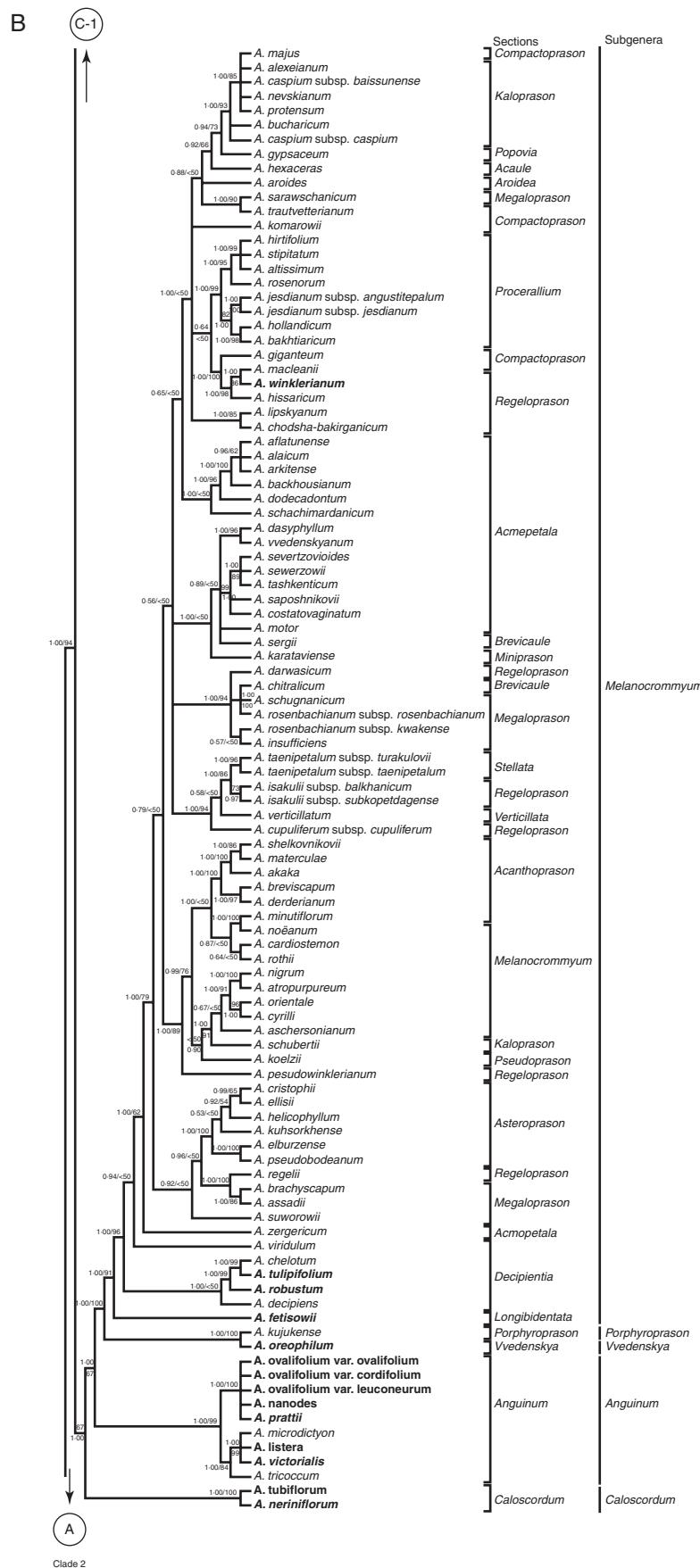


FIG. 1. Continued

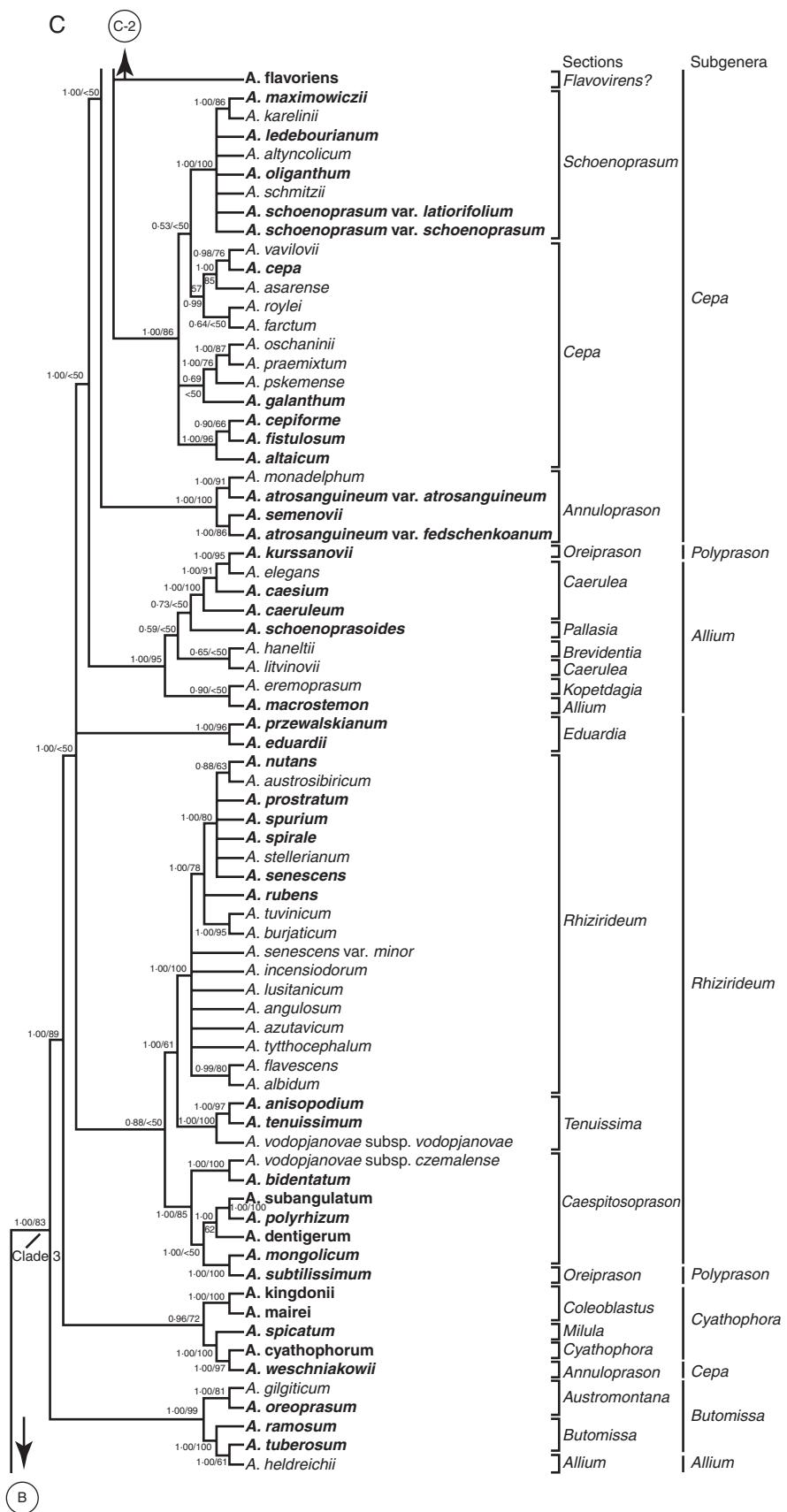


FIG. 1. Continued

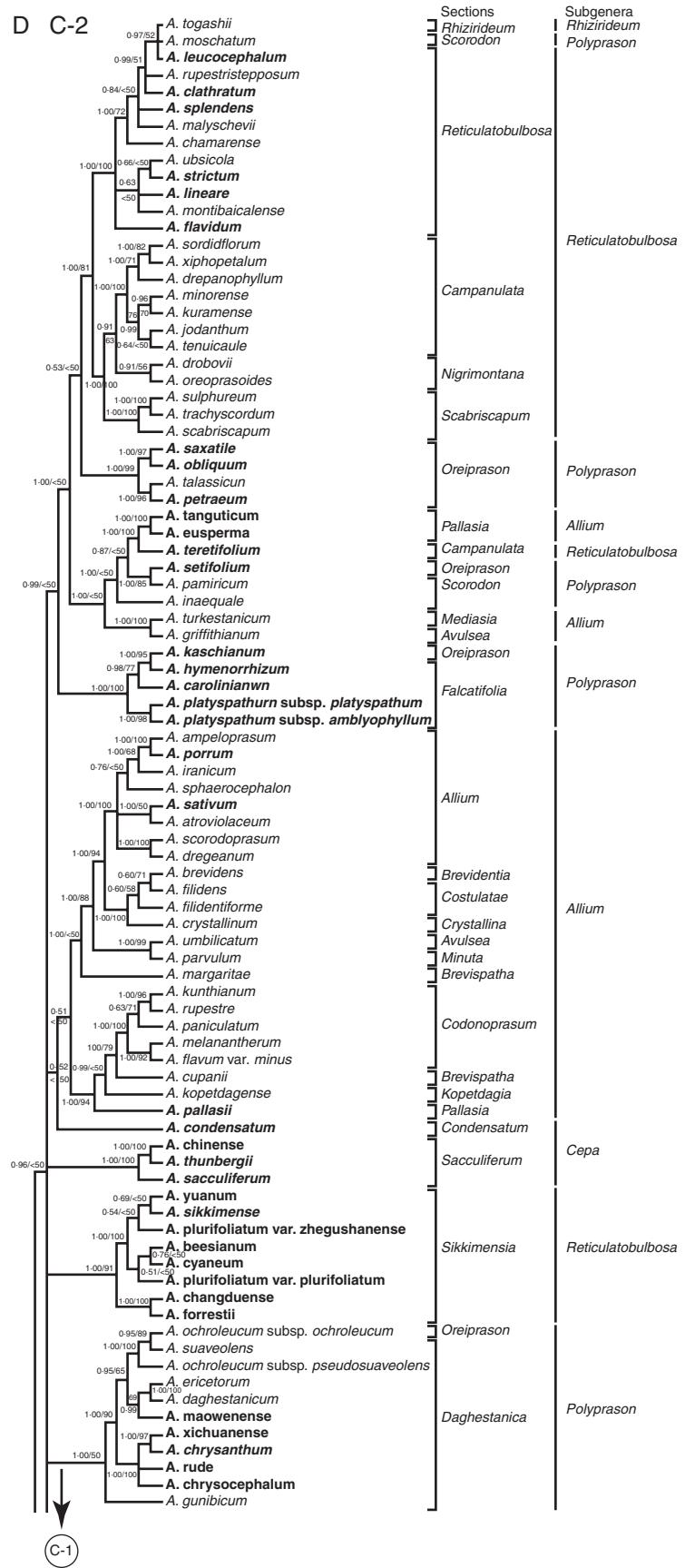


FIG. 1. Continued

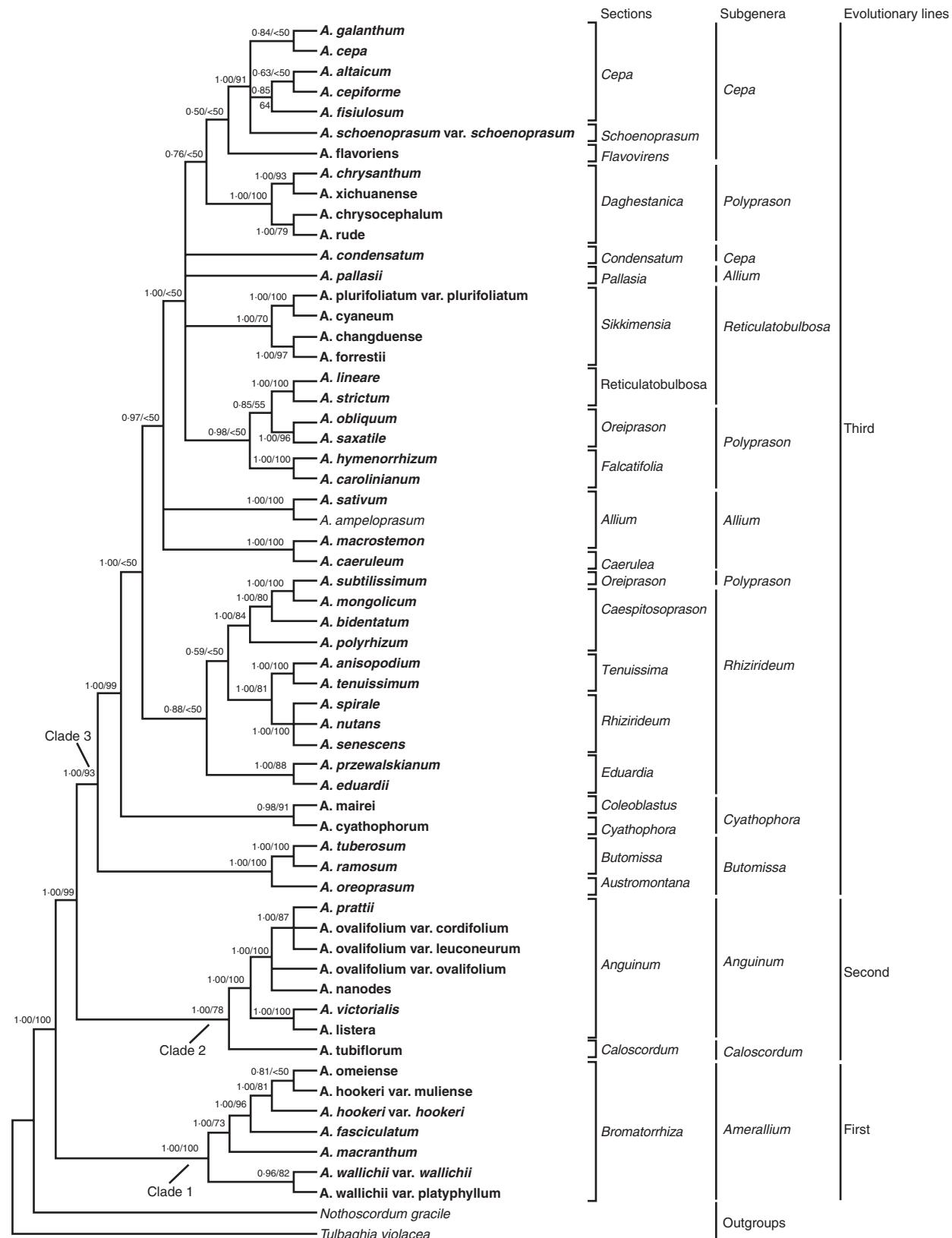


FIG. 2. Phylogenetic tree resulting from a Bayesian analysis of combined sequence data (ITS and *rps16*) focusing on Chinese *Allium*. The subgeneric and sectional classification according to Hanelt *et al.* (1992), Dubouzet and Shinoda (1999), Friesen *et al.* (2006), Nguyen *et al.* (2008), Kovtonyuk *et al.* (2009) and our own results is indicated on the right. Values along branches represent Bayesian posterior probabilities (PP) and parsimony bootstrap (BS), respectively. Scientific names given in bold are those endemic to China, in bold italics those distributed in China and other areas, and in italics species distributed in other areas of the world.

and long-distance dispersals are inferred after the analysis by considering area relationships (Calviño *et al.*, 2008) as in S-DIVA. For those subgenera not subject to a statistical dispersal-vicariance analysis (because resolution of relationships was poor), we speculate on the possible distribution of ancestors.

RESULTS

Molecular datasets

Two different datasets were generated: dataset 1 (only ITS) with a worldwide sampling of *Allium* species, and dataset 2 (ITS and *rps16*) focusing on Chinese *Allium*. Among the *Allium* sequences analysed, the ITS region varied in length from 589 bp (*A. shevockii*) to 665 bp (*A. oreoprasoides*), and the *rps16* sequences ranged from 556 bp (*A. caeruleum*) to 901 bp (*A. eduardii*). The outgroup species had relatively long sequences, with ITS sequences ranging from 643 bp (*Dichelostemma volubile*) to 672 bp (*Nothoscordum bivalve*) and *rps16* sequences ranging from 836 bp (*N. gracile*) to 876 bp (*T. violacea*). After introducing the necessary gaps, the ITS alignment (dataset 1) was 774 bp in length and resulted in 102 constant characters and 672 variable characters, 606 of which were potentially parsimony-informative. The mean G + C content of the ITS region was 46.67 %. Dataset 2 is a taxonomic subset of dataset 1 with a focus on Chinese *Allium* with the ITS data realigned where necessary and the addition of *rps16* data. The subset of ITS sequences produced a matrix 706 bp in length, and for which 179 characters were constant, 92 autapomorphic and 435 potentially parsimony-informative. The mean G + C content of the *rps16* region was 46.99 %. The aligned *rps16* sequences produced a matrix 1148 bp in length, and for which 703 characters were constant, 208 autapomorphic and 237 potentially parsimony-informative. The mean G + C content of the *rps16* region was 30.47 %. The combined aligned dataset was 1854 bp in length, with 882 characters constant, 300 autapomorphic and 672 potentially parsimony-informative; the mean G + C content was 37.69 %.

Phylogenetic analyses

For dataset 1, trees inferred from BI and MP showed no significant difference in their topologies, and therefore only the Bayesian tree with posterior probabilities (PP) and bootstrap support values (BS) is shown in Fig. 1. In all analyses, the genus *Allium* proved to be monophyletic and robustly separated from the outgroup species (PP = 1.00, BS = 100 %). Three major clades were found within *Allium*: the first major clade (PP = 1.00, BS = 82 %) comprising subgenera *Nectaroscordum* (Lindl.) Asch. & Graebn. ($x = 9$), *Amerallium* ($x = 7, 8, 9, 10, 11$) and *Microcordum* (Maxim.) N.Friesen ($x = 8$); the second major clade (PP = 1.00, BS = 67 %) comprising subgenera *Caloscordum*, *Anguinum*, *Vvedenskya*, *Porphyroprason* and *Melanocrommyum* (all $x = 8$); and the third major clade (PP = 1.00, BS = 83 %) comprising subgenera *Butomissa* (Salisb.) N.Friesen, *Cyathophora* (R.M.Fritsch) R.M.Fritsch, *Rhizirideum* (G.Don ex Koch) Wendelbo *sensu stricto* (s.s.), *Allium*, *Cepa* (Mill.) Radić, *Reticulatobulbosa*

(Kamelin) N.Friesen and *Polyprason* Radić (all $x = 8$). For dataset 2, the ILD test conducted on the combined data matrix of common ITS and *rps16* accessions was significant (ILD probability value = 0.01), indicating that the two data sets are heterogeneous. The ITS and *rps16* trees placed some species (mainly those in the third evolutionary line) in contradictory positions, which may reflect reticulate events in the evolutionary history of these species, the potential mechanism of which is beyond the scope of the present paper. However, the combined tree was better resolved than any of the separate ITS and *rps16* trees, and trees derived from the combined dataset were mostly consistent with respect to their major groupings; we therefore describe the combined tree here. These results corroborated the irrelevance of the significant ILD values in terms of combinability, as proposed or confirmed by previous authors (Reeves *et al.*, 2001; Barker and Lutzoni, 2002; Inda *et al.*, 2008; Mitsui *et al.*, 2008). The topology of the Bayesian tree was similar to that of the MP consensus tree. The 50 % majority-rule consensus tree from BI is presented in Fig. 2, with PP and BS support values. The monophyly of *Allium* was also recovered by the combined analysis (PP = 1.00, BS = 100 %). Three major clades were detected in this tree: the first major clade (PP = 1.00, BS = 100 %) containing taxa from subgenera *Amerallium*, the second major clade (PP = 1.00, BS = 78 %) with species from subgenera *Caloscordum* and *Anguinum*, and the third major clade (PP = 1.00, BS = 93 %) with species from subgenera *Butomissa*, *Cyathophora*, *Rhizirideum*, *Allium*, *Cepa*, *Reticulatobulbosa* and *Polyprason*.

The major clades resulting from the combined analysis were similar to those based on ITS, and are therefore not discussed separately. In the first major clade, the *Nectaroscordum* clade including two species was well supported (Fig. 1A, PP = 1.00, BS = 100 %) and the *Microcordum* clade was represented by *A. monanthum*. The *Amerallium* clade is the largest in the first major clade. Within this clade, the New World *Amerallium* clade is sister to the Old World *Amerallium* clade (Fig. 1A, PP = 1.00, BS = 75 %). The New World *Amerallium* clade (Fig. 1A, PP = 1.00, BS = 82 %) contains two groups. One group includes several subclades corresponding to sections *Amerallium* Traub + *Caulorhizideum* Traub + *Rhopetoprasón* Traub with species native to the mid-west and south-west USA (with a few exceptions, e.g. *A. validum*). The other group includes the monophyletic section *Lophioprasón* Traub (Fig. 1A, PP = 1.00, BS = 51 %) with species restricted to western North America (the only exceptions are *A. cernuum* and *A. stellatum*). Within the Old World *Amerallium* clade (Fig. 1A, PP = 1.00, BS = 53 %), two sister subclades are evident, one with species from the Mediterranean region and the other with species from the Himalayas and south-west China. In the Mediterranean subclades, section *Narkissoprasón* Kam. is sister to a clade containing sections *Briseis* (Salisb.) Stearn, *Arctoprasum* Kirsch. and *Molium* G.Don ex Koch (Fig. 1A, PP = 1.00, BS = 84 %). In the Himalayas and south-west China clade, *A. wallichii* var. *wallichii*, *A. wallichii* var. *platyphyllum* and *A. macranthum* are sister to a clade containing other species of section *Bromatorrhiza* Ekberg (Fig. 1A, PP = 0.77, BS < 50 %). In the combined (ITS and *rps16*) analyses, *A. wallichii* var. *wallichii* and *A. wallichii* var. *platyphyllum* are sister to a clade comprising *A. macranthum*, *A. omeiene*,

A. fasciculatum, *A. hookeri* var. *hookeri* and *A. hookeri* var. *muliense* (Fig. 2, PP = 1·00, BS = 100 %).

In the second major clade, the *Caloscordum* clade was represented by *A. neriniflorum* and *A. tubiflorum* in the ITS analysis and by *A. tubiflorum* in the combined analysis. The *Anguinum* clade contains two sister groups (Fig. 1B, PP = 1·00, BS = 99 %). In one sister group, species from Eurasia (*A. listera*, *A. microdictyon* and *A. victorialis*) form a trichotomy (Fig. 1B, PP = 1·00, BS = 99 %) and this trichotomy is sister to the north-eastern North American species *A. tricoccum* (Fig. 1B, PP = 1·00, BS = 84 %). In the other sister group, species from eastern Asia (*A. ovalifolium* var. *ovalifolium*, *A. ovalifolium* var. *cordifolium*, *A. ovalifolium* var. *leuconeurum*, *A. nanodes* and *A. prattii*) form a polytomy (Fig. 1B, PP = 1·00, BS = 100 %; Fig. 2, PP = 1·00, BS = 100 %). The *Vvedenskya* and *Porphyroprason* clades were represented by *A. kujukense* and *A. oreophilum*, respectively. In the *Melanocrommyum* clade, *Allium fetisowii* from section *Longibidentata* (R.M.Fritsch) R.M.Fritsch is at the base of the clade and is sister to the remaining species of *Melanocrommyum*. Then, *A. decipiens*, *A. robustum*, *A. tulipifolium* and *A. chelotum* from section *Decipientia* (Omelczuk) R.M.Fritsch, *A. viridulum* from section *Decipientia* and *A. zergericum* from section *Acnopetala* R.M.Fritsch form three successive lineages sister to the remaining clade (Fig. 1B, PP = 1·00, BS = 79 %).

Splits within the third major clade are complex. In the *Butomissa* clade, section *Butomissa* is sister to section *Austromontana* (Fig. 1C, PP = 1·00, BS = 99 %; Fig. 2, PP = 1·00, BS = 100 %). Noteworthy here is that *A. heldreichii* from subgenus *Allium* section *Allium* is sister to *A. tuberosum* (Fig. 1C, PP = 1·00, BS = 61 %). As only one sequence of *A. heldreichii* from GenBank was included in the present study, additional sequences from multiple exemplars would be necessary to test this relationship. In the *Cyathophorum* clade, section *Coleoblastus* is sister to a clade comprising section *Cyathophora* and the monotypic section *Milula* (Fig. 1C, PP = 0·96, BS = 72 %). However, unexpected relationships were recovered for *A. cyathophorum* and *A. weschniakowii* from subgenus *Cepa* section *Annulopraspon* T.V.Egorova (Fig. 1C, PP = 1·00, BS = 97 %). A larger sample of species and more molecular markers would have to be analysed to exclude technical causes for the incongruence between molecular data and the morphology-based taxonomy and to test the relationships further. In the *Rhizirideum* clade, section *Eduardia* is sister to a group comprising *Rhizirideum*, *Caespitosoprason* and *Tenuissima* (Fig. 2, PP = 0·88, BS < 50 %). Section *Caespitosoprason* is sister to the clade consisting of sections *Tenuissima* and *Rhizirideum* (Fig. 1C, PP = 0·88, BS < 50 %; Fig. 2, PP = 0·59, BS < 50 %). Species from section *Rhizirideum* form polytomies (Fig. 1C, PP = 1·00, BS = 100 %) and their relationship is beyond the resolution of the ITS. *Allium subtilissimum* from subgenus *Polyprason* section *Oreipraspon* F.Herm. is sister to *A. mongolicum* in our study (Fig. 1C, PP = 1·00, BS = 100 %; Fig. 2, PP = 1·00, BS = 100 %), and additional sequences from multiple exemplars of this species would have to be analysed to exclude technical causes. Species from subgenera *Allium*, *Cepa*, *Reticulatobulbosa* and *Polyprason* form a large clade (Fig. 1C, PP = 1·00, BS < 50 %; Fig. 2, PP =

0·97, BS < 50 %). Within this large clade, *A. macrostemon*, *A. eremoprasum*, *A. haneltii*, *A. schoenoprasoides*, *A. kurssanovii*, four species from section *Caerulea* (Omelcz.) F.O.Khassanov and four species from section *Annulopraspon* form two successive lineages sister to all other examined members of these four subgenera (Fig. 1C, PP = 1·00, BS < 50 %). The remaining members are divided into seven subclades and form a large polytomy.

Biogeographical analysis

The following five areas were considered for biogeographical analysis of *Amerallium* and its allies: (A) eastern Asia, (B) the Mediterranean region, (C) central North America, (D) western North America and (E) eastern North America. The optimal reconstruction (Fig. 3) required eight dispersals to explain the present distribution of *Amerallium* and their allies and favoured the ancestor of *Amerallium* as having originated in eastern Asia (node 1; A: 64·91 %, AB: 35·08 %). The most favoured reconstructions at node 2 (AD: 63·31 %, BD: 36·69 %) indicated both eastern Asia and North America as ancestral areas for *Amerallium*. For *Anguinum*, *Vvedenskya*, *Porphyroprason*, *Melanocrommyum* and their allies, the following seven areas were defined: (A) eastern Asia, (B) eastern North America, (C) western North America, (D) Europe and Siberia, (E) West Asia and adjacent areas, (F) Central Asia and (G) the Mediterranean. The optimal reconstruction (Fig. 4) required 26 dispersals to explain the present distribution of *Anguinum*, *Vvedenskya*, *Porphyroprason*, *Melanocrommyum* and their allies and indicated that their ancestor originated in eastern Asia (node 1). The reconstruction of ancestral areas suggested an eastern Asia origin for *Anguinum* (node 13) and a central Asian origin for *Vvedenskya*, *Porphyroprason* and *Melanocrommyum* (node 3).

DISCUSSION

Variation in the ITS and rps16 intron sequences

Genetic distances in ITS and *atpB-rbcL* sequences are apparently high (Klaas and Friesen, 2002) and in genetic variation the genus *Allium* resembles plant families in other groups of the angiosperms (Baldwin *et al.*, 1995). Our analysis suggests large genetic distances are also found in the plastid *rps16* intron (up to 9 %). These findings imply that the genus *Allium* is of ancient origin and molecular evolution has not been accompanied with a rise in pronounced morphological divergence (Friesen *et al.*, 2006). The wide area of distribution and pronounced molecular differences of *Allium* indicate that this genus was probably already well differentiated in the early Tertiary (Hanelt *et al.*, 1992; Dubouzet and Shinoda, 1999). Janssen and Bremer (2004) reported that divergence within the crown group Amaryllidaceae began about 87 Mya, providing additional evidence. *Allium* could be one of the many herbaceous groups that, according to Tiffney (1985), formed part of the floor of the boreotropical forests that covered northern latitudes during the Eocene.

In contrast, our data show some species (e.g. *A. ovalifolium* var. *ovalifolium*, *A. ovalifolium* var. *cordifolium*, *A. ovalifolium*

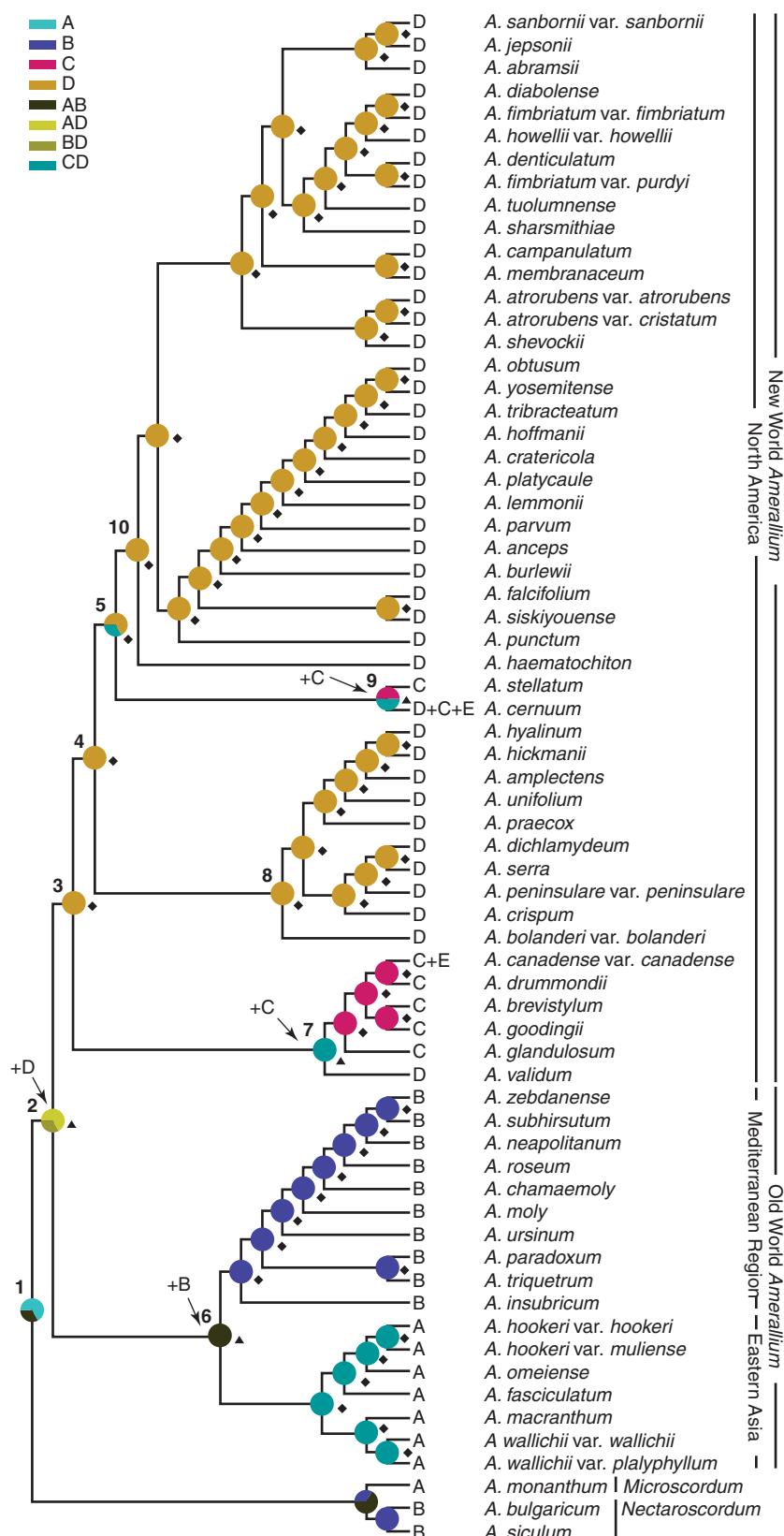


FIG. 3. Dispersal–vicariance scenarios for *Amerallium* and its allies reconstructed by statistical dispersal–vicariance (S-DIVA) optimization with the maximum number of area units set to two. The phylogeny is a Bayesian tree with ambiguities resolved arbitrarily. Pie charts at internal nodes represent the marginal probabilities for each alternative ancestral area derived by using S-DIVA. Triangle: vicariance event; rhomb: duplication event (speciation within the area); arrow (+): dispersal event. Letters denote area units as described in the text.

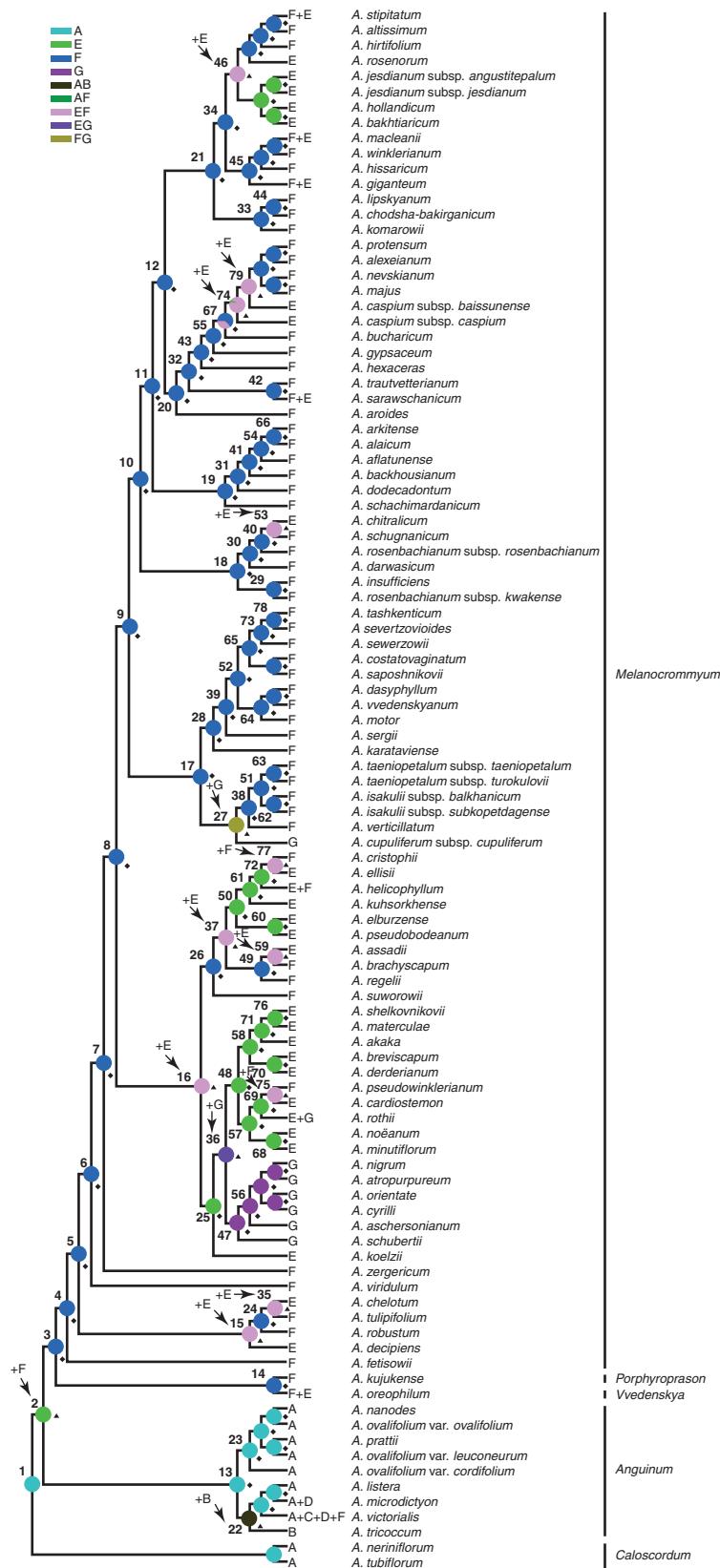


FIG. 4. Dispersal–vicariance scenarios for *Anguinum*, *Vvedenskya*, *Porphyroprason*, *Melanocrommyum* and their allies reconstructed by statistical dispersal–vicariance (S-DIVA) optimization with the maximum number of area units set to two. The phylogeny is a Bayesian tree with ambiguities resolved arbitrarily. Pie charts at internal nodes represent the marginal probabilities for each alternative ancestral area derived by using S-DIVA. Triangle: vicariance event; rhomb: duplication event (speciation within the area); arrow (+): dispersal event. Letters denote area units as described in the text.

var. *leuconeum*, *A. nanodes* and *A. prattii*) share almost identical ITS and/or *rps16* intron sequences, and the low average pairwise genetic distances in terminal groups consisting of several species suggest that these groups underwent recent radiation.

Phylogeny within the genus Allium

The results presented here robustly support the earlier finding that *Allium* is monophyletic (Friesen *et al.*, 2006). Previous evidence for three main evolutionary lines in the evolution of *Allium* reported in Fritsch (2001), Fritsch and Friesen (2002) and Friesen *et al.* (2006) has been largely corroborated in our present work. Our molecular data allow us to conclude that *Allium* evolution proceeded along three separate evolutionary lines. The most ancient line consists of only bulbous plants from subgenera *Nectaroscordum*, *Microscordum* and *Amerallium* (Fig. 1A), which rarely produce a notable rhizome (Fritsch and Friesen, 2002). The second evolutionary line comprises subgenera *Caloscordum*, *Anguinum*, *Vvedenskya*, *Porphyroprason* and *Melanocrommyum* (Fig. 1B), the third evolutionary line comprises subgenera *Butomissa*, *Cyathophora*, *Rhizirideum*, *Allium*, *Cepa*, *Reticulatobulbosa* and *Polyprason* (Fig. 1C), and these two evolutionary lines contain both rhizomatous and bulbous taxa.

Phylogeny in the first evolutionary line

In the first evolutionary line (Fig. 1A), *Amerallium* is sister group to either *Microscordum* or *Nectaroscordum* and their close affinities are also supported by some common characters of leaf anatomy (Fritsch, 1988).

Nectaroscordum comprises species distributed in the eastern Mediterranean. Lindley (1836) recognized this group as the segregated genus *Nectaroscordum*; this taxonomy induced a great deal of controversy. Some authors (Vvedensky, 1935; Traub, 1968, 1972; Dahlgren *et al.*, 1985; Mes *et al.*, 1997; Friesen *et al.*, 2006) considered *Nectaroscordum* to be a subgenus of *Allium*, while others (Stearn, 1978; Davis, 1984; Hanelt *et al.*, 1992) excluded *Nectaroscordum* from classifications of the genus *Allium*. Our phylogenetic analysis supports inclusion of *Nectaroscordum* as a separate subgenus in *Allium* and indicates its close relationship with *Amerallium* and *Microscordum*. Its subgeneric level was also suggested in previous molecular and morphological studies. Phylogenetic analysis of *rbcL* sequences (Fay and Chase, 1996) and ITS sequences (Dubouzet and Shinoda, 1998; Friesen *et al.*, 2006) affirmed that *Nectaroscordum* was closely related to *Allium*. The vascular bundles of *Nectaroscordum* and *Amerallium* are arranged in one row (Traub, 1968) and the laticifers in subgenera *Amerallium* and *Nectaroscordum* are hypodermal in the bulb scale (Traub, 1972). The joint occurrence of several specific characters such as large and three- to seven-veined tepals, a wider than long ovary, multiovulate locules, heavy seed with three sharp edges, a wide secretory channel and a basic chromosome number of $x = 9$ implies a long and separate phylogenetic history of this subgenus (Stearns, 1955, 1978; Mathew and Baytop, 1984; Fritsch, 1992b; Friesen *et al.*, 2006).

Microscordum is characterized by one- or two-flowered inflorescences, feathery ends of the stigmatic lobes and the occurrence of dioecy. This monotypic eastern Asian group shares similar morphological characters of bulbs, bulb tunics, leaves and flowers with species of subgenus *Amerallium* (Friesen *et al.*, 2006). Plants of *A. monanthum* were shown to constitute a polyploid complex, consisting of diploid, triploid and tetraploid individuals based on the basic chromosome number $x = 8$. Such cytological peculiarities form the background of the complex sexuality and predominant asexual reproduction of *A. monanthum* (Noda and Kawano, 1988; Kawano *et al.*, 2005). Our molecular data verify its systematic position close to *Amerallium* and *Nectaroscordum*.

Amerallium is the largest subgenus in the first evolutionary line and is extremely diverse morphologically and ecologically. Morphological synapomorphies for *Amerallium* include one row of vascular bundles, absence of palisade parenchyma and a subepidermal position of laticifers (Fritsch, 1988). Furthermore, strong serological affinities and the predominant basic chromosome number of $x = 7$ strongly support its separate status, although $x = 8, 9, 10$ and 11 (Traub, 1968; Huang *et al.*, 1995; Xu *et al.*, 1998) also occur in several groups. The results provide additional support for earlier findings that *Amerallium* is monophyletic (Samoylov *et al.*, 1995; Dubouzet and Shinoda, 1999) and further verify its close relationships with subgenus *Microscordum* and *Nectaroscordum* (Friesen *et al.*, 2006). In accordance with studies of Dubouzet and Shinoda (1999), our molecular data underline the existence of two distinct biogeographical clades, namely the Old World clade and the New World clade. This also agrees with a uniform electrophoretic banding pattern of salt-soluble seed storage proteins (Maass, 1992). Furthermore, the present results indicate that *Amerallium* comprises three isolated geographical groups: one consisting mainly of all *Allium* species native to North America (New World) and the remainder containing two smaller groups from the Mediterranean region and eastern Asia (Old World).

Phylogeny in the second evolutionary line

In the second evolutionary line (Fig. 1B), subgenus *Caloscordum* is sister to *Anguinum*, *Vvedenskya*, *Porphyroprason* and *Melanocrommyum*. *Anguinum* is sister to *Vvedenskya*, *Porphyroprason* and *Melanocrommyum*. *Vvedenskya* and *Porphyroprason* form a clade sister to the large clade consisting of the *Melanocrommyum* species.

Caloscordum is an oligotypic group with three species restricted to eastern Asia (Hanelt *et al.*, 1992). Its different geographical distribution, anatomy of the scape and root and structure of septal nectaries (Fritsch, 1992a, b, 1993), three-lobed stigma, peculiar ultrastructure of testa (Kruse, 1992b) and winter dormancy (Pistrick, 1992) justify its delimitation as a separate subgenus near *Melanocrommyum* (Ohri *et al.*, 1998). Our molecular data indicate that *Anguinum*, *Vvedenskya*, *Porphyroprason* and *Melanocrommyum* are equally related to *Caloscordum* through their common ancestor and *Caloscordum* is monophyletic. It shows an affinity with *Melanocrommyum* on the basis of multiovulate locules, seed weight, leaf arrangement, subterranean leaf sheaths,

partially joined tepals and the presence of relatively large inner vascular bundles in the scapes (Friesen *et al.*, 1986; Hanelt *et al.*, 1992; Fritsch, 1993; Hanelt and Fritsch, 1994). Simple characters of seed testa cells (Kruse, 1984, 1988) support its relationship with subgenus *Anguinum* morphologically.

Anguinum has a disjunct distribution in high mountains from south-western Europe to eastern Asia and in north-eastern North America (Fritsch and Friesen, 2002). It is characterized by specific root anatomical characters (Fritsch, 1992a), leaf and bulb structure (Pastor and Valdes, 1985), hypogeal seed germination and *A. victorialis*-type seedlings (Druselmann, 1992), uniovulate locules (Hanelt, 1992), narrow, branched and lengthwise-twisted septal nectaries (Fritsch, 1992b) and a short vegetative period with adaptation to the light regime under deciduous forest conditions (Pistrick, 1992). Species of *Anguinum* also share the basic chromosome number $x = 8$, the 2A type karyotype and similar metaphase chromosomes (Jing *et al.*, 1999). Based on the consistency of its morphological, anatomical and cytological characteristics, it is thus a rather distinct and specialized group. According to our molecular studies, *Anguinum* is monophyletic and shares a most recent common ancestor with *Vvedenskya*, *Porphyroprason* and *Melanocrommyum* and is the sister group to *Caloscordum*. Its simple seed testa sculpture (Kruse, 1984, 1988), which shares most characters with *Caloscordum*, implies their close relationships, and serological data reveal its affinity with *Melanocrommyum* (Hanelt *et al.*, 1992). In agreement with Friesen *et al.* (2006), two sister groups exist in this subgenus: one with a Eurasian-American distribution and the other restricted to eastern Asia. The polytomy formed by the eastern Asia species was interpreted as a rapid radiation that coincided with the colonization of the Hengduan Mountains and adjacent areas.

Our molecular data indicate that subgenera *Vvedenskya* and *Porphyroprason* have a recent common ancestor and those two monotypic groups form a clade sister to *Melanocrommyum*. Multiovulate locules and the narrowly campanulate flowers of *Vvedenskya* indicate its affinity with *Melanocrommyum*, and the lax inflorescence with rather few flowers and the small subglobose bulbs with several stalked side bulbs and membranous tunics also imply its close relationship with *Porphyroprason* (Friesen *et al.*, 2006). The scape and the cylindrical and tubular leaves of *Vvedenskya* are densely ribbed and bear short hairs differing considerably from *Porphyroprason*, which may support its delimitation as a separate subgenus. *Porphyroprason* is characterized by several specific morphological characters, including planar venation of leaf blades, occurrence of up to three veins in the outer tepals, a tripartite stigma, three or four ovules per locule, and evenly granulous periclinal walls and only slightly undulate anticlinal walls of the seed testa cells (Friesen *et al.*, 2006). Shape and position of nectaries and excretory tubes and serological characters underline its close relationship with *Melanocrommyum* (Hanelt *et al.*, 1989).

Melanocrommyum is the largest subgenus in the second evolutionary line and is extremely diverse morphologically. Species of *Melanocrommyum* occur from the Mediterranean to the Near and Middle East, reaching north-western China and Pakistan in the east, and southern Siberia in the north,

with Central Asia being the important centre of evolution (Fritsch, 1990; Hanelt *et al.*, 1992; Khassanov and Fritsch, 1994; Mes *et al.*, 1999). Well-developed leaf sheaths restricted to subterranean parts, the extremely short developmental period and several anatomical characters, including two opposite rows of vascular bundles in leaf blades and true palisade parenchyma composed of radial-only, slightly elongated cells, are considered synapomorphies for this subgenus (Fritsch, 1992a; Hanelt *et al.*, 1992). Furthermore, all members show epigeal germination with seedlings of the *A. karataviense* type (Druselmann, 1992) and share uniform karyotypes without any clear species-specific or section-specific characteristics (Fritsch and Astanova, 1998). A strongly unreduced, salt-soluble seed storage protein with molecular weight of 65 000–70 000 was found in this subgenus only (Maass, 1992). None of the above character states occurs in any other subgenus of *Allium*, and it is obviously a monophyletic group. Our ITS data corroborate the monophyly of *Melanocrommyum* and indicate its close affinity with subgenera *Vvedenskya* and *Porphyroprason*.

Phylogeny in the third evolutionary line

The evolutionary history in the third evolutionary line is complex. Subgenera *Butomissa* and *Cyathophora* form two successive lineages sister to the clade formed by the remaining species. Subgenus *Rhizirideum* is sister to subgenera *Allium*, *Cepa*, *Reticulatobilbosa* and *Polyprason*, and relationships among the last four subgenera are not well resolved (Figs 1C and 2).

Butomissa forms the first branching group in the third evolutionary line. This small subgenus includes about four species (Friesen *et al.*, 2006), some of which inhabit the Siberian–Mongolian–North Chinese steppes, and the others are distributed in mountains from eastern to central Asia and into the eastern Mediterranean area (Fritsch and Friesen, 2002). Position, shape and excretory tube of the nectaries show rather simple character states (Fritsch, 1992b), which may imply it is the earliest-branching group in the third evolutionary line. Its growth form (Kruse, 1992a) and chromosome morphology are as simple as in section *Rhizirideum* G.Don ex Koch (Friesen, 1988), but multiovulate locules, serological data and rather high TKW (thousand kernel weight) (Hanelt, 1992) show relationships to subgenera *Melanocrommyum* and *Anguinum*. Our phylogenetic analysis suggests that *Butomissa* occupies a position between these subgenera closer to subgenera *Cyathophora* and *Rhizirideum*.

Cyathophora includes about five species mainly distributed in Tibet and the Himalayas (Friesen *et al.*, 2006). All species have biovulate locules (Hanelt, 1992). Furthermore, all species share only one row of identically orientated vascular bundles in the leaf blades combined with the presence of palisade parenchyma and subcortical laticifers, which is perhaps the most ancient character combination in *Allium* (Fritsch, 1988). Our results indicate *Milula* has close relationships with *Cyathophora* and *Coleoblastus*, which is also suggested by Friesen *et al.* (2000) based on a molecular study and anatomical investigations of leaf characters.

Species of *Rhizirideum* are Eurasian steppe taxa showing the most diversity in southern Siberia and Mongolia. The simple

form of nectaries without an excretory tube (Fritsch, 1992b) and differing karyotypes in every section (Friesen, 1988) reveal its phylogenetically rather ancient state. Short periods of time between speciation events would readily explain the polytomies observed in section *Rhizirideum*, and the occurrence of the polyploid complex in the *A. senescens* alliance (Friesen, 1992; He, 1999) could be connected with the recent origin of these species.

Species from subgenera *Allium*, *Cepa*, *Reticulatobulbosa* and *Polyprason* form the largest clade in the third evolutionary line. Our molecular data suggest that these subgenera are not monophyletic. Furthermore, the systematic position of some species should also be reconsidered. For example, on the basis of our molecular results, *A. kaschianum*, formerly attributed to section *Oreiprason*, fell in a clade comprising species from section *Falcatifolia* N.Friesen and *A. togashii*, formerly attributed to section *Rhizirideum*, clustered with species from section *Reticulatobulbosa* Kamelin s.s. However, better sampling, multiple unlinked loci and improved analyses would be desirable before suggesting any taxonomic change for these species and would be necessary to better understand the evolutionary history of these *Allium* species. We propose that the large polytomy formed by species from these subgenera reflects a rapid diversification of their ancestors. Similarly, the reason that species from section *Schoenoprasum* are placed in a polytomy could be a recent origin.

Implications for biogeography

Amerallium. The separation between the Old and New World branches of subgenus *Amerallium*, evidenced by geographical and molecular data, has fuelled doubts in many researchers (Dubouzet and Shinoda, 1999), and the origin and migration about *Amerallium* has been long in dispute. Hanelt *et al.* (1992) postulated that *Amerallium* had its origins in Asia and spread to North America via the Bering Land Bridge, but they did not discuss the origins of the Mediterranean *Amerallium* species. The alternative hypothesis is a predominantly unidirectional migration via the Bering and North Atlantic land bridges (Dubouzet and Shinoda, 1999). Although Dubouzet and Shinoda (1999) made analyses of every possible route and provided a time frame for every route in their research on the relationships among Old and New World *Allium* species, the migration routes in *Amerallium* are still not clear.

Using a molecular phylogenetic reconstruction, the optimal solution and the current geographical distribution pattern of species (Fig. 3), we suggest the following biogeographical hypotheses for *Amerallium*. The sister group of *Amerallium* is *Nectaroscordum* and *Microscordum*, which are confined to the Mediterranean and eastern Asia, respectively. Thus, the Old World is implicated as the centre of origin for *Amerallium*. The basal Old World–New World split (node 2) in *Amerallium* might reflect an early vicariance event that did not impact the other taxa, perhaps because transcontinental distributions had not yet been established in these groups (Donoghue, 2001). The ancestor of *Amerallium* originated at high latitudes of eastern Asia during the transition from Cretaceous to Tertiary (node 1); this was followed by an early dispersal to western North America (+D, the internode

leading to node 2). The dispersion and vicariance (node 2) may have occurred first, before diversification within either the Old World or the New World. One lineage of *Amerallium* probably spread eastward to North America via the Bering Land Bridge and expanded their range southward. Two separate groups (sections *Lophioprason* and *Rhophetoprason* + *Caulorhizideum* + *Amerallium*) were isolated and this isolation resulted in the divergence of North America *Amerallium* (Nguyen *et al.*, 2008). Western North America has acted as an important centre of diversification within North America *Amerallium*. S-DIVA optimal reconstructions suggest that western North America was the ancestral area in the basal duplication events, where it underwent duplication (speciation within the area), and gave rise to two different lineages. One of them, the ancestor of section *Lophioprason*, remained in western North America, where it diversified, and afterward dispersed to central North America (+C) followed by vicariance between west and central North American (node 9). Then for *A. cernuum*, dispersal events occurred from western North America into central North America (+C) and eastern North America (+E). The second North America lineage (sections *Rhophetoprason* + *Caulorhizideum* + *Amerallium*) probably dispersed from western North America to central North America (+C) followed by vicariance between western and central North America (node 7) and resulted in local diversification in central North America. Then for *A. canadense* var. *canadense*, a dispersal event occurred from central North America into eastern North America (+E). Dispersal events for *A. canadense* var. *canadense* and *A. cernuum* occurred at terminal tips (i.e. they are not inferred as ancestral areas at terminal nodes), indicating that these events correspond to recent range expansions (Sanmartín, 2003). The other lineage of *Amerallium* expanded its range from east to west and ended up in the Mediterranean region, not across the North Atlantic. Diversification within Old World *Amerallium* presumably involved dispersal to the Mediterranean region (+B), followed by a vicariance event (node 6). One part of this lineage (eastern Asian *Amerallium*) survived in the Himalayan region and the area south of the Qinglin Mountains (China) and diverged into section *Bromatorrhiza* – comprising about eight species and two varieties – whereas the other part of this lineage (Mediterranean region *Amerallium*) originated in the Mediterranean region (B) by duplication, probably after dispersal from eastern Asia. Successive duplication events within the Mediterranean region gave rise to several sections including *Narkissoprason*, *Briseis*, *Arctoprasum* and *Molium*. The unidirectional migration is less probable, because it involves two intercontinental dispersals, whereas the first route requires only one.

Anguinum. Two distinct groups exist in *Anguinum*, one with a Eurasian–American distribution and the other restricted to the Hengduan Mountains and adjacent areas. For *Anguinum*, the basal sister group is *Caloscordum*. Thus, eastern Asia is implicated as the centre of origin for the ancestor of *Anguinum*. Using molecular phylogenetic reconstruction, the optimal solution and the current distribution of species (Fig. 4), we suggest the following biogeographical hypotheses for *Anguinum*. The ancestral reconstruction for node 13 suggests

eastern Asian as the ancestral area for *Anguinum*, where it underwent duplication, and gave rise to two different lineages. One of them, the ancestor of the eastern Asia alliance clade (node 23), remained in eastern Asia (eastern Himalaya and areas south of the Qinlin Mountains), and successive duplication events within eastern Asia gave rise to several closely related taxa (*A. ovalifolium* var. *ovalifolium*, *A. ovalifolium* var. *cordifolium*, *A. ovalifolium* var. *leuconeurm*, *A. funckiiifolium*, *A. nanodes* and *A. prattii*). The low average pairwise genetic distances (0.00–0.31 % for ITS; 0.07–0.41 % for *rps16*) among these species and polytomies formed by these species in the Bayesian tree (Figs 1B and 2) suggest that this lineage underwent a recent radiation, and the region from the Hengduan Mountains to the Qinling Range is the centre of the present distribution and source of differentiation of this lineage (Jing *et al.*, 1999). The second eastern Asian lineage (the ancestor of the Eurasian–American alliance clade, node 22) probably dispersed westward and across the North Atlantic Land Bridge to north-eastern North America (+B), where *A. tricoccum* originated and diverged into two varieties (*A. tricoccum* var. *tricoccum* and *A. tricoccum* var. *burdickii*). The other descendant of this lineage perhaps stayed in eastern Asia and diverged into several species including *A. listera*, *A. microdictyon*, *A. ochotense* and *A. victorialis* and then several dispersal events occurred at terminal tips. For *A. microdictyon*, a dispersal event occurred from eastern Asia to Siberia (+D). For *A. victorialis*, dispersal events occurred from eastern Asia to western North America (+C), Europe and Siberia (+D) and Central Asia (+F). Furthermore, *A. victorialis sensu lato* (*s.l.*) is found in North America only on Attu Island, where it is reported to be native, and on Unalaska Island, where it is reported to have been introduced from Attu Island (Dale *et al.*, 2002). We agree with Hultén (1933) and propose that *A. victorialis* *s.l.* from the Kamchatka Peninsula was dispersed to Attu Island by natural means.

Vvedenskya, *Porphyroprason* and *Melanocrommyum*. Phylogenetic reconstruction suggests that *Vvedenskya*, *Porphyroprason* and *Melanocrommyum* have close affinities with *Caloscordum* and *Anguinum* and together they comprise the second evolutionary line in the evolution of *Allium*. *Vvedenskya*, *Porphyroprason* and *Melanocrommyum* are late-branching groups, whereas *Caloscordum* and *Anguinum* are early branching. *Caloscordum* is restricted to eastern Asia and *Anguinum* is mainly distributed in eastern Asia. Thus, eastern Asia is implicated as the ancestral distribution area for the ancestor of *Vvedenskya*, *Porphyroprason* and *Melanocrommyum*. S-DIVA to the second evolutionary lineage indicates that the ancestor of *Porphyroprason*, *Vvedenskya* and *Melanocrommyum* originated in eastern Asia (Fig. 4, node 1), then dispersed to Central Asia (+F, the internode leading to node 2) along the north coast of the Tethys sea, namely the Cayan–Alai Mountain ranges. The ancestral reconstruction for node 3 including all *Porphyroprason*, *Vvedenskya* and *Melanocrommyum* species favours Central Asia as the ancestral area. We propose the following biogeographical hypotheses for *Vvedenskya* and *Porphyroprason*. The two subgenera evolved in Central Asia and differentiated into two species, *A. kujikense* and *A. oreophilum*, respectively. Then

for *A. oreophilum*, a dispersal event occurred from Central Asia into western Asia and adjacent areas (+E). The upheaval of the Tien-shan/Alai mountain range and retreat of the former Tethys sea during the late Oligocene and early Miocene (Guo *et al.*, 2002) would have left the Asian interior more arid and would also have formed many new ecological niches. Based on the optimal solution and palaeogeographical data, we propose the following biogeographical hypotheses for *Melanocrommyum*. *Melanocrommyum* evolved in Central Asia, where it underwent duplication, and quickly colonized the territories of the former Tethys sea and radiated in Central Asia. The resulting taxa expanded their ranges via dispersal into other areas such as western Asia (+E, i.e. the internode leading to nodes 15, 16, 35, 37, 46, 53, 59, 74 and 79 and another four dispersal events occurred at terminal tips) and the Mediterranean (i.e. +G, the internode leading to node 27) and diversified in these regions, followed by dispersion from western Asia to Central Asia (+F, i.e. the internode leading to nodes 75 and 77 and another dispersal event occurred at terminal tip) and from western Asia to the Mediterranean (+G, i.e. the internode leading to node 36 and the other dispersal event occurred at terminal tip). The striking morphological variability of the species is hardly reflected by molecular data (Linne von Berg *et al.*, 1996), which may also indicate that the radiation is a relatively recent phenomenon.

Because of the lack of resolution within the third evolutionary line of *Allium*, we could not analyse the biogeography using S-DIVA. Species of this evolutionary line have a wide distribution in Eurasia, which may indicate that the ancestor of these species originated in Asia. *Butomissa* is the first branching group and is now distributed in Siberia, eastern Asia, central Asia and the eastern Mediterranean area. *Cyathophora* is a solely Asian (Tibet and the Himalayas) group and is sister to the remaining groups including *Rhizirideum*, *Allium*, *Cepa*, *Polyprason* and *Reticulatobulbosa*. The lack of resolution among these remaining groups suggests that subsequent dispersal events and speciation were rapid, and they eventually spread into Eurasia, extending into the Mediterranean and temperate north-eastern and subarctic regions of America (Hanelt *et al.*, 1992).

These analyses imply that *Allium* may have originated in Asia or more exactly in eastern Asia and that various subgenera underwent different biogeographical pathways that involved different numbers of vicariant and dispersal events, and further studies should be carried out to test and verify this hypothesis. We also hope that it will be possible to sort clades into categories for comparison based directly on age estimates and to infer which palaeoclimatic or palaeogeographical events impacted on the dispersal and vicariance of *Allium*.

Taxonomic implications and intrageneric classification of Chinese Allium

Allium spp. have adapted to diverse habitats and display a remarkable polymorphism, which is the main reason for the widely recognized difficulties in taxonomy and classification of *Allium*. Moreover, the traditional infrageneric classifications are based on some homoplasious characters (Fritsch and Friesen, 2002). Both these factors make it difficult to select natural evolutionary lineages using easily discernible

characteristics. Today, biologists are adopting a system of classification based on phylogenetics, which reflects the evolutionary processes that have shaped life, including *Allium* spp.

The division of the included species in clades (Figs 1 and 2) is for the most part in accordance with the accepted taxonomic division based on traditional taxonomy or the new classification of Friesen *et al.* (2006). However, our phylogenetic analysis (Figs 1C and 2) provides some new findings. Both ITS and combined analyses strongly support placing *A. przewalskianum* in section *Eduardii*, and their close similarities in morphology (Xu, 1980) also indicate a close relationship. In both ITS and combined analyses, *A. flavovirens* forms an isolated branch, which provides evidence that it deserves sectional rank. Nevertheless, the circumscription of the new section and the identification of diagnostic characters are premature and further morphological and molecular studies are required.

Our taxon sample covers roughly 70 % of the known *Allium* spp. in China assembled to represent almost all taxonomic groups. Based on previous morphological studies and molecular data and our own results, we propose a synopsis (Appendix 3) which divides Chinese *Allium* into 13 subgenera and 34 sections (containing all the species recorded in *Allium* in *Flora of China* plus *A. spicatum*). The subgenera are listed according to their position in the phylogenetic tree (Figs 1 and 2), and a list of species is given for every section and subgenus. We believe this to be helpful because this is the first intrageneric classification treatment of *Allium* in China since the *Flora of China* for *Allium* was published. Unfortunately, we did not obtain ITS or *rps16* sequences of some species and we were not able to conduct phylogenetic analysis on all the described taxa in China. We could only deduce the true position of these species from morphological characteristics and geographical distribution; in the appendices below, species for which the systematic position needs to be further tested and verified are marked with question marks.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of a phylogenetic tree resulting from a Bayesian analysis of a subset of ITS matrix (Fig. S1) and a phylogenetic tree resulting from a Bayesian analysis of the *rps16* matrix (Fig. S2), both including 60 taxa focusing on the Chinese *Allium*.

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APPENDIX 1

New accessions of *Allium* and outgroups from which ITS and *rps16* intron sequences were obtained, with corresponding voucher information and GenBank reference numbers. A dash indicates the region was not sampled. The information is listed as follows: taxon — ITS, *rps16* intron; voucher information.

Allium: *A. altaicum* Pall. —GQ181094, GU566637; Burqin, Xinjiang, China; He X-J & Zhang X-L 97643. *A. anisopodium* Ledeb. —GQ181095, GU566650; Zhongwei, Ningxia, China; He X-J et al. 97834. *A. bidentatum* Fisch. ex Prokh. & Ikonn.-Gal. —GQ181096, GU566655; Ximeng, Nei Mongol, China; He X-J et al. 97813. *A. caeruleum* Pall. —GQ181064, GU566645; Yumin, Xinjiang, China; He X-J & Zhang X-L 97609. *A. carolinianum* DC. —GQ181097, GU566627; Wulumuqi, Xinjiang, China; He X-J & Zhang

X-L 97656. *A. cepiforme* G.Don—GU566611, GU566635; Chengdu Botanical Garden, China; Li Q-Q 09011511. *A. changduense* J.M.Xu—GQ181065, GU566631; Changdu, Tibet, China; Zhang Y-C B19. *A. chrysanthum* Regel—GQ181066, GU566639; Huzhu, Xining, China; Xu J-M 97–5–663. *A. chrysocephalum* Regel—GU566612, GU566640; Qinhai Lake, Qinhai, China; Ma X-G 09080401. *A. condensatum* Turcz. —GQ181098, GU566643; Daqing Mountain, Nei Mongol, China; He X-J & Zhang X-L 97827. *A. cyanum* Regel—GQ181099, GU566632; Queer Mountain, Sichuan, China; Zhang Y-C B25. *A. cyathophorum* Bureau & Franch.—GQ181093, GU566659; Zhongdian, Yunnan, China; Xu J-M 93–17. *A. eduardii* Stearn ex Airy Shaw—GQ181100, GU566657; Daqing Mountain, Nei Mongol, China; He X-J et al. 97829. *A. eupherma* Airy Shaw—GQ181067, —; Batang, Sichuan, China; Zhang Y-C B03. *A. fasciculatum* Rendle—GQ181068, GU566674; Dazi, Xizang, China; Tu Y-L et al. 94-9. *A. fistulosum* L. — —, GU566638; Chengdu Botanical Garden, China; Li Q-Q 091201. *A. flavovirens* Regel—GQ181069, GU566634; Bayanhoti, Nei Mongol, China; He X-J et al. 97830. *A. forrestii* Diels—GQ181070, GU566633; Queer Mountain, Sichuan, China; Zhang Y-C B24. *A. galanthum* Kar. & Kir.—GQ181101, GU566636; Altai, Xinjiang, China; He X-J & Zhang X-L 97631. *A. hookeri* Thwaites var. *hookeri*— —, GU566672; Lijiang, Yunnan, China; Li Q-Q 09072702. *A. hookeri* var. *muliense* Airy Shaw—GQ181071, GU566671; Zhongdian, Yunnan, China; Xu J-M 93 —25. *A. hymenorrhizum* Ledeb.—GQ181102, GU566626; Burqin, Xinjiang, China; He X-J & Zhang X-L 97641. *A. lineare* L.—GQ181103, GU566629; Wulumuqi, Xinjiang, China; He X-J & Zhang X-L 97606. *A. listera* Stearn—GQ181063, GU566668; Dongfeng, Jilin, China; Tuliguer 91001. *A. macranthum* Baker—GQ181072, GU566675; Zhongdian, Yunnan, China; Xu J-M 93–23. *A. macrostemon* Bunge—GU566613, GU566646; Wenchuan, Sichuan, China; Li Q-Q 09060601. *A. mairei* H.Lévl. —GU566614, GU566658; Lijiang, Yunnan, China; Ma X-G 09102407. *A. maowenense* J. M.Xu—GQ181073, —; Wenchuan, Sichuan, China; Yu H 97836. *A. mongolicum* Regel—GQ181074, GU566649; Saihan, Nei Mongol, China; He X-J et al 97802. *A. nanodes* Airy Shaw—GU566615, GU566666; Daocheng, Sichuan, China; Li Q-Q 09080101. *A. nutans* L.—GQ181075, GU566652; Burqin, Xinjiang, China; He X-J & Zhang X-L 97635. *A. obliquum* L.—GQ181104, —; Tacheng, Xinjiang, China; He X-J & Zhang X-L 97604. *A. omeiense* Z.Y.Zhu—GQ181076, GU566673; Emei Mountain, Sichuan, China; Xu J-M 91–01. *A. oreoprasum* Schrenk—GQ181105, GU566660; Tuoli, Xinjiang, China; He X-J & Zhang X-L 97619. *A. ovalifolium* Hand.-Mazz. var. *ovalifolium*—GQ181084, GU566663; Kangding, Sichuan, China; Li Q-Q 2008081202. *A. ovalifolium* var. *cordifolium* (J. M. Xu) J.M.Xu—GQ181085, GU566664; Xiaojin, Sichuan, China; Li Q-Q 2008081703. *A. ovalifolium* var. *leuconeurum* (J.M.Xu) J.M.Xu—GU566616, GU566665; Lixian, Sichuan, China; Li Q-Q 09060901. *A. pallasi* Murr. —GQ181077, GU566644; Emin, Xinjiang, China; He X-J & Zhang X-L 97603. *A. petraeum* Kar. & Kir.—GQ181106, —; Yumin, Xinjiang, China; He X-J & Zhang X-L 97610. *A. platyspathum* Schrenk—GQ181078, —; Wulumuqi, Xinjiang, China; He X-J

& Zhang X-L 97628. *A. plurifolium* Rendle var. *plurifoliatum*—GU566617, GU566625; Taibai Mountain, Shanxi, China; Li Q-Q 09080807. *A. plurifoliatum* var. *zhegushanense* J.M.Xu—GQ181086, —; Maerkang, Sichuan, China; Li Q-Q 2008081905. *A. polyrhizum* Turcz. ex Regel—GQ181107, GU566648; Saihan, Nei Mongol, China; He X-J *et al.* 97804. *A. prattii* C.H.Wright ex Hemsley—GQ181087, GU566667; Xiaojin, Sichuan, China; Li Q-Q 2008081603. *A. przewalskianum* Regel—GU566618, GU566656; Lintan, Ganshu, China; Wei X-Q 09072802. *A. ramosum* L.—GQ181079, GU566661; Daqing Mountain, Nei Mongol, China; He X-J *et al.* 97812. *A. rude* J.M.Xu—GQ181080, GU566641; Xiaojin, Sichuan, China; Li Q-Q 2008081701. *A. saxatile* M..Bieb. —GQ181108, GU566625; Wulumuqi, Xinjiang, China; He X-J & Zhang X-L 97626. *A. senescens* L.—GU566619, GU566653; Manzhouli, Nei Mongol, China; Wang C-B 09062. *A. sikkimense* Baker—GQ181088, —; Maerkang, Sichuan, China; Li Q-Q 2008082001. *A. spirale* Willd.—GU566620, GU566654; Helingeer, Nei Mongol, China; Li Q-Q 20080903. *A. strictum* Schrad.—GU566621, GU566628; Tuoli, Xinjiang, China; He X-J & Zhang X-L 97636. *A. subtilissimum* Ledeb.—GQ181081, GU566647; Wulumuqi, Xinjiang, China; He X-J & Zhang X-L 97650. *A. tanguticum* Regel—GQ181089, —; Zuodao, Qinhai, China; Feng T *et al.* 20080701. *A. tenuissimum* L.—GQ181090, GU566651; Helingeer, Nei Mongol, China; Li Q-Q 20080902. *A. tuberosum* Rottler ex Spreng. —, GU566662; Foping, Shanxi, China; Li Q-Q 2008092501. *A. tubiflorum* Rendle—GU566622, GU566670; Hua Mountain, Shanxi, China; Liao C-Y C072804. *A. victorialis* L.—GQ181082, GU566669; Dunhua, Jilin, China; Tuliguer 91002. *A. wallichii* Kunth var. *wallichii*—GQ181091, GU566676; Kangding, Sichuan, China; Li Q-Q 2008081203. *A. wallichii* var. *platyphyllum* (Diels) J.M.Xu—GU566624, GU566677; Lijiang, Yunnan, China; Li Q-Q 09072503. *A. xichuanense* J.M.Xu—GQ181083, GU566642; Queer Mountain, Sichuan, China; Zhang Y-C B29. *A. yuanum* F.T.Wang & Tang—GQ181092, —; Maerkang, Sichuan, China; Li Q-Q 2008082003.

Outgroups: *Nothoscordum gracile* (Aiton) Stearn—, GU566678; Kunming Botanical Garden, China; Liao C-Y *et al.* 091117. *Tulbaghia violacea* Harv.—GU566623, GU566679; Chengdu Botanical Garden, China; Li Q-Q *et al.* 091110.

APPENDIX 2

Previously published ITS and *rps16* accessions obtained from GenBank

ITS accessions of *Allium* and outgroups obtained from GenBank. ¹Dubouzet and Shinoda (1998); ²Dubouzet and Shinoda (1999); ³Friesen *et al.* (2000); ⁴Ricroch *et al.* (2005); ⁵Friesen *et al.* (2006); ⁶Gurushidze *et al.* (2007); ⁷Gurushidze *et al.* (2008); ⁸Nguyen *et al.* (2008); ⁹Sinitsyna and Friesen (2008).

Allium: *A. abramsii* (Ownbey & Aase) McNeal EU096131⁸; *A. aflatunense* B.Fedtsch. FM177239⁷; *A. akaka* S.G.Gmel. ex Schult. & Schult.f. FM177242⁷; *A. alaicum* Vved. FM177250⁷; *A. albidum* Fisch. ex Bieb. AJ411892⁵; *A. alexeianum* Regel FM177247⁷; *A. altissimum* Regel

FM177251⁷; *A. altynolicum* N.Friesen AJ411939⁵; *A. ampeloprasum* L. AY427530⁴; *A. amplexens* Torr. AF055097²; *A. anceps* Kellogg EU096134⁸; *A. angulosum* L. EU096136⁸; *A. arkitense* R.M.Fritsch FM177256⁷; *A. aroides* Popov & Vved. FM177259⁷; *A. asarense* R.M.Fritsch & Matin AM418365⁶; *A. aschersonianum* Barbey FM177260⁷; *A. assadii* Seisums FM177261⁷; *A. atropurpureum* Waldst. & Kit. FM177262⁷; *A. atrorubens* S.Watson var. *atrorubens* EU096137⁸; *A. atrorubens* var. *cristatum* (S.Watson) McNeal EU096138⁸; *A. atrosanguineum* Schrenk var. *atrosanguineum* AJ411864⁵; *A. atrosanguineum* var. *fedschenkoanum* (Regel) G.H.Zhu & Turland AJ411844⁵; *A. atroviolaceum* Boiss. AJ411884⁵; *A. austrosibiricum* N.Friesen AJ411832⁵; *A. azutavicum* Kotukhov AM949600⁹; *A. backhousianum* Regel FM177264⁷; *A. bakhtiaricum* Regel FM177269⁷; *A. beesianum* W.W.Sm. AJ411860⁵; *A. bolanderi* S.Watson var. *bolanderi* EU096139⁸; *A. brachyscapum* Vved. FM177273⁷; *A. brevidens* Vved. AJ412721⁵; *A. breviscapum* Staph FM177274⁷; *A. brevistylum* S.Watson AJ412763⁵; *A. bucharicum* Regel FM177275⁷; *A. bulgaricum* (Janka) Prodan AJ412747⁵; *A. burjaticum* N.Friesen AM949602⁹; *A. burlewii* Davidson EU096142⁸; *A. caesium* Schrenk AJ412731⁵; *A. campanulatum* S.Watson EU096143⁸; *A. canadense* L. var. *canadense* EU096145⁸; *A. cardiostemon* Fisch. & C.A.Mey. FM177277⁷; *A. caspium* subsp. *baissunense* FM177267⁷; *A. caspium* (Pall.) M.Bieb. subsp. *caspium* FM177280⁷; *A. cepa* L. AM418370⁶; *A. cernuum* Roth AF037622¹; *A. chamaemoly* L. AF055109²; *A. chamarensis* M.M.Ivanova AJ411957⁵; *A. chelotom* Wendelbo FM177464⁷; *A. chinense* G.Don AJ411848⁵; *A. chitralicum* F.T.Wang & Tang FM177283⁷; *A. chodsha-bakirganicum* Gaffarov & Turak. FM177285⁷; *A. christophii* Trautv. FM177272⁷; *A. clathratum* Ledeb. AJ411855⁵; *A. costatovaginatum* Kamelin & Levichev FM177286⁷; *A. cratericola* Eastw. EU096146⁸; *A. crispum* Greene EU096147⁸; *A. crystallinum* Vved. AJ412724⁵; *A. cupaniifolium* Raf. AJ412737⁵; *A. cupuliferum* Regel subsp. *cupuliferum* FM177292⁷; *A. cyrilli* Ten. FM177462⁷; *A. daghestanicum* Grossh. AJ411850⁵; *A. darwasicum* Regel FM177452⁷; *A. dasypyllyum* Vved. FM177305⁷; *A. decipiens* Fisch. ex Schult. & Schult.f. FM177306⁷; *A. denticulatum* (Ownbey & Aase ex Traub) McNeal EU096149⁸; *A. dentigerum* Prokh. AJ411958⁵; *A. derderianum* Regel FM177308⁷; *A. diabolense* (Ownbey & Aase) McNeal EU096150⁸; *A. dichlamydeum* Greene EU096151⁸; *A. dodecadontum* Vved. FM177461⁷; *A. dregeanum* Kunth AJ411962⁵; *A. drepanophyllum* Vved. AJ411854⁵; *A. drobowii* Vved. AJ411895⁵; *A. drummondii* Regel AJ411908⁵; *A. elburzense* Wendelbo FM177311⁷; *A. elegans* Drobow AJ412730⁵; *A. ellisi* Hook.f. FM177384⁷; *A. eremoprasum* Vved. AJ412726⁵; *A. ericetorum* Thore AJ311867⁵; *A. falcifolium* Hook. & Arn. EU096153⁸; *A. farctum* Wendelbo AM492184⁷; *A. fetisowii* Regel FM177316⁷; *A. filidens* Regel AJ412723⁵; *A. filidentiforme* Vved. AJ412722⁵; *A. fimbriatum* S.Watson var. *fimbriatum* EU096155⁸; *A. fimbriatum* var. *purdyi* (Eastw.) McNeal EU096156⁸; *A. fistulosum* L. AM418371⁶; *A. flavescens* Besser AJ411842⁵; *A. flavidum* Ledeb. AJ411956⁵; *A. flavum* L. var. *minus* AJ411926⁵; *A. giganteum* Regel FM177320⁷

A. gilgiticum F.T.Wang & Tang AJ412762⁵; *A. glandulosum* Link & Otto AJ412746⁵; *A. goodingii* Ownbey AF055095²; *A. griffithianum* Boiss. AJ411862⁵; *A. gunibicum* Miscz. ex Grossh AM418361⁶; *A. gypsaceum* Popov & Vved. FM177322⁷; *A. haematochiton* S.Watson EU096157⁸; *A. haneltii* F.O.Khass. & R.M.Fritsch AJ412725⁵; *A. heldreichii* Boiss. AY427539⁴; *A. helicophyllum* Vved. FM177324⁷; *A. hexaceras* Vved. FM177326⁷; *A. hickmanii* Eastw. EU096159⁸; *A. hirtifolium* Boiss. AF037612¹; *A. hissaricum* Vved. FM177328⁷; *A. hoffmannii* Ownbey EU096160⁸; *A. hollandicum* R.M.Fritsch FM177333⁷; *A. hookeri* Thwaites AJ412740⁵; *A. howellii* Eastw. var. *howellii* EU096161⁸; *A. hyalinum* Curran EU096162⁸; *A. inaequale* Janka AJ412735⁵; *A. incensiodorum* Radic AJ411866⁵; *A. insubricum* Boiss. & Reut. AJ250291³; *A. insufficiens* Vved. FM177334⁷; *A. iranicum* (Wendelbo) Wendelbo AJ411961⁵; *A. isakulii* subsp. *balkhanicum* R.M.Fritsch & F.O.Khass. FM177271⁷; *A. isakulii* subsp. *subkopetdagense* R.M.Fritsch & F.O.Khass. FM177427⁷; *A. jepsonii* (Ownbey & Aase) S.S.Denison & McNeal EU096163⁸; *A. jesdianum* subsp. *angustitepalum* (Wendelbo) F.O.Khass. & R.M.Fritsch FM177253⁷; *A. jesdianum* Boiss. & Buhse subsp. *Jesdianum* FM177337⁷; *A. jodanthum* AJ411902⁵; *A. karataviense* Regel FM177341⁷; *A. karelinii* Poljakov AJ411876⁵; *A. kaschianum* Regel AJ412754⁵; *A. kingdonii* Stearn AJ250286⁵; *A. koelzii* (Wendelbo) Perss. & Wendelbo FM177313⁷; *A. komarovianum* Vved. AJ412760⁵; *A. komarovii* Lipsky FM177342⁷; *A. kopetdagense* Vved. AJ411950⁵; *A. kuhsorkhense* R.M.Fritsch & Joharchi FM177386⁷; *A. kujukense* Vved. AJ411947⁵; *A. kunthianum* Vved. AJ412734⁵; *A. kuramense* F.O.Khass. & N.V.Friesen AJ411868⁵; *A. kurssanovii* Popov AJ311869⁵; *A. ledebourianum* Schult. & Schult.f. AJ411925⁵; *A. lemmonii* S.Watson EU096164⁸; *A. leucocephalum* Turcz. ex Ledeb. AJ412757⁵; *A. lipskyanum* Vved. FM177348⁷; *A. litvinovii* Drobow ex Vved. AJ412727⁵; *A. lusitanicum* Lam. AJ411831⁵; *A. macleanii* Baker FM177351⁷; *A. majus* Vved. FM177355⁷; *A. malyshevii* N.Friesen AJ412758⁵; *A. margaritae* B.Fedtsch. AJ412732⁵; *A. materculae* Bordz. FM177356⁷; *A. maximowiczii* Regel AJ411877⁵; *A. melanantherum* Pancic AJ412739⁵; *A. membranaceum* Ownbey ex Traub EU096165⁸; *A. microdictyon* Prokh. AJ411859⁵; *A. minorense* ined. AJ412748⁵; *A. minutiflorum* Regel FM177446⁷; *A. moly* L. AF055108²; *A. monadelphum* Turcz. ex Kar. et Kir. AJ411955⁵; *A. monanthum* Maxim. AJ412745⁵; *A. montibaicalense* N.Friesen AJ411838⁵; *A. moschatum* L. AJ411872⁵; *A. motor* Kamelin & Levichev FM177364⁷; *A. neapolitanum* Cirillo AF055104²; *A. neriniflorum* (Herb.) G.Don AJ411920⁵; *A. nevskianum* Vved. FM177365⁷; *A. nigrum* L. FM177368⁷; *A. noéanum* Reut. ex Regel FM177374⁷; *A. obtusum* Lemmon EU096166⁸; *A. ochroleucum* Waldst. et Kit. subsp. *ochroleucum* AJ412755⁵; *A. ochroleucum* subsp. *pseudosuaveolens* Zahar. AJ411863⁵; *A. oliganthum* Kar. & Kir. AJ411835⁵; *A. oreophilum* C.A.Mey. AF037620¹; *A. oreoprasoides* Vved. AJ411896⁵; *A. orientale* Boiss. FM177377⁷; *A. oschaninii* O.Fedtsch. AM418376⁶; *A. pamiricum* Wendelbo AJ412736⁵; *A. paniculatum* L. AJ411949⁵; *A. paradoxum* (M.Bieb.) G.Don AJ412741⁵; *A. parvulum* Vved. AJ412720⁵; *A. parvum* Kellogg EU096169⁸;

A. peninsulare Lemmon ex Greene var. *peninsulare* EU096170⁸; *A. platycarpe* S.Watson EU096171⁸; *A. platyspathum* subsp. *amblyophyllum* (Kar. & Kir.) N.Friesen AJ411875⁵; *A. platyspathum* Schrenk subsp. *platyspathum* AJ411878⁵; *A. porrum* L. AY427543⁴; *A. praecox* Brandege EU096173⁸; *A. praemixtum* Vved. AM418379⁶; *A. prostratum* Trevir. AM949604⁹; *A. protensum* Wendelbo FM177380⁷; *A. pseudobodeanum* R.M.Fritsch & Matin FM177381⁷; *A. pseudowinklerianum* R.M.Fritsch & F.O.Khass. FM177387⁷; *A. pskemense* B.Fedtsch. AM418382⁶; *A. punctum* L.F.Hend. EU096174⁸; *A. regelii* Trautv. FM177389⁷; *A. robustum* Kar. & Kir. FM177391⁷; *A. rosenbachianum* subsp. *kwakense* R.M.Fritsch FM177345⁷; *A. rosenbachianum* Regel subsp. *rosenbachianum* FM177393⁷; *A. rosenorum* R.M.Fritsch FM177395⁷; *A. roseum* L. AF055105²; *A. rothii* Zucc. FM177400⁷; *A. roylei* Stearn AM492189⁶; *A. rubens* Schrad. ex Willd. AM949619⁹; *A. rupestre* Steven AJ412733⁵; *A. rupestristepposum* N.Friesen AJ411869⁵; *A. sanbornii* Alph.Wood var. *sanbornii* EU096177⁸; *A. saposhnikovii* Nikitina FM177405⁷; *A. sarawchanicum* Regel FM177406⁷; *A. sativum* L. AF037621¹; *A. scabriscapum* Boiss. AJ411881⁵; *A. schachimardanicum* Vved. FM177410⁷; *A. schmitzii* Cout. AJ412761⁵; *A. schoenoprasoides* Regel AJ412728⁵; *A. schoenoprasum* L. subsp. *schoenoprasum* AF055112²; *A. schoenoprasum* subsp. *latrixifolium* (Pau) Rivas Mart. AJ411837⁵; *A. schubertii* Zucc. FM177411⁷; *A. schugnanicum* Vved. FM177412⁷; *A. scorodoprasum* L. AJ412713⁵; *A. semenovii* Regel AJ411897⁵; *A. sergii* Vved. AJ411936⁵; *A. serra* McNeal & Ownbey EU096178⁸; *A. setifolium* Schrenk AJ411898⁵; *A. severzovioides* R.M.Fritsch FM177414⁷; *A. sewerzowii* Regel FM177403⁷; *A. sharsmithiae* (Ownbey & Aase) McNeal EU096179⁸; *A. shelkovnikovii* Grossh. FM177413⁷; *A. shevockii* McNeal EU096180⁸; *A. siculum* Ucria AJ250299³; *A. siskiyouense* Munz & Keck ex Ownbey EU096181⁸; *A. sordidiflorum* Vved. AJ411899⁵; *A. sphaerocephalon* L. AJ412717⁵; *A. spicatum* (Prain) N.Friesen AJ250285³; *A. splendens* Willd. ex Schult. & Schult.f. AJ411927⁵; *A. spurium* G.Don AM949635⁹; *A. stellatum* Nutt. ex Ker Gawl. EU096183⁸; *A. stellarianum* Willd. subsp. *splendens* AJ411963⁵; *A. stipitatum* Regel AJ411911⁵; *A. suaveolens* Jacq. AJ411874⁵; *A. subangulatum* Regel AJ411870⁵; *A. subhirsutum* L. AF055106²; *A. sulphureum* Vved. AJ412759⁵; *A. suworowii* Regel FM177430⁷; *A. taeniopetalum* Popov & Vved. var. *taeniopetalum* FM177433⁷; *A. taeniopetalum* subsp. *turakulovii* R.M.Fritsch & F.O.Khass. FM177443⁷; *A. talassicum* Regel AJ411865⁵; *A. tashkenticum* F.O.Khass. & R.M.Fritsch FM177434⁷; *A. tenuicaule* Regel AJ411887⁵; *A. teretifolium* Regel AJ411886⁵; *A. thunbergii* G.Don AJ411849⁵; *A. togashii* H.Hara AJ411843⁵; *A. trachyscordum* Vved. AJ411857⁵; *A. trautvetterianum* Regel FM177438⁷; *A. tribracteatum* Torr. EU096184⁸; *A. tricoccum* Sol. AJ411917⁵; *A. triquetrum* L. AJ412742⁵; *A. tuberosum* Rottler ex Spreng. AJ411914⁵; *A. tulipifolium* Ledeb. FM177442⁷; *A. tuolumnense* (Ownbey & Aase) S.S.Denison & McNeal EU096185⁸; *A. turkestanicum* Regel AJ411968⁵; *A. tuvinicum* (N.Friesen) N.Friesen AM949609⁹; *A. tyttocephalum* Schult. & Schult.f. AM949632⁹;

A. ubsicola Regel AJ411960⁵; *A. umbilicatum* Boiss. AJ412719⁵; *A. unifolium* Kellogg EU096186⁸; *A. ursinum* L. AJ412744⁵; *A. validum* S.Watson EU096188⁸; *A. vavilovii* Popov & Vved. AM418383⁶; *A. verticillatum* Regel FM177447⁷; *A. viridulum* Ledeb. FM177449⁷; *A. vodopjanovae* N.Friesen subsp. *vodopjanovae* AJ411845⁵; *A. vodopjanovae* subsp. *czemalense* N.Friesen AJ311868⁵; *A. vvedenskyanum* Pavlov FM177451⁷; *A. weschniakowii* Regel AJ411946⁵; *A. winklerianum* Regel FM177455⁷; *A. xiphopetalum* Aitch. et Baker AJ411858⁵; *A. yosemitense* Eastw. EU096189⁸; *A. zebdanense* Boiss. & Noë AY427552⁴; *A. zergericum* F.O.Khass. & R.M.Fritsch FM177456⁷.

Outgroups: *Dichelostemma capitatum* (Benth.) Alph. Wood subsp. *capitatum* EU096190⁸; *Dichelostemma congestum* (Smith) Kunth EU096191⁸; *Dichelostemma ida-maia* (Alph.Wood) Greene EU096192⁸; *Dichelostemma multiflorum* (Benth.) A. Heller EU096193⁸; *Dichelostemma volubile* (Kellogg) A.Heller EU096194⁸; *Ipheion uniflorum* (Graham) Raf. AJ412715⁵; *Nothoscordum gracile* (Ait.) Stearn AJ412716⁵; *Nothoscordum bivalve* (L.) Britton AJ250301³; *Tulbaghia fragrans* Verdoorn AJ250300³.

rps16 accessions obtained from GenBank

¹⁰Umeshara et al. (unpublished); ¹¹Ryzhova et al. (2009).

Allium: *A. ampeloprasum* FJ653700¹¹; *A. cepa* L. AB292300¹⁰; *A. obliquum* FJ653671¹¹; *A. sativum* FJ653688¹¹; *A. schoenoprasum* FJ653705¹¹.

APPENDIX 3

Taxonomic synopsis of the genus *Allium* L. in China

First evolutionary line

1. Subgenus *Microcordum* (Maxim.) N.Friesen.—Type: *A. monanthum* Maxim. (monotypic).

1.1. Section *Microcordum* Maxim.—Type: *A. monanthum* Maxim.

1.1.1. *A. monanthum* Maxim.

2. Subgenus *Amerallium* Traub.—Type: *A. canadense* L.

2.1. Section *Bromatorrhiza* Ekberg.

2.1.1. *A. guanxianense* J.M.Xu

2.1.2. *A. xiangchengense* J.M.Xu

2.1.3. *A. hookeri* Thwaites

2.1.3a. *A. hookeri* var. *hookeri*

2.1.3b. *A. hookeri* var. *muliense* Airy Shaw

2.1.4. *A. omeiense* Z.Y.Zhu

2.1.5. *A. chienchuanense* J.M.Xu

2.1.6. *A. fasciculatum* Rendle

2.1.7. *A. wallichii* Kunth

2.1.7a. *A. wallichii* var. *wallichii*

2.1.7b. *A. wallichii* var. *platyphyllum* (Diels) J.M.Xu

2.1.8. *A. macranthum* Baker

Second evolutionary line

3. Subgenus *Caloscordum* (Herb.) R.M.Fritsch.—Type:

A. neriniflorum (Herbert) G.Don

3.1. Section *Caloscordum* (Herb.) Baker.—TYPE:

A. neriniflorum (Herbert) G.Don

3.1.1. *A. tubiflorum* Rendle

3.1.2. *A. inutile* Makino

3.1.3. *A. neriniflorum* (Herbert) G.Don

4. Subgenus *Anguinum* (G.Don ex Koch) N.Friesen.—Type:

A. victorialis L.

4.1. Section *Anguinum* G.Don ex Koch.—Type:

A. victorialis L.

4.1.1. *A. victorialis* L.

4.1.2. *A. listera* Stearn

4.1.3. *A. ovalifolium* Hand.-Mazz.

4.1.3a. *A. ovalifolium* var. *ovalifolium*

4.1.3b. *A. ovalifolium* var. *leuconeum* J.M.Xu

4.1.3c. *A. ovalifolium* var. *cordifolium* (J.M.Xu) J.M.Xu

4.1.4. *A. funckii* Hand.-Mazz.

4.1.5. *A. nanodes* Airy Shaw

4.1.6. *A. prattii* C. H. Wright ex Hemsley

5. Subgenus *Porphyroprason* (Ekberg) R.M.Fritsch.—Type:

A. oreophilum C.A.Mey. (monotypic).

5.1. Section *Porphyroprason* Ekberg.—TYPE:

A. oreophilum C.A.Mey.

5.1.1. *A. oreophilum* C.A.Mey.

6. Subgenus *Melanocrommyum* (Webb & Berth.) Rouy.—Type: *A. nigrum* L.

6.1. Section *Melanocrommyum* Webb & Berth.—Type:

A. nigrum L.

6.1.1. *A. tulipifolium* Ledeb.

6.1.2. *A. roborowskianum* Regel

6.1.3. *A. robustum* Kar. & Kir.

6.2. Section *Acmopetala* R. M. Fritsch.—Type:

A. backhousianum Regel

6.2.1. *A. fetisowii* Regel

6.3. Section *Regeloprason* Wendelbo.—Type: *A. regelii* Trautv.

6.3.1. *A. winklerianum* Regel

Third evolutionary line

7. Subgenus *Butomissa* (Salisb.) N.Friesen.—Type:

A. ramosum L.

7.1. Section *Butomissa* (Salisb.) Kamelin.—Type:

A. ramosum L.

7.1.1. *A. tuberosum* Rottler ex Spreng.

7.1.2. *A. ramosum* L.

7.2. Section *Austromontana* N.Friesen.—Type:

A. oreoprasum Schrenk

7.2.1. *A. oreoprasum* Schrenk

8. Subgenus *Cyathophora* (R.M.Fritsch) R.M.Fritsch.—Type: *A. cyathophorum* Bur. & Franch.

8.1. Section *Cyathophora* R.M.Fritsch.—Type:

A. cyathophorum Bur. & Franch.

8.1.1. *A. cyathophorum* Bur. & Franch.

8.1.1a. *A. cyathophorum* var. *cyathophorum*

8.1.1b. *A. cyathophorum* var. *farreri* (Stearn) Stearn

8.1.2. *A. trifurcatum* (F.T.Wang & Tang) J.M.Xu?

8.2. Section *Coleoblastus* Ekberg.—Type: *A. mairei* H.Lévl.

8.2.1. *A. mairei* H.Lévl.

8.2.2. *A. kingdonii* Stearn

8.2.3. *A. rhynchogynum* Diels

8.3. Section *Milula* (Prain) N.Friesen.—Type: *A. spicatum* (Prain) N.Friesen.

8.3.1. *A. spicatum* (Prain) N.Friesen

9. Subgenus *Rhizirideum* (G.Don ex Koch) Wendelbo s.s.—Type: *A. senescens* L.

9.1. Section *Rhizirideum* G.Don ex Koch s.s.—Type:

A. senescens L.

9.1.1. *A. prostratum* Trevir.

- 9.1.2. *A. rubens* Schrad. ex Willd.
 9.1.3. *A. brevidentatum* F.Z.Li
 9.1.4. *A. taishanense* J.M.Xu
 9.1.5. *A. chiwui* F.T.Wang & Tang
 9.1.6. *A. spurium* G.Don
 9.1.7. *A. spirale* Willd.
 9.1.8. *A. senescens* L.
 9.1.9. *A. nutans* L.
 9.2. Section *Caespitosoprason* N.Friesen.—Type:
A. polyrrhizum Siev.
 9.2.1. *A. mongolicum* Regel
 9.2.2. *A. yongdengense* J.M.Xu?
 9.2.3. *A. subangulatum* Regel
 9.2.4. *A. polyrhizum* Turcz. ex Regel
 9.2.5. *A. bidentatum* Fisch. ex Prokh. & Ikon.-Gal.
 9.2.6. *A. dentigerum* Prokh.
 9.3. Section *Rhizomatosa* Egor.—Type: *A. caespitosum* Siev. ex Bong. & C.A.Mey.
 9.3.1. *A. caespitosum* Siev. ex Bong. & C.A.Mey.
 9.4. Section *Tenuissima* (Tzagolova) Hanelt.—Type:
A. tenuissimum L.
 9.4.1. *A. tenuissimum* L.
 9.4.2. *A. elegantulum* Kitag.
 9.4.3. *A. anisopodium* Ledeb.
 9.4.3a. *A. anisopodium* var. *anisopodium*
 9.4.3b. *A. anisopodium* var. *zimmermannianum* (Gilg) F.T.Wang & Tang
 9.5. Section *Eduardia* N. Friesen.—Type: *A. eduardii* Stearn ex Airy Shaw
 9.5.1. *A. eduardii* Stearn ex Airy Shaw
 9.5.2. *A. przewalskianum* Regel
 9.5.3. *A. siphonanthum* J.M.Xu?
 10. Subgenus *Allium*.—Type: *A. sativum* L.
 10.1. Section *Allium* L. —Type: *A. sativum* L.
 10.1.1. *A. porrum* L.
 10.1.2. *A. sativum* L.
 10.1.3. *A. macrostemon* Bunge
 10.2. Section *Caerulea* (Omelcz.) F.O.Khassanov.—Type:
A. caeruleum Pall.
 10.2.1. *A. caeruleum* Pall.
 10.2.2. *A. caesium* Schrenk
 10.2.3. *A. sairamense* Regel?
 10.2.4. *A. jacquemontii* Kunth?
 10.2.5. *A. juldusicola* Regel?
 10.3. Section *Pallasia* (Tzagolova.) F.O.Khassanov, R.M.Fritsch & N.Friesen.—Type: *A. pallasii* Murr.
 10.3.1. *A. delicatulum* Siev. ex Schult. & Schult.f.
 10.3.2. *A. eusperma* Airy Shaw
 10.3.3. *A. pallasii* Murr.
 10.3.4. *A. glomeratum* Prokh.?
 10.3.5. *A. schoenoprasoides* Regel
 10.3.6. *A. songpanicum* J.M.Xu?
 10.3.7. *A. tanguticum* Regel
 10.4. Section *Eremoprasum* (Kamelin) F.O.Khassanov, R.M.Fritsch & N.Friesen.—Type: *A. sabulosum* Steven ex Bunge
 10.4.1. *A. sabulosum* Steven ex Bunge
 11. Subgenus *Cepa* (Mill.) Radić.—Type: *A. cepa* L.
 11.1. Section *Cepa* (Mill.) Prokh.—Type: *A. cepa* L.
 11.1.1. *A. altaicum* Pall.
 11.1.2. *A. fistulosum* L.
 11.1.3. *A. cepa* L.
 11.1.3a. *A. cepa* var. *cepa*
 11.1.3b. *A. cepa* var. *proliferum* (Moench) Regel
 11.1.3c. *A. cepa* var. *aggregatum* G.Don
 11.1.4. *A. cepiforme* G.Don
 11.1.5. *A. galanthum* Kar. & Kir.
 11.2. Section *Annuloprasum* T.V.Egorova.—Type:
A. fedschenkoanum Regel.
 11.2.1. *A. semenovii* Regel
 11.2.2. *A. atrosanguineum* Schrenk
 11.2.2a. *A. atrosanguineum* var. *atrosanguineum*
 11.2.2b. *A. atrosanguineum* var. *fedschenkoanum* (Regel) G.Zhu & Turland
 11.2.2c. *A. atrosanguineum* var. *tibeticum* (Regel) G.Zhu & Turland
 11.2.3. *A. weschniakowii* Regel
 11.3. Section *Condensatum* N. Friesen.—Type:
A. condensatum Turcz.
 11.3.1. *A. longistylum* Baker?
 11.3.2. *A. alabasicum* Y.Z.Zhao?
 11.3.3. *A. condensatum* Turcz.
 11.4. Section *Sacculiferum* P.P.Gritz.—Type:
A. sacculiferum Maxim.
 11.4.1. *A. grisellum* J.M.Xu?
 11.4.2. *A. chinense* G.Don
 11.4.3. *A. yanchiense* J.M.Xu?
 11.4.4. *A. sacculiferum* Maxim.
 11.4.5. *A. thunbergii* G.Don
 11.5. Section *Schoenoprasum* Dumort.—Type:
A. schoenoprasum L.
 11.5.1. *A. schoenoprasum* L.
 11.5.1a. *A. schoenoprasum* var. *schoenoprasum*
 11.5.1b. *A. schoenoprasum* var. *scaberrimum* Regel
 11.5.2. *A. oliganthum* Kar. & Kir.
 11.5.3. *A. maximowiczii* Regel
 11.5.4. *A. ledebourianum* Schult. & Schult.f.
 11.6. Section *Flavovirens* Q.Q.Li & X.J.He—Type:
A. flavovirens Regel (monotypic).?
 11.6.1. *A. flavovirens* Regel
 12. Subgenus *Reticulatobulbosa* (Kamelin) N.Friesen.—Type: *A. lineare* L.
 12.1. Section *Reticulatobulbosa* Kamelin s.s.—Type:
A. lineare L.
 12.1.1. *A. humile* Kunth?
 12.1.2. *A. lineare* L.
 12.1.3. *A. schrenkii* Regel?
 12.1.4. *A. amphibolum* Ledeb.
 12.1.5. *A. strictum* Schrad.
 12.1.6. *A. splendens* Willd. ex Schult. & Schult.f.
 12.1.7. *A. maackii* (Maxim.) Prokh. ex Kom.
 12.1.8. *A. clathratum* Ledeb.
 12.1.9. *A. flavidum* Ledeb.
 12.1.10. *A. leucocephalum* Turcz. ex Ledeb.
 12.2. Section *Campanulata* Kamelin.—Type:
A. xiphopetalum Aitch.
 12.2.1. *A. teretifolium* Regel
 12.2.2. *A. tekesicola* Regel?
 12.2.3. *A. korolkowii* Regel?

- 12.3. Section *Sikkimensia* (Traub) N.Friesen.—Type:
A. sikkimense Baker.
 12.3.1. *A. forrestii* Diels
 12.3.2. *A. changduense* J.M.Xu
 12.3.3. *A. beesianum* W.W.Smith
 12.3.4. *A. yuanum* F.T.Wang & Tang
 12.3.5. *A. sikkimense* Baker
 12.3.6. *A. cyaneum* Regel
 12.3.7. *A. aciphyllum* J.M.Xu
 12.3.8. *A. henryi* C.H.Wright
 12.3.9. *A. heteronema* F.T.Wang & Tang
 12.3.10. *A. paepalanthoides* Airy Shaw
 12.3.11. *A. plurifoliatum* Rendle
 12.3.11a. *A. plurifoliatum* var. *plurifoliatum*
 12.3.11b. *A. plurifoliatum* var. *zhegushanense* J.M.Xu
 12.3.12. *A. stenodon* Nakai & Kitag.
 13. Subgenus *Polyprason* Radić.—Type: *A. moschatum* L.
 13.1. Section *Oreiprason* F.Herm.—Type: *A. saxatile* M.Bieb.
 13.1.1. *A. obliquum* L.
 13.1.2. *A. saxatile* M.Bieb.
 13.1.3. *A. petraeum* Kar. & Kir.
 13.1.4. *A. tianschanicum* Rupr.?
 13.1.5. *A. megalobulbon* Regel?
 13.1.6. *A. pevtzovii* Prokh.?
 13.1.7. *A. caricoides* Regel?
- 13.1.8. *A. kurssanovii* Popov
 13.1.9. *A. setifolium* Schrenk
 13.1.10. *A. subtilissimum* Ledeb.
 13.2. Section *Falcatifolia*
 N.Friesen.—Type: *A. carolinianum* DC.
 13.2.1. *A. hymenorhizum* Ledeb.
 13.2.1a. *A. hymenorhizum* var. *hymenorhizum*
 13.2.1b. *A. hymenorhizum* var. *dentatum* J.M.Xu
 13.2.2. *A. kaschianum* Regel
 13.2.3. *A. carolinianum* DC
 13.2.4. *A. blandum* Wall.?
 13.2.5. *A. phariense* Rendle?
 13.2.6. *A. platyspathum* Schrenk
 13.2.6a. *A. platyspathum* subsp. *platyspathum*
 13.2.6b. *A. platyspathum* subsp. *amblyophyllum* (Kar. & Kir.) N.Friesen
 13.3. Section *Daghestanica* (Tscholok.)
 N.Friesen.—Type:
A. daghestanicum Grossh.
 13.3.1. *A. rude* J.M.Xu
 13.3.2. *A. chrysocephalum* Regel
 13.3.3. *A. xichuanense* J.M.Xu
 13.3.4. *A. chrysanthum* Regel
 13.3.5. *A. maowenense* J.M.Xu
 13.3.6. *A. herderianum* Regel?