Systematics of disjunct northeastern Asian and northern North American *Allium* (Amaryllidaceae)

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Abstract: This study was undertaken to better understand *Allium* infrageneric taxonomy, character evolution, species diversification, and patterns of radiation in disjunct species between the New and Old World using morphological and molecular data. Taxonomic sampling focused on northeastern Asian (mainly Korean and northeastern Chinese) and representative disjunct northern North American (Canadian) species. Pistil and seed testa morphology was investigated using light and scanning electron microscopy, respectively. These characters were useful to assess degree of relationship at different taxonomic levels in *Allium*. Phylogenetic studies included nrDNA ITS and cpDNA *trnL–trnF* sequence data analyzed using maximum parsimony approaches. Our molecular phylogeny recovers a similar topology to that published in recent studies and confirms three major evolutionary lines and patterns of radiation regarding the ancestors of subgenera *Amerallium* and *Anguinum* in the genus. The northeastern Asian and northern North American disjunction in this genus is inferred to be the result of multiple intercontinental migrations. Seed testa sculpture attributes in combination with seed shape provide key characters to distinguish *Allium*'s major clades in the molecular phylogeny. The two types of ovarian processes, basal hood-like and apical crest-like in disjunct Old and New World species, respectively, are newly derived characters in each continent. Most infrageneric *Allium* groups are monophyletic, while subgenus *Cepa* is polyphyletic.

Key words: Allium, Canada, disjunct taxa, ITS, Korea, molecular phylogeny, northeastern China, pistil evolution, seed testa, *trnL–trnF*.

Résumé : Les auteurs ont étudié les *Allium* afin de mieux en comprendre la taxonomie infragénérique, l'évolution des caractères, la diversification des espèces et les partons de radiation chez les espèces disjointes du Nouveau et de l'Ancien Monde, en utilisant des données morphologiques et moléculaires. Ils ont centré l'échantillonnage taxonomique sur l'Asie du Nord-est (surtout en Corée et au nord-est de la Chine) ainsi que des espèces disjointes de l'Amérique du Nord (Canada). Ils ont examiné la morphologie des pistils et des téguments de la graine, en utilisant la microscopie photonique et électronique par balayage, respectivement. Ces caractères se sont avérés utiles pour évaluer le degré de relation aux différents degrés taxonomiques des *Allium*. Les études phylogénétiques ont porté sur les données des séquences des ITS du nrADN et cpADN *trnL–trnF*, analysées en utilisant des approches de parcimonie maximum. La phylogénie moléculaire obtenue par les auteurs conduit à une topologie similaire à celle publiée dans de récentes études et confirme trois lignées évolutives et patrons de radiation en ce qui a trait aux ancêtres des sous-genres *Amerallium* et *Anguinum*, dans le genre. On déduit que la disjonction du genre entre le Nord-est asiatique et le Nord-Américain serait le résultat de multiples migrations intercontinentales. Les attributs sculpturaux des téguments combinés avec la forme des graines fournissent des caractères clés pour distinguer les principaux clades provenant de la phylogénie moléculaire. Les deux types de processus ovariens avec base en forme de capuchon et apical en forme de crête chez les espèces de l'Ancien et du Nouveau Monde, respectivement, constituent des caractères récemment dérivés sur chaque continent. La plupart des groupes infragénériques d'*Allium* sont monophylétiques, alors que le sous-genre *Cepa* est polyphylétique.

Mots-clés : Allium, Canada, taxons disjoints, ITS, Corée, phylogénie moléculaire, nord-est de la Chine, évolution du pistil, téguments de la graine, *trnL–trnF*.

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Introduction

The genus *Allium* includes several ornamental and edible plants, such as onion, garlic, and leek, among others. It is characterized by the presence of bulbs enclosed in membranous (sometimes becoming fibrous) tunics, free to almost free tepals, and often a subgynobasic style (Friesen et al. 2006). Most taxa have a characteristic odor and taste produced by cysteine sulphoxides (Fritsch and Keusgen 2006). Historically, humans have exploited over 20 cultivated *Allium*

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species (van der Meer 1997), and in the last decades both Old and New World *Allium* are becoming popular worldwide because of the health benefits and medical properties (Rabinowitch and Currah 2002) and its edible and culinary species (Choi and Cota-Sánchez 2010).

Allium is a member of the expanded family Amaryllidaceae, subfamily Allioideae, tribe Allieae (Fay and Chase 1996; The Angiosperm Phylogeny Group 2009; Chase et al. 2009). With over 800 species, this genus (including *Caloscordum* Herb., *Milula* Prain, and *Nectaroscordum* Lindl.) is distributed naturally in the northern hemisphere and South Africa, mostly in regions with dry seasons (de Sarker et al. 1997; Friesen et al. 2006; Nguyen et al. 2008; Neshati and Fritsch 2009). The greatest diversity occurs in southwestern and central Asia and the Mediterranean region, the primary center of diversification, but a smaller secondary area of diversification is found in western North America (Friesen et al. 2006; Nguyen et al. 2008).

The genus *Allium* has a great deal of taxonomic complexity owing to discrepancies regarding its generic subdivision and the proliferation of synonyms. The lack of a comprehensive monograph, with the exception of that of Regel (1875), has led to a classification that is rather unstable because past studies have used few reliable characters in *Allium* taxonomy, yielding controversial viewpoints. At first, von Linné (1753) recognized 30 species in three alliances. Later, infrageneric groups with an increased number of species were recognized, e.g., six sections and 262 species (Regel 1875); nine sections and 228 species for the former USSR alone (Vvedensky 1935); three subgenera, 36 sections and subsections, and ca. 600 species (Traub 1968); six subgenera, 30 sections, and 14 subsections (Kamelin 1973); and six subgenera, 50 sections and subsections for 600–700 species (Hanelt et al. 1992). The most recent *Allium* classification includes ca. 800 species, 15 subgenera, and 56 sections (Friesen et al. 2006).

Several sources of taxonomic characters have been used in previous classifications of *Allium*, including sexuality of plants, macro- and micromorphology, anatomy of vegetative and reproductive parts, and cytology (Choi and Cota-Sánchez 2010 and references therein), and the analysis of seed coat in over 250 *Allium* species has yielded a useful terminology (Neshati and Fritsch 2009). However, most literature deals with taxa from Europe, central Asia, and North America. To our knowledge, an extensive study of seed testa in *Allium* species from northeastern Asia has yet to be conducted. With the advent of molecular systematics, the first generic reassessment of *Allium* based on molecular data was that of Linne von Berg et al. (1996), which marked the beginning of modern *Allium* systematics. Recent data from both nuclear and chloroplast genomes (e.g., Samoylov et al. 1995, 1999; Linne von Berg et al. 1996; Mes et al. 1997, 1999; Dubouzet and Shinoda 1998, 1999; Friesen et al. 2000, 2006; Gurushidze et al. 2007, 2008, 2010; Nguyen et al. 2008; Li et al. 2010) have refined the delineation of several taxonomic assemblages proposed based on morphological, anatomical, and cytological characters.

The utility of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) in the reconstruction of *Allium* phylogeny is evident at the generic and subgeneric levels (Dubouzet and Shinoda 1998, 1999; Friesen et al. 2000; Gurushidze et al. 2007, 2008; Li et al. 2010). The lat-

est data representing about 30% of *Allium* diversity have confirmed its monophyly and provided new insights into the evolutionary history of the genus in the Californian center of diversity (Friesen et al. 2006; Nguyen et al. 2008), supporting the systematic value of this marker. Likewise, noncoding regions of the chloroplast DNA (cpDNA) have also been effective to assess degree of relationships in plants, among them, the *trnL* (UAA) – *trnF* (GAA) intergenic region (Bakker et al. 1999; Beardsley and Olmstead 2002 and references therein). Nevertheless, the evolutionary rate of this marker and other of chloroplast noncoding regions is not fully understood in the Alliaceae s.s. and (or) Amaryllidaceae alliance (Gurushidze et al. 2010; Li et al. 2010). Similarly, Li et al. (2010) provide ample views into the phylogenetic relationships and evolutionary and biogeographic pathways of *Allium* using chloroplast and nuclear sequence data of numerous Chinese endemic species, but their taxonomic sampling has fallen short in the inclusion of representative taxa from other regions of northeastern Asia. The same premise applies to other *Allium* phylogenies including species from all major taxonomic groups within the genus but focused on European and central Asian (Friesen et al. 2006; Gurushidze et al. 2008, 2010) or western North American (Californian) taxa (Nguyen et al. 2008).

Notwithstanding the broad distribution and taxonomic diversity of *Allium* in northeastern Asia and northern North America in the neighboring areas of the Bering Strait, studies involving disjunct species of the genus are scarce. The investigation of *Allium*'s historical separations between northeastern Asia and northern North America has double significance: first, assisting in the inference of the ancestral area of intercontinental generic disjunction and, second, tracking down character evolution since the entities found in the edges of the Old World and the secondary center of diversity include the precursors of the commercial onion species. At present, 19 species are known from the Russian Far East (Vvedensky 1935; Barkalov 1987; Kovtonyuk et al. 2009), 32 from northeastern China (defined here as the provinces of Heilongjiang, Jilin, and Liaoning) and Korea (Xu and Kamelin 2000; Choi and Oh 2011), and 13 from Japan (Ohwi 1984; Hotta 1998; Takahashi and Hotta 2009) for a total of ca. 40 *Allium* species in northeastern Asia. In turn, North America contains 96 species, 12 of which occur in Canada (McNeal and Jacobsen 2002). Only *A. schoenoprasum* is common in the native floras of both northeastern Asia and northern North America (McNeal 1992; McNeal and Jacobsen 2002). *Allium victorialis* L. s.l. is also shared between Eurasia and North America; however, its North American range is limited to the westernmost island (Attu) of the Aleutian archipelago (McNeal and Jacobsen 2002).

In general, a basic understanding of *Allium* phylogeny exists, but previous studies have inadvertently excluded the northeastern Asian and northern North American *Allium* species from their analyses. There is, in fact, a general consensus that the global *Allium* phylogeny is a work in progress and that several critical species should be included in future studies (Gurushidze et al. 2008; Nguyen et al. 2008; Li et al. 2010). In addition, the morphological component is missing in most molecular studies conducted thus far. To deal with this knowledge gap, in this study we combine macro- and micromorphological reproductive characters in concert with

nuclear and chloroplast DNA sequence data to investigate *Allium*'s infrageneric classification, character evolution, diversification, and disjunct patterns between the New and Old World. Our sampling includes taxa from other regions of northeastern Asia (mainly Korea and northeastern China) as well as representative disjunct northern North American (Canadian) *Allium* species. Our goals are (*i*) to expand the current knowledge on pistil morphology and seed coat sculpture and (*ii*) to re-evaluate the recently proposed infrageneric classification of *Allium* (Friesen et al. 2006) using ITS and *trnL–trnF* sequence data collectively with pistil and seed testa traits. We also discuss the evolution and taxonomic implications of these morphological characters traditionally used to identify various infrageneric groups in relation to the molecular phylogenetic hypothesis.

Materials and methods

Taxon sampling

A total of 46 *Allium* accessions, representing 35 taxa, circumscribed in six sections (Fig. 1; Appendix A) were collected in the field from 2000 to 2010. Of these, 36 accessions are from Korea and northeastern China (Figs. 1A, 1F, 1G, and 1I–1X), one from Mongolia, and seven from Canada (Figs. 1B–1E, and 1H). The remaining two accessions are *A. caeruleum* (Fig. 1Q) and *A. fistulosum* (Fig. 1T), which were obtained from the living collection of the Korea National Arboretum (Pocheon, Gyeonggi, South Korea).

This study includes a total of 35 *Allium* taxa investigated for pistil morphology and phylogenetic analyses. We generated 13 nrDNA ITS sequences and 34 cpDNA *trnL–trnF* sequences. For seed testa morphology, we analyzed a total of 33 *Allium* taxa, 27 of which were analyzed in this study. Seed testa data of six *Allium* species were obtained from previous studies, namely Kruse (1984, 1988) and Choi and Cota-Sánchez (2010). Eight ITS sequences and one *trnL–trnF* sequence of *Allium* species were obtained from the GenBank database. DNA sequence data of *Dichelostemma capitatum* subsp. *capitatum*, *Ipheion dialystemon*, *Ipheion uniflorum*, *Nothoscordum bivalve*, *Tulbaghia simmleri*, and *Tulbaghia violacea* were also obtained from GenBank and included as outgroup genera in the molecular phylogenetic analyses (Fay and Chase 1996; Mes et al. 1997; Friesen et al. 2006). Voucher information and GenBank accession numbers are listed in Appendix A.

Analyses of pistil morphology

The investigation of pistil shape encompassed a minimum of 10 open fresh flowers of each accession, which were fixed in formalin – acetic acid – alcohol (FAA) according to Johansen (1940). The pistil was gently separated from each flower, examined to characterize its components, and photographed using an Olympus SZX7 stereoscope with a Canon A630 camera or a TESSOVAR Photomacrographic Zoom System with a Nikon D100 camera. Line drawings were generated from these observations and photos of voucher specimens using Adobe Photoshop 7.01 software.

Analyses of seed testa sculptures

Mature seeds were removed from fruits in the field and fixed using FAA (Johansen 1940). The seeds were rinsed twice with 0.1 mol·L^{-1} phosphate buffer (pH 6.8), refixed in 2.5% glutaraldehyde, dehydrated through an alcohol–isoamylacetate series, critical-point dried, mounted on stubs, and coated with gold in an ion sputter coater with 200–250 nm. In all cases, the seeds of at least five samples per accession were analyzed, characterized, and photographed with an LEO 1420 scanning electron microscope (SEM) at the Plant Taxonomy Laboratory of the Andong National University, South Korea.

DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from about 10 mg silica gel-dried leaf tissue using the E.Z.N.A. plant DNA mini kit (Omega Bio-tek, Norcross, Ga., USA). The concentration of the extracted DNA was checked on a 1% agarose gel and then estimated using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Del., USA). Isolated DNA was used directly in polymerase chain reaction (PCR) amplifications. The nuclear ITS region (ITS-1, 5.8S nrDNA subunit, ITS-2) was amplified using previously published universal primers (White et al. 1990). We amplified the *trnL* (UAA) intron, the *trnL* (UAA) partial 3′ exon, and the intergenic spacer between the *trnL* (UAA) 3′ exon and the *trnF* (GAA) gene (*trnL–trnF* region) using primers C, D, E, and F as described in Taberlet et al. (1991). We used a 50 μL PCR containing 40 ng·μL^{-1} of template and a final concentration of 1× PCR buffer minus MgCl$_2$ 5 mmol·L^{-1}, MgCl$_2$ 0.025 mmol·L^{-1}, and dNTPs 0.2 mmol·L^{-1} each, primer(s), and 1.25–3 U *Taq* polymerase (Promega). PCR amplifications were performed on a thermal cycler (PTC-200, MJ research) using the following conditions: (*i*) for the ITS: initial denaturation for 4 min at 94 °C, followed by 30 cycles of 20 s at 95 °C, 40 s at 50 °C, 1 min at 72 °C, with a final extension of 72 °C for 7 min; (*ii*) for the *trnL–trnF*: initial denaturation was 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 1 min at 50 °C, 1 min 30 s at 72 °C, with a final extension of 72 °C for 7 min. The PCR products were cleaned using the QIAquick PCR purification kit (QIAGEN, Valencia, Calif., USA) following the manufacturer's protocol. Sequencing reactions were performed using BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif., USA). Both forward and reverse strands were sequenced with a minimum overlap of 90% for every taxon on an ABI 377 automated sequencer using Long Ranger acrylamide gels (FMC Bioproducts, Rockland, Maine, USA). All sequences generated in this study were submitted to GenBank (Appendix A).

Sequence alignment and phylogenetic analyses

Forward and reverse sequence fragments for each species were edited and assembled in contigs using Sequencher software (ver. 4.9; Gene Codes Corporation, Ann Arbor, Mich., USA). Ambiguous bases were corrected by visual inspection of chromatograms to generate consensus sequences. Consensus sequences for each region were aligned manually using BioEdit Sequence Alignment Editor (ver. 5.09; Hall 2001); the ends of sequences were trimmed from each data set to maintain complementary data between taxa.

Parsimony-based analyses of sequence data were performed to assess phylogenetic relationships among species. Phylogenetic searches were carried out independently for

Fig. 1. Inflorescence diversity in selected *Allium* species used in this study. (A) sect. *Microscordum*, (B, C) sect. *Amerallium*, (D, E) sect. *Lophioprason*, (F) sect. *Caloscordum*, (G, H) sect. *Anguinum*, (I, J.) sect. *Butomissa*, (K) sect. *Caespitosoprason*, (L) sect. *Tenuissima*, (M, N) sect. *Rhizirideum*, (O, P) sect. *Reticulatobulbosa*, (Q) sect. *Caerulea*, (R) sect. *Scorodon* s.l., (S) sect. *Condensatum*, (T) sect. *Cepa*, (U, V) sect. *Schoenoprasum*, (W, X) sect. *Sacculiferum*.

Fig. 2. Diagrams of pistil shapes in *Allium* species. Dark areas indicate ovarian processes. (A) *A. monanthum*, (B) *A. textile*, (C) *A. stellatum*, (D) *A. cernuum*, (E) *A. neriniflorum*, (F) *A. microdictyon*, (G) *A. ramosum*, (H) *A. bidentatum*, (I) *A. tenuissimum*, (J) *A. pseudosenescens*, (K) *A. splendens*, (L) *A. caeruleum*, (M) *A. condensatum*, (N) *A. fistulosum*, (O) *A. schoenoprasum*, (P) *A. longistylum*. (Scale bar = 0.5 mm for all diagrams).
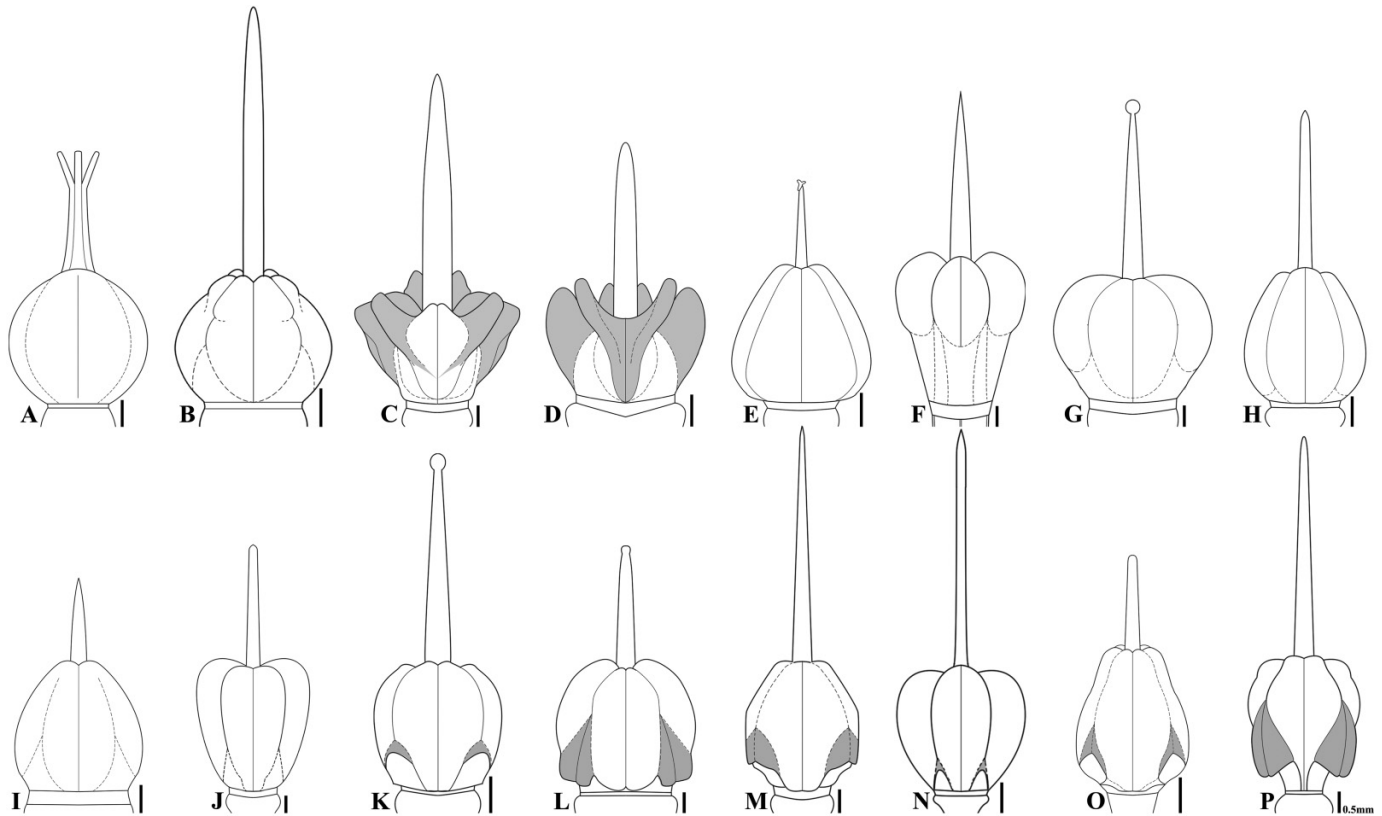
the ITS and the *trnL–trnF* data matrices and then with the combined molecular data sets (see Supplementary data[1]). The analysis of each individual genomic marker was made to explore phylogenetic signal and to detect consistent clades and possible phylogenetic incongruence with the competitive phylogeny, i.e., between the ITS and *trnL–trnF* data sets. Heuristic searches were conducted using TNT ver. 1.1 (Goloboff et al. 2003, 2008). We used equal weights and nonadditive characters, and gaps and (or) indels were treated as missing data. Before searches, all uninformative characters were deactivated. The searches involved 1000 replicates, each of which generated a Wagner tree using a random addition sequence of taxa from the data matrix, swapping the initial tree with TBR (tree bisection and reconnection) and retaining a maximum of two trees in each replicate. Subsequently, all optimal trees were swapped using TBR holding a maximum of 100 000 trees. Ten thousand fast bootstrap (BS) replicates (Felsenstein 1985) were used to assess confidence limits for the resulting tree topologies. Congruence between ITS and *trnL–trnF* data sets was tested using the incongruence length difference (ILD) test (Farris et al. 1994, 1995), as implemented by the partition homogeneity test in PAUP 4.0b for 50 replicates (heuristic search, simple addition, TBR branching swapping), each saving a maximum of 1000 most parsimonious trees per replicate.

Results

Pistil morphology

Typically, *Allium* pistil grows from the receptacle borne on a small gynophore, forming a distinct stalk from the base of the androecium to the ovary. The ovary is superior and the shape in the species investigated varies from globose to obovoid and may have or lack ovarian processes (appendages), which can be basal or apical. Based on the presence or absence of these ovarian processes, the ovaries are classified in three types: naked, with crest-like processes, and with hood-like processes (Fig. 2; Table 1), the presence or absence of which show some correlation with biogeographic patterns. The ovary with apical crest-like processes is characteristic of northern North American species (Figs. 2C and 2D), while the ovary with basal hood-like processes is an attribute of the northeastern Asian species (Figs. 2K–2P). The number of ovules per locule is typically two. Some exceptions include one ovule in *A. microdictyon*, *A. ochotense*, and *A. tricoccum*, two to four in *A. ramosum*, and five to eight in *A. neriniflorum* (Table 1). On the other hand, the style of *A. monanthum* is trigonous (Fig. 2A), while in the remaining species it is terete (Figs. 2B–2P). Four types of stigmas were observed in the species studied (Fig. 2; Table 1): tripartite in *A. monanthum* (Fig. 2A), trilobed in *A. neriniflorum* (Fig. 2E), capitate in *A. canadense* var. *canadense*, *A. ramo-*

Table 1. Morphological characters of pistil and seed in *Allium* species investigated.

Cn	Subgenus / section	Species	Pistil					Seed			
			Ovary			Style	Stigma	Shape	Testa cell wall		
			Shape	Op	On				Periclinal	Channel	Anticlinal
1	*Microscordum /Microscordum*	A. monanthum	Globose	—	2	Trigonous	Tripartite	Elliptical to oval-angular	?	?	?
2	*Amerallium / Amerallium*	A. canadense var. canadense	Subglobose	—	2	Terete	Capitate	Oval-hemispherical	Verrucate	Simple	Straight
2		A. geyeri var. tenerum	Subglobose	—	2	Terete	Conical	Unknown	?	?	?
2		A. textile	Subglobose	—	2	Terete	Conical	Oval-hemispherical	Granulate[c]	Simple[c]	Straight[c]
2	*Amerallium / Lophioprason*	A. cernuum	Subglobose	C	2	Terete	Conical	Oval-hemispherical	Verrucate[c]	Simple[c]	Straight[c]
2		A. stellatum	Subglobose	C	2	Terete	Conical	Oval-hemispherical	Minutely roughened[c]	Simple[c]	Straight[c]
3	*Caloscordum / Caloscordum*	A. neriniflorum	Ovoid	—	5–8	Terete	trilobed	Globose or nearly so	Smooth	Terraced	Straight
4	*Anguinum / Anguinum*	A. microdictyon	Obconical	—	1	Terete	Conical	Globose or nearly so	Smooth	Convex	Straight
4		A. ochotense	Obconical	—	1	Terete	Conical	Globose or nearly so	Smooth	Convex	Straight
4		A. tricoccum	Obconical	—	1	Terete	Conical	Globose or nearly so	Smooth[A]	Convex[A]	Straight[A]
5	*Butomissa / Butomissa*	A. ramosum	Obovoid	—	2–4	Terete	Capitate	Oval-hemispherical	Granulate	Simple	Irregularly curved
5		A. tuberosum	Obovoid	—	2	Terete	Capitate	Oval-hemispherical	Granulate	Simple	Irregularly curved
6	*Rhizirideum / Caespitosoprason*	A. bidentatum	Ovoid	—	2	Terete	Conical	Oval-hemispherical	Granulate	Simple	Undulated (S-like)
6	*Rhizirideum / Tenuissima*	A. anisopodium	Ovoid	—	2	Terete	Conical	Oval-angular	Granulate	Simple	Undulated (S-like)
6		A. tenuissimum	Ovoid	—	2	Terete	Conical	Oval-angular	Granulate	Simple	Undulated (S-like)
6	*Rhizirideum / Rhizirideum*	A. minus	Ovoid	—	2	Terete	Conical	Oval-hemispherical	Granulate	Simple	Straight
6		A. pseudosenescens	Ovoid	—	2	Terete	Conical	Oval-hemispherical	Granulate	Simple	Straight
6		A. senescens	Ovoid	—	2	Terete	Conical	Oval-hemispherical	Granulate	Simple	Straight
6		A. spirale	Ovoid	—	2	Terete	Conical	Oval-hemispherical	Granulate	Simple	Straight
7	*Reticulatobulbosa / Reticulatobulbosa*	A. koreanum	Obovoid	H	2	Terete	Conical	Elliptical-angular	Verrucate	Simple	Straight
7		A. splendens	Obovoid	H	2	Terete	Capitate	Elliptical-angular	Verrucate	Simple	Straight
7		A. strictum	Obovoid	H	2	Terete	Capitate	Elliptical-angular	Verrucate[B]	Simple[B]	Straight[B]
7	*Allium / Caerulea*	A. caeruleum	Subcubical	H	2	Terete	Capitate	Oval-hemispherical	Verrucate	Simple	Undulated (U- to Omega-like)
7	*Allium / Scorodon* s.l.	A. macrostemon	Subcubical	H	2	Terete	Conical	Oval-hemispherical	Verrucate	Simple	Undulated (U- to Omega-like)
7	*Cepa / Condensatum*	A. condensatum	Ovoid	H	2	Terete	Conical	Elliptical-angular	Verrucate	Simple	Straight
7	*Cepa / Cepa*	A. fistulosum	Obovoid	H	2	Terete	Conical	Elliptical-angular	Minutely roughened	Simple	Straight
7	*Cepa / Schoenoprasum*	A. maximowiczii	Ellipsoid	H	2	Terete	Conical	Elliptical-angular	Minutely roughened	Simple	Straight
7		A. schoenoprasum	Ellipsoid	H	2	Terete	Conical	Elliptical-angular	Granulate[c]	Simple[c]	Straight[c]
7	*Cepa / Sacculiferum*	A. linearifolium	Obovoid	H	2	Terete	Conical	Oval-flattened	Granulate	Simple	Straight
7		A. longistylum	Obovoid	H	2	Terete	Conical	Oval-flattened	Granulate	Simple	Straight
7		A. sacculiferum	Obovoid	H	2	Terete	Conical	Oval-flattened	Granulate	Simple	Straight
7		A. pseudojaponicum	Obovoid	H	2	Terete	Conical	Oval-flattened	Granulate	Simple	Straight
7		A. taquetii	Obovoid	H	2	Terete	Conical	Oval-flattened	Granulate	Simple	Straight
7		A. thunbergii var. thunbergii	Obovoid	H	2	Terete	Conical	Oval-flattened	Granulate	Simple	Straight
7		A. thunbergii var. teretifolium	Obovoid	H	2	Terete	Conical	Oval-flattened	Granulate	Simple	Straight

Note: Arrangement of taxa follows the putative basal to more derived position showing potential evolutionary trends and character states. Superscript A–C indicate data from previously published results (A, Kruse 1984; B, Kruse 1988; C, Choi and Cota-Sánchez 2010). Cn, clade number in molecular phylogenetic tree of Fig. 6; Op, ovarian process (C, crest-like apical; H, hood-like basal); On, ovule number per locule.

sum (Fig. 2G), *A. tuberosum*, *A. splendens* (Fig. 2K), and *A. caerulerum* (Fig. 2L), and conical, the most common stigma type distinguishing the remaining species (Figs. 2B–2D, 2F, 2H–2J, and 2M–2P).

Seed testa sculptures

Allium seeds display some degree of variability with potential taxonomic value. The seeds investigated are black or nearly so in color but vary in shape from globose to oval-flattened (Table 1). The epidermal cells of the seed coat, rather than fitting tightly together, form small voids or channels between them. *Allium* testa topography usually consists of anticlinal cell walls, boundary relief, undulation pattern, and microrelief of the periclinal cell wall, which is, at times, divided into a central and a peripheric anticlinal field (Figs. 3 and 4).

Periclinal cell walls

Our survey indicates that the moderately flat periclinal walls of the *Allium* seed coat can be divided in four types: smooth, minutely roughened, granulate, and verrucate (Figs. 3 and 4; Table 1). The smooth type, distinguished by the lack of a microrelief, is characteristic of *A. neriniflorum*, *A. microdictyon*, *A. ochotense*, and *A. tricoccum* (Figs. 3B–3D and 4B–4D; Table 1). Among these, *A. microdictyon* and *A. ochotense* are distinguished by a "glossy" surface evident with the naked eye. The minutely roughened type, differentiated by the lack of a relief (though a minor microrelief may be evident), is an attribute of *A. stellatum*, *A. fistulosum*, and *A. maximowiczii* (Figs. 3Q, 3R, 4Q, and 4R; Table 1). The granulate type is characteristic of most species (18) examined (Figs. 3E–3K, 3S, 3T, 4E–4K, 4S, and 4T; Table 1). In this type, the sculpture of the seed testa in *A. anisopodium* and *A. tenuissimum* is indistinctly granulate with stripe-like superficial surfaces (Figs. 3H, 3I, 4H, and 4I), whereas the remainder of the species investigated have obvious granulate periclinal walls. The verrucate type is present in eight species (Figs. 3A, 3L–3P, 4A, and 4L–4P; Table 1). Among these, the first five species listed in Table 1 are distinguished from the others by having a rather obscure large verruca, e.g., *A. canadense* var. *canadense*, *A. cernuum*, and *A. splendens* (Figs. 3A, 3M, 4A, and 4M), to several small verrucae, e.g., *A. koreanum* and *A. strictum* (Figs. 3L and 4L), per cell wall. Among these five taxa, *A. canadense* var. *canadense* and *A. cernuum* have a fairly smooth verrucae (Fig. 4A), as opposed to the granulate verrucae in the other species (Fig. 4L). The cell walls of *A. caeruleum*, *A. macrostemon*, and *A. condensatum* seem to be the most interesting in terms of verrucae development (Figs. 3N–3P and 4N–4P). *Allium caeruleum* and *A. macrostemon* have a coarse and prominent verruca in the central region of each periclinal cell wall and several smaller verrucae along the peripheral region. The verrucae of these species are also covered with tiny verrucae (Figs. 3N, 3O, 4N, and 4O). In turn, *A. condensatum* possesses five to eight evident verrucae similar in size with a finely granulated surface in the central and peripheral regions of periclinal walls (Figs. 3P and 4P).

Anticlinal cell walls

The boundary relief of anticlinal walls in *Allium* is shallowly to deeply channelled. The species investigated are distinguished by three types of channel patterns, namely simple, terraced, and convex (Figs. 3 and 4; Table 1). The simple and depressed type is found in most of the species (29) examined (Figs. 3A, 3E–3T, 4A, and 4E–4T; Table 1), with the exception of *A. neriniflorum*, a species with terraced type (Figs. 3B and 4B), and *A. microdictyon*, *A. ochotense*, and *A. tricoccum*, characterized by the convex type (Figs. 3C, 3D, 4C, and 4D). Among the taxa with simple and depressed boundaries, *A. ramosum*, *A. tuberosum*, *A. linearifolium*, *A. longistylum*, *A. sacculiferum*, *A. pseudojaponicum*, *A. taquetii*, and *A. thunbergii* show shallow channels (Figs. 3E, 3 F, 3S, 3T, 4E, 4F, 4S, and 4T), and *A. anisopodium* and *A. tenuissimum* exhibit comparatively narrower channels (Figs. 3H, 3I, 4H, and 4I) than the other species with deep and widely channeled boundaries. *Allium condensatum* is a distinct member because of the presence of channels of intermediate depth (Figs. 3P and 4P).

The types of anticlinal cell boundaries in *Allium* seed coats are straight, irregularly curved, or variously undulated with different amplitudes and wavelengths (Figs. 3 and 4). In most cases (26 taxa), the anticlinal walls are straight or gently curved (Figs. 3A–3D, 3J–3M, 3P-3T, 4A–4D, 4J–4M, and 4P–4T; Table 1). Among these, *A. canadense* var. *canadense*, *A. cernuum*, *A. stellatum*, *A. textile*, *A. minus*, *A. pseudosenescens*, *A. senescens*, *A. spirale*, *A. koreanum*, *A. splendens*, *A. strictum*, *A. fistulosum*, *A. maximowiczii*, and *A. schoenoprasum* exhibit a well-developed anticlinal layer of unknown origin, perhaps cuticle, waxy material, or mucopolysaccharides secreted by the seed coat (Figs. 3A, 3J–3M, 3Q, 3R, 4A, 4J–4M, 4Q, and 4R). This matter creates striation patterns varying from obscure to prominent, making the observation of the actual boundaries difficult.

The undulation patterns of the anticlinal walls are of three types: irregularly curved, S-like, and U- to Omega-like undulated. The irregularly curved walls are present in *A. ramosum* and *A. tuberosum* (Figs. 3E, 3F, 4E, and 4F). The S-like undulating pattern is found in *A. bidentatum*, *A. anisopodium*, and *A. tenuissimum* (Figs. 3G–3I and 4G–4I), but *A. bidentatum* displays a poorly defined undulated pattern considered an intermediate type between the straight and S-like configuration (Fig. 4G). The other two aforementioned species with S-like undulation exhibit higher and more distinct amplitudes with moderate wavelengths (Figs. 3H, 3I, 4H, and 4I). Lastly, *A. caeruleum* and *A. macrostemon* show a combination of U-like and Omega-like coarse undulation with remarkably high amplitudes and short wavelengths (Figs. 3N, 3O, 4N, and 4O), one of the most striking patterns of anticlinal walls among the taxa investigated.

Sequence analyses

The length of the ITS sequences in the *Allium* species investigated ranged from 623 in *A. strictum* to 661 base pairs (bp) in *A. stellatum*. The aligning of individual sequences, including outgroup taxa, resulted in a data matrix of 751 bp in length, of which 439 characters (58.46%) were parsimony informative. In the *trnL–trnF* region, the length of the sequences ranged from 578 in *A. canadense* var. *canadense*, *A. ramosum*, and *A. tuberosum* to 610 bp in *A. condensatum*. The alignment of individual sequences, including outgroup taxa, yielded a data matrix of 1048 bp in length, of which only 63 characters (6.01%) were parsimony informative. The

Fig. 3. Epidermal cells of the seed coat in *Allium.* (A) *A. canadense* var. *canadense*, (B) *A. neriniflorum*, (C) *A. microdictyon*, (D) *A. ochotense*, (E) *A. ramosum*, (F) *A. tuberosum*, (G) *A. bidentatum*, (H) *A. anisopodium*, (I) *A. tenuissimum*, (J) *A. minus*, (K) *A. spirale*, (L) *A. koreanum*, (M) *A. splendens*, (N) *A. caeruleum*, (O) *A. macrostemon*, (P) *A. condensatum*, (Q) *A. fistulosum*, (R) *A. maximowiczii*, (S) *A. linearifolium*, (T) *A. taquetii.* V, verrucae. (Scale bar = 20 μm for all photos).
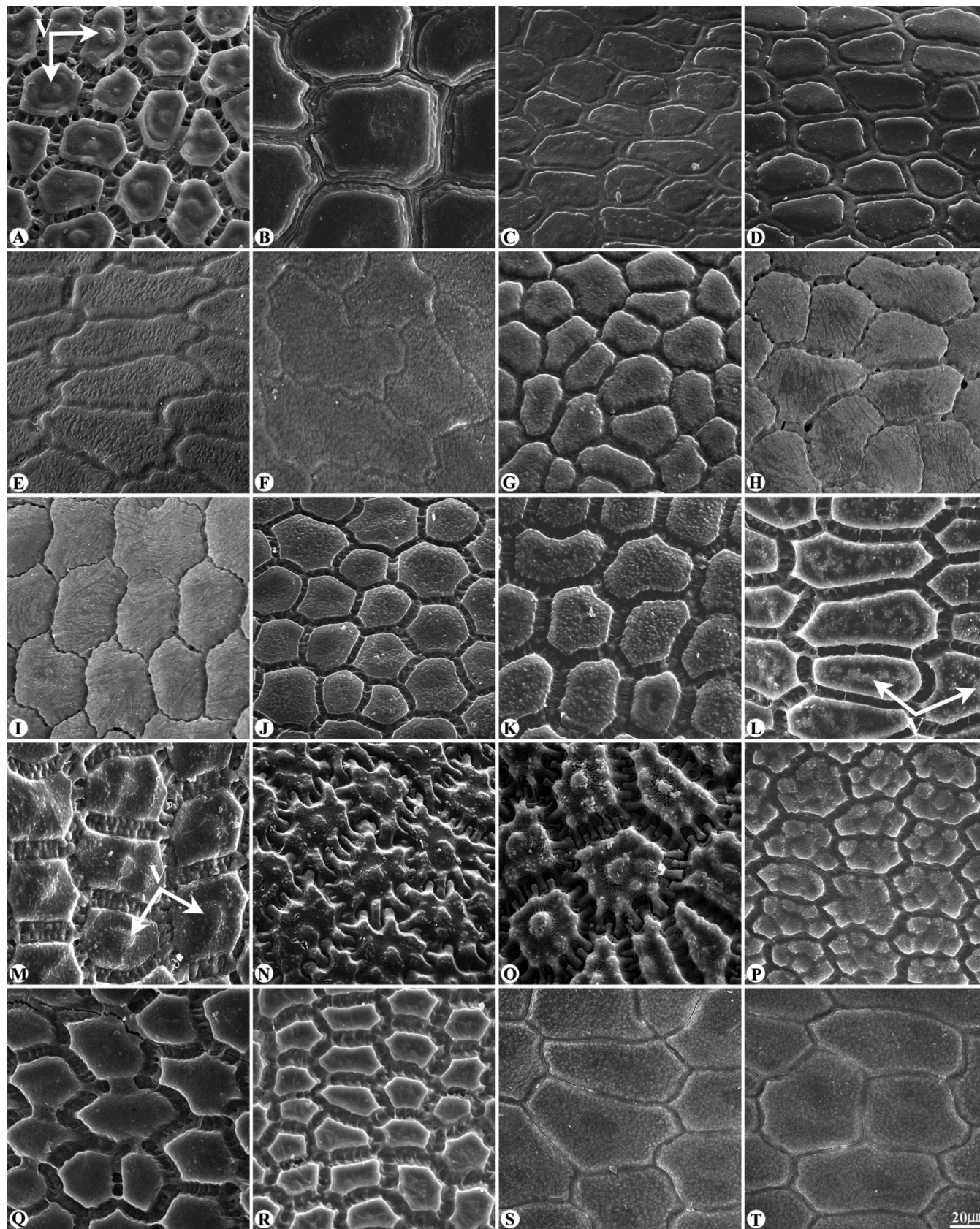
Fig. 4. Details of epidermal cells of the seed coat in *Allium.* Acronyms for each taxon are the same as Fig. 3. Cb, curved boundary; Cc, convex channel; Dc, deep channel; Gv, granulate verruca; Ic, intermediate type of shallow and deep channel; Ou, Omega-like undulation; PSu, poor S-like undulation; Sb, straight boundary; Sc, shallow channel; Su, S-like undulation; Sv, smooth verruca; Tc, terraced channel; Uu, U-like undulation; Vv, verrucate verruca. (Scale bar = 10 μm for all photos).
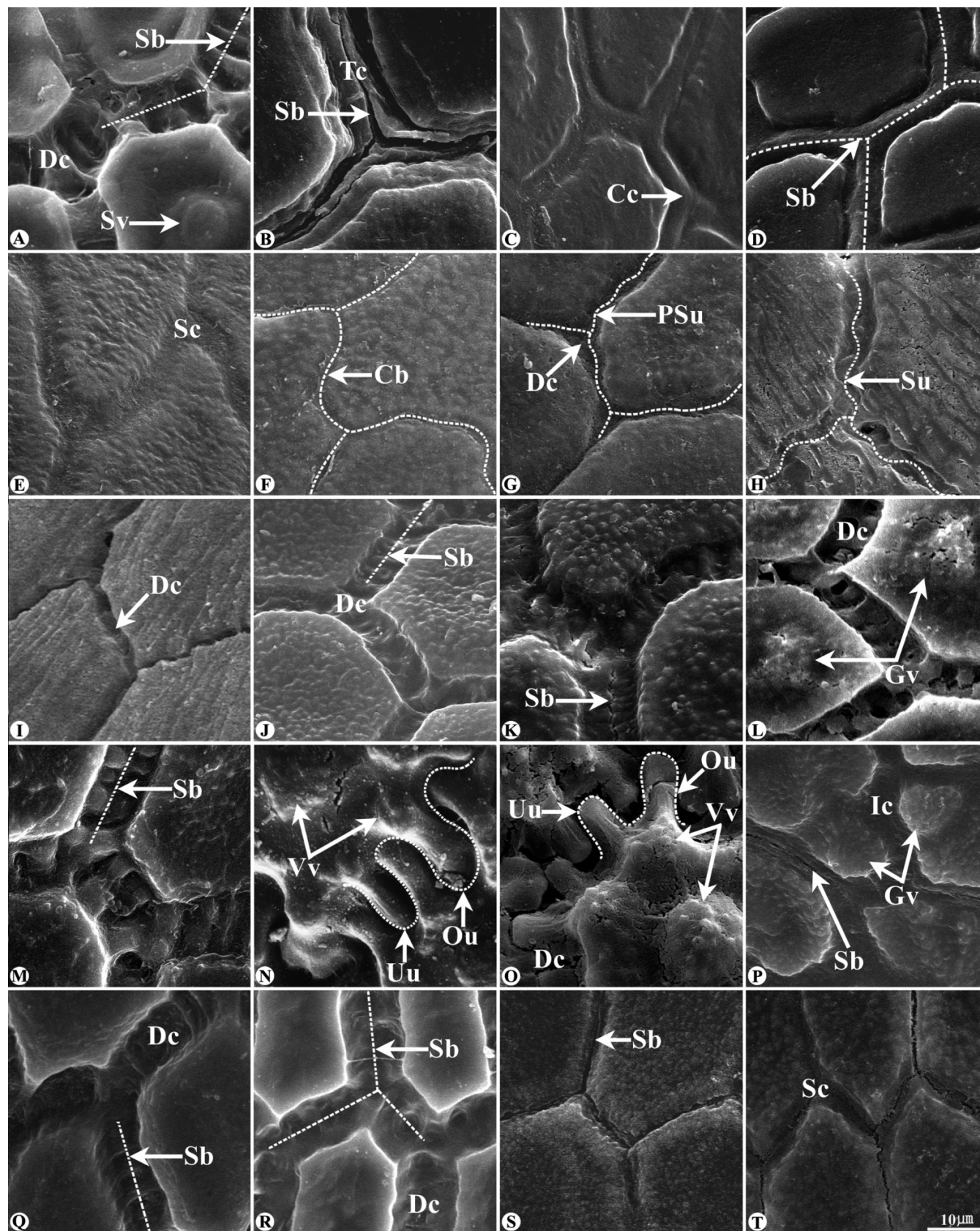
Fig. 5. A comparison of competing phylogenies showing strict consensus trees obtained from ITS (A: length, 1412; CI, 0.57; RI, 0.79) and *trnL–trnF* (B: length, 104; CI, 0.76; RI, 0.90) data sets. Both trees were each derived from two most-parsimonious trees. Bootstrap values >50% are provided below branches. Numbers 1–7 represent major clades (sometimes represented by a branch) as discussed in the text, and the arrows indicate the non-monophyletic nature (and (or) polytomy) of infrageneric grouping recognized by Friesen et al. (2006).
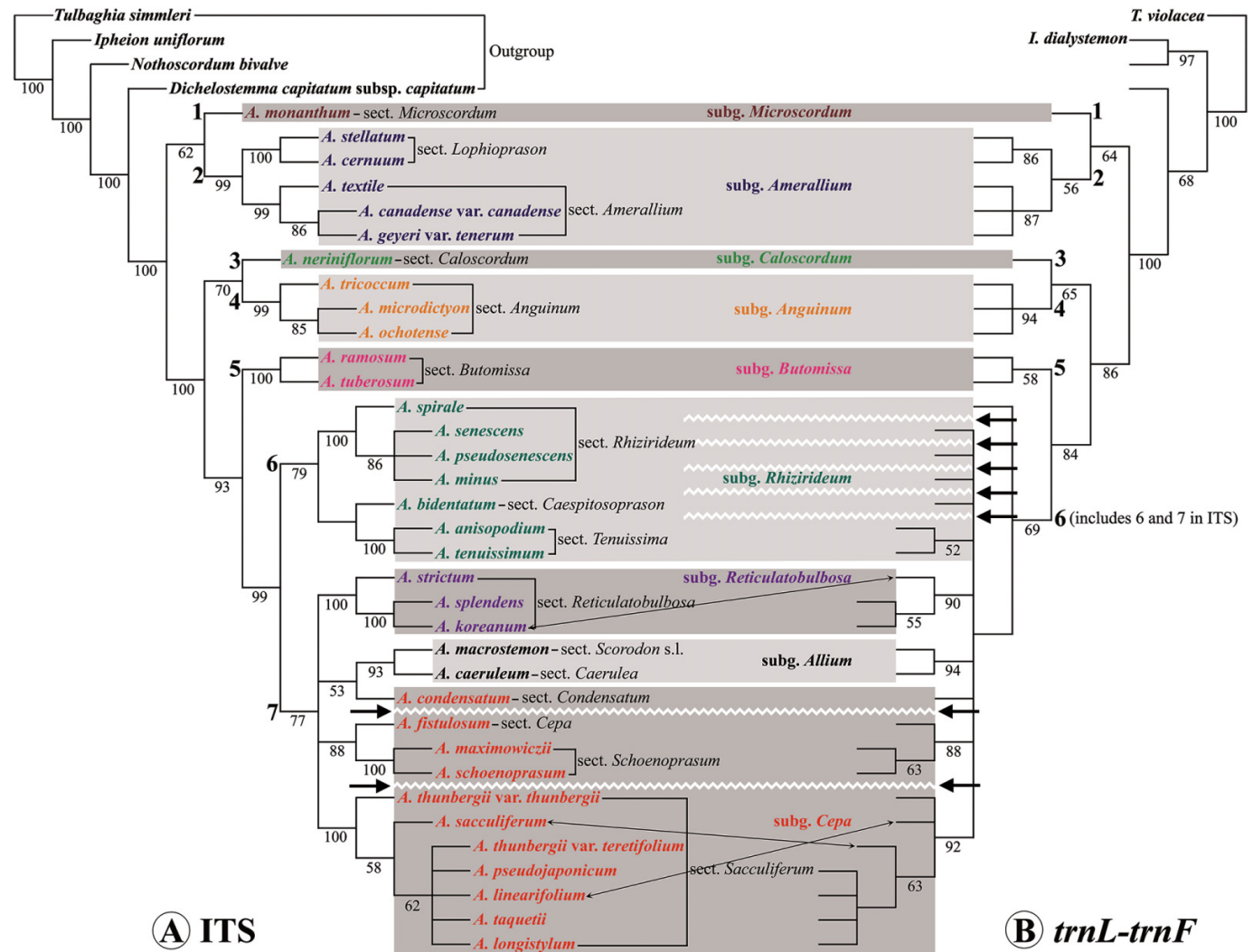
ITS region was more variable and provided the vast majority of phylogenetically informative data compared with the *trnL–trnF* region.

Phylogenetic analysis of the ITS data set

Maximum parsimony (MP) analysis of ITS data produced two most parsimonious trees, with a tree length of 1412 steps, a consistency index (CI) of 0.57, and a retention index (RI) of 0.79, when all 39 accessions were included. We generated the strict consensus tree from the two most parsimonious trees (Fig. 5A). There are six nodes collapsing in the consensus tree. The strict consensus strongly supports the monophyly of *Allium* with high BS value (100%) (Fig. 5A); however, the phylogeny moderately upholds previous taxonomic schemes. A significant finding in this analysis is the polyphyly of the subgenus *Cepa*, represented here by sections *Cepa*, *Condensatum*, *Sacculiferum*, and *Schoenoprasum* in polytomic clade 7. The remaining taxa formed several well-supported infrageneric clades: the monotypic subgenus *Microscordum*, represented by *A. monanthum* (clade 1), sister

group to the New World subgenus *Amerallium* (clade 2); the oligotypic subgenus *Caloscordum* (clade 3), which is sister to the broad leaved subgenus *Anguinum* (clade 4); and the last three well-supported groups represented by subgenus *Butomissa* (clade 5), subgenus *Rhizirideum* (clade 6), and subgenera *Reticulatobulbosa* and *Allium* (clade 7).

Phylogenetic analysis of the *trnL–trnF* data set

MP analysis of *trnL–trnF* data produced two most parsimonious trees with a length of 104 steps (CI, 0.76; RI, 90), from which we generated a strict consensus tree (Fig. 5B). There are 13 nodes collapsing in the consensus tree, quite likely as a result of the limited phylogenetic signal. Despite the fact that the *trnL–trnF* data yielded a less resolved topology compared with the ITS data set, the chloroplast phylogeny recovers most of the major subgenera and clades found in the ITS tree, that is, clades 1–5 (Fig. 5). As in the ITS data set, *Allium* forms a strong monophyletic group with 100% BS support. The position of members in clades 6 and 7 of the ITS phylogeny is inconsistent with that of the *trnL–*

trnF, and unlike the ITS phylogeny, the *trnL–trnF* data set yielded an unresolved subgenus *Rhiziriedum* (Fig. 5).

Phylogenetic analysis of the combined ITS and *trnL–trnF* data set

Our results from the ITS and *trnL–trnF* data sets also show little heterogeneity and the partition homogeneity test for the ITS and *trnL–trnF* indicated, as per the ILD test result, that there is no significant conflict between these two partitions ($P = 0.73$). In our study, there are several clades collapsed in the *trnL–trnF* consensus tree owing to the absence of informative characters but not by incongruent characters. Also, *A. sacculiferum* and *A. linearifolium* are included in the same clade, but internal relationships are not well supported within the clade. The position of these species in both trees (ITS and *trnL–trnF*) varies, but they are not highly supported; hence, it is not considered as true incongruency between trees. In fact, combining trees helped to resolve these polytomies. As stated by Huelsenbeck et al. (1996), it is likely unreasonable to expect that any test of data incongruence would be capable of indentifying cases in which combining data would increase phylogenetic accuracy.

Discussion

Phylogenetic inferences of disjunct *Allium* species

The independent and combined analyses from ITS and *trnL–trnF* support the monophyly of *Allium*, also supported by Friesen et al. (2006), Nguyen et al. (2008), and Li et al. (2010), and yielded sufficient resolution to delineate most sectional and subgeneric boundaries, as proposed by Friesen et al. (2006) (Figs. 5 and 6). This study also substantiates the three main phylogenetic lines in the evolutionary history of *Allium*, as reported by Fritsch (2001), Friesen et al. (2006), and Li et al. (2010). The basal-most and presumably ancestral lineage (I of Fig. 6) consists of subgenera *Microscordum* and *Amerallium* (clades 1 and 2 of Fig. 6). The second evolutionary lineage (II of Fig. 6) includes subgenera *Caloscordum* and *Anguinum* (clades 3 and 4 of Fig. 6), while the third evolutionary lineage (III of Fig. 6) comprises subgenera *Butomissa*, *Rhizirideum*, *Reticulatobulbosa*, *Allium*, and *Cepa* (clades 5–7 of Fig. 6). Among these evolutionary lines, the third one is more complex as the subgeneric relationships, particularly in clade 7, are not well resolved (Figs. 5 and 6).

Our combined analyses of sequence data reveal rather distinct lineages corresponding to the phytogeographic disjunctions in northeastern Asia (subgenera *Microscordum*, *Caloscordum*, *Anguinum*, *Butomissa*, *Rhizirideum*, *Reticulatobulbosa*, *Allium*, and *Cepa*) and northern North America (subg. *Amerallium*) (Figs. 5 and 6), which are, for the most part, consistent with the disjunct geographic patterns existing in most extant and extinct genera of flowering plants (Wen 1998, 1999, 2001; Han et al. 2010). A sister-taxa connection is formed between northeastern Asian and northern North American groups in the first evolutionary lineage (I of Fig. 6): northeastern Asian subgenus *Microscordum* ($x = 8$; clade 1) and subgenus *Amerallium* in North America ($x = 7$; clade 2), albeit weakly supported by BS values (62%, 64%, and 72%, respectively, Figs. 5A, 5B, and 6). Several shared or similar morphological characters of bulbs, bulb tunics, leaves, and flowers support the close affinity between these two subgenera (Friesen et al. 2006). This correlation supports the idea that the ancestor of the New World *Amerallium* originated in eastern Asia (Li et al. 2010), and we believe that migration took place via the Bering Land Bridge, in which exchanges of temperate deciduous plants between eastern Asia and North America were possible throughout most of the Tertiary, as suggested by Hopkins (1967) and Raven and Axelrod (1978). Our phylogenetic hypothesis also suggests that ancestral species existing on both continents diversified early during the origin and diversification of the genus, implying that multiple independent speciation events occurred after the separation and restricted genetic exchange and (or) extinction of species between these regions.

In the second evolutionary lineage (II of Fig. 6), subgenera *Caloscordum* and *Anguinum* are recovered as sister groups with moderate BS support (70%, 65%, and 74%, respectively, in Figs. 5A, 5B, and 6), which is consistent with Friesen et al. (2006), Nguyen et al. (2008), and Li et al. (2010). In spite of this apparent degree of phylogenetic relatedness, these two subgenera rarely share morphological and anatomical characters, except the simple testa cells character in seeds (Figs. 3 and 4). In fact, recent studies involving morphological and anatomical data of Korean and northeastern Chinese *Allium* species do not support a close relationship between these two subgenera (Choi 2009). The lack of morphological support for this subgeneric alliance suggests that the evolutionary rate of morphological and anatomical characters in the *Allium* groups is not parallel to the rate of molecular change, but the possibility of morphological convergent evolution should not be ruled out. Subgenus *Caloscordum* is considered an ancestral entity of the second evolutionary line in *Allium* (Friesen et al. 2006; Li et al. 2010), which concurs with our phylogeny. In addition, *A. tricoccum*, although considered a North American species, represents a special case and falls outside of the $x = 7$ subgenus *Amerallium* (Nguyen et al. 2008). Furthermore, *A. tricoccum* is sister to the northeastern Asian species, *A. microdictyon* and *A. ochotense* ($x = 8$, subg. *Anguinum*) (Nguyen et al. 2008), which suggests an independent radiation event to North America from the dispersal episode leading to the main North American clade of subgenus *Amerallium* (Figs. 5 and 6). In fact, the biogeographic disjunctions between eastern Asia and North America have involved multiple historical events (Wen 1998, 1999). Current distribution ranges of *A. tricoccum* (eastern North America) and its most closely related *A. ursinum* L. (western and central Europe) also suggest that the migration of the subgenus *Anguinum* to North America was most likely via the North Atlantic Land Bridge (Wen 1998, 1999). It has been also proposed that the floristic disjunction involving eastern Asia and eastern and western North America probably arose at different geological times in different genera (Li 1952), an idea supported by Xiang et al. (1998), who proposed that some of the most remarkable examples of intercontinental disjunction are those between eastern Asia and eastern and western North America.

Subgenus *Butomissa* is the first lineage arising in the third evolutionary lineage (III of Fig. 6). Pistil morphology, ovule number, and seed testa cell structures (Figs. 2–4 and 6) exhibit rather plesiomorphic character states in this genus, supporting its basal position and early branching in the third evolutionary line, an idea also proposed by Li et al. (2010).

Fig. 6. Distribution of pistil and seed testa characters onto the strict consensus tree (length, 1526; CI, 0.58; RI, 0.79) of four most-parsimonious trees obtained from the combined ITS and *trnL–trnF* data sets. Bootstrap values >50% are shown below branches, and the systematic position of each ovary type (Fig. 2) is found above branches (indicated with letters A–P). The asterisk (*) and star (★) indicate the first appearance of the crest-like and hood-like processes in the ovary, respectively. Numbers 1–7 and I–III represent major clades (sometimes represented by a branch) and three evolutionary lineages discussed in the text. The arrows indicate the non-monophyletic nature (and (or) polytomy) of infrageneric grouping recognized by Friesen et al. (2006).
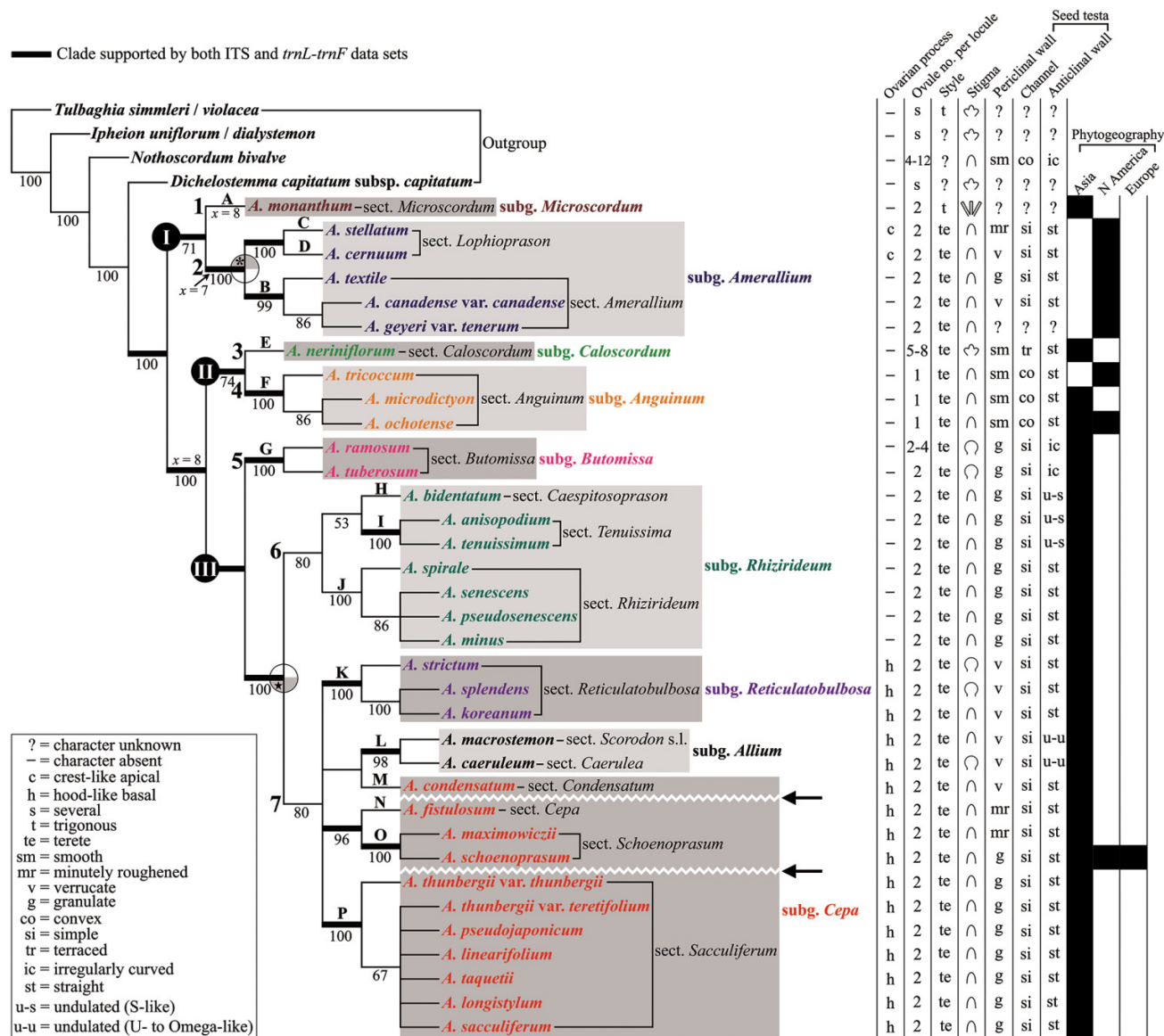
Species from subgenera *Reticulatobulbosa*, *Allium*, and *Cepa* form a large and complex sister clade to subgenus *Butomissa* in this third evolutionary lineage (Figs. 5 and 6). Our results agree with those of Li et al. (2010), indicating that the large polytomy in these subgenera reflects one or more events of rapid radiation and diversification from a common ancestor. Although the taxonomic position and phylogenetic relationship of most infrageneric taxa of the genus *Allium* are well-supported by our molecular data (Figs. 5 and 6), the *trnL–trnF* data set (Fig. 5B), on the other hand, lacks phylogenetically informative sites to adequately delimit section *Rhizirideum* and subgenera *Rhizirideum* and *Cepa*, whose members from northeastern Asia exhibit a high degree of morphological and cytological variability, i.e., chromosome number and karyotype (Ko et al. 2009; Choi and Oh 2010). The ITS and combined phylogenies, in turn, reveal a non-monophyletic subgenus *Cepa* (Figs. 5A and 6). Recent studies (Nguyen et al. 2008; Li et al. 2010) have indicated that some subgenera, including *Cepa*, are not monophyletic. In view of the polyphyletic origin and the lack of molecular synapomorphies and unifying morphological characters, we propose the reappraisal of subgenus *Cepa* involving an extensive taxonomic sampling and comparative morphological analyses.

Our phylogenetic analyses yielded some topological incongruence between the nuclear and chloroplast phylogenies, especially regarding subgenus *Rhizirideum* (clade 6 of Fig. 5). The *trnL–trnF* phylogeny does not support the monophyly and (or) relationships recovered from the ITS data set; instead, the plastid sequence data recovered a large polytomy encompassing species from subgenera *Rhizirideum*, *Reticula-*

tobulbosa, *Allium*, and *Cepa*. It is clear that this result in relation to the topological incongruence between the nuclear and chloroplast phylogenies is the result of the lack of informative characters in the *trnL–trnF* data set; however, hybridization and introgression have been viewed as important processes in plant evolution and speciation (Rieseberg et al. 2003; Mallet 2007) and should not be ruled out. It is feasible that the conflictive position of some species is associated with the biparental (nuclear) and uniparental (chloroplast) nature of these genomes and markers and that hybridization and lineage sorting may be operating in *Allium* (Kim et al. 2008). In fact, lineage sorting obscures the phylogenetic inference especially when the number of loci used to estimate the phylogeny and the number of individuals sampled per species is insufficient (Maddison and Knowles 2006). Forthcoming studies including larger sample size and multiple independent loci from the nucleus, chloroplast, or even mitochondrion are required to more accurately infer the species tree and determine whether any of these processes is involved in the evolution of *Allium*.

Systematic implications of pistil morphology in the genus *Allium*

The major clades in the combined molecular phylogeny represent infrageneric taxa and are strongly correlated with pistil characters (Fig. 6). The systematic utility of pistil characters is significant in tracking down character state changes of this structure and supporting some monophyletic lineages recovered in the phylogeny. For instance, the first evolutionary lineage, clade 1, representing the monotypic subgenus *Microscordum*, is characterized by a trigonous style and a tripartite stigma (Figs. 2A and 6), two characters presumably plesiomorphic (or ancestral) in *Allium* with relation to outgroup taxa. In turn, clade 2, which includes North American species, is clearly divided into two groups: (*i*) section *Amerallium*, a monophyletic assemblage in which the pistil lacks ovarian processes (Figs. 2B and 6) and (*ii*) section *Lophioprason*, another monophyletic group with a pistil bearing crest-like ovarian processes (Figs. 2C, 2D, and 6). Clades 3 and 4 of the second evolutionary lineage include two morphologically divergent groups, namely subgenera *Caloscordum* and *Anguinum*. Each of these subgenera possesses a characteristic type of pistil in the lower part of the ovary, different ovule number, and stigma shapes. That is, the pistil of *A. neriniflorum* (subg. *Caloscordum*; clade 3) has a condensed ovary base, five to eight ovules per locule, and trilobed stigma (Figs. 2E and 6), while *A. microdictyon*, *A. ochotense*, and *A. tricoccum* (subg. *Anguinum*; clade 4) have a pistil with an elongated ovarian base, one ovule per locule, and a whole or unlobed conical stigma (Figs. 2F and 6).

The presence of two types (crest-like apical type in the New World taxa and hood-like basal type in the Old World taxa) of ovarian processes (Figs. 2C, 2D, and 2K–2P) is a unique characteristic of the northern North American (assemblage in the first evolutionary lineage) and northeastern Asian (group in the third evolutionary lineage) *Allium* species (Fig. 6). These processes are sometimes quite prominent, such as in sections *Lophioprason* (with crest-like type; Figs. 2C and 2D) and *Sacculiferum* (with hood-like type; Fig. 2P), and their sizes, shapes, and ornamentations are valuable in classification and phylogenetic inference (Choi et al.

2011). For instance, McNeal (1992) described the ovarian crests as a plesiomorphism in North American *Allium*. However, we infer that the presence of the two types of ovarian processes probably represents an apomorphic character that originated from simple ovaries lacking processes. This hypothesis is based on the optimization of the pistil type on the molecular phylogeny in relation to outgroup taxa (letters A–P on branches in Fig. 6). Taxa with ovarian processes in the northern North American (sect. *Lophioprason*) and northeastern Asian (subgenera *Reticulatobulbosa*, *Allium*, and *Cepa*) lineages are clearly distinct from the other groups having naked ovaries, i.e., lacking processes. In addition, each section or subgenus is also distinguishable by pistil morphology, such as the shape and (or) size of style, ovary, and ovarian processes (Figs. 2 and 6; Choi et al. 2007).

At each putative diverging point (indicated by the circled asterisk and circled star in Fig. 6), the optimization of ovary morphology leads to two groups. In one the ovary lacks processes (sect. *Amerallium* of clade 2 and clade 6), while in the other the ovary has crest- or hood-like processes (sect. *Lophioprason* of clade 2 and clade 7). Our data suggest that the northern North America (sect. *Lophioprason*) and northeastern Asia (subgenera *Reticulatobulbosa*, *Allium*, and *Cepa*) groups (two groups purportedly more recently derived in *Allium*) have crest-like apical and hood-like basal processes, respectively. In contrast, the basal-most group (subg. *Microscordum*) lacks ovarian processes, a condition also present in the basal outgroup lineages, such as *Dichelostemma* Kunth (Pires 2002), *Ipheion* Raf. (Traub and Moldenke 1955), *Nothoscordum* Kunth (Jacobsen and McNeal 2002), and *Tulbaghia* L. (H.J. Choi, personal observation), further suggesting the prevalence of ovary without processes in *Allium* ancestors (Fig. 6; Choi et al. 2011). We also posit that the different types of ovarian processes represent structural traits and modifications probably associated with floral isolation and different pollination syndromes adapted to suit specific pollinators in disjunct *Allium* species between the New and Old World.

Systematic significance of testa sculpture in the genus *Allium*

This study reveals that seed testa sculpture attributes are of systematic value and in combination with seed shape provide key characters to distinguish sections in *Allium* (Figs. 3 and 4; Table 1). The variability of testa sculpture in combination with the molecular data is of reasonable systematic value at the subgeneric classification level. Subgenus *Cepa*, recognized as polyphyletic in molecular phylogenies, also lacks unifying character states, but the periclinal cell walls of seed testa vary from minutely roughened to verrucate (Figs. 3P–3T, 4P–4T, and 6). The optimization of seed characters on the strict consensus (Fig. 6) supports previous ideas, indicating that the prominent design patterns of the periclinal walls with distinct verrucate sculptures along with S- to Omega-like undulated anticlinal walls have evolved from relatively simple sculptures, which are characterized by straight anticlinal walls and nearly smooth to minutely granulate periclinal walls (Fritsch et al. 2006). The verrucate periclinal walls represent a homoplasious autapomorphic character in *A. canadense* var. *canadense* (sect. *Amerallium*) and *A. cernuum* (sect. *Lophioprason*) of the New World subgenus *Ameral-*

lium, whereas this trait is a synapomorphy in some Old World taxa (sections *Caerulea*, *Condensatum*, *Reticulatobulbosa*, and *Scorodon* s.l.) (Fig. 6).

Another taxonomic inference arising from seed testa information involves *A. macrostemon*, a species traditionally placed in section *Scorodon* s.l. of subgenus *Allium* (Hanelt et al. 1992). Friesen et al. (2006) did not classify this species at the sectional level but rather treated it within subgenus *Allium*; the possibility of erecting a new section was not considered back then. Based on our data and considering other reports on testa sculptures (Kruse 1984, 1986, 1988, 1992, 1994; McNeal 1992; Fritsch et al. 2006; Neshati and Fritsch 2009; Choi and Cota-Sánchez 2010), the testa sculpture of *A. macrostemon* (Figs. 3O and 4O) has more similarities with members of section *Caerulea* (subg. *Allium*), i.e., *A. caeruleum* (Figs. 3N and 4N), type species of section *Caerulea*, and *A. caesium* Schrenk (Fig. 12 of Kruse 1986). Other morphological characters such as the development of bulbils in the inflorescence, pistil morphology, and general shape of seed are quite similar between *A. macrostemon* and *A. caeruleum* (Fig. 6; Table 1). Furthermore, restriction site data of cpDNA also revealed their close relationship (Mes et al. 1999). However, considering that subgenus *Allium* comprises ca. 300 species (Friesen et al. 2006), we recommend maintaining the sectional status of *A. macrostemon* in *Scorodon* s.l. (subg. *Allium*) until wider range surveys, including various members of subgenus *Allium*, are evaluated.

Friesen et al. (2006) described the monotypic section *Condensatum* from the traditional classification of section *Oreiprason* F. Herm. (subg. *Polyprason* Radić) and treated it as an additional member of a re-circumscribed subgenus *Cepa*. Here, for the first time, we report verrucate periclinal walls and straight anticlinal walls (Figs. 3P and 4P) in section *Condensatum* (*Allium condensatum*). This trait supports *A. condensatum* in its own section, which was also proposed by Friesen et al. (2006). Based on testa sculpture, *A. condensatum* is clearly distinguished from the other members of section *Oreiprason* (e.g., *A. hymenorrhizum* Ledeb.; Fig. 15 of Kruse 1986 and *A. obliquum* L.; H.J. Choi, personal observation) and subgenus *Cepa* (Figs. 3Q–3T and 4Q–4T). However, comparative work with previous studies indicates that *A. condensatum*'s testa is more closely related to *A. carinatum* L. (Fig. 18 of Kruse 1984), *A. flavum* L. (Figs. 19–23 of Kruse 1984), and *A. melanantherum* Pančić (Fig. 18 of Kruse 1988), members of section *Codonoprason* Rchb. of subgenus *Allium*, instead of sections *Cepa*, *Sacculiferum*, and *Schoenoprasum* of subgenus *Cepa*. This morphological information supports our molecular phylogenetic groupings from ITS and combined data sets. That is, section *Condensatum* is more closely related to subgenus *Allium* than *Cepa* (Figs. 5A and 6).

Testa sculptures with rather convex channels have been reported in seeds of *Agapanthus orientalis* Leighton (Amaryllidaceae s.s) (Fig. 46 of Kruse 1986), the sister group of Alliaceae s.s., as well as *Nothoscordum bivalve* (Fig. 48 of Kruse 1986) and *Nothoscordum inodorum* (Aiton) Nicholson (Fig. 47 of Kruse 1986) of the Alliaceae s.s. In this study we observed the convex type boundary in the relatively early-evolved subgenus *Anguinum* (*A. microdictyon*, *A. ochotense*, and *A. tricoccum*; Figs. 3C, 3D, 4C, and 4D). Testa sculptures in these taxa are very similar and share the nearly smooth periclinal walls and the convex channel. Even though this is a homoplasious character, it may suggest a phylogenic relationship among the three genera as members of the recently expanded Amaryllidaceae s.l. (sensu The Angiosperm Phylogeny Group).

In addition to the systematic significance of seed morphology, we postulate that various aspects of seed structure in *Allium* species are correlated with ecological distribution. For instance, one of the most important adaptive factors for seed germination in *Allium* is the retention of water in the seed coat surface area (Finch-Savage and Phelps 1993). From this perspective, it makes sense to think that the complex testa architecture from granulate to verrucate patterns on periclinal walls and curved to undulated outlined anticlinal walls increase seed surface area. These features, in conjunction with a deep and wide channel region, possibly represent an adaptation facilitating the retention of water enhancing germination rate in the usually dry and sunny habitats in which *Allium* species propagate. In contrast, species growing in the moist and shaded habitat of deciduous forest, such as *A. microdictyon*, *A. ochotense*, and *A. tricoccum*, have smooth periclinal walls and straight anticlinal boundaries with slightly convex channels (Figs. 3C, 3D, 4C, and 4D).

Concluding remarks

Pistil and seed coat morphology provides systematic information to assess the degree of relationship at different taxonomic levels in *Allium*. Seed testa patterns, in particular, offer key characters supporting major clades recovered in the molecular phylogeny, and the two types of ovarian processes in the Old and New World species are recently derived features in each continent. Our molecular phylogeny recovers a similar topology to that reported in previous studies (Friesen et al. 2006; Nguyen et al. 2008; Li et al. 2010), with most infrageneric groups being monophyletic, except the polyphyletic subgenus *Cepa*. We also confirmed three major evolutionary lineages (Friesen et al. 2006; Li et al. 2010) and the radiation patterns and ancestry of subgenera *Ameralliam* and *Anguinum* (Li et al. 2010) and hypothesize that the northeastern Asian and northern North American disjunction of *Allium* is the result of multiple ancient intercontinental migrations. Additional wider range studies including species from both the Old and New World not included in this study nor in previous investigations will be useful in the elucidation of the infrageneric relationships and evolutionary trends in *Allium*.

Acknowledgements

References

Angiosperm Phylogeny Group. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of

flowering plants: APG III. Bot. J. Linn. Soc. **161**(2): 105–121. doi:10.1111/j.1095-8339.2009.00996.x.

Bakker, F.T., Culham, A., Daugherty, L.C., and Gibby, M. 1999. A *trn*L–F based phylogeny for species of *Pelargonium* (Geraniaceae) with small chromosomes. Plant Syst. Evol. **216**(3–4): 309–324. doi:10.1007/BF01084405.

Barkalov, V.Ju. 1987. *Allium* L. *In* Plantae vasculares orientis extremi Sovietici. Vol. 2. *Edited by* S.S. Charkevicz. Nauka, Leningrad, Russia. pp. 376–393.

Beardsley, P.M., and Olmstead, R.G. 2002. Redefining Phrymaceae: the placement of *Mimulus*, tribe Mimuleae and *Phryma*. Am. J. Bot. **89**(7): 1093–1102. doi:10.3732/ajb.89.7.1093. PMID: 21665709.

Chase, M.W., Reveal, J.L., and Fay, M.F. 2009. A subfamilial classification for the expanded asparagalean families Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae. Bot. J. Linn. Soc. **161** (2): 132–136. doi:10.1111/j.1095-8339.2009.00999.x.

Choi, H.J. 2009. Systematics of the genus *Allium* (Alliaceae) in Korea and northeastern China. Ph.D. thesis, Department of Biology, The Chungbuk National University, Cheongju, Chungbuk, Korea.

Choi, H.J., and Cota-Sánchez, J.H. 2010. A taxonomic revision of *Allium* (Alliaceae) in the Canadian prairie provinces. Botany, **88**(9): 787–809. doi:10.1139/B10-056.

Choi, H.J., and Oh, B.U. 2010. A new species and a new combination of *Allium* sect. *Rhizirideum* (Alliaceae) from northeastern China and Korea. Brittonia, **62**(3): 199–205. doi:10.1007/s12228-009-9124-6.

Choi, H.J., and Oh, B.U. 2011. A partial revision of *Allium* (Amaryllidaceae) in Korea and north-eastern China. Bot. J. Linn. Soc. **167**(2): 153–211. doi:10.1111/j.1095-8339.2011.01166.x.

Choi, H.J., Jang, C.G., Lee, Y.M., and Oh, B.U. 2007. A taxonomic study of Korean *Allium* L. based on morphological characters. Korean J. Plant Taxonomy, **37**(3): 275–308.

Choi, H.J., Davis, A.R., and Cota-Sánchez, J.H. 2011. Comparative floral structure of four New World *Allium* (Amaryllidaceae) species. Syst. Bot. **36**(4): 870–882. doi:10.1600/036364411X604895.

de Sarker, D., Johnson, M.A.T., Reynolds, A., and Brandham, P.E. 1997. Cytology of the highly polyploid disjunct species, *Allium dregeanum* (Alliaceae), and of some Eurasian relatives. Bot. J. Linn. Soc. **124**(4): 361–373. doi:10.1006/bojl.1997.0109.

Dubouzet, J.G., and Shinoda, K. 1998. Phylogeny of *Allium* L. subgenus *Melanocrommyum* (Webb et Berth.) Rouy based on DNA sequence analysis of the internal transcribed spacer region of nrDNA. Theor. Appl. Genet. **97**(4): 541–549. doi:10.1007/s001220050929.

Dubouzet, J.G., and Shinoda, K. 1999. Relationships among Old and New World *Alliums* according to ITS DNA sequence analysis. Theor. Appl. Genet. **98**(3–4): 422–433. doi:10.1007/s001220051088.

Farris, J.S., Källersjö, M., Kluge, A.G., and Bult, C. 1994. Testing significance of incongruence. Cladistics, **10**(3): 315–319. doi:10.1111/j.1096-0031.1994.tb00181.x.

Farris, J.S., Källersjö, M., Kluge, A.G., and Bult, C. 1995. Constructing a significance test for incongruence. Syst. Biol. **44**(4): 570–572.

Fay, M.F., and Chase, M.W. 1996. Resurrection of Themidaceae for the *Brodiaea* alliance, and recircumscription of Alliaceae, Amaryllidaceae and Agapanthoideae. Taxon, **45**(3): 441–451. doi:10.2307/1224136.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, **39**(4): 783–791. doi:10.2307/2408678.

Finch-Savage, W.E., and Phelps, K. 1993. Onion (*Allium cepa* L.) seedling emergence patterns can be explained by the influence of soil temperature and water potential on seed germination. J. Exp. Bot. **44**(2): 407–414. doi:10.1093/jxb/44.2.407.

Friesen, N., Fritsch, R.M., Pollner, S., and Blattner, F.R. 2000. Molecular and morphological evidence for an origin of the aberrant genus *Milula* within Himalayan species of *Allium* (Alliaceae). Mol. Phylogenet. Evol. **17**(2): 209–218. doi:10.1006/mpev.2000.0844. PMID:11083935.

Friesen, N., Fritsch, R.M., and Blattner, F.R. 2006. Phylogeny and new intrageneric classification of *Allium* (Alliaceae) based on nuclear ribosomal DNA ITS sequences. Aliso, **22**: 372–395.

Fritsch, R.M. 2001. Taxonomy of the genus *Allium*: contribution from IPK Gatersleben. Herbertia, **56**: 19–50.

Fritsch, R.M., and Keusgen, M. 2006. Occurrence and taxonomic significance of cysteine sulphoxides in the genus *Allium* L. (Alliaceae). Phytochemistry, **67**(11): 1127–1135. doi:10.1016/j.phytochem.2006.03.006. PMID:16626766.

Fritsch, R.M., Kruse, J., Adler, K., and Rutten, T. 2006. Testa sculptures in *Allium* L. subg. *Melanocrommyum* (Webb & Berth.) Rouy (Alliaceae). Feddes Repert. **117**(3–4): 250–263. doi:10.1002/fedr.200611094.

Goloboff, P.A., Farris, J.S., and Nixon, K.C. 2003. TNT: tree analysis using new technology, version 1.1. Program and documentation. Available from http://www.zmuc.dk/public/phylogeny/tnt/.

Goloboff, P.A., Farris, J.S., and Nixon, K.C. 2008. TNT, a free program for phylogenetic analysis. Cladistics, **24**(5): 774–786. doi:10.1111/j.1096-0031.2008.00217.x.

Gurushidze, M., Mashayekhi, S., Blattner, F.R., Friesen, N., and Fritsch, R.M. 2007. Phylogenetic relationships of wild and cultivated species of *Allium* section *Cepa* inferred by nuclear rDNA ITS sequence analysis. Plant Syst. Evol. **269**(3–4): 259–269. doi:10.1007/s00606-007-0596-0.

Gurushidze, M., Fritsch, R.M., and Blattner, F.R. 2008. Phylogenetic analysis of *Allium* subg. *Melanocrommyum* infers cryptic species and demands a new sectional classification. Mol. Phylogenet. Evol. **49**(3): 997–1007. doi:10.1016/j.ympev.2008.09.003. PMID:18824112.

Gurushidze, M., Fritsch, R.M., and Blattner, F.R. 2010. Species-level phylogeny of *Allium* subgenus *Melanocrommyum*: incomplete lineage sorting, hybridization and *trnF* gene duplication. Taxon, **59**(3): 829–840.

Hall, T.A. 2001. BioEdit: a user-friendly biological sequence alignment editor and analysis, version 5.09. Department of Micobiology, North Carolina State University, Raleigh, N.C.

Han, J.E., Chung, K.-H., Nemoto, T., and Choi, B.-H. 2010. Phylogenetic analysis of eastern Asian and eastern North American disjunct *Lespedeza* (Fabaceae) inferred from nuclear ribosomal ITS and plastid region sequences. Bot. J. Linn. Soc. **164**(3): 221–235. doi:10.1111/j.1095-8339.2010.01084.x.

Hanelt, P., Schulze-Motel, J., Fritsch, R.M., Kruse, J., Maass, H., Ohle, H., and Pistrick, K. 1992. Infrageneric grouping of *Allium* — the Gatersleben approach. *In* The genus *Allium* — taxonomic problems and genetic resources. Proceedings of an International Symposium, Gatersleben, Germany, 11–13 June 1991. *Edited by* P. Hanelt, K. Hammer, and H. Knupffer. Gatersleben, Germany. pp. 107–123.

Hopkins, D.A. 1967. The Bering Land Bridge. Stanford University Press, Stanford, Calif.

Hotta, M. 1998. Taxonomical notes on plants of southern Japan. IV. The *Allium thunbergii* group (Liliaceae) distributed in southern Kyushu and Ryukyu Island. Acta Phytotaxon. Geobot. **49**(1): 57–66.

Huelsenbeck, J.P., Bull, J.J., and Cunningham, C.W. 1996. Combining data in phylogenetic analysis. Trends Ecol. Evol. **11**(4): 152–158. doi:10.1016/0169-5347(96)10006-9. PMID:21237790.

Jacobsen, T.D., and McNeal, D.W. 2002. *Nothoscordum* Kunth. *In* Flora of North America. Vol. 26. *Edited by* Editorial Committee of Flora of North America. Oxford University Press, New York, N.Y. pp. 276–277.

Johansen, D.A. 1940. Plant microtechnique. McGraw-Hill, New York, N.Y.

Kamelin, R.V. 1973. Florogeneticheskij analiz estestvennoj flory gornoj Srednej Azii. Nauka, Leningrad, Russia.

Kim, S.-C., Mejías, J.A., and Lubinsky, P. 2008. Molecular confirmation of the hybrid origin of the critically endangered western Mediterranean endemic *Sonchus pustulatus* (Asteraceae: Sonchinae). J. Plant Res. **121**(4): 357–364. doi:10.1007/s10265-008-0166-8. PMID:18491031.

Ko, E.M., Choi, H.J., and Oh, B.U. 2009. A cytotaxonomic study of *Allium* (Alliaceae) sect. *Sacculiferum* in Korea. Korean J. Plant Taxonomy, **39**(3): 170–180.

Kovtonyuk, N.K., Barkalov, V.Ju., and Friesen, N.V. 2009. Synopsis of the family Alliaceae Borkh. (Onions) of Asian part of Russia. Turczaninowia, **12**(3–4): 31–39.

Kruse, J. 1984. Rasterelektronenmikroskopische untersuchungen an samen der gattung *Allium* L. Kulturpflanze, **32**(2): 89–101. doi:10.1007/BF02002072.

Kruse, J. 1986. Rasterelektronenmikroskopische untersuchungen an samen der gattung *Allium* L. II. Kulturpflanze, **34**(2): 207–228. doi:10.1007/BF02112802.

Kruse, J. 1988. Rasterelektronenmikroskopische untersuchungen an samen der gattung *Allium* L. III. Kulturpflanze, **36**(3): 355–368. doi:10.1007/BF02034817.

Kruse, J. 1992. Variability of testa sculptures in the genus *Allium* L. *In* The genus *Allium* — taxonomic problems and genetic resources. Proceedings of an International Symposium, Gatersleben, Germany, 11–13 June 1991. *Edited by* P. Hanelt, K. Hammer, and H. Knupffer. Gatersleben, Germany. pp. 181–182.

Kruse, J. 1994. Rasterelektronenmikroskopische untersuchungen an samen der gattung *Allium* L. IV. Feddes Repert. **105**(7–8): 457–471.

Li, H.L. 1952. Floristic relationships between eastern Asia and eastern North America. Trans. Am. Philos. Soc. **42**(2): 371–429. doi:10.2307/1005654.

Li, Q.-Q., Zhou, S.-D., He, X.-J., Yu, Y., Zhang, Y.-C., and Wei, X.-Q. 2010. Phylogeny and biogeography of *Allium* (Amaryllidaceae: Allieae) based on nuclear ribosomal internal transcribed spacer and chloroplast *rps*16 sequences, focusing on the inclusion of species endemic to China. Ann. Bot. (Lond.), **106**(5): 709–733. doi:10.1093/aob/mcq177. PMID:20966186.

Linne von Berg, G., Samoylov, A., Klaas, M., and Hanelt, P. 1996. Chloroplast DNA restriction analysis and the infrageneric grouping of *Allium* (Alliaceae). Plant Syst. Evol. **200**(3–4): 253–261. doi:10.1007/BF00984939.

Maddison, W.P., and Knowles, L.L. 2006. Inferring phylogeny despite incomplete lineage sorting. Syst. Biol. **55**(1): 21–30. doi:10.1080/10635150500354928. PMID:16507521.

Mallet, J. 2007. Hybrid speciation. Nature, **446**(7133): 279–283. doi:10.1038/nature05706. PMID:17361174.

McNeal, D.W. 1992. Taxonomy of North American species of *Allium*. *In* The genus *Allium* — taxonomic problems and genetic resources. Proceedings of an International Symposium, Gatersleben, Germany, 11–13 June 1991. *Edited by* P. Hanelt, K. Hammer, and H. Knupffer. Gatersleben, Germany. pp. 195–204.

McNeal, D.W., and Jacobsen, T.D. 2002. *Allium* L. *In* Flora of North America. Vol. 26. *Edited by* Editorial Committee of Flora of North America. Oxford University Press, New York, N.Y. pp. 224–276.

Mes, T.H.M., Friesen, N., Fritsch, R.M., Klaas, M., and Bachmann, K. 1997. Criteria for sampling in *Allium* based on chloroplast DNA PCR–RFLPs. Syst. Bot. **22**(4): 701–712. doi:10.2307/2419436.

Mes, T.H.M., Fritsch, R.M., Pollner, S., and Bachmann, K. 1999. Evolution of the chloroplast genome and polymorphic ITS regions in *Allium* subg. *Melanocrommyum*. Genome, **42**(2): 237–247. doi:10.1139/g98-123. PMID:10231958.

Neshati, F., and Fritsch, R.M. 2009. Seed characters and testa sculptures of some Iranian *Allium* L. species (Alliaceae). Feddes Repert. **120**(5–6): 322–332. doi:10.1002/fedr.200911112.

Nguyen, N.H., Driscoll, H.E., and Specht, C.D. 2008. A molecular phylogeny of the wild onions (*Allium*; Alliaceae) with a focus on the western North American center of diversity. Mol. Phylogenet. Evol. **47**(3): 1157–1172. doi:10.1016/j.ympev.2007.12.006. PMID:18226928.

Ohwi, J. 1984. Flora of Japan. Smithsonian Institution, Washington, D.C.

Pires, J.C. 2002. *Dichelostemma* Kunth. *In* Flora of North America. Vol. 26. *Edited by* Editorial Committee of Flora of North America. Oxford University Press, New York, N.Y. pp. 328–331.

Rabinowitch, H.D., and Currah, L. 2002. *Allium* crop science: recent advances. CABI Publishing, New York, N.Y.

Raven, P.H., and Axelrod, D.I. 1978. Origin and relationships of the Californian flora. Univ. Calif. Publ. Bot. **72**: 1–134.

Regel, E. 1875. Alliorum adhuc cognitorum monographia. Trudy Imper. S.-. Peterburgsk. Bot. Sada, **3**: 1–266.

Rieseberg, L.H., Raymond, O., Rosenthal, D.M., Lai, Z., Livingstone, K., Nakazato, T., Durphy, J.L., Schwarzbach, A.E., Donovan, L. A., and Lexer, C. 2003. Major ecological transitions in wild sunflowers facilitated by hybridization. Science, **301**(5637): 1211–1216. doi:10.1126/science.1086949. PMID:12907807.

Samoylov, A., Klaas, H., and Hanelt, P. 1995. Use of chloroplast polymorphism for the phylogenetic study of subgenera *Amerallium* and *Bromatorrhiza* (genus *Allium*). Feddes Repert. **106**(3–4): 161–167.

Samoylov, A., Friesen, N., Pollner, S., and Hanelt, P. 1999. Use of chloroplast polymorphism for the phylogenetic study of *Allium* subgenera *Amerallium* and *Bromatorrhiza* (Alliaceae) II. Feddes Repert. **110**(1–2): 103–109.

Taberlet, P., Gielly, L., Pautou, G., and Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. **17**(5): 1105–1109. doi:10.1007/BF00037152. PMID:1932684.

Takahashi, H., and Hotta, M. 2009. A taxonomic revision of the *Allium virgunculae* complex (Alliaceae). Acta Phytotaxon. Geobot. **60**(2): 79–86.

Traub, H.P. 1968. The subgenera, sections and subsections of *Allium* L. Plant Life, **24**: 147–163.

Traub, H.P., and Moldenke, H.N. 1955. The genus *Ipheion*, diagnosis, key to species and synonyms. Plant Life, **11**: 125–130.

van der Meer, Q.P. 1997. Old and new crops within edible alliums. Acta Hortic. **433**: 17–31.

von Linné, C. 1753. Species plantarum. L. Salvii, Stockholm, Sweden.

Vvedensky, A.I. 1935. *Allium* L. *In* Flora U.S.S.R. Vol. 4. *Edited by* V.L. Komarov. Botanical Institute of Academy of Science, Leningrad, Russia. pp. 12–280.

Wen, J. 1998. Evolution of the eastern Asian and eastern North American disjunct pattern: insight from phylogenetic studies. Korean J. Plant Taxonomy, **28**(1): 63–81.

Wen, J. 1999. Evolution of eastern Asian and eastern North American disjunct distributions in flowering plants. Annu. Rev. Ecol. Syst. **30**(1): 421–455. doi:10.1146/annurev.ecolsys.30.1.421.

Wen, J. 2001. Evolution of eastern Asian-eastern North American biogeographic disjunctions: a few additional issues. Int. J. Plant Sci. **162**(S6): S117–S122. doi:10.1086/322940.

White, T.J., Bruns, T.D., Lee, S.B., and Taylor, J.W. 1990.

Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* PCR protocols: a guide to method and applications. *Edited by* M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White. Academic Press, Sand Diego, Calif. pp. 315–322.

Xiang, Q.Y., Soltis, D.E., and Soltis, P.S. 1998. The eastern Asian and eastern and western North American floristic disjunction: congruent phylogenetic patterns in seven diverse genera. Mol. Phylogenet. Evol. **10**(2): 178–190. doi:10.1006/mpev.1998.0524. PMID:9878229.

Xu, J.-M., and Kamelin, R.V. 2000. *Allium* L. *In* Flora of China. Vol. 24. *Edited by* Z.Y. Wu and P.H. Raven. Science Press, Beijing, China, and Missouri Botanical Garden Press, St. Louis, Mo. pp. 165–202.

Appendix A

List of species investigated, including taxonomic authorities, voucher information, and GenBank accession numbers. The information is listed as follows; taxon, locality, collection number (herbarium acronym), identity of Figs. 1–6, and GenBank accession (ITS, *trnL–trnF*). The asterisk (*) indicates DNA sequences obtained from GenBank.

Allium L.—subg. *Allium*: sect. *Caerulea* (Omelcz.) F.O. Khassanov, *A. caeruleum* Pall., Korea National Arboretum, Gyeonggi, Korea (Cultivated), *H.J.Choi 090001* (KH), Figs. 1Q, 2L, 3N, 4N, -JF262659; *ITS-AJ412729. sect. *Scorodon* Koch s.l., *A. macrostemon* Bunge, Soheul-eup, Pocheon-si, Gyeonggi, Korea, *H.J.Choi 080002* (KH), Figs. 1R, 3O, 4O, *trnL–trnF*-JF262658; *ITS-AJ412738. subg. *Amerallium* Traub: sect. *Amerallium* Traub, *A. canadense* L. var. *canadense*, Sherbrooke, Quebec, Canada, *H.J.Choi-QC-1* (SASK), Figs. 3A, 4A, -JF262637; *ITS-EU096145. *A. geyeri* S. Watson var. *tenerum* M.E. Jones, Waternton Lakes National Park, Alberta, Canada, *H.J.Choi-AB-1* (SASK), Fig. 1B, ITS-JF262634, *trnL–trnF*-JF262638. *A. textile* A. Nelson & J.F. Macbride, Beaver creek, Saskatoon, Saskatchewan, Canada, *H.J.Choi-SK-17* (SASK), Figs. 1C, 2B, ITS-JF262635, *trnF*-JF262639. sect. *Lophioprason* Traub, *A. cernuum* Roth, Cypress Hills, Saskatchewan, Canada, *H.J.Choi-SK-10* (SASK), Figs. 1D, 2D, -JF262641; *ITS-AJ250289. *A. stellatum* Ker Gawler, Macdowell, Saskatchewan, Canada, *H.J.Choi-SK-12* (SASK), Figs. 1E, 2C; Martensville, Saskatchewan, Canada, *H.J.Choi-SK-16* (SASK), *trnL–trnF*-JF262640; *ITS-AF055102. subg. *Anguinum* (G. Don ex Koch) N. Friesen: sect. *Anguinum* G. Don ex Koch, *A. microdictyon* Prokh., Bukdae, Odaesan, Gangwon, Korea, *H.J.Choi et al. 010008* (CBU), ITS-GQ412216; Gariwangsan, Pyeongchang-gun, Gangwon, Korea, *H.J.Choi 040002* (KH), Figs. 2F, 3C, 4C, -JF262643. *A. ochotense* Prokh., Seonginbong, Ulleungdo-Gyeongbuk, Korea, *H.J.Choi 020056* (CBU), Figs. 1G, 3D, 4D, ITS-GQ412224, *trnL–trnF*-JF262644. *A. tricoccum* Solander, Ottawa, Ontario, Canada, *H.J.Choi-ON-1* (SASK), Fig. 1H, -JF262645; *ITS-AJ411917. subg. *Butomissa* (Salisb.) N. Friesen: sect. *Butomissa* (Salisb.) Kamelin, *A. ramosum* L., Qiqihar, Heilongjiang, China, *L-61237* (KH), Figs. 1I, 2G, 3E, 4E; Tahe, Heilongjiang, China, *Y.M.Lee & H.J.Choi 080001* (KH), *trnL–trnF*-JF262646; *ITS-AJ250295. *A. tuberosum* Rottler ex Sprengel, Seodaesan, Geumsan-gun, Chungnam, Korea, *Y.Y.Kim et al. 020075* (CBU), Figs. 3F, 4F; Daecheongdo, Incheon-si, Gyeonggi, Korea, *H.J.Choi 080254* (KH), Fig. 1J, *trnL–trnF*-JF262647; *ITS-AJ411914.

subg. *Caloscordum* (Herb.) R.M. Fritsch: sect. *Caloscordum* Herb., *A. neriniflorum* (Herb.) Baker, Dagosan, Dandong-shi, Liaoning, China, *CBU-037* (KH, CBU), Figs. 1F, 2E, 3B, 4B, -JF262642; *ITS-AJ411913. subg. *Cepa* (Mill.) Radić: sect. *Cepa* (Mill.) Prokh., *A. fistulosum* L., Soheul-eup, Pocheon-si, Gyeonggi, Korea (Cultivated), *S.H.Park 027565* (KH), Figs. 1T, 2N, 3Q, 4Q; *ITS-AJ411918; *trnL–trnF*-FJ628604. sect. *Condensatum* N. Friesen, *A. condensatum* Turcz., Gunhamsan, Hwaryong, Jilin, China, *B.U.Oh et al. 030012* (CBU), Figs. 1S, 2M, 3P, 4P, -JF262660; *ITS-AJ412752. sect. *Sacculiferum* P.P. Gritzenko, *A. linearifolium* H.J. Choi & B.U. Oh, Woraksan, Jecheon-si, Chungbuk, Korea, *H.J.Choi et al. 020001* (CBU-isotype), Figs. 1W, 3S, 4S, ITS-GQ412207, *trnL–trnF*-JF262666. *A. longistylum* Baker, Bukhangang, Hwacheon-gun, Gangwon, Korea, *B.U.Oh et al. 020038* (CBU), Fig. 2P; Donggang, Jeongseon-gun, Gangwon, Korea, *E.S.Jeon & H.J.Choi 070001* (KH), ITS-GQ412209, -JF262668. *A. pseudojaponicum* Makino, Geomundo, Yeosu-si, Jeonnam, Korea, *H.J.Choi 50377* (KH), ITS-GQ412227, *trnL–trnF*-JF262665. *A. sacculiferum* Maxim., Duwibong, Jeongseon-gun, Gangwon, Korea, *H.J.Choi 080167* (KH), ITS-GQ412233; Ganwoljae, Ulju-gun, Gyeongnam, Korea, *S.H. Park 73970* (KH), -JF262669. *A. taquetii* H. Lév., 1100 Goji Seupji, Hallasan, Jeju, Korea, *H.J.Choi et al. 020063* (CBU-topotype), Figs. 3T, 4T; Yeongsil, Hallasan, Jeju, Korea, *G. H.Nam 06125* (KH), Fig. 1X, ITS-GQ412244, *trnL–trnF*-JF262667. *A. thunbergii* G. Don var. *teretifolium* H.J. Choi & B.U. Oh, Jungbong, Jirisan, Gyeongnam, Korea, *C.S.Jang 49475* (CBU), ITS-GQ412251, -JF262664. *A. thunbergii* G. Don var. *thunbergii*, Taebaeksan, Taebaek-si, Gangwon, Korea, *H.J.Choi s.n.* (KH), *trnL–trnF*-JF262663; *ITS-AJ411849. sect. *Schoenoprasum* Dumort., *A. maximowiczii* Regel, Mohe, Heilongjiang, China, *D.G.Jo et al. 070070* (KH), Figs. 1U, 3R, 4R; Tahe, Heilongjiang, China, *Y.M.Lee & H.J.Choi 080002* (KH), -JF262661; *ITS-AJ411877. *A. schoenoprasum* L. Mohe, Heilongjiang, China (Cultivated), *D.G.Jo et al. 070072* (KH), Fig. 2O; Candle Lake, Saskatchewan, Canada, *H.J.Choi-SK-4* (SASK), Fig. 1V; Red rock Canyon, Waterton Lakes National Park, Alberta, Canada, *H.J.Choi-AB-3* (SASK), *trnL–trnF*-JF262662; *ITS-AJ411836. subg. *Microscordum* (Maxim.) N. Friesen: sect. *Microscordum* Maxim., *A. monanthum* Maxim., Woraksan, Jecheon-si, Chungbuk, Korea, *H.J.Choi et al. 010006* (CBU), Fig. 1A; Soheul-eup, Pocheon-si, Gyeonggi, Korea, *H.J.Choi 080001* (KH), Fig. 2A, -JF262636; *ITS-AJ412745. subg. *Reticulatobulbosa* (Kamelin) N. Friesen: sect. *Reticulatobulbosa* Kamelin, *A. koreanum* H.J. Choi & B.U. Oh, Maisan, Jinan-gun, Jeonbuk, Korea, *H.J.Choi 020057* (CBU-isotype), Figs. 3L, 4L; Ganwoljae, Ulju-gun, Gyeongnam, Korea, *ParkSH 73933* (KH), Fig. 1O, ITS-GQ412205, *trnL–trnF*-JF262649. *A. splendens* Willd. ex Schult. f., Roem. & Schult., Seopa, Jangbaeksan, Jilin, China, *B.U.Oh et al. 030005* (CBU), Fig. 2K, *trnL–trnF*-JF262648; Seopa, Jangbaeksan, Jilin, China, *H.J.Choi & J.W.Han 070050* (KH), Figs. 1P, 3M, 4M; *ITS-AJ411927. *A. strictum* Schrad., Mongolia, *H.J.Choi s.n.* (KH), *trnL–trnF*-JF262650; *ITS-AJ411951. subg. *Rhizirideum* (G. Don ex Koch) Wendelbo: sect. *Caespitosoprason* N. Friesen, *A. bidentatum* Fisher ex Prokh., Héngsan, Daeryeon-shi, Liaoning, China, *CBU-280* (KH), Figs. 1K, 2H, 3G, 4G, *trnL–trnF*-

JF262653; *ITS-AJ411861. sect. *Rhizirideum* G. Don ex Koch, ***A. minus*** (S. Yu, W. Lee & S. Lee) H.J. Choi & B.U. Oh, Wolhaksam-ri, Inje-gun, Gangwon, Korea, *H.J. Choi 080063* (KH-topotype), Figs. 1M, 3J, 4J, ITS-GQ412218, *trnL–trnF*-JF262657. ***A. pseudosenescens*** H. J. Choi & B.U. Oh, Talin Linchang, Tahe, Heilongjiang, China, *H.J.Choi 080199* (KH-isotype), Fig, 2J, ITS-GQ412222, *trnL–trnF*-JF262655. ***A. senescens*** L., Do-dong, Ulleungdo, Gyeongbuk, Korea, *H.J.Choi 070001* (KH), *trnL–trnF*-JF262654; *ITS-AJ411834. ***A. spirale*** Willd., Ip-beopsan, Gyoha-shi, Jilin, China, *Jilin23–060902–007* (CBU), Figs. 1N, 3K, 4K; Dandong-shi, Jilin, China, *H.J. Choi & J.W.Han 070012* (KH), *trnL–trnF*-JF262656; *ITS-AJ411833. sect. *Tenuissima* (Tzag.) Hanelt, ***A. anisopodium*** Ledeb., Ilsongjeong, Yongjeong-shi, Jilin, China, *B.U.Oh et*

al. 030009 (CBU), Figs. 3H, 4H; Daegosan, Dandong-shi, Liaoning, China, *CBU-047* (KH), *trnL–trnF*-JF262651; *ITS-AJ411847. ***A. tenuissimum*** L., Baengnyeongdo, Incheon-si, Gyeonggi, Korea, *H.J.Choi 030008* (CBU), Figs. 1L, 3I, 4I; Daecheongdo, Incheon-si, Gyeonggi, Korea, *H.J.Choi 080255* (KH), Fig. 2I, *trnL–trnF*-JF262652; *ITS-AJ411846.

Outgroup—***Dichelostemma capitatum*** (Benth.) Alph. Wood subsp. ***capitatum***, *ITS-EU096190; *trnL–trnF*-AF508476. ***Ipheion dialystemon*** Guagl., *trnL–trnF*-AF508517. ***I. uniflorum*** (Graham) Raf., *ITS-AJ412715. ****Nothoscordum bivalve*** (L.) Britton, *ITS-AJ250301; *trnL–trnF*-AF117024, AF117052. ****Tulbaghia simmleri*** Beauverd, *ITS-AJ250300. ***T. violacea*** Harv., *trnL–trnF*-AF116999, AF117030.