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Research paper

Flower morphology of *Allium* (Amaryllidaceae) and its systematic significance



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ABSTRACT

Allium is a complicated genus that includes approximately 1000 species. Although its morphology is well studied, the taxonomic importance of many morphological traits, including floral traits, are poorly understood. Here, we examined and measured the floral characteristics of 87 accessions of 74 *Allium* taxa (belonging to 30 sections and nine subgenera) from Central to Eastern Asian countries. We then examined the taxonomic relationships between select flower characteristics and a phylogenetic tree based on ITS sequences. Our results confirm that floral morphology provides key taxonomic information to assess species delimitation in *Allium*. We found that perianth color is an important characteristic within the subg. *Melanocrommyum*, *Polyprason*, and *Reticulatobulbosa*. In subg. *Allium*, *Cepa,* and *Rhizirideum*, significant characteristics include ovary shape, perianth shape, and inner tepal apex. For species in subg. *Angunium*, the key taxonomic character is ovule number (only one ovule in per locule). In the subg. *Allium*, *Cepa*, *Polyprason*, and *Reticulatobulbosa*, which belong to the third evolutionary line of *Allium*, hood-like appendages occur in the ovary, although these do not occur in subg. *Rhizirideum*. Our results also indicated that the flower morphology of several species in some sections are not clearly distinguished, e.g., sect. *Sacculiferum* (subg. *Cepa*) and sect. *Tenuissima* (subg. *Rhizirideum*). This study provides detailed photographs and descriptions of floral characteristics and information on general distributions, habitats, and phenology of the studied taxa.

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1. Introduction

The genus *Allium* L. is one of the largest genera in the Amaryllidaceae family, with approximately 1000 species that are naturally distributed in the Northern Hemisphere (Fritsch and Friesen, 2002; Friesen et al., 2020; Govaerts et al., 2021). Its main center of diversity is located between Southwest and Central Asia and the Mediterranean regions (Choi and Oh, 2011). Molecular phylogenies have demonstrated the monophyly of *Allium* (Friesen et al., 2006; Nguyen et al., 2008; Li et al., 2010). The most recent classification divided *Allium* into 15 subgenera and 72 sections based on molecular evidence (nrITS; Friesen et al., 2006). Subsequent studies have focused on the morphology, molecular-based phylogeny, evolutionary history, and biogeography of various ambiguous subgenera and sections of *Allium* (Seregin et al., 2015; Herden and Friesen, 2016; Li et al., 2016, 2021; Sinitsyna et al., 2016; Friesen et al., 2020; Xie et al., 2020a, 2020b; Yang et al., 2023), including several novel sections and subsections of *Allium* (Friesen et al., 2020, 2021; Khan et al., 2022; Yang et al., 2023). Several characteristics important for the taxonomy of *Allium* have been investigated in detail, including seed testa sculptures (Celep et al., 2012; Baasanmunkh et al., 2020, 2021; Yusupov et al., 2022), pollen morphology (Namin et al., 2009), bulb and scape anatomy (Khorasani et al., 2018), pistil morphology (Choi et al., 2012), septal nectary anatomy (Gurushidze et al., 2008), leaf anatomy (Mashayekhi and Columbus, 2014), and the chromosomes (Peruzi

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et al., 2016; Han et al., 2020). However, due to the enormous number of *Allium* species worldwide, studies that combine both morphological and molecular data of many species are still lacking.

Allium is characterized by the presence of bulbs enclosed in membranous tunics, flowers with six free, or almost free, tepals, and often a subgynobasic style (Fritsch and Friesen, 2002; Friesen et al., 2006; Choi et al., 2011; Choi and Oh, 2011). Recently, a number of *Allium* species have been described as new to science based on their morphology (e.g., Armağan, 2021; Khan et al., 2021), or mixed morphological, including molecular and karyological properties (e.g., Koçyiğit et al., 2016; Choi et al., 2019; Xie et al., 2020c; Friesen et al., 2020, 2022; Jang et al., 2021; Pandey et al., 2021; Bartolucci et al., 2022; Khan et al., 2022). In general, the major morphological traits used to assess *Allium* taxonomy include rhizome and bulb tunics, stamens, tepals, and filaments. Rhizome and bulb tunics are the most useful at the subgeneric level (Friesen et al., 1999; Friesen et al., 2006; Choi and Oh, 2011); pistil morphology is useful for clades within subgenera (Choi et al., 2012), and the floral characteristics of tepals and filaments are useful at the species level (Xu and Kamelin, 2000; Choi et al., 2011, 2012; Jang et al., 2021).

Floral structures of *Allium* are characterized by a fasciculate to umbel or head-like inflorescence, hypogynous flower, free or almost free tepals in two slightly differentiated whorls, and trilocular ovaries with single style (Fritsch and Friesen, 2002; Choi and Oh, 2011). However, there are very few studies that have focused on the flower morphology of the *Allium* genus (Zuraw, 2009; Choi et al., 2011, 2012; Aryakia et al., 2016). Fritsch (1992) reported various shapes and positions of the nectaries and their openings in more than 160 species. Gurushidze et al. (2008) investigated nectary anatomy in the subgenus *Melanocrommyum* with molecular analysis. In addition, several studies examined nectaries in various species (Zuraw et al., 2009, 2010). Choi et al. (2011) presented a micro- and macro-morphological comparative analysis of floral characteristics of four New World *Allium* species. Furthermore, Choi et al. (2012) studied pistil morphology of northeast Asian and northern North American *Allium* using molecular data. Recently, Jang et al. (2021) investigated floral parts specifically for species of section *Rhizirideum* in South Korea and northeast China. However, due to over-reliance on dried specimens, disagreements regarding the taxonomic importance of specific morphological traits, and the proliferation of synonyms (Hanelt et al., 1992; Khassanov and Fritsch, 1994; Mes et al., 1997; Gregory et al., 1998; Choi and Oh, 2011), the infrageneric taxonomy and evolution in the genus are still not entirely understood.

Here, we investigated the extensive floral morphology of *Allium* from the central and the east Asian countries, using quantitative and qualitative morphological characteristics. The aims of this study were to: (1) analyze floral morphology of previously unstudied species and different accessions of previously studied taxa, and (2) evaluate the taxonomic implications of the analyzed floral characters in *Allium*. This study, together with that of Choi et al. (2011, 2012), provides a strong basis for a future monograph and the systematic understanding of *Allium*.

2. Materials and methods

2.1. Plant materials

A total of 87 accessions of 74 taxa belonging to 30 sections and nine subgenera were evaluated in this study (Table S1). Field surveys were conducted between 2005 and 2021 in Asian countries, namely, Mongolia (26 accessions), South Korea (24), Kyrgyzstan (20), Uzbekistan (13), China (3), Russia (2), and Japan (1). In addition, we collected three cultivated species from South Korea (Table S1). All samples were stored in 70% ethanol during the field surveys and were subsequently deposited in the herbarium of Changwon National University (CWNU) (Table S1). The subgenera and sections were provided by Friesen et al. (2006). All taxa names were ordered alphabetically followed by subgenus and section (Table S1).

2.2. Morphological observation and measurement

The size and shape of flowers (at least 30–50 for each accession) were measured and photographed using an Olympus SZ61 stereoscope (Olympus Co., Tokyo, Japan) with a TrueChrome II camera (Tucsen Photonics Co., Ltd., Fuzhou, China). Quantitative characters of flowers, including length and width of perianth, tepal, stamen, and pistil, were measured (Fig. 1). Furthermore, we examined qualitative characteristics of flowers, including color of perianth, shape of tepals, filaments, ovaries, and anthers. The colors of perianth were determined using RHS color chart (Royal Horticultural Society, 2015).

2.3. Statistical analysis

All statistical analyses were conducted using FeatureImpCluster, flexclust, clustMixType, factoextra, cluster packages in R v.4.0.4 (R Core Team, 2020). FeatureImpCluster package offers a feature importance for partitional clustering. For clustering of mixed type data, the clustMixType package was used in R by using k-prototypes algorithm. The analysis was conducted with two raw data sets, one including only quantitative factor and another that included a combination of quantitative and qualitative factors. After calculating feature importance values, a second cluster plot was generated by adding certain qualitative traits with high values. We applied this analysis to all accessions and each subgenus. The cluster analysis for all accessions was performed with an average value of quantitative characteristics. All quantitative and qualitative characteristics are summarized in Table 1.

2.4. Phylogenetic analyses

We obtained ITS sequences of 67 species belonging to 30 sections and nine subgenera from NCBI (Table S4). Two species including *Nothoscordum bivalve* and *Tulbaghia violacea* were selected as an outgroup according to Friesen et al. (2006) and Li et al. (2010). The sequences were aligned using ClustalW (Thompson et al., 1994) and the default settings and manual adjustments were made using BioEdit (Hall, 1999). Ambiguous nucleotide bases were corrected using the corresponding base of the sequence that was obtained using the reverse primer. The phylogenetic analyses were conducted using the Bayesian inference (BI), Maximum Likelihood (ML), and Maximum Parsimony (MP) methods. The BI analysis was conducted using MrBayes 3.2.2 (Ronquist et al., 2012), with two independent runs of four simultaneous chains, executed for 5,000,000 generations using the Markov chain Monte Carlo algorithm. ML was performed using RAxML v.8.2.11 (Stamatakis, 2006, 2014) as implemented in the Geneious v.10.2.2 (Kearse et al., 2012) search for the best-scoring ML tree algorithm, which included 1000 bootstrap replicates. MP analysis was conducted using PAUP v.4.0b10 (Swofford, 1989). The trees were determined from a 50% majority-rule consensus to estimate the posterior probabilities. The reconstructed trees were visualized using FigTree v.1.4.2 (Rambaut, 2012).

3. Results and discussion

We studied the floral morphology of 87 accessions of 74 *Allium* taxa belonging to 30 sections and nine subgenera from Central to

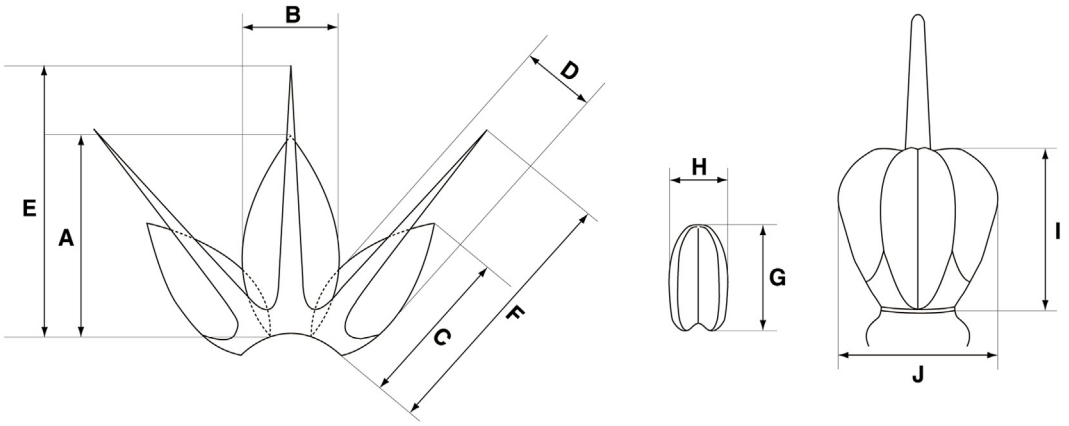


Fig. 1. Illustration of flower structure in *Allium* with quantitative characteristics. (A) Inner tepal length (B) inner tepal width (C) outer tepal length (D) outer tepal width (E) inner filament length (F) outer filament length (G) anther length (H) anther width (I) ovary length (J) ovary width.

Table 1
Morphological traits of quantitative and qualitative characteristics used in statistical analysis.

No.	Abbreviations (Quantitative)	Morphological characteristics	No.	Abbreviations (Qualitative)	Morphological characteristics
1	ITL	inner tepal length	1	PSP	perianth shape
2	ITW	inner tepal width	2	PCO	perianth color
3	OTL	outer tepal length	3	ITS	inner tepal shape
4	OTW	outer tepal width	4	ITA	inner tepal apex
5	IFL	inner filament length	5	OTS	outer tepal shape
6	OFL	outer filament length	6	OTA	outer tepal apex
7	AL	anther length	7	RFP	reflexed point of tepal apex
8	AW	anther width	8	RL	filament relative length compared with perianth
9	OL	ovary length	9	IFS	inner filament shape
10	OW	ovary width	10	OFS	outer filament shape
11	OVU	number of ovules	11	MEN	filament margin entire
			12	MTT	filament margin toothed
			13	TP	filament toothed position
			14	OVS	ovary shape
			15	APH	appendages on ovary
			16	STY	style type
			17	STG	stigma type

East Asian countries. The shapes and sizes of floral morphology are provided in Tables S2 and S3. The detailed flower description of each taxon is provided based on our fresh samples (Supplementary data). In addition, we provide the global distribution and flowering season for each species based on extensive literatures and our own field information (Supplementary data).

3.1. Quantitative and qualitative characteristics

3.1.1. Inflorescence color

The inflorescence colors varied among the investigated species. In general, we classified colors into five categories: blue, purple and pink, red, white, and yellow (Figs. 2–16; Table S2). For example, species of the subgenus *Reticulatobulbosa* have inflorescence of various colors, including reddish purple (*Allium jodanthum*, Fig. 11D), pale yellow (*A. flavidum*, Fig. 12B), and white (*A. leucocephalum*, Fig. 12D).

3.1.2. Perianth shape

The perianth shapes are typically categorized as campanulate, semi-stellate, and stellate (Figs. 2–16; Table S2).

3.1.3. Tepal shape and size

The tepals of *Allium* species comprise inner and outer parts (Figs. S1–S6). In general, tepal shapes (inner and outer) can be categorized as ovate (*Allium condensatum*, Fig. S1X), lanceolate (*A. karelinii*, Fig. S2D), elliptic (*A. giganthum*, Fig. S2H), oblong (*A. monanthum*, Fig. S2IM), linear (*A. fetisowii*, Fig. S2E), or obovate (*A. anisopodium*, Fig. S3O). Tepal apex is categorized as rounded (*A. oschaninii*, Fig. S1W), obtuse (*A. koreanum*, Fig. S2W), acute (*A. scabriscapum*, Fig. S3B), mucronate (*A. oreoprasum*, Fig. S1P), acuminate (*A. kirilovii*, Fig. S2N), retuse (*A. sabulosum*, Fig. S1J), or truncate (*A. vodopjanovae*, Fig. S3Q). Detailed information on the shapes of each species can be found in the Supplementary data.

We measured inner and outer tepals for each taxon (Table S3). Based on the mean value (mean ± standard deviation), inner tepal length/width ratio ranged from 1.48 ± 0.16 to 6.94 ± 1.66, with *Allium taguetii* having the smallest (1.23; Fig. S1AA) and *A. karataviense* having the biggest (10.68; Fig. S2G). Similarly, outer tepal length/width ratio ranged from 1.34 ± 0.07 to 7.4 ± 1.88, with *A. taguetii* having the smallest (1.14; Fig. S4AA) and *A. karataviense* having the biggest (10.4; Fig. S5G). However, the length of inner tepals ranged from 2.72 ± 0.26 to 11.64 ± 0.85 mm, with *A. logicuspis* having the shortest (2.46 mm; Fig. S1D) and *A. schoenoprasum* var. *schoenoprasum* having the longest (13.46 mm; Fig. S2C). The inner tepal width ranged from 1.01 ± 0.1 to 4.00 ± 0.3 mm, with *A. fetisowii* having the narrowest (0.74 mm; Fig. S2E) and *A. semenovii* having the widest (5.19 mm; Fig. S1S). In addition, the outer tepal length ranged from 2.69 ± 0.12 to 14.16 ± 1.18 mm, with

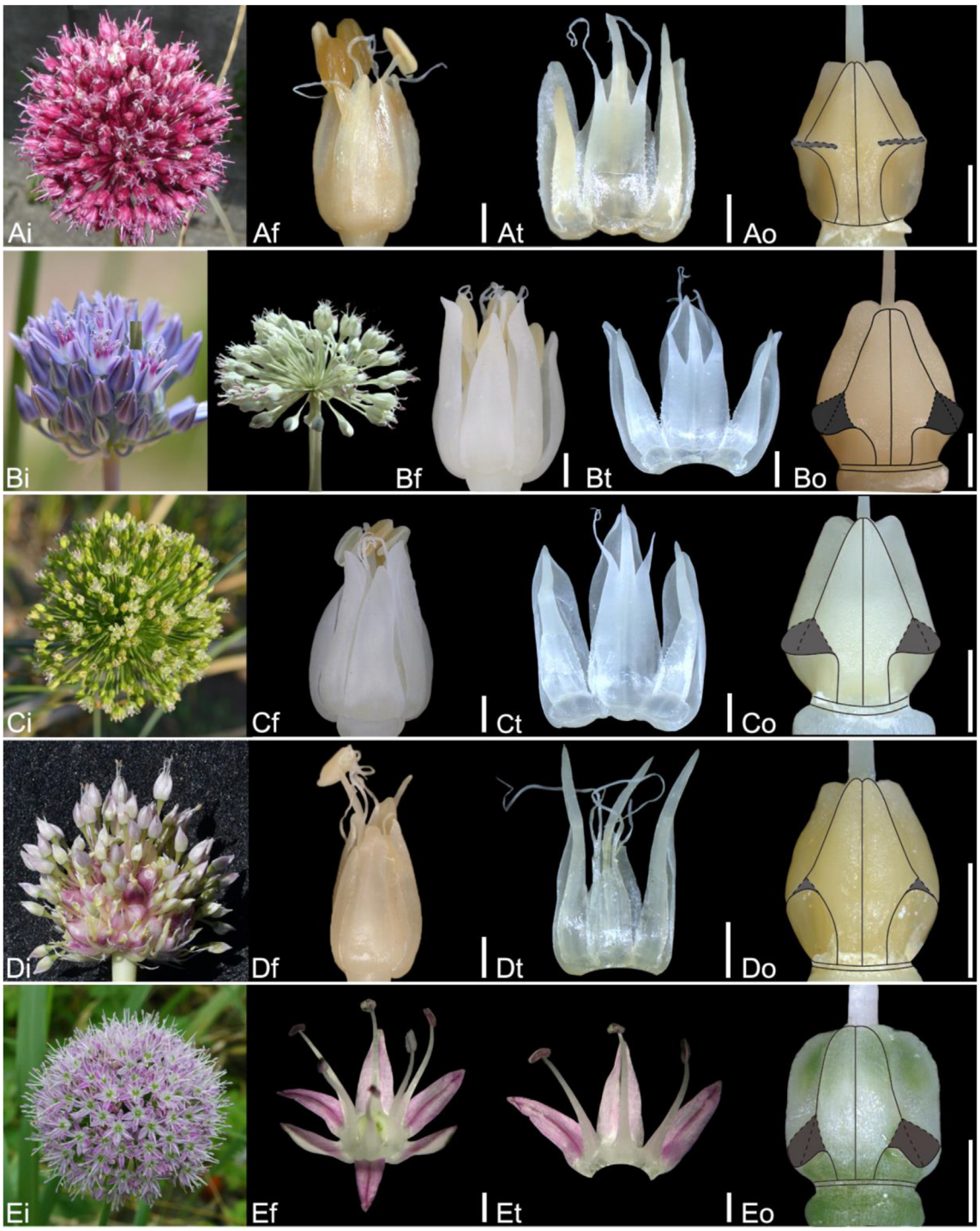


Fig. 2. Floral morphology in *Allium* (A–E) Subg. *Allium* (A) *A. atroviolaceum* (B) *A. filidens* (C) *A. filidentiforme* (D) *A. longicuspis* (E) *A. macrostemon* (i – inflorescence, f – flower, t – tepal and filament arrangement, o – ovary). Scale bars = 1 mm.

A. sabulosum having the shortest (2.32 mm; Fig. S4J) and *A. semenovii* having the longest (15.96 mm; Fig. S4S). Finally, the outer tepal width ranged from 0.85 ± 0.07 to 4.22 ± 0.38 mm, *A. logicuspis* having the narrowest (0.73 mm; Fig. S4D) and *A. semenovii* having the widest (4.88 mm; Fig. S4S).

3.1.4. Stamen

The relative length of the filament with perianth can be categorized into four types: non-exserted (e.g. *Allium griffithianum*, Fig. 3A), slightly non-exserted (e.g. *A. maximowiczii*, Fig. 8A), slightly exserted (e.g. *A. subtilissimum*, Fig. 11B), and exserted (e.g. *A. leucocephalum*, Fig. 12D). The filament shapes are represented as triangular (e.g. *A. filidens*, Fig. 2B), narrowly triangular (e.g. *A. sabulosum*, Fig. 4A), broadened for length (e.g. *A. bidentatum*, Fig. 15E), subulate (e.g. *A. thunbergii* var. *thunbergii*, Fig. 7E), and subulate from a triangular base (e.g. *A. stipitatum*, Fig. 9B). In addition, the margin of inner filament was entire (e.g. *A. macrostemon*, Fig. 2E) or toothed (e.g. *A. malyschevii*, Fig. 12E). Based on

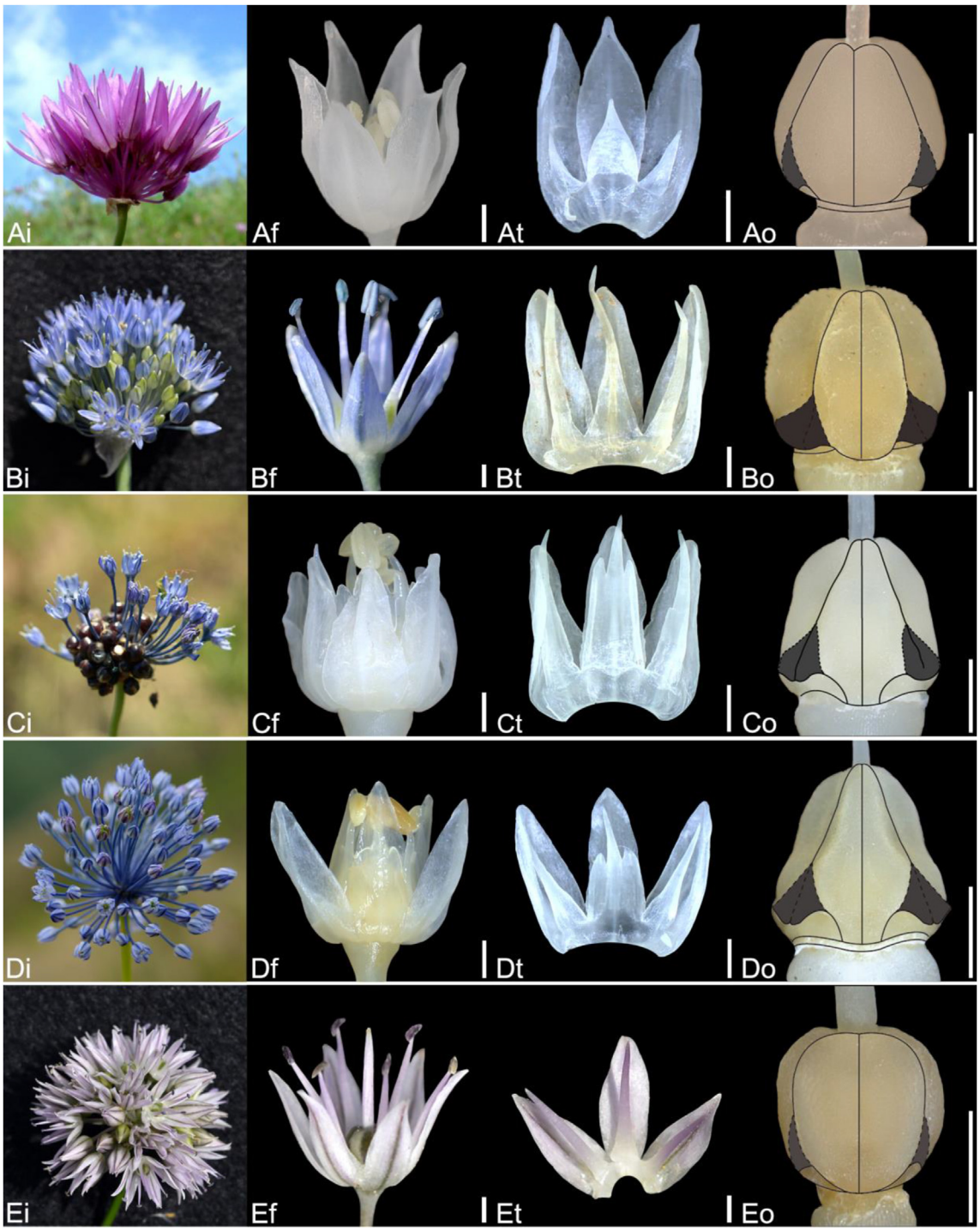


Fig. 3. Floral morphology in *Allium* (A–E) Subg. *Allium* (A) *A. griffithianum* (B) *A. caeruleum* (C–D) *A. caesium* (E) *A. glomeratum* (i – inflorescence, f – flower, t – tepal and filament arrangement, o – ovary). Scale bars = 1 mm.

the length/width ratio, the shape of anthers was classified into three types: ovate (e.g. *A. vodopjanovae*), elliptic (e.g. *A. prostratum*), and oblong (e.g. *A. ulleungense*). More detailed information on the stamen is presented in Figs. 2–16 and Table S2.

We measured two filaments (inner and outer) for each taxon, which are summarized in Table S3. Based on the mean value (mean ± standard deviation), the length of inner filament ranged from 2.21 ± 0.14 to 12.79 ± 0.73 mm, with *Allium griffithianum* having the shortest (1.31 mm; Fig. 3A) and *A. altaicum* having the longest (13.96 mm; Fig. 6A). Similarly, the length of outer filament ranged from 1.83 ± 0.21 to 10.37 ± 1.21 mm, with *A. griffithianum* having the shortest (1.31 mm; Fig. 3A) and *A. altaicum* having the longest (13.42 mm; Fig. 6A). The anther length ranged from 0.88 ± 0.06 to 2.48 ± 0.19 mm, with *A. monanthum* having the shortest (0.74 mm) and *A. ramosum* having the longest (2.71 mm); whereas the anther width ranged from 0.49 ± 0.04 to 1.03 ± 0.06 mm, with *A. monanthum* having the narrowest (0.36 mm) and *A. pseudojaponicum* having the widest (1.18 mm).

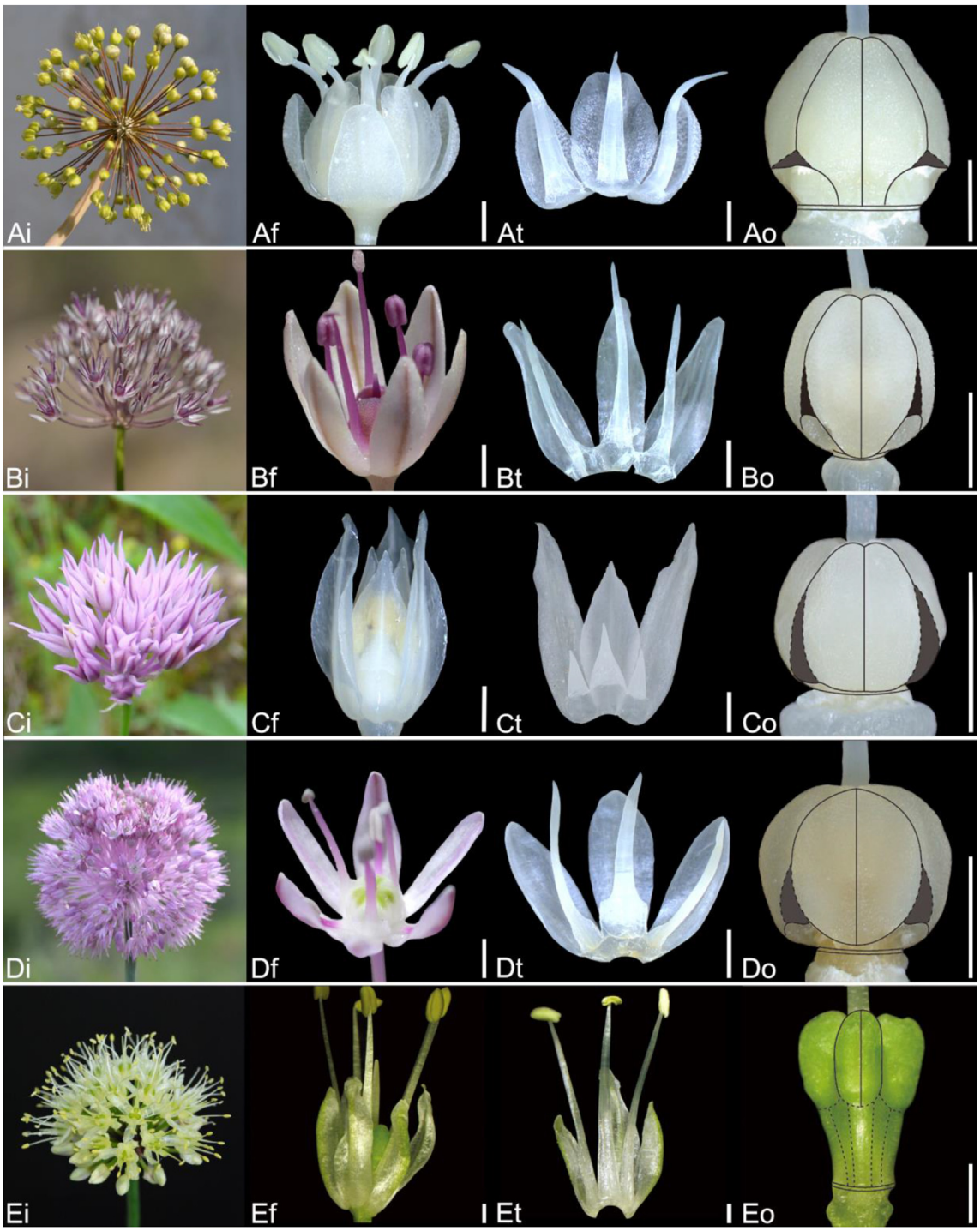


Fig. 4. Floral morphology in *Allium* (A–D) Subg. *Allium* (A) *A. sabulosum* (B) *A. haneltii* (C) *A. anisotepalum* (D) *A. caricifolium* (E) Subg. *Anguinum* (E) *A. microdictyon* (i – inflorescence, f – flower, t – tepal and filament arrangement, o – ovary). Scale bars = 1 mm.

3.1.5. Pistil shape and size

The shape of ovaries can be divided in eight types: ellipsoid, subglobose, globose, oblate spheroid, ovoid, obovoid, obconical, subcubical (Figs. 2–16). Two types of ovarian processes, with and without hood-like appendages, were observed (Figs. 2–16). The number of ovules per locule was typically two. Some exceptions include zero or one in the male of *Allium monanthum*, one ovule in *Allium microdictyon*, *A. ulleungense*, two to four in *A. ramosum*, and four to six in *A. fetisowii*. In contrast, the style of *A. semenovii* and *A. monanthum* is trigonous (Figs. 5E and 9D), while the remaining species are terete. Three types of stigmas were observed in the species studied: slightly three-cleft in *A. oreoprasum* (Fig. 5B), three-cleft in *A. semenovii* and *A. monanthum* (Figs. 5E and 9D), and smooth, the most common stigma type.

We measured the length and width of the ovary for each species (Table S3). Based on the mean value (mean ± standard deviation), the length of the ovary ranged from 1.2 ± 0.18 to 3.6 ± 0.15 mm, with *Allium anisotepalum* having the shortest (0.99 mm; Fig. 4C)

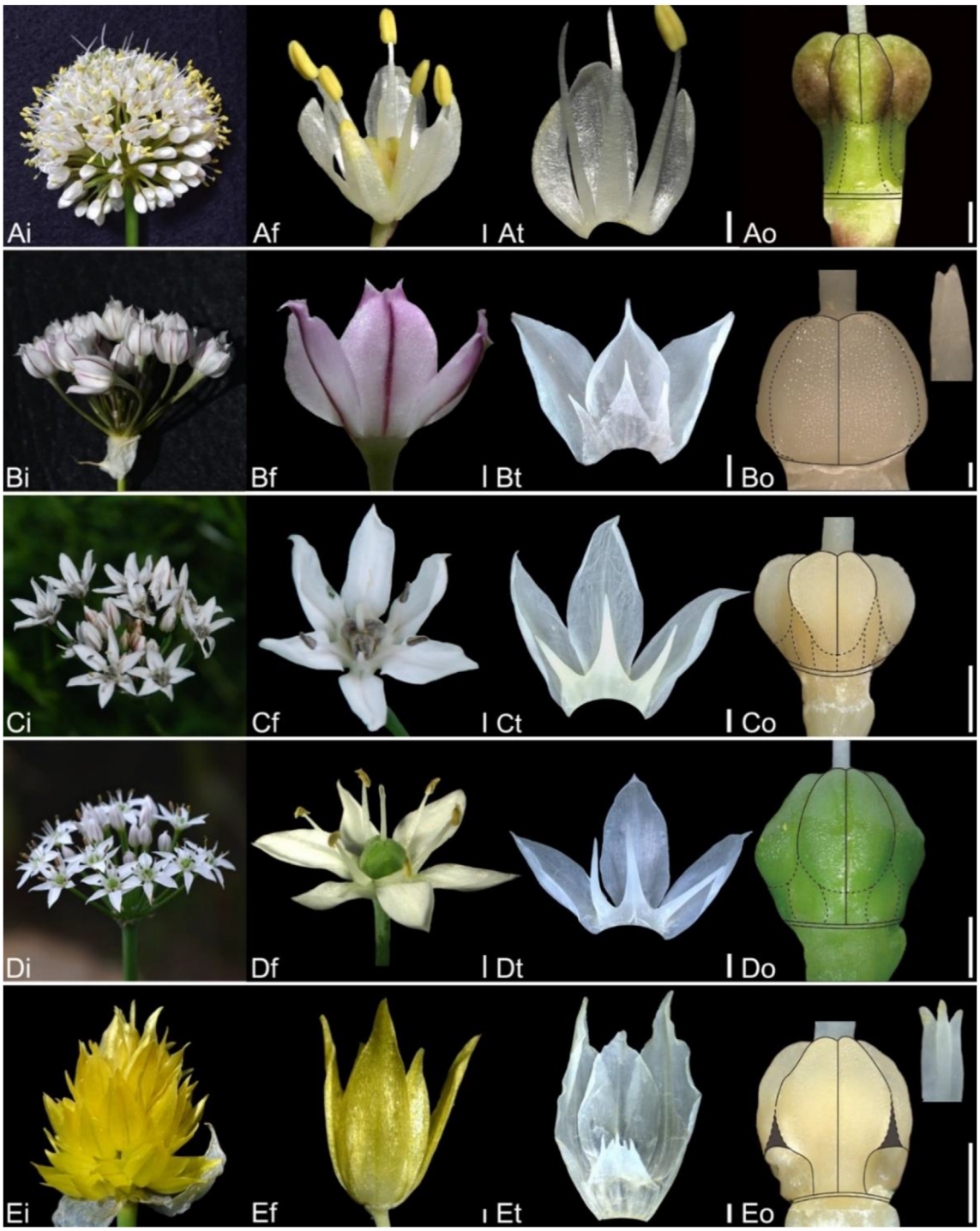


Fig. 5. Floral morphology in *Allium* (A) Subg. *Anguinum* (A) *A. ulleungense* (B–D) Subg. *Butomissa* (B) *A. oreoprasum* (C) *A. ramosum* (D) *A. tuberosum* (E) Subg. *Cepa* (E) *A. semenovii* (i – inflorescence, f – flower, t – tepal and filament arrangement, o – ovary). Scale bars = 1 mm.

and *A. ulleungense* having the longest (4.1 mm; Fig. 5A). The width of the ovary ranged from 1.24 ± 0.09 to 3.51 ± 0.25 mm, with *A. anisotepalum* having the narrowest (1.06 mm; Fig. 4C) and *A. karataviense* having the widest (4.36 mm; Fig. 9C).

3.2. Statistical analysis at the subgenus level

Cluster analysis was conducted using 11 quantitative and 17 qualitative floral morphological characteristics. We present the two different cluster plots: one based on only quantitative data, and one based on combined quantitative and selected qualitative characteristics at the species level within each subgenus of *Allium* (Figs. 17–20). According to quantitative and qualitative results, the species are relatively well separated from each other in the subgenera *Anguinum* (Fig. 17C and D), *Butomissa* (Fig. 18A), and *Melanocrommym* (Fig. 19A and B). Further, species are not well distinguished based on quantitative characteristics in subgenera *Allium* (Fig. 17A), *Cepa* (Fig. 18B), *Polyprason* (Fig. 19C), *Reticulatobulbosa* (Fig. 20A), and *Rhizirideum* (Fig. 20C). However, species within the four subgenera, which were difficult to distinguish

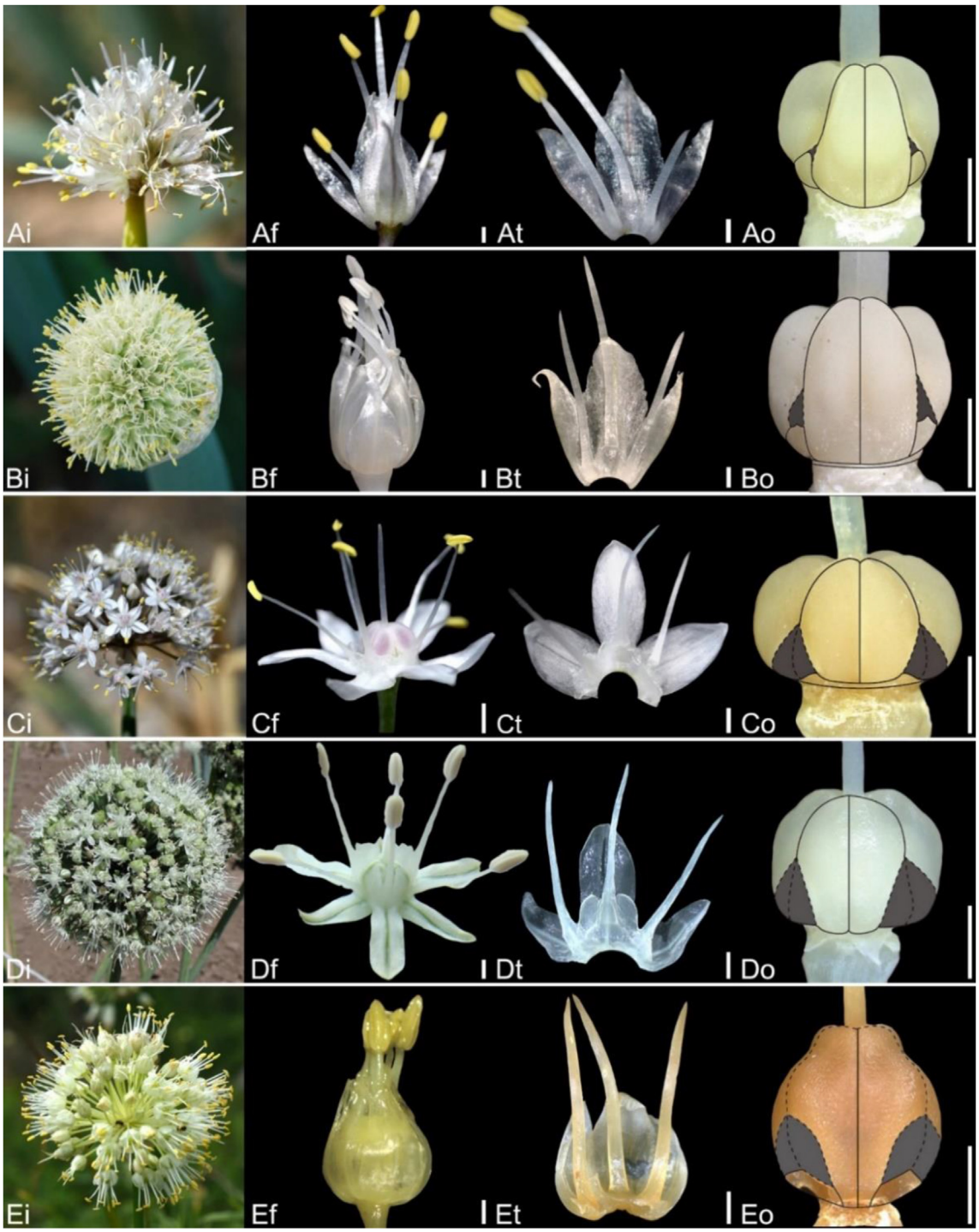


Fig. 6. Floral morphology in *Allium* (A–E) Subg. *Cepa* (A) *A. altaicum* (B) *A. fistulosum* (C) *A. galanthum* (D) *A. oschaninii* (E) *A. condensatum* (i – inflorescence, f – flower, t – tepal and filament arrangement, o – ovary). Scale bars = 1 mm.

only by quantitative characteristics, were more clearly distinguished when analyzed with qualitative characteristics (Figs. 17B, 18C, 19D, and 20B and D).

3.3. Flower morphology at the subgenus level

According to statistical analysis, flower morphology is not sufficiently significant to distinguish each subgenus accessed in this study (Fig. S8). However, evidently, the flower morphology is the key diagnoses for the species within each subgenus; shape and size of floral organs such as perianth, tepals, filament, and pistils are useful diagnostic characteristics at the specific level (Figs. 17–20). In addition, the flower character has been consistently noted to be the most important key characteristics to distinguish species in the genus *Allium* (Choi and Oh, 2011; Choi et al., 2012; Xie et al., 2020c; Jang et al., 2021; Bartolucci et al., 2022). In this study, we present several important diagnoses between closely related taxa at the section and subgenus levels.

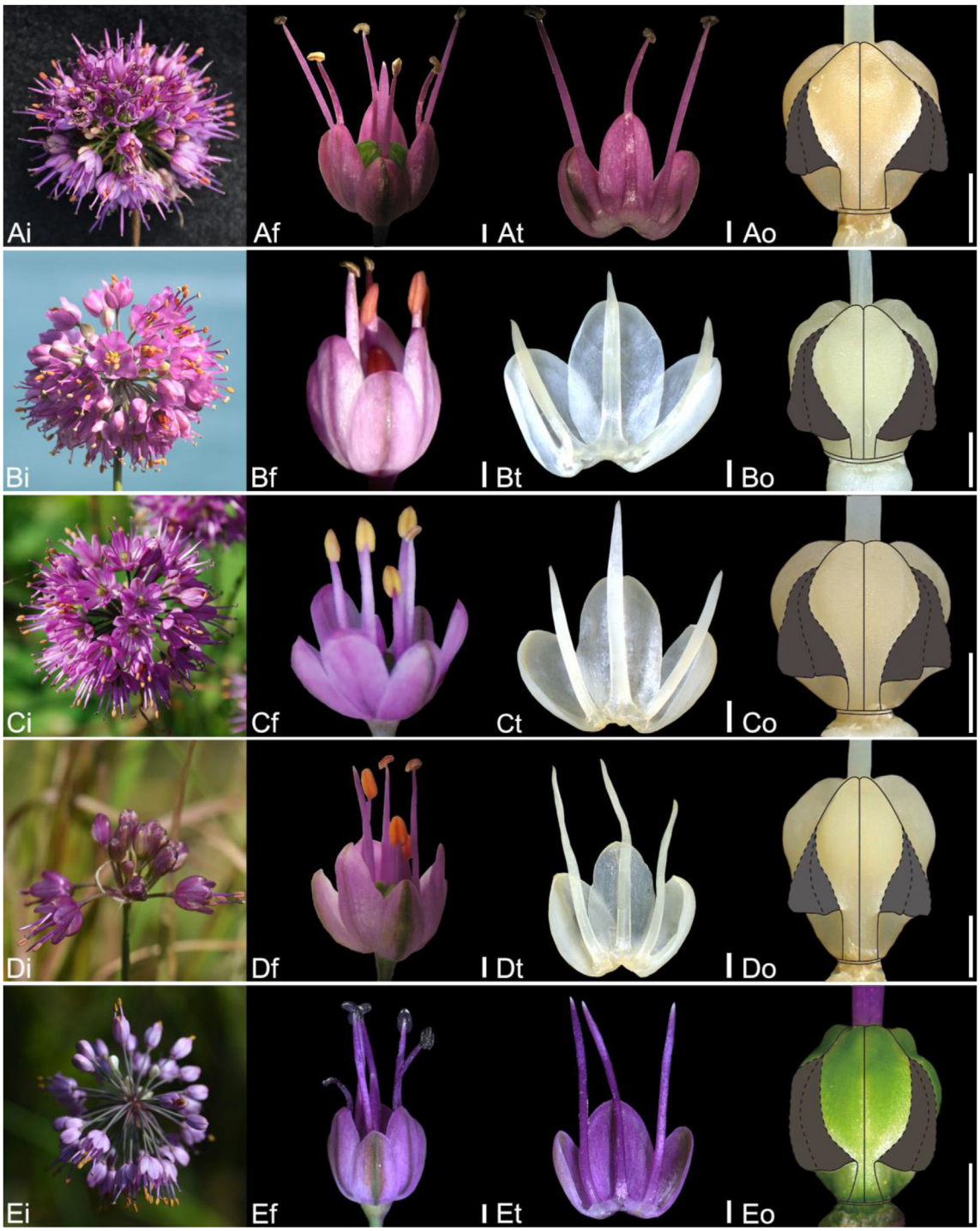


Fig. 7. Floral morphology in *Allium* (A–E) Subg. *Cepa* (A) *A. linearifolium* (B) *A. pseudojaponicum* (C) *A. taguetii* (D) *A. thunbergii* var. *teretifolium* (E) *A. thunbergii* var. *thunbergii* (i – inflorescence, f – flower, t – tepal and filament arrangement, o – ovary). Scale bars = 1 mm.

3.3.1. *Subgenus* Allium

The subgenus *Allium* is a large group including over 375 taxa divided into 19 sections, which is placed in the third evolutionary line (Friesen et al., 2006; Khassanov, 2018; Yang et al., 2023). However, a detailed phylogenetic classification of this taxonomic group remains incomplete (Khassanov, 2018; Munavvarov et al., 2022). In this study, 15 accessions of 13 species representing eight sections (*Allium*, *Avulsea*, *Caerulea*, *Eremoprasum*, *Haneltia, Macrostemon, Minuta*, and *Pallasia*) were investigated. The quantitative cluster plot revealed that 75.3% of the total variation is not adequately separated from each other (Fig. 17A). However, the combined cluster plot, which included five important qualitative characteristics (ovary shape, inner tepal shape, inner tepal apex, inner filament shape and perianth shape), indicated that species were almost separated from one another in 74% of the total variation (Fig. 17B). In this subgenus, some species in the combined cluster plot were clustered overlapping. For example, *Allium atroviolaceum, A. filidens,* and *A.*

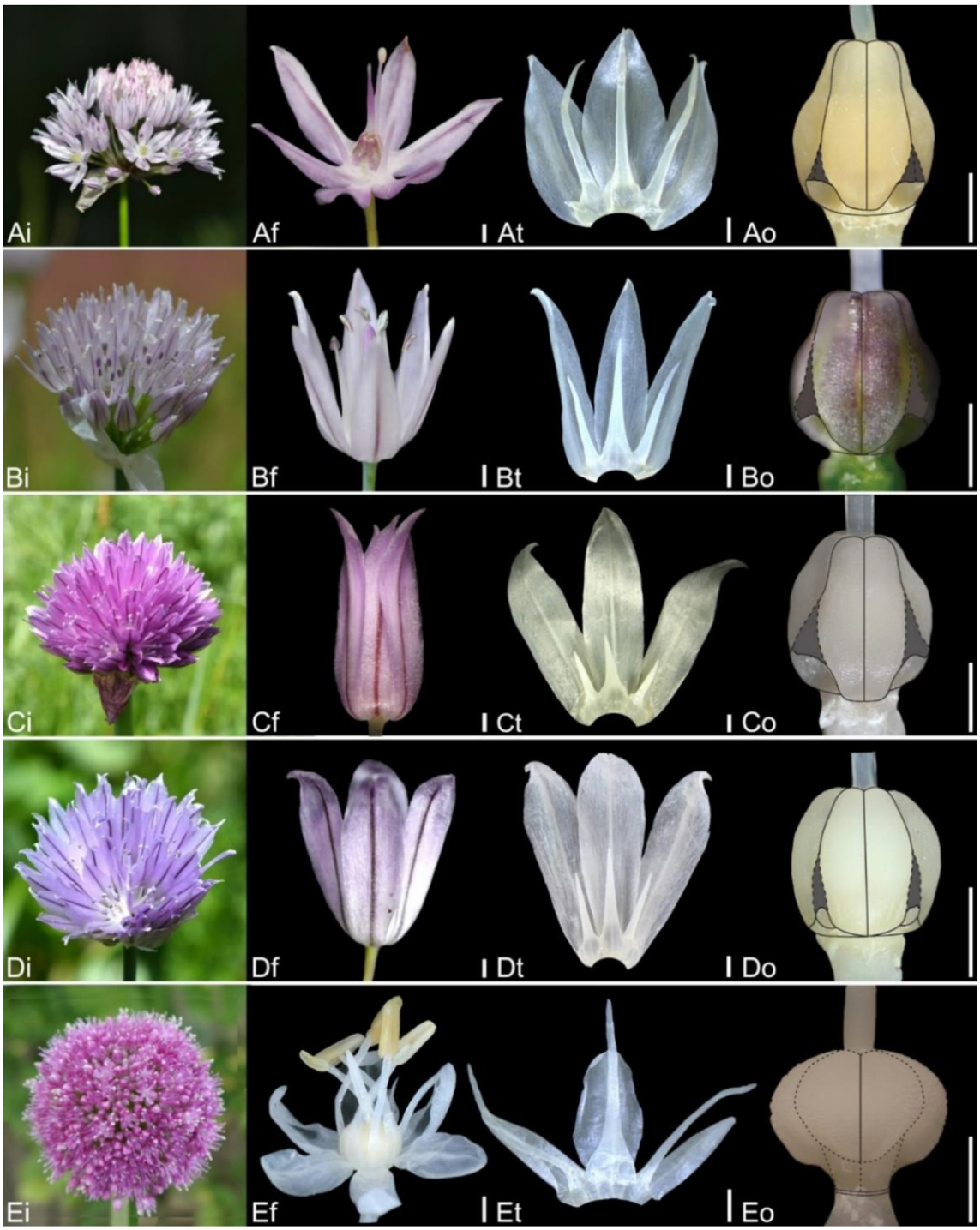


Fig. 8. Floral morphology in *Allium* (A–D) Subg. *Cepa* (A) *A. maximowiczii* (B) *A. schoenoprasum* var. *foliosum* (C) *A. schoenoprasum* var. *schoenoprasum* (D) *A. karelinii* (E) Subg. *Melanocrommyum* (E) *A. giganteum* (i – inflorescence, f – flower, t – tepal and filament arrangement, o – ovary). Scale bars = 1 mm.

filidentiforme (all species belongs to sect. *Allium*) are closely placed (Fig. 17B), but are distinguished mostly by perianth color, the relative length of filament with perianth and tepal size (Fig. 2A–C). Previous research has suggested that *A. filidens* s. str. is a rather polymorphic taxon with respect to the form of flowers and tepals (Khassanov, 1997). Moreover, *A. anisotepalum* (sect. *Minuta*; Fig. 4C) and *A. griffithianum* (sect. *Avulsea*; Fig. 3A) were mixed in the combined cluster plot (Fig. 17B), but the former is distinguished by the lanceolate and purplish pink perianth. Based on our results, both species may belong to the same section because they were not included in the recent phylogenetic studies (Friesen et al., 2006; Li et al., 2010). Recently, a monotypic section *Macrostemon* of subg. *Allium* was newly categorized based on a complete chloroplast genome (Yang et al., 2023). According to our results, the flower morphology of *A. macrostemon* (Fig. 2E) can be clearly distinguished from the other species of subg. *Allium* (Figs. 2–4), in line with the findings of Yang et al. (2023).

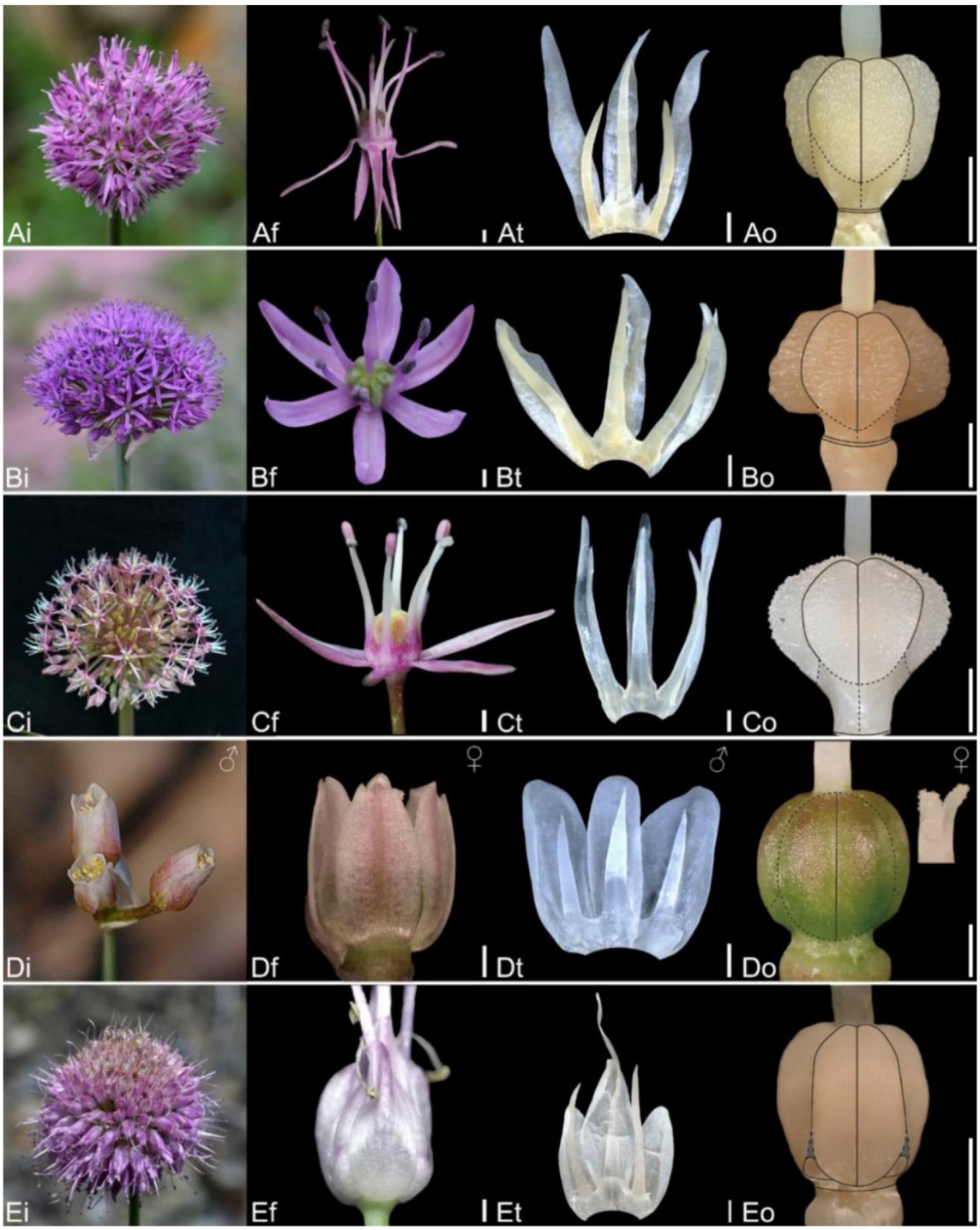


Fig. 9. Floral morphology in *Allium* (A–C) Subg. *Melanocrommyum* (A) *A. fetisowii* (B) *A. stipitatum* (C) *A. karataviense* (D) Subg. *Microscordum* (D) *A. monanthum* (E) Subg. *Polyprason* (E) *A. carolinianum* (i – inflorescence, f – flower, t – tepal and filament arrangement, o – ovary). Scale bars = 1 mm.

3.3.2. *Subgenus* Anguinum *(G. Don ex Koch) N. Friesen*

This subgenus consists of ten species and several varieties and is part of the second of three main evolutionary lines of *Allium* (Friesen et al., 2006; Li et al., 2010; Choi et al., 2012). Three accessions of two species from sect. *Anguinum* were studied. Two species (*A. microdictyon* and *A. ulleungense*) were well distinguished from each other based on qualitative (86.3% of the total variation) and combined qualitative and selected quantitative characteristics (90.7% of the total variation) (Fig. 17C and D). The perianth color is the most important characteristic among the two species (Figs. 4E and 5A). In addition, the molecular phylogenetic analysis clearly indicated that *A. ulleungense* is distinct from other species of the subg. *Anguinum* (Choi et al., 2019; Yang et al., 2023).

3.3.3. *Subgenus* Butomissa *(Salisb.) N. Friesen*

The subg. *Butomissa* is placed in the third evolutionary line within the *Allium* genus, including only two sections (Friesen et al., 2006). In this study, four accessions of three species

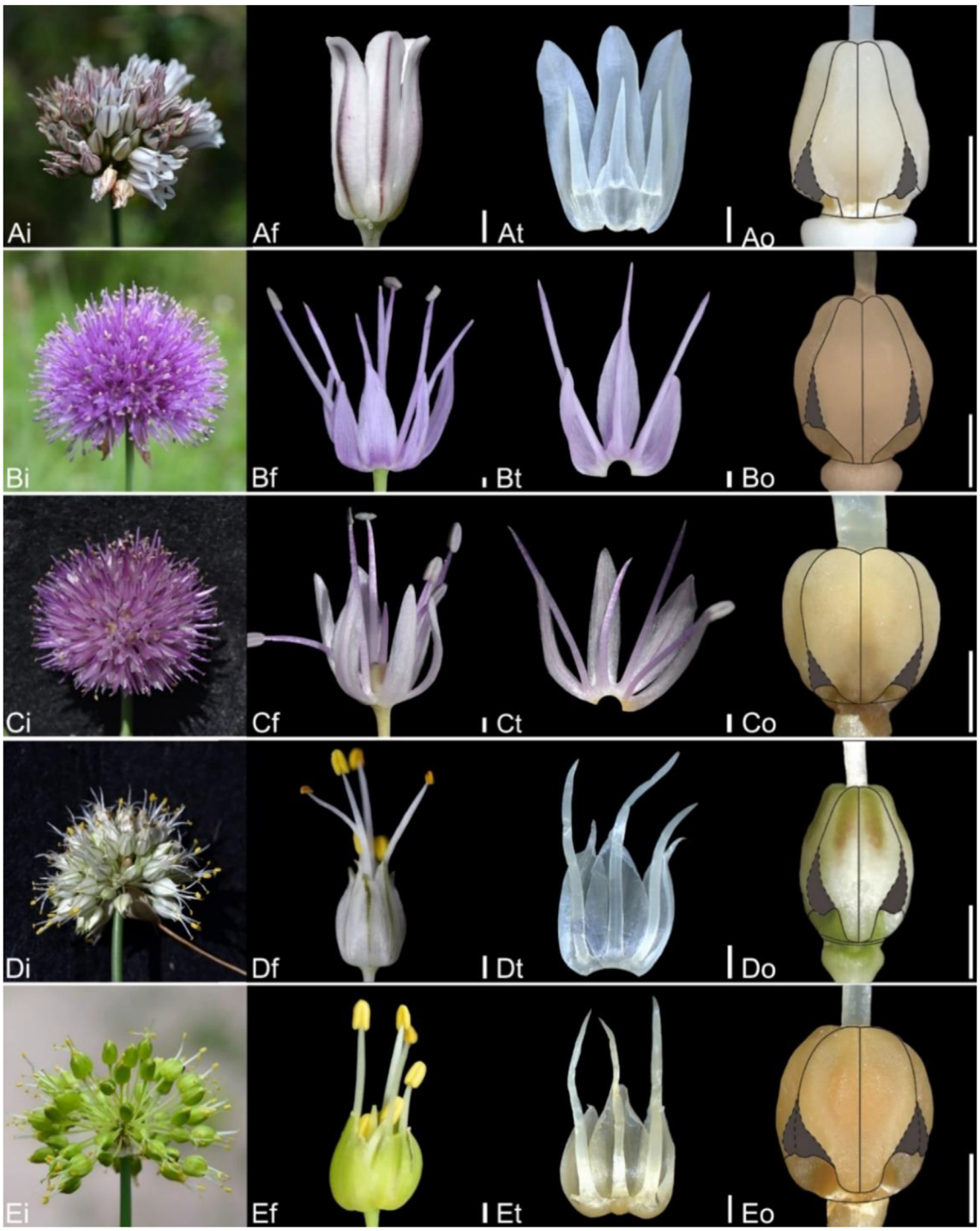


Fig. 10. Floral morphology in *Allium* (A–E) Subg. *Polyprason* (A) *A. korolkowii* (B) *A. platyspathum* subsp. *amblyophyllum* (C) *A. platyspathum* subsp. *platyspathum* (D) *A. kirilovii* (E) *A. obliquum* (i – inflorescence, f – flower, t – tepal and filament arrangement, o – ovary). Scale bars = 1 mm.

from two sections (*Austromontana* and *Butomissa*) were investigated. We only provide finding from the quantitative cluster plot because informative qualitative characteristics were not significant (Fig. S7D). Based on the quantitative plot, all taxa are well separated (83.9% of the total variation) among the studied species (Fig. 18A). Additionally, two accessions of *A. oreoprasum* are separated into sub-cluster owing to size of floral morphology (Fig. 18A).

3.3.4. *Subgenus* Cepa *(Mill.) Radić*

This subgenus includes approximately 30 taxa and five sections (Friesen et al., 2006). We investigated 22 accessions of 15 taxa, including two varieties belonging to five sections (*Annuloprason, Cepa, Condensatum, Sacculiferum, Schoenoprasum*). The quantitative cluster plot revealed approximately 76.9% of the total variation and the species that were generally gathered, excluding sect. *Annuloprason* (*Allium semenovii*) and sect. *Schoenoprasum* (four taxa)

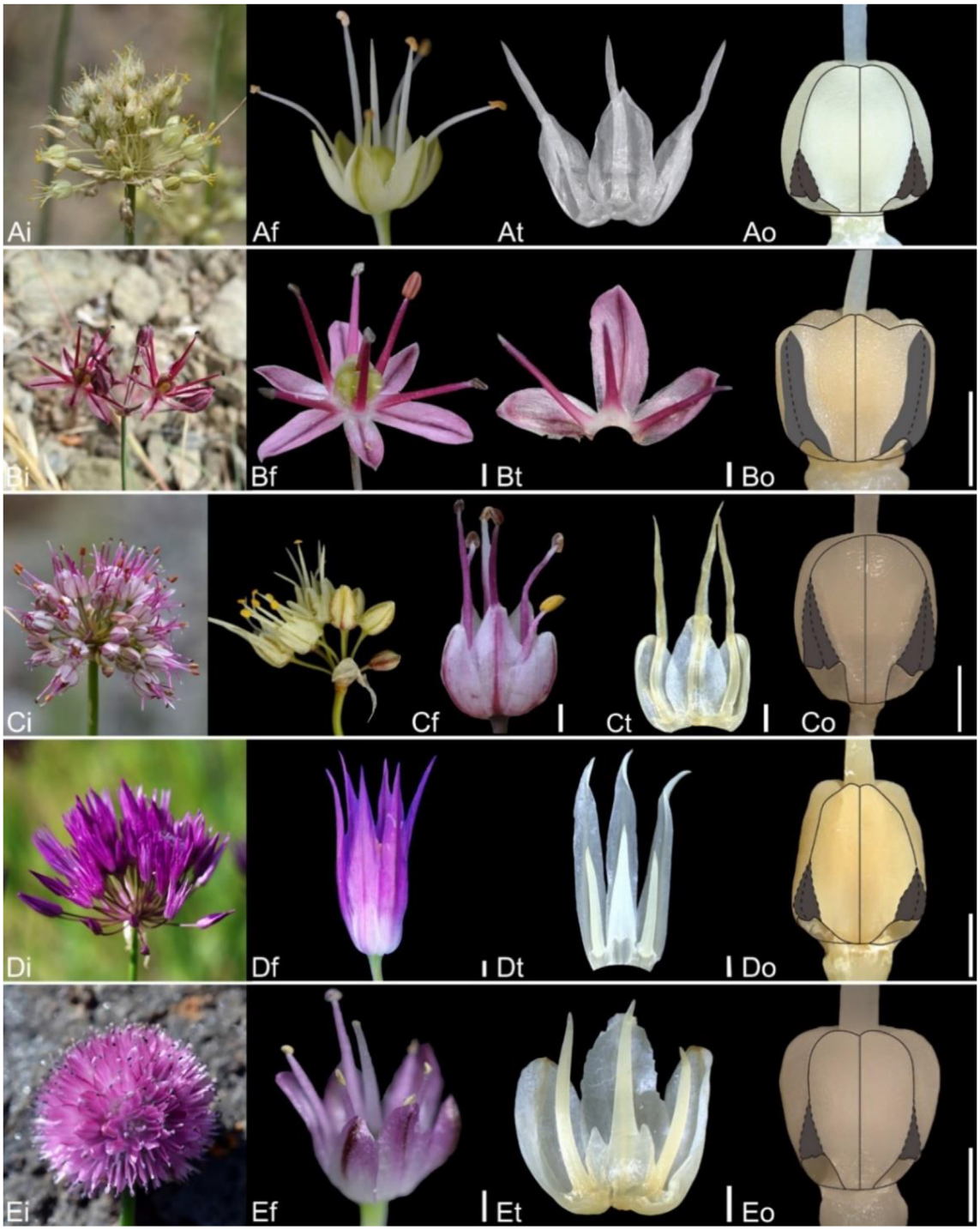


Fig. 11. Floral morphology in *Allium* (A–C) Subg. *Polyprason* (A) *A. petraeum* (B) *A. subtilissimum* (C) *A. tianschanicum* (D–E) Subg. *Reticulatobulbosa* (D) *A. jodanthum* (E) *A. amphibolum* (i – inflorescence, f – flower, t – tepal and filament arrangement, o – ovary). Scale bars = 1 mm.

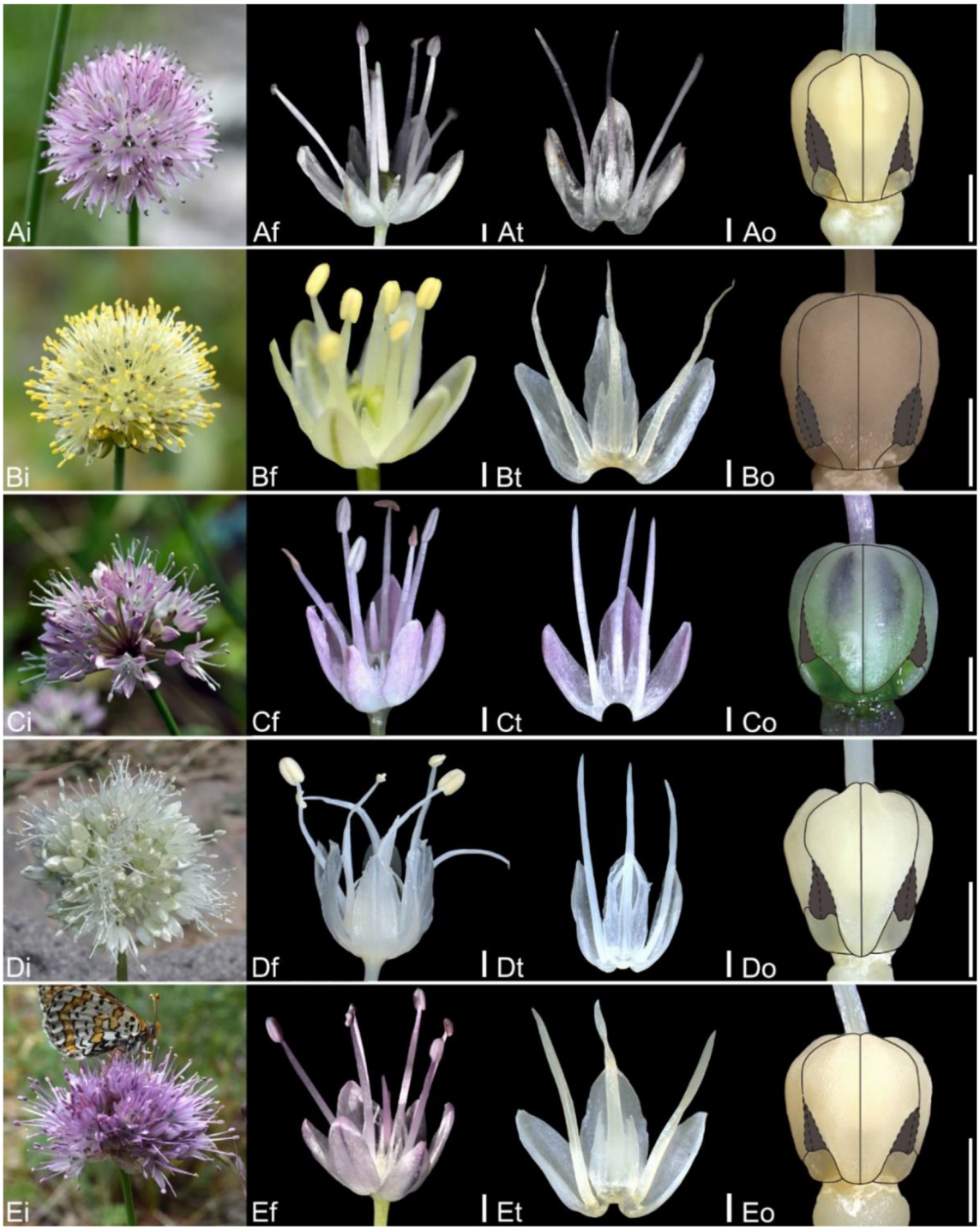


Fig. 12. Floral morphology in *Allium* (A–E) Subg. *Reticulatobulbosa* (A) *A. clathratum* (B) *A. flavidum* (C) *A. koreanum* (D) *A. leucocephalum* (E) *A. malyschevii* (i – inflorescence, f – flower, t – tepal and filament arrangement, o – ovary). Scale bars = 1 mm.

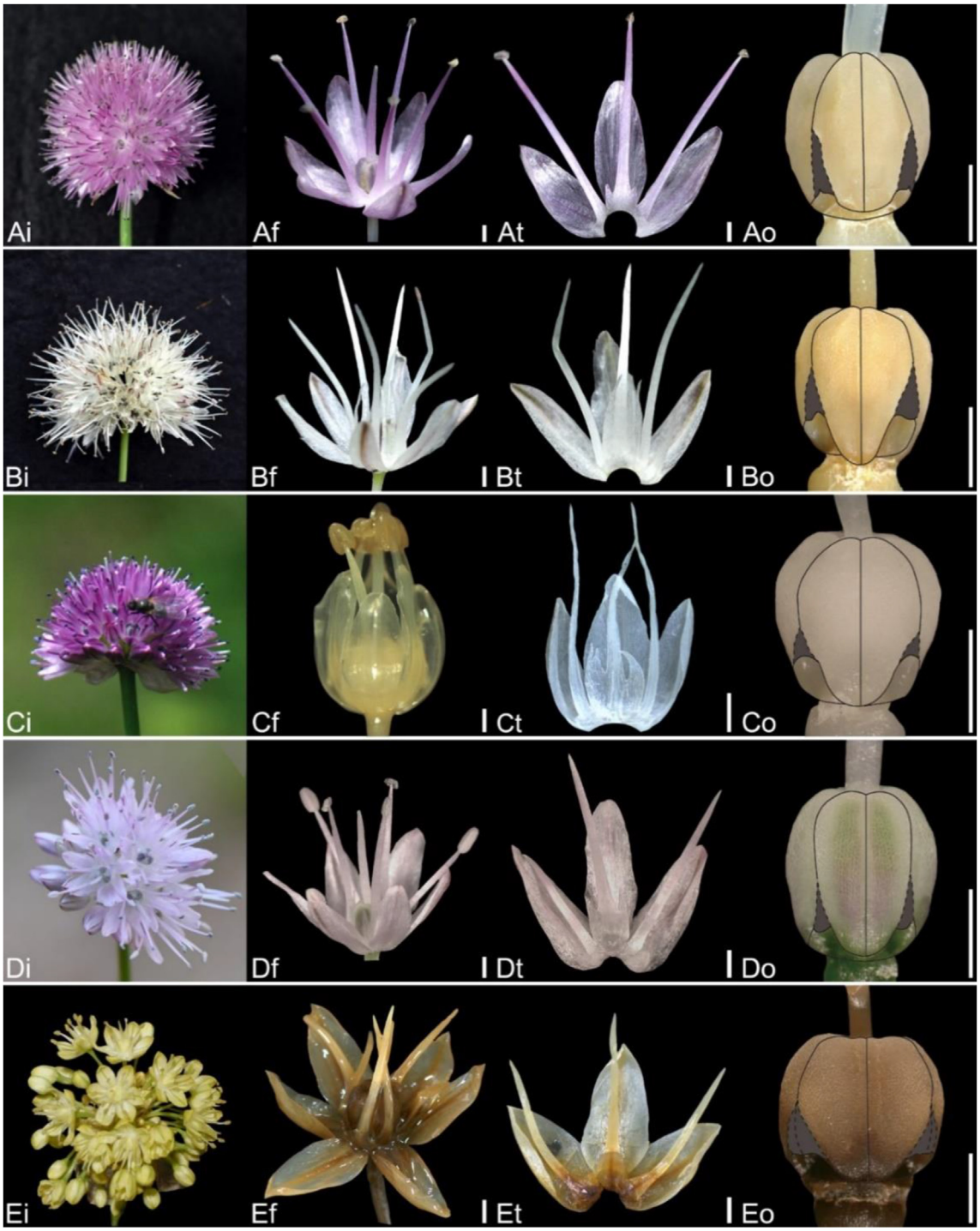


Fig. 13. Floral morphology in *Allium* (A–E) Subg. *Reticulatobulbosa* (A) *A. pumilum* (B) *A. schischkinii* (C) *A. splendens* (D) *A. ubsicola* (E) *A. scabriscapum* (i – inflorescence, f – flower, t – tepal and filament arrangement, o – ovary). Scale bars = 1 mm.

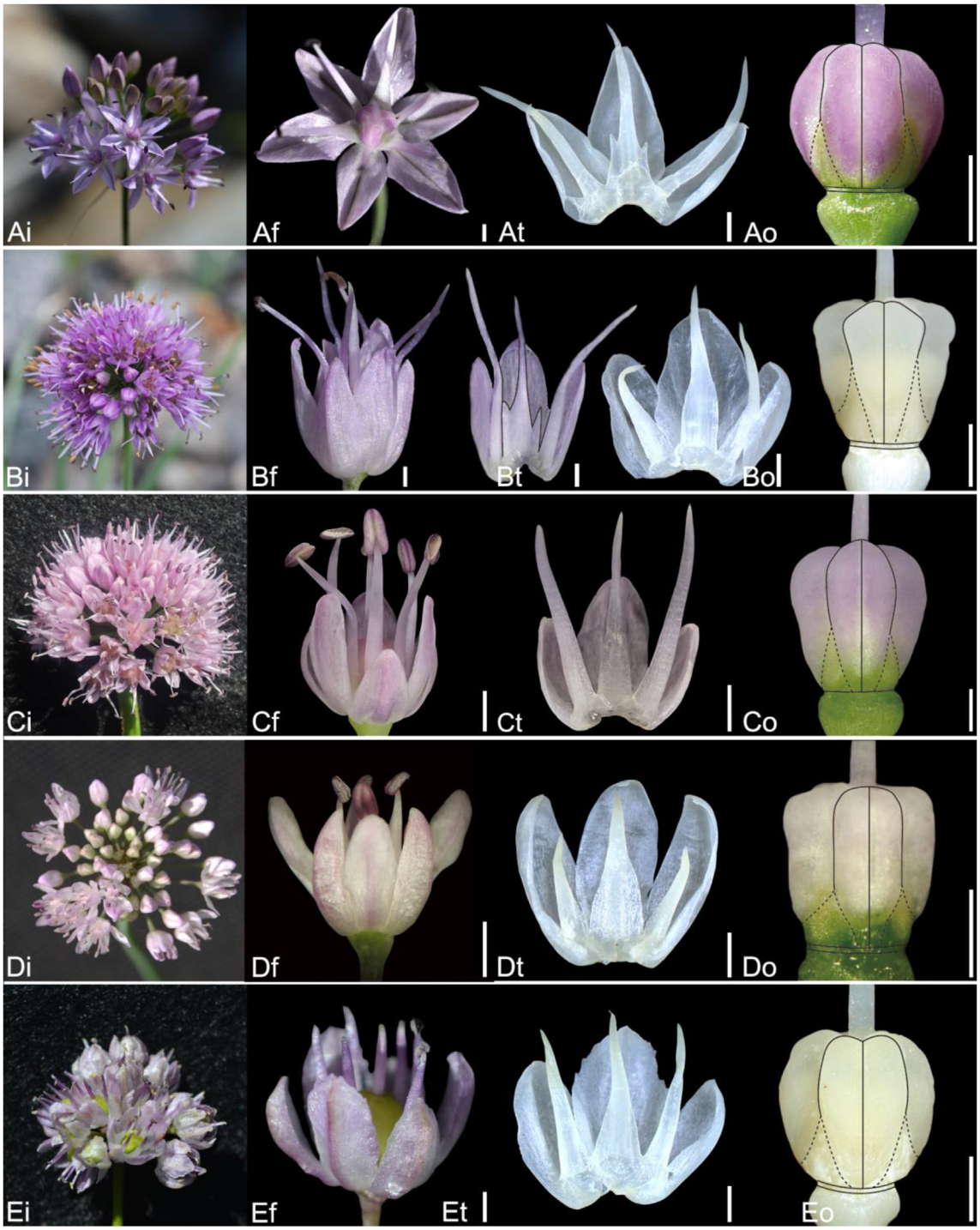


Fig. 14. Floral morphology in *Allium* (A–E) Subg. *Rhizirideum* (A) *A. eduardii* (B) *A. austrosibiricum* (C) *A. dumebuchum* (D) *A. minus* (E) *A. prostratum* (i – inflorescence, f – flower, t – tepal and filament arrangement, o – ovary). Scale bars = 1 mm.

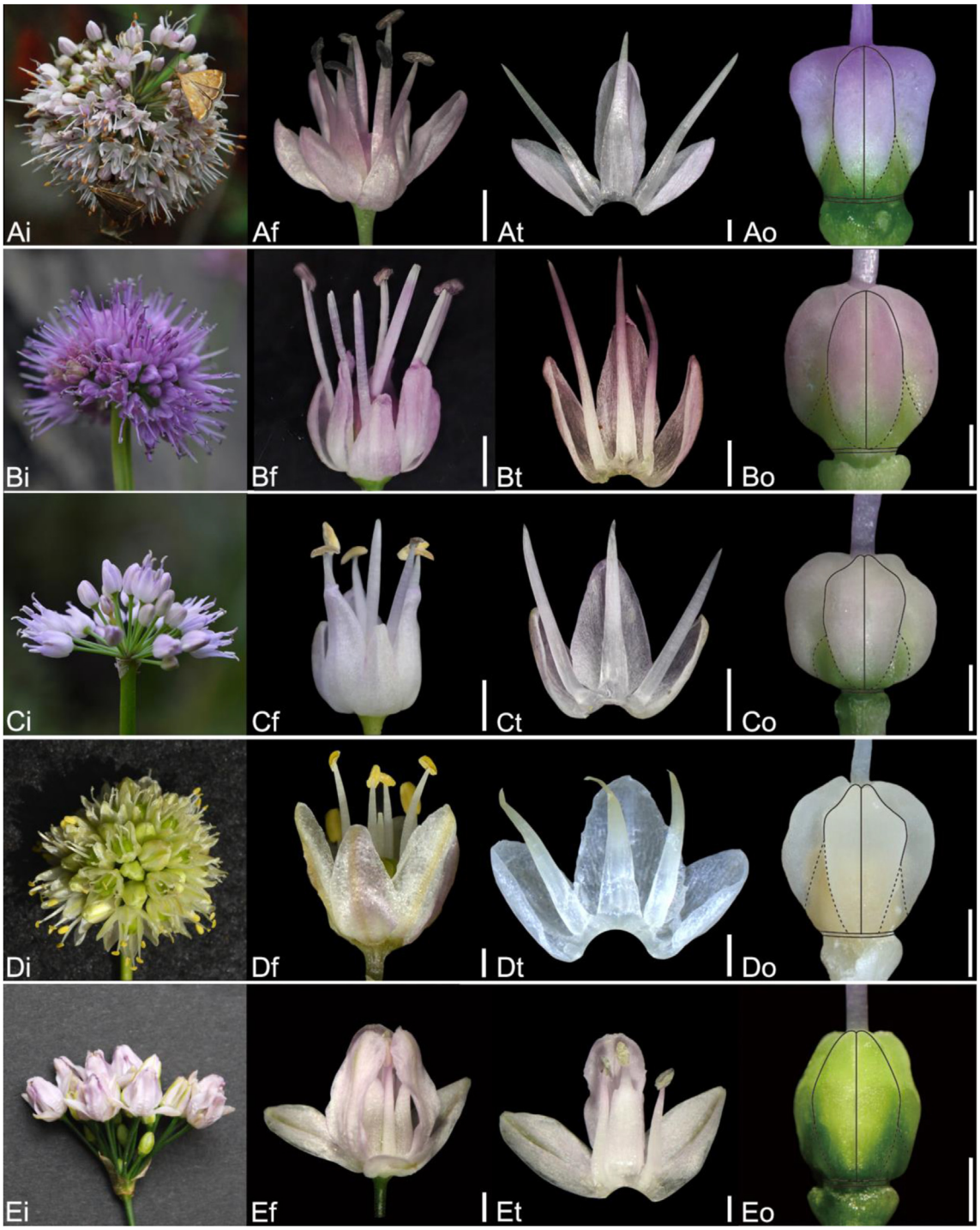


Fig. 15. Floral morphology in *Allium* (A–E) Subg. *Rhizirideum* (A) *A. senescens* (B) *A. spirale* (C) *A. spurium* (D) *A. tuvinicum* (E) *A. bidentatum* (i – inflorescence, f – flower, t – tepal and filament arrangement, o – ovary). Scale bars = 1 mm.

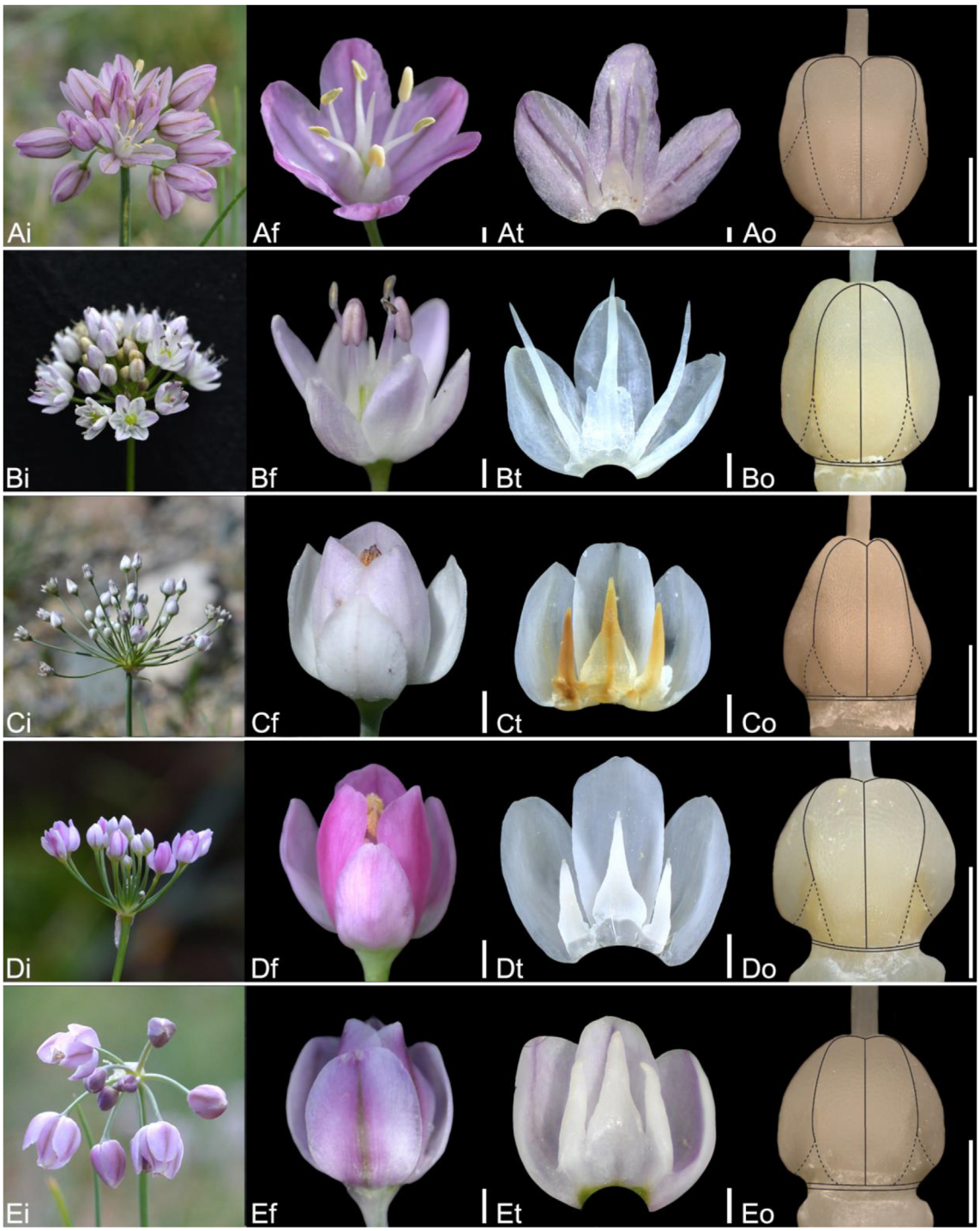


Fig. 16. Floral morphology in *Allium* (A–E) Subg. *Rhizirideum* (A) *A. mongolicum* (B) *A. polyrhizum* (C) *A. anisopodium* (D) *A. tenuissimum* (E) *A. vodopjanovae* (i – inflorescence, f – flower, t – tepal and filament arrangement, o – ovary). Scale bars = 1 mm.

(Fig. 18B). However, the combined cluster plot displays 83% of the total variation and the species that were better separated (Fig. 18C). In this subgenus, four quantitative characteristics, including perianth shape, reflexed point of tepal apex, filament margin toothed, and filament toothed position, are most important (Fig. 18C).

In sect. *Schoenoprasum, A. karelinii* is more similar to *A. schoenoprasum* var. *schoenoprasum* than to its variety, *A. schoenoprasum* var. *foliosum,* with 9–14 mm long tepals, pedicels usually shorter than perianth, tepals often with a reflexed point at the tip (Vvedensky, 1935; Ohwi, 1965); however, there is a slight difference between them, for example, *A. karelinii* has a purplish violet perianth (vs reddish purple) and obovate-lanceolate tepals (vs oblong-lanceolate) (Fig. 8C and D).

Molecular studies have failed to clearly distinguish species within sect. *Sacculiferum* (Choi et al., 2012). We found that floral morphology does not provide a statistically significant approach to delimiting species within this section either (Fig. 7). According to Choi (2009), the floral characteristics of this section show

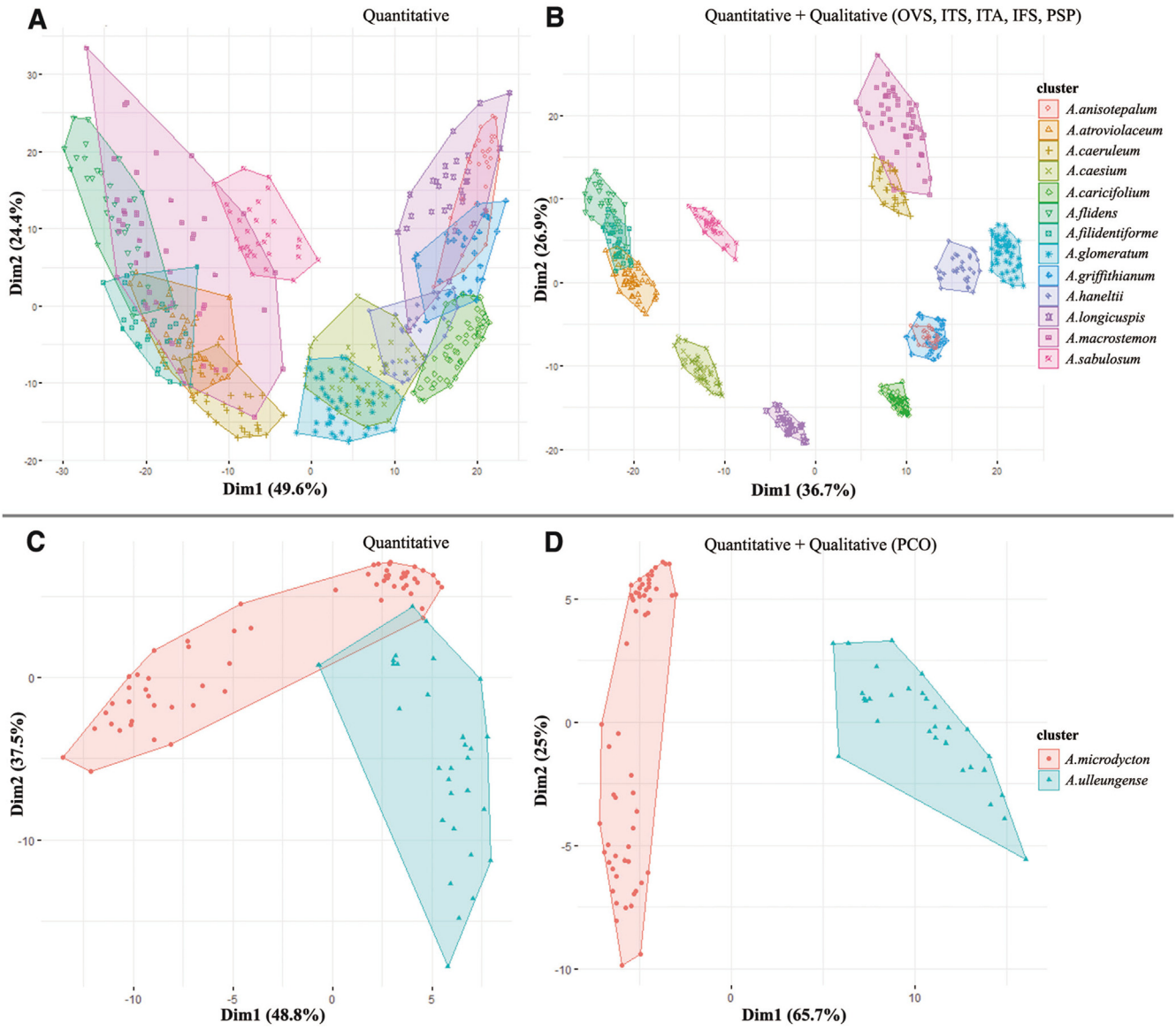


Fig. 17. Cluster analyses at the specific level within subgenera (A, B) Subg. *Allium* (A) cluster plot with only quantitative factors (B) cluster plot with combined quantitative and qualitative factors strongly associated with clustering (C, D) Subg. *Anguinum* (C) cluster plot with only quantitative factors (D) cluster plot with combined quantitative and qualitative factors strongly associated with clustering.

continuous variation among taxa. In addition, the polyploid is especially common in this group (Baasanmunkh et al., 2018). The taxa of sect. *Sacculiferum* can be distinguished by features such as the leaf blade (the texture, the color, the shape in cross-section) and the position of scape from bulbs during development (Kim et al., 2021). However, the main characteristics for classification are limited to vegetative organs that are affected by the environment. In addition, it has been difficult to distinguish between two species in this section: *A. pseudojaponicum* (Fig. 7B) and *A. thunbergii* (Fig. 7D and E), and their reproductive characteristics are not distinctly discontinuous (Choi, 2009). Similarly, Baasanmunkh et al. (2018) also suggested that *Allium sacculiferum* be considered as a synonym of *A. thunbergii,* because it does not clearly fulfill the requirements of any species concept. Therefore, further comprehensive studies of sect. *Sacculiferum* are needed to better understand their taxonomic rank.

3.3.5. *Subgenus* Melanocrommyum *(Webb & Berth.) Rouy*

This subgenus comprises over 150 species and represents one of the largest subgenera in *Allium* (Gurushidze et al., 2008; Fritsch, 2012; Friesen et al., 2021). In this study, four species belonging to four sections (*Compactoprason, Longibidentata, Megaloprason,* and *Miniprason*) were investigated. The quantitative (84% of the total variation) and combined cluster plots (90% of the total variation) separated *A. karataviense* (sect. *Miniprason*) from *A. fetisowii* (sect. *Longibidentata*), while *Allium giganteum* (sect. *Compactoprason*) and *A. stipitatum* (sect. *Megaloprason*) remained overlapping (Fig. 19A and B). The most important characteristic among the studied species of this subgenus is perianth color (Fig. S7).

3.3.6. *Subgenus* Polyprason *Radić*

The subg. *Polyprason* belongs to the third evolutionary line, which includes over 50 species and four sections (Friesen et al.,

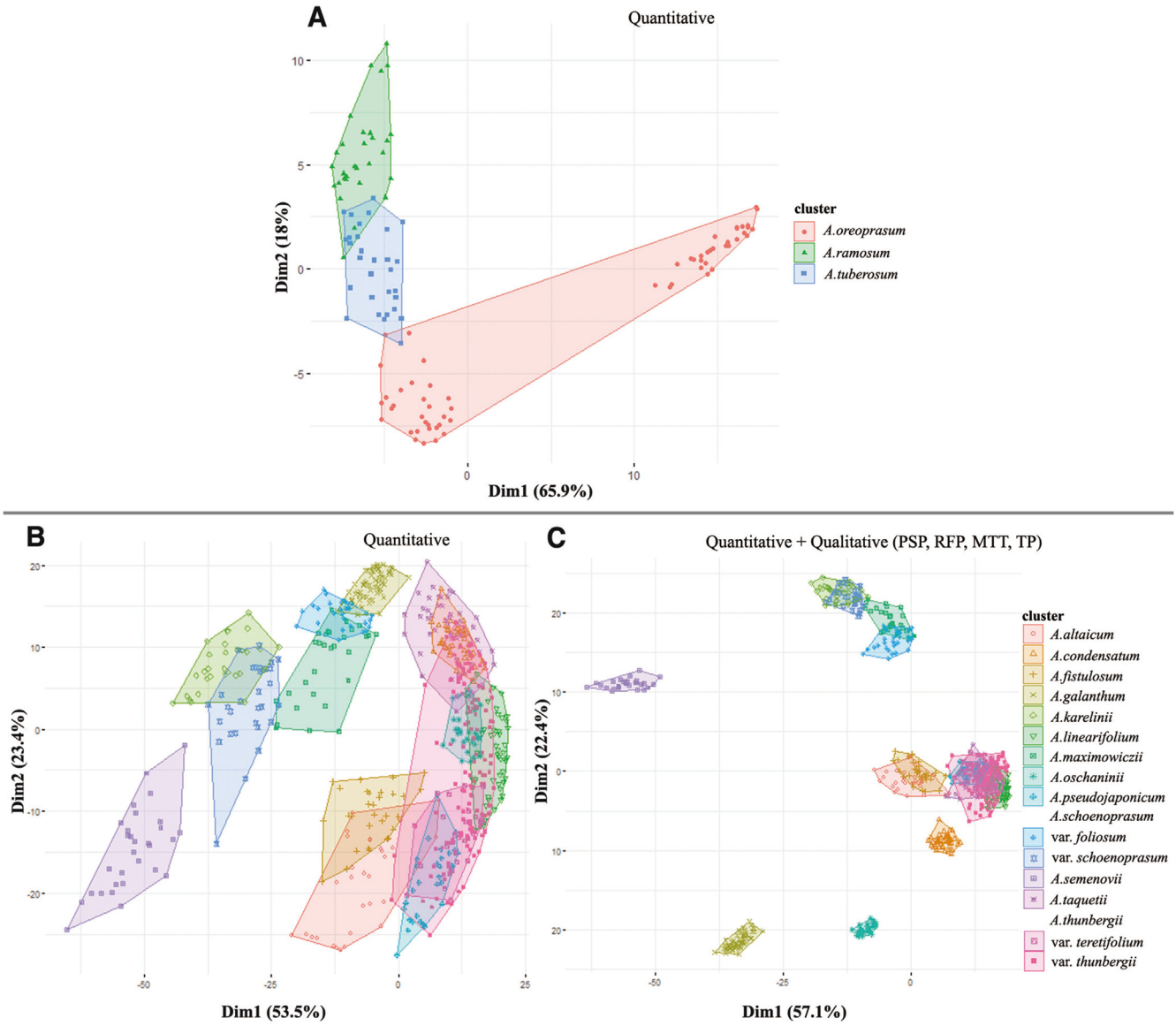


Fig. 18. Cluster analyses at the specific level within subgenera (A) Subg. *Butomissa*: cluster plot with only quantitative factors (B, C) Subg. *Cepa* (B) cluster plot with only quantitative factors (C) cluster plot with combined quantitative and qualitative factors strongly associated with clustering.

2006; Bartolucci et al., 2022). Nine taxa (including one subspecies) belonging to two sections (*Falcatifolia* and *Oreiprason*) were investigated. The quantitative cluster plot shows approximately 73.8% of the total variation, wherein species were not clearly separated, except for *Allium korolkowii* (Fig. 19C). For the studied taxa here, 73.4% of the total variation can be explained by quantitative characteristics and five important qualitative characteristics, specifically, perianth shape, perianth color, inner tepal shape, reflexed point of tepal apex, and inner filament shape, except for in *A. obliquum* and *A. petraeum* (sect. *Oreiprason*). The flower morphology of both species is quite similar (Figs. 10E and 11A).

3.3.7. *Subgenus* Reticulatobulbosa *(Kamelin) N. Friesen*

This subgenus comprises over 80 species belonging to four sections and is placed in the third evolutionary line (Friesen et al., 2006; Li et al., 2010). In this study, 12 species belonging to three sections (*Campanulata, Reticulatobulbosa,* and *Scabriscapum*) were investigated. The quantitative cluster plot showed approximately 72.7% of the total variation where species are generally gathered (Fig. 20A); in contrast, the combined quantitative and qualitative cluster plot showed 79.1% of the total variation wherein most species are well separated (Fig. 20B). In this subgenus, the most important characteristics among the studied taxa are perianth shape, perianth color, inner tepal shape, filament relative length compared with perianth, and inner filament shape (Fig. S7). Notably, in subg. *Reticulatobulbosa*, *Allium koreanum* and *A. malyschevii* (sect. *Reticulatobulbosa*) have similar floral morphology; however, it differs by the color and shape of perianth (Fig. 12C and E).

3.3.8. *Subgenus* Rhizirideum *(G. Don ex Koch) Wendelbo*

The subgenus *Rhizirideum* includes over 40 species belonging to six sections and is placed in the third evolutionary line (Friesen et al., 2006, 2020; Jang et al., 2021). In this study, 19 accessions of

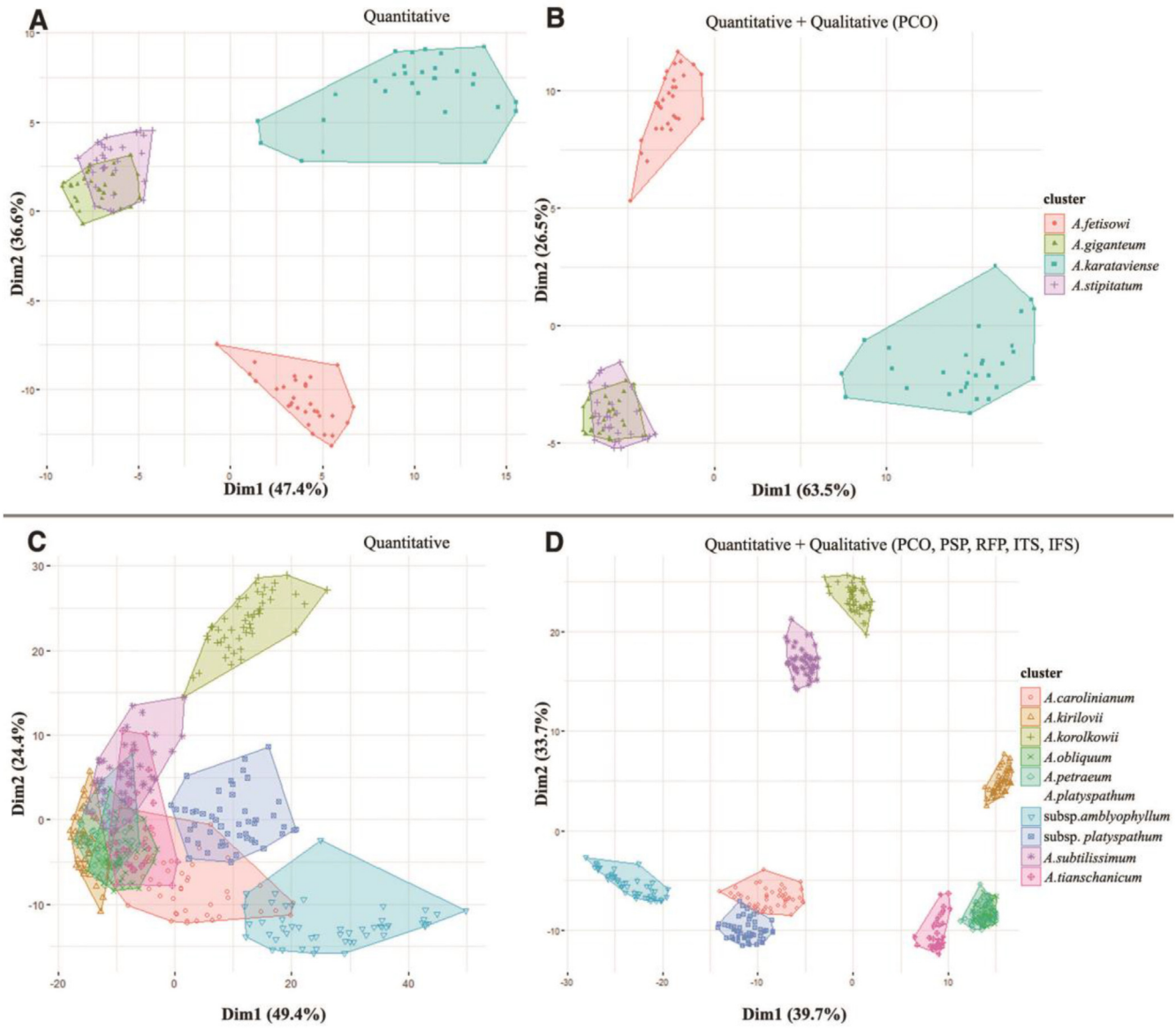


Fig. 19. Cluster analyses at the specific level within subgenera (A, B) Subg. *Melanocrommyum* (A) cluster plot with only quantitative factors (B) cluster plot with combined quantitative and qualitative factors strongly associated with clustering (C, D) Subg. *Polyprason* (C) cluster plot with only quantitative factors (D) cluster plot with combined quantitative and qualitative factors strongly associated with clustering.

15 species belonging to four sections (*Caespitosoprason, Eduardia, Rhizirideum,* and *Tenuissima*) were investigated. Based on a quantitative cluster plot, almost all species (87.5% of the total variation) were distributed on the left side except *Allium dumebuchum* (Fig. 20C). The combined cluster plot shows that 62.6% of the total variation was well separated, except *A. spirale, A. spurium* (sect. *Rhizirideum*), *A. tenuissimum, A. anisopodium,* and *A. vodopjanovae* (sect. *Tenuissima*) (Fig. 20D). In this subgenus, the most important characteristics are perianth shape and color, inner tepal apex and shape, filament relative length, inner filament shape eight, filament toothed position, and ovary shape (Fig. S7).

In sect. *Tenuissima*, the flower morphology of *Allium tenuissimum, A. anisopodium,* and *A. vodopjanovae* are remarkably similar and all have obovoid inner tepals, non-exserted filaments, and an ovoid ovary; however, they are slightly distinguished by shape, size, and apex of outer tepals (Fig. 16C–E). In addition, these species are possibly inter-species hybridizations in the wild (Friesen, personal observation).

3.4. Morphological characteristics of selected flowers traits in the phylogenetic tree

Previous phylogenetic studies have shown that *Allium* is monophyletic and includes three main evolutionary lines (Friesen et al., 2006; Li et al., 2010). However, phylogenetic evidence has been unable to confirm that species from subgenera *Allium*, *Cepa*, *Reticulatobulbosa*, and *Polyrason* (i.e., species in the third evolutionary line) are monophyletic (Friesen et al., 2006; Li et al., 2010; Xie et al., 2020a; Yang et al., 2023). Species in these subgenera differ in a variety of ways (Khan et al., 2022). In this study, we examined how five important morphological traits of flowers differ between these species and whether those traits mapped onto a phylogenetic tree (Fig. 21). Our findings indicate that the morphological characters of flowers also do not highlight monophyletic problems in the above-mentioned subgenera (Fig. 21). There is also no consensus on the phylogenetic significance of testa cell characteristics and whether they have been reflected in the different

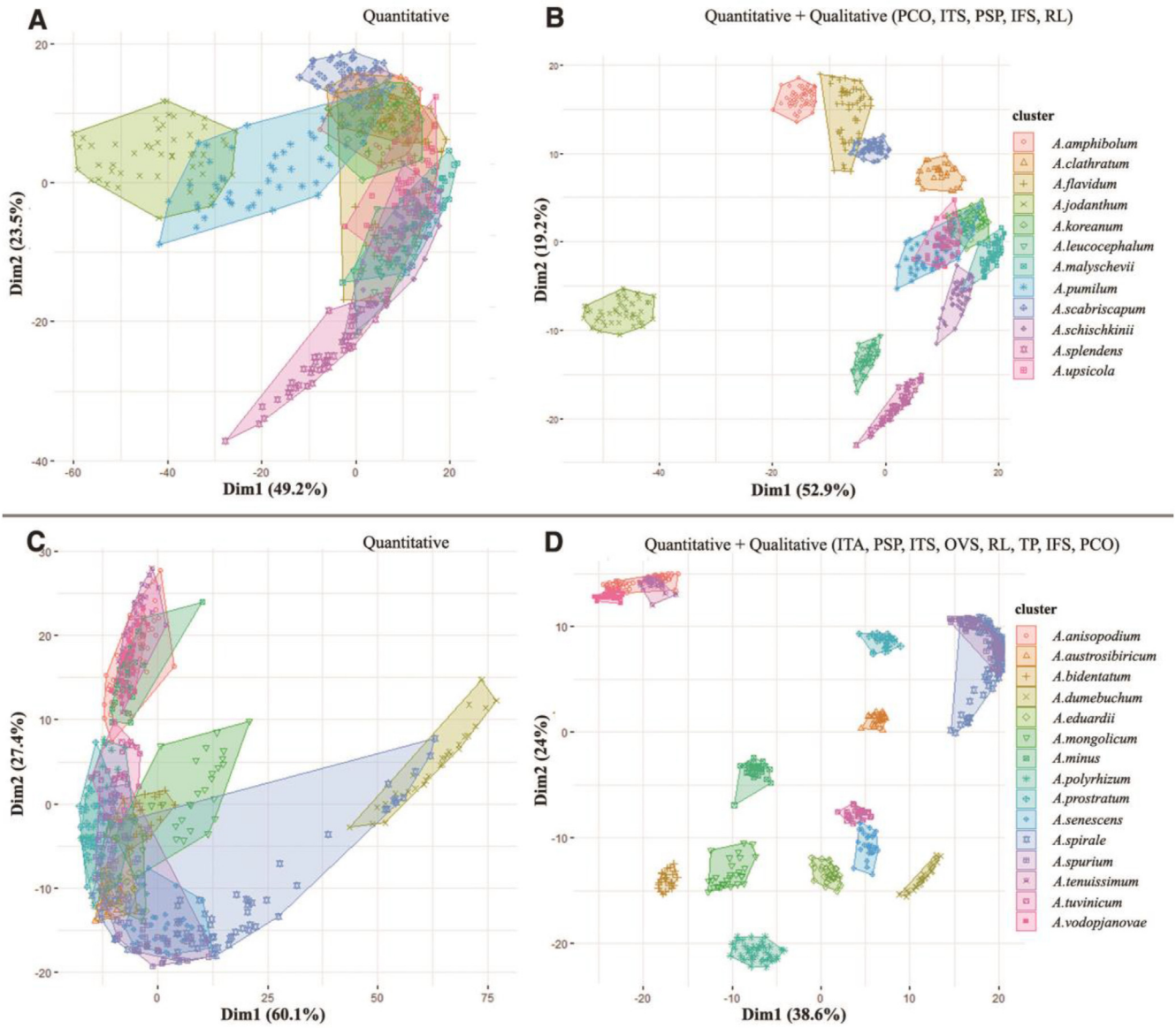


Fig. 20. Cluster analyses at the specific level within subgenera (A, B) Subg. *Reticulatobulbosa* (A) cluster plot with only quantitative factors (B) cluster plot with combined quantitative and qualitative factors strongly associated with clustering (C, D) Subg. *Rhizirideum* (C) cluster plot with only quantitative factors (D) cluster plot with combined quantitative and qualitative factors strongly associated with clustering.

evolutionary levels recognized in *Allium* (see Yusupov et al., 2022). However, pistil characteristics have been strongly correlated with three major evolutionary lines (Fig. 21). For instance, except for subgenera *Butomissa* and *Rhizirideum*, members of the third evolutionary lineage possess hood-like appendages in the ovary (Fig. 21). Ovule numbers are key taxonomic characters for subg. *Angunium* species, which is placed in the second evolutionary line and which have only one ovule per locule, as expected in all the taxa studied (Fig. 21). Reticulate-fibrous bulb tunics of *Allium eduardii* (subg. *Rhizirideum*) are similar to those of subg. *Reticulatobulbosa*, but are distinct from those subg. *Reticulatobulbosa*, which have ovaries without appendages. *A. monanthum* and *A. semenovii*, which are placed at the first and third evolutionary lines, have only trigonous ovary styles and 3-cleft stigma among the taxa studied, respectively (Fig. 21). Therefore, the pistil characters of *Allium semevonii* require further study. Furthermore, this study has shown that the diversity and richness of pistil morphology (e.g., the shape of style and ovary) are useful for elucidating the specific species of *Allium*.

4. Conclusion

This comparative study on floral morphology of 74 *Allium* taxa confirmed that floral morphology provides useful taxonomic information for species delimitation in *Allium*. The quantitative characteristics of floral traits, which refer to their size, have been used to identify *Allium* species. However, we observed high variability in the size of the floral factors, showing a wide range of clusters and overlapping with other groups in the cluster plot with only quantitative factors. Therefore, a combination of qualitative and quantitative factors is required to address the taxonomy of *Allium*. In this study, we determined which qualitative characteristics influence clustering in each subgenus. Four quantitative characteristics—ovary shape, perianth color, perianth shape, and inner tepal apex—were strongly

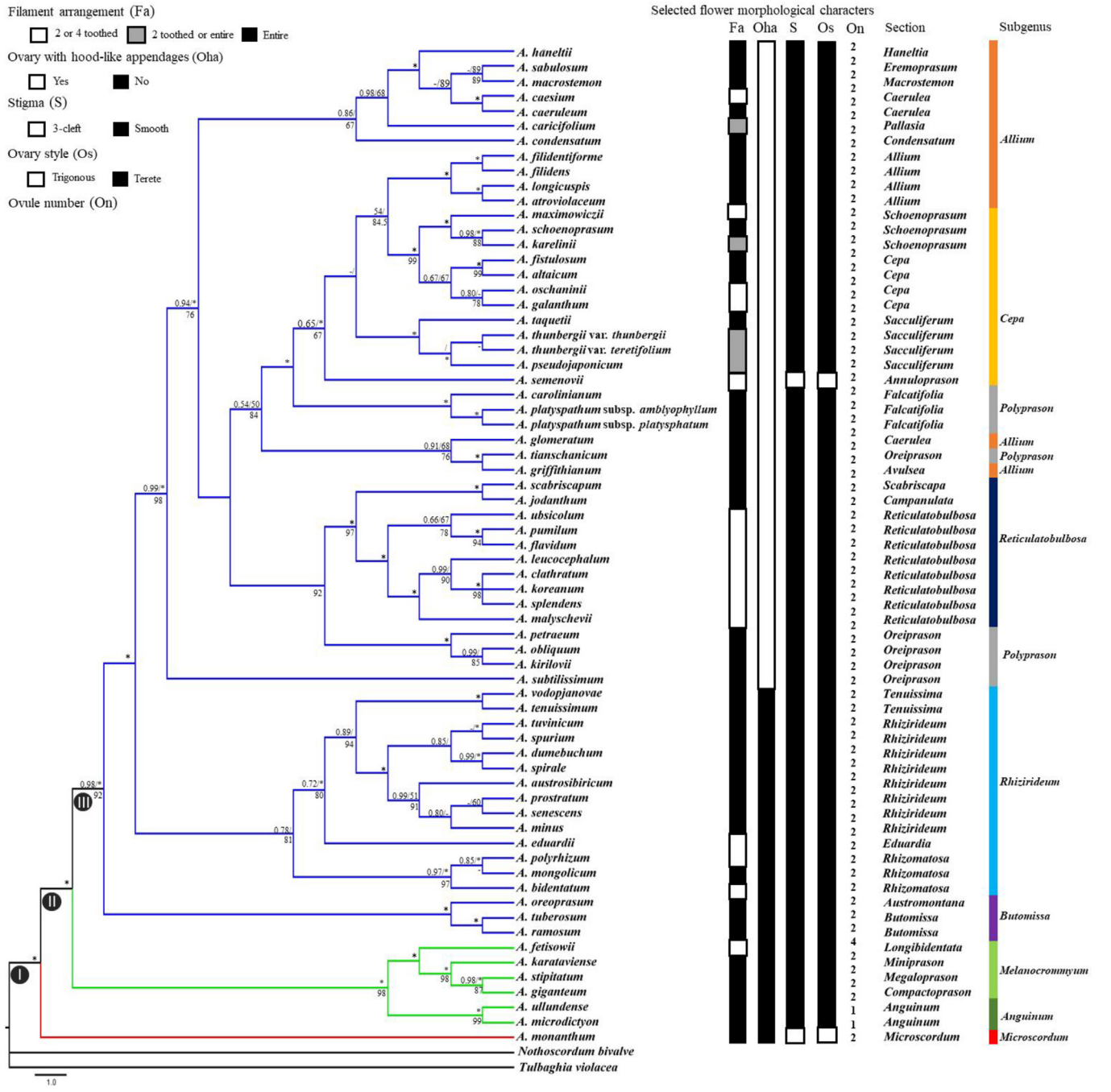


Fig. 21. Selected flower morphological characteristics on phylogenetic tree. Phylogenetic tree was constructed with Bayesian inference posterior probability/maximum parsimony, which are given on each branch; maximum likelihood is shown below branches. Asterisks indicate maximum support values, bold asterisks indicate all maximum values of each values, and values lower than 50% are excluded. I–III and branch colors represent major clades.

associated with clustering among the studied subgenera. In particular, perianth color was shown to be a key characteristic within subg. *Melanocrommyum*, *Polyprason*, and *Reticulatobulbosa*. Ovary shape, perianth shape, and inner tepal apex were significant characteristics within subg. *Allium*, *Cepa*, *Rhiziridium*. Our results also indicated that flower morphology of several species within various sections are not clearly distinguished, such as sect. *Sacculiferum* (subg. *Cepa*) and sect. *Tenuissima* (subg. *Rhiziridium*). Despite their importance, floral studies have been limited because observations on fresh material must be made in a specific season, and dry specimens do not provide satisfactory results (Pandey et al., 2022). Our findings suggest that understanding *Allium* phylogeny requires that more attention be paid to floral organs during field expeditions. In particular, inflorescences must be photographed in the field using a color chart and be preserved in 70% ethanol for subsequent measurement. The present study provides a basis for future studies on infrageneric relationships and evolutionary trends in *Allium* and other genera with a complicated taxonomy.

Author contributions

HJC designed research. HJC, SB, KT and ZY collected samples. JEJ, SB, NN, SOY and HJC analyzed data. SB and JEJ writing – original draft. All the authors revised and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pld.2023.06.009.

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